ROLE OF PHYSICAL ACTIVITY, PHYSICAL FITNESS, AND EXERCISE ON IMMUNOMETABOLISM DURING PREGNANCY

DOCTORAL PROGRAMME IN BIOMEDICINE

Pedro Acosta-Manzano

UNIVERSIDAD DE GRANADA

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Pedro Acosta-Manzano

Director de la Tesis Doctoral [Doctoral Thesis Supervisor]

Virginia A. Aparicio García Molina
PhD
Profesora Titular
Universidad de Granada,
España

Víctor Segura Jiménez
PhD
Profesor Ayudante Doctor
Universidad de Cádiz
España

Miembros del Tribunal [Doctoral Thesis Comittee]

Jesús F. Rodríguez Huertas
PhD
Catedrático de Universidad
Universidad de Granada,
España

Luis Gracia Marco
PhD
Investigador Senior
Universidad de Granada,
España

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PhD
Professor
University of Western Ontario,
Canada

Kristi B. Adamo
PhD
Associate Professor
University of Ottawa,
Canada

Rubén Barakat Carballo
PhD
Profesor Titular y Vicedecano de
Investigación y Doctorado
Universidad Politécnica de Madrid,
España

Granada, 12 de Diciembre de 2020

A mis padres, hermanos y amigos cercanos. En especial, a aquellas personas que me ayudaron a descubrir mi pasión por la ciencia

To my parents, siblings, and close friends. Specially, to those who helped me to keep the flame of science alive

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RESEARCH PROJECTS AND FUNDING

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ABSTRACT/RESUMEN

ABSTRACT

Pregnancy induces extraordinary immunometabolic changes in women's physiology to support maternal, placental and foetal demands during gestation, and thereby ensure a successful pregnancy. However, this period is also very susceptible to dyshomeostasis, and in some women characterized by an unfavourable genotype *(e.g. diabetic family history)*, or exposed to an adverse lifestyle *(e.g. obesity, sedentary lifestyle)*, these alterations might lead to short and long-term adverse outcomes. Indeed, the adverse consequences related to a dysfunctional metabolic machinery in pregnancy have the potential to negatively affect not only one life, but two (the mother and offspring), and possibly next generations. Thus, the spotlight of reference institutions nowadays is on searching effective strategies to promote an optimal maternal and intrauterine environment, and break the maternal-foetal intergenerational diabesity cycle. In this regard, physical activity (PA), physical fitness (PF) and exercise could be promising tools to optimise metabolic control during pregnancy, and thus avoid potential complications and future diseases. Unfortunately, evidence is very scarce and elusive, and many questions remain still unrevealed. In the current Doctoral Thesis, we address knowledge gaps, and provide a greater insight on i) the role of sedentary time (ST), PA, PF and exercise in immunometabolism during pregnancy; and ii) the underlying mechanisms by which these stimuli might induce metabolic changes. We show that:

Increasing moderate-to-vigorous PA levels, or meeting PA recommendations, could be of utility to modulate the cytokine profile of women in early to middle pregnancy; but not reducing ST (**Study I**). Additionally, lower ST in early to middle pregnancy is related to higher expression of placental genes related to lipid transport in overweight-obese women (**Study II**); but moderate-to-vigorous PA has little effect. However, we could not identify any metabolic factor underlying the relationship between lifestyle and placental metabolism (**Study II**). Increased PF, especially CRF and muscle strength in early to middle pregnancy, is related to an improved metabolic phenotype, and may confer a cardio-protector effect in maternal metabolism (**Study III**); also indirectly via potentially reducing excessive gestational weight-gain (**Study VI**). A concurrent exercise program during pregnancy appears to be effective to modulate cytokines in pregnant women without metabolic disruptions and their foetuses (**Study IV**). However, its direct effects

on other immunometabolic markers such as glucose, lipids, C-reactive protein, etc. are hardly appreciable (**Study V**). Of note, few cytokines appear to mediate some of the effects of exercise into maternal metabolism (**Study V**). Moreover, exercise robustly reduces maternal weight-gain during pregnancy and postpartum weight-retention, independently of other lifestyle behaviours and PF (**Study VI**). Although exercise is not able to avoid excessive gestational weight-gain, it appears to protect against the impaired metabolic phenotype related to exacerbated weight-gain (**Study VI**).

Thus, the findings from the present Doctoral Thesis increase our knowledge on the role of ST, PA, PF and exercise on immunometabolism during pregnancy, and on the underlying mechanisms by which these stimuli might be translated into metabolic changes.

RESUMEN

El embarazo induce adaptaciones metabólicas importantes en las mujeres embarazadas que son necesarias para cumplir con las demandas maternas, placentarias y fetales durante la gestación, y asegurar un embarazo exitoso. Sin embargo, este periodo es muy susceptible a desregulaciones metabólicas, y en algunas mujeres con genotipo adverso (ej. historial familiar de diabetes) o hábitos inadecuados (ej. obesidad, sedentarismo), dichas alteraciones pueden ser perjudiciales y dar lugar a consecuencias adversas a corto y largo plazo. De hecho, un metabolismo disfuncional durante el embarazo podría afectar negativamente no solamente a la vida de la madre y al feto, sino también posiblemente a las futuras generaciones. De ahí que las principales instituciones de referencia a día de hoy sigan buscando estrategias efectivas para favorecer un ambiente materno e intrauterino óptimo, y romper así el ciclo intergeneracional de obesidad-diabetes. En este sentido, la actividad física (AF), condición física y el ejercicio físico son herramientas potenciales para optimizar el control metabólico durante el embarazo, y así evitar posibles complicaciones y enfermedades futuras. Desafortunadamente, la evidencia científica al respecto es muy escasa e imprecisa a día de hoy, y muchas preguntas permanecen sin respuesta. En la presente Tesis Doctoral, proporcionamos un mayor conocimiento acerca del papel que el sedentarismo, actividad física, condición física y ejercicio desempeñan en el inmunometabolismo durante el embarazo, y de los mecanismos por los cuales dichos estímulos podrían inducir cambios metabólicos. Mostramos que:

Mayores niveles de AF moderada-vigorosa y cumplir con las recomendaciones de AF (pero no menor tiempo de sedentarismo), parecen ser herramientas útiles para modular las concentraciones de citoquinas en mujeres embarazadas durante la gestación temprana (**Estudio I**). Además, un menor tiempo de sedentarismo durante la gestación temprana se relaciona con una mayor expresión placentaria de genes relacionados con el transporte lipídico en mujeres con sobrepeso y obesidad (**Estudio II**); mientras que la AF moderada-vigorosa apenas tiene efectos. Sin embargo, no pudimos identificar ningún factor metabólico subyaciendo la relación entre el estilo de vida y metabolismo placentario (**Estudio II**). Una mejor condición física, específicamente capacidad cardiorrespiratoria y fuerza muscular en la gestación temprana, se relaciona con un

mejor fenotipo metabólico, y parece conferir un efecto cardioprotector en el metabolismo materno (**Estudio III**); también indirectamente a través de reducir potencialmente las ganancias de peso excesivo (**Estudio VI**). Un programa de ejercicio concurrente durante el embarazo parece ser efectivo para modular positivamente las citoquinas en las mujeres embarazadas sin disrupciones metabólicas, y en sus fetos (**Estudio IV**). Sin embargo, los efectos directos del ejercicio sobre otros marcadores inmunometabólicos como la glucosa, lípidos, proteína C-reactiva, etc. son casi nulos o difícilmente apreciables (**Estudio V**). Cabe destacar que algunas citoquinas parecen mediar algunos de los efectos del ejercicio sobre el metabolismo materno (**Estudio V**). Además, el ejercicio reduce robustamente las ganancias de peso maternas durante el embarazo y la retención de peso postparto, independientemente del estilo de vida y de la condición física (**Estudio VI**). Aunque el ejercicio no sea capaz de prevenir las ganancias de peso excesivo, parece que puede proteger contra el fenotipo metabólico adverso relacionado con dichas ganancias (**Estudio VI**).

Por lo tanto, en la presente Tesis Doctoral proporcionamos evidencia científica sobre el papel del sedentarismo, AF, condición física y ejercicio sobre el inmunometabolismo durante el embarazo, y sobre los mecanismos subyacentes por los cuales estos estímulos podrían dar lugar a cambios metabólicos.

ABBREVIATIONS

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ACOG: American College of Obstetrics and Gynaecology

Acox: peroxisomal acyl-coenzyme A oxidase 1

Acsl1: acyl-coA synthetase long chain

Aldoa: aldolase, fructose-bisphosphate A

B: unstandardized regression coefficient

BMI: body mass index

CI: confidence interval

Cpt1: carnitine palmitoyltransferase I

Crat: carnitine O-acetyltransferase

CRF: cardiorespiratory fitness

CRF85%MHR: time to reach the 85%MHR (Bruce treadmill test)

CRF85%THR: time to reach the 85%THR (Bruce treadmill test)

CRP: c-reactive protein

DALI: Vitamin D And Lifestyle Intervention for GDM prevention

DBP: diastolic blood pressure

DNA: deoxyribonucleic acid

DXA: dual-energy X-ray absorptiometry

ELISA: enzyme linked immunosorbent assays

Enol: enolase

Ex-NS: exercise (wheel) pre-conception & exercise gestation

Ex-S: exercise (wheel) pre-conception & exercise+stress gestation

Ex-Tr: exercise (treadmill) pre-conception & exercise gestation

F2F: face to face sessiones

FABP: fatty acid binding protein

FATP: fatty acid transport protein

Fbp: fructose-bisphosphatase

FFA: free fatty acids

FTO: fat mass and obesity associated gene (FTO alpha-ketoglutarate dependent dioxygenase)

G6PB: glucose-6-phosphate dehydrogenase

Abbreviations

GDM: gestational diabetes mellitus

GESTAFIT: Gestation and Fitness

GLUT: glucose transporter

Gpd1: glycerol-3-phosphate dehydrogenase 1

Gpi: glucose-6-phosphate isomerase

GWG: gestational weight gain

Gyk: glycerol kinase

HbA1c: glycated hemoglobin

HDL-C: high density lipoprotein-cholesterol

HE: healthy eating

HOMA:homeostasis model assessment

IFN-γ: interferon gamma

IGF: insulin like growth factor

IL: interlukin

IOM: Institute of Medicine

IQR: interquartile range

IR: insulin resistance

Lcad: long chain acly-CoA dehydrogenase

LDL-C: low density lipoprotein-cholesterol

Lipe: lipase E, hormone sensitive type

LIPP: Lifestyle Intervention in Preparation for Pregnancy

LPA: light physical activity

Lpl: lipoprotein lipase

MAR: missing at random

Mcad: medium-chain acyl-CoA dehydrogenase

MCAR: missing completely at random

MCR4: melanocortin 4 receptor

MDS: mediterranean diet score

MHR: maximum heart rate

MPA: moderate physical activity

mRNA: messenger ribonucleic acid

mTOR: mechanistic target of rapacycin complex

MVPA: moderate-to-vigorous physical activity

OAZ1: ornithine decarboxylase antizyme 1

OGTT: oral glucose tolerance test

PA: physical activity

PARmed-X: physical activity readiness medical examination

PCK: phosphoenolpyruvate carboxykinase

PF: physical fitness

Pfkl: phosphofructokinase

Pgam1: phosphoglycerate mutase

Pgc1a: peroxisome proliferator-activated receptor-γ coactivator 1 alpha

Pgk1: phosphoglycerate kinase

Pklr: pyruvate kinase

PPAR-γ: peroxisome proliferator-activated receptor gamma

PRKAB: protein kinase AMP-activated non-catalytic subunit beta

RCT: randomized controlled trial

RXR: retinoid X receptor

SBP: systolic blood pressure

SD: standard deviation

SE: standard error

Sed: sedentary pre-conception & sedentary gestation

Sed-S: sedentary pre-conception & sedentary+stress gestation

SLC25A20: solute carrier family 25 member 20 (acylcarnitine carrier protein)

SNAT: small neutral amino acid transporters

SNPs: single nucleotide polymorphism

ST: sedentary time

T2DM: type 2 diabetes mellitus

TBP: tata-box-binding protein

THR: target heart rate

TNF-α: tumour necrosis factor-α

Tpi1: triosephosphate Isomerase 1

VAT: visceral adipose tissue

VO2max: maximal oxygen uptake

Abbreviations

VPA: vigorous physical activity

WDR45L: WD repeat-containing protein 45-like

β: B standardized regression coefficient

Δ: delta (change)

GENERAL INTRODUCTION

GENERAL INTRODUCTION

THE COMPLEX NATURE OF PREGNANCY: THE IMPORTANCE OF AN ADEQUATE AND TIMELY INTRAUTERINE PROGRAMMING

Pregnancy is a critical physiological period for women, which implies conspicuous metabolic changes and adaptations[1-4]. Its characteristic endocrine and immunometabolic plasticity is indeed necessary to reprogram maternal physiology during this stage, and promote an adequate maternal-foetal homeostasis[1-5].

Metabolic changes in lean healthy women

In early pregnancy (largely anabolic), lean women with normal glucose tolerance appear to undergo a decrease in insulin sensitivity compared to pre-conception[6], along with an increase in insulin secretion[6] and maternal fat stores (lipogenesis)[7]. These pregnancy-induced changes are necessary to store nutrients, and subsequently be able to meet the maternal-placental-foetal demands in late gestation and lactation[2,3].

In late pregnancy, this predominant anabolic metabolism changes towards a catabolic state, which is characterized by a more accentuated decrease in insulin sensitivity[6], and lower systemic glucose, free fatty acids (FFA) and amino acids[2,7]. Additionally, there is a considerable increase in systemic insulin and insulin release[1,6], endogenous glucose production[8,9], lipolysis, and fat and lean mass[7].

But, *how are these late metabolic responses connected with each other? And why are they important for the maternal-foetal homeostasis?*

The progressive decrease in maternal insulin sensitivity from early pregnancy, which is mainly dependent on the pre-conception insulin sensitivity and B-cell function[1], leads to an increase in insulin secretion, which influences placental phenotype[7]. In the normal course of pregnancy, this continuous mother-placenta-foetus crosstalk appears to be necessary to release placental factors such as hormones and cytokines[1,7]. These placental factors[3] along with other mechanisms (e.g. impaired insulin signalling, lipid metabolism), “negatively” modulate maternal peripheral insulin sensitivity (in liver, skeletal muscle, and adipose tissue), which facilitates nutrients availability towards the foetus[1,3,10]. Thus, these metabolic adaptations are essential to provide the foetuses with

substrates for an optimal development[1-3], while maintaining fuel requirements for mothers, who are preparing for parturition.

Noteworthtily, these changes in maternal metabolism occur simultaneously with fluctuations in immune responses (immunometabolism)[5,11]. Traditionally, it was believed that pregnancy was associated with immune suppression, and thus with increased susceptibility to infectious diseases[5]. However, recent evidence has overcome this myth, showing that the maternal-placental-foetal immune interface is fundamental for biological processes and homeostasis during pregnancy[5,12]. This immune condition is actually very active and well-controlled during pregnancy[12]. Thus, the maternal, placental and foetal immune systems represent a unique-coordinated system that modulate anti- and pro-inflammatory responses according to the trimester of pregnancy, to ensure the maternal and foetal well-being[5,11] (i.e. a successful pregnancy). The first and early second trimesters of pregnancy -*1st immunological phase*- are accompanied by a maternal pro-inflammatory state that is necessary for blastocyst implantation, decidualization and initial placentation[13] (vasculogenesis and formation of capillary networks)[5,12]. Noteworthily, this is a key vulnerability period very susceptible to aberrations and disruptions[11,14]. In fact, this traditionally unperceived period is the origin of multiple common pregnancy complications that arise during late pregnancy[11] (e.g. preeclampsia). This phase is followed by an anti-inflammatory state -*2nd immunological phase*-, during which placental development (angiogenesis[13]) and foetal growth occur[5,12]. Lastly, late pregnancy -*3rd immunological phase*- is characterized by a pro-inflammatory state to prepare the mother and foetus for the parturition (increased myometrium cell contraction; softening of the cervical extracellular matrix; foetal membranes rupture)[15]. Of note, cytokines from maternal, placental and foetal origin have a vital role in all these processes. They are continuously interacting among them, together with other factors such as exosomes and hormones, to balance the pro- and anti-inflammatory states[4,15-17]. Unfortunately, the origin, metabolism and clearance of these cytokines, and their interplay with the maternal-placental-foetal crosstalk, is poorly understood.

Although the mechanisms underlying metabolic alterations still remain a mystery, what is clear is that tightly-coordinated and timely maternal, placental and foetal immunometabolic responses are required during gestation for a healthy

pregnancy[4,5,11,15]. Any error in this complex molecular and biological machinery (e.g. exacerbated pro-inflammatory responses, defects in signalling pathways) could lead to birth defects[11,17], pregnancy complications[4,11,15], and future metabolic diseases[4,7,10].

A DYSFUNCTONAL METABOLIC MACHINERY: MATERNAL AND OFFSPRING SHORT AND LONG-TERM ADVERSE CONSEQUENCES

Obesity and gestational diabetes mellitus

Adverse phenotypes such as obesity and gestational diabetes mellitus (GDM) are closely related to exacerbated immunometabolic alterations, which predispose pregnant women to an increased risk for birth complications and future maternal and offspring diseases[4,7,10]. This situation is especially worrisome if we consider that overweight, obesity[18,19] and GDM[10,19] prevalence is increasing worldwide.

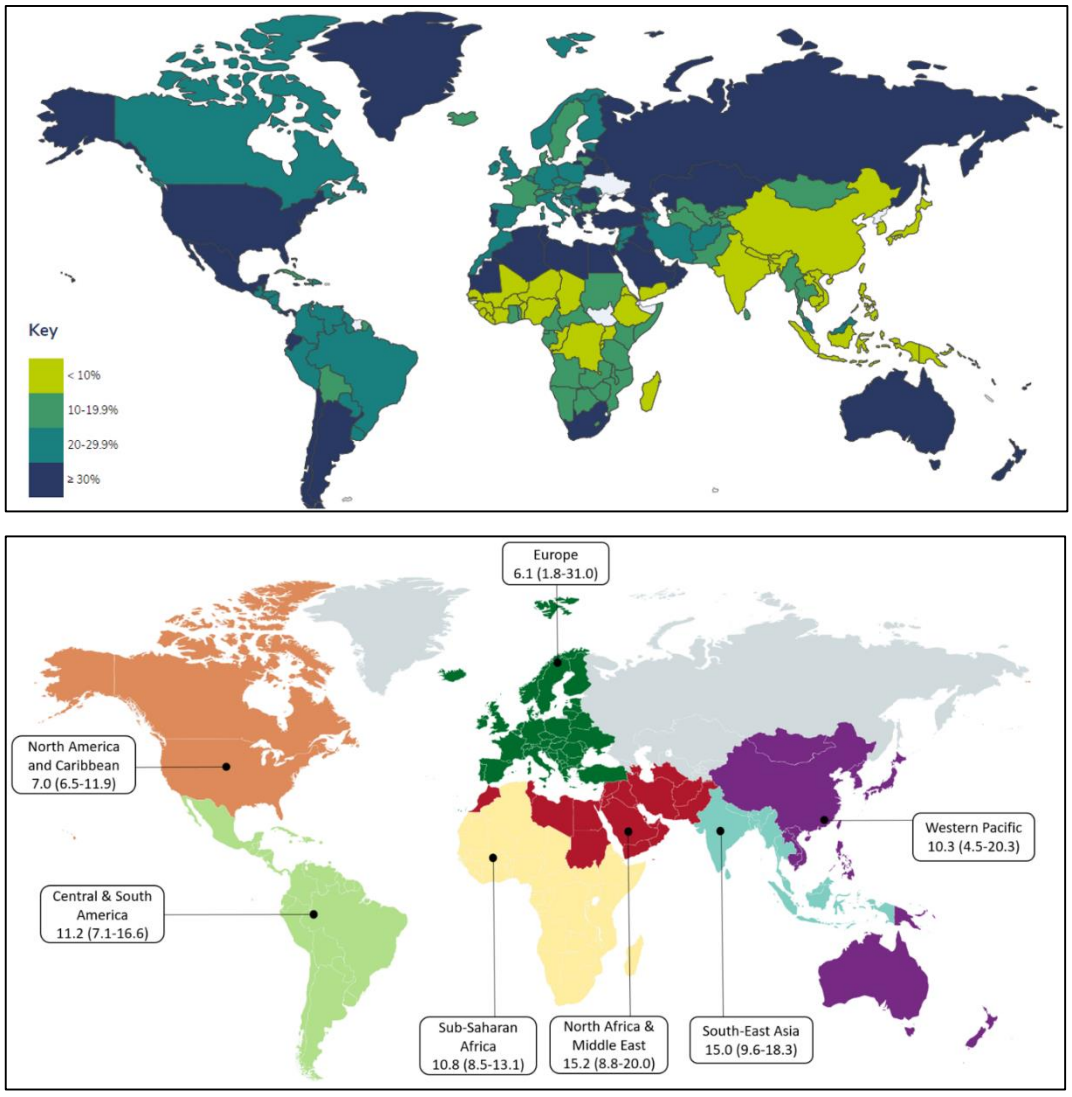

Figure 1. Worldwide prevalence (%) of obesity (upper image: general population) and gestational diabetes mellitus in women (lower image). Upper image obtained from *https://data.worldobesity.org/maps/.*

Lower image adapted from McIntyre et al.[10], and created with *mapchart.net. Of note, the figures indicated above might be inaccurate regarding the screening of GDM, especially in developing countries.*

Below, the main immunometabolic alterations associated with these adverse conditions are introduced.

Metabolic alterations in obesity and GDM

From pre-pregnancy to early-pregnancy, obese women experience small increases in insulin sensitivity, hepatic glucose production and insulin secretion[20]. In late pregnancy, they show higher hepatic production (gluconeogenesis) and insulin levels than lean women with normal glucose tolerance[1], which is indicative of the impaired ability of insulin to suppress endogenous glucose production (i.e. obesity further induces hepatic insulin resistance). However, the increases in insulin levels and total insulin resistance from pre-pregnancy are more pronounced in lean women than in obese women with normal glucose tolerance[1], since lean women usually begin their pregnancies with better insulin sensitivity.

Concerning the lipid metabolism, lipolysis is predominant in early and late gestation in obese women[7,20], which supports the inability of insulin to suppress lipolysis[1]. These changes in lipid metabolism are accompanied by increased triglycerides, total cholesterol, low-density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) with the advance of gestation[2,7,19,20]. Regarding body composition, obese women have similar changes in lean and fat mass compared to lean women, but more accentuated[7].

As pregnancy progresses, obese women with GDM present similar changes to obese women with normal glucose tolerance, but more pronounced: considerable higher insulin resistance, glucose and insulin levels, and suppression of hepatic glucose production[20]. Interestingly, they also show an impaired ability of insulin to supress FFA levels[1]. This evidence indicates that the impaired insulin sensitivity in specific tissues, along with defects in B-cell function (lower insulin secretion relative to the decrease in insulin sensitivity), leads to lower glucose tolerance[1] and maternal hyperglycaemia. Moreover, women with obesity and GDM are usually characterized by low grade tissue-specific and systemic inflammation that affect insulin signalling, and contribute to insulin resistance and dysfunctional metabolism[7,19,21].

Metabolic dysfunction in obesity and GDM - Adverse outcomes for the maternal-foetal health

But how are the above mentioned metabolic derangements *(e.g. hyperglycaemia and insulin resistance) connected with pregnancy complications?*

In 1952, Jorgen Perdersen was one of the first researchers linking gestational metabolic derangements and pregnancy complications, when he stated that maternal hyperglycaemia resulted in foetal hyperglycaemia and hyperinsulinemia (foetal islet tissue hypertrophy), and subsequent macrosomia[19]. This hypothesis was an incredible stimulus for subsequent generations of researchers to investigate the metabolic dysfunction and related-pregnancy disorders. Indeed, from then on, smart evidence proposing related interconnected mechanisms has been generated.

To comprehend the pathophysiology of these adverse conditions, one needs first to recognize the metabolic changes occurring during "healthy and non-healthy" pregnancies (see the previous section). Additionally, it is critical to understand that pregnancies initiated with pre-gravid risk factors such as obesity, excessive inflammation, prediabetes, etc. are characterized by unperceived metabolic dysfunction (e.g. decreased insulin sensitivity and B-cell defects) that manifest later in pregnancy.

In these metabolically dysfunctional women, with the onset of pregnancy and the associated immunometabolic changes, insulin is less effective in boosting glucose uptake by skeletal muscle, liver and adipose tissue, and in supressing endogenous glucose production[8,20]. This, along with the influence of placental factors[21,22], provoke an excessive endogenous glucose production and peripheral insulin resistance (defective insulin signalling[23-29]), which leads to maternal hyperglycaemia and exacerbated availability of nutrients for the placental-foetal growth[7,10]. This situation is indeed potentially harmful for both the mother and foetus[21].

Of note, both excessive maternal fasting and postprandial glucose levels, have been strongly associated with pregnancy complications and future maternal-offspring metabolic disruptions[30-37]. Regarding the fetoplacental unit, these adverse metabolic alterations create an environment of excessive nutrients in which the placenta and foetus develop[19]. The excessive availability of nutrients lead to greater transport of glucose, lipids and amino acids through the placenta, as well as greater insulin levels which induce foetal hyperinsulinemia[7,10]. The exacerbated insulin levels in the foetal

compartment stimulate the use of excessive glucose to synthesize foetal FFA (hepatic lipogenesis); and these foetal FAA together with maternal FFA (transported via placenta) are used to synthesize triglycerides, which are stored as fat in white adipocytes (i.e. foetal overgrowth)[10,21].

In summary, maternal hyperglycaemia and foetal hyperinsulinemia negatively modulate the intrauterine environment, leading to short and long-term consequences for both the mother (e.g. preeclampsia, type 2 diabetes mellitus) and the foetus (e.g. neonatal adiposity, obesity)[7,10,21].

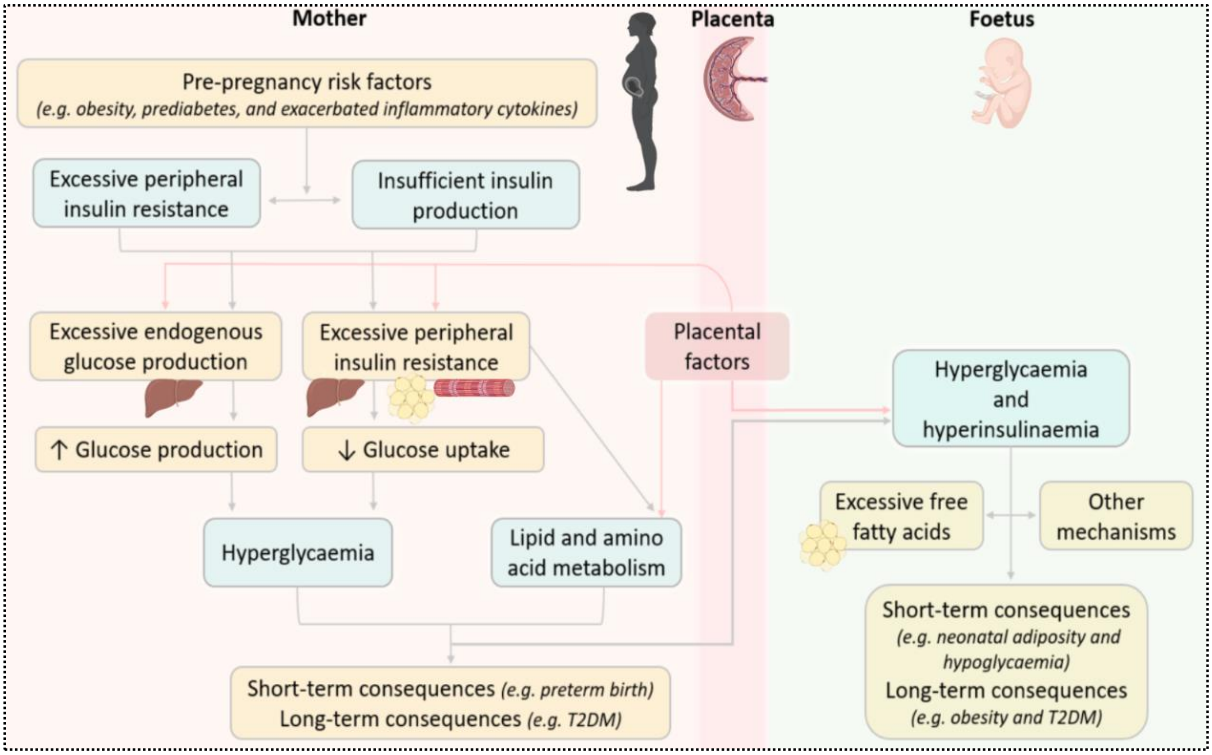

Figure 2. Metabolic dysfunction in obesity and gestational diabetes mellitus: short and long-term consequences. Adapted from Catalano et al.[10].

Moreover, there are other additional mechanisms/factors that underline the link between these adverse phenotypes and pregnancy complications.

For instance, there is an accentuated increase in lipids (hyperlipidaemia) in women with obesity and GDM, which might contribute to insulin resistance and foetal adiposity[4,19]. The maternal amino acid metabolism also appears to mediate foetal growth[4]. This might be related to the capacity of amino acids to stimulate insulin secretion in the B-cells[38] (hyperinsulinemia).

Placental mechanisms from early pregnancy for sub-optimal foetal development

Importantly, the obesity and GDM-associated early metabolic dysfunction does not only influence maternal metabolism, but also dictates the placental phenotype from early pregnancy[22,39-41], and consequently the foetal phenotype by direct interaction. Among the different placental mechanisms involved in this metabolic dysfunction, hormones synthesized and secreted by the placenta can affect negatively its own metabolism[22,39], and induce maternal insulin resistance[2]. Moreover, the expression of placental genes related to glucose, lipid and amino acid uptake and transport [e.g. glucose transporters (GLUT), fatty acid transport and binding proteins (FATP and FABP, respectively), amino acid transporters, etc.] are upregulated in these adverse conditions, which affects nutrients uptake and their intracellular transport[22,39-41]. Upstream placental signalling molecules [peroxisome proliferator-activated receptor gamma (PPARgamma), retinoid X receptor (RXR), mechanistic target of rapacycin complex (mTOR), etc.] expression is also dysregulated, which affect placental signalling pathways and metabolic homeostasis[22,39-41]. These placentas from obese and diabetic women are also characterized by low-grade inflammation [e.g. higher expression of interlukin-6 (IL-6) and tumour necrosis factor-α (TNFα)], which postulates a negative role for the inflammatory cytokines and mediators in this context[19]. Whether this maternal-placental inflammatory state directly translates into a pro-inflammatory environment in the foetus, remains still under debate[42,43]. Moreover, placental and maternal reactive oxygen species, which might be transmitted to the foetus, are also increased with maternal obesity and diabetes[14,22]. Hence, all these placental alterations modulate placental growth and metabolism, contributing to an increased flux of nutrients into the placental-foetal circulation -among other changes-, and leading to non-optimal foetal development (e.g. foetal overgrowth)[22]. Thus, early maternal and placental phenotype (conditioned by pre-gravid metabolic dysfunction) will symbiotically programme the intrauterine environment from early pregnancy.

Other emerging mechanisms

We cannot either forget about epigenetics changes (e.g. DNA methylation) occurring in obesity and GDM, since these changes could alter developmental pathways (especially in pre- and peri-conception)[19,44], and thus determine health later in life. Moreover, alterations in the microbiome and human milk composition are also emerging

mechanisms in obesity and diabetes that could regulate the intrauterine programming and early neonatal health[19].

Metabolic dysfunction in mild adverse phenotypes

It is necessary to highlight that not only severe adverse phenotypes such as those that could be observed in obesity and GDM are susceptible to disease. In the presence of unfavourable environmental (e.g. sedentary lifestyle[45,46], unhealthy diet[47], smoking[48], pollutants and endocrine disruptors[49,50]) and predisposing factors (e.g. genetic mutations[10,51-53]), the physiological changes that accompany normal pregnancies can be dysregulated, thereby inducing metabolic alterations capable of provoking pregnancy complications and metabolic disorders[3,7,10,54,55]. For instance, some studies have shown that "a priori" healthy lean women, but with a previous GDM diagnosis or family history of diabetes, can develop abnormal glucose tolerance and impaired metabolism during pregnancy[3,8,56]. In fact, the remarkable HAPO study has recently showed that the contribution of mild hyperglycaemia to maternal and neonatal adverse outcomes is independent of other conditions such as obesity and GDM[31,32,57]; although its impact is stronger when combined with them[30,57]. Thus, a priori lower-risk groups such as normal-weight women, who are characterized by less glucose intolerance than diabetic women and represent a substantial proportion of Western women, might also manifest higher susceptibility to negative outcomes under certain contexts.

Another potential mechanism predisposing women towards adverse metabolic consequences, even in normal-weight women, is excessive gestational weight-gain and postpartum weight retention, which are strong determinants for birth complications[58], and maternal and offspring diseases[58-61]. Hence, a major spotlight of some institutions[58,62] (e.g. Institute of Medicine) nowadays is on achieving the recommended gestational weight-gain not only in obese, but also in lean and overweight women.

Additionally, previous evidence has clearly shown that those women who present individual cardiometabolic risk factors [dyslipidemia, increased glucose and insulin levels, pre-pregnancy overweight, high waist circumference, high blood pressure] are predisposed to an increased risk for adverse outcomes in pregnancy[54,55,63]. Of note, this risk is considerably higher with the presence of more/grouped cardiometabolic risk factors[54,55,63]. Furthermore, exacerbated inflammation or an imbalance between pro-

and anti-inflammatory responses, could alter maternal metabolism and placental-foetal developmental pathways, leading to preterm birth, foetal loss and brain disorders, congenital diseases, etc[7,11,12,17,64]. Therefore, it is clear that any alteration in these mechanisms which are related to a mild adverse phenotype, could negatively influence the maternal-placental-foetal crosstalk, and therefore be fatal for the maternal-foetal health[5,11,17].

Although we have mentioned some of the most studied mechanisms, there are others less known (e.g. stem cells programming[44], exosomes[16], cytokines interaction[65]), and many others waiting to be discovered. Despite the remarkable progress in this field, the link connecting mild and severe dysfunctional metabolism with adverse consequences still remain poorly understood.

APPROACHES TO MODULATE METABOLISM AND PONTETIALLY AVOID PREGNANCY COMPLICATIONS AND FUTURE DISEASES - A CLINICAL PERSPECTIVE

So far, we have exposed the mechanisms showing how mild and severe adverse phenotypes can negatively impact immunometabolism, and the gravity of this issue for the maternal and foetal health. Indeed, the negative consequences associated with metabolic dysfunction during pregnancy, have the potential to affect not only one life, but two, and possibly next generations.

Thus, it is of primordial importance to break the maternal-foetal intergenerational diabesity cycle, and to promote an optimal maternal and intrauterine environment. It should be a priority to find appropriate strategies to optimise metabolic control during pregnancy, aimed at avoiding potential metabolic disruptions and related short and long-term adverse consequences. Paradoxically, pharmacological drugs and anti-inflammatory modalities have been previously employed for controlling hyperglycaemia and exacerbated inflammation once that complications have arisen in pregnancy[17,66-68], instead of paying more attention to its prevention. This is an important point to consider since although unperceived, some drugs might have side effects, as occurred with the thalidomide -*teratogenic effects*- in the past century[69]. In this regard, physical activity (PA), physical fitness (PF) and physical exercise could be promising tools -without side effects- to optimise metabolic control during pregnancy. The potential utility of these approaches to regulate immunometabolic responses is supported by the

extensive evidence in the general population[70-80], and several studies in pregnant women and rodents (see below).

The importance of sedentary time and physical activity in pregnancy

Scientific evidence has clearly demonstrated the beneficial role of reducing sedentary time (ST)[71,77] and increasing PA[74] for metabolic health in the general population[81]. However, in pregnancy, evidence regarding the role of these lifestyle behaviours in immunometabolism is equivocal and elusive. For instance, previous literature has suggested that reducing sedentary behaviours and increasing PA are not effective strategies for the prevention of GMD[82-85], whereas others have defended their effectiveness for reducing prevalence of GDM and preeclampsia[86-88]. These discrepancies between studies are likely due to methodological differences (use of self-reported instruments to assess ST and PA levels vs. accelerometry[89], different metabolic phenotypes, gestational ages, lifestyle interventions, supervision, etc.). Of note, recent literature has suggested that PA is undoubtedly effective to manage GDM when started before or earlier in pregnancy[7,87,90,91]; which might be explained via gestational weight-gain control, improved early placental phenotype[65,92], and enhanced insulin signalling and GLUT-4 translocation[83,93], among other mechanisms. Interestingly, a recent study using accelerometry has also shown that reducing ST over the course of pregnancy is more beneficial on the glucose-insulin axis than increasing moderate-vigorous physical activity (MVPA) in obese women[46]. Since the change in these behaviours appeared to have limited effects on maternal metabolism, they also stated that lifestyle interventions should target pre-pregnancy and early pregnancy. Indeed, using accurate device-measures of ST and PA in pregnancy instead of self-reported questionnaires, would be of high utility to reach a solid conclusion[89].

Bearing all above in mind, and considering that early pregnancy is a key vulnerability period where most pregnancy complications arise[5,11], reducing ST and increasing PA earlier in pregnancy might be a useful approach to better control immunometabolic responses in this context. Unfortunately, only two studies so far have explored the relationship of objectively measured ST and PA with individual immunometabolic markers specifically in early pregnancy. One of them by Nayak et al. [45], observed that ST at early pregnancy was not associated with any glycaemic and lipid

marker, or cytokine, in obese women. The other one, by van Poppel et al. [94], showed that higher MVPA at early pregnancy was associated with higher concentrations of specific pro- and anti-inflammatory cytokines, and with lower fasting insulin and insulin secretion in obese women. Thus, as appreciable, evidence regarding the role of ST and PA on immunometabolism continues to be scant and equivocal in early pregnancy[87].

Noteworthily, it is possible that ST and PA, which are potential tools to better regulate these immunometabolic responses *(mainly in early pregnancy*), could also modulate positively placental development and metabolism. This indeed appears to be of utmost relevance for an optimal intrauterine programming and foetal development. In fact, in previous analyses of the DALI lifestyle trial[95], sedentary behaviour mediated the lifestyle intervention effects on offspring adiposity in obese women. This further supports the possibility that placental metabolism could play an intermediary role between lifestyle and foetal outcomes, and open new opportunities for lifestyle to enhance in utero perinatal metabolic programming. However, only three studies so far have analysed the role of PA on the placental function and metabolism[96-98], despite its strong relevance. These studies have shown that PA in middle gestation is able to modulate the expression of relevant placental molecules involved in glucose, fatty acid, amino acid and water transport, and insulin and mTOR signalling. Unfortunately, none of these studies have explored by which mechanisms ST or PA could potentially alter the expression of placental genes involved in the transport of nutrients and metabolism regulation, or could impact the foetal health.

Considering the scarce evidence, and the potential of these lifestyle behaviours to impact immunometabolic responses and placental development, especially in early pregnancy, further studies are indeed necessary to comprehend their role and the underlying mechanisms. This is of basic and clinical interest to target regulation of placental transcripts that could directly affect maternal and foetal health.

The unexplored role of physical fitness in maternal and neonatal metabolism

Physical fitness represents the individual's ability to carry out daily tasks with vigour and alertness, without undue fatigue[78]. It mainly consists of several measurable health and skill-related components: cardiorespiratory fitness (CRF), muscular strength, flexibility, balance, and agility[78].

In the general population, and across all ages, PF has shown an extraordinary potential to confer a cardiometabolic-protector role [71,73,79,80,93,99,100], and improve the impaired phenotype associated with obesity[72]. However, and despite its clinical relevance, whether PF has a similar effect in maternal and foetal metabolism during pregnancy has not been explored so far. Although some studies have investigated how exercise affects PF (contradictory results)[101-103], they did not explore its association with maternal and foetal metabolism. Only few studies have focused on delivery outcomes, showing that increasing PF might favour better new-born and birth outcomes[104-106]. Thus, it is of clinical relevance to explore whether increasing PF could be a useful strategy to optimise cardiometabolic markers during this period, and potentially confer a protector role in the maternal and foetal metabolism. This information would be also of great utility to design more effective and tailored exercise programs -*focused on specific or combined PF components*- concerning the metabolic control in pregnancy.

The promising but poorly understood role of physical exercise in maternal and neonatal metabolism

It is well-known that the investigation of exercise offers an extraordinary potential in the discovery of new therapeutic interventions for diseases: immunometabolic, pulmonary and congenital diseases, among others[70,75]. Such are the benefits that exercise has been postulated as the real polypill[76], with comparable if not further benefits than pharmacological therapies in the treatment of certain pathologies[107].

However, in pregnancy, exercise has been a "taboo" in the clinical practise for a long time[108]. Historically, pregnant women were advised to increase their energy intake and avoid exercise due to concerns regarding foetal risk[109]. Moreover, those women with contraindications for exercise were traditionally prescribed with bed-rest[110] (i.e. severe form of sedentary behaviour with harmful consequences), without any advice for rehabilitative exercise. Fortunately, this trend is changing over time[108] thanks to the countless evidence emphasizing the safety and benefits of exercise on metabolic, physical, and mental aspects of health during pregnancy[62,110-113]. However, this traditional issue is still a matter of debate, because most of the "medical contraindications for exercise in pregnancy" listed in the guidelines, are derived from

expert opinions with scarce and poor evidence supporting the benefits of inactivity or harm of exercise[110].

Indeed, pre-gravid and prenatal exercise are first-line[111] strategies to reduce the prevalence of GDM, excessive weight gains, pregnancy and birth complications, mental disorders, and lumbar pain, among others[62,109-115]. However, while most evidence is based on preventing GDM and excessive gestational weight-gain, experiments investigating the effects of exercise on maternal and foetal immunometabolism are scarce. In this regard, exercise appears to positively modulate maternal-foetal metabolism in normal-weight, overweight and obese women[62,92,116-125]. Nonetheless, scientific evidence provides contradictory results, with some aerobic, concurrent (aerobic+resistance), and mixed (exercise+diet) exercise interventions showing limited or no effects on maternal metabolism[126-132]. Additionally, the effects of exercise (and its underlying mechanisms) on foetal glucose and lipid metabolism have not been explored. In view of the weak and scarce evidence, future studies exploring the influence of concurrent exercise (which is apparently more effective[133]) on the maternal-foetal metabolism, and the underlying mechanisms, are necessary.

Similarly to the general population, skeletal muscle might play a pivotal role on maternal and foetal metabolism. In the general population, muscle contractions during and after an acute exercise stimulus induce a milieu of homeostatic perturbations within the contracting muscle (depending on dose of exercise)[93]. These perturbations activate/inhibit specific signalling pathways that regulate transcription and translation processes (*excitation-transcription coupling*)[93,134], and if challenged regularly, produce chronic adaptations that dictate the muscle phenotype and related metabolic adaptations. In pregnancy, normoglycaemic and hyperglycaemic pregnancies are characterized by specific alterations and defects in signalling pathways and transcription-translation processes in the skeletal muscle[10,24,26,27], which means that the skeletal muscle may be a key regulator of metabolic homeostasis in pregnancy. Thus, it is plausible that similar pathways to those observed in the general population, could explain some of the effects of exercise in pregnancy.

Moreover, the emerging role of skeletal muscle as a relevant endocrine organ[135,136], and its characteristic interplay with other organs via muscle contraction-induced factors (myokines)[65,135-137], could also partially explain the beneficial effects of

exercise on immunometabolic health[135,136]. Actually, previous literature (few studies in pregnant rodents[92], and one in pregnant women[65]), have indirectly suggested that myokines might play a relevant role on the mother-placenta-foetus interface. Unfortunately, none study has directly explored the role of myokines in pregnancy. Only one previous study[138], focused on systemic cytokines, has shown that prenatal exercise modulates systemic TNF-α. Surprisingly, none study has either explored the capacity of cytokines to translate the effects of exercise into metabolic changes in pregnant women. Thus, further studies are necessary to better understand the influence of exercise on cytokines, and the role of cytokines as potential messengers of the exercise-induced effects.

Preventing and limiting excessive gestational weight-gain and postpartum weight retention, via exercise, might be another potential mechanism for improved metabolic control. This is plausible since excessive weight-gain and retention are strong determinants of impaired metabolism, pregnancy complications and future diseases[58,59,139-142]; and exercise appears to be effective to control weight-gain and weight retention[114,120,143,144], and avoid excessive weight-gain[114,143,145]. However, evidence is equivocal[114,129,143,145,146], and has not considered whether the effects of exercise on gestational weight-gain could be confounded by other lifestyle behaviours – *such as ST, PA, sleep and diet quality*– or PF capacities. Moreover, little is known about how these lifestyle behaviours and PF relate to gestational weight-gain, and if they could partially explain the effects of exercise on weight-gain and metabolism. Additionally, whether exercise protects maternal and foetal metabolism against the adverse alterations related to excessive weight-gain, which might represent another indirect via to avoid metabolic disruptions, remain also undermined. Taken together, further studies with an integrative approach are necessary to understand the role of lifestyle (including exercise) on the maternal and foetal metabolism.

Finally, to mention is that exercise could affect the maternal and foetal/neonatal metabolism via modulating the placental metabolism and development[65,92], and via other potential mechanisms not specifically addressed in this Doctoral Thesis: epigenetic changes[22], oxidative stress[147], breast milk composition[119], microbiome[7], lipokines and hepatokines[148], exosomes[148] and circadian rhythmicity[149], among others.

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AIMS

AIMS

Overall aim

The overall aim of the present International Doctoral Thesis is to understand the role of PA, PF and exercise on immunometabolism during pregnancy. This overall aim is addressed in six specific aims which correspond to six different studies:

Part I. Role of sedentary and physical activity on immunometabolism

- **Specific aim I:** to analyse the association of ST and PA levels with immunometabolic markers during early pregnancy; and to examine if meeting the PA recommendations is associated with the immunometabolic profile of pregnant women **(Study I).**
- **Specific aim II:** to analyse whether placental expression of GLUT1 as well as of PPAR-γ and its downstream targets FATP2, FATP3 and FABP4 are involved in the association of sedentary behaviour with neonatal adiposity in offspring of obese women; and to explore which maternal metabolic factors mediate changes in these placental transcripts, and which cord blood metabolites related to these placental mRNAs mediate neonatal adiposity **(Study II).**

Part II. Role of physical fitness on maternal and foetal metabolism

- **Specific aim III:** to examine the association of PF with maternal and foetal cardiometabolic biomarkers, and with clustered cardiometabolic risk in pregnancy; and to explore whether being fit during pregnancy is a determinant for improved metabolic control, and might counteract some of the adverse alterations related to overweight and obesity **(Study III).**

Part III. Role of physical exercise on immunometabolism

- **Specific aim IV:** to analyse the influence of a supervised concurrent exercise-training program on inflammatory markers in maternal, and arterial and venous cord serum **(Study IV).**

- **Specific aim V:** to analyse the influence of a concurrent exercise program on immunometabolic parameters in maternal, and arterial and venous cord serum; to investigate whether exercise-induced changes in cytokines are related to these maternal-foetal immunometabolic parameters during pregnancy, and if these associations are dependent on exercise; and to explore the role of these cytokines as mediators of the effects of exercise on immunometabolic parameters **(Study V).**

PART IV. Lifestyle and physical fitness: strategies to manage gestational weight-gain

- **Specific aim VI:** to analyse the independent influence of lifestyle and PF on maternal weight-gain and postpartum weight-retention, and their potential to prevent excessive weight-gain during pregnancy; and to explore if exercise might play a protector role attenuating the adverse outcomes related to exacerbated weight-gain **(Study VI).**

METHODS

METHODS

The present International Doctoral Thesis is composed of six studies classified within four different parts: **Part I** focuses on the influence of ST and PA on maternal and placental immunometabolism; **Part II** focuses on the role of PF on maternal and foetal immunometabolism; **Part III** focuses on the influence of physical exercise on immunometabolism; and **Part IV** focuses on the search of strategies to manage gestational weight-gain during pregnancy, and their potential to attenuate adverse outcomes related to excessive weight-gain. All these parts address knowledge gaps under the framework of two projects in pregnant women: the GESTAFIT and DALI projects.

THE GESTAFIT PROJECT: PART I-IV

Study design and population

The GESTAtion and FITness (GESTAFIT) project was initially a randomized controlled trial that was carried out in Granada (southern Spain) between November 2015 and April 2018. The main aim of the GESTAFIT randomized controlled trial was to evaluate the effects of a supervised concurrent exercise intervention on maternal and foetal health. It was conducted at the "*Sport and Health University Research Institute*", and at the "*San Cecilio and Virgen de las Nieves University Hospitals*", and was approved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15). Three hundred and eighty-four pregnant women attended to their first gynaecological visit at the hospital at 12th week of gestation, and were informed about the current project. Finally, a total of 159 women were recruited after showing interest in participating. All participants signed a personal informed consent. The inclusion and exclusion criteria are detailed in **Table 1.**

The number of individuals to be included in the study was estimated based on the change in maternal body weight. We employed the difference in weight-gain changes (between the control and exercise group) from Ruiz et a.[1] as the expected effect size. Thus, to detect a mean difference of 1.04 and standard deviation of 1.15 Kg in the weight-gain change, with a 90% of statistical power and α=0.05, a total of 52 women (i.e. 26 per group) was necessary. At the onset of the research project, the participants were randomized to either the control or exercise group after the baseline assessments. In order to allocate participants into the control or exercise group, a computer generated simple randomization sequence was used (before participants enrolled in the intervention). However, the randomized component was not possible in all the waves of participants due to some difficulties related to the adherence of control women to the intervention. Hence, roughly half of the women were finally allocated to the control/exercise group according to their personal preference and convenience to attend the exercise sessions. Thus, the GESTAFIT project was finally characterized by a quasi-experimental design.

Table 1. Inclusion and exclusion criteria in the GESTAFIT project

Inclusion criteria
- Pregnant women aged 25-40 years old with a normal pregnancy course.
- Answering "no" to all questions on the PARmed-X for pregnancy.
- Being able to walk without assistance.
- Being able to read and write properly.
- Informed consent: Being capable and willing to provide written consent.
Exclusion criteria
- Having acute or terminal illness.
- Having malnutrition.
- Being unable to conduct tests for assessing physical fitness or exercise during pregnancy.
- Having pregnancy risk factors (such as hypertension, type 2 diabetes, etc.).
- Having a multiple pregnancy.
- Having chromosopathy or foetal malformations.
- Having uterine growth restriction.
- Having foetal death.
- Having upper or lower extremity fracture in the past 3 months.
- Suffering neuromuscular disease or presence of drugs affecting neuromuscular function.
- Being registered in another exercise program.
- Performing more than 300 minutes of at least moderate physical activity per week.
- Being engaged in another physical exercise program
- Being unwilling either to complete the study requirements or to be randomized into the control or intervention group.

Procedures

Women were evaluated at several time points during and after pregnancy by experienced researchers: at 16th and 33rd gestational weeks (2 days/assessment), delivery (2 days/assessment), and postpartum (1 day/assessment). At 16th week (early-middle pregnancy), an initial anamnesis was conducted by face-to-face interviews with experienced personnel to collect data related to sociodemographic and clinical characteristics, reproductive history, history of illness (hypertension, diabetes, obesity, etc.), and alcohol and smoking habits. Other self-administered questionnaires were also employed to collect health information related to sleep and diet quality, among others. Additionally, anthropometrics (weight, height, and waist and hip circumference) and PF (flexibility, muscle strength and CRF) were assessed. Before leaving, participants were given accelerometers (along with a diary to daily report in-bed time, water activities, etc.) to wear until the following appointment. At 17th week, the accelerometers along and diaries were returned, and maternal blood was extracted by a trained nurse. After the baseline assessment, the concurrent (aerobic+resistance) exercise intervention (3 days/week, 60 minutes/session, moderate with peaks of vigorous intensity) was

initiated and performed until delivery. At 33rd-34th week (late pregnancy), the above assessments were performed with identical timing. Just after delivery, umbilical cord blood samples (from artery and vein) were gathered by midwives, and the placenta and perinatal obstetrics records were collected. Subsequently (one day after delivery), the colostrum was obtained from mothers at the hospital. One month postpartum, the mature milk from mothers was collected, maternal and neonatal buccal mucosa cells were extracted, and anthropometrics, body composition, sleep, diet quality, and PF were evaluated. Further information is provided in the methodological study of the project[2]. The whole procedures of the project are presented in **Figure 3.**

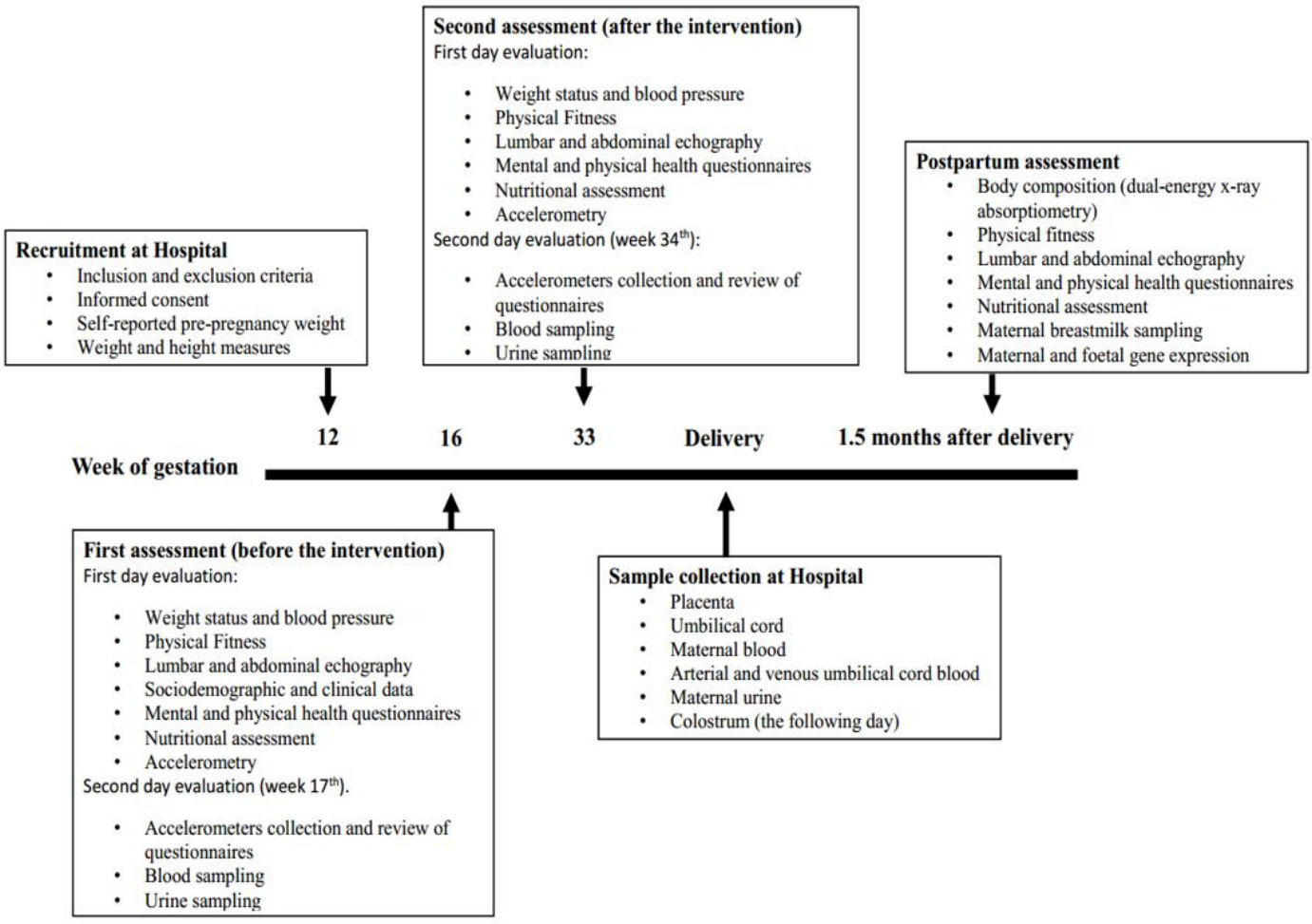

Figure 3. Assessments conducted along the GESTAFIT Project

THE DALI STUDY: PART I

Study design and population

The DALI (Vitamin D and lifestyle intervention for GDM prevention) Lifestyle study was a multicentre randomized controlled trial (2x2 factorial design) conducted between 2012 and 2015 in nine European countries: Austria, Belgium, Denmark, Ireland, Italy, Netherlands, Poland, Spain and United Kingdom. The general aim was to make overweight and obese women conscious about their capacity to influence their weight, and minimize their gestational weight gain during pregnancy, which might help to prevent GDM, among others maternal and foetal outcomes. The study was registered as a randomized controlled trial on November-2011 (ISRCTN70595832) and was individually approved by the local Clinical-Research Ethic Committees of each country.

From the 2009 women assessed for eligibility, 406 women were randomized into lifestyle interventions, and 189 women with placental data were considered for our analyses. Before getting involved in the project, they signed a personal informed consent. Only pregnant women with a pre-pregnancy body mass index (BMI) ≥29 kg/m^2, with singleton pregnancy and aged ≥18 years, and who were assessed before than 19+6 days of gestation, were eligible to be included in the project. Regarding the exclusion criteria, those pregnant women who were diagnosed with GDM before randomization (using the International Association of Diabetes and Pregnancy Study criteria), had pre-existing diabetes, were unable to walk 100 meters safely, had complex diets, were characterized by serious medical conditions, or were unable to converse with the lifestyle coach in another language for which translated materials existed, were excluded from the DALI study. The number of women to be included in the study were calculated based on the primary outcomes: gestational weight gain, fasting glucose and insulin sensitivity in late pregnancy. A 20% drop out was considered when calculating the participants necessary for each arm (80% power, α=5%). To detect a weight-gain change of 4 kg (standard deviation of 6.5 kg), 80 women were needed in each arm. To assess a fasting glucose difference of 0.3 mmol/L (standard deviation of 0.5 mmol/L), 85 women were needed in each arm. To detect a difference of 0.44 in the HOMA-IR (standard deviation of 0.8), 101 women were needed in each arm.

In the lifestyle trial, eligible women were randomly allocated to one of the four following intervention arms: healthy eating (HE), physical activity (PA), HE+PA, and control group. A computerized random number generator (pre-stratified for intervention centre and 2x2 trial) was employed for the random allocation of participants. The DALI team involved within measurements was kept blinded of the intervention allocation of the participants. The trial schedule is shown in **Figure 4.**

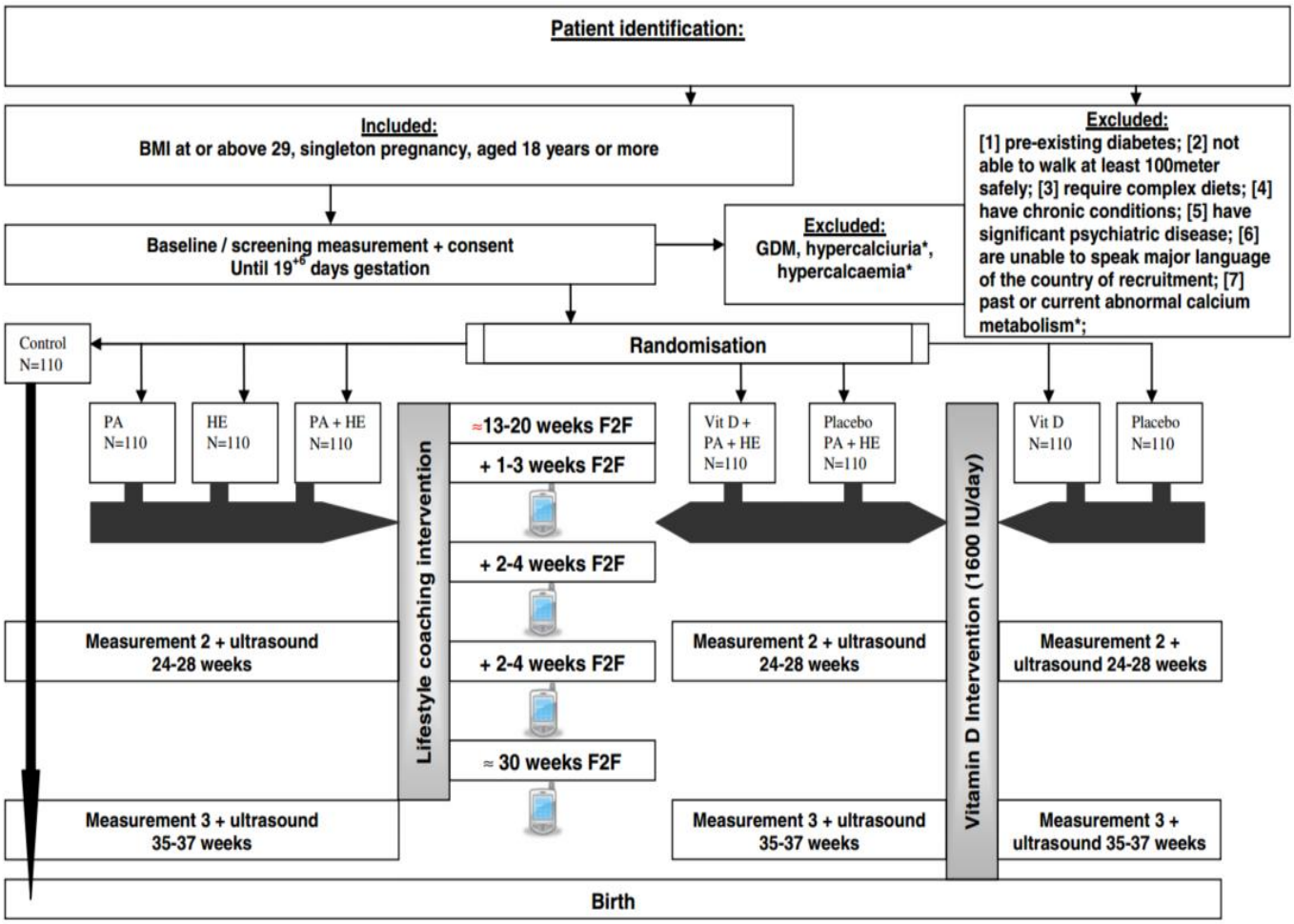

Figure 4. Trial schedule of the whole DALI study. Adapted from Jelsma et al.[3].

Procedures

Pregnant women were evaluated three times during pregnancy, and once after delivery, by the research personnel (time points: baseline <20 gestational weeks, 24-28 weeks, 35-37 weeks, and delivery). At baseline, the sociodemographic and clinical characteristics and sleep habits (questionnaires), and anthropometry and body composition, were evaluated. Additionally, pregnant women underwent an oral glucose tolerance test (OGTT). Blood samples in the OGTT were collected at 0, 60 and 120 minutes. Similarly to the GESTAFIT study, before leaving, women were given an activity log and an accelerometer along with a food diary, to assess ST and PA levels, and nutritional intake, respectively. These data and instruments were sent back with a reply-paid envelope which was provided to them previously.

After baseline measurements, women were randomly allocated to the aforementioned lifestyle-counselling intervention groups. Overall, the counselling interventions consisted in five face to face and four optional booster telephone coaching sessions during the course of pregnancy until 35th week. Personal coaching involved discussion of PA and/or HE habits. These lifestyle interventions were inspired on motivational interviewing methods. Participants were not blinded for the intervention, but were asked not to reveal their intervention group to the research team.

At 24-28 and 35-37 weeks, the aforementioned assessments with identical timing were performed. At delivery, placental biopsies and cord blood samples were collected and processed just after birth. Additionally, information about delivery was obtained from perinatal obstetric records. Until 2 days after delivery, maternal and neonatal measurements were conducted. The assessment procedures are further detailed elsewhere[3].

METHODOLOGICAL OVERVIEW OF THE STUDIES INCLUDED

Table 2 shows the methodological overview of all studies included in the present International Doctoral Thesis.

Methods

Study	Design	Project	Participants	Main predictor/independent var (instruments)
Study I *Association of sedentary time and physical activity levels with immumometabolic markers in early pregnancy: The GESTAFIT project*	Cross-sectional	GESTAFIT	50 Caucasian pregnant women (age: 33±5 years, body mass index: 24.2±4.1 kg/m^2) from Granada, Spain	Time spent in sedentary behaviou intensity levels (triaxial accelerometry)
Study II *Association of sedentary time and physical activity with placental mRNAs related to glucose and lipid metabolism in overweight-obese pregnant women: The DALI Lifestyle study*	Randomized Controlled Trial (2x2 factorial)	DALI	183 European pregnant women (age: 32±5 years, pre-pregnancy body mass index: 33.6±3.9 kg/m^2) from Austria, Belgium, Denmark, Ireland, Italy, Netherlands, Poland, Spain and United Kingdom	Lifestyle intervention (4 groups counselling and recommendations: PA, and control) Sedentary time and PA level accelerometry), maternal anthropome measurements), cardiometabolic (standard methods), and insulin and lep
Study III *Association of physical fitness during pregnancy with maternal and foetal metabolism. The GESTAFIT project*	Longitudinal	GESTAFIT	151 Caucasian pregnant women [age: 33±5 years, body mass index: 22.8 (20.7, 26.5) kg/m^2] from Granada, Spain	Flexibility (back scratch test), lower-b strength (chair stand test), upper-b strength (handgrip test) and cardi fitness (Bruce test)

Study IV *Influence of a concurrent exercise training intervention during pregnancy on maternal and arterial and venous cord serum cytokines: The GESTAFIT project*	Quasi-experimental	GESTAFIT	58 Caucasian pregnant women [age: 34±5 years, pre-pregnancy body mass index: 23.2±3.8 kg/m^2) from Granada, Spain	Supervised exercise intervention Exercise group (n=21): (aerobic+resistance) training program f week until delivery (3 days/ minutes/session) of moderate-to-vigor Control group (n=37): usual care
Study V *The effects of prenatal exercise on maternal and foetal immunometabolism during pregnancy: the GESTAFIT project*	Quasi-experimental	GESTAFIT	88 Caucasian pregnant women [age: 34±5 years, pre-pregnancy body mass index: 22.5 (20.5, 25.9) kg/m^2] from Granada, Spain	Supervised exercise intervention Exercise group (n=44): (aerobic+resistance) training program f week until delivery (3 days/ minutes/session) of moderate-to-vigor Control group (n=44): usual care
Study VI *Influence of lifestyle and physical fitness on gestational weight-gain and postpartum weight retention. The GESTAFIT project*	Quasi-experimental, and longitudinal	GESTAFIT	121 Caucasian pregnant women [age: 33±5 years, pre-pregnancy body mass index: 23.7±3.9 kg/m^2] from Granada, Spain	Supervised exercise intervention (sumn control group n=54, exercise group n=4 Upper-body muscle strength cardiorespiratory fitness (Bruce test) time and PA levels (triaxial accelerome habits (food frequency questionnaire duration and quality (triaxial acceler the Pittsburgh Sleep Quality Index)

DALI, Diabetes and Pregnancy Vitamin D And Lifestyle Intervention for Gestational Diabetes Mellitus Prevention; DXA, dual-en and fitness; HE, healthy eating; mRNA, messenger ribonucleic acid; PA, physical activity.

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3. Jelsma JG, van Poppel MN, Galjaard S, et al. DALI: Vitamin D and lifestyle intervention for gestational diabetes mellitus (GDM) prevention: an European multicentre, randomised trial - study protocol. BMC Pregnancy Childbirth 2013;13:142.

RESULTS AND DISCUSSION

PART I. Role of sedentary time and physical activity on immunometabolism

STUDY I

Association of sedentary time and physical activity levels with immunometabolic markers in early pregnancy: The GESTAFIT project

ABSTRACT

Objectives: To analyze the association of sedentary time and physical activity (PA) intensity levels with immunometabolic markers during early pregnancy; and to examine if meeting the PA recommendations is associated with the immunometabolic profile of pregnant women.

Methods: Fifty Caucasian pregnant women (age: 32.8±4.7years old, body mass index: 24.2±4.1kg/m2, gestational age: 17±1.5weeks) participated in this cross-sectional study (from September-2015 through May-2016). Sedentary time and PA intensity levels were objectively measured with triaxial accelerometry (7 consecutive valid days). Fasting serum glucose, total cholesterol, phospholipids, and triglycerides were assessed with standard methods. Serum pro-inflammatory and anti-inflammatory cytokines (fractalkine, interleukin-1β, interleukin-6, interleukin-8, interleukin-10, interferon-γ, and tumor necrosis factor–α) were measured using Luminex xMAP technology.

Results: Sedentary time and PA were not correlated with any glycemic or lipid marker ($p>0.05$). After adjusting for the potential confounders, vigorous PA showed a positive non-significant association with interleukin-6 ($p=0.06$), and bouts of moderate-vigorous PA was inversely associated with interleukin-1β and interferon-γ ($p=0.02$ and $p=0.04$, respectively). Meeting the PA guidelines was inversely associated with interleukin-1β and positively associated with interleukin-8 ($p=0.01$ and $p=0.04$, respectively). These associations disappeared after controlling for multiplicity.

Conclusions: Increasing the time spent in moderate-vigorous PA, or meeting the PA recommendations, is associated with the cytokine profile of women without metabolic disruptions in early pregnancy. However, sedentary time and PA do not seem to be associated with glucose or lipids levels. These results should be interpreted cautiously in view of the discrepancies after adjusting for multiple comparisons. Future studies in this novel field of research are warranted before reaching any conclusion.

INTRODUCTION

Pregnancy is a state characterized by significant endocrine and immunometabolic plasticity, in which maternal physiology is reprogrammed and modulated for maintaining an adequate maternofetal homeostasis.[1-6] During early pregnancy (largely anabolic), non-obese pregnant women with normal glucose tolerance seem to undergo a slight decrease in insulin sensitivity along with an increase in lipogenesis.[1,4,6] Moreover, pregnant women are predisposed to a mild pro-inflammatory state, with elevated concentrations of cytokines.[2-4] An uncontrolled exacerbation of glycemic and lipid alterations and an imbalance between pro-inflammatory and anti-inflammatory cytokines during early pregnancy, might lead to complicated pregnancies and adverse outcomes.[2,6-8] These adverse consequences include impaired fetal development[1,2,8] and future maternal and child health diseases.[1,2,5,9]

Paradoxically, pharmacological drugs and anti-inflammatory modalities have been previously used for controlling aberrant inflammation once that complications have been developed during pregnancy,[2,7,10] instead of paying more attention to its prevention. In this regard, other potential targets such as lifestyle behaviors [sedentary time (ST) and physical activity (PA)] might help pregnant women to control and regulate immunometabolic responses during early pregnancy.[4,11-14]

To date, two studies[12,15] have suggested that ST is not associated with any immunometabolic marker during early pregnancy. Furthermore, maintaining appropriate PA levels during early pregnancy seems to be associated with lower risk of developing gestational diabetes mellitus (GDM), better insulin sensitivity, and lower plasma triglycerides and total cholesterol concentrations.[4,13,14] However, the role of PA on inflammatory cytokines during early pregnancy is not clear.[12] Thereby, previous literature[4,16] has highlighted that more evidence is imperative regarding the impact of ST and PA (intensity, frequency, and duration) on immunometabolic health. Considering the potential influence of ST and PA on the intrauterine environment and the short-long term maternal, fetal, and newborn health,[4,5,11,12] it is of clinical interest to determine whether ST, PA intensity levels, and meeting the PA recommendations for pregnancy are associated with glycemic, lipid, and inflammatory markers during early pregnancy. Moreover, to the best of our knowledge, no previous studies have analyzed this relationship in early pregnant women without metabolic impairments, and

independently of maternal age, obesity, ST, and PA. This study might guide future research to focus on alternative non-side-effect therapeutic targets and related-specific lifestyle interventions aimed at promoting a healthy physiological course of pregnancy.

Therefore, the main aims of this study were i) to analyze the association of ST and PA intensity levels with immunometabolic markers during early pregnancy; and ii) to examine if meeting the PA recommendations is associated with the immunometabolic profile of pregnant women.

MATERIAL AND METHODS

Study design and participants

The procedures, along with the inclusion-exclusion criteria (**Table S1**) of the present cross-sectional study, have been published elsewhere.[17] From the 109 pregnant women contacted at "San Cecilio" University Hospital, Granada (southern Spain) during early pregnancy (12th week of gestation), we recruited 90 pregnant women (**Figure S1**). All interested participants signed a written informed consent after being informed about the study aims and procedures. This study was approved by the Clinical Research Ethics Committee of Granada, Regional Government of Andalusia, Spain (code: GESFIT-0448-N-15).

Procedures

The evaluation procedures were performed on 2 non-consecutive days. On the first appointment (15th-17th gestational weeks), the recruited participants came to the research center, and sociodemographic-clinical data, blood pressure, and body composition were assessed. Before leaving, each participant was given an accelerometer to wear during 9 consecutive days. One week later, participants attended our research center for the extraction of blood samples in fasting conditions.

Measurements

Sociodemographic and clinical data

A clear and concise self-reported printed survey [with standardized questions and answers (mostly for qualitative data) to ensure accuracy and consistency of data recording] was used to collect sociodemographic (age, marital, and professional status and educational level) and clinical (clinical history of CVD risk makers and drugs) data. The participants were provided with instructions on how to complete such self-reported

survey by our research team. The approximate duration for most pregnant women to finish the survey was 15-20 minutes.

Body composition

Body weight and height were assessed using a scale (InBody R20; Biospace, Seoul, Korea) and a stadiometer (Seca 22, Hamburg, Germany), respectively. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m^2).

Blood pressure and resting heart rate

A blood pressure monitor (M6 upper arm blood pressure monitor Omron, The Netherlands) was employed to assess systolic and diastolic blood pressure and resting heart rate, while women were seated in a relaxed state. Measurements were taken twice, five minutes apart, and the lowest value of the two measurements of each parameter was used for posterior analyses.

Sedentary time and physical activity

Sedentary time and PA were objectively assessed with triaxial accelerometry (ActiGraph GT3X+, Florida, US), using an epoch length of 60 seconds and a frequency rate of 30 Hz. The participants wore the accelerometer on their waist during 9 consecutive days, 24 hours/day (for waking and sleeping hours, excepting water-based activities). A total of 7 days of recording with a minimum registration of ≥10hours/day was necessary to be included in the analyses. "Accelerometer wear time" was calculated by deducting the sleeping and non-wear time from the total registered time during the whole day (usually 1440 min). Bouts of 90 continuous minutes of 0 activity intensity counts were excluded from the analyses.[18] Sedentary time was calculated as the amount of time accumulated below 200 counts/min (minimum length of 10 continuous minutes)[19] and was expressed in min/day. The time involved in different PA intensity levels (light, moderate, vigorous, and moderate-vigorous) was calculated based on the recommended PA vector magnitude cut points ≥200-2690, ≥2690-6166, ≥6167 counts/min, and ≥2690,[20] respectively, and was expressed in min/day. The time spent in bouted moderate-vigorous physical activity (MVPA) was also calculated, and it was expressed in min/week.

Bouted MVPA was defined as the minutes spent in MVPA when accumulated in periods of ≥10 consecutive minutes (up to 2 minutes below the cut point allowance). PA categories were established according to the PA recommendations for pregnant women: not meeting the PA recommendations (<150min/week of bouted MVPA) vs.

meeting the PA recommendations (≥150min/week of bouted MVPA). Data download, cleaning, and analyses were performed using ActiGraph software (ActiLife v. 6.13.3).

Immunometabolic markers

Glycemic and lipid markers

Serum glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, phospholipids, and triglycerides were assessed following standard methods using an autoanalyzer (Hitachi-Roche p800, Switzerland).

Pro- and anti-inflammatory markers

Maternal pro-inflammatory and anti-inflammatory cytokines (fractalkine, interleukin (IL)-1β, IL-6, IL-8, IL-10, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α were measured using Luminex xMAP technology. More detailed information about blood samples analyses is shown in Appendix S1.

Statistical analysis

Descriptive statistics for continuous and categorical variables was used to show the sociodemographic and clinical characteristics of pregnant women. Pearson´s partial correlations along with *p*-value and 95% bias corrected and accelerated confidence intervals (1000 bootstrap samples) were used to examine whether ST, PA intensity levels, and meeting the PA recommendations were correlated with immunometabolic markers after adjusting for age and BMI.

Given the asymmetry of the outcome variables, linear regression models were adjusted. Data preparation was employed for those outcome variables which were statistically significantly (or showed borderline statistical significance) associated with predictors in the Pearson´s partial correlation analyses. Particularly, optimum Box-Cox transformations and censor of extreme outliers (for the outcomes) were performed to improve the linear relation between the predictor and outcome variables. Subsequently, linear regression analysis (enter method) was performed to analyze the association between predictors (ST, PA intensity levels, and meeting the PA recommendations) and transformed outcomes (inflammatory markers)**,** after adjusting for potential confounders. Model 1 was adjusted for maternal age and BMI; model 2 was adjusted for maternal age, BMI, average accelerometer wear time, and bouted MVPA (for ST) or ST (for PA predictors); model 3 was adjusted for maternal age, BMI, average accelerometer wear time, and total PA (only for PA predictors).

Adjustments for multiple comparisons were performed with the Hochberg procedure (in view of the assumptions met) to control the overall type I error rate[21,22]. The analyses were performed using Statistica 12.0 (Statsoft Inc.) for the inference of the Box-Cox transformations and SPSS 22.0 (IBM, NY, USA) for the rest of the analyses. The level of significance was set at $p \leq 0.05$.

RESULTS

From all the interested participants (n=109), the final study sample was composed of 50 Caucasian pregnant women (age 32.8±4.7 years old, gestational age at measurement 17±1.5 weeks, BMI 24.2±4.1 kg/m^2) (**Figure S1**). The average BMI of pregnant women by weight-status was: lean 21.6±2.2; overweight 26.7±1.4; and obese 32.8±1.8. The sociodemographic and clinical characteristics of the study sample during early pregnancy are presented in **Table 1**. Roughly, half of the study sample had not had children previously, worked full time, and had received high education. Overall, pregnant women spent ~54% of daytime in sedentary behaviors and 76% of them did not attain the recommendations of MVPA levels. None of the pregnant women consumed any medication.

Pearson´s partial correlations of ST and PA intensity levels with immunometabolic markers (after adjusting for maternal age and BMI) during early pregnancy are shown in **Table 2**. ST was not associated with any alteration of glycemic, lipid, or inflammatory marker [$r_{partial}$ coefficient, ($r_{partial}$ 95% confidence interval), *p-value*: -0.232 to 0.163, (-0.468 to 0.422), $p>0.05$]. Light PA showed some evidence of statistical significance with phospholipids [-0.249, (-0.487, 0.020), $p=0.09$]. Moderate PA showed some evidence of statistical significance with IL-1β [-0.256, (-0.501, -0.017), $p=0.08$] and IL-8 [0.272 (-0.070, 0.520), $p=0.06$]. Vigorous PA showed evidence of statistical significance with IL-6 [0.269 (-0.020, 0.481), $p=0.06$]. Bouted MVPA was positively associated with IL-8 [0.293 (-0.106, 0.541, $p=0.04$] and inversely associated with IL-1β [-0.338 (-0.584, -0.162), $p=0.02$] and INF-γ [-0.283 (-0.470, -0.006), $p=0.05$]. Meeting the PA recommendations was associated with IL-1β [-0.352, (-0.587, -0.096), $p=0.02$] and IL-8 [0.330, (0.025, 0.601), $p=0.02$]. When additionally adjusting the analyses for accelerometer wear time, the results did not change. Pearson´s partial correlation coefficients, along with the 95% confidence intervals, are shown in **Table S2**.

Table 1. Sociodemographic and clinical characteristics, accelerometer data and immunometabolic markers concentrations in early pregnancy (n=50).

Age (years)	32.8	(4.7)
Average gestational age (weeks)	17	(1.5)
Body mass index (kg/m^2)	24.2	(4.1)
Cohabitation, n (%)		
Living alone	0	(0)
Living accompanied	50	(100.0)
Number of children, n (%)		
0	27	(54.9)
1-2	22	(43.1)
>3	1	(2.0)
Professional status, n (%)		
Worked full/part time	29	(58.0)
Unemployed/Retired/Housekeeper	21	(42.0)
Education level, n (%)		
Non-university degree	24	(48.0)
University degree	26	(52.0)
Cardiometabolic disruptions/medication, n (%)		
Hypertension diagnosis/Antihypertensive medication	0	(0.0)
Heart disease diagnosis/Medication for heart diseases	0	(0.0)
Diabetes diagnosis/Glycemic lowering medication or insulin treatment	0	(0.0)
High cholesterol diagnosis/Lipid lowering medication	0	(0.0)
Cardiovascular function		
Systolic blood pressure (mmHg)	105.8	(9.1)
Diastolic blood pressure (mmHg)	61.7	(7.3)
Resting heart rate (bpm)	81.6	(10.7)
Sedentary lifestyle and PA		
Sedentary time (min/day)	500.2	(94.1)
Light PA (min/day)	395.7	(85.9)
Moderate PA (min/day)	35.0	(21.9)
Vigorous PA (min/day)	1.0	(3.2)
Moderate-vigorous PA (min/day)	36.0	(22.2)
Bouted moderate-vigorous PA (min/week)	91.1	(116.0)
Average vector magnitude counts (counts/day)	504569.8	(121894.7)
Steps (steps/day)	7578	(2600.7)
Average accelerometer wear time (min/week)	6523.1	(395.9)
Serum glycemic and lipid markers		
Glucose (mg/dL)	86.5	(9.0)
Cholesterol (mg/dL)	184.8	(32.7)
High Density Lipoprotein (mg/dL)	77.6	(14.7)
Low Density Lipoprotein (mg/dL)	134.2	(43.9)
Phospholipids (mg/dL)	187.9	(37.7)
Triglycerides (mg/dL)	118.8	(55.9)
Serum inflammatory markers		
Fractalkine (pg/mL)	380.8	(145.5)
Interleukin 1 beta (pg/mL)	6.8	(3.0)
Interleukin 6 (pg/mL)	5.9	(2.8)
Interleukin 8 (pg/mL)	20.3	(9.3)
Interleukin 10 (pg/mL)	22.5	(10.1)
Interferon gamma (pg/mL)	24.0	(11.5)
Tumor necrosis factor alpha (pg/mL)	5.6	(2.1)

Continuous variables are presented as Mean (Standard Deviation) and categorical variables as Number (Percentage); PA, physical activity.

Table 2. Partial correlations of sedentary time and physical activity intensity levels with serum glycemic, lipid, and inflammatory markers during early pregnancy (n = 50).

	Sedentary time	LPA	MPA	VPA	MVPA	Bouted MVPA
Glucose	-0.127	0.087	-0.021	-0.186	-0.047	-0.046
Cholesterol	-0.018	-0.053	0.056	-0.103	0.041	0.066
HDL	0.009	-0.002	0.165	-0.034	0.158	0.121
LDL	-0.232	0.037	-0.019	-0.078	-0.030	0.012
Phospholipids	0.163	**-0.249**	0.136	0.065	0.144	0.128
Triglycerides	0.000	0.067	-0.067	-0.101	-0.080	0.048
Fractalkine	0.007	0.120	-0.087	0.014	-0.084	-0.156
Interleukin-1β	0.013	0.135	**-0.256**	-0.073	**-0.263**	**-0.338***
Interleukin-6	0.021	-0.022	-0.041	**0.269**	-0.002	-0.100
Interleukin-8	-0.067	0.014	**0.272**	-0.178	**0.243**	**0.293***
Interleukin-10	0.010	0.044	-0.047	0.195	-0.019	-0.129
Interferon gamma	0.005	0.090	-0.116	-0.029	-0.119	**-0.283***
Tumor necrosis factor alpha	-0.147	0.178	0.068	0.080	0.078	-0.006

LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous physical activity; MVPA, moderate-to-vigorous physical activity; HDL, high density lipoprotein; LDL, low density lipoprotein. All values are presented as Pearson r coefficient; *p*-value and 95% bias corrected and accelerated confidence intervals are based on 1000 bootstrap samples (the complete table with 95% confidence intervals is shown in Supplementary material-**TableS2**). Partial correlations were performed using age and body mass index as covariates. The accelerometer wear time was also added as confounder and the results did not change (data not shown); numbers in bold indicate evidence of statistical significance (p≤0.09); numbers in bold with asterisks indicate statistical significance * p<0.05.

The association of ST and PA intensity levels with transformed inflammatory markers during early pregnancy is shown in **Table 3**. After adjusting for maternal age and BMI (model 1), ST was not associated with any alteration of any transformed inflammatory marker (*p*>0.05). When considering model 1, vigorous PA showed evidence of statistical significance with IL-6 [B (standard error), β; *p-value*: 0.099 (0.052), 0.261; *p*=0.06], and bouted MVPA was inversely associated with IL-1β [-0.002 (0.001), -0.325; *p*=0.02] and IFN-γ [-0.003 (0.001), -0.307; *p*=0.04]. Meeting the PA guidelines was inversely associated with IL-1β [-0.666 (0.259), -0.355; *p*=0.01] and positively associated with IL-8 [0.150 (0.069), 0.31; *p*=0.04]. Overall, the results did not change when considering the rest of the potential confounders (models 2 and 3). The rest of the potential confounders for each model are described in the legend for **Table 3**.

Table 3. Linear regression analysis assessing the association of sedentary time and physical activity intensity levels with transformed serum inflammatory markers during early pregnancy (n=50).

		B*	SE*	β*	p-value			Adjusted
					Model 1	Model 2	Model 3	R^2*
IL-1β	Sedentary time	0.000	0.001	-0.025	0.86	0.40		-0.007
	Light PA	0.002	0.001	0.179	0.21	0.07	0.07	0.027
	Moderate PA	-0.009	0.005	-0.24	0.09	0.08	0.08	0.053
	Vigorous PA	-0.015	0.037	-0.057	0.70	0.70	0.69	-0.004
	Bouted MVPA	-0.002	0.001	-0.325	**0.02**	**0.02**	**0.02**	**0.100**
	Meeting PA guidelines	-0.666	0.259	-0.355	**0.01**	**0.01**	**0.01**	**0.120**
IL-6	Sedentary time	0.000	0.002	0.011	0.94	0.96		0.034
	Light PA	0.000	0.002	0.006	0.97	0.10	0.10	0.034
	Moderate PA	-0.002	0.008	-0.036	0.80	0.81	0.81	0.035
	Vigorous PA	0.099	0.052	0.261	**0.06**	**0.07**	**0.07**	**0.104**
	Bouted MVPA	-0.001	0.001	-0.075	0.61	0.62	0.62	0.039
	Meeting PA guidelines	-0.369	0.953	-0.056	0.70	0.90	0.90	0.028
IL-8	Sedentary time	0.000	0.000	-0.040	0.78	0.84		-0.049
	Light PA	0.000	0.000	0.007	0.96	0.30	0.30	-0.051
	Moderate PA	0.002	0.001	0.197	0.18	0.20	0.20	-0.010
	Vigorous PA	-0.014	0.010	-0.218	0.14	0.15	0.15	-0.002
	Bouted MVPA	0.000	0.000	0.22	0.14	0.15	0.15	-0.002
	Meeting PA guidelines	0.150	0.069	0.31	**0.04**	**0.04**	**0.04**	**0.046**
INF-γ	Sedentary time	0.000	0.002	-0.006	0.97	0.52		-0.047
	Light PA	0.001	0.002	0.125	0.395	0.36	0.36	-0.030
	Moderate PA	-0.005	0.007	-0.121	0.42	0.38	0.38	-0.032
	Vigorous PA	-0.016	0.046	-0.052	0.73	0.73	0.73	-0.044
	Bouted MVPA	-0.003	0.001	-0.307	**0.04**	**0.04**	**0.04**	**0.049**
	Meeting PA guidelines	-0.476	0.337	-0.208	0.17	0.16	0.16	-0.003

B, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient; PA, physical activity; MVPA, moderate-vigorous physical activity; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; IFNγ, interferon gamma. Model 1 was adjusted for maternal age and body mass index; Model 2 was adjusted for maternal age, body mass index, accelerometer wear time, and sedentary time (for PA predictors) or bouted MVPA (for sedentary time); Model 3 was adjusted for maternal age, body mass index, accelerometer wear time, and total physical activity (only for PA predictors). *The values shown are derived from Model 1. Optimum Box-Cox transformations and/ a subtle variation of winsorizing (convert back from a z-score: replacing extreme scores (z>2.58) with a score equivalent to ±2.58 standard deviations from the mean) were performed on inflammatory markers.

Given that there is no consensus regarding the best cut-off point for estimating ST in adults (validated with triaxial accelerometers),[23] we additionally examined the associations between ST and transformed cytokines using the cut-point provided by

Kozey-Keadle, et al.[24] (postulated as one of the best approaches to estimate ST in adults[23]). The results remained similar after these sensitivity analyses (**Table S3**). Of note, after controlling for multiplicity, all previous significant associations became non-significant.

DISCUSSION

A major finding of the present study is that higher levels of bouted MVPA and meeting the PA recommendations were associated with lower circulating IL-1β in early pregnancy. Similarly, higher levels of bouted MVPA and meeting the PA recommendations were associated with lower concentrations of IFN-γ and higher concentrations of IL-8, respectively. Pregnant women with greater levels of vigorous PA showed a trend to have higher IL-6 concentrations, although this association was non-significant. Neither ST nor PA intensity levels were associated with any glycemic or lipid marker.

To facilitate the interpretation of the results in this novel field of research, we firstly aimed at comparing the inmunometabolic status of pregnant women from our sample with non-pregnant women from other studies (similar age range: 22-40 years old, and BMI: 23.5-26.3 kg/m^2). Overall, we could observe that pregnant women presented similar glucose and total cholesterol levels[25-27]; and greater LDL and HDL-cholesterol[25,27], and triglycerides concentrations[25]. Regarding the inflammatory markers, pregnant women showed higher levels of IL-1[28], IL-6 [25-29], IL-8[28], IL-10[28], IFN-γ[28], and TNF–α[25,27,29] than non-pregnant women of similar characteristics. These comparisons and previous evidence[1,6,30,31] seem to support the idea that early pregnant women are predisposed to a mild pro-inflammatory profile, despite the fact that changes on cardiometabolic markers are not very accentuated.

Previous studies have stated that ST is not associated with any glycemic or lipid marker, or with any cytokine in the 15th week of gestation,[12,15] which is in agreement with the results of the current study. This lack of association between ST with glycemic and lipid markers has been also observed in non-pregnant women of similar characteristics.[32,33] By contrast, Loprinzi, et al.[34] found a positive association of ST with LDL-cholesterol in pregnant women. This might be explained by the fact that they combined data across the 3 trimesters of pregnancy, which might have led to equivocal

conclusions. Hence, we hypothesize that ST stimulus might not be enough to induce immunometabolic changes compared to the anabolic and pro-inflammatory alterations predisposed by early pregnancy.

Our results also showed that PA intensity levels were not associated with any glycemic or lipid marker. Overall, these results were in agreement with those reported by Loprinzi, et al.[34] However, previous literature has observed that PA is inversely associated with plasma triglycerides and total cholesterol concentrations during early pregnancy.[4,13,14] These discrepancies might be explained by the fact that previous studies did not distinguish between gestational ages,[13,34] assessed PA with self-reported questionnaires, or presented an appreciable greater statistical power.[13,14,34] Moreover, the normative values observed in our study on several metabolic outcomes (related to the healthier status of our participants), might also partially explain these differences. Interestingly, in non-pregnant women of similar age (age range: 24-32 years, BMI: 27 kg/m^2), previous evidence has shown that light PA is inversely associated with triglycerides and total cholesterol concentrations,[33] and MVPA is positively associated with HDL-cholesterol.[32]

PA intensity levels were not associated either with TNF-α, IL-10 or fractalkine in our study. Overall, these results are supported by previous studies in non-pregnant women[33,35,36], except for MVPA, which was inversely associated with TNF-α[33]. Regarding exercise in the general population, most studies have shown that acute strenuous exercise normally has either no effect or a slight effect on circulating TNF-α,[37,38] and increases IL-10 concentrations (partly mediated by IL-6).[37-39] However, in pregnancy, regular exercise might suppress the alterations observed in TNF-α along each trimester of pregnancy.[40] Hence, due to the scarce evidence, it remains controversial whether these trends are similar for PA stimulus during early pregnancy. Only one previous study[11] has observed that MVPA is positively associated with serum TNF-α and IL-10 during early pregnancy, contrary to our results. This difference might be explained by the overweight-obese status of their sample compared to the more predominant normal-weight phenotype of the pregnant women included in our study. Finally, findings regarding the associations of ST and PA with fractalkine,[41] cannot be commented with regard to other studies, since it has never been explored in pregnant women.

In the current study, bouted MVPA and meeting the PA recommendations were inversely associated with IL-1β during early pregnancy, independently of maternal age, obesity, and ST. By contrast, van Poppel, et al.[11] observed that physically active pregnant women (based on MVPA categorization) presented greater IL-1β concentrations. They hypothesized that this result might have been caused by the more pronounced pro-inflammatory status of pregnant women due to their overweight-obese status and metabolic profile, differently to our study, in which most women were normal-weight. This finding is relevant since IL-1β plays a meaningful role in the pathogenesis of obesity-associated morbidity[42] and metabolic-inflammatory abnormalities.[42,43] Therefore, strategies targeting IL-1β blockage are of clinical relevance. Several biological pharmacological approaches targeting IL-1β (such as anakinra or canakinumab) have been used in the prevention of cardiovascular and inflammatory events.[43,44] However, evidence on maternal-fetal outcomes and risks when applying these medications during pregnancy is insufficient to claim safety.[10] In the light of our results, it seems that increasing bouted MVPA levels is related to reduced IL-1β, which might contribute to regulate immunometabolic responses and avoid dysregulations of the autoimmune inflammation, in early pregnant women without metabolic impairments. This idea is supported by van Poppel, et al.[11] who showed that changes in circulating IL-1β were inversely associated with fasting insulin and the first-phase insulin response in more physically active pregnant women.

IL-6 has been usually considered a pro-inflammatory cytokine. However, in response to muscle contraction, this myokine also stimulates the release of anti-inflammatory cytokines such as IL-10 and IL-1 receptor antagonist.[37] In non-pregnant women of similar characteristics to our participants, no association between levels of PA and plasma IL-6 has been previously observed[33,35]. In early pregnancy, van Poppel, et al.[11] observed that more physically active pregnant women presented greater circulating IL-6. Similarly, our findings suggest that pregnant women with greater levels of vigorous PA might present higher IL-6 concentrations, although this association is non-statistically significant (p=0.06). Regarding the other PA intensity levels, light, moderate, and MVPA were not associated with IL-6 concentrations in our study. When interpreting these results, despite the fact that vigorous PA seems to explain 10% of the variance in this regression model (its adjusted R^2 is similar to those of the significant associations), we

should consider that the levels of vigorous PA performed by pregnant women were generally very low. If future studies in pregnant women with higher heterogeneity (i.e. greater levels of vigorous PA) verify a positive association between vigorous PA and IL-6 levels during early pregnancy, the plausible hypotheses discussed in the **Appendix S2** might be of interest. However, more studies are necessary first to better characterize this association.

In the current study, light, moderate, vigorous, and bouted MVPA were not associated with serum IL-8 concentrations. In young non-pregnant women (age 22 years, BMI 23,5 kg/m^2; unpublished data from the ACTIBATE project)[45], these associations were also found to be non-significant. These results might be plausible since muscle-derived IL-8 (at mRNA and protein levels), during and after strenuous exercise,[37,38,46] exerts its effects locally in the myocytes and endothelium vascular cells (in the contracting muscle) rather than in systemic circulation.[39,46] Paradoxically, our results showed that meeting the PA recommendations was positively associated with circulating IL-8. We speculated that this finding might be explained through different mechanisms. Similarly to the exhaustive exercise pathway (which involves eccentric muscle contractions),[38,46] the increased circulating IL-8 observed in people meeting the PA recommendations, might be related to chemo-attraction of neutrophils and macrophages. However, as aforementioned, PA intensity levels were not associated with IL-8. Hence, we hypothesized that meeting the PA recommendations implies a substantial strenuous stimulus (similar intensity, but greater and more maintained duration compared to PA intensity levels) for triggering an inflammatory response. Another explanation to the increased levels of IL-8 might be related to the first immunological phase, predisposed by early pregnancy and necessary for a proper implantation, and decidual and placental development.[2,3] In the implantation site, the differentiated immune cells play a pronounced role on angiogenesis, and on cytokine secretion-regulation at the maternal-fetal interface.[2,3] Moreover, exercise can enhance placental growth[47] and angiogenesis[48] through the upregulation of circulating endothelial progenitor cells[49] and CXCR2 mRNA and protein expression in myocytes and endothelium cells (IL-8-induced angiogenesis).[37,46] This led us to contemplate that, during implantation and placentation, meeting the PA recommendations might be related to a more vascularized placenta (greater placental angiogenesis), and an

increased release of circulating IL-8 facilitated by the maternal-fetal interface. Unfortunately, these results and hypotheses have not been confirmed by other studies, since these associations have never been explored.

Our results showed that IFN-γ was inversely associated with bouted MVPA in pregnant women. These results could not be confirmed, since there is no evidence regarding this association. However, it has been previously suggested that physical exercise has little effect on circulating IFN-γ or that it attenuates IFN-γ synthesis.[39] Therefore, increasing MVPA could be related to lower levels of IFN-γ, which might contribute to regulate an excessive chronic inflammatory environment during early pregnancy.

Importantly, it is necessary to acknowledge that all significant associations disappeared after adjusting for multiples comparisons. Hence, the results from the present study should be interpreted cautiously. However, when interpreting these analyses, it is also imperative to consider that: i) multiplicity adjustments primarily apply to confirmatory hypotheses and corresponding analyses, not for exploratory analyses[22,50,51]; ii) the lack of statistical power have limited us to handle the necessary α-adjustments[50,51], and iii) the Hochberg procedure is more conservative and less powerful than other semi-parametric and parametric tests[21,22].

Some limitations need to be mentioned: (i) the cross-sectional design of the study does not allow us to make causal inferences; (ii) the results should be interpreted with caution given the small size of the studied sample; iii) only interested participants were involved in the study; iv) a self-reported survey was used to collect sociodemographic and clinical data. On the other hand, some strengths need to be mentioned: (i) ST and PA intensity levels were objectively measured with accelerometry (although the use of non-validated cut-points, unstandardized processing criteria, etc. represent a weakness of this study, and an inherent limitation of the current evidence in pregnancy); (ii) such a strict criteria (7 days of ≥10hours/day) for including data from ActiGraph accelerometers in the analysis had never been used in previous studies; (iii) the complete set of inflammatory markers assessed is noteworthy; (iv) this is the first time that the association of ST and PA intensity levels with some cytokines (such as fractalkine, IL-8, and IFN-γ) has been explored during early pregnancy; and (v) we

adjusted the analyses for essential confounders to avoid overrating the independent influence of ST and PA on these metabolic outcomes.[11,12]

CONCLUSIONS

The present study provides a novel and greater insight suggesting that increasing the time in MVPA is associated with the cytokine profile of women without metabolic disruptions in early pregnancy. PA could be an alternative-complementary therapeutic target to control immunometabolic responses, which might favor the prevention of any potential metabolic disruption. However, these results should be interpreted cautiously in view of the discrepancies after controlling for multiplicity. Futures studies providing a wider insight on how the intensity, duration, and frequency of PA (discriminating properly from physical exercise constructs-mechanisms) influence all these immunometabolic markers in early pregnant women are warranted.

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SUPPLEMENTARY MATERIAL

Appendix S1. *Detailed information of blood samples analyses.*

In standardized fasting conditions (8-9 a.m.) at our research center, venous blood samples (5mL) of all pregnant women were extracted from the antecubital vein and collected in EDTA vacuum tubes and serum tubes. Then, the samples were centrifuged at 1750 rpm for 10 minutes at 4°C in a refrigerated centrifuge (GS-6R Beckman, Fullerton, CA, USA) to separate serum from formed elements. Subsequently, serum was aliquoted and frozen at -80º C to avoid breaking the cold chain before the analysis in the laboratory.

Serum glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, phospholipids, and triglycerides were assessed following standard methods using an autoanalyzer (Hitachi-Roche p800, F. Hoffmann-La Roche Ltd. Switzerland). We employed Luminex xMAP technology based on MILLIPLEX MAP kits to assess the cytokine profile from the collected serum in pregnant women. Luminex xMAP technology (Millipore, Darmstadt, Germany) is a mix of three existing and proved technologies: use of microspheres, flow cytometry, and laser technology, mixing digital signal processing and traditional chemistry immunoassay. Because of robust multiplexing, xMAP technology potentially delivers more data in less time than other bioassay products, with comparable results with enzyme linked immunosorbent assay and microarray. The technology offers several other distinct advantages over traditional methods such as speed and high throughput, versatility, flexibility, accuracy, and reproducibility. Particularly, for maternal pro-inflammatory and anti-inflammatory (fractalkine, interleukin-1β, interleukin-6, interleukin-8, interleukin-10, interferon-γ and tumor necrosis factor-α) determination, we used Human Sepsis Magnetic Bead Panel 3 Multiplex Assay (cat. No. HTH17MAG-14K). We prepared samples, reagents, and standards by following the manufacturer's instructions. Equipment settings: 50 events per bead, gate settings: 8,000-15,000, time out 60 seconds. Plate was read on LABScan 100 analyzer (Luminex Corporation, Texas, USA) with xPONENT software for data acquisition. The average values for each set of duplicate samples or standards were within 15% of the mean. We determined cytokine concentrations by comparing the mean of duplicate samples with the standard curve for each assay.

Appendix S2. *Association between vigorous PA and IL-6 concentrations.*

As aforementioned in the main manuscript, this cytokine might present a relevant role during early pregnancy. In our study, we could observe that vigorous PA showed evidence of statistical significance (positive relationship, p=0.06) with serum IL-6 concentrations. However, in view of the non-significant association (p>0.05), and aimed at avoiding the over-interpretation of these results which might provoke misleading conclusions, we have discussed this association separately in this section. Moreover, this will allow readers to focus on the real-significant findings in the manuscript. If futures studies confirm a positive association between vigorous PA and serum IL-6 levels, the hypotheses stated below might help to partially understand this association.

Since circulating IL-6 is not related to local muscle damage,[1,2] we hypothesized that the increased IL-6 observed with greater vigorous PA might be explained via similar anti-inflammatory exercise-induced mechanisms.[1,3] Given that IL-6 response is sensitive to exercise intensity,[1,2] vigorous PA might contribute to greater systematic release of IL-6 (as mRNA and protein levels increase largely within myocytes) compared to lower intensities. Nonetheless, in disagreement with our results, previous literature[1,2] has indicated that PA is inversely associated with plasma IL-6 in several non-pregnant populations. Since muscle-IL-6 is glycogen-dependent,[1,2] they suggested that usual muscle work (training adaptation), which leads to increased intramuscular glycogen capacity, might explain the reduced circulating IL-6 levels and the upregulation of muscular IL-6 receptors (via enhanced muscular IL-6 sensitivity). Therefore, differences in glycogen stores in myocytes, predisposed by this complex anabolic-catabolic transition,[4-6] might explain the discrepancies between studies.[1,2] Additionally, elevated estrogen levels predisposed by this anabolic period,[7] might also partially explain the greater IL-6 related to higher vigorous PA.[8,9] These hypotheses should be considered carefully given that the association between vigorous PA and IL-6 was borderline. Thus, the underlying related-mechanisms remain controversial in pregnancy, and more studies are necessary to verify these findings.

Table S1. Inclusion and exclusion criteria in the GESTAFIT project.

Inclusion criteria
- Pregnant women aged 25-40 years old with a normal pregnancy course.
- Answering “no” to all questions on the PARmed-X for pregnancy.
- Being able to walk without assistance.
- Being able to read and write properly.
- Informed consent: Being capable and willing to provide written consent.
Exclusion criteria
- Acute or terminal illness.
- Malnutrition.
- Inability to conduct tests for assessing physical fitness or exercise during pregnancy.
- Underweight.
- Pregnancy risk factors (such as hypertension, type 2 diabetes, etc.).
- Multiple pregnancy.
- Chromosopathy or fetal malformations.
- Uterine growth restriction.
- Fetal death.
- Upper or lower extremity fracture in the past 3 months.
- Presence of neuromuscular disease or drugs affecting neuromuscular function.
- Being registered in another exercise program.
- Doing more than 300 minutes of at least moderate physical activity per week.
- Unwillingness either to complete the study requirements or to be randomized into the control or intervention group.

Table S2. Pearson r coefficients (with p-value and 95% bias corrected and accelerated confidence intervals based on 1000 bootstrap samples) of sedentary time and physical activity intensity levels with serum glycemic, lipid, and inflammatory markers (n = 50).

		Sedentary time	LPA	MPA	VPA	MVPA	Bouted MVPA
Glucose	Pearson´s r	-0.127	0.087	-0.021	-0.186	-0.047	-0.046
	95% CI Lower	-0.367	-0.198	-0.255	-0.631	-0.281	-0.268
	95% CI Upper	0.077	0.362	0.229	0.291	0.228	0.187
Cholesterol	Pearson´s r	-0.018	-0.053	0.056	-0.103	0.041	0.066
	95% CI Lower	-0.260	-0.326	-0.233	-0.393	-0.247	-0.251
	95% CI Upper	0.207	0.216	0.319	0.221	0.306	0.359
HDL	Pearson´s r	0.009	-0.002	0.165	-0.034	0.158	0.121
	95% CI Lower	-0.217	-0.251	-0.104	-0.335	-0.123	-0.205
	95% CI Upper	0.228	0.243	0.442	0.264	0.450	0.454
LDL	Pearson´s r	-0.232	0.037	-0.019	-0.078	-0.030	0.012
	95% CI Lower	-0.468	-0.214	-0.326	-0.293	-0.334	-0.266
	95% CI Upper	0.041	0.271	0.305	0.266	0.292	0.308
Phospholipids	Pearson´s r	0.163	**-0.249**	0.136	0.065	0.144	0.128
	95% CI Lower	-0.102	-0.487	-0.248	-0.217	-0.236	-0.251
	95% CI Upper	0.422	0.020	0.553	0.410	0.556	0.602
Triglycerides	Pearson´s r	0.000	0.067	-0.067	-0.101	-0.080	0.048
	95% CI Lower	-0.288	-0.247	-0.325	-0.276	-0.343	-0.188
	95% CI Upper	0.281	0.382	0.265	0.029	0.260	0.354
Fractalkine	Pearson´s r	0.007	0.120	-0.087	0.014	-0.084	-0.156
	95% CI Lower	-0.283	-0.207	-0.390	-0.180	-0.376	-0.459
	95% CI Upper	0.315	0.382	0.123	0.257	0.127	0.115
IL-1β	Pearson´s r	0.013	0.135	**-0.256**	-0.073	**-0.263**	**-0.338***
	95% CI Lower	-0.410	-0.207	-0.501	-0.249	-0.513	-0.584
	95% CI Upper	0.321	0.496	-0.017	0.142	-0.015	-0.162
IL-6	Pearson´s r	0.021	-0.022	-0.041	**0.269**	-0.002	-0.100
	95% CI Lower	-0.291	-0.251	-0.245	-0.020	-0.213	-0.308
	95% CI Upper	0.284	0.198	0.153	0.481	0.220	0.113
IL-8	Pearson´s r	-0.067	0.014	**0.272**	-0.178	**0.243**	**0.293***
	95% CI Lower	-0.324	-0.264	-0.070	-0.380	-0.118	-0.106
	95% CI Upper	0.156	0.274	0.520	0.228	0.503	0.541
IL-10	Pearson´s r	0.010	0.044	-0.047	0.195	-0.019	-0.129
	95% CI Lower	-0.324	-0.228	-0.322	-0.059	-0.298	-0.363
	95% CI Upper	0.292	0.295	0.202	0.451	0.231	0.072
IFNγ	Pearson´s r	0.005	0.090	-0.116	-0.029	-0.119	**-0.283***
	95% CI Lower	-0.296	-0.191	-0.367	-0.293	-0.360	-0.470
	95% CI Upper	0.237	0.360	0.213	0.306	0.203	-0.006
TNFα	Pearson´s r	-0.147	0.178	0.068	0.080	0.078	-0.006
	95% CI Lower	-0.412	-0.083	-0.172	-0.161	-0.157	-0.232
	95% CI Upper	0.077	0.503	0.290	0.316	0.310	0.286

LPA, light physical activity; MPA, moderate physical activity; VPA, vigorous; MVPA, moderate-vigorous physical activity; HDL, high density lipoprotein; LDL, low density lipoprotein; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IFNγ, interferon gamma; TNFα, tumor necrosis factor alpha; 95% CI, 95% confidence interval. Partial correlations were performed using age and body mass index as covariates. Accelerometer wear time was also added as confounder and the results did not change (data not shown); Numbers in bold indicate evidence of statistical significance; Numbers in bold with asterisks indicate statistical significance * p<0.05.

Table S3. Linear regression sensitivity analysis assessing the association of sedentary time (cut-point: Kozey-Keadle, et al.[10]) with transformed serum inflammatory markers during early pregnancy (n=50).

		B*	SE*	β*	p-value	
					Model 1	Model 2
Sedentary time	IL-1β	0.000	0.001	-0.028	0.85	0.411
	IL-6	0.000	0.002	0.011	0.94	0.97
	IL-8	0.000	0.000	-0.037	0.80	0.85
	INF-γ	0.000	0.002	-0.013	0.93	0.52

B, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient; IL-1β, interleukin-1β; IL-6, interleukin-6; IL-8, interleukin-8; IFNγ, interferon gamma. Model 1 was adjusted for maternal age and body mass index; Model 2 was adjusted for maternal age, body mass index, accelerometer wear time, and bouted MVPA (for sedentary time). *The values shown are derived from Model 1. Optimum Box-Cox transformations and/ a subtle variation of winsorizing (convert back from a z-score: replacing extreme scores (z>2.58) with a score equivalent to ±2.58 standard deviations from the mean) were performed on inflammatory markers.

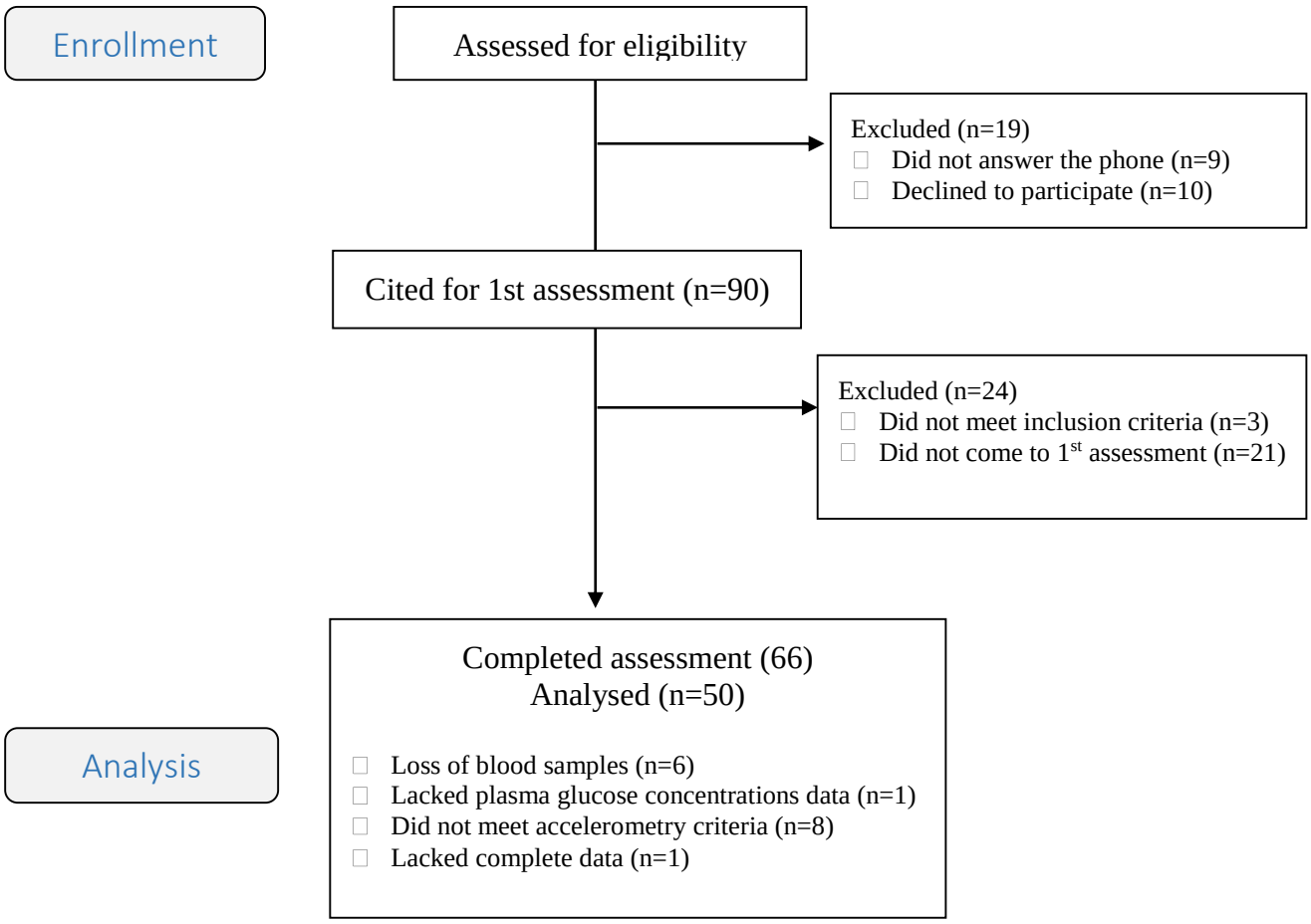

Figure S1. Flowchart of the participants for the specific study aims

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STUDY II

Association of sedentary time and physical activity with placental mRNAs related to glucose and lipid metabolism in overweight-obese pregnant women: The DALI lifestyle study

ABSTRACT

Objectives: To explore i) the association of sedentary time (ST) and physical activity (PA) levels during pregnancy with placental expression of genes related to glucose and lipid metabolism in overweight-obese pregnant women; ii) maternal metabolic factors mediating changes in placental transcripts; and iii) cord blood metabolites related to these mRNAs mediating neonatal adiposity.

Methods: A subsample of the DALI trial encompassing 183 pregnant women (age 32±5y, BMI at baseline 33.7 (31.7, 36.6) kg/m^2) with placental tissue available was analysed. ST and moderate-to-vigorous PA (MVPA) levels were objectively measured with accelerometry at three time periods in pregnancy. Placental mRNAs (PPAR-γ, FATP2, FATP3, FABP4 and GLUT1) were measured with Nanostring technology using three reference genes for normalization (OAZ1, TBP, and WDR45L).

Results: ST in early to middle pregnancy was inversely associated with placental FATP2 and FATP3 expression (p<0.05). At 24-28 weeks, maternal fasting insulin and homeostatic model assessment-insulin resistance (HOMA-IR) index were inversely associated with FATP2 mRNA. Higher fasting glucose and lower HOMA-B index (beta-cell function) were related to greater FATP3 mRNA (all, p<0.05). FATP2 mRNA was inversely associated with cord blood triglycerides and free fatty acids (p<0.01). MVPA at baseline was inversely associated with GLUT1 mRNA, which was related to cord blood glucose (all, p<0.05).

Conclusions: ST in early to middle pregnancy is associated with the expression of placental genes linked to lipid transport. PA is hardly related to the expression of placental molecules involved in glucose and lipid metabolism. It seems plausible that strategies aimed at reducing sedentary behaviours during pregnancy can modulate placental gene expression, which might help prevent unfavourable foetal and maternal pregnancy outcomes.

INTRODUCTION

The placenta is a multifunctional organ that regulates key aspects of pregnancy maintenance and fetal development[1-4]. Under pathological conditions such as obesity[5] and gestational diabetes mellitus (GDM)[1,2], placental metabolism is often dysregulated[6-8]. Impaired placental development and function, especially in early pregnancy, is closely related to pregnancy complications and future maternal and child diseases[1,2,4,5]. Unfortunately, the mechanisms connecting an obesogenic intrauterine environment to short and long-term consequences in the offspring have remained elusive[5,9,10]. Previous literature has emphasized that maternal obesity is associated with changes in the expression and activity of placental transporters such as glucose transporter 1 (GLUT1)[9,11,12], fatty acid transport protein (FATP) 2[9,11] and FATP3[13], and fatty acid binding protein 4 (FABP4)[9,14,15].

Interestingly, GLUT1, which is the main placental glucose transporter, and FATP2, FATP3 and FABP4, which are relevant proteins for cellular free fatty acids (FFA) uptake and intracellular transport, associate with excessive fat accumulation in offspring born to obese women[9-12,14,15]. Peroxisome proliferator-activated receptor gamma (PPAR-γ) is the master regulator of fatty acids related transcripts including FATP2, FATP3 and FABP4[14,16-18]. It is associated with maternal obesity[9,13], and also plays a fundamental role in fatty acid metabolism, adipogenesis, and hence, in foetal development[9,14,18]. Thus, obesity-related changes of these placental transporter isoforms could potentially alter placental uptake and, by inference, transport of nutrients into the placental-foetal circulation, thereby contributing to sub-optimal foetal growth (e.g. overgrowth).

Lifestyle behaviours [sedentary time (ST) and physical activity (PA)] can counteract some obesity-related metabolic disruptions during pregnancy[6,19-22], and modulate concentrations of relevant maternal and cord serum molecules[20,23-27]. However, there is a paucity of evidence about the influence of lifestyle on placental transporters[28,29]. Since improving lifestyle behaviours may represent a promising strategy to prevent placental dysregulations and inadequate foetal development in obese pregnant women, this information is imperative to guide clinical practice.

In previous analyses of the DALI lifestyle trial, sedentary behaviour, but not MVPA, mediated intervention effects on offspring adiposity[30]. Whether placental transport of glucose, as well as placental lipid uptake and metabolism, could be involved

in the negative association of sedentary behaviour with neonatal adiposity in obese women, remains unknown. Thus, the main aims of the current study were to assess whether placental expression of GLUT1 as well as of PPAR-γ and its downstream targets FATP2, FATP3 and FABP4 are involved in the association of sedentary behaviour with neonatal adiposity in offspring of obese women. A secondary study aim was to explore potential i) maternal metabolic factors mediating changes in these placental transcripts, and ii) cord blood metabolites related to these placental mRNAs mediating neonatal adiposity.

MATERIAL AND METHODS

Study design and population

The DALI lifestyle study was a multicentre randomized controlled trial (RCT) using a 2x2 factorial design, and performed in nine European countries (Austria, Belgium, Denmark, Ireland, Italy, Netherlands, Poland, Spain and United Kingdom) between 2012-2015. The study was prospectively registered as RCT on November 2011 (ISRCTN70595832) and was individually approved by local Clinical-Research Ethic Committees in each country. All pregnant women with a pre-pregnancy body mass index (BMI) ≥29 kg/m^2 (eligible for inclusion) provided signed informed consent. The rationale, along with the procedures and inclusion-exclusion criteria of the DALI lifestyle study, have been previously described elsewhere[31]. Of note, women diagnosed with GDM using IADPSG criteria were excluded from the trial.

Sample size

The required sample size for the main DALI trial was determined for the primary outcomes (gestational weight gain, glucose and insulin sensitivity) but, given the exploratory nature, not for the secondary outcomes analysed in this study.

Procedures

Women were assessed three times by the research midwife/nurse (time points: baseline <20 gestational weeks, 24-28 weeks, 35-37 weeks). At baseline, sociodemographic and clinical characteristics, body composition and anthropometry, sleep patterns were assessed by questionnaire, and women underwent a 75g oral glucose tolerance test, with samples taken at 0, 60 and 120 minutes. Before leaving, women were given an activity log and an accelerometer along with a food diary, to assess ST and PA levels, and

nutritional intake, respectively. After baseline measurements, women were randomly allocated to the HE (healthy eating), PA (physical activity), HE&PA, or control group (**Figure S1**; additional information about the lifestyle interventions can be found in **Appendix A**). At 24-28 and 35-37 weeks, the same assessments were performed. At delivery, placental tissue and cord blood samples were taken and processed within 30 minutes after birth, and data related to the delivery were obtained from perinatal obstetric records. After delivery (<48h), maternal and neonatal measurements were performed. The assessment procedures are further detailed elsewhere[31].

Exposures, Outcomes and Confounders

Sociodemographic-clinical data, obstetric history and other outcomes

Sociodemographic (age, ethnicity, among others) and clinical (e.g. pre-existing conditions, co-morbidities, medications) data, reproductive history, adverse events from the mother and neonate, and tobacco, alcohol and sleep habits, were obtained from questionnaires and medical files.

Maternal body weight and height

Pre-pregnancy weight was self-reported. Maternal body weight was measured twice (no shoes, light clothes) at each time point by calibrated electronic scales (SECA-888; SECA-877). Height was measured once at baseline with stadiometers (SECA-206, Birmingham, UK). Body mass index (BMI) was calculated [weight (Kg)/height(m)^2].

Dietary habits

The frequency and amount of specified foods were used to estimate the servings per week of foods rich in fibre, protein, fat and carbohydrates[31].

Sedentary time and physical activity

ST and PA levels at <20, 24-28, and 35-37 weeks were objectively measured with Actigraph uniaxial/triaxial accelerometers (GT3X+ or GT1M; Pensacola, Florida, USA), using an epoch length of 60 seconds, and sampling frequency of 60-80 Hz. Women waist-wore the devices for at least 3 days (for sleeping and waking hours, excepting water-based activities). A minimum register of 3 days (2 weekdays and 1 weekend day; 8 hours/day) was required to be included in the analyses. Considering the information filled in activity logs by the participants, "accelerometer wear time" was estimated by deducting the non-wear and sleeping time from the time registered during the whole day. The time spent in sedentary, light and moderate-to-vigorous PA (MVPA) behaviours

was calculated based on the vertical axis cut points ≤100, 101-1951, ≥1952 counts/min (respectively) provided by Freedson, et al.[32], and was expressed in min/day. Data download, cleaning, and analyses were performed using ActiGraph software (ActiLife version 6.8.1).

Neonatal adiposity[30]

Triceps, subscapular, supra-iliac, and quadriceps skinfold thickness was measured (within 48 hours after birth); and values were summated. All skinfold measurements were performed twice.

Laboratory methods

Placental tissues collection

At delivery, placental biopsies were collected from each of the four quadrants from the central part in relation to the cord insertion. Each of these placental biopsies were equally divided into maternal and foetal parts and stored in cryotubes filled with RNA-later (Sigma-Aldrich, St. Louis, MO, USA). These cryotubes were stored in freezer at -20ºC until shipped to the central lab in Graz for analyses.

RNA isolation

After removing RNA-later at the central lab, two pieces from each of maternal and fetal side (approx. 20mg/piece) were pooled. Subsequently, 700µL of Quiazol were added to each pooled sample, and then the homogenization was done using the MagNa Lyser Instrument (Roche: 2-3runs, 6500rpm, 20s). Standard procedures were performed with the miRNeasy Mini Kit (Qiagen, #217004) for RNA isolation and DNAse digestion in the lysates. RNA concentration and quality were determined using the QIAexpert System (Qiagen) and Agilent 2100 Bioanalyses System, respectively. An RNA integrity number ≥4 for lysates was required to be included in the analyses.

Gene expression analysis by nCounter system

Overall, the quantification of the different placental mRNAs (PPAR-γ, FATP2, FATP3, FABP4 and GLUT-1) were analysed by molecular counting using the NanoString nCounter Analysis Technology (NanoString Technologies, Seattle, WA). The probes for the investigated genes were part of a customized CodeSet (nCounterTM PlexSetTM) with in total 24 probes, including probes for three validated housekeeping genes [ornithine decarboxylase antizyme 1 (OAZ1), WD repeat-containing protein 45-like (WDR45L) and tata-box-binding protein (TBP)], that was used for hybridization according to the

manufacturer's protocol (**Appendix B**). A total of 490 ng RNA per sample was applied for hybridization. Quality control and normalization was done utilizing the NanoString nSolver Analysis Software v4.0 (NanoString Technologies).

Blood samples - Laboratory analyses

Maternal blood was collected at baseline, 24-28 and 35-37 weeks. From maternal fasting blood samples, plasma glucose, insulin, glycated haemoglobin (HbA1c), insulin, lipids (triglycerides, FFA, total cholesterol, high- and low-density lipoprotein cholesterol (HDL-C; LDL-C), and leptin concentrations were measured. Cord blood (from placental chorionic vessels) was processed within 30 minutes of birth. From cord venous blood samples, plasma glucose, C-peptide, the aforementioned lipids, and leptin were determined. All the analytes were quantified by conventional clinical chemistry methods, but insulin and leptin (ELISA).

Insulin resistance and beta-cell function

For insulin resistance and beta-cell function, the homeostasis model assessment (HOMA)-IR and HOMA-B were calculated, respectively, according to standard formulas[33].

Statistical analysis

Descriptive statistics were performed to show the characteristics (**Table 1**), and ST and PA levels of participants during pregnancy (**Table 2**). To detect differences between groups, analyses of variance (normal distribution, homoscedasticity) and Kruskal-Wallis tests (non-normal distribution) were performed for quantitative variables, and Chi-square tests for qualitative variables. Few influential outliers in some outcome variables were handled (**Appendix C**). Subsequently, Box-Cox transformations were used for models characterized by asymmetry of placental mRNA. Interaction between offspring sex and the independent/predictor variables (intervention and ST/MVPA) was assessed in linear regression analyses. Afterwards, multilevel analyses were used to take into account the clustering effect of the different countries. All multilevel analyses were based on a two-level hierarchy (country and individual), with random intercept and slope.

To address the first aim, linear regression analyses (multilevel models) were employed to assess the effects of a PA-counselling intervention on placental mRNAs (per-protocol basis; Table S1). Multilevel linear regression analyses were also used to

examine the association of ST and PA levels at the different time points (baseline, 24-28 and 35-37 weeks), and changes in ST and PA levels (from baseline to 24-28 and 35-37 weeks), with placental mRNAs content (**Table 3**). For the secondary aims, linear regression, moderation and mediation analyses (see **Appendix D**) were used to explore maternal lifestyle-related metabolic factors mediating placental transcript changes, and measured mRNA-related cord blood metabolites mediating neonatal adiposity (Tables 4-5, Tables S2-S11, Figures S3-S6).

Potential confounders identified from previous literature that modified the relationship between the predictors and outcomes (change in the regression coefficient >15%) were included in the models. Specifically, the main cofounders included in the analyses (specified in each table), besides site, were: the intervention group, gestational week at delivery, smoking at baseline, the relative percentage of daily ST [(ST/accelerometer wearing time)*100; when analysing MVPA] or MVPA (when exploring ST), and the requirement for prostaglandins at delivery (induction of labour)[34]. All the assumptions related to the generalization of the results were met. The statistical analyses were conducted using SPSS 22.0 (IBM, NY, USA). The statistical level of significance was set at $p \leq 0.05$. False discovery rate corrections were made using Benjamini-Hochberg´s step-up procedure[35].

RESULTS

Placental mRNAs were analysed in a subsample of the DALI cohort (n=183 for intervention analyses, and n=112 for "ST and PA" analyses; see Flow Chart in **Figure S1**). Sociodemographic and clinical characteristics, and ST and MVPA levels of the participants during pregnancy are shown in the **Tables 1** and **2**. The expression of placental genes and their bivariate Pearson correlations are shown in **Figure S2**.

Associations of sedentary time and physical activity with placental mRNAs

No differences (all $p \geq 0.05$) in placental mRNAs were found between the HE & PA, HE or PA groups compared to the control group (**Table S1**). Given that there were no differences between intervention groups (**Table S1**), all intervention groups were combined to one cohort to assess the associations between ST and MVPA with placental mRNAs. This provides the opportunity to explore greater variation in placental mRNAs, and increases statistical power.

Table 1. Sociodemographic and clinical characteristics of pregnant women (n=112).

Maternal age (years)	32.7	5.3
Gestational age (weeks)		
At baseline	14.7	2.3
At delivery	39.7	1.3
Ethnicity, n (%)		
Maternal European descent	85	(75.9)
Living with a partner, n (%)	108	(96.4)
High educational level, n (%)	62	(55.4)
Working, n (%)	91	(81.3)
Body composition		
BMI pre-pregnancy (kg/m^2)	33.6	3.9
GWG, baseline to 35-37 weeks (kg)	8.1	4.6
Dietary behaviour (baseline) ***(n=107)***		
Fibre (number consumed per week)	29.5	(20.3, 42.8)
Protein (number consumed per week)	7	(5, 12)
Fat (number consumed per week)	4	(2, 8)
Carbohydrates (number consumed per week)	39	(26, 58)
Multiparous, n (%)	56	(50)
Female offspring sex, n (%)	55	(49.1)
Active smoking at baseline, n (%)	15	(13.4)
Developed GDM during pregnancy, n (%)	40	(37.7)
Placental weight (g) ***(n=103)***	634.4	151.4
Weight of the neonate (g)	3540.9	500.4
Sum of skinfolds (mm) ***(n=103)***	20.4	4.4
Cord blood parameters ***(n=89)***		
C-peptide (µg/L)	0.7	(0.5, 0.9)
Glucose (mmol/L)	4.6	(3.6, 5.4)
Triglycerides (mmol/L)	0.4	(0.3, 0.7)
Free fatty acids (mmol/L)	0.3	(0.2, 0.4)
Leptin (µg/L)	8.5	(4.2, 12.4)

BMI, body mass index; GWG, gestational weight gain; GDM, gestational diabetes mellitus. Continuous variables are presented as mean - standard deviation, or median (interquartile range), unless otherwise indicated.

Table 2. Sedentary time and physical activity levels during pregnancy (n=112).

Sedentary time and PA levels		
Baseline (<20 weeks)		**n=112**
Sedentary time (min/day)	577.7	(102.5)
MVPA (min/day)	40.0	(24.3, 56.0)
Relative percentage of daily sedentary time (%)	71.2	(64.8, 79.4)
Relative percentage of daily MVPA (%)	4.6	(3.0, 6.8)
24-28 weeks		**n=72**
Sedentary time (min/day)	596.8	(100.9)
MVPA (min/day)	39.0	(24.2, 56.9)
Relative percentage of daily sedentary time (%)	72.8	8.4
Relative percentage of daily MVPA (%)	4.6	(2.9, 7.0)
35-37 weeks		**n=64**
Sedentary time (min/day)	593.2	(102.1)
MVPA (min/day)	31.2	(16.8, 46.8)
Relative percentage of daily sedentary time (%)	74.0	(7.1)
Relative percentage of daily MVPA (%)	4.3	(2.8)
Changes in sedentary time and PA levels from baseline		
24-28 weeks minus baseline		**n=72**
Sedentary time (min/day)	12.4	(88.5)
MVPA (min/day)	-2.4	(25.4)
35-37 weeks minus baseline		**n=64**
Sedentary time (min/day)	-12.6	(-65.0, 67.2)
MVPA (min/day)	-13.9	(-27.9, 4.6)

PA, physical activity; MVPA, moderate-to-vigorous physical activity. Data are mean (standard deviation) or median (interquartile range=Q3, Q1).

The associations of ST and MVPA levels at each time point, and absolute changes in ST and MVPA levels from baseline to either 24-28 or 35-37 weeks with placental mRNAs, are shown in **Table 3**. Notably, most of the significant associations were found with ST, and not with MVPA. After adjusting for confounders (model 2), ST at baseline was inversely associated with FATP2 and FATP3 mRNA levels (p=0.03 and p=0.05). At 24-28 or 35-37 weeks, no statistically-significant associations were found, except for PPAR-γ mRNA which was inversely associated with ST at 24-28 weeks (model 1, p=0.05; model 2, p=0.06). The change in ST from baseline to 24-28 weeks was inversely associated with FABP4 mRNA (model 2, p=0.05). From baseline to 35-37 weeks, the change in ST showed an inverse association with GLUT1 mRNA (model 1, p=0.01), which was not significant after adjusting for additional confounders (model 2). MVPA at baseline was inversely associated with GLUT1 mRNA (model 1, p<0.05), but not when additionally adjusted for ST (model 2). No further associations with (changes in) MVPA were found.

Table 3. Linear regressions assessing the association of objectively measured sedentary time and physical activity levels at each time point with targeted placental transporters mRNAs (n=112).

Placental mRNAs	ST/PA (min/day)	Baseline <20 weeks (n=112)				24-28 weeks (n=88)				35-37 weeks (n=79)			
		Model 1			Model 2 (n=97)	Model 1			Model 2 (n=80)	Model 1			Model 2 (n=70)
		B	SE	p-value	p-value	B	SE	p-value	p-value	B	SE	p-value	p-value
PPAR-γ (au)[c]	ST	0.000	0.000	0.63	0.27	-0.001	0.000	**0.05**	**0.06**	0.000	0.000	0.56	0.45
	MVPA	-0.001	0.002	0.62	0.19	0.000	0.002	0.96	0.62	0.000	0.002	0.96	0.94
FATP2 (au)	ST	-0.001	0.001	**0.05**	**0.03**	-0.002	0.001	0.06	0.31	-0.001	0.001	0.29	0.48
	MVPA	0.002	0.003	0.48	0.48	-0.001	0.003	0.82	0.35	0.003	0.004	0.54	0.78
FATP3 (au)	ST	-0.002	0.001	0.09	**0.05**	-0.002	0.002	0.30	0.13	0.000	0.002	0.92	0.55
	MVPA	0.002	0.006	0.78	0.43	-0.006	0.006	0.33	0.28	-0.003	0.007	0.67	0.36
FABP4 (au)[abc]	ST	0.001	0.001	0.56	0.41	-0.001	0.001	0.43	0.89	0.000	0.001	0.78	0.34
	MVPA	0.000	0.004	0.94	0.40	0.001	0.004	0.91	0.97	-0.009	0.005	0.06	0.08
GLUT1 (au)[ac]	ST	0.001	0.000	0.10	0.67	0.000	0.000	0.73	0.35	0.000	0.000	0.77	0.37
	MVPA	-0.005	0.002	**0.01**	0.14	-0.002	0.002	0.23	0.43	-0.003	0.002	0.20	0.21
Placental mRNAs	**Changes in ST/PA (min/day)**					**Baseline to 24-28 weeks (n=72)**				**Baseline to 35-37 weeks (n=64)**			
						Model 1			Model 2	Model 1			Model 2
						B	SE	p-value	p-value	B	SE	p-value	p-value
PPAR-γ (au)[de]	ST					-0.001	0.001	0.10	**0.10**	0.000	0.001	0.81	0.69
	MVPA					0.000	0.002	0.85	0.89	0.000	0.002	0.86	0.82
FATP2 (au)	ST					0.000	0.001	0.88	0.70	0.000	0.001	0.77	0.40
	MVPA					0.001	0.003	0.68	0.45	-0.001	0.004	0.89	0.52
FATP3 (au)	ST					0.000	0.002	0.79	0.58	0.001	0.002	0.60	0.97
	MVPA					-0.011	0.006	0.09	0.27	-0.011	0.007	0.15	0.11
FABP4 (au)[de]	ST					-0.002	0.001	0.08	**0.05**	-0.001	0.001	0.47	0.39
	MVPA					-0.001	0.005	0.78	0.76	-0.004	0.005	0.45	0.61
GLUT1 (au)[de]	ST					-0.001	0.001	0.15	0.25	-0.001	0.001	**0.01**	0.10
	MVPA					0.000	0.002	0.88	0.76	0.002	0.002	0.45	0.49

B, unstandardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; GLUT1, glucose transporter 1; PA, physical activity; PPAR-γ, peroxisome proliferator-activated receptor gamma; ST: sedentary time. Winsorizing of extreme outliers and/or optimum Box-Cox transformations were performed on mRNAs at the different time point analyses (baseline[a], 24-28th week[b], 35-38th week[c], changes baseline to 24-28th week[d], and changes baseline to 35-37th week[e]). The models 1 were adjusted for the intervention group, smoking at baseline, and gestational week at delivery. In the single time point analyses, the model 2 was additionally adjusted for the relative % of daily ST or MVPA at the respective time point, and the requirement of prostaglandins at delivery. In the analyses of changes from baseline, the model 2 was adjusted for the intervention group, gestational age at delivery, and the relative percentage of ST (35-37th week) or MVPA (baseline).

None of these results changed when multilevel analyses were adjusted in a stepwise manner for the relative percentage of daily ST, light PA or MVPA at baseline, maternal age, BMI, sleep, fat percentage, gestational weight-gain, development of GDM, protein, fat and carbohydrate consumption, maternal/paternal ethnicity, parity, or mode of delivery (data not shown). Only when additionally adjusting for development of GDM, ST at 24-28 weeks was inversely associated with PPAR-γ mRNA ($p<0.05$).

Association of maternal metabolic and adiposity parameters with placental mRNAs

Since maternal ST was highly related to insulin and insulin sensitivity in this cohort[22], the hypothesis that maternal metabolic parameters drive the associations between ST and placental mRNAs was tested (**Table 4)**. To assess whether associations between maternal metabolic parameters and mRNAs were independent of ST, linear regression models were additionally adjusted for ST (model 2).

Table 4. Linear regression associations of maternal metabolic parameters (at 24-28 weeks, and 35-37 weeks) with placental mRNAs (n=171).

		Predictors 24-28 weeks					**Predictors 35-37 weeks**				
Outcomes	**Predictors**	**Model 1**				**Model 2**	**Model 1**				**Model 2**
		B	**SE**	**β**	**p-value**	**p-value**	**B**	**SE**	**β**	**p-value**	**p-value**
PPAR-γ	Fasting glucose	-0.06	0.0	-0.06	0.48	0.09	0.04	0.07	0.04	0.63	0.63
	Fasting insulin	0.00	0.0	-0.09	0.26	0.64	0.00	0.00	-0.07	0.38	0.16
	HbA1c	0.09	0.0	0.08	0.31	0.92	0.03	0.09	0.03	0.71	0.28
	Insulin resistance (HOMA-IR)	-0.03	0.0	-0.10	0.21	0.60	-0.01	0.01	-0.05	0.51	0.20
	B-cell function (HOMA-B)	0.00	0.0	-0.03	0.71	0.54	0.00	0.00	-0.09	0.24	0.71
	Triglycerides	0.03	0.0	0.04	0.62	0.59	0.00	0.04	0.01	0.95	0.55
	Free fatty acids	-0.15	0.1	-0.08	0.33	0.39	-0.04	0.14	-0.02	0.79	0.85
	Leptin	0.00	0.0	0.00	0.96	0.78	0.00	0.00	0.01	0.95	0.85
FATP2	Fasting glucose	-0.23	0.1	-0.11	0.16	0.06	-0.03	0.15	-0.02	0.85	0.57
	Fasting insulin	-0.02	0.0	-0.16	**0.04**	0.67	-0.01	0.01	-0.09	0.27	0.36
	HbA1c	0.14	0.1	0.07	0.41	0.08	-0.21	0.18	-0.10	0.24	0.71
	Insulin resistance (HOMA-IR)	-0.07	0.0	-0.17	**0.03**	0.37	-0.03	0.03	-0.08	0.31	0.45
	B-cell function (HOMA-B)	0.00	0.0	-0.11	0.14	0.93	0.00	0.00	-0.04	0.58	1.00
	Triglycerides	-0.12	0.1	-0.10	0.22	0.08	-0.07	0.09	-0.06	0.46	0.09
	Free fatty acids	-0.05	0.3	-0.01	0.88	0.34	-0.26	0.27	-0.07	0.35	0.26
	Leptin	0.00	0.0	-0.09	0.24	0.87	-0.01	0.00	-0.11	0.18	0.26
FATP3	Fasting glucose	-0.64	0.2	-0.18	**0.02**	**0.003**	-0.27	0.24	-0.09	0.27	0.19
	Fasting insulin	0.00	0.0	0.03	0.75	0.16	0.01	0.01	0.11	0.19	0.43
	HbA1c	0.63	0.2	0.18	**0.03**	0.23	0.47	0.29	0.13	0.11	0.15
	Insulin resistance (HOMA-IR)	-0.02	0.0	-0.02	0.79	0.46	0.04	0.04	0.08	0.30	0.58
	B-cell function (HOMA-B)	0.00	0.0	0.18	**0.02**	**0.008**	0.00	0.00	0.11	0.16	0.16
	Triglycerides	0.29	0.1	0.14	0.07	0.56	0.31	0.15	0.17	**0.03**	0.69
	Free fatty acids	-0.29	0.5	-0.04	0.58	0.99	0.48	0.46	0.08	0.30	0.09
	Leptin	-0.01	0.0	-0.10	0.21	0.28	-0.00	0.01	-0.05	0.55	0.40
FABP4	Fasting glucose	-0.02	0.1	-0.01	0.93	0.11	-0.23	0.15	-0.13	0.12	0.15
	Fasting insulin	0.01	0.0	0.07	0.37	0.20	0.00	0.01	0.06	0.44	0.20
	HbA1c	0.17	0.1	0.08	0.35	0.10	-0.15	0.18	-0.07	0.41	0.84
	Insulin resistance (HOMA-IR)	0.03	0.0	0.07	0.38	0.33	0.02	0.03	0.05	0.53	0.35
	B-cell function (HOMA-B)	0.00	0.0	0.06	0.42	0.25	0.00	0.00	0.12	0.12	0.07
	Triglycerides	0.05	0.1	0.04	0.61	0.28	-0.05	0.09	-0.04	0.61	0.65
	Free fatty acids	-0.11	0.3	-0.03	0.74	0.31	0.31	0.28	0.09	0.27	0.19
	Leptin	0.00	0.0	0.01	0.94	0.69	-0.00	0.00	-0.01	0.94	0.86
Glucose transporter 1[a]	Fasting glucose	0.10	0.0	0.09	0.28	0.42	0.05	0.08	0.05	0.54	0.40
	Fasting insulin	0.00	0.0	0.06	0.46	0.95	0.00	0.00	0.00	0.96	0.60
	HbA1c	-0.05	0.1	-0.05	0.58	0.53	0.11	0.10	0.09	0.28	0.09
	Insulin resistance (HOMA-IR)[b]	0.01	0.0	0.05	0.52	0.81	0.00	0.01	0.00	0.98	0.75
	B-cell function (HOMA-B)	0.00	0.0	0.11	0.18	0.24	-0.00	0.00	-0.05	0.56	0.45
	Triglycerides[b]	0.02	0.0	0.03	0.68	0.90	0.06	0.05	0.10	0.25	0.23
	Free fatty acids	-0.06	0.1	-0.03	0.70	0.52	0.15	0.15	0.08	0.32	**0.05**
	Leptin	0.00	0.0	0.04	0.60	0.96	0.00	0.00	0.14	0.09	0.38

The results remained similar when only women with ST and PA data (n=112) were included in the analyses, except for the association of FATP2 with insulin and HOMA-IR, and of FATP3 with HbA1c, which showed the same trend but non-significant (p≤0.1); and the association of FATP2 with HbA1c, which become significant (p=0.04). B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; HbA1c, glycated hemoglobin; PPAR-γ, peroxisome proliferator-activated receptor gamma. Subtle variation of winsorizing was performed on extreme outliers of mRNAs[a] and metabolic parameters[b]. The results remained similar after handling outliers. Model 1 was adjusted for the intervention group, smoking at baseline, and gestational week at delivery; model 2 was additionally adjusted for the relative percentage of ST at baseline, and the requirement of prostaglandins at delivery (N=93). All the coefficients of determination were equal o below to 0.07. Moderation analyses showed that glucose was inversely associated with FATP2 in male foetuses (data not shown).

At 24-28 weeks, higher fasting insulin and HOMA-IR were associated with lower FATP2 mRNA (only model 1, p<0.05). Higher glucose levels and lower HbA1c and HOMA-B were related to lower FATP3 mRNA (p<0.05). Higher HDL-C was associated with higher GLUT1 mRNA (p<0.05). At 35-37 weeks, higher triglycerides were related to higher FATP3 mRNA (p<0.05), and higher HDL-C was associated with higher GLUT1 mRNA (p<0.05). After controlling for false discovery rates, only the association between glucose and FATP3 (24-28 weeks) remained significant.

Maternal adiposity was not related to mRNAs (Table S2, p>0.05). In mediation analyses, maternal metabolic parameters did not mediate the relationship of ST or MVPA with placental mRNAs (Table S3, p>0.05).

Association of placental mRNAs with cord blood metabolic parameters, and neonatal adiposity after birth

Subsequently, the hypothesis that placental mRNAs are associated with glucose and lipid transport related foetal metabolites, and proxy measures of neonatal adiposity (sum of skin folds, cord blood leptin) was tested (**Table 5)**. FATP2 mRNA was inversely associated with cord triglycerides and FFA (*p*<0.01). After separating the analyses by foetal sex, the association of FATP2 mRNA with cord FFA was only observed in female foetuses (Table S4, *p*=0.01). GLUT1 mRNA was positively associated with cord glucose (model 1, *p*=0.02). Greater FABP4 and PPAR-γ mRNAs were associated with higher cord leptin (*p*<0.05); these associations were not observed with the sum of skinfolds. When controlling for false discovery rates, only the associations of cord triglycerides and FFA with FATP2 remained significant. Mediation analyses showed that PPAR-γ mRNA exerted an indirect effect on neonatal sum of skinfolds via cord blood leptin (positive

association, $p<0.05$; data not shown). No other cord metabolic parameter mediated the association of placental mRNAs with neonatal adiposity (Table S5, $p>0.05$).

Table 5. Linear regression associations of placental mRNAs with cord blood glycaemic (n=142) and lipid parameters (n=146), and neonatal adiposity (n=161).

Outcomes	Predictors	Model 1				Model 2	Adjusted	Adjusted
		B	SE	β	p-value	p-value	R2[c]	R2[d]
C-peptide[a]	PPAR-γ	0.08	0.09	0.08	0.35	0.72	0	0
	FATP2	0.04	0.05	0.07	0.42	0.63	0	0
	FATP3	0.01	0.03	0.03	0.71	0.86	0	0
	FABP4	0.04	0.04	0.09	0.33	0.51	0	0
	Glucose transporter 1	-0.03	0.08	-0.03	0.75	0.75	0	0
Glucose[ab]	PPAR-γ	0.37	0.21	0.15	0.08	0.76	0.01	0.04
	FATP2	-0.08	0.11	-0.06	0.50	0.84	0	0.02
	FATP3	-0.04	0.07	-0.05	0.55	0.17	0	0.02
	FABP4	-0.01	0.09	-0.01	0.92	0.80	0	0.33
	Glucose transporter 1	0.46	0.20	0.19	**0.02**	0.22	0.04	0.06
Triglycerides[a]	PPAR-γ	0.03	0.07	0.04	0.61	0.66	0	0.04
	FATP2	-0.14	0.03	-0.34	**<0.001**	**<0.001**	0.13	0.14
	FATP3	-0.02	0.02	-0.10	0.24	0.21	0	0.05
	FABP4	0.02	0.03	0.06	0.50	0.43	0	0.03
	Glucose transporter 1	0.10	0.06	0.15	**0.07**	0.14	0.02	0.04
Free fatty acids[a]	PPAR-γ	0.04	0.04	0.09	0.28	0.94	0	0.05
	FATP2	-0.06	0.02	-0.26	**<0.001**	**0.005**	0.07	0.10
	FATP3	0.01	0.01	0.04	0.60	0.86	0	0.04
	FABP4	0.01	0.02	0.04	0.61	0.43	0	0.04
	Glucose transporter 1	0.07	0.04	0.17	**0.04**	0.21	0.03	0.07
Sum of skinfolds (thickness)	PPAR-γ	-0.33	0.99	-0.03	0.74	0.89	0	0.04
	FATP2	0.34	0.48	0.06	0.47	0.69	0	0.04
	FATP3	0.15	0.28	0.04	0.59	0.41	0	0.04
	FABP4	0.10	0.48	0.02	0.84	0.83	0	0.04
	Glucose transporter 1	-0.30	0.85	-0.03	0.72	0.97	0	0.04
Cord blood leptin[a]	PPAR-γ	3.56	1.36	0.21	**0.01**	**0.02**	0.04	0.13
	FATP2	0.20	0.72	0.02	0.78	0.86	0	0.09
	FATP3	0.66	0.40	0.13	0.10	**0.05**	0.01	0.11
	FABP4	1.42	0.65	0.18	**0.03**	**0.05**	0.04	0.12
	Glucose transporter 1	1.76	1.28	0.11	0.17	0.31	0.01	0.10

The results did not change when only those women with ST and PA data (n=112) were included in the analyses. B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; PPAR-γ, peroxisome proliferator-activated receptor gamma. [a] A subtle variation of winsorizing was performed on extreme outliers of cord blood parameters. [b] Optimum Box-Cox transformations were conducted on cord blood markers. The results remained similar after handling outliers. Model 1 was adjusted for the intervention group, smoking at baseline, and gestational week at delivery; and model 2 was additionally adjusted for maternal age, consumption of food rich in protein and delivery route (metabolic parameters N=137; neonatal adiposity, N=152). The coefficient of determination values shown are derived from unadjusted [c] and model 1[d].

Sensitivity analyses

Additional sensitivity analyses of groups with high/low ST or MVPA and obese class I-III are shown in Tables S6-S11**.** The association between FATP2 mRNA and cord triglycerides was more noticeable in women with higher ST levels at early pregnancy ($p<0.05$).

DISCUSSION

This is the first large-scale study examining the influence of objectively measured ST and MVPA at different time periods in pregnancy on targeted placental mRNAs in overweight-obese pregnant women. The overall result was that MVPA had little, if any, effect on placental mRNAs. Strikingly, however, more time spent sedentary, especially in early pregnancy, was associated with lower FATP2 and FATP3 mRNA in term placenta samples. Although higher maternal insulin and insulin resistance (24-28 weeks) were associated with lower FATP2, and higher glucose, poorer beta-cell function (24-28 weeks), and lower triglycerides (35-37 weeks) with lower FATP3 expression, none of these metabolic parameters mediated the relationship of ST or MVPA with transporter mRNAs. This might be due to lack of power in our mediation analyses. Contrary to our expectations[10,14], FATP2 mRNA was inversely associated with cord blood triglycerides and FFA, and was not associated with neonatal adiposity. In addition to trans-placental transport, FFA uptake into foetal tissues contributes to the steady-state levels in cord blood, which might account for the inverse association of FATP2 mRNA with cord blood triglycerides and FFA. Moreover, other placental transporters and transcripts/proteins could play a role in determining cord blood levels of triglycerides and FFA.

It is worth noting that PPAR-γ was not associated with ST or MVPA. This is surprising since it is a transcriptional regulator of FATPs and FABPs acting upstream of FATP2, FATP3 and FABP4[14,16-18]; and these transporters were related to ST. A possible explanation is that we measured mRNAs only, and not proteins. However, PPAR-γ expression was positively correlated with FATP2, FATP3 and FABP4 mRNA (see Figure S2). The higher levels of these placental transporters with lower ST levels, although at various time periods, prompted the hypothesis that PPAR-γ upregulation could indirectly explain lifestyle-induced changes on FATP2, FATP3 and FABP4 mRNA.

However, we did not find PPAR-γ mediating the association between ST and FATP2, FATP3 or FABP4 (data not shown), which might be due to a lack of statistical power.

In agreement with an earlier study[29], our findings also suggested that higher MVPA during early pregnancy was related to down-regulated placental GLUT1 expression; although this association was dependent on ST levels. However, another study[28] did not observe any association. Methodological differences (e.g. measurements/devices employed, statistical power, or maternal phenotype) could explain discrepancies in findings. Given that most of the associations were reported with ST during early to middle gestation, a potential explanation is that reducing ST during this period might have induced diverse structural, metabolic and molecular changes[28,29,36,37] in placental cells that remain throughout pregnancy until parturition, and might dictate placental phenotype[5,20,28,29,37]. However, we cannot fully exclude that there might also be an acute influence of currently unknown drivers in later pregnancy that account for placental alterations. If future studies confirm our findings, strategies aimed at reducing ST during this vulnerable tipping point during which pregnancy complications arise[1,2,4], might be of high relevance for targeting placental regulation of these transcripts.

LIMITATIONS

The main strength of the current study is that it included objective device-based measurements of ST and MVPA at three time points in pregnancy. Furthermore, the cohort is very well phenotyped, with important information on maternal and cord blood metabolic parameters available. In addition, maternal diet and sleep duration were considered for the analyses, since they could be important confounders of the association between ST/MVPA and placental mRNA. The sample size was large enough to assess sex differences in associations of placental mRNAs with both maternal and foetal metabolites. Some limitations need to be acknowledged as well. First, the representativeness of the sample might be compromised because we only analysed placental samples from a subgroup of women (Figure S1). Hence, some selection biases might be present. We preferentially selected women for mRNA analyses from the intervention groups with PA counselling, since we expected the most relevant changes

in ST and MVPA levels in these groups. Thus, women from the HE groups are underrepresented in this study. Another limitation of the current study is that only gene expression, and not protein, was analysed in pooled placental tissues. Furthermore, statistical power might have been too limited for mediation analyses.

CONCLUSIONS

The present study showed that ST at specific periods of pregnancy, and changes in ST from baseline, were associated with the expression of different placental genes linked to intracellular lipid transport. However, PA levels during pregnancy were hardly related to transporter mRNAs. Therefore, the role of PA on these placental mRNAs of overweight-obese women is debatable. Strategies aimed at lowering ST behaviours are more likely to regulate neonatal growth and adiposity by modulating the expression of relevant placental molecules, which is of clinical interest for the prevention of future maternal and offspring-adult diseases. Future studies i) earlier in pregnancy[5], ii) considering the crosstalk between muscle, placenta and other organs[38], iii) and epigenetic changes[39], and iv) distinguishing clearly normal and pathological conditions during pregnancy, are necessary to better understand the role of the placenta in linking maternal lifestyle with neonatal outcomes.

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SUPPLEMENTARY MATERIAL

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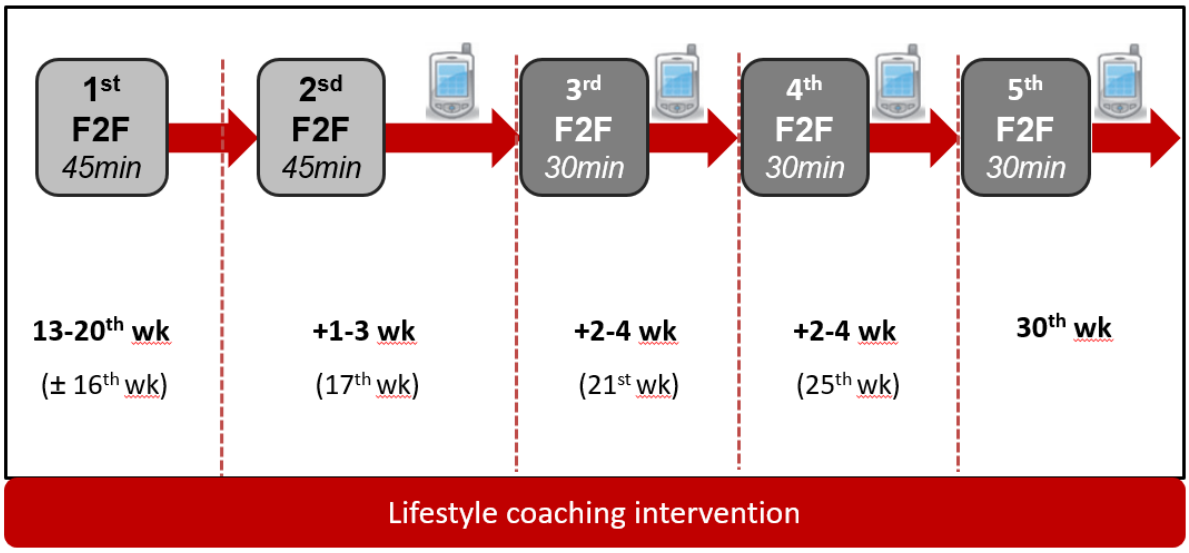
Appendix A. ***Lifestyle interventions***

The general goal of lifestyle interventions was to make women conscious about their capacity to influence their weight, and minimize their gestational weight gains during pregnancy. Accordingly, they were advised not to exceed a weight gain greater than 5kg, or to maintain their weight if they have already gained ≥5kg before starting the interventions. As previously further described[1], after the randomization and baseline measurements, five face to face and four optional booster telephone coaching sessions (**see in the figure below**) were scheduled for each participant with the same lifestyle coach through the intervention. Importantly, four of these five face-to-face coaching appointments should have taken place before the second measurement (24-28 weeks), and the different interventions should have finished before 35 weeks. Lifestyle interventions were inspired on motivational interviewing methods. Depending on the lifestyle group, personal coaching involved discussion of PA and/or HE habits (**see below**), and specific material was provided to the participants to support them to progress and change their behaviour.

Lifestyle messages used during coaching sessions

Physical activity (PA) intervention
1) "Be active every day": Incorporate light and moderate PA as much as possible into daily life (e.g. by parking further away from destination or undertake special activities for pregnant women).
2) "Sit less": Reduce sedentary time.
3) "Build your strength": Incorporate upper and/or lower limb resistance exercise as PA.
4) "Take more steps": increase the number of steps taken per day.
5) "Be more active at weekends": Be more active during the weekends.
Healthy Eating (HE) intervention
1) "Replace sugary drinks": Reduce intake of sugary drinks (e.g. replace with water).
2) "Eat more non-starchy vegetables": Eat more non-starchy vegetables.
3) "Increase fibre consumption": Choose high-fibre, over low fibre products (≥5 g fibre/100 g).
4) "Watch portion size": Be conscious about the amount of food eaten each meal.
5) "Eat protein": Increase intake of proteins (e.g. meat, fish, beans).
6) "Reduce fat intake": Reduce fat intake (e.g. snack, fast food, fried foods).
7) "Eat less carbohydrates": Reduce intake of carbohydrates (e.g. potatoes, pasta, rice, snacks, candy).

In the PA group, participants were encouraged to increase PA levels based on previous American College of Obstetrics and Gynaecology recommendations[2], and received information manuals, pedometers (Yamax Digiwalker SW-200), and flexible elastic dynabands (Thera-Band, Akron, USA) along with a training video. In the HE group, the action plan aimed at improving dietary habits, and participants were provided with information manuals along with additional data about healthy eating and related myths. The PA & HE group consisted of a combination of both PA and HE groups. The control group did not receive any lifestyle intervention, but usual care from their midwives/obstetricians. Lifestyle coaches attended meetings, and were provided with personal digital assistants and manuals, presentations in English, training courses about motivational interviewing, and feedback by experts, to achieve the maximum standardization of interventions across the different study centres.



Life coaching intervention schedule. F2F, face to face sessions. Picture phone, booster telephone call sessions.

Appendix B. ***Target sequence of placental genes***

Nanostring technology: customized CodeSet (nCounterTM PlexSetTM)

Specie: Homo sapiens

Gene	Target Sequence	Gene (HUGO)
FATP2	ACACCATTGAGATCACTGGAACTTTTAAACACCGCAAAATGACCCTGGTGGAGGAGGGCTTTAACCCTGCTGTCATCAAAGATGCCTTGTATTTCTTGGA	SLC27A2
FATP3	TGCTAAAGGATGTCTTCCGGCCTGGGGATGTTTTCTTCAACACTGGGGACCTGCTGGTCTGCGATGACCAAGGTTTTCTCCGCTTCCATGATCGTACTGG	SLC27A3
FABP4	GGTGGAATGCGTCATGAAAGGCGTCACTTCCACGAGAGTTTATGAGAGAGCATAAGCCAAGGGACGTTGACCTGGACTGAAGTTCGCATTGAACTCTACA	FABP4
GLUT1	AGGCTCCATTAGGATTTGCCCCTTCCCATCTCTTCCTACCCAACCACTCAAATTAATCTTTCTTTACCTGAGACCAGTTGGGAGCACTGGAGTGCAGGGA	SLC2A1
OAZ1	GGTGGGCGAGGGAATAGTCAGAGGGATCACAATCTTTCAGCTAACTTATTCTACTCCGATGATCGGCTGAATGTAACAGAGGAACTAACGTCCAACGACA	OAZ1
PPAR-γ	CAGATCCAGTGGTTGCAGATTACAAGTATGACCTGAAACTTCAAGAGTACCAAAGTGCAATCAAAGTGGAGCCTGCATCTCCACCTTATTATTCTGAGAA	PPARG
TBP	ACAGTGAATCTTGGTTGTAAACTTGACCTAAAGACCATTGCACTTCGTGCCCGAAACGCCGAATATAATCCCAAGCGGTTTGCTGCGGTAATCATGAGGA	TBP
WDR45L	CTGCCCAGGGACCTTGGTCTCGAAGCCATACGTGGTTGTCTGCTTTCCTAAGGACTCCCATTTCCAGTATTAAAGAGAGAATCATCATCAAGGCACCGTA	WDR45B

Appendix C. Outlier detection and management

Nowadays, the presence of outliers is one of the most enduring and pervasive methodological changes in biomedical science research[3-5]. Worryingly, there is a lack of consensus about how addressing outliers (i.e. how defining, identifying and handling them). Since the decisions that researchers make about this issue have important implications, we have included this section to promote transparency and the critical interpretation of the results, as previously recommended by several authors[3-5]. Although no specific guidelines exist about how addressing outliers, several studies[3-10] (especially that one from Aguinis, et al. [5]) have previously provided smart advices and recommendations to address them in the best possible way. Accordingly, the different steps to address outliers in the present study have been performed proceeding with the following recommendations. We have identified and handled outliers according to the basis for multilevel (primary aims) and regression analyses (secondary aims).

Error outliers

Singles construct techniques (box plots, descriptive statistics, percentage analyses, etc.) were performed to initially identify error outliers. Subsequently, we also employed multiple construct techniques to identify error outliers. Particularly, we identified error outliers based on the outlyingness of the observation in term of its residual score and

scores of predictors (standardized residuals and studentized residuals). When it was not possible/appropriate to correct these data points, and we were sure that their inaccuracy was related to human errors, device malfunction, miscalculations or similar circumstances (i.e. we had determined the cause of the identified outlying observation), these error outliers were removed from the respective database. Since these potential error outliers could have been caused by inherent variability in the data (in this case they would represent a legitimate part of the population), we were very prudent when identifying and handling them. We paid special attention to the reasoning behind the classification of data points as error outliers.

Interesting outliers

After the application of this first filter to the database, there were several remaining interesting outliers, which required additional analyses in depth. Thereby, we aimed at analysing these interesting outliers with quantitative approaches (e.g., we tried to analyse differences in how predictors were able to predict high and low outlier scores). However, the number of outliers was minimum, and only appreciable in few outcomes, which prevented us from performing these analyses properly. As consequence, we did not finally perform these analyses.

Influential outliers

Since it is not legitimate to simply drop the remaining potential outliers from the analyses (they tend to increase error variance, reduce the power of statistical test, etc.), nor plainly deleting them without any basis (they could be part of the inherent variability of the distribution of data), we analysed more in depth the influence of these outliers in the model. Aimed at checking their influence, we analysed how the deletion of specific outliers could affect the change of the model fit (e.g., changes in R^2; model fit outliers), parameters estimates (intercept, slope, regression coefficients, etc.; prediction outliers) and the assumptions of the model. If these remaining unusual cases were not finally identified as influential outliers, or they were identified but influenced the model slightly, these potential outliers were not handled (as observed in some mRNAs, and maternal glycaemic/lipid and neonatal adiposity markers: **Tables 3-5** and **Tables S1-S4**).

In this case, these unusual data points were dropped in the analyses since they did not affect either the results or assumptions of the tests, and they could be caused by inherent variability in the data. By contrast, if these remaining unusual cases were

confirmed as influential outliers which affected the model fit and parameter estimates (as observed in some outcomes variables in the **Tables 3-5** and **Table S1-S11**), those influential outliers we handled. As general rule (some exceptions such as in **Table 4** and **Table S3**), no handling of outliers was performed in predictor variables since residual values from models were small, and/or potential extreme values from predictors did not/scarcely influence the fit and coefficients of the model[5] (checked with changes in the coefficient of determinations, changes in the intercepts and slopes, Cook´s distances, centered leverage values, DFBETAS values, and the studentized residuals). In order to handle influential outliers (when identified), a subtle variation of winsorizing [convert back from a z-score: replacing extreme scores (z>2.58; value equivalent to a probable outlier) with a score equivalent to ±2.58 standard deviations from the mean] was employed to handle these outliers. After handing these outliers, data distribution improved, and some of the problematic issues related to the assumptions of some models disappeared. Subsequently, data preparation was employed for those models characterized by asymmetry (skewness, kurtosis, etc.) of placental mRNAs, and the violation of some assumptions related to the generalization of the results. Specifically, optimum Box-Cox transformations were used to reduce the impact of potential source of bias, and improve the goodness of fit of the data. After dealing with these "problematic" outcomes, the results remained similar (but with better and more symmetrical distribution of data) to the analyses without data preparation.

Appendix D. Statistical analyses (for the secondary aims)

Several extreme values of some outcome variables were confirmed as influential outliers in these sensitivity analyses. Hence, these influential outliers were handled. Particularly, a subtle variation of winsorizing was employed to handle these outliers (**Appendix C**). Subsequently, data preparation was employed for those models (specified in the **Tables 4-5 and Tables S4-S11**) characterized by asymmetry of outcome variables, and the violation of some assumptions related to the generalization of the results. Specifically, optimum Box-Cox transformations were used to reduce the impact of potential source of bias and improve the goodness of fit of the data.

After considering relevant confounders suggested by previous literature, bivariate correlations and stepwise linear regressions were employed to identify

potential confounders in the different sensitivity analyses. Particularly, those statistically significant confounders, which were strongly related to the outcomes and might influence the model, were chosen.

Firstly, linear regressions analyses were performed to explore the association of maternal glycaemic and lipid parameters (at 24-28 and 35-37 weeks), and maternal adiposity (35-37 weeks), with placental mRNAs (**Table 4** and **Table S2**). The maternal metabolic and adiposity parameters were introduced in the models as predictors, and the placental mRNAs as outcomes. The model 1 was adjusted for the intervention group, and smoking at baseline and gestational week at delivery; model 2 was additionally adjusted for the relative percentage of daily ST at baseline, and the requirement of prostaglandins at delivery. Linear regression associations grouped by foetal sex were performed for those models in **Table 4 and Table S2** which showed foetal sex dependency. Additionally, simple mediation analyses (**Table S3**, and **Figure S3**) were conducted to investigate the potential role of maternal glycaemic and lipid markers (at 24-28 and 35-37 week) as mediators of the association between ST (at baseline and 24-28 week), MVPA (at baseline) and changes in ST (from baseline to 35-37 week), with placental mRNAs. These mediation analyses were only conducted for those associations which were statistically significant in previous analyses and were especially related to our main hypotheses. All the models were adjusted for lifestyle intervention, smoking at baseline and gestational week at delivery. Foetal sex dependency of all pathways in the mediation models from **Figure S3** were tested through conditional process analyses (moderated mediation) (see **Figure S4**). No potential effect modification by foetal sex was found in any pathway. Therefore, sexes were combined in all mediation models.

Secondly, linear regression analyses were used to examine the individual association of placental mRNAs with cord serum glycaemic and lipid parameters (**Table 5**), neonatal adiposity (**Table 5**), and maternal metabolic markers (data not shown: none significant association) after birth. The placental mRNAs were introduced in the models as predictors, and the aforementioned maternal and neonatal variables as outcomes. The model 1 was adjusted for the intervention group, smoking at baseline, and gestational week at delivery; and the model 2 was additionally adjusted for maternal age, consumption of food rich in protein and delivery route. Linear regression associations grouped by foetal sex (**Table S4**; analyses of simple slopes) were performed

for those models in **Table 5** which showed foetal sex dependency (i.e. a statistically significant interaction-term; presence of potential effect modification). Additionally, simple mediation analyses (**Table S5**, and **Figure S5**) were conducted to investigate the potential role of cord blood metabolic parameters as mediators of the association between placental mRNAs and neonatal adiposity. These mediation analyses were only conducted for those associations which were statistically significant in previous analyses, and/or might allow us to better understand some mechanisms related to neonatal adiposity. All the models were adjusted for the intervention group, smoking at baseline, and gestational week at delivery. We did not employ parallel multiple mediator models to avoid problems with collinearity, which in turn might increase sample variance and affect the indirect effect and confidence interval coefficients. Foetal sex dependency was also tested in all the pathways of these mediation models from **Figure S5** through conditional process analyses (moderated mediation – see schematic diagram **Figure S6**). No potential effect modification by foetal sex was found in any pathway. Therefore, sexes were combined in all mediation models.

Lastly, simple slope analyses (moderation analyses) were performed to analyse if the associations between placental mRNAs and maternal/neonatal outcomes (at/after birth) differed depending on the level of ST and MVPA, and the weight status category (**Table S6-S11**). These linear regressions were only conducted for those models which were statistically significant (or showed evidence of statistical significance) in **Table 5**, and **Tables S4-S5**, and were of clinical interest to better understand our main results (**Table 3** and **Table S1**). Specifically, these associations were tested separately in the following groups (the groups were categorized according the median, except for weight status):

i) Women with low ST vs. high ST levels at baseline *(low ST <584min/day; high ST ≥584min/day)* **(Table S6)**.

ii) Women with low ST vs. high ST levels at 24-28 weeks *(low ST <590min/day; high ST ≥590min/day)* **(Table S7)**.

iii) Women who increased less ST vs. women who increased more ST, from baseline to 24-28 weeks *(increased less ST from baseline, <20min/day; increased more ST from baseline, ≥20min/day)* **(Table S8)**.

iv) Women with low MVPA vs. high MVPA levels at baseline *(low MVPA <40min/day; high MVPA ≥40min/day)* **(Table S9)**.

v) Women with low MVPA vs. high MVPA levels at 24-28 weeks *(low MVPA <33min/day; high MVPA ≥33min/day)* **(Table S10)**.

vi) Overweight-obese class-1 women vs. obese class-2 women *(overweight-obese class-1, BMI pre-pregnancy<35; obese class-2 and 3, BMI pre-pregnancy ≥35,* **Table S11)**.

The model 1 was adjusted for the intervention group and gestational week at delivery; and the model 2 was adjusted for gestational week at delivery, and delivery route. All the assumptions related to the generalization of the results (linearity, normal distribution of the residuals, no perfect multicollinearity, etc.) were met in the different models from all the analysis addressing the secondary aims. The statistical analyses were conducted using SPSS 22.0 (IBM, NY, USA). The simple mediation and conditional process analyses were performed with the PROCESS version 3.4.1. The statistical significance was set at $p \leq 0.05$.

Study II

Table S1. Linear regression analyses assessing the effects of the lifestyle counselling intervention on placental m

Placental mRNAs	HE+PA (n=54)		HE (n=23)		PA (n=52)		Control (n=54)		HE+PA vs. control				HE vs.	
									Model 1			Model 2	Model 1	
									B	SE	p-value	p-value	B	SE
PPAR-γ	7.01	0.42	7.13	0.34	7.02	0.40	6.95	0.37	0.07	0.07	0.31	0.42	0.17	0.09
FATP2	3.32	0.74	3.41	0.87	3.17	0.78	3.19	0.85	0.10	0.15	0.49	0.68	0.17	0.19
FATP3[a]	3.92	(2.63, 4.55)	3.79	(2.94, 4.6)	3.91	(2.72, 4.78)	3.35	(2.75, 3.86)	0.22	0.25	0.37	0.25	0.42	0.32
FABP4	7.72	0.80	8.02	0.95	7.59	0.84	7.80	0.79	-0.09	0.15	0.57	0.48	0.20	0.20
GLUT 1[a]	11.4	0.45	11.3	0.38	11.43	0.45	11.3	0.42	0.07	0.08	0.39	0.50	-0.05	0.10

Data are mean (standard deviation) or median (interquartile range=Q3, Q1). B, unstandardized regression coeffi
acid binding protein; HE, healthy eating; PA, physical activity; PPAR-γ, peroxisome proliferator-activated
coefficients indicate the raw differences in means on placental mRNAs between conditions. [a] Winsorizing of ex
optimum Box-Cox transformations were performed on placental mRNAs. Model 1 was unadjusted; and model
and requirement of prostaglandins at delivery. The sample size involved in the comparison of differences betw
2 (HE+PA, n=47; HE, n=21; PA, n=48; control, n=47).

Table S2. Linear regression associations of maternal adiposity parameters (at 35-37th week of gestation) with placental mRNAs (n=173).

Outcomes	Predictors	Model 1				Model	Adjusted	Adjusted
		B	SE	β	p-value	p-value	R2[b]	R2[c]
PPAR-γ	Sum of skinfolds	0.00	0.00	-0.08	0.35	0.80	0	0
	Fat percentage	-0.01	0.01	-0.10	0.19	0.97	0	0
	Weight	0.00	0.00	0.04	0.58	0.57	0	0
	Body mass index	-0.01	0.01	-0.11	0.17	0.68	0	0.01
FATP2	Sum of skinfolds	-0.00	0.00	-0.08	0.31	0.50	0	0.06
	Fat percentage	-0.00	0.02	-0.02	0.84	0.46	0	0.06
	Weight	-0.00	0.00	-0.07	0.35	0.50	0	0.06
	Body mass index	-0.01	0.02	-0.04	0.63	0.59	0	0.05
FATP3	Sum of skinfolds	0.00	0.00	0.09	0.29	0.50	0	0.01
	Fat percentage	0.03	0.03	0.08	0.31	0.49	0	0.01
	Weight	0.00	0.01	0.02	0.83	0.23	0	0
	Body mass index	-0.02	0.03	-0.06	0.44	0.48	0	0
FABP4	Sum of skinfolds	0.00	0.00	0.09	0.28	0.41	0	0.02
	Fat percentage	0.02	0.02	0.06	0.47	0.73	0	0.01
	Weight	0.00	0.00	0.05	0.50	0.69	0	0.01
	Body mass index	-0.01	0.02	-0.06	0.48	0.65	0	0.01
Glucose transporter 1[a]	Sum of skinfolds	0.00	0.00	-0.05	0.56	0.61	0	0
	Fat percentage	-0.01	0.01	-0.06	0.45	0.44	0	0
	Weight	-0.00	0.00	-0.06	0.46	0.20	0	0
	Body mass index	-0.00	0.01	-0.03	0.67	0.44	0	0

The results did not change when only those women with ST and PA data (n=112) were included in the analyses. B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; PPAR-γ, peroxisome proliferator-activated receptor gamma. [a]Subtle variation of winsorizing was performed on extreme outliers (z value>2.58) of placental mRNAs. The coefficient of determination values shown are derived from unadjusted [b] and model 1[c]. The results remained similar after handling outliers. Model 1 was adjusted for the intervention group, smoking at baseline, and gestational week at delivery; and model 2 was additionally adjusted for the percentage of ST at baseline, and the requirement of prostaglandins (N=91).

Study II

Table S3. Simple mediation analyses assessing the potential role of glycaemic (n=107) and lipid (n=102) param
mediators of the relationship between sedentary time and physical activity levels with placental mRNAs. Schen

			Effect of the predictor			Effect of mediator on the			D
Outcome (Y)	**Predictor** (X)	**Mediator** (M)	**B**	**95% CI**		**B**	**95% CI**		**E**
FATP2	ST (baseline)	Insulin (24-28 weeks)	0.013	-0.004	0.030	-0.009	-0.030	0.001	-0.0
		HOMA-IR (24-28 weeks)	0.003	-0.001	0.007	-0.049	-0.127	0.030	-0.0
FATP3	ST (baseline)	Glucose (24-28 weeks)	0.000	-0.000	0.001	**-0.860**	**-1.620**	**-0.091**	-0.0
		HbA1c (24-28 weeks)	-0.000	-0.001	0.000	0.503	-0.292	1.298	-0.0
		HOMA-B (24-28 weeks)	0.397	-0.361	1.156	**0.001**	**0.000**	**0.001**	-0.0
		Triglycerides (35-37 weeks)	-0.000	-0.002	0.001	0.257	-0.132	0.647	-0.0
Glucose transporter 1[a]	MVPA (baseline)	Glucose (24-28 weeks)	0.002	-0.001	0.004	0.139	-0.104	0.383	**-0.0**
		Insulin (24-28 weeks)	**-0.083**	**-0.156**	**-0.001**	0.002	-0.008	0.012	**-0.0**
		HDL-C (24-28 weeks)	0.002	-0.001	0.004	**0.350**	**0.068**	**0.634**	**-0.0**
		HDL-C (35-37 weeks)	0.001	-0.002	0.004	**0.349**	**0.091**	**0.606**	**-0.0**
	Change ST	HDL-C (35-37 weeks)	**-0.002**	**-0.003**	**-0.001**	0.168	-0.183	0.519	-0.0

B, unstandardized regression coefficient; CI, confidence interval; FATP, fatty acid transport protein; HbA1
homeostasis model assessment (insulin resistance); HOMA-B, homeostasis model assessment (B-cell function)
time. Confidence intervals are shown as 95% bias corrected and accelerated CI, and are based on 5000 boots
winsorizing was performed on extreme outliers (z value>2.58) of placental mRNAs. [b] N=64. All models were
smoking at baseline and gestational week at delivery. When additionally adjusting for the relative percentage c
point, the results remained similar. Conditional analyses (**Figure S4**) showed a significant inverse association of
in male fetuses.

Table S4. Linear regression associations (grouped by fetal sex) of placental mRNAs concentrations with neonatal (n=147) and maternal metabolic (n=110) parameters.

Outcomes	Predictors	Male fetuses					Female fetuses				
		B	SE	β	p-value	p-value[c]	B	SE	B	p-value	p-value[d]
Cord serum parameters				**(N=78)**					**(N=69)**		
Glucose[ab]	FATP2	-0.08	0.17	-0.06	0.62	0.56	-0.06	0.16	-0.05	0.71	0.89
Triglycerides[a]	FATP2	-0.11	0.05	-0.26	**0.02**	**0.02**	-0.15	0.05	-0.39	**0.002**	**0.00**
Free fatty acids[a]	FATP2	-0.05	0.03	-0.19	0.10	0.10	-0.08	0.03	-0.34	**0.01**	**0.01**
	FATP3	0.01	0.02	0.04	0.70	0.70	0.00	0.02	0.03	0.85	0.92
Maternal lipids				**(N=61)**					**(N=49)**		
Free fatty acids	PPAR-γ	0.57	0.26	0.27	**0.03**	**0.03**	-0.53	0.39	-0.19	0.18	0.10
	PPAR-γ	-0.13	0.11	-0.16	0.24	0.25	-0.05	0.15	-0.06	0.73	0.85

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FATP, fatty acid transport protein; GLUT1, glucose transporter; HDL-C, high density lipoprotein-cholesterol; IL, interleukin; LDL-C, low density lipoprotein-cholesterol; PPAR-γ, peroxisome proliferator-activated receptor gamma. [a] Subtle variation of winsorizing was performed on extreme outliers (z value>2.58) of cord blood parameters. [b] Optimum Box-Cox transformations were conducted on cord blood parameters. The analyses were adjusted for the intervention group, smoking at baseline, and gestational week at delivery. These models were additionally adjusted for maternal age, consumption of food rich in protein and delivery route ([c] Nmale=73 and Nfemale=64 for cord serum lipids; [d] Nmale=59, Nfemale=46 for maternal lipids).

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Table S5. Simple mediation analyses assessing the potential role of cord glycaemic and lipid parameters as mediators mRNAs and neonatal adiposity (n=140). Schematic diagram in **Figure S5**.

			Effect of the predictor			Effect of mediator on the			Direct effect of th		
Outcome (Y)	**Predictor** (X)	**Mediator** (M)	**B**	**95% CI**		**B**	**95% CI**		**B**	**95% CI**	
Sum of skinfolds	PPAR-γ	Glucose[ab]	0.445	-0.010	0.900	0.343	-0.503	1.188	-0.695	-2.865	1.
	FATP2	Cholesterol[ab]	-0.210	-0.440	0.019	0.086	-0.648	1.020	0.322	-0.786	1.
		Triglycerides[a]	**-0.142**	**-0.208**	**-0.076**	-2.271	-5.133	0.590	-0.039	-1.198	1.
		Free fatty acids[a]	**-0.066**	**-0.107**	**-0.025**	-2.351	-7.037	2.335	0.127	-1.001	1.
	FATP3	FFA[a]	0.016	-0.009	0.040	-2.660	-7.190	1.870	0.204	-0.423	0.
	GLUT1	Glucose[ab]	**0.446**	**0.305**	**0.861**	0.365	-0.482	1.212	-0.858	-2.850	1.
		Free fatty acids[a]	**0.082**	**0.010**	**0.155**	-2.249	-6.840	2.342	-0.533	-2.469	1.
	PPAR-γ	Glucose[ab]	0.366	-0.080	0.813	0.857	-0.264	1.978	**3.617**	**0.806**	**6.**
		Triglycerides[a]	0.066	-0.072	0.203	3.454	-0.251	7.158	**3.381**	**0.501**	**6.**
		Free fatty acids[a]	0.067	-0.018	0.152	-2.243	-8.326	3.839	**3.759**	**0.827**	**6.**
	FATP2	Cholesterol[ab]	-0.227	-0.466	0.012	**1.154**	**0.614**	**2.246**	0.196	-1.293	1.
		Triglycerides[a]	**-0.135**	**-0.199**	**-0.070**	**4.271**	**0.260**	**8.2884**	0.509	-1.055	2.
		Free fatty acids[a]	**-0.063**	**-0.105**	**-0.022**	-1.316	-7.715	5.083	-0.149	-1.696	1.
	FATP3	Free fatty acids[a]	0.007	-0.018	0.032	-1.456	-7.548	4.636	0.837	-0.009	1.
	GLUT1	Glucose[ab]	**0.493**	**0.083**	**0.904**	0.925	-0.234	2.084	1.576	-1.126	4.
		Free fatty acids[a]	**0.077**	**0.002**	**0.152**	-2.028	-8.250	4.194	2.075	-0.593	4.

The results did not change when only those women with ST and PA data (n=112) were included in the analyses. B, unstandardize interval; FATP, fatty acid transport protein; GLUT1, glucose transporter 1; PPAR-γ, peroxisome proliferator-activated rec shown as 95% bias corrected and accelerated CI, and are based on 5000 bootstrap samples. [a] A subtle variation of winsori (z value>2.58) cord blood markers. [b] Optimum Box-Cox transformations were conducted on cord blood parameters. intervention group, smoking at baseline, and gestational week at delivery. When additionally adjusting for the delive Conditional analyses (Figure S6) showed a significant direct effect of PPAR-γ on sum of skinfolds (inverse association whe foetuses; and a positive significant association of cord cholesterol with cord leptin only in male foetuses.

Table S6. Associations of placental mRNAs with neonatal and maternal metabolism parameters, and neonatal adiposity, in women with low or high ST levels at **baseline (<20 weeks).**

Outcomes	Predictors	Low sedentary time (n=52)					High sedentary time (n=52)				
		B	SE	β	p-value[c]	p-value[d]	B	SE	β	p-value[c]	p-value[d]
Cord blood (at birth)				(N=41)					(N=44)		
C-peptide[a]	PPAR-γ	-0.01	0.14	-0.01	0.95	0.85	0.12	0.13	0.16	0.34	0.60
	FATP2	0.12	0.08	0.22	0.13	0.35	0.07	0.07	0.17	0.33	0.56
	FATP3	-0.01	0.04	-0.06	0.72	0.73	-0.02	0.04	-0.08	0.63	0.38
	FABP4	0.04	0.07	0.10	0.56	0.51	-0.04	0.06	-0.10	0.53	0.43
	GLUT1	-0.25	0.12	-0.33	0.05	0.05	0.15	0.14	0.17	0.30	0.69
Glucose[ab]	PPAR-γ	0.25	0.47	0.08	0.61	0.31	0.47	0.31	0.22	0.14	0.79
	FATP2	-0.03	0.26	-0.02	0.92	0.60	-0.14	0.19	-0.11	0.44	0.96
	FATP3	-0.10	0.15	-0.11	0.50	0.17	-0.03	0.09	-0.05	0.72	0.10
	FABP4	-0.34	0.24	-0.23	0.16	0.31	0.13	0.16	0.12	0.42	0.76
	GLUT1	0.53	0.43	0.20	0.23	0.58	0.46	0.36	0.18	0.21	0.58
HDL-C[a]	PPAR-γ	-0.10	0.07	-0.22	0.17	0.07	0.03	0.08	0.05	0.76	0.71
	FATP2	0.05	0.04	0.20	0.21	0.22	-0.06	0.05	-0.20	0.21	0.31
	FATP3	-0.03	0.02	-0.24	0.14	0.16	-0.01	0.03	-0.06	0.69	0.46
	FABP4	0.00	0.04	-0.01	0.96	0.81	-0.01	0.04	-0.03	0.87	0.86
	GLUT1	-0.12	0.06	-0.33	**0.03**	**0.01**	0.05	0.10	0.08	0.61	0.70
Triglycerides[a]	PPAR-γ	0.05	0.12	0.07	0.67	0.78	0.08	0.12	0.11	0.48	0.43
	FATP2	-0.12	0.06	-0.28	0.07	**0.05**	-0.17	0.07	-0.38	**0.01**	**0.02**
	FATP3	-0.06	0.03	-0.27	0.08	0.18	-0.01	0.04	-0.05	0.76	0.95
	FABP4	0.01	0.06	0.04	0.82	0.63	0.06	0.06	0.16	0.29	0.29
	GLUT1	-0.01	0.10	-0.01	0.95	0.85	0.24	0.13	0.28	0.07	0.10
Free fatty acids[a]	PPAR-γ	0.00	0.07	0.01	0.96	0.46	0.01	0.07	0.03	0.86	0.79
	FATP2	-0.04	0.04	-0.17	0.28	0.22	-0.08	0.04	-0.33	**0.04**	**0.09**
	FATP3	-0.01	0.02	-0.07	0.64	0.62	0.01	0.02	0.07	0.68	0.79
	FABP4	-0.03	0.03	-0.16	0.32	0.51	0.04	0.04	0.15	0.34	0.37
	GLUT1	0.03	0.05	0.09	0.56	0.96	0.11	0.08	0.23	0.16	0.21
Maternal blood (<48h postnatal)				(N=52)					(N=52)		
Glucose[a]	PPAR-γ	-0.63	0.78	-0.12	0.42	0.45	-0.03	0.51	-0.01	0.95	0.87
	FATP2	-0.62	0.42	-0.21	0.15	0.28	-0.18	0.29	-0.09	0.53	0.51
	FATP3	-0.45	0.22	-0.29	**0.04**	0.10	-0.32	0.14	-0.30	**0.03**	**0.02**
	FABP4	-0.85	0.41	-0.28	0.04	0.04	-0.06	0.25	-0.03	0.82	0.69
	GLUT1	-0.23	0.60	-0.06	0.70	0.87	-0.94	0.58	-0.23	0.11	0.31
Leptin[a]	PPAR-γ	-2.52	9.13	-0.05	0.78	0.99	10.45	6.55	0.26	0.12	0.63
	FATP2	2.16	5.09	0.08	0.67	0.93	-4.42	3.28	-0.21	0.19	0.17
	FATP3	2.66	2.09	0.22	0.21	0.22	0.29	1.81	0.03	0.87	0.95
	FABP4	1.86	4.77	0.07	0.70	0.60	3.40	3.39	0.16	0.32	0.32
	GLUT1	-0.61	6.21	-0.02	0.92	0.93	2.79	6.91	0.07	0.69	0.99
Neonatal adiposity (at birth)				(N=41)					(N=41)		
Cord blood leptin[a]	PPAR-γ	2.50	2.15	0.16	0.25	0.51	3.71	2.26	0.24	0.11	0.07
	FATP2	1.72	1.15	0.22	0.15	0.11	-1.05	1.43	-0.11	0.47	0.54
	FATP3	0.76	0.59	0.18	0.21	0.23	0.24	0.71	0.05	0.74	0.67
	FABP4	0.81	1.10	0.11	0.47	0.27	1.92	1.12	0.25	**0.09**	0.12
	GLUT1	-1.07	1.99	-0.08	0.59	0.25	3.63	2.48	0.22	0.15	0.28

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; GLUT1, glucose transporter 1; HDL-C, high density lipoprotein-cholesterol; PPAR-γ, peroxisome proliferator-activated receptor gamma. Low sedentary time <584min/day; high sedentary time ≥584min/day. [a] A subtle variation of winsorizing was performed on extreme outliers (z value>2.58) of outcomes. [b] Optimum Box-Cox transformations were conducted on outcomes. [c] Model 1 was adjusted for the intervention group and gestational week at delivery; [d] model 2 was adjusted for gestational week at delivery, and delivery route (low ST, N=52; high ST, N=51). The results remained similar when adjusting for foetal sex and the relative percentage of MVPA at 35-37 weeks.

Table S7. Associations of placental mRNAs with neonatal and maternal metabolism parameters, and neonatal adiposity, in women with low or high ST levels at **24-28 weeks.**

Outcomes	Predictors	Low sedentary time (n=43)					High sedentary time (n=40)				
		B	SE	β	p-value[c]	p-value[d]	B	SE	β	p-value[c]	p-value[d]
Cord blood (at birth)				(N=37)					(N=37)		
C-peptide[a]	PPAR-γ	0.07	0.17	0.07	0.69	0.84	0.09	0.20	0.09	0.65	0.36
	FATP2	0.02	0.10	0.03	0.86	0.80	-0.09	0.09	-0.20	0.32	0.59
	FATP3	0.02	0.04	0.10	0.60	0.83	-0.07	0.05	-0.26	0.19	0.55
	FABP4	-0.05	0.09	-0.09	0.61	0.39	-0.04	0.07	-0.11	0.57	0.89
	GLUT1	0.20	0.15	0.24	0.18	0.42	-0.01	0.19	-0.01	0.96	0.78
Glucose[ab]	PPAR-γ	0.74	0.50	0.27	0.15	0.81	-0.08	0.47	-0.03	0.87	0.90
	FATP2	-0.28	0.31	-0.17	0.37	0.29	-0.17	0.24	-0.14	0.49	0.68
	FATP3	0.07	0.13	0.09	0.63	0.53	-0.07	0.13	-0.10	0.61	0.25
	FABP4	-0.07	0.28	-0.05	0.80	0.62	0.02	0.18	0.02	0.92	0.97
	GLUT1	0.88	0.42	0.37	0.05	0.17	0.12	0.47	0.05	0.80	0.42
HDL-C[a]	PPAR-γ	-0.07	0.09	-0.14	0.44	0.11	0.02	0.12	0.03	0.88	0.88
	FATP2	-0.01	0.06	-0.03	0.88	0.96	0.01	0.05	0.02	0.93	0.92
	FATP3	0.00	0.02	-0.01	0.95	0.39	0.03	0.03	0.20	0.28	0.30
	FABP4	0.04	0.05	0.16	0.39	0.26	0.01	0.04	0.02	0.91	0.81
	GLUT1	0.06	0.08	0.13	0.50	0.90	-0.06	0.11	-0.10	0.58	0.38
Triglycerides[a]	PPAR-γ	0.06	0.11	0.09	0.59	0.66	0.14	0.17	0.13	0.43	0.28
	FATP2	-0.03	0.07	-0.06	0.73	0.53	-0.19	0.07	-0.44	**0.01**	**0.01**
	FATP3	-0.05	0.03	-0.27	0.13	0.04	-0.04	0.04	-0.14	0.41	0.60
	FABP4	0.06	0.06	0.16	0.35	0.40	0.01	0.06	0.02	0.93	0.82
	GLUT1	-0.03	0.10	-0.06	0.75	0.85	0.23	0.16	0.23	0.15	0.14
Free fatty acids[a]	PPAR-γ	0.12	0.08	0.25	0.13	0.75	0.00	0.10	0.00	0.99	0.81
	FATP2	-0.08	0.05	-0.29	0.10	0.09	-0.07	0.04	-0.31	0.10	0.20
	FATP3	-0.01	0.02	-0.05	0.80	0.89	0.02	0.03	0.12	0.51	0.30
	FABP4	0.03	0.04	0.10	0.56	0.35	0.04	0.04	0.16	0.35	0.24
	GLUT1	0.12	0.07	0.27	0.11	0.10	0.06	0.09	0.11	0.54	0.60
Maternal blood (<48h postnatal)				(N=43)					(N=40)		
Glucose[a]	PPAR-γ	-0.01	0.82	0.00	0.99	0.66	0.35	0.80	0.07	0.67	0.88
	FATP2	-0.43	0.47	-0.15	0.38	0.17	-0.41	0.37	-0.19	0.28	0.30
	FATP3	-0.15	0.22	-0.12	0.50	0.41	-0.39	0.18	-0.34	0.03	0.03
	FABP4	-0.54	0.42	-0.21	0.21	0.17	-0.23	0.29	-0.13	0.44	0.32
	GLUT1	0.06	0.65	0.02	0.93	0.52	-1.11	0.72	-0.24	0.13	0.16
Leptin[a]	PPAR-γ	7.05	12.05	0.11	0.57	0.31	5.62	8.96	0.12	0.54	0.74
	FATP2	-1.62	6.38	-0.05	0.80	0.67	-0.58	4.03	-0.03	0.89	0.41
	FATP3	2.21	2.11	0.19	0.31	0.28	0.92	2.00	0.08	0.65	0.93
	FABP4	2.28	5.73	0.07	0.70	0.51	5.96	3.44	0.30	0.09	0.12
	GLUT1	1.92	6.57	0.06	0.77	0.84	-5.14	8.43	-0.11	0.55	0.30
Neonatal adiposity (at birth)				(N=37)					(N=34)		
Cord blood leptin[a]	PPAR-γ	3.15	3.12	0.17	0.32	0.85	7.32	2.72	0.41	**0.01**	**0.00**
	FATP2	3.51	1.85	0.32	0.07	0.08	-1.37	1.32	-0.18	0.31	0.64
	FATP3	1.14	0.75	0.27	0.14	0.40	0.32	0.78	0.08	0.69	0.43
	FABP4	1.46	1.69	0.15	0.40	0.41	1.22	1.03	0.20	0.24	0.18
	GLUT1	4.07	2.73	0.26	0.15	0.24	0.88	2.81	0.05	0.76	0.99

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; GLUT1, glucose transporter 1; HDL-C, high density lipoprotein-cholesterol; PPAR-γ, peroxisome proliferator-activated receptor gamma. Low sedentary time <590min/day; high sedentary time ≥590min/day). [a] A subtle variation of winsorizing was performed on extreme outliers (z value>2.58) of outcomes. [b] Optimum Box-Cox transformations were conducted on outcomes. [c] Model 1 was adjusted for the intervention group and gestational week at delivery; [d] model 2 was adjusted for gestational week at delivery, and delivery route (low ST, N=43; high ST, N=40). The results remained similar when adjusting for foetal sex and the relative percentage of MVPA at 35-37 weeks.

Table S8. Associations of placental mRNAs with neonatal and maternal metabolism parameters, and neonatal adiposity, in women who increased less or more ST from **baseline to 24-28 weeks.**

Outcomes	Predictors	Increased less ST from baseline (n=43)					Increased more ST from baseline (n=40)				
		B	SE	β	p-value[c]	p-value[d]	B	SE	β	p-value[c]	p-value[d]
Cord blood (at birth)					(N=30)					(N=30)	
C-peptide[a]	PPAR-γ	0.14	0.16	0.17	0.39	0.62	0.10	0.20	0.10	0.64	0.40
	FATP2	-0.09	0.08	-0.23	0.26	0.12	0.06	0.14	0.10	0.67	0.76
	FATP3	0.02	0.05	0.07	0.73	0.80	-0.03	0.06	-0.11	0.63	0.91
	FABP4	-0.01	0.09	-0.02	0.92	0.62	0.03	0.09	0.07	0.75	0.28
	GLUT1	0.25	0.20	0.30	0.22	0.12	-0.33	0.19	-0.33	0.10	0.10
Glucose[ab]	PPAR-γ	0.57	0.46	0.24	0.23	0.94	0.25	0.61	0.09	0.68	0.46
	FATP2	-0.11	0.24	-0.09	0.64	0.74	-0.71	0.43	-0.40	0.12	0.79
	FATP3	0.06	0.14	0.10	0.65	0.36	-0.20	0.19	-0.24	0.29	0.17
	FABP4	0.17	0.25	0.14	0.50	0.91	-0.13	0.29	-0.11	0.65	0.53
	GLUT1	1.01	0.57	0.39	0.09	0.11	0.05	0.68	0.02	0.94	0.23
HDL-C[a]	PPAR-γ	0.05	0.09	0.10	0.58	1.00	-0.13	0.11	-0.25	0.23	0.43
	FATP2	-0.03	0.05	-0.13	0.50	0.49	0.09	0.08	0.26	0.26	0.06
	FATP3	-0.02	0.03	-0.13	0.52	0.97	0.00	0.03	-0.01	0.96	0.93
	FABP4	0.07	0.05	0.26	0.18	0.19	-0.04	0.05	-0.18	0.41	1.00
	GLUT1	-0.05	0.12	-0.09	0.67	0.57	-0.12	0.11	-0.22	0.32	0.06
Triglycerides[a]	PPAR-γ	-0.01	0.15	-0.02	0.94	0.86	0.16	0.16	0.18	0.33	0.37
	FATP2	-0.16	0.07	-0.44	**0.02**	**0.02**	-0.19	0.11	-0.34	**0.09**	0.14
	FATP3	-0.04	0.04	-0.17	0.44	0.87	-0.03	0.05	-0.13	0.52	0.81
	FABP4	0.07	0.08	0.18	0.40	0.23	0.03	0.07	0.08	0.69	0.59
	GLUT1	0.08	0.18	0.10	0.69	1.00	0.11	0.17	0.12	0.53	0.40
Free fatty acids[a]	PPAR-γ	0.10	0.10	0.18	0.34	0.47	-0.06	0.08	-0.15	0.45	0.75
	FATP2	-0.14	0.04	-0.53	**0.00**	**0.02**	-0.07	0.05	-0.29	0.20	0.81
	FATP3	0.00	0.03	0.02	0.93	0.47	-0.01	0.02	-0.09	0.68	0.94
	FABP4	0.08	0.05	0.27	0.17	0.06	-0.07	0.03	-0.41	0.04	0.35
	GLUT1	0.01	0.12	0.01	0.97	1.00	0.17	0.07	0.44	**0.03**	0.18
Maternal blood (<48h postnatal)					(N=34)					(N=34)	
Glucose[a]	PPAR-γ	0.23	0.83	0.05	0.79	0.94	0.10	0.89	0.02	0.91	0.89
	FATP2	-0.44	0.43	-0.18	0.32	0.39	-1.06	0.61	-0.34	0.10	0.13
	FATP3	-0.54	0.24	-0.41	**0.04**	**0.04**	-0.58	0.23	-0.46	**0.02**	**0.03**
	FABP4	-0.01	0.43	-0.01	0.98	0.96	-0.56	0.39	-0.26	0.16	0.10
	GLUT1	-0.84	0.69	-0.23	0.24	0.44	-1.37	0.94	-0.27	0.16	0.20
Leptin[a]	PPAR-γ	-6.60	13.09	-0.11	0.62	0.92	1.72	9.96	0.04	0.86	0.91
	FATP2	-6.05	4.54	-0.29	0.20	0.09	5.80	7.30	0.20	0.44	0.30
	FATP3	0.40	2.85	0.04	0.89	0.77	3.28	2.50	0.28	0.20	0.30
	FABP4	-5.33	5.90	-0.21	0.38	0.55	5.29	5.12	0.21	0.31	0.29
	GLUT1	9.26	7.42	0.33	0.23	0.74	-16.76	11.03	-0.31	0.14	0.07
Neonatal adiposity (at birth)					(N=30)					(N=27)	
Cord blood leptin[a]	PPAR-γ	1.32	2.41	0.10	0.59	0.85	10.06	3.07	0.50	0.00	0.02
	FATP2	1.19	1.19	0.17	0.33	0.31	-1.79	2.52	-0.15	0.48	0.47
	FATP3	0.49	0.70	0.13	0.49	0.38	0.45	1.00	0.09	0.66	0.36
	FABP4	1.53	1.23	0.22	0.23	0.25	1.86	1.56	0.23	0.25	0.22
	GLUT1	2.34	3.02	0.17	0.45	0.28	-3.82	3.55	-0.20	0.29	0.50

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; GLUT1, glucose transporter 1; HDL-C, high density lipoprotein-cholesterol; PPAR-γ, peroxisome proliferator-activated receptor gamma; ST, sedentary time. Women who increased less ST from baseline, <20min/day; who increased more ST from baseline, ≥20min/day. [a] A subtle variation of winsorizing was performed on extreme outliers (z value>2.58) of outcomes. [b] Optimum Box-Cox transformations were conducted on outcomes. [c] Model 1 was adjusted for the intervention group and gestational week at delivery; [d] model 2 was adjusted for gestational week at delivery, and delivery route (increased less ST, N=34; increased more ST N=34). The results remained similar when adjusting for foetal sex and the relative percentage of MVPA at 35-37 weeks.

Table S9. Associations of placental mRNAs with neonatal and maternal metabolism parameters, and neonatal adiposity, in women with low or high MVPA levels at **baseline (<20 weeks).**

Outcomes	Predictors	Low MVPA (n=52)					High MVPA (n=52)				
		B	SE	β	p-value[c]	p-value[d]	B	SE	β	p-value[c]	p-value[d]
Cord blood (at birth)				(N=44)					(N=46)		
C-peptide[a]	PPAR-γ	0.05	0.12	0.06	0.69	0.85	0.14	0.14	0.16	0.30	0.59
	FATP2	-0.06	0.08	-0.12	0.47	0.66	0.01	0.07	0.02	0.88	1.00
	FATP3	-0.05	0.05	-0.18	0.25	0.12	0.01	0.03	0.05	0.74	0.58
	FABP4	0.02	0.06	0.05	0.76	0.80	0.03	0.08	0.05	0.75	0.80
	GLUT1	0.15	0.15	0.16	0.31	0.78	-0.17	0.11	-0.22	0.14	0.11
Glucose[ab]	PPAR-γ	0.14	0.33	0.07	0.68	0.47	0.52	0.49	0.16	0.29	0.76
	FATP2	-0.03	0.20	-0.02	0.91	0.85	-0.26	0.24	-0.16	0.30	0.84
	FATP3	-0.05	0.12	-0.06	0.70	0.20	-0.05	0.12	-0.07	0.67	0.06
	FABP4	0.08	0.16	0.08	0.62	0.95	-0.35	0.29	-0.19	0.23	0.33
	GLUT1	0.58	0.42	0.23	0.17	0.93	0.59	0.41	0.21	0.16	0.65
HDL-C[a]	PPAR-γ	0.08	0.08	0.16	0.32	0.67	-0.14	0.08	-0.26	0.09	0.13
	FATP2	-0.03	0.05	-0.10	0.58	0.41	-0.03	0.05	-0.09	0.57	0.75
	FATP3	0.02	0.03	0.10	0.55	0.92	-0.04	0.02	-0.30	**0.05**	0.09
	FABP4	0.02	0.04	0.09	0.56	0.69	-0.04	0.05	-0.14	0.40	0.71
	GLUT1	0.02	0.09	0.04	0.83	0.62	-0.06	0.08	-0.11	0.48	0.41
Triglycerides[a]	PPAR-γ	0.02	0.10	0.02	0.88	0.82	0.09	0.13	0.11	0.50	0.55
	FATP2	-0.15	0.06	-0.38	**0.01**	**0.04**	-0.17	0.07	-0.37	**0.01**	**0.02**
	FATP3	0.02	0.04	0.10	0.52	0.55	-0.08	0.03	-0.34	**0.03**	**0.04**
	FABP4	0.06	0.05	0.17	0.24	0.21	-0.03	0.08	-0.06	0.72	0.98
	GLUT1	0.23	0.11	0.30	0.04	0.30	0.04	0.13	0.05	0.75	0.85
Free fatty acids[a]	PPAR-γ	-0.01	0.06	-0.02	0.91	0.24	0.03	0.07	0.06	0.67	0.49
	FATP2	-0.06	0.04	-0.27	0.14	0.15	-0.10	0.04	-0.36	0.01	0.06
	FATP3	0.02	0.02	0.16	0.36	0.53	-0.02	0.02	-0.12	0.43	0.52
	FABP4	0.00	0.03	-0.01	0.97	0.87	0.00	0.04	-0.01	0.94	0.38
	GLUT1	0.11	0.07	0.28	0.11	0.54	0.07	0.07	0.15	0.33	0.37
Maternal blood (<48h postnatal)				(N=51)					(N=53)		
Glucose[a]	PPAR-γ	-0.60	0.52	-0.16	0.26	0.26	0.20	0.70	0.04	0.78	0.78
	FATP2	-0.02	0.30	-0.01	0.95	0.78	-0.44	0.40	-0.15	0.28	0.23
	FATP3	-0.13	0.18	-0.11	0.46	0.49	-0.48	0.18	-0.36	**0.01**	**0.01**
	FABP4	-0.26	0.26	-0.14	0.32	0.21	-0.22	0.40	-0.08	0.59	0.37
	GLUT1	-0.31	0.55	-0.08	0.58	0.83	-0.56	0.65	-0.12	0.39	0.51
Leptin[a]	PPAR-γ	3.07	5.72	0.09	0.60	0.63	1.24	9.43	0.02	0.90	0.94
	FATP2	-5.90	3.36	-0.31	**0.09**	**0.08**	3.52	4.39	0.13	0.43	0.80
	FATP3	-0.49	1.99	-0.04	0.81	0.89	0.61	1.94	0.05	0.76	0.52
	FABP4	2.50	3.31	0.13	0.46	0.53	3.23	4.77	0.11	0.50	0.69
	GLUT1	11.70	5.65	0.34	**0.05**	**0.05**	-8.94	6.84	-0.21	0.20	0.12
Neonatal adiposity (at birth)				(N=42)					(N=42)		
Cord blood leptin[a]	PPAR-γ	2.52	1.95	0.17	0.20	0.29	3.31	2.62	0.20	0.21	0.14
	FATP2	0.01	1.32	0.00	1.00	0.91	1.00	1.25	0.12	0.43	0.41
	FATP3	0.66	0.77	0.12	0.40	0.61	0.38	0.60	0.10	0.53	0.42
	FABP4	1.44	0.93	0.21	0.13	0.11	0.96	1.54	0.10	0.54	0.40
	GLUT1	5.18	2.34	0.30	**0.03**	0.32	-2.54	2.20	-0.17	0.26	0.34

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; GLUT1, glucose transporter 1; HDL-C, high density lipoprotein-cholesterol; MVPA, moderate-to-vigorous physical activity; PPAR-γ, peroxisome proliferator-activated receptor gamma. Low MVPA <40min/day; high MVPA≥40min/day. [a] A subtle variation of winsorizing was performed on extreme outliers (z value>2.58) of outcomes. [b] Optimum Box-Cox transformations were conducted on outcomes. [c] Model 1 was adjusted for the intervention group and gestational week at delivery; [d] model 2 was adjusted for gestational week at delivery, and delivery route (low ST, N=51; high ST, N=52). The results remained similar when adjusting for foetal sex and the relative percentage of ST at baseline.

Table S10. Associations of placental mRNAs with neonatal and maternal metabolism parameters, and neonatal adiposity, in women with low or high MVPA levels at **35-37 weeks.**

Outcomes	Predictors	Low MVPA (n=45)					High MVPA (n=29)				
		B	SE	β	p-value[c]	p-value[d]	B	SE	β	p-value[c]	p-value[d]
Cord blood (at birth)					(N=37)					(N=25)	
C-peptide[a]	PPAR-γ	0.19	0.18	0.19	0.30	0.42	0.47	0.21	0.42	0.04	0.42
	FATP2	-0.10	0.09	-0.20	0.27	0.46	0.02	0.10	0.04	0.85	0.87
	FATP3	-0.01	0.05	-0.02	0.92	0.71	0.02	0.05	0.10	0.63	0.88
	FABP4	0.01	0.08	0.02	0.93	0.75	0.21	0.15	0.27	0.17	0.73
	GLUT1	0.39	0.19	0.35	**0.06**	**0.03**	0.03	0.15	0.03	0.87	0.75
Glucose[ab]	PPAR-γ	0.00	0.34	0.00	1.00	0.24	0.54	0.79	0.17	0.50	0.93
	FATP2	-0.19	0.17	-0.21	0.25	0.50	0.14	0.30	0.11	0.66	0.71
	FATP3	-0.18	0.10	-0.35	**0.06**	**0.03**	0.05	0.16	0.08	0.75	0.55
	FABP4	-0.11	0.15	-0.14	0.46	0.36	-0.50	0.51	-0.23	0.34	0.31
	GLUT1	0.73	0.37	0.33	0.06	0.23	0.28	0.53	0.13	0.61	0.78
HDL-C[a]	PPAR-γ	0.02	0.13	0.04	0.85	0.82	0.05	0.14	0.08	0.74	0.57
	FATP2	0.01	0.05	0.03	0.87	0.90	0.02	0.06	0.09	0.71	0.73
	FATP3	0.03	0.03	0.20	0.32	0.43	-0.03	0.03	-0.21	0.35	0.20
	FABP4	0.00	0.05	0.01	0.96	0.93	0.11	0.09	0.27	0.24	0.50
	GLUT1	0.09	0.13	0.14	0.48	0.58	-0.06	0.10	-0.14	0.56	0.30
Triglycerides[a]	PPAR-γ	0.35	0.17	0.36	**0.05**	**0.08**	0.06	0.22	0.06	0.81	0.59
	FATP2	-0.19	0.07	-0.44	**0.01**	0.07	-0.18	0.09	-0.44	0.06	0.07
	FATP3	0.00	0.05	-0.01	0.94	0.93	-0.10	0.05	-0.43	0.05	0.06
	FABP4	0.02	0.07	0.06	0.76	0.74	0.17	0.15	0.25	0.28	0.25
	GLUT1	0.34	0.18	0.34	0.06	0.12	0.06	0.16	0.08	0.73	0.72
Free fatty acids[a]	PPAR-γ	0.09	0.09	0.19	0.29	0.33	0.17	0.12	0.28	0.19	0.06
	FATP2	-0.05	0.04	-0.21	0.21	0.53	-0.09	0.05	-0.36	0.10	0.12
	FATP3	0.02	0.02	0.20	0.28	0.14	-0.05	0.03	-0.35	0.08	0.30
	FABP4	0.01	0.04	0.06	0.73	0.33	0.05	0.09	0.13	0.55	0.18
	GLUT1	0.11	0.09	0.21	0.22	0.79	0.13	0.09	0.31	0.15	**0.05**
Maternal blood (<48h postnatal)					(N=45)					(N=29)	
Glucose[a]	PPAR-γ	0.08	0.83	0.02	0.92	0.86	1.05	1.02	0.18	0.31	0.21
	FATP2	-0.19	0.34	-0.09	0.59	0.48	0.09	0.45	0.04	0.84	0.62
	FATP3	-0.38	0.20	-0.31	**0.07**	**0.02**	0.00	0.22	0.00	1.00	0.72
	FABP4	-0.13	0.34	-0.06	0.71	0.23	-0.66	0.68	-0.17	0.34	0.26
	GLUT1	-0.52	0.67	-0.13	0.44	0.88	-0.59	0.77	-0.14	0.46	0.39
Leptin[a]	PPAR-γ	7.68	8.21	0.16	0.36	0.27	-30.55	18.67	-0.51	0.13	0.48
	FATP2	-2.78	3.27	-0.14	0.40	0.67	-4.85	6.82	-0.21	0.49	0.79
	FATP3	1.45	2.09	0.13	0.49	0.11	-0.52	3.27	-0.05	0.88	0.90
	FABP4	-0.36	3.87	-0.02	0.93	0.18	13.48	17.68	0.24	0.46	0.87
	GLUT1	6.56	6.07	0.19	0.29	0.86	7.02	14.90	0.16	0.65	0.84
Neonatal adiposity (at birth)					(N=34)					(N=25)	
Cord blood leptin[a]	PPAR-γ	7.17	3.09	0.35	**0.03**	**0.01**	2.48	4.14	0.14	0.56	0.96
	FATP2	-0.83	1.58	-0.09	0.60	0.99	-0.18	1.76	-0.02	0.92	0.88
	FATP3	1.51	0.92	0.28	0.11	0.10	0.15	0.83	0.04	0.86	0.98
	FABP4	1.55	1.36	0.20	0.26	0.37	-0.97	2.69	-0.08	0.72	0.71
	GLUT1	6.60	3.43	0.30	0.07	**0.04**	0.04	2.72	0.00	0.99	0.84

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; GLUT1, glucose transporter 1; HDL-C, high density lipoprotein-cholesterol; MVPA, moderate-to-vigorous physical activity; PPAR-γ, peroxisome proliferator-activated receptor gamma. Low MVPA <33min/day; high MVPA ≥33min/day). [a] A subtle variation of winsorizing was performed on extreme outliers (z value>2.58) of outcomes. [b] Optimum Box-Cox transformations were conducted on outcomes. [c] Model 1 was adjusted for the intervention group and gestational week at delivery; [d] model 2 was adjusted for gestational week at delivery, and delivery route (low MVPA, N=44; high MVPA, N=29). The results remained similar when adjusting for the sex of the foetus and the relative percentage of sedentary time at baseline.

Table S11. Associations of placental mRNAs with neonatal and maternal metabolism parameters, and neonatal adiposity, in obese class-1, and class-2 and -3 women (body mass index at pre-pregnancy).

Outcomes	Predictors	Obese class-1 women (n=122)					Obese class-2 and 3 women (n=49)				
		B	SE	β	p-value[c]	p-value[d]	B	SE	β	p-value[c]	p-value[d]
Cord blood (at birth)				**(N=101)**					**(N=47)**		
C-peptide[a]	PPAR-γ	0.14	0.10	0.14	0.18	0.19	0.00	0.15	0.00	0.99	0.79
	FATP2	0.00	0.05	0.01	0.96	0.61	0.10	0.08	0.19	0.22	0.28
	FATP3	0.00	0.03	0.00	0.99	0.91	0.01	0.05	0.02	0.89	0.79
	FABP4	0.03	0.05	0.07	0.51	0.30	0.04	0.08	0.08	0.61	0.67
	GLUT1	0.05	0.09	0.05	0.62	0.92	-0.30	0.15	-0.30	0.05	0.11
Glucose[ab]	PPAR-γ	0.32	0.27	0.12	0.23	0.52	0.61	0.36	0.26	0.10	0.49
	FATP2	-0.13	0.13	-0.10	0.34	0.67	0.11	0.21	0.08	0.60	0.50
	FATP3	-0.07	0.07	-0.09	0.35	0.13	0.06	0.17	0.05	0.75	0.75
	FABP4	0.00	0.12	0.00	0.97	0.36	0.04	0.19	0.03	0.84	0.21
	GLUT1	0.51	0.23	0.22	0.03	0.10	0.50	0.39	0.20	0.21	0.97
HDL-C[a]	PPAR-γ	0.12	0.06	0.21	0.04	0.14	-0.16	0.07	-0.35	0.03	0.08
	FATP2	-0.02	0.03	-0.05	0.62	0.64	-0.02	0.04	-0.09	0.56	0.48
	FATP3	0.02	0.02	0.10	0.36	0.50	-0.04	0.03	-0.23	0.12	0.19
	FABP4	0.06	0.03	0.22	0.04	0.06	-0.08	0.04	-0.32	0.04	0.06
	GLUT1	0.00	0.05	0.01	0.96	0.79	0.01	0.07	0.02	0.88	0.85
Triglycerides[a]	PPAR-γ	0.10	0.08	0.12	0.23	0.21	-0.06	0.12	-0.08	0.61	0.62
	FATP2	-0.13	0.04	-0.32	**0.00**	**0.003**	-0.14	0.06	-0.34	**0.02**	**0.02**
	FATP3	0.00	0.02	0.01	0.91	0.75	-0.13	0.04	-0.44	**0.002**	**0.003**
	FABP4	0.05	0.04	0.14	0.18	0.11	-0.05	0.06	-0.14	0.36	0.40
	GLUT1	0.11	0.07	0.17	0.10	0.15	0.14	0.11	0.18	0.24	0.31
Free fatty acids[a]	PPAR-γ	0.02	0.05	0.04	0.68	0.86	0.08	0.06	0.19	0.21	0.31
	FATP2	-0.07	0.03	-0.26	0.01	0.04	-0.06	0.03	-0.27	0.06	0.04
	FATP3	0.01	0.02	0.04	0.70	0.61	0.00	0.02	0.02	0.90	0.88
	FABP4	0.03	0.03	0.11	0.31	0.28	-0.02	0.03	-0.09	0.55	0.79
	GLUT1	0.06	0.05	0.13	0.20	0.22	0.12	0.06	0.29	0.04	0.24
Maternal blood (<48h postnatal)				**(N=122)**					**(N=49)**		
Glucose[a]	PPAR-γ	0.36	0.46	0.07	0.44	0.90	0.14	0.53	0.04	0.80	0.96
	FATP2	-0.26	0.22	-0.11	0.23	0.32	0.05	0.27	0.03	0.85	0.87
	FATP3	-0.16	0.12	-0.12	0.19	0.20	-0.09	0.19	-0.07	0.63	0.70
	FABP4	-0.08	0.21	-0.04	0.71	0.35	-0.13	0.27	-0.07	0.62	0.51
	GLUT1	0.19	0.39	0.04	0.64	0.54	-0.65	0.51	-0.17	0.21	0.17
Leptin[a]	PPAR-γ	3.20	5.26	0.07	0.55	0.59	11.37	12.02	0.18	0.35	0.32
	FATP2	-1.48	2.40	-0.07	0.54	0.62	1.50	4.97	0.06	0.77	0.57
	FATP3	1.32	1.30	0.12	0.31	0.25	-0.88	3.02	-0.05	0.77	0.75
	FABP4	6.41	2.46	0.29	0.01	0.01	0.53	5.18	0.02	0.92	0.73
	GLUT1	5.08	4.32	0.14	0.24	0.37	-13.76	8.73	-0.28	0.13	0.07
Neonatal adiposity (at birth)				**(N=99)**					**(N=42)**		
Cord blood leptin[a]	PPAR-γ	4.06	1.70	0.23	0.02	0.02	2.76	2.29	0.18	0.24	0.32
	FATP2	-0.50	0.88	-0.06	0.57	0.85	1.49	1.23	0.17	0.23	0.37
	FATP3	0.81	0.45	0.17	0.08	0.05	-0.44	0.86	-0.07	0.61	0.86
	FABP4	0.93	0.78	0.12	0.24	0.16	2.08	1.14	0.25	0.08	0.16
	GLUT1	1.88	1.48	0.12	0.21	0.36	-0.07	2.54	0.00	0.98	0.41

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; FABP, fatty acid binding protein; FATP, fatty acid transport protein; GLUT1, glucose transporter 1; HDL-C, high density lipoprotein-cholesterol; PPAR-γ, peroxisome proliferator-activated receptor gamma. Overweight-obese class-1 women, BMI pre-pregnancy 29 to 35 kg/m^2; obese class-2 and 3 women, BMI pre-pregnancy ≥35 kg/m^2. [a] A subtle variation of winsorizing was performed on extreme outliers (z value>2.58) of outcomes. [b] Optimum Box-Cox transformations were conducted on outcomes. [c] Model 1 was adjusted for the intervention group and gestational week at delivery; [d] model 2 was adjusted for gestational week at delivery and delivery route (obese-1 women, N=122; obese-2 and -3 women, N=48). The results remained similar when adjusting for foetal sex and the relative percentage of MVPA at 35-37 week.

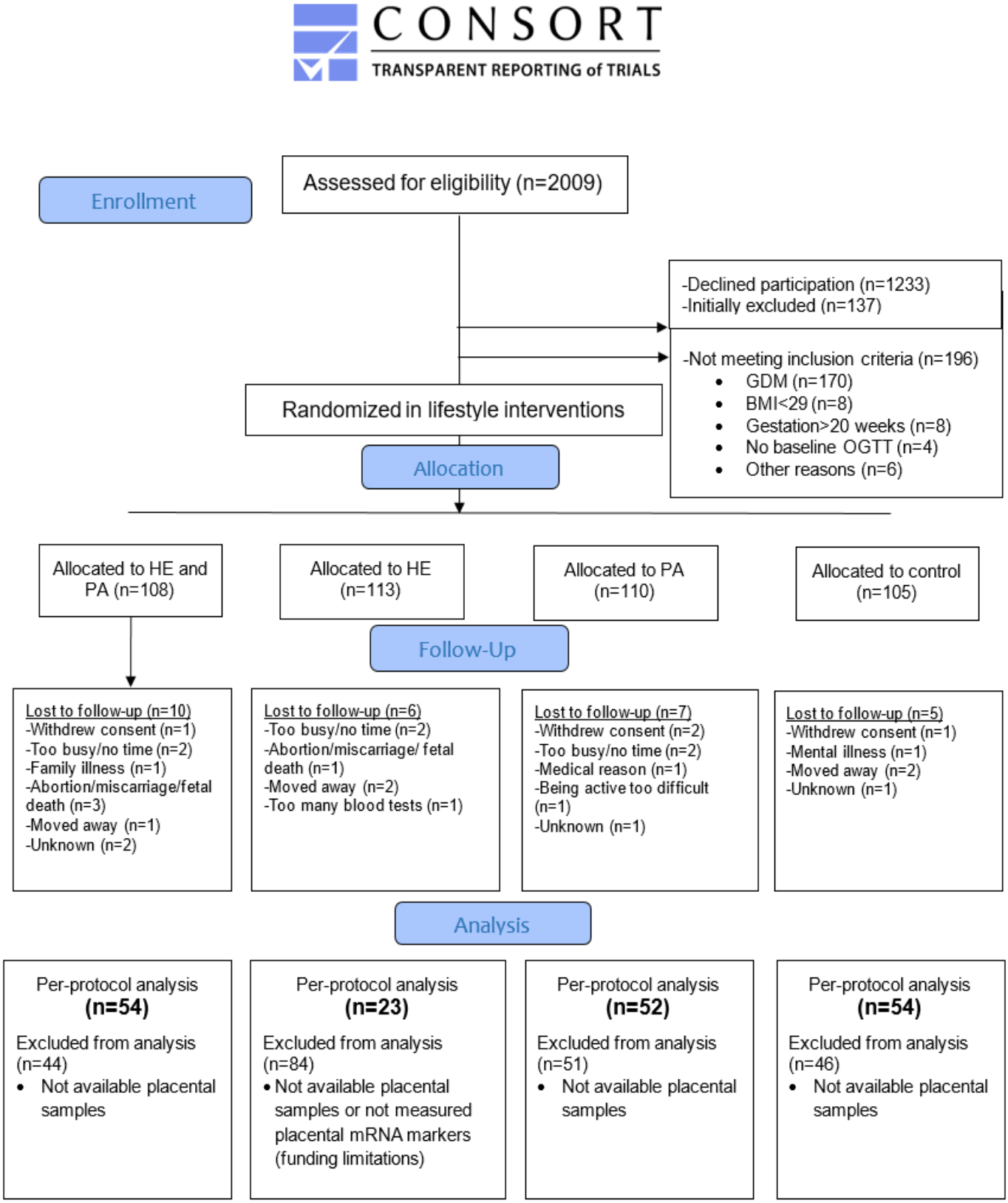
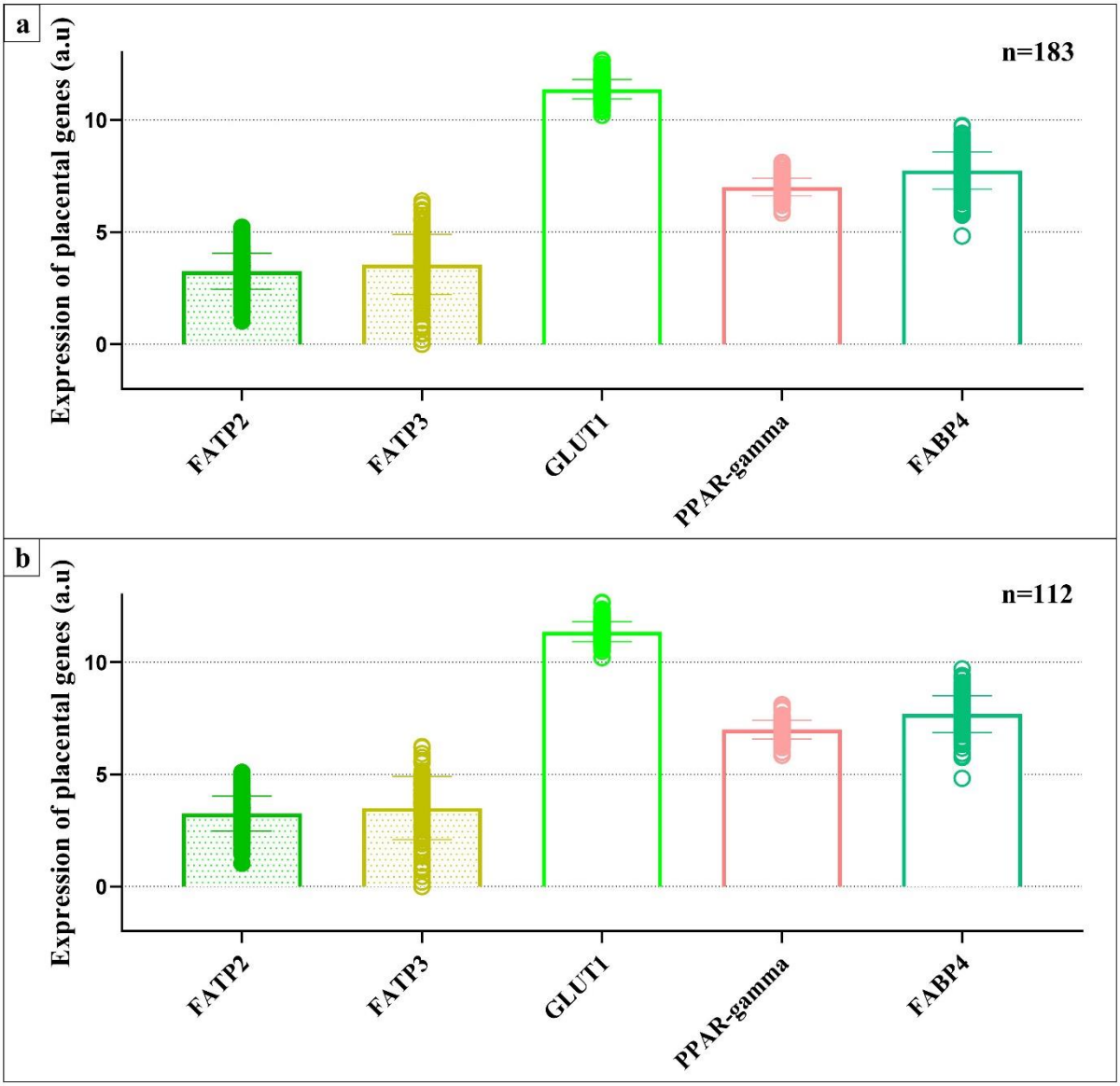


Figure S1. CONSORT flow chart diagram for the DALI lifestyle trial (n=183).



	FATP2	FATP3	FABP4	GLUT1
PPAR-γ	0.19*	0.26**	0.53**	-0.02
FATP2		0.25**	0.21*	-0.38**
FATP3			0.27**	0.14
FABP4				-0.32**

Figure S2. Graphical representation of placental gene expression in pregnant women (**a**: n=183, **b**: n=112), and bivariate Pearson correlations between placental mRNA levels (n=112). a.u, arbitrary units. FABP, fatty acid binding protein; FATP, fatty acid transport protein; GLUT, glucose transporter; PPAR-γ, peroxisome proliferator-activated receptor gamma. Numbers with asterisks indicate statistical significance * p≤0.05, ** p<0.01.

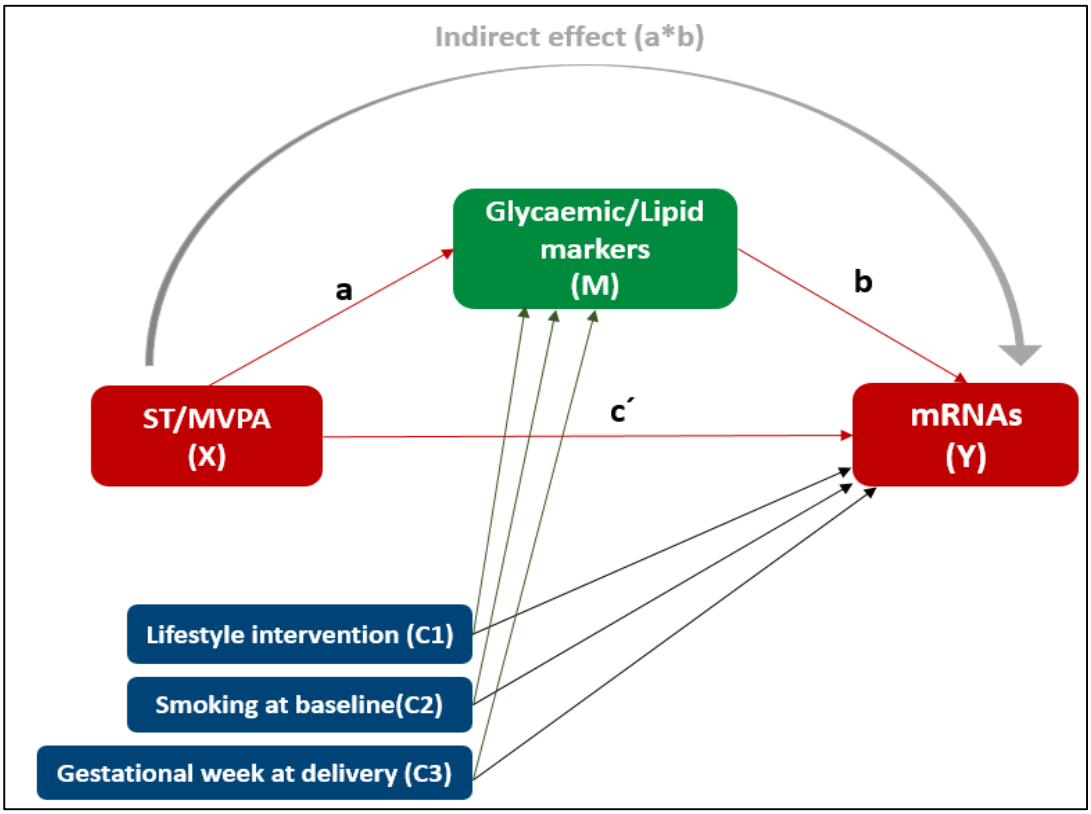


Figure S3. Schematic diagram of the simple mediation analyses investigating the mediator role of maternal glycaemic and lipid parameters (at 24-28 and 35-37 weeks) on the association between ST and MVPA with placental mRNAs. X, predictor; M, mediator; Y, outcome; C, confounder.

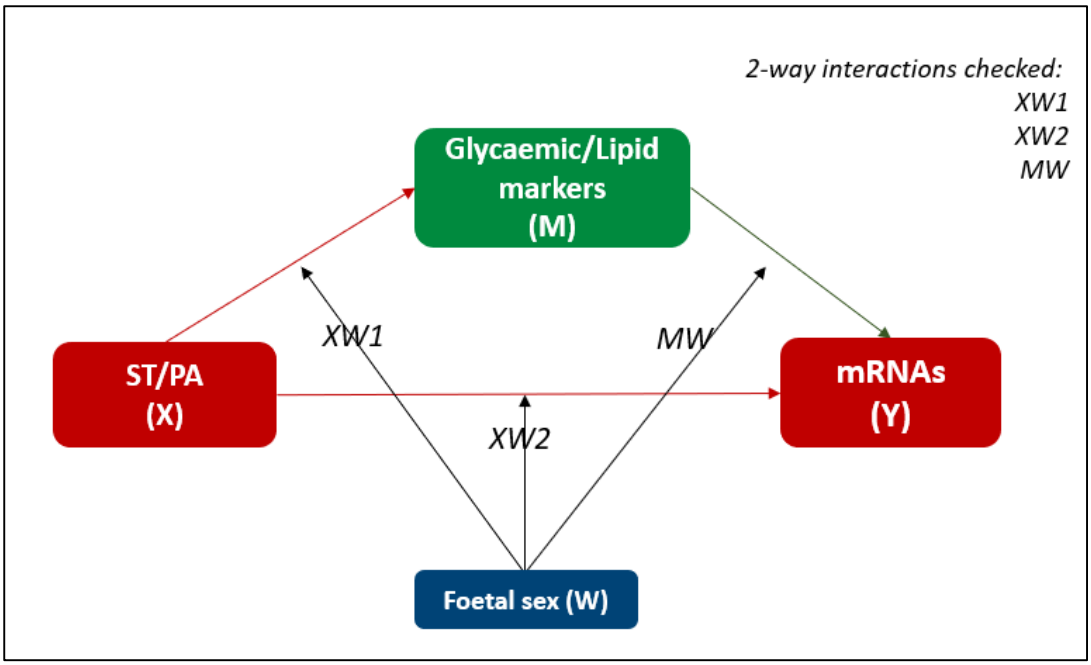


Figure S4. Schematic diagram of the conditional process analyses testing the moderator role of fetal sex in all pathways of the mediation models from **Figure S3**. X, predictor; M, mediator; Y, outcome; W, moderator.

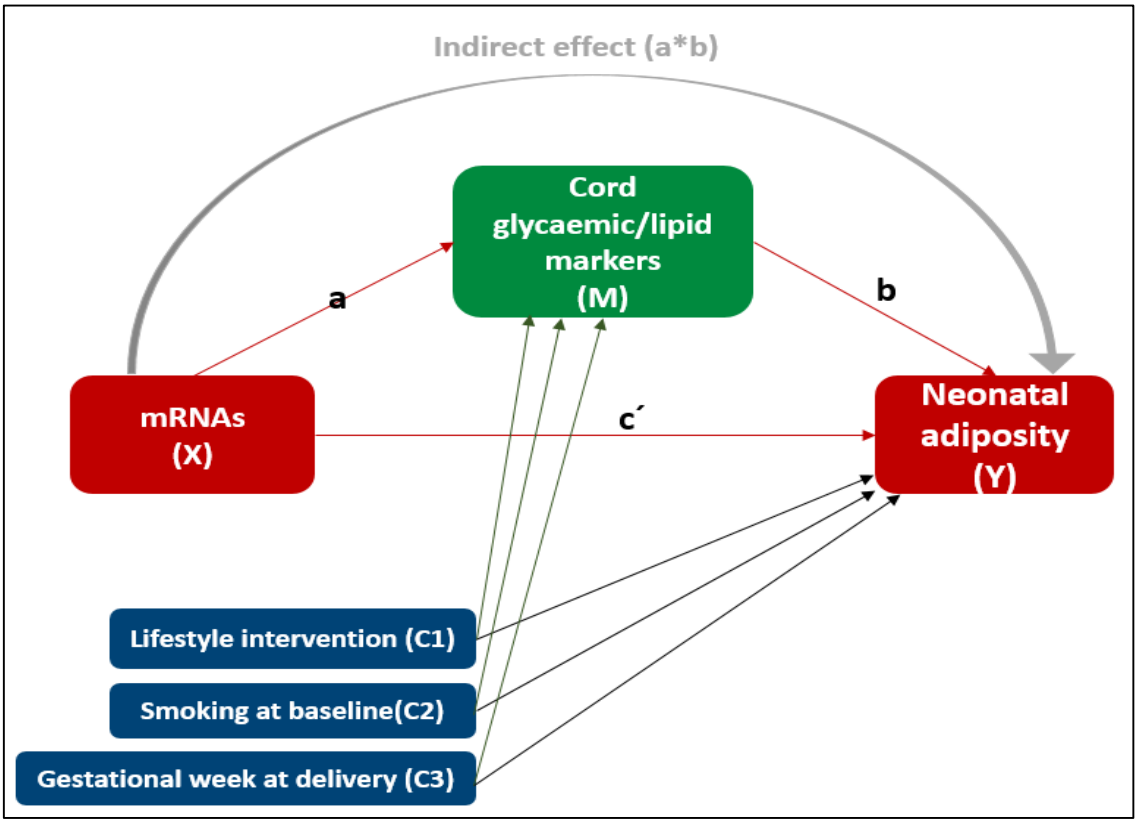


Figure S5. Schematic diagram of the simple mediation analyses investigating the mediator role of cord blood metabolic parameters on the association of placental mRNAs with neonatal adiposity (<48 hours after delivery). X, predictor; M, mediator; Y, outcome; C, confounder.

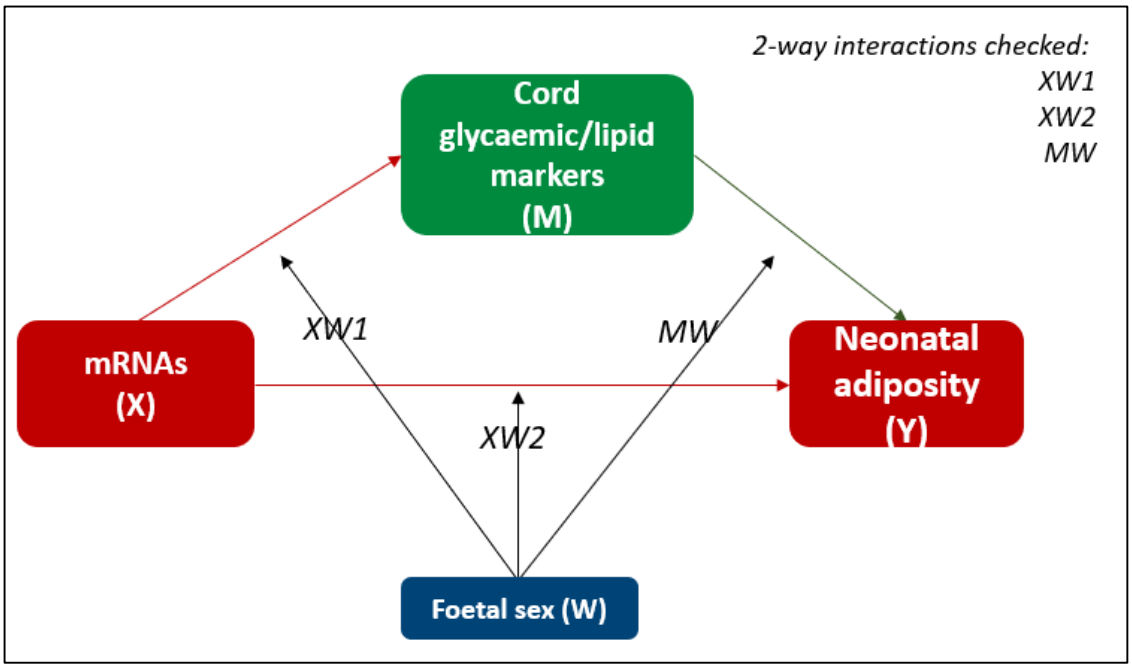


Figure S6. Schematic diagram of the conditional process analyses testing the moderator role of fetal sex in all pathways of the mediation models from **Figure S5**. X, predictor; M, mediator; Y, outcome; W, moderator.

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PART II. Role of physical fitness on maternal and foetal metabolism

STUDY III

Association of physical fitness during pregnancy with maternal and foetal metabolism. The GESTAFIT project

ABSTRACT

Background: Physical fitness (PF) is a cornerstone of metabolic health across all ages. However, its role on maternal and foetal metabolism during pregnancy remains unexplored.

Objectives: To analyse i) the association of PF with maternal and foetal cardiometabolic markers, and with clustered cardiometabolic risk during pregnancy; and ii) whether being fit might counteract the adverse alterations related to overweight-obesity ("Fat but Fit paradox").

Methods: A total of 151 pregnant women (age 33±5 years) participated in this study. Several PF components (test) were objectively assessed at 16th and 33rd gestational week: flexibility (back-scratch), lower-body (chair stand) and upper-body muscle strength (handgrip), and cardiorespiratory fitness (CRF; modified Bruce); and an overall PF cluster was computed. Maternal venous and arterial, and venous cord serum glycaemic and lipid markers, cortisol and C-reactive protein (CRP) were measured with standard biochemical methods. Blood pressure was also assessed. A cardiometabolic risk cluster was created.

Results: PF was associated with several maternal, but not foetal, cardiometabolic markers (p<0.05). Additionally, lower-body and upper-body muscle strength, CRF, overall PF (16th week), and CRF changes (16th-33rd week) were inversely associated with clustered cardiometabolic risk (p<0.05). Normal-weight and fit women showed lower insulin and insulin resistance, triglycerides, low density lipoprotein-cholesterol, CRP, and diastolic blood pressure than overweight-obese and unfit women at 16th week (p<0.05).

Conclusions: Greater PF, especially muscle strength and CRF in early-middle pregnancy, is related to a better metabolic phenotype, and might provide a protector effect on maternal metabolism. "Keep yourself fit and normal-weight" should be a key message for pregnant women.

INTRODUCTION

Pregnancy induces well-known physiological and metabolic adaptations to meet placenta and foetal demands[1]. However, dysregulated metabolic changes (e.g. exacerbated systemic glucose and lipids) in a priori healthy, and adverse phenotypes, can lead to pregnancy complications[1-5] and negative consequences for the mother and the child[3-7]. In fact, previous studies have shown that those women who present more cardiometabolic risk factors are predisposed to an increased risk for adverse outcomes in pregnancy (e.g. GDM, preterm birth, foetal demise)[3,8,9].

Accordingly, it should be a priority in pregnancy to find appropriate tools to optimise metabolic control, and avoid exacerbated cardiometabolic markers. In this regard, the potential of physical fitness (PF) to confer a cardio-protector role in metabolism[10-13], and improve the impaired phenotype associated with obesity[14] is undeniable, at least in the general population (across all ages). Whether PF has a similar effect in maternal and foetal metabolism during pregnancy has not been explored so far, despite its clinical relevance. Of note, this information is also imperative to design more tailored and effective exercise programs *–focused on specific or combined PF components*– regarding metabolic control during pregnancy, an unperceived "ingredient in the recipe" of exercise programming until now.

Therefore, the main aim of this study was to analyse the association of PF with maternal and foetal cardiometabolic biomarkers *-glycaemic and lipid markers, cortisol, C-reactive protein and blood pressure,* and with clustered cardiometabolic risk in pregnancy. A secondary aim was to explore whether being fit during pregnancy was a determinant for improved metabolic control, and might counteract some of the adverse alterations related to overweight and obesity (the "Fat but Fit" paradox).

MATERIAL AND METHODS

Study design and population

The GESTAFIT project was a quasi-experimental trial performed at the "San Cecilio and Virgen de las Nieves University Hospitals" and at the "Sport and Health University Research Institute" (Granada, Spain) between November-2015 and April-2018. Briefly, pregnant women were divided into a control or exercise group. Exercisers performed a supervised concurrent (aerobic+resistance) exercise training from the 17th week until

delivery (3 days/week, 60 minutes/session), and controls continued with their daily lifestyle[15,16]. The inclusion criteria (**Table S1**) and the general procedures have been previously described[16,17]. From those participants visiting their gynaecologist at 12th week, 159 pregnant women who showed interest were finally enrolled in the study. After being individually informed about the methodology, and before starting the project, women signed a personal written consent. The GESTAFIT project was approved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15).

General procedure

The participants were evaluated by experienced researchers at several time points: 16-17th week (2 days), 33-34th week (2 days) and delivery (1 day). At 16th week (early-middle pregnancy), clinical characteristics, blood pressure, height and weight, sleep and dietary habits, and PF were evaluated. Before leaving the research facilities, each woman was provided with two accelerometers to wear in the wrist and waist until the next week. At 17th week, these devices were personally returned, and maternal fasting blood was extracted by a nurse. At 33rd week (late pregnancy), these assessments were conducted with the same timing than 16th week. Just after delivery, maternal, and arterial and venous cord blood samples were collected by the hospital personnel, and obstetric information was gathered. The general procedures of the GESTAFIT project are shown in **Figure S1**.

Outcomes

Clinical data, obstetric history and perinatal records

Sociodemographic (e.g. educational level) and clinical (e.g. medications, diseases) data, reproductive and obstetric history, maternal-neonatal adverse events, and smoking habits, were collected from the medical history and questionnaires. Information about the offspring sex, abortions, type of delivery, etc. was obtained from perinatal records (partogram).

Cardiovascular function

Systolic and diastolic blood pressure (SBP; DBP), and resting heart rate, were assessed twice using a digital sphygmomanometer (M6 upper-arm Omron Health-Care Europe, the Netherlands), with women seated and relaxed at rest, without talking. The lowest score of two correct assessments was used for the analyses.

Height and weight

Height was assessed with a calibrated stadiometer (Seca 22, Hamburg, Germany). Pre-conception weight was self-reported, and weight through pregnancy (16th and 33rd week) was measured, with subjects in light clothes and wearing no shoes, using an electronic scale (InBody R20; Biospace, Seoul, Korea). Body mass index (BMI) was calculated as: weight (Kg) /height (m^2).

Dietary habits and Mediterranean diet adherence

A 105-items food frequency questionnaire was employed by a trained nutritionist to assess the consumption and frequency of different foods[18]. This information, along with the grams consumed of these products, were employed to estimate total energy intake (kcal/day) using the Evalfinut software. The Mediterranean diet score index[19] (MDS: lower punctuation indicates lower adherence to the Mediterranean dietary pattern) was also calculated.

Sedentary time and physical activity

Triaxial accelerometry (ActiGraph GT3X+, Florida, US) placed in the waist was employed to objectively evaluate sedentary time (ST) and moderate-to-vigorous physical activity (MVPA), as previously done[20]. A minimum register of 7 days (10 hours/day) was required to use this data for the analyses. Sedentary time (<200 counts/min) and MVPA (2690-6166 counts/min) were estimated according to the recommended vector magnitude counts cut-points[21].

Sleep duration and quality

Triaxial accelerometers (ActiSleep, ActiGraph GT3X+, Florida, US), located in the non-dominant wrist, were used to assess sleep efficiency and duration (Cole-Kripke algorithm)[21]. The filters, analyses criteria (e.g. 7 days, 10 hours/day), etc. were similar to those described previously[20]. Self-reported sleep quality was assessed using the Pittsburgh Sleep Quality Index Questionnaire[22,23]; lower score indicates better sleep quality.

Physical fitness

All PF tests were performed within the same day for each woman to avoid unnecessary burdens –this will have increased drop-out from the study. The order followed was as shown below, in order to minimize the potential carry-over effects (e.g. fatigue) induced

by PF tests, and to optimise recovery between tests. All women were encouraged to do their best when performing the tests.

Lower-body muscle strength was assessed with the Chair stand test[24], which consists of standing up from a seated position (back straight and feet flat) to a full stand the maximum number of times within 30 seconds. More repetitions are indicative of better strength. To avoid women pushing with their arms, these were crossed at the chest level. This PF component was expressed in relative terms (i.e. divided by individual weight).

Upper-body flexibility (i.e. overall shoulder range of motion) was assessed with the back scratch test[16,24], which consists of measuring the distance or overlap (in millimetres) between the middle fingers of both hands behind the back, with a measuring tape. When the middle fingers overlapped in the back, the score was positive (+mm); if they did not, the score was negative (-mm). The largest score (i.e. highest overlap or minimum distance between fingers) obtained from two trials for each arm was registered, and the average was used for analyses.

Upper-body muscle strength was measured with the handgrip strength test[12,16], which consist of squeezing the grip (adapted to participants' hand size[25]) of a digital dynamometer (TKK5101 Grip-D; Takey, Japan) as strong as possible. Women were emphasized to use the correct technique (straight bipedal position, arm completely extended, and without other body movements) to facilitate standardized measures. The best score of two trials for each hand was chosen, and the average was employed for the analyses. This PF component was expressed in relative terms (divided by weight). Of note, hand-grip is usually employed in clinical studies as a proxy of overall muscle strength[12].

Cardiorespiratory fitness (CRF) was assessed with the submaximal modified Bruce protocol[26,27]. This treadmill test consists in increasing the slope and speed during 5 progressive workload stages each 3 minutes (stage 1: 2.7km/h, 10% inclination; stage 2: 4km/h, 12%; stage 3: 5.5km/h, 14%; stage 4: 6.8km/h, 16%; stage 5: 8km/h, 18%). During the trial, women were encouraged to first reach the 85% of the age-predicted maximum heart rate ($85\%_{MHR}$), and subsequently the 85% of the target heart rate ($85\%_{THR}$). The $85\%_{THR}$ was calculated according to the heart rate reserve (Karvonen formula)[28] to consider the within-individual basal heart rate. The test was finished when

women reached the 85%$_{THR}$, or when they reported to reach volitional fatigue. If women did not reach at least the 85%$_{MHR}$, their data was not considered for the quantitative analyses. Previous authors have shown that not only time to exhaustion during the maximal Bruce treadmill test, but also time to 85%$_{THR}$ during the submaximal modified Bruce treadmill test are highly correlated with the direct measurement of the maximal volume of oxygen consumption (Vo_{2max}) in women (r=0.92, r=0.82; respectively)[28]. Hence, and considering that exercising until volitional exhaustion might be an unsafe and unethical practice in pregnant women (potential burden to maternal/foetal health), time to 85%$_{MHR}$ and 85%$_{THR}$ were regarded as proxies of cardiorespiratory fitness (hereinafter CRF$_{85\%MHR}$ and CRF$_{85\%THR}$). CRF$_{85\%MHR}$ and CRF$_{85\%THR}$ were highly correlated (r≈0.9, see **Figure S2**). Heart rate was continuously controlled with a monitor (Polar V800, Finland). Although cardiopulmonary submaximal exercise testing is usual and safe in pregnancy[29], a harness was employed to secure women (not for support) during the test to prevent any potential fall and the consequent risk. None complication or adverse consequence led us to stop the tests.

Overall physical fitness

A clustered PF index (overall PF) was created as the mean of the z-scores [(value-mean)/standard deviation)] of upper-body flexibility, upper-body muscle strength, and CRF$_{85\%MHR}$. Higher scores indicate better PF. Lower-body muscle strength was not considered for this cluster due to the reduced sample size (only assessed in a subsample of women).

Laboratory methods

Blood collection

In standardized fasting conditions (8-9 a.m.), maternal venous blood samples -5mL- were extracted from the antecubital vein. Immediately after delivery, maternal and cord arterial and venous blood samples were extracted by midwives. All blood samples were collected in serum tubes, and subsequently centrifuged, aliquoted and frozen (80ºC) until posterior analyses.

Cardiometabolic markers

Glucose, lipids and C-reactive protein

At 16th-33rd week, maternal glucose, total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and C-

reactive protein (CRP) levels were assessed with spectrophotometric enzyme assays (AU5822-Clinical Chemistry Analyser, Beckman-Coulter, CA, USA). Maternal, and arterial and venous cord serum glucose, total cholesterol, triglycerides, HDL-C, and LDL-C concentrations were measured with spectrophotometric determination (BS-200 Chemistry Analyzer, Mindray Bio-medical Electronics, Shenzen, China) as well.

Insulin and cortisol

Paramagnetic-particle-based chemiluminescence immunoassays (UniCel-Dxl800 Access Immunoassay analyser, Beckman Coulter, CCA, USA) were employed to measure maternal insulin and cortisol levels.

Insulin resistance

Conventional formulas[30] were used to estimate the homeostasis model assessment (HOMA)-IR (insulin resistance).

Clustered cardiometabolic risk

A clustered cardiometabolic risk score[3] was created from the z-scores of BMI at pre-pregnancy, and fasting glucose, triglycerides, HDL-C (inverted score), and blood pressure ((SBP+DBP)/2) at 16th and 33rd week. Higher scores indicate greater cardiometabolic risk.

Statistical analysis

Descriptive statistics were employed to show the clinical characteristics (**Table 1**), and maternal-foetal metabolic markers levels (**Table S2**) of women. Important confounders according to previous evidence, and which were statistically related to the outcomes, were considered for the main analyses: pre-pregnancy BMI, maternal age, and specific gestational week at first/second assessment or birth, MDS, baseline value of the respective outcome, and type of delivery. Additional confounders (e.g. PA, sleep) were employed (specified in tables). Few extreme values confirmed as influential outliers were adjusted (**Appendix A**). In some instances, optimum Box-Cox transformations were used.

For the first aim, linear regressions were employed to analyse the associations of the individual PF components and the overall PF with glycaemic and lipid markers, cortisol, and CRP -*in maternal, and/or arterial and venous cord serum-,* and with blood pressure at 16^{th} and 33^{rd} week (**Table 2** and **Table S4**). The associations of changes in PF (16^{th}-33^{rd} week) with changes in cardiometabolic outcomes (16^{th}-33^{rd} week) were also

explored (**Table 2**). Additionally, linear regressions were conducted to analyse the associations of individual PF components, and overall PF with clustered cardiometabolic risk during pregnancy (**Figure 1**). The PF variables were introduced as predictors in all the analyses, and the cardiometabolic markers as outcome variables.

To address the secondary aim, analyses of covariance were used to test differences in maternal metabolic markers (at 16th and 33rd week) according to pre-pregnancy BMI and overall PF (**Figure 2a**). Of note, PF groups, which were defined according to the median values (below and above median), will be named hereinafter "unfit" and "fit" to facilitate the understanding. Specifically, metabolic outcomes were compared across four women´s groups: combined i) normal-weight & unfit; ii) normal-weight & fit; iii) overweight-obese & unfit; and iv) overweight-obese & fit. Normal-weight and overweight-obesity were defined as pre-pregnancy BMI 18-25kg/m^2 and >25kg/m^2, respectively. Unfit and fit were defined based on the median of overall PF: <-0.001 and ≥-0.001 at 16th week, and <-0.005 and ≥-0.005 at 33rd week, respectively. Additionally, as traditionally observed in the "Fit but Fat paradox" studies[14], these analyses were replicated but defining fit and unfit according to CRF (**Figure 2b**): median $CRF_{85\%MHR}$ <382 and ≥382 seconds at 16th week, and <295 and ≥295 seconds at 33rd week, respectively. The median cut-points were chosen because specific cut-points for the time to $85\%_{MRH}$ and $85\%_{TRH}$ in the Bruce test do not exist for pregnant women – neither for $VO2_{max}$– and the distribution of groups was more balanced.

All the assumptions related to the generalization of the results were met. The analyses were conducted using SPSS 22.0 (IBM, NY, USA). The statistical significance was set at p≤0.05.

RESULTS

From all the participants willing to get involved (n=384), 151 Caucasian southern Spanish women (age 33±5 years, pre-pregnancy BMI 22.8 (20.7, 26.5) kg/m^2) without diagnosed cardiometabolic illnesses, were considered for these study aims (see **Figure S3**). Participants´ sociodemographic and clinical characteristics are provided in **Table 1**. The metabolic markers concentrations during pregnancy are shown in **Table S2**.

Table 1. Sociodemographic and clinical characteristics of pregnant women (n=151).

	n	Mean*	SD*
Age (years)		33	5
Gestational age – 1st assessment		15.9	1.7
Gestational age – 2nd assessment		33.0	1.9
Gestational age - delivery		40	(39, 41)
Educational level, n (%)			
Non university degree		62	41.3
University degree		88	58.7
Parity status (primiparious), n (%)		90	59.6
Female offspring sex, n (%)	137	69	50.4
Use of oxytocin, n (%)	127	42	33.1
Use of epidural anaesthesia	132	91	68.9
Number of abortions		0	(0, 1)
Type of deliver, n (%)	139		
Spontaneous		81	58.3
Vacuum extraction		18	12.9
Forceps		5	3.6
Caesarean Section		35	25.2
Anthropometry			
Pre-pregnancy body mass index (kg/m^2)	142	22.8	(20.7, 26.5)
Gestational weight-gain 16th-33rd week (kg)	120	8.7	3.4
Cardiovascular function, 16th week			
Systolic blood pressure (mmHg)		108	9.2
Diastolic blood pressure (mmHg)		64	7.7
Dietary habits, 16th week			
Adherence to the Mediterranean Diet Score (0-50)		29	3.9
Energy intake (kcal/day)		2566	772.9
Physical fitness, 16th week			
Flexibility (cm)		4.2	6.3
Lower body-muscle strength (rep)	85	16	2.1
Upper-body muscle strength (kg)		26.2	3.3
$CRF_{85\%MHR}$ (s)	122	379	99
Women who finished the Bruce test ($CRF_{85\%MHR}$), n (%)	127	122	96.9
$CRF_{85\%THR}$ (s)	54	438	102
Women who finished the Bruce test ($CRF_{85\%THR}$), n (%)	77	54	68.9
Physical fitness, 33rd week			
Flexibility (cm)	122	4	6.0
Lower body-muscle strength (rep)	64	16	2.4
Upper-body muscle strength (kg)	122	26.9	3.4
$CRF_{85\%MHR}$ (s)	93	303	89
Women who finished the Bruce test ($CRF_{85\%MHR}$), n (%)	115	93	80.8
$CRF_{85\%THR}$ (s)	29	371	80
Women who finished the Bruce test ($CRF_{85\%THR}$), n (%)	62	29	46.8
Sleep (accelerometry), 16th week			
Sleep time (min/day)		429	46.8
Sleep quality (Pittsburgh questionnaire)	133	5	(3.8, 9.0)
Sedentary lifestyle and PA (waist), 16th week			
Sedentary time (min/day)	134	510	96.4
Moderate-vigorous PA (min/day)	134	33	(20.9, 49.2)

$CRF_{85\%MHR}$, time to reach the 85% of the maximum heart rate in the submaximal Bruce treadmill test (a proxy of cardiorespiratory fitness); $CRF_{85\%THR}$, time to reach the 85% of the target heart rate; PA, physical activity. The sample size was 151 for all variables, unless otherwise indicated in the table. ***** Continuous variables are presented as mean -standard deviation- or median (interquartile range), and qualitative variables as number of women -%-.

Association of physical fitness with cardiometabolic markers

The associations of PF components and overall PF with maternal metabolic markers are shown in **Table 2**. Upper-body flexibility was positively associated with DBP at 16th week (p=0.01), and changes in upper-body flexibility (16th-33rd week) were inversely associated with changes in total cholesterol and LDL-C levels (p<0.05). Lower-body muscle strength was inversely associated with insulin levels (p<0.001) and HOMA-IR (p=0.004) at 33rd week, and changes in lower-body muscle strength was inversely associated with changes in triglycerides (p=0.03). Upper-body muscle strength was inversely associated with insulin and HOMA-IR (p<0.05), and HDL-C levels (p=0.01) at 16th week, and positively associated with glucose levels (p=0.001) and SBP at 33rd week (p=0.01). Changes in this component were inversely related to triglycerides concentrations (p=0.02). $CRF_{85\%MHR}$ was inversely associated with cholesterol and LDL-C levels at 16th week, and glucose and cortisol at 33rd week (all, (p≤0.05). $CRF_{85\%THR}$ was inversely associated with HOMA-IR at 16th week (p=0.03). Overall PF was inversely associated with total cholesterol concentrations at 16th week (p=0.02). The rest of associations were non-statistically significant (p>0.05). After additionally adjusting *–in a stepwise manner–* for sleep quality and duration, energy intake, intervention group, ST, and the different PF components, all the results remained similar, except for few associations. Specifically, the associations of upper-body flexibility with DBP at 16th week, and upper-body muscle strength with glucose levels and SBP at 33rd week (*confounders: sleep time and ST*), became non-significant (p>0.05). Overall, no effect modification of PF by foetal sex was found, except for few associations (see legend **Table 2**). Regarding foetal metabolic markers (**Table S3**), only an increase in upper-body flexibility (16th-33rd week) was slightly associated with decreased maternal LDL-C levels at birth, and with reduced arterial cord serum total cholesterol, HDL-C, and LDL-C levels (all, p<0.05).

When controlling these analyses (**Table 2** and **Table S3**) for the familywise error rate (Hochberg procedure), only the associations of lower-body muscle strength with insulin and HOMA-IR, and of upper-body muscle strength with glucose levels, remained statistically significant (33rd week).

Table 2. Associations of physical fitness with maternal cardiometabolic markers during pregnancy (n=151)

	Flexibility *16th week; n=134*				**Lower-body muscle strength [c]** *16th week; n=81*				**Upper-body muscle strength [c]** *16th week; n=137*				**$CRF_{85\%MHR}$** *16th week; n=114*			
	Model 1			**Mo.2**	**Model 1**			**Mo.2**	**Model 1**			**Mo.2**	**Model 1**			**Mo.2**
16th week	B	SE	p	p	B	SE	p	p	B	SE	p	p	B	SE	p	p
Glucose	0.22	0.17	0.20	0.30	-58.3	41.03	0.16	0.16	-11.9	16.34	0.47	0.35	0.00	0.01	0.93	0.92
Insulin[b]	0.02	0.02	0.29	0.29	-0.77	3.29	0.82	0.84	-2.98	1.38	**0.03**	**0.02**	0.00	0.00	0.22	0.24
HOMA-IR[b]	0.01	0.02	0.37	0.42	-1.56	3.31	0.64	0.65	-2.79	1.39	**0.05**	**0.03**	0.00	0.00	0.20	0.22
Cholesterol	-0.18	0.47	0.70	0.72	-38.6	87.41	0.66	0.67	-83.3	43.37	0.06	**0.05**	-0.07	0.03	**0.03**	**0.03**
Triglycerides	0.76	0.70	0.28	0.35	-195	135.99	0.16	0.16	50.71	64.69	0.44	0.43	-0.05	0.05	0.27	0.30
HDL-C	-0.13	0.17	0.43	0.37	10.1	32.36	0.76	0.78	-43.5	15.02	**0.01**	**0.01**	-0.01	0.01	0.64	0.53
LDL-C	-0.20	0.40	0.63	0.70	-9.70	73.17	0.90	0.92	-48.3	37.11	0.20	0.15	-0.05	0.03	**0.05**	**0.05**
Cortisol	-0.02	0.07	0.75	0.79	2.77	14.34	0.85	0.76	6.50	6.91	0.35	0.50	0.00	0.01	0.38	0.39
CRP[a]	-0.01	0.01	0.42	0.24	-1.49	1.11	0.19	0.19	-0.21	0.58	0.72	0.80	0.00	0.00	0.35	0.35
SBP	0.11	0.13	0.42	0.35	-6.06	25.17	0.81	0.77	4.22	12.41	0.74	0.75	-0.01	0.01	0.45	0.53
DBP	0.24	0.11	**0.03**	**0.01**	15.4	19.03	0.42	0.43	-11.1	10.24	0.28	0.41	0.00	0.01	0.61	0.65
33rd week	**Flexibility** *33rd week; n=115*				**Lower-body muscle strength [c]** *33rd week; n=64*				**Upper-body muscle strength [c]** *33rd week; n=115*				**$CRF_{85\%MHR}$** *33rd week; n=89*			
Glucose	0.26	0.19	0.17	0.12	47.2	46.58	0.32	0.27	52.43	21.27	**0.02**	**0.001**	-0.02	0.01	**0.05**	**0.04**
Insulin[b]	0.02	0.02	0.29	0.09	-6.66	3.68	0.08	**<0.001**	-0.73	1.96	0.71	0.84	0.00	0.00	0.22	0.38
HOMA-IR[b]	0.02	0.02	0.19	0.06	-5.08	3.93	0.20	**0.004**	0.17	1.95	0.93	0.97	0.00	0.00	0.20	0.33
Cholesterol	-0.30	0.65	0.65	0.86	194.	119.65	0.11	0.11	-34.3	74.19	0.65	0.28	0.00	0.05	0.98	0.40
Triglycerides	0.16	1.25	0.90	0.89	14.8	285.24	0.96	0.64	-20.0	144.16	0.89	0.05	-0.01	0.09	0.96	0.10
HDL-C	0.07	0.17	0.69	0.51	-28.6	29.74	0.34	0.86	-2.95	19.87	0.88	0.46	0.00	0.01	0.78	0.67
LDL-C	-0.46	0.61	0.46	0.82	220.	116.92	0.07	0.11	-40.2	70.10	0.57	0.26	0.01	0.05	0.88	0.38
Cortisol	-0.07	0.07	0.36	0.26	-4.86	13.70	0.72	0.49	4.94	8.26	0.55	0.99	-0.01	0.01	0.17	**0.04**
CRP	0.00	0.01	0.56	0.91	-1.49	1.11	0.19	0.48	0.67	0.81	0.41	0.26	0.00	0.00	0.51	0.44
SBP	-0.02	0.20	0.92	0.53	77.8	45.22	0.09	0.18	51.34	22.75	**0.03**	**0.01**	-0.01	0.02	0.42	0.97
DBP	0.03	0.13	0.81	0.27	37.8	26.02	0.15	0.60	11.02	14.48	0.45	0.29	-0.01	0.01	0.37	0.89
Δ 16th-33rd week	**Δ Flexibility** *16th-33rd week; n=103*				**Δ Lower-body muscle strength [c]** *16th-33rd week; n=62*				**ΔUpper-body muscle strength[c]** *16th-33rd week; n=103*				**Δ $CRF_{85\%MHR}$** *16th-33rd week; n=78*			
Glucose[a]	-0.03	0.46	0.95	0.83	14.3	49.93	0.78	0.61	6.43	28.04	0.82	0.61	-0.03	0.02	0.05	0.10
Insulin[ab]	0.04	0.04	0.39	0.31	-1.20	5.00	0.81	0.79	-3.47	2.60	0.19	0.74	0.00	0.00	0.15	0.07
HOMA-IR[ab]	0.03	0.04	0.45	0.47	-0.56	5.02	0.91	0.86	-3.49	2.67	0.20	0.85	0.00	0.00	0.22	0.08
Cholesterol[a]	-2.99	1.18	**0.01**	**0.02**	-135	101.99	0.19	0.32	30.47	74.23	0.68	0.93	0.02	0.05	0.70	0.87
Triglycerides[a]	-0.20	1.79	0.91	0.78	-194	173.34	0.27	**0.03**	-222	106.31	**0.04**	**0.02**	-0.09	0.07	0.20	0.26
HDL-C[a]	-0.47	0.34	0.16	0.15	0.27	26.63	0.99	0.88	44.74	19.75	**0.03**	0.10	0.01	0.01	0.51	0.76
LDL-C[a]	-2.49	1.11	**0.03**	**0.04**	-93.7	102.47	0.37	0.50	31.07	69.88	0.66	0.98	0.03	0.04	0.52	0.61
Cortisol	-0.28	0.22	0.21	0.93	4.72	20.46	0.82	0.23	12.74	14.19	0.37	0.54	-0.01	0.01	0.26	0.65
CRP[a]	0.02	0.02	0.49	0.57	1.48	1.40	0.30	0.41	1.94	1.21	0.11	0.26	0.00	0.00	0.79	0.34
SBP	0.84	0.42	0.05	0.07	69.7	48.00	0.15	0.08	-14.9	26.34	0.57	0.49	-0.03	0.02	0.13	0.12
DBP	-0.16	0.25	0.52	0.42	11.2	26.21	0.67	0.43	-13.9	15.29	0.36	0.50	0.00	0.01	0.86	0.70

$CRF_{85\%MHR}$, time to reach the $85\%_{MHR}$ in the Bruce test; $CRF_{85\%THR}$, time to reach the $85\%_{THR}$; CRP, C-reactive protein, DBP, diastolic blood pre
cholesterol; HOMA, homeostasis model assessment; IR, insulin resistance; LDL-C, low density lipoprotein-cholesterol; Mo., model; p, p-value;
variation of winsorizing, and optimum Box-Cox transformations [b] were performed on metabolic outcomes. Similar results were observed w
expressed in relative terms (muscle strength/weight), and thus B coefficients have to be interpreted accordingly. [d] The physical fitness index wa
of the back scratch, handgrip, and Bruce $85\%_{MHR}$ tests. All <u>models 1</u> were adjusted for pre-pregnancy body mass index, maternal age, and sp
week/33rd week). In analyses at 16th week, all <u>models 2</u> were additionally adjusted for Mediterranean diet score at baseline. In analyses at 34th
2 were additionally adjusted for the Mediterranean diet score at 34th week (and difference from baseline), and for the baseline values of the resp
rate (Hochberg procedure), only the associations of lower-body muscle strength with insulin and HOMA-IR, and of upper-body muscle strengt
When grouping the results at 16th week <u>by foetal sex</u>, the association of upper-body muscle strength with insulin and HOMA-IR was only o
associations of lower-body muscle strength with glucose, of $CRF_{85\%MHR}$ with insulin and insulin resistance, and of overall physical fitness with tot
with female foetuses. At 33rd week, lower-body muscle strength was inversely associated with HOMA-IR only in mothers with male foetuses
changes in flexibility with cholesterol and LDL-C, and of upper-body muscle strength with triglycerides, were only in mothers with female foetus

Associations of physical fitness with clustered cardiometabolic risk

At 16th week (**Figure 1a**), relative lower-body (B=-5.41, SE=1.68, β=-0.50, *p*=0.003) and *upper-body (B=-2.41, SE=0.77, β=-0.34, p=0.003) muscle strength, CRF85%MHR (B=-0.001, SE=0.001, β=-0.28, p=0.02) and overall PF (B=-0.28, SE=0.09, β=-0.36, p=0.004) were inversely associated* with clustered cardiometabolic risk. At 33rd week (**Figure 1b**), neither PF components, nor overall PF, were associated with clustered cardiometabolic risk (*p*>0.05). Regarding the analyses of changes (16th-33rd week; **Figure 1c**), only changes in $CRF_{85\%MHR}$ were inversely associated with changes in clustered cardiometabolic risk (B=-0.001, SE=0.001, β=-0.32, *p*=0.02). These results remained similar after adjusting for sleep quality and time, energy intake, intervention group and ST.

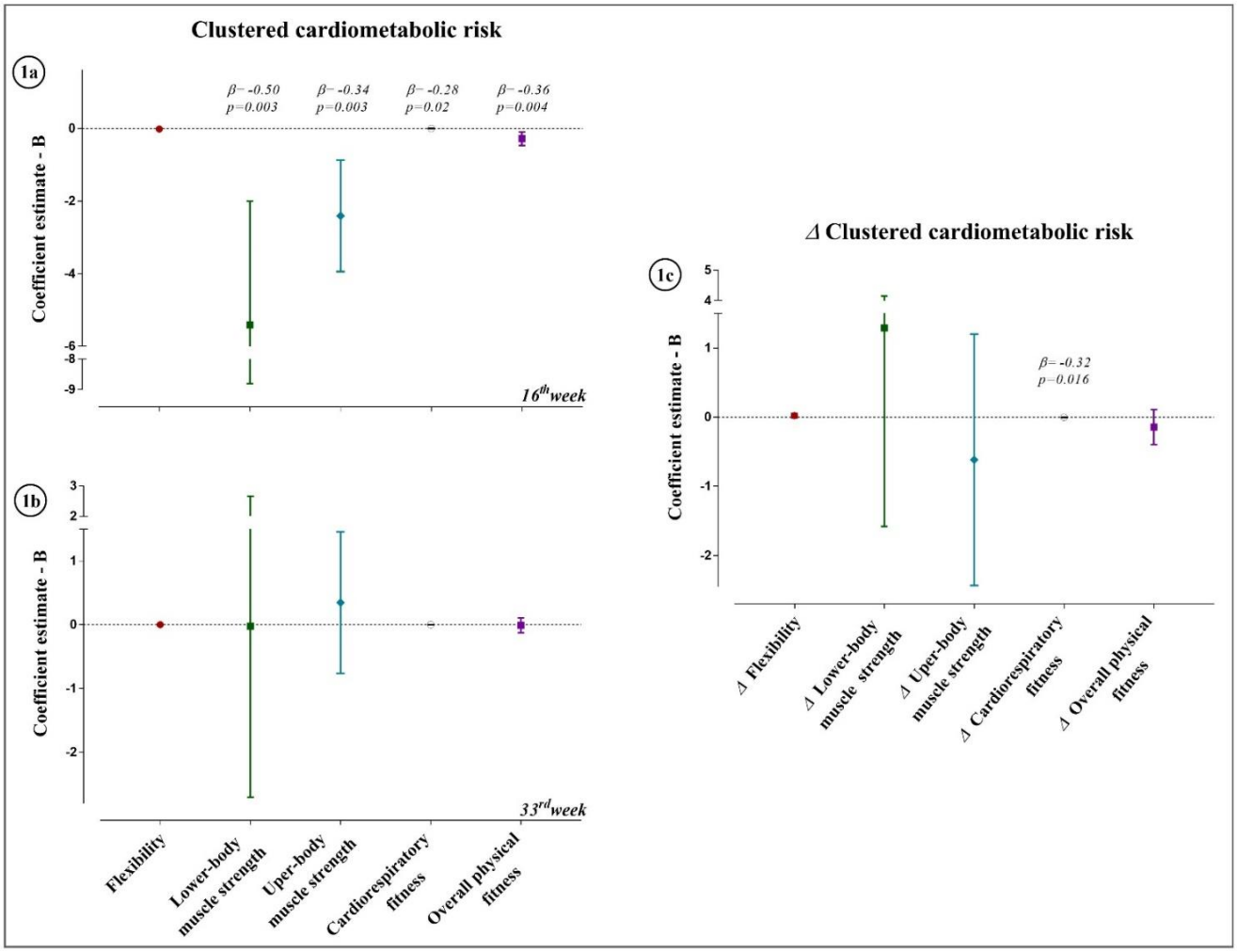
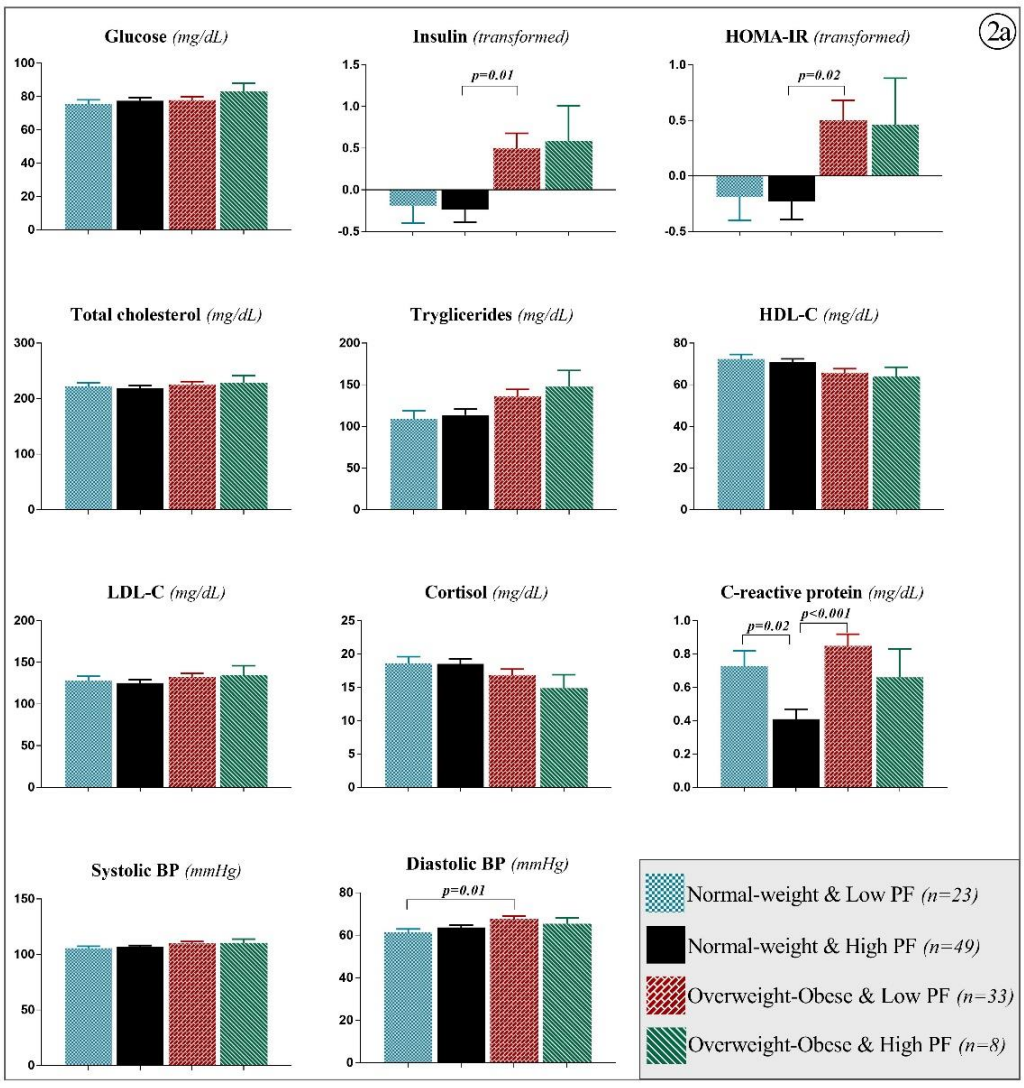


Figure 1. Associations of physical fitness with clustered cardiometabolic risk: 1a) at 16th week (n=78); 1b) at 33rd week (n=78); 1c) changes in physical fitness and clustered cardiometabolic risk (16th-33rd week; n=64). B, unstandardized regression coefficient, Δ, change. Higher scores indicate greater cardiometabolic risk. Standardized coefficients, along with p-values, were only provided for the significant associations

(above the dashed line). Muscle strength is expressed in relative terms (muscle strength/weight). Cardiorespiratory fitness reflects the CRF85%MHR. Overall physical fitness was created from z scores [(value-mean)/standard deviation)] of the back scratch, handgrip, and Bruce85%MHR tests. In the analyses 1a (16^{th} week) and 1b (33^{rd} week), all models were adjusted for maternal age, specific week of gestation at 16th and 33rd week, the Mediterranean diet score at 16^{th} or 33^{rd} week, and for the clustered cardiometabolic risk at 16^{th} week (only for 1b). In the analyses 1c, all models were adjusted for maternal age, specific week of gestation at second assessment, change in Mediterranean diet score (16^{th}-33^{rd} week), and for the clustered cardiometabolic risk at 16^{th} week.

Cardiometabolic profile according to pre-pregnancy BMI and PF

Differences in maternal cardiometabolic markers according to combined pre-pregnancy BMI (normal-weight or overweight-obese) and overall PF (unfit or fit) are shown in **Figure 2a**. At 16^{th} week, normal-weight & fit women were characterized by lower insulin (mean difference=-0.74, SE=0.24, *p*=0.01), HOMA-IR (-0.72, 0.28, *p*=0.02) and CRP (-0.44, 0.09, *p*<0.001) levels than overweight-obese & unfit women, and by lower CRP (-0.32, 0.11, *p*=0.02) than normal-weight & unfit women. Additionally, normal-weight & unfit women showed lower DBP than overweight-obese & unfit women (-6.24, 1.99, *p*=0.01). At 33^{rd} week, none difference was found between groups. Similarly to the traditional approach of the "Fat but Fit" paradox, the analyses were replicated (**Figure 2b**) considering $CRF_{85\%MHR}$ (instead of overall PF) to define unfit and fit women. At 16^{th} week, normal-weight & fit women were characterized by lower insulin (-0.736, 0.236, *p*=0.04), HOMA-IR (-0.70, 0.26, *p*=0.05), triglycerides (-35.01, 12.15, *p*=0.03), CRP (-0.42, 0.11, *p*=0.002), and DBP (-5.71, 0.1.9, *p*=0.02) than overweight-obese & unfit women, and by lower total cholesterol (-21.84, 7.14, *p*=0.02) than normal-weight & unfit women. Although non-significant, normal-weight & fit (-16.67, 6.65, *p*=0.08) and overweight-obese & fit (-15.26, 6.20, *p*=0.09) women showed a trend towards reduced LDL-C levels than overweight-obese & unfit women. None significant association was observed at 33^{rd} wee



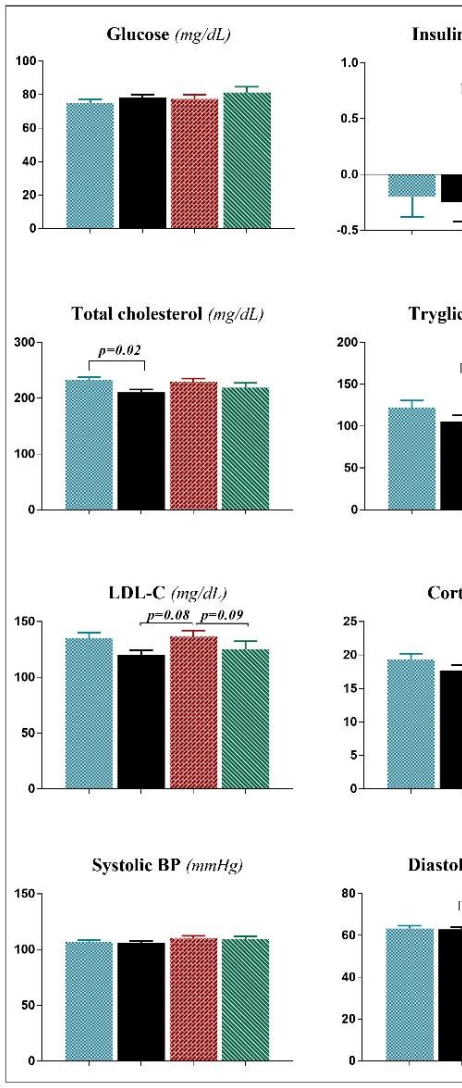


Figure 2. Differences in maternal metabolic markers at 16th week according to: **2a)** level of overall fitness and pre-pregnancy body mass index at 16th week; **2b)** level of o
pre-pregnancy body mass index at 16th week. BP, blood pressure; HDL-C, high density lipoprotein-cholesterol; HOMA, homeostasis model assessment; IR, insulin res
whiskers indicate means and standard error, respectively. All the models were adjusted for maternal age, specific week of gestation at 16th week and the Mediterranean
step procedure) was employed for pairwise comparisons between groups.

DISCUSSION

Despite the vast array of studies emphasizing the protector role of PF on metabolism in the general population, evidence regarding its influence in maternal and foetal metabolism during pregnancy is inexistent. This is the first study showing that PF is associated with several cardiometabolic markers during pregnancy, and might potentially confer a protector role in maternal metabolism –especially in early pregnancy. Of note, the current findings also showed that normal-weight women who were fit (but not those unfit) had an improved metabolic phenotype compared to overweight-obese and unfit women, which emphasizes a prominent role for PF in pregnancy.

Physical fitness and maternal-foetal metabolism

When the spotlight was on the individual PF components, our findings robustly showed that higher lower-body muscle strength was related to lower insulin levels and insulin resistance in late pregnancy. Of note, these associations were also observed in upper-body muscle strength, but earlier in pregnancy. Similarly, we observed that greater $CRF_{85\%THR}$ and $CRF_{85\%MHR}$ were related to lower insulin resistance in early-middle pregnancy, and lower glucose levels in late pregnancy, respectively. Altogether, this might suggest a beneficial role of muscle strength and CRF in glucose-insulin metabolism, as previously observed in the general population[12,13,31]. Although some might attribute this favourable glucose-insulin axis to a less sedentary lifestyle[32] or healthier dietary habits during pregnancy, we accounted for both behaviours in these associations. If confirmed by future studies, this might be of special interest for pregnant women with obesity and GDM, since both adverse conditions are usually characterized by excessive peripheral insulin resistance and endogenous glucose production[2,6], which can lead to hyperglycaemia, and thus to short/long-term consequences for the mother and offspring[2,4-6].

Our findings also suggested that greater CRF and overall PF were related to lower cholesterol in early-middle pregnancy. Additionally, it appeared that those women who increased flexibility and muscle strength also improved lipid metabolism (reduced cholesterol and LDL-C, and triglycerides, respectively). These results concur with previous evidence in the general population[12,13], and might be explained via improved muscle phenotype and lipid metabolism (e.g. increased lipoprotein lipase activity and

fatty acid oxidation, mitochondrial biogenesis), lower abdominal obesity, greater transport of systemic lipids to the liver, etc.[11,13,33]. Whether the association of upper-body flexibility with lipids was spurious, or might be related to increased relaxin[34], weight status or vascular adaptations[35], remains undetermined. Indeed, future studies are necessary to better understand the role of PF in the complex nature of pregnancy.

Regarding foetal metabolism, only flexibility at late pregnancy appeared to show a slight relationship with foetal lipid metabolism. Whether PF did not influence directly foetal metabolism, or if the methodological design/limitations might have masked some of its effects (e.g. reduced sample size to detect effect sizes, lack of mechanistic studies), needs to be further addressed. All these associations should be interpreted cautiously since most of them disappeared after controlling for the familywise error rate.

Physical fitness and cardiometabolic risk

Aimed at better understanding the role of PF during pregnancy, we analysed its influence on the clustered cardiometabolic risk. This is of particular interest if we consider that pregnant women exposed to various cardiometabolic risk factors (i.e., greater cardiometabolic risk) are predisposed to a greater risk for adverse pregnancy outcomes[3,8,9]. Noteworthily, our findings showed that women with higher PF *(lower- and upper-body muscle strength, CRF and overall PF)* presented lower cardiometabolic risk in early-middle pregnancy, but not in late gestation. Additionally, those women who increased CRF from early-middle pregnancy until late pregnancy also reduced cardiometabolic risk during pregnancy.

Thus, based on our results and previous evidence in the general population[10,12,13], increasing PF could be a potential strategy to regulate metabolic markers during pregnancy, and potentially confer a cardio-protector effect to maternal metabolism, especially in early pregnancy. From a clinical standpoint, the PF-related optimised metabolic control and cardio-protector effect, might contribute to decrease the prevalence of pregnancy complications[2-5,7,8,36]. However, it is important to mention that our sample was relatively "healthy", and thus the link between this protector effect and adverse complications, need to be interpreted cautiously. Larger studies, investigating the potential of PF screening early in pregnancy to detect women at higher risk of cardiometabolic disruptions and birth complications, are necessary.

The prominent role of physical fitness in early pregnancy

Intrigued by the idea that PF might contribute to better control maternal metabolism, we wondered whether: i) being normal-weight but unfit was synonymous of being "healthy", and might be enough to avoid potential metabolic alterations related to overweight-obesity; ii) being fit was an indispensable determinant for improved metabolic control in normal-weight women; and iii) overweight-obese but fit women could have an improved metabolic profile and be at lower cardiometabolic risk than normal-weight and unfit individuals (the Fat but Fit paradox).

First, we observed that normal-weight and unfit women generally presented similar metabolic markers concentrations than overweight-obese and unfit women in early-middle pregnancy, except for diastolic blood pressure and HDL-C concentrations; i.e. being only normal-weight hardly attenuated the potential metabolic alterations related to the overweight-obese status when women were unfit. Second, normal-weight but fit women showed lower insulin levels, HOMA-IR, triglycerides, LDL-C, CRP, and DBP than overweight-obese and unfit women in early-middle pregnancy; i.e. being fit in normal-weight women appears to be essential to improve metabolic control[14], and potentially avoid impaired metabolic alterations related to overweight-obesity. This was further supported by the lower CRP and total cholesterol levels observed in fit vs. unfit normal-weight women. Third, none significant difference was found between overweight-obese but fit women and normal-weight and unfit individuals, or between fit and unfit overweight-obese women. We only observed some trends in the overweight-obese and fit group towards lower LDL-C and CRP levels than the overweight-obese and unfit group; i.e. being fit might not be enough in overweight-obese women to confer a protective effect against metabolic alterations in early pregnancy. This is in contrast with the famous "Fat but Fit" paradox[13,14] in the general population, which has shown that obese but fit individuals could be at lower risk than normal-weight and obese unfit individuals. However, in our study, the overweight-obese but fit women group was limited by the small sample, which might have hindered the detection of small-medium effect sizes.

Bearing all above in mind, healthcare and educational actions prompting women to be normal-weight, and more importantly keeping fit, are primordial to optimise metabolic phenotype in early pregnancy. From a practical perspective, the design of exercise interventions so far has been mainly oriented at reducing the prevalence of

GDM and birth complications, and excessive gestational weight-gains[37,38]. However, when considering the "ingredients" for designing effective exercise programs, little attention has been paid to the control of metabolic markers during pregnancy. This is of primordial relevance considering that exacerbated metabolic markers are well-known to influence the course of pregnancy, and directly contribute to birth complications[2-8]. Thus, exercise interventions mainly aimed at improving muscle strength and CRF, before or early in pregnancy, might be effective strategies to regulate maternal metabolism, and avoid potential adverse outcomes. This supports the previous notion that concurrent (aerobic+strength) exercise programs are more beneficial than those focused on individual PF components[38].

LIMITATIONS

A major limitation of our study is that these PF tests have not been validated in pregnancy. This represents an inherent limitation of pregnancy studies, since none PF test battery has been validated yet. However, these PF tests are characterized by good psychometric properties[24,26,27], and are adaptable, viable and safe for clinical populations[10,12,13,24,26,27]; specially, to avoid potential risks during the evaluation processes. Although more feasible, submaximal testing might overestimate $Vo2_{max}$, and thus induce some error when estimating women´s CRF compared to maximal exercise testing. However, maximal testing is limited by ethical considerations, since safety and utility issues are still emerging[29]. Thus, we employed $CRF_{85\%MHR}$, and additionally $CRF_{85\%THR}$ to consider heart rate reserve and better account for individual changes in cardiovascular function[28].

Although we did our best to avoid carry-over fatigue, and ensure women´s recover, this test was performed after other PF tests, and thus we cannot dismiss a potential influence on women´s physical capacity. Moreover, at 33rd week, women´s abdomen could be a mechanic barrier for the chair stand test (although they did not generally complain about this). However, these potential biases were systematic for all women at 16th and 33rd week, and thus our results are hardly likely to be affected by this fact. Additionally, our study was not originally powered to address these aims, and the sample size was "relatively" small. However, the meaningful findings support enough statistical power and sensitivity to detect effect sizes in these exploratory analyses. The

present study also has some strengths: i) this is the first study addressing (and providing such a compressive insight) the role of objectively measured PF in maternal-foetal metabolism; ii) the metabolic markers were evaluated at multiple time points, and in both arterial and venous cord serum; and iii) we have considered imperative confounders such as objectively measured ST/PA (7 days, ≥10hours/day), sleep and dietary habits, etc.

CONCLUSIONS

Increased PF, especially muscle strength and CRF in early pregnancy, is associated with a better metabolic phenotype, and might potentially provide a cardio-protector effect in maternal metabolism. “Keep yourself fit and normal-weight before and during pregnancy” should be a key message for women. Muscle strength and CRF are relevant targets to consider when designing concurrent exercise interventions to better regulate maternal metabolism.

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SUPPLEMENTARY MATERIAL

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Appendix A. *Outlier detection and management*

Nowadays, the presence of outliers is one of the most enduring and pervasive methodological changes in biomedical science research[1-3]. Worryingly, there is a lack of consensus about how addressing outliers (i.e. how defining, identifying and handling them). Since the decisions that researches make about this issue have important implications, we have included this section to promote transparency and the critical interpretation of the results, as previously recommended by several authors[1-3]. Although no specific guidelines exist about how addressing outliers, several studies[1-8] (especially that one from Aguinis, et al. [3]) have previously provided smart advices and recommendations to address them in the best possible way. Accordingly, the different steps to address outliers in the present study have been performed proceeding with the following recommendations. We have identified and handled outliers according to the basis for regressions, which are the main analyses involved in this study.

Error outliers

During the assessments at the different time points, questionnaires and tests (where errors related to data recording, coding, manipulation, etc. were likely and easily observed) were checked to identify clear error outliers, and correct them immediately by asking women, repeating the corresponding test, etc. When lacking, misleading or inaccurate data, was identified posteriori (up to 2 weeks after the assessments), women were contacted to ensure the accuracy of these data points, or to correct these potential outliers (whenever appropriate for data) in the respective database. Singles construct techniques (box plots, descriptive statistics, percentage analyses, etc.) were performed to initially identify error outliers. Subsequently, we also employed multiple construct techniques to identify error outliers. Particularly, we identified error outliers based on the outlyingness of the observation in term of its residual score. When it was not possible/appropriate to correct these data points, and we were sure that their inaccuracy was related to human errors, device malfunction, miscalculations or similar circumstances (i.e. we had determined the cause of the identified outlying observation), these error outliers were removed from the respective database. Since these potential error outliers could have been caused by inherent variability in the data (in this case they would represent a legitimate part of the population), we were very prudent when

identifying and handling them. We paid special attention to the reasoning behind the classification of data points as error outliers.

Interesting outliers

After the application of this first filter to the database, there were several remaining interesting outliers, which required additional analyses in depth. Thereby, we aimed at analysing these interesting outliers with quantitative approaches (e.g., we tried to analyse differences in how predictors were able to predict high and low outlier scores). However, the number of outliers was minimum, and only appreciable in few outcomes, which prevented us from performing these analyses properly. As consequence, we did not finally perform these analyses.

Influential outliers

Since it is not legitimate to simply drop the remaining potential outliers from the analyses (they tend to increase error variance, reduce the power of statistical test, etc.), nor plainly deleting them without any basis (they could be part of the inherent variability of the distribution of data), we analyzed more in depth the influence of these outliers in the model. Aimed at checking their influence, we analyzed how the deletion of specific outliers could affect the change of the model fit (e.g., changes in R^2; model fit outliers), parameters estimates (intercept, slope, regression coefficients, etc.; prediction outliers) and the assumptions of the model. If these remaining unusual cases were not finally identified as influential outliers, or they were identified but influenced the model slightly, these potential outliers were not handled (as observed in some outcomes the **Table 2**). In this case, these unusual data points were dropped in the analyses since they did not affect either the results or assumptions of the tests, and they could be caused by inherent variability in the data. By contrast, if these remaining unusual cases were confirmed as influential outliers which affected the model fit and parameter estimates (as appreciable in the **Table 2**), those influential outliers we handled.

In order to handle the aforementioned influential outliers (when identified), a subtle variation of winsorizing [convert back from a z-score: replacing extreme scores ($z>2.58$; value equivalent to a probable outlier) with a score equivalent to ±2.58 standard deviations from the mean] was employed to handle these outliers. After handing these outliers, data distribution improved, and some of the problematic issues related to the assumptions of some models disappeared. Subsequently, data preparation was

employed for those characterized by remaining asymmetry (skewness, kurtosis, etc.) of outcomes, and the violation of some assumptions related to the generalization of the results. Specifically, optimum Box-Cox transformations were used to reduce the impact of potential source of bias, and improve the goodness of fit of the data. After dealing with these "problematic" outcomes, the results remained similar (but with better and more symmetrical distribution of data) to the analyses without data preparation (i.e. without handling of outliers or/and applying Box-Cox transformations).

Table S1. Inclusion and exclusion criteria in the GESTAFIT project

Inclusion criteria
- Pregnant women aged 25-40 years old with a normal pregnancy course.
- Answering "no" to all questions on the PARmed-X for pregnancy.
- Being able to walk without assistance.
- Being able to read and write properly.
- Informed consent: Being capable and willing to provide written consent.
Exclusion criteria
- Having acute or terminal illness.
- Having malnutrition.
- Being unable to conduct tests for assessing physical fitness or exercise during pregnancy.
- Having pregnancy risk factors (such as hypertension, type 2 diabetes, etc.).
- Having a multiple pregnancy.
- Having chromosopathy or foetal malformations.
- Having uterine growth restriction.
- Having foetal death.
- Having upper or lower extremity fracture in the past 3 months.
-Suffering neuromuscular disease or presence of drugs affecting neuromuscular function.
- Being registered in another exercise program.
- Performing more than 300 minutes of at least moderate physical activity per week.
-Being engaged in another physical exercise program
- Being unwilling either to complete the study requirements or to be randomized into the control or intervention group.

Study III

Table S2. Metabolic markers concentrations at 16th and 33rd gestational weeks, and delivery.

	16th week of gestation ***(n=139)***		**33rd week of gestation** ***(n=115)***		**Delivery** ***(n=46)***		**Arter**
Maternal serum	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**
Glucose (mg/dL)	77.7	11.3	74.3	11.4	79.2	25.4	59.5
Insulin (microIU/dL)	4.5	(3.3, 8.2)	5.9	(4.4, 8.4)			
HOMA-IR	0.8	(0.6, 1.5)	1.1	(0.8, 1.5)			
Total cholesterol (mg/dL)	219.7	32.2	275.9	38.4	201.7	57.0	59.2
Triglycerides (mg/dL)	109.0	(87.0, 148.8)	196.3	(155, 254)	170.0	(127.8, 205.8)	43.8
HDL-C (mg/dL)	67.9	11.5	67.1	10.8	77.1	28.1	27.5
LDL-C (mg/dL)	127.6	26.5	166.9	36.1	46.8	15.5	7.2
Cortisol (mg/dL)	17.9	5.3	22.1	4.3			
C-reactive protein (mg/dL)	0.5	(0.3, 0.9)	0.4	(0.2, 0.7)			

HDL-C, high density lipoprotein-cholesterol; HOMA-IR, homeostatic model assessment-insulin resistance; cholesterol; SD, standard deviation. Data are mean (standard deviation) or median (interquartile range=0

Table S3. Associations of physical fitness with maternal and foetal metabolic markers at birth (n=44).

	Δ Flexibility *16th-33rd week; n=44*					**Δ Upper-body muscle strength [c]** *16th-33rd week; n=42*					**Δ $CRF_{85\%MHR}$ [c]** *16th-33rd week; n=32*				
	Model 1				**Model 2**	**Model 1**				**Model 2**	**Model 1**				**M**
Maternal serum (birth)	B	SE	Beta	p value	p value	B	SE	Beta	p value	p value	B	SE	Beta	p value	p v
Glucose[ab]	0.01	0.06	0.04	0.82	0.86	-4.21	4.68	-0.16	0.37	0.34	0.00	0.00	-0.10	0.58	0
Cholesterol	-1.65	3.30	-0.08	0.62	0.58	-270.84	270.46	-0.17	0.32	0.30	0.05	0.15	0.05	0.76	0
Triglycerides[a]	-0.16	3.60	-0.01	0.96	0.97	-414.43	287.96	-0.25	0.16	0.17	0.15	0.15	0.19	0.31	0
HDL-C	2.64	1.55	0.25	0.10	0.10	-47.80	130.79	-0.06	0.72	0.67	0.01	0.07	0.02	0.91	0
LDL-C[b]	-0.12	0.06	-0.33	**0.03**	**0.03**	-3.19	4.78	-0.12	0.51	0.49	0.00	0.00	0.10	0.60	0
Arterial cord serum			***n=26***					***n=26***					***n=19***		
Glucose[a]	0.06	0.11	0.11	0.61	0.68	-7.37	7.01	-0.26	0.30	0.34	0.00	0.00	-0.04	0.89	0
Cholesterol[ab]	-0.22	0.11	-0.40	0.06	**0.04**	-0.13	7.66	0.00	0.99	0.95	-0.01	0.00	-0.51	0.06	0
Triglycerides[b]	-0.02	0.11	-0.04	0.86	0.61	10.51	6.61	0.36	0.13	0.05	0.00	0.00	-0.22	0.32	0
HDL-C	-1.95	0.90	-0.41	**0.04**	**0.04**	-39.22	64.06	-0.15	0.55	0.58	-0.04	0.03	-0.37	0.16	0
LDL-C[ab]	-0.17	0.10	-0.32	0.11	**0.03**	2.35	7.19	0.08	0.75	0.57	0.00	0.00	-0.25	0.30	0
Venous cord serum			***n=38***					***n=37***					***n=30***		
Glucose	-0.28	1.33	-0.04	0.84	0.92	-68.42	110.80	-0.13	0.54	0.55	0.06	0.05	0.26	0.18	0
Cholesterol[ab]	-0.05	0.07	-0.12	0.46	0.30	5.22	5.53	0.19	0.35	0.35	0.00	0.00	-0.07	0.70	0
Triglycerides[a]	-2.09	1.37	-0.25	0.14	0.09	156.39	116.06	0.26	0.19	0.19	0.00	0.05	-0.01	0.95	0
HDL-C[a]	-0.84	0.77	-0.19	0.28	0.34	69.64	64.97	0.22	0.29	0.29	0.01	0.03	0.04	0.83	0
LDL-C[a]	-0.29	0.33	-0.15	0.39	0.39	16.25	28.20	0.12	0.57	0.57	0.01	0.01	0.11	0.55	0

$CRF_{85\%MHR}$, time to reach the 85%$_{MHR}$ in the submaximal Bruce treadmill test; Δ, delta (change); HDL-C, high densit
density lipoprotein-cholesterol; MHR, maximum heart rate; Mo., model; SBP, systolic blood pressure; SE, sta
winsorizing, and optimum Box-Cox transformations [b] were performed on metabolic outcomes. Similar results were
unhandled [c] Muscle strength is expressed in relative terms (muscle strength/weight), and thus B coefficients have
physical fitness index was created from z scores [(value-mean)/standard deviation] of the back scratch, handgrip, a
were adjusted for pre-pregnancy body mass index and week of gestation at birth. All models 2 were additionally ad
controlling for the familywise error rate (Hochberg procedure), none association remained statistically significant (

Study III

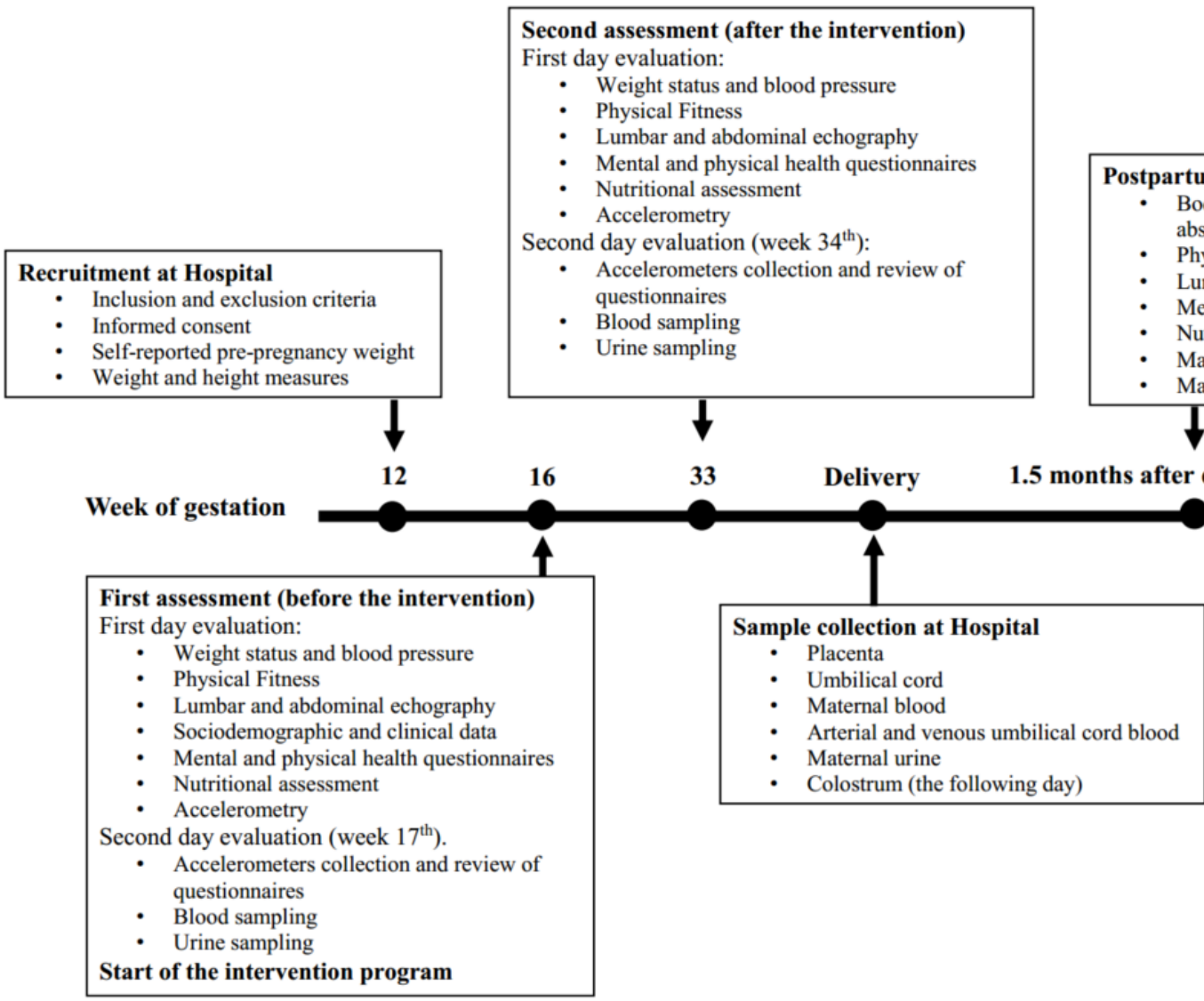

Figure S1. Assessments conducted along the GESTAFIT Project

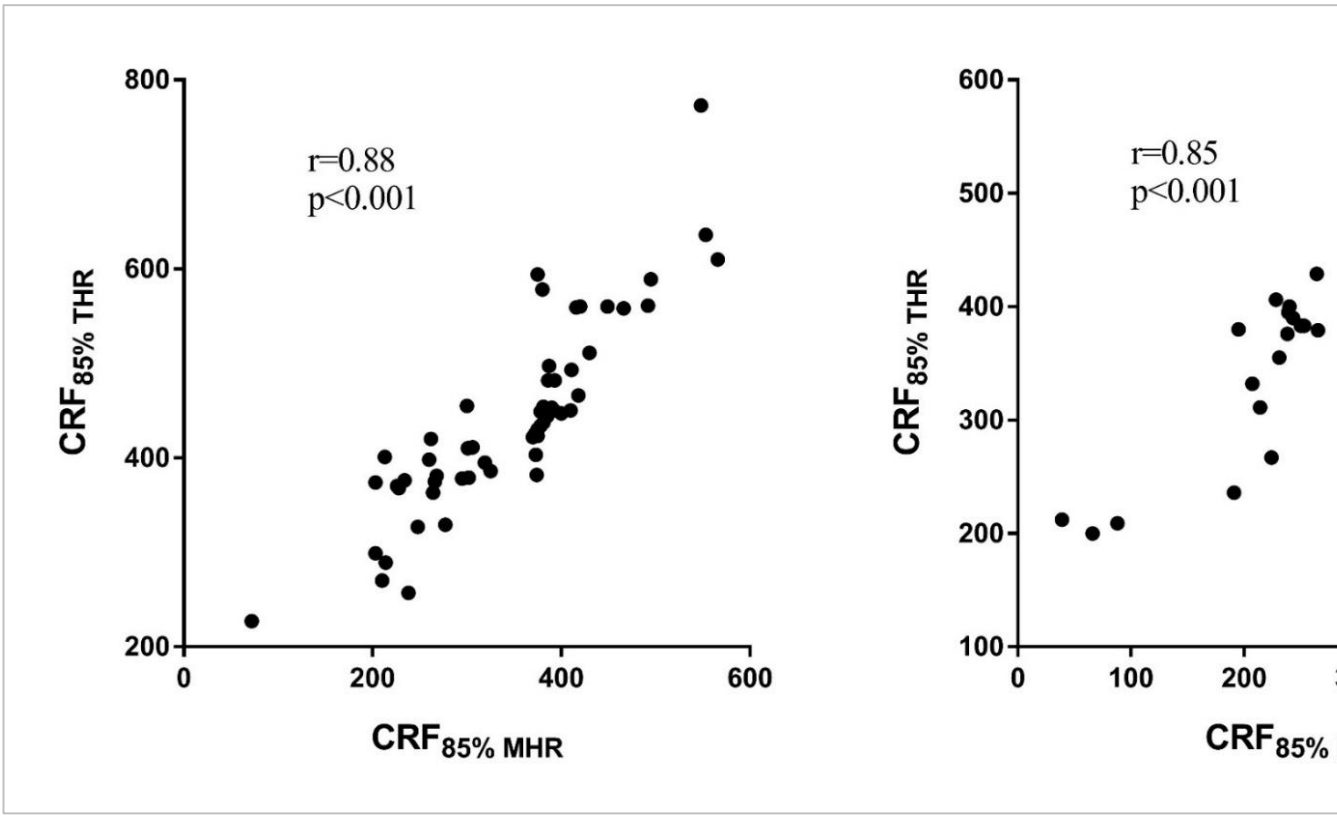

Figure S2. Correlations between $CRF_{85\%MHR}$ and $CRF_{85\%THR}$ at 16th week (left figure) and 33rd week (right figure). CRF
heart rate (proxy of cardiorespiratory fitness); $CRF_{85\%THR}$, time to 85% of the target heart rate.

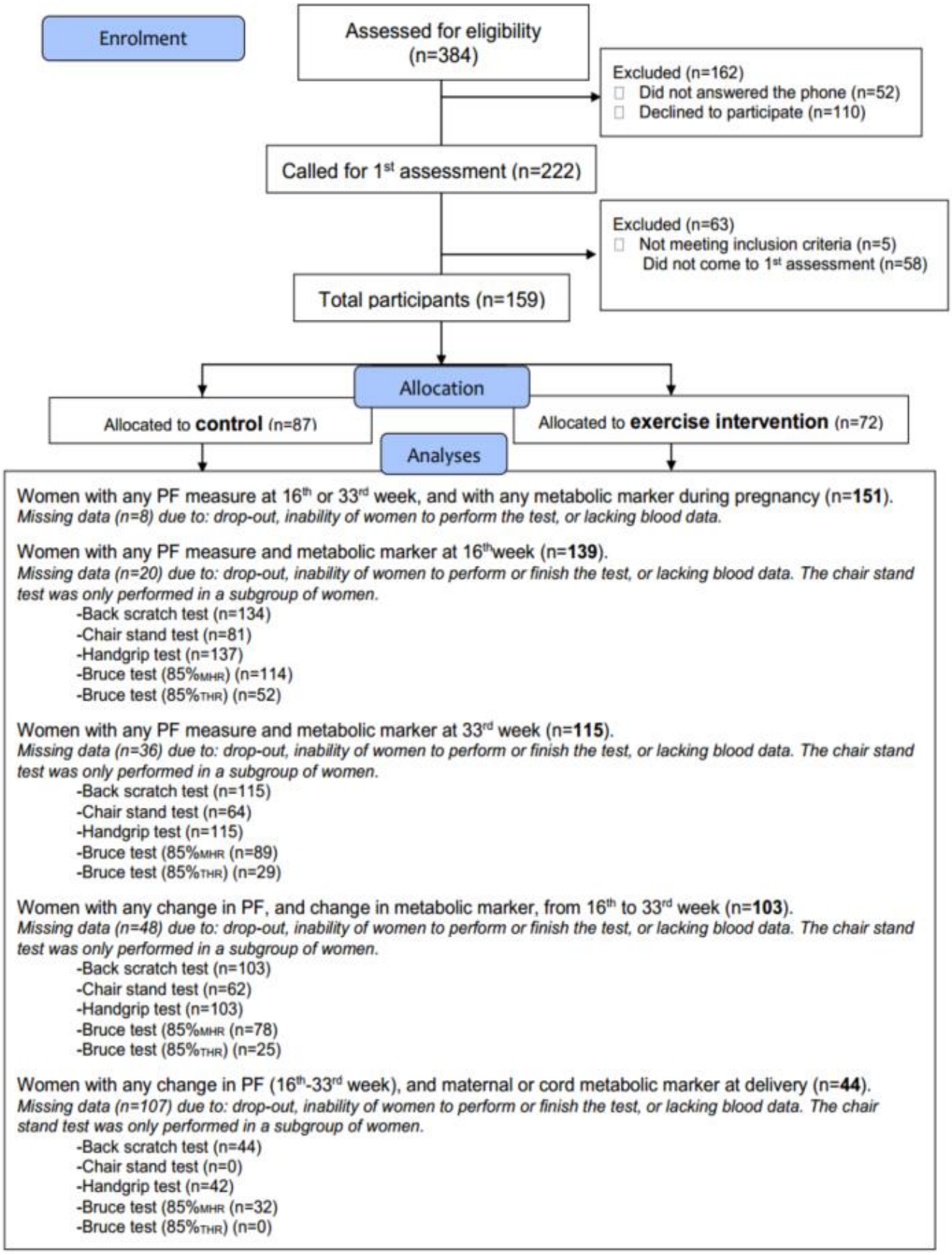

Figure S3. CONSORT flow chart diagram for the present study

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PART III. Role of physical exercise on immunometabolism

STUDY IV

Influence of a concurrent exercise training intervention during pregnancy on maternal and arterial and venous cord serum cytokines: The GESTAFIT project

ABSTRACT

Objectives: To analyze the influence of a supervised concurrent exercise-training program, from the 17th gestational week until delivery, on cytokines in maternal (at 17th and 35th gestational week, and at delivery) and arterial and venous cord serum.

Methods: Fifty-eight Caucasian pregnant women (age: 33.5±4.7 years old, body mass index: 23.6±4.1kg/m^2) from the GESTAFIT Project (control [n=37] and exercise [n=21] groups) participated in this quasi-experimental study (per-protocol basis). The exercise group followed a 60-min 3 days/week concurrent (aerobic-resistance) exercise training from the 17th gestational week to delivery. Maternal, and arterial and venous cord serum cytokines (fractalkine, interleukin [IL]–1β, IL-6, IL-8, IL-10, interferon [IFN]–γ, and tumour necrosis factor [TNF]–α) were assessed using Luminex xMAP technology. Sedentary time and physical activity intensity levels were objectively-measured with triaxial accelerometry (7 days).

Results: In maternal serum (after adjusting for the baseline values of cytokines), the exercise group decreased TNF-α (from baseline to 35th week, p=0.02), and increased less IL-1β (from baseline to delivery, p=0.03) concentrations than controls. When adjusting for other potential confounders, these differences became non-significant (evidence of statistical significance). In cord blood, the exercise group showed reduced arterial IL-6 and venous TNF-α (p=0.03 and p=0.001, respectively), and higher concentrations of arterial IL-1β (p=0.03) compared to controls.

Conclusion: The application of concurrent exercise training programs could be a strategy to modulate inflammatory responses in pregnant women and their foetuses. However, future research is needed to better understand the origin and clearance of these cytokines, their role in the maternal-placental-foetus crosstalk, and the influence of exercise interventions on them

INTRODUCTION

Pregnancy is a critical period of women´s life characterized by different immunometabolic responses depending on the trimester of pregnancy.[1-3] The fluctuations on these inflammatory responses are essential for an adequate maternofetal homeostasis, and thus, for a healthy and in-term pregnancy.[1-3] The first trimester of pregnancy is a highly anabolic phase accompanied by a pro-inflammatory state. This phase is followed by an anti-inflammatory state during the growth of the fetus, which finally turns into a pro-inflammatory state during late pregnancy (preparation for parturition).[1,2] Nonetheless, an exacerbation or dysregulation of pro and anti-inflammatory cytokines might lead to higher risk of developing pregnancy complications.[1,3-7]

Importantly, not only the maternal immune system, but also the placenta and fetus are sources of cytokines during pregnancy, and are continuously interacting between them to balance pro and anti-inflammatory states.[1-3,5,6,8-11] Unfortunately, the metabolism, origin and clearance of the different cytokines, and their role and contribution to the maternal-placental-fetus crosstalk, are currently poorly understood.[1,3,7,8,10,12] Hence, it seems important to better explore these underlying mechanisms, since it would facilitate the search of more adequate strategies aimed at preventing related disruptions.

In this regard, exercise might be a promising clinical tool to modulate inflammatory responses and prevent complicated pregnancies.[13] The emerging role of skeletal muscle as a primordial endocrine organ,[14,15] and its characteristic interplay with other organs via muscle contraction-induced factors (myokines),[9,14-16] could partially explain the beneficial effects of acute exercise (stress-like response) and long-term exercise (chronic adaptive responses) on immunometabolic health.[14,15] However, this issue remains currently unperceived in pregnancy, where these cytokines could play a key/relevant function at the maternal-fetal interface.[13,17] Thus far, the majority of exercise interventions during pregnancy have mainly focused on few classical markers, such as maternal plasma C-reactive protein and leptin. However, no studies to date have analyzed the effect on the inflammatory profile [including relevant cytokines such as fractalkine, interleukin (IL)-1β, IL-6, IL-8, IL-10, interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-alpha)] of healthy pregnant women (without metabolic

dysregulations) during pregnancy. Hence, it is of clinical interest to determine if exercise could be a potential strategy to modulate inflammatory responses of pregnant women and their fetuses.

The aim of this study was to analyze the influence of a supervised concurrent exercise-training program from the 17th gestational week until delivery, on cytokines in maternal (at 17th and 35th gestational week, and at delivery), and arterial and venous cord serum.

MATERIAL AND METHODS

Study population

The procedures, along with the inclusion-exclusion criteria (**Table S1**) of the GESTAFIT Project, are described elsewhere.[18] Three-hundred and eighty-four pregnant women attending their gynecologist at the 12th gestational week were informed about the project in the San Cecilio and Virgen de las Nieves University Hospitals (Granada, Spain). The recruitment was performed in three different waves. From all these initially interested participants, 159 women were finally recruited. All participants signed an informed consent after being individually informed about the study aims and procedures. The GESTAFIT project was approved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15).

Sample size

The required sample size was only determined for the primary outcomes (maternal weight gains and maternal/neonatal glycemic profile) of the GESTAFIT Project, and it was 52 pregnant women (26 per group).[18]

Randomization

Initially, this study was based on a randomized control trial design. However, the randomization design was finally broken because of some difficulties related to the complexity of maintaining women in the control group (avoiding high rates of withdrawal). These methodological and ethical barriers are frequent in antenatal exercise research, as previously argued.[19] Hence, it was decided to subsequently allocate pregnant women to the exercise/control group depending on their personal preference and convenience to attend the intervention sessions, and the wave in which they were recruited.

Procedures

Women were assessed twice (2 different days/assessment) during the study. Socio-demographic and clinical characteristics, dietary patterns, blood pressure, pre-gravid self-reported weight, body weight and height were assessed on the first day at 16th week (±2 weeks). Each participant was given an accelerometer to wear until next appointment. One week later (17th week), blood samples of the mothers were collected by a nurse, and accelerometers were returned. At the 34th week, the same assessments (all but socio-demographic and clinical characteristics; blood samples collected at 35th week) were performed, with identical timing to the 16th week. On the delivery day, maternal, and arterial and venous umbilical cord blood samples were collected moments after the delivery, and obstetric and gynecological histories were collected through the "Pregnancy Health Document". The responsible of the training sessions were the only personnel not blinded to the allocation of participants to the training/control groups. The assessments procedures are further explained in **Figure S1**.

Intervention

Exercise group

Pregnant women into the exercise group participated in a concurrent-training program from the 17th week until delivery (3 days/week, 60 minutes/session) consisting in a combination of aerobic-resistance exercises of moderate-to-vigorous intensity. This exercise protocol was designed by an expert multidisciplinary team, following the recommendations from the American College of Obstetrics and Gynecology.[20] The exercise group started with an informative and movement learning phase (3 sessions). In this initial phase, fundamental basic movement patterns were taught (hip and knee dominant, pull and push movements), and theoretical explanations were provided to the participants. Subsequently, the main exercise training phase lasted from the 18th until 34th week, and was focused on improving or maintaining physical fitness. The final phase during the last weeks of pregnancy was focused on the pelvic mobilization (preparation for the delivery). The detailed exercise sessions (**Appendix A**) and protocol, along with specific exercises, can be found elsewhere (Supplementary material section).[21] The attendance to the training sessions was recorded. During this period, the research team also gave 7 talks to the participants aimed at providing them with basic pregnancy health-related information (detailed in **Appendix B**).

Control group

The participants in the control group were requested to continue with their daily activities. Because of ethical considerations, we also invited them to these 7 talks. We also used these meetings to maintain their fidelity until the end of the program.

Outcomes

Gynecologists and midwives from the hospitals, and expert physiologists responsible for the assessment of these secondary outcomes, were blinded to the allocated treatment of the participants.

Sociodemographic and clinical data

Women completed a self-reported questionnaire of sociodemographic (age, number of children, cohabitation, marital, and educational status, among others), reproductive history and clinical (suffering or having suffered specific diseases, and drug consumption) data, and smoking and alcohol habits. All instructions needed to properly understand and complete the self-reported survey were given by the research team.

Perinatal outcomes

Data related to the type of delivery (natural, instrumental, or caesarean), its duration, number of abortions, and offspring sex were obtained from perinatal obstetric records (partogram).

Weight status

Pre-pregnancy weight was self-reported. The height and weight were assessed using a stadiometer (Seca 22, Hamburg) and scale (InBody R20; Biospace, Seoul), respectively. Body mass index was calculated [weight(Kg)/height(m^2)].

Blood pressure and resting heart rate

A blood pressure monitor (M6 monitor Omron, The Netherlands) was employed to assess systolic and diastolic blood pressure, and resting heart rate.

Mediterranean Diet Score

The Mediterranean Diet Score (MDS)[22] is an index created to evaluate the degree of adherence to the Mediterranean dietary pattern. The consumption of each of those foods for further calculations was assessed with a food frequency questionnaire.[23]

Sedentary time and physical activity

Sedentary time and physical activity were objectively assessed with triaxial accelerometry (ActiGraph GT3X+, Florida, US). Detailed information is provided in a previous published article[24].

Blood collection

In standardized fasting conditions (8–9 a.m.) at our research center, venous blood samples of all women (in a rested state) were extracted from the antecubital vein and collected in serum vacutainers. Immediately after the delivery, maternal (from the antecubital vein) and arterial and venous (from the umbilical cord) blood samples were also extracted and stored in serum tubes. Then, the samples were centrifuged to separate serum from formed elements. Subsequently, serum was frozen at −80°C to avoid breaking the cold chain before the analysis in the laboratory. More detailed information is shown in **Appendix C**.

Inflammatory markers

Maternal, and umbilical arterial and venous serum cytokines (fractalkine, IL-1β, IL-6, IL-8, IL-10, IFN-γ, and TNF-α) were measured using Luminex xMAP technology (detailed in **Appendix C**).

Statistical analysis

As initially designed,[21] the statistical analysis was conducted on per-protocol basis. Only women who attended ≥75% of the exercise sessions, and completed both baseline and follow-up assessments, were included in the per-protocol analyses.

Descriptive statistics for continuous and categorical variables were performed to show the sociodemographic and clinical characteristics (**Table 1**), along with the cytokines concentrations of pregnant women (**Table 2**). To detect potential differences on these outcomes between the groups, the following statistical tests were performed: independent sample Student's t-test (normal distribution, homoscedasticity), Welch´s test (normal distribution, heteroscedasticity) and Mann-Whitney U test (non-normal distribution) for continuous variables, and the Chi-square test for categorical variables.

Considering the asymmetry of some cord serum cytokines, and the violation of some assumptions related to the generalization of the results, data preparation was employed for those models. Particularly, optimum Box-Cox transformations and a subtle variation of winsorizing were used to reduce the impact of potential source of bias. Additional

information, along with an "Outlier detection-management" section, is provided in **Appendix D**.

Subsequently, linear regression analyses were used to analyze the differences on cytokines concentrations between the control and exercise group at the different time points. In the multiple time point analyses (**Table 3**), the changes in maternal serum cytokines concentrations (from baseline to 34th gestational week and delivery) were included in the regression analyses as dependent variables, and the group (control=0, exercise=1) as independent variable. In the single time point analyses (**Table 4**), the arterial and venous cord serum cytokines concentrations were included in the regressions as dependent variables, and the group as independent variable.

After considering relevant confounders suggested by previous literature, mostly those confounders which were statistically-significantly related to the outcomes, and did influence the relation between the independent and dependent variable (i.e. meaningful change in the coefficient B of the independent variable when added), were included in the models. In the multiple point analyses for 34th week, the model 1 was adjusted for baseline values of the particular cytokine and adherence to the MDS; and the model 2 was additionally adjusted for the relative percentage of daily total PA (total PA/accelerometer wearing time). In multiple point analyses for delivery, the model 1 was adjusted for baseline values of the particular cytokine; and the model 2 was additionally adjusted for parity status and weeks of gestation at delivery. In the single point time analyses, the model 1 was adjusted for adherence to the MDS; and the model 2 was additionally adjusted for parity status and gestational age at delivery. Since important variables (maternal age, BMI, tobacco and daily total PA, among others) according to the literature showed a weak or no relationship with the outcomes, and we wanted to enhance the parsimony of the main models, these variables were only tested as additional confounders in secondary sensitive analyses.

All the assumptions related to the generalization of the results were met in the different analyses. Multiple imputation was performed for those cases with missing data in specific outcomes. Subsequently, the aforementioned statistical analyses were conducted on intention-to-treat basis to evaluate more realistically the effectiveness of this concurrent exercise-training program when applied to the clinical practice (**Table S2**

and **Table S3**), according to the CONSORT guidelines. The statistical analyses were conducted using SPSS 22.0 (IBM, NY, USA). The statistical significance was set at p≤0.05.

RESULTS

From all the initially interested participants (n=384) between November-2015 and November-2017, the final study sample included in the per-protocol analyses (>75% of attendance) was 58 Caucasian southern Spanish pregnant women (age 33.5±4.7 years old, BMI 23.6±4.1 kg/m^2). These women were divided into control (n=37) and exercise (n=21) groups. The follow-up for the last wave of participants was completed in April-2018. Further information about the allocation and analysis process, along with reasons for losses/exclusions, is provided in **Figure 1** and **Appendix E**.

The sociodemographic and clinical characteristics of pregnant women are shown in **Table 1**. Baselines differences were found for the time spend in light and total PA between the control and exercise group (p=0.01 and p=0.03, respectively). The mean exercise training adherence was approximately 84%. The unadjusted differences in cytokines concentrations at the three time points are shown in **Table 2**. In maternal serum, differences were found in TNF–α (35th week) and IL-10 (delivery) concentrations (p=0.03 and p=005, respectively). Regarding the cord serum, the control group showed lower arterial serum IL-1β levels, and greater arterial serum IL-6 and venous TNF-α concentrations than the exercise group (p-values between 0.002-0.05).

The effects of the exercise intervention on maternal serum cytokines are shown in the **Table 3.** In the regression analyses for changes from baseline to 35th week (model 1), the exercise group decreased 1.03pg/mL (-1.89 to -0.18, p=0.02) TNF-α concentrations compared to the control group. When analyzing changes from baseline to delivery (model 1), the exercise group was associated with a lower increase in IL-1β (-2.38pg/mL, -4.53 to -0.22, p =0.03), and a greater increase in IL-10 (9.40pg/mL, 0.15 to 18.64, p=0.05) compared to the control group. When additionally adjusting these analyses (model 2), the aforementioned differences for maternal TNF-α, IL-1β and IL-10 became non-significant, but showed evidence of statistical significance.

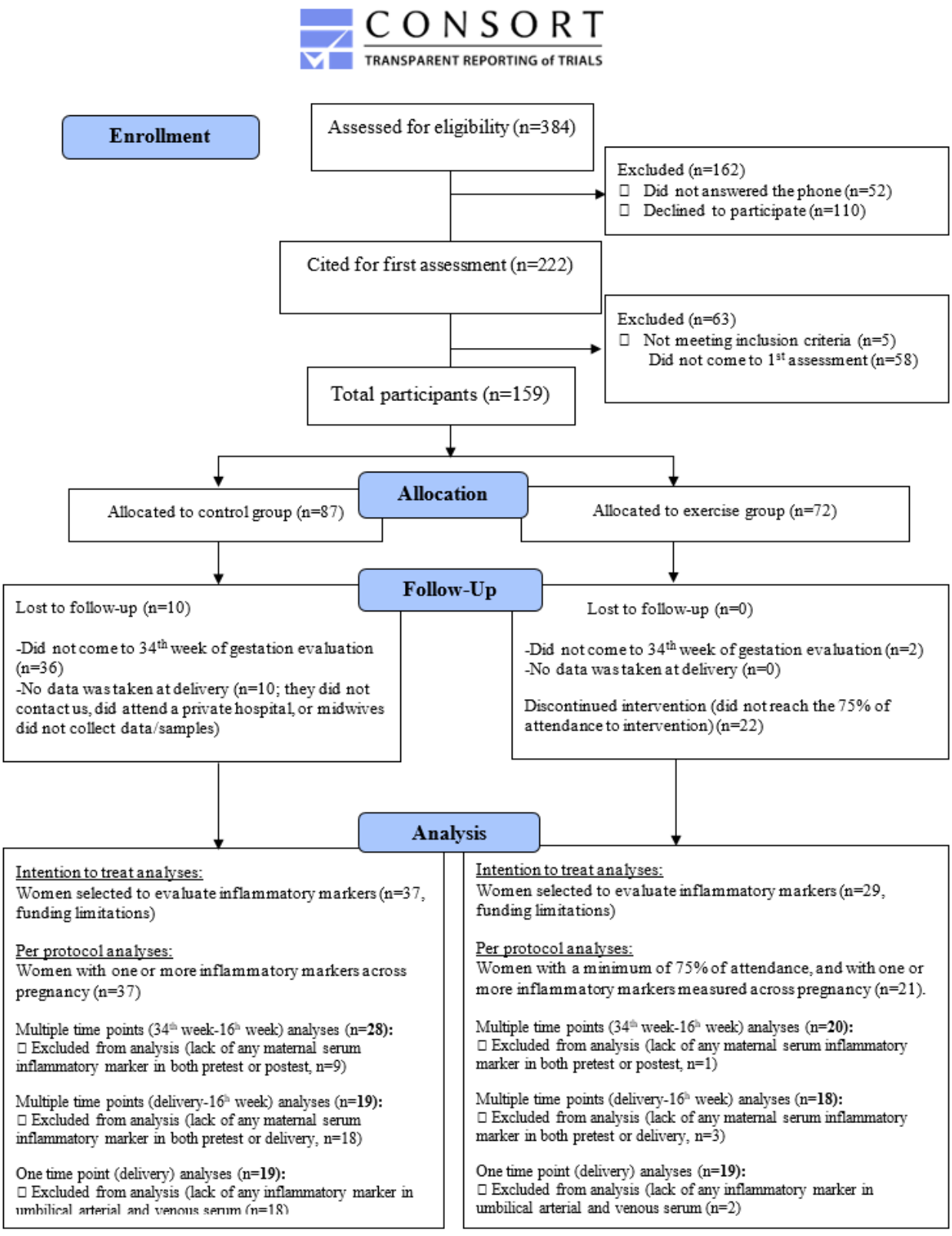

Figure 1. Flowchart of pregnant women through each stage of the study.

Table 1. Sociodemographic and clinical characteristics of pregnant women (n=48).

	Total (n=48)		Control (n=28)		Intervention (n=20)		p-value
Age (years)	33.5	(4.7)	33.5	(4.7)	33.5	(4.8)	0.97
Gestational age in the 1st assessment (weeks), 16th week	16.8	(1.4)	16.9	(1.1)	16.6	(1.7)	0.68
Gestational age in the 2nd assessment (weeks), 34th week	33.0	(1.7)	32.1	(1.7)	31.6	(1.7)	0.28
Gestational age at delivery (weeks)	39.4	(1.4)	39.1	(1.6)	39.8	(1)	0.16
Percentage of attendance					83.9	(8.2)	
Cohabitation, n (%)							
Living alone	0	(0)	0	(0)	0	(0)	
Living with partner	48	(100)	28	(100)	20	(100)	
Educational level, n (%)							
Non-university degree	20	(41.7)	11	(39.3)	9	(45)	0.92
University degree	28	(58.3)	17	(60.7)	11	(55)	
Professional status, n (%)							
Work full/part time	31	(64.6)	21	(75)	10	(50)	0.14
Unemployed/Retired/Housekeeper	17	(35.4)	7	(25)	10	(50)	
Parity status, n (%)							
Primarious	28	(58.3)	14	(50)	14	(70)	0.28
Multiparious	20	(41.7)	14	(50)	6	(30)	
Number of abortions	0.5	(0.8)	0.5	(0.8)	0.5	(0.8)	0.64
Type of deliver[a], n (%)							
Spontaneous	27	(57.4)	16	(59.3)	11	(55)	
Vacuum extraction	9	(19.1)	3	(11.1)	6	(30)	0.20
Caesarean section	11	(23.4)	8	(29.6)	3	(15)	
Offspring sex[b], n (%)							
Male	24	(52.2)	13	(50)	11	(55)	0.97
Female	22	(47.8)	13	(50)	9	(45)	
Body mass index pre-pregnancy[e] (kg/m²)	23.2	(3.8)	22.8	(3.5)	24.0	(4.4)	0.32
Body mass index (kg/m²), 16th week	23.6	(4.1)	23.3	(3.5)	24.0	(4.9)	0.98
Gestational weight gain from pre-pregnancy to 16th week[e] (kg)	1.1	(2.8)	1.1	(3.2)	0.9	(2)	0.81
Gestational weight gain from 16th to 34th week[c] (kg)	9.5	(3.2)	10.1	(2.8)	8.7	(3.5)	0.234
Cardiovascular function[b], 16th week							
Systolic blood pressure (mmHg)	105.2	(8.8)	106.1	(9.1)	103.8	(8.4)	0.38
Diastolic blood pressure (mmHg)	61.9	(7.5)	61.5	(7.9)	62.4	(7)	0.70
Resting heart rate (bpm)	81.7	(10.8)	81.9	(10.7)	81.5	(11.3)	0.57
Smoking during pregnancy (cigarettes per day), 16th week	0.4	(1.6)	0.5	(2)	0.2	(0.9)	0.50
Adherence to the Mediterranean Diet Score (0-55), 16th week	29.1	(3.8)	29.3	(3.9)	28.8	(3.9)	0.73
Sedentary lifestyle and physical activity[d], 16th week							
Sedentary time (min/day)	503.9	(98.5)	486.0	(116.5)	526.4	(65.7)	0.19
Light PA (min/day)	392.5	(89.9)	420.8	(99.2)	356.7	(61.9)	**0.01**
Moderate-vigorous PA (min/day)	37.8	(23)	37.2	(26.1)	38.6	(19.0)	0.48
Bouted moderate-vigorous PA (min/week)	99.4	(120.1)	106.1	(141.6)	90.8	(89.1)	0.95
Total PA (min/day)	430.3	(93.0)	458.0	(99.1)	395.3	(73.0)	**0.03**
Average accelerometer wear time (min/day)	934.2	(53.5)	944.0	(61.5)	921.7	(39.3)	0.18
Relative percentage of daily sedentary time (%)	53.9	(9.7)	51.3	(10.7)	57.2	(7.4)	**0.05**
Relative percentage of total daily PA (%)	46.1	(9.7)	48.7	(10.7)	42.8	(7.4)	**0.05**

PA, physical activity. Continuous variables are presented as mean (standard deviation) and categorical variables as number (percentage). Superscripts in outcomes indicate lower sample size when considering all participants: [a] n=47, [b] n=46, [c] n=45, [d] n=43, [e] n=41. P values were calculated using independent sample Student's t-test (normal distribution, homoscedasticity), Welch´s test (normal distribution, heteroscedasticity) and Mann-Whitney U test (non-normal distribution) for continuous variables, and the Chi-square test for categorical variables.

Table 2. Cytokines concentrations at three time points (n=48).

	17th week of gestation (n=48)					35th week of gestation (n=48)				
Inflammatory markers	**Control**		**Intervention**		p-value	**Control**		**Intervention**		p-value
Maternal serum	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Fractalkine (pg/ml)	376.1	149.0	371.4	152.7	0.85	375.8	107.3	391.4	107.2	0.74
Interleukin 1 beta (pg/ml)	6.8	2.6	6.1	3.2	0.17	7.5	3.0	6.3	3.6	0.20
Interleukin 6 (pg/ml)	5.6	2.8	5.9	3.0	0.70	6.3	2.5	5.2	2.6	0.15
Interleukin 8 (pg/ml)	21.5	10.7	18.6	7.6	0.50	19.8	8.5	21.6	10.7	0.62
Interleukin 10 (pg/ml)	24.0	9.6	22.3	12.2	0.59	24.5	8.8	29.1	9.8	0.10
Interferon gamma (pg/ml)	23.5	11.0	22.6	12.8	0.57	22.9	11.5	18.4	9.3	0.15
Tumor necrosis factor alpha (pg/ml)	5.6	1.7	5.2	2.7	0.11	7.1	1.7	6.1	1.4	**0.03**
Umbilical arterial serum [a]										
Fractalkine (pg/ml)										
Interleukin 1 beta (pg/ml)										
Interleukin 6 (pg/ml)										
Interleukin 8 (pg/ml)										
Interleukin 10 (pg/ml)										
Interferon gamma (pg/ml)										
Tumor necrosis factor alpha (pg/ml)										
Umbilical venous serum										
Fractalkine (pg/ml)										
Interleukin 1 beta (pg/ml)										
Interleukin 6 (pg/ml)										
Interleukin 8 (pg/ml)										
Interleukin 10 (pg/ml)										
Interferon gamma (pg/ml)										
Tumor necrosis factor alpha (pg/ml)										

SD, standard deviation. [a] indicate lower sample size of the control group (n=15) in all the umbilical arterial serum infla
calculated using independent sample Student's t-test (normal distribution, homoscedasticity), Welch´s test (normal di
Mann-Whitney U test (non-normal distribution) for continuous variables.

Table 3. Per-protocol analyses showing the effect of the concurrent exercise-training program on maternal serum inflammatory r

	Changes in control group		Changes in exercise group		Model unadjusted				Model 1		
	Mean	SD	Mean	SD	B	SE	β	p	B	SE	β
35th week-17th week (maternal serum, n=48)	(n=28)		(n=20)								
Fractalkine	-0.35	101.10	19.98	91.67	20.33	28.49	0.11	0.48	17.92	20.72	0.09
Interleukin 1 beta	0.67	3.13	0.17	2.12	-0.50	0.81	-0.09	0.54	-0.79	0.76	-0.14
Interleukin 6	0.74	3.27	-0.67	3.11	-1.41	0.94	-0.22	0.14	-1.19	0.73	-0.18
Interleukin 8	-1.68	9.48	3.05	7.40	4.73	2.54	0.26	**0.07**	3.38	2.23	0.19
Interleukin 10	0.55	13.74	6.80	8.88	6.25	3.50	0.25	**0.08**	4.66	2.47	0.19
Interferon gamma	-0.55	9.97	-4.21	9.65	-3.65	2.88	-0.18	0.21	-4.11	2.50	-0.21
Tumor necrosis factor alpha	1.51	2.29	0.86	2.52	-0.66	0.70	-0.14	0.35	-1.03	0.43	-0.22
Delivery-17th week (maternal serum, n=37)	(n=19)		(n=18)								
Fractalkine	-3.22	69.74	4.36	101.34	7.57	28.47	0.05	0.79	24.09	20.9	0.14
Interleukin 1 beta	3.24	2.86	0.86	3.78	-2.38	2.10	-0.34	**0.04**	-2.38	1.06	-0.34
Interleukin 6	26.91	9.86	27.06	18.93	0.15	4.92	0.01	0.98	0.23	4.97	0.01
Interleukin 8	14.53	14.57	19.31	16.10	4.78	5.04	0.16	0.35	3.33	3.91	0.11
Interleukin 10	18.65	13.98	27.30	16.93	8.65	0.10	0.28	**0.10**	9.40	4.55	0.30
Interferon gamma	-2.50	9.08	-8.26	12.21	-5.76	3.53	-0.27	0.11	-3.97	2.03	-0.18
Tumor necrosis factor alpha	4.66	2.72	3.62	2.97	-1.04	0.94	-0.19	0.27	-1.03	085	-0.18

SD, standard deviation; B, unstandardized regression coefficient; p, p-value; SE, standard error; β, standardized regression coeffici
including only women who attended ≥75% of the exercise sessions. Linear regression analyses (enter method) were used to examin
between the control and exercise group. The within-group post-pre intervention changes (from the exercise training grou
concentrations were included in the linear regression analyses as dependent variables, and the group (control=0 and exercise=1)
the "35th week-17th week" multiple point analyses, the model 1 was adjusted for baseline values of the particular cytokine and a
and the model 2 was additionally adjusted for the relative percentage of daily total physical activity (total physical activity/accel
the "delivery-17th week" multiple point analyses, the model 1 was adjusted for baseline values of the particular cytokine; and the
status and gestational age at birth. * The adjusted R^2 values shown are derived from the unadjusted model. All the assumptions
have been reasonably met, and non-transformations or data preparation of the outcomes were needed.

The effects of the exercise intervention on arterial and venous cord serum cytokines are shown in the **Table 4**. In the model 1, the exercise group was associated with higher arterial cord serum IL-1β (0.69pg/mL, 0.30 to 0.08, p=0.03), and lower arterial cord serum IL-6 (-0.79pg/mL, -1.48 to -0.11, p-value=0.02) compared to the control group. Regarding the venous cord cytokines (model 1), the exercise group was associated with lower TNF-α (-5.53pg/mL, -8.47 to -2.60, p=0.001) concentrations as compared with the control group. In the model 2, the results remained similar.

When additionally adjusting the single/multiple point analyses for BMI, maternal age, tobacco and daily total PA, the results did not change.

Because of the substantial percentage of missing data (average=42.7%), multiple imputation was not possible for some outcomes. Intention-to-treat analyses have been added to Supplementary material (**Table S2** and **Table S3)** to be as transparent as possible. Considering that some authors do not recommend to perform imputation when more than 20% of cases are missing,[25] we have not considered this data for the discussion.

The moderate-to-vigorous exercise intervention was shown to be safe. Non-adverse, potentially harmful, or unintended effects were observed in none group.

Study IV

Table 4. Per-protocol analyses showing the effect of the concurrent exercise-training program on arterial and venous cord serum

	Model unadjusted				Model 1				
	B	**SE**	**β**	**p-value**	**B**	**SE**	**β**	**p-value**	**B**
Umbilical arterial serum (delivery)[a]									
Fractalkine*	0.63	0.32	0.33	**0.06**	0.53	0.32	0.28	0.11	0.52
Interleukin 1 beta*	0.66	0.29	0.38	**0.03**	0.69	0.30	0.39	**0.03**	0.72
Interleukin 6*	-0.83	0.32	-0.42	**0.02**	-0.79	0.33	-0.40	**0.02**	-0.80
Interleukin 8	4.83	9.56	0.09	0.61	6.67	9.85	0.12	0.50	7.25
Interleukin 10	2.32	1.20	0.32	**0.06**	2.14	1.24	0.30	**0.10**	2.14
Interferon gamma	-0.65	0.44	-0.25	0.15	-0.70	0.46	-0.27	0.14	-0.68
Tumor necrosis factor alpha	-1.55	1.10	-0.24	0.17	-1.63	1.14	-0.25	0.17	-1.63
Umbilical venous serum (delivery)									
Fractalkine	34.58	37.38	0.15	0.36	27.29	36.93	0.12	0.47	23.68
Interleukin 1 beta*	0.21	0.32	0.11	0.53	0.18	0.33	0.10	0.58	0.17
Interleukin 6	-0.77	1.67	-0.08	0.65	-0.90	1.70	-0.09	0.60	-1.03
Interleukin 8*	0.20	0.32	0.11	0.53	0.22	0.33	0.11	0.50	0.16
Interleukin 10	0.37	1.28	0.05	0.78	0.31	1.31	0.04	0.82	0.23
Interferon gamma	0.34	0.41	0.14	0.41	0.24	0.39	0.10	0.54	0.29
Tumor necrosis factor alpha	-5.07	1.54	-0.48	**0.002**	-5.53	1.45	-0.53	**0.001**	-5.21

B, unstandardized regression coefficient; SD, standard deviation; SE, standard error; β, standardized regression coefficient. Per-p
only women who attended ≥75% of the exercise sessions. Linear regression analyses (enter method) were used to examine the diff
the control and exercise group. The umbilical arterial serum cytokines concentrations were included in the linear regression analy
(control=0 and exercise=1) as independent variable. The model 1 was adjusted for adherence to the Mediterranean Diet score; an
parity status and gestational age at birth. * Optimum Box-Cox transformations and a subtle variation of winsorizing (convert bac
with a score equivalent to ±2.58 SDs from the mean) were performed on inflammatory markers. [a] indicate lower sample size of the
serum inflammatory markers. [b] the adjusted R2 values shown are derived from the unadjusted model (i.e. it assesses the indivi
without confounders). All the assumptions related to the generalization of the results have been reasonably met. After dealing w
remained similar (but with better and more symmetrical distribution of data) to the analyses without data preparation, exceptin
statically significant.

DISCUSSION

Under the framework of the GESTAFIT project, the present study shows, for the first time, the effects of a novel, well-designed and supervised individually-tailored concurrent exercise program18 (based on the latest guidelines in pregnancy[13]) on maternal, and arterial and venous cord serum cytokines. The main findings suggest that a concurrent exercise-training program might reduce arterial cord IL-6 and venous cord TNF-α concentrations. Unexpectedly, pregnant women from the exercise group showed higher concentrations of arterial cord IL-1β.

Until now, only two previous studies have presented similar results to those shown in the current study. Clapp, et al.[26] conducted a weight-bearing exercise intervention from pregravid, but only focused on maternal serum TNF-α and leptin concentrations. Otherwise, Aparicio et al.[21] also showed similar results to those described above in breast milk.

Interlukin-6 is a pleiotropic well-known pro and anti-inflammatory cytokine[15,27] with relevant influence on the immunometabolic homeostatic responses during pregnancy.[1,2] Our results indicated that arterial cord serum IL-6 concentrations were reduced in the exercise compared to the control group, with a similar non-significant trend in maternal (at 34th week and delivery) and venous cord serum IL-6. Moran, et al.[28] showed that a dietary-PA counselling was not associated with either maternal or cord blood IL-6 concentrations. When comparing with the general population, some studies have suggested that exercise might reduce IL-6 expression in the skeletal muscle and plasma levels.[14,15,29] However, these results are inconclusive according to a recent systematic review.[17] Interestingly, we observed greater concentrations of arterial than venous cord serum IL-6 (**Table S4**), which might suggest that IL-6 synthesis during parturition is mainly induced by the fetus.[8,12] Hence, given that we found differences in arterial cord IL-6 (but not in maternal or cord venous IL-6) concentrations between the exercise and control group, we hypothesize that exercise might modulate fetal synthesis of IL-6 and placental clearance during parturition.[8,9,12] However, in spite of the fact that induced pro-inflammatory responses are necessary for the normal physiological course of pregnancy and birth,[1-3,8] exacerbated IL-6 concentrations have been related to pregnancy-related inflammatory complications.[1,3] Finally yet importantly, it has been suggested that exercise-induced IL-6 might facilitate an optimal fetal growth and

neonatal body composition,[9] via modulating the expression and activity of placental nutrient transporters. In light of the above, strategies targeting IL-6 regulation during pregnancy are of clinical relevance.[1,3,27] Within this context, this concurrent exercise intervention might facilitate IL-6 regulation, favoring an optimal pregnancy and fetal development, and the prevention of potential immunometabolic dysregulations.

Regarding TNF-α, Clapp, et al.[26] have previously suggested that weight-bearing exercise attenuates the increase in TNF-α concentrations across pregnancy. Remarkably, our results also showed that maternal (at 34th week) and venous cord TNF-α concentrations were much lower in the exercise compared to the control group. These findings are specially relevant if we consider that TNF-α is a major driver for metabolic disruptions (e.g. gestational diabetes mellitus)[7,15] and complicated diseases during pregnancy.[1,3,6] It is noteworthy that depending on the concentrations, receptor distribution and duration of its stimulation, this pro-inflammatory cytokine has an imperative-bimodal physiological-pathological role mediating beneficial/adverse effects on female reproduction and pregnancy.[6] Hence, its seems that exercise could be a promising target to modulate TNF-α concentrations at the maternal-fetal interface during pregnancy, which might help to prevent immunometabolic dysregulations and reproductive diseases. However, when interpreting this data, we should consider that most studies addressing the maternal-fetal/fetal-maternal transfer of TNF-α have been performed in deliveries without labors[10,12] (unlike our study), and TNF-α was under detection limit in the immunoassays of vaginal labors.[8] Moreover, we found comparable concentrations of cord arterial and venous TNF-α in our participants (**Table S4**). Therefore, it is not possible/unsuitable to conclude any exercise-induced underlying mechanism related to maternal, placental or fetal TNF-α.

Interleukin-1β is a pro-inflammatory cytokine highly involved in the pathogenesis of immunometabolic abnormalities, with a recently discovered role as physiological-metabolic mediator.[30] During implantation and parturition, adequate induced-IL-1β responses are imperative in the maternal-fetal communication to promote healthier pregnancies.[1,3] Surprisingly, our results showed that pregnant women from the exercise group presented higher concentrations of arterial cord IL-1β, with a similar but non-significant trend in venous cord IL-1β. By contrast, maternal IL-1β serum levels (at delivery) were slightly reduced in the exercise compared to the control group (evidence

of statistical significance). Unfortunately, we could not compare these results with previous studies in pregnant women. Notwithstanding, one similar study by Moran, et al.[28] observed that dietary-PA counselling did not affect maternal or cord blood IL-1β. In the general population, evidence regarding the influence of exercise interventions on IL-1β is also scarce and inconclusive.[17] To explain the raise observed, we hypothesized that higher arterial cord IL-1β in the exercise group could be related to greater exercise-induced placental volume and vascularization,[9] which in turn might lead to a higher proportional release of IL-1β into maternal-fetal circulation. However, we dismissed this hypothesis since: i) we did not observed significant changes in either maternal or venous cord serum IL-1β, which should be logical assuming an IL-1β-interplay between the placenta and fetus; ii) IL-1β was not detectable in previous uncomplicated in-term pregnancies in the absence of labor (suggesting the absence of any inflammatory fetal-placental response),[8] or was not able to cross the placenta (suggesting that the inflammatory response in fetal blood and amniotic fluid might be of fetal origin);[12] and iii) it is likely that unnoticed factors[11] (even if we have considered the most relevant confounders such as duration of delivery, type of delivery, etc.) related to parturition, which is an acute phase with huge influence on immune system,[3] might partially explain these differences.

Therefore, when interpreting these results, we should consider that labor might play a role in the acute elevation of some cytokines at term, and is not a simple process itself. Different mechanisms (fetal membrane cell senescence, circadian endocrine clocks, inflammatory, and mechanical factors, etc.), are coordinated in a sequential and progressive manner, to initiate and provoke the parturition[31]. However, to date, our understanding of the pathways of parturition is limited, and many of the labor phenotypes observed at term have not been fully characterized biologically.

To facilitate an easier interpretation of the findings, those associations which showed evidence of statistical significance have been discussed separately in the **Appendix F**. Therefore, getting conclusions from the comparison with previous studies in pregnant women is difficult since the scarce existing interventions are based on PA-dietary counselling (different kind of metabolic stimuli-responses), and/or have not measured the cytokines included in this study. Moreover, the interpretation of the results is even more complicated given the discrepancies aggravated by: i) assessments

are usually performed in different gestational weeks; ii) variable immunometabolic and weight statuses; iii) different methodologies and tissues when analyzing cytokines; iv) different single/multiple time point statistical analyses, and small statistical power; v) distinct type of deliveries, etc.

LIMITATIONS

Some limitations need to be highlighted. The group randomization was broken. However, the presence/absence of a randomized design itself is unlikely to be as determinant as the methodological quality of the study.[32] The results should be interpreted cautiously given the small sample size, and considering that no correction for multiple comparisons were made (as usual in exploratory/secondary outcomes analyses). The lack of statistically significant differences might be related to reduced statistical power. Only interested women participated in the study. Some strengths also deserve to be mentioned: i) this exercise program is a novel individually-tailored intervention designed by an expert multidisciplinary team, based on the latest Guidelines in pregnancy;[13] ii) the exercise program was strictly supervised during the whole study, and the attendance, intensity and other related parameters, were monitored periodically; iii) this is the first time that the effect of exercise has been analyzed in all these cytokines (excepting TNF-α); iv) the cytokines were measured at multiple time points (including delivery), and in both the artery and vein cord serum; v) and we have not only adjusted the analyses for baseline values, but also for powerful confounders such as objectively measured PA (with such a tight criterion, 7 days of ≥10hours/day) and the MDS (among others).

CONCLUSIONS

This concurrent exercise program might be a complementary-alternative tool to modulate inflammatory responses of pregnant women and their newborns. The development of similar exercise programs might avoid potential immunometabolic impairments, and prevent pregnancy complications. However, further research focused on the origin and clearance of these cytokines, their role in the maternal-placental-fetus crosstalk, and the influence of exercise interventions on them (along with the underlying mechanisms), is warranted before reaching any certain conclusion.

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SUPPLEMENTARY MATERIAL

Appendix A. *Exercise sessions.*

Each exercise session included a 10-min warm-up period with walks, mobility and activation exercises. The main part of the first and last weekly sessions consisted of 40 minutes of exercises organized in two resistance circuits of 15 exercises (40" work/20" rest), alternating with cardiovascular blocks (concurrent training). The second session of the week was focused on aerobic training through dancing, proprioceptive and coordinative circuits, and interval walks. The sessions finished with a 10-min cool-down period of stretching, breathing, relaxation and myofascial relief.[1]

Appendix B. *Talks provided to pregnant women.*

During the duration of the intervention, the research team gave 7 lectures to pregnant women from both groups (exercise and control group) about: 1) the benefits of physical exercise for a better pregnancy, prevention and treatment of cardiovascular diseases and excessive weight gain; 2) ergonomic advises, exercises to perform at home and strategies to increase their daily physical activity levels; 3) the benefits of the Mediterranean Diet and nutritional education during pregnancy; 4) how to avoid toxics and chemicals during the pregnancy and breastfeeding; 5) pregnancy, postpartum and sex; 6) physical and mental preparation for the labor, what to expect; 7) nutritional education towards breastfeeding. We also used these conferences to maintain control group fidelity until the end of the program.

Appendix C. *Detailed information of blood samples analyses.*

In standardized fasting conditions (8-9 a.m.) at our research center (at the 17th and 35th gestational week), venous blood samples (5 mL) of all pregnant women were extracted from the antecubital vein and collected in serum vacutainers Immediately after the delivery, maternal (from the antecubital vein), and arterial and venous (from the umbilical cord) blood samples were also extracted and stored in serum tubes. For the umbilical cord blood sampling, a trained midwife performed a double clamping of the umbilical cord in the first three minutes of the newborn's life, with a minimum distance between both clamps of 10 centimeters. A 1 mL syringe was used for the blood

extraction. Then, the samples were centrifuged at 1750 rpm for 10 minutes at 4°C in a refrigerated centrifuge (GS-6R Beckman, Fullerton, CA, USA) to separate serum from formed elements. Subsequently, serum was aliquoted and frozen at -80º C to avoid breaking the cold chain before the analysis in the laboratory.

We employed Luminex xMAP technology based on MILLIPLEX MAP kits to assess the cytokine profile from the collected serum in pregnant women. Luminex xMAP technology (Millipore, Darmstadt, Germany) is a mix of three existing and proved technologies: use of microspheres, flow cytometry, and laser technology, mixing digital signal processing and traditional chemistry immunoassay. Because of robust multiplexing, xMAP technology potentially delivers more data in less time than other bioassay products, with comparable results with enzyme linked immunosorbent assay and microarray. The technology offers several other distinct advantages over traditional methods such as speed and high throughput, versatility, flexibility, accuracy, and reproducibility. Particularly, for maternal pro-inflammatory and anti-inflammatory (fractalkine, interleukin-1β, interleukin-6, interleukin-8, interleukin-10, interferon-γ and tumor necrosis factor-α) determination, we used Human Sepsis Magnetic Bead Panel 3 Multiplex Assay (cat. No. HTH17MAG-14K). We prepared samples, reagents, and standards by following the manufacturer's instructions. Equipment settings: 50 events per bead, gate settings: 8,000-15,000, time out 60 seconds. Plate was read on LABScan 100 analyzer (Luminex Corporation, Texas, USA) with xPONENT software for data acquisition. The average values for each set of duplicate samples or standards were within 15% of the mean. We determined cytokine concentrations by comparing the mean of duplicate samples with the standard curve for each assay.

Appendix D. *Outlier detection and management.*

Nowadays, the presence of outliers is one of the most enduring and pervasive methodological changes in biomedical science research.[2-4] Worryingly, there is a lack of consensus about how addressing outliers (i.e. how defining, identifying and handling them). Since the decisions that researches make about this issue have important implications, we have included this section to promote transparency and the critical interpretation of the results, as previously recommended by several authors.[2-4] Although no specific guidelines exist about how addressing outliers, several studies[2-9]

(especially that one from Aguinis, et al.[4]) have previously provided smart advices and recommendations to address them in the best possible way. Accordingly, the different steps to address outliers in the present study have been performed proceeding with the following recommendations. We have identified and handled outliers according to the basis for regressions, which are the main analyses involved in this study.

Error outliers

During the assessments at the different time points, questionnaires and tests (where errors related to data recording, coding, manipulation, etc. were likely and easily observed) were checked to identify clear error outliers, and correct them immediately by asking women, repeating the corresponding test, etc. When lacking, misleading or inaccurate data, was identified posteriori (up to 2 weeks after the assessments), women were contacted to ensure the accuracy of these data points, or to correct these potential outliers (whenever appropriate for data) in the respective database. Singles construct techniques (box plots, descriptive statistics, percentage analyses, etc.) were performed to initially identify error outliers.

Subsequently, we also employed multiple construct techniques to identify error outliers. Particularly, we identified outlyingness based on predictor (leverage values, Cook´s distance and standardized differences in beta) and residual scores (standardized residuals). When it was not possible/appropriate to correct these data points, and we were sure that their inaccuracy was related to human errors, device malfunction, miscalculations or similar circumstances (i.e. we had determined the cause of the identified outlying observation), these error outliers were removed from the respective database. Since these potential error outliers could have been caused by inherent variability in the data (in this case they would represent a legitimate part of the population), we were very prudent when identifying and handling them. We paid special attention to the reasoning behind the classification of data points as error outliers.

Interesting outliers

After the application of this first filter to the database, there were several remaining interesting outliers, which required additional analyses in depth. Thereby, we aimed at analyzing these interesting outliers with quantitative approaches (e.g., we tried to analyze differences in how predictors were able to predict high and low outlier scores). However, the number of outliers was minimum, and only appreciable in few outcomes,

which prevented us from performing these analyses properly. As consequence, we did not finally perform these analyses.

Influential outliers

Since it is not legitimate to simply drop the remaining potential outliers from the analyses (they tend to increase error variance, reduce the power of statistical test, etc.), nor plainly deleting them without any basis (they could be part of the inherent variability of the distribution of data), we analyzed more in depth the influence of these outliers in the model. Aimed at checking their influence, we analyzed how the deletion of specific outliers could affect the change of the model fit (e.g., changes in R^2; model fit outliers), and we paid attention to the Cook´s Distance and standardized DFBETAs to identify prediction outliers. If these remaining unusual cases were not finally identified as influential outliers, or they were identified but influenced the model slightly, these potential outliers were not handled (as observed in the **Table 3**). In this case, these unusual data points were dropped in the analyses since they did not affect either the results or assumptions of the tests, and they could be caused by inherent variability in the data. By contrast, if these remaining unusual cases were confirmed as influential outliers which affected the model fit and parameter estimates (as appreciable in the **Table 4**), those influential outliers we handled.

Considering the asymmetry of some cord serum cytokines and the violation of some assumptions related to the generalization of the results (which were partially caused by these influential outliers), data preparation was employed for those models. First, optimum Box-Cox transformations were employed to correct distributional problems, non-linearity, outliers, non-normality, etc. Subsequently, in those few outcomes where outliers were/remained influential, a subtle variation of winsorizing (convert back from a z-score: replacing extreme scores (z>2.58/3.29) with a score equivalent to ±2.58/3.29 standard deviations from the mean) was employed to handle these outliers. After dealing with the problematic outcomes, the results remained similar (but with better and more symmetrical distribution of data) to the analyses without data preparation (i.e. without applying Box-Cox transformations or handling of outliers), excepting for the interleukin 1 beta, which became statically significant.

Appendix E. *Reasons for losses and exclusions during the enrollment and follow-up.*

From the 159 women that participated in the study and were allocated to the control (n=87) or intervention (n=72) group, 10 controls dropped out of the study because of: moving to another city (n=1), unwillingness to continue (n=7) or unknown reasons (n=2). In the control group, 36 women did not come to the evaluation (34th week) because of personal reasons. Data loss (n=10) at delivery was related to women who did not contact us, attended private hospitals, or midwives who did not collect data/samples. Because of funding limitations, the inflammatory markers could only be analyzed in a subsample (n=66). Hence, 37 and 29 women from the control and exercise group (respectively), were included the intention-to-treat analyses (**Table S2**). From the 29 women in the exercise group, only 21 attended 75% of the sessions.

Appendix F. *Effect of the concurrent exercise-training program on maternal, and arterial and venous cord serum cytokines (non-statistically significant associations, but evidence of statistical significance).*

To facilitate an easier interpretation of the results, and provide a more complete and transparent description of the findings, we have decided to discuss this section separately. However, considering the magnitude of the effects (along with the confidence interval/standard error), caution must be paid in order not to over-interpret these results, and to avoid misleading conclusions.

Overall, a non-significant trend characterized by higher arterial IL-10 concentrations in pregnant women from the intervention group was noticed. When focusing on the changes in maternal serum cytokines from baseline to 34th week and delivery, exercise showed a non-significant but clinically meaningful[10] association with greater IL-8 and IL-10, and lower IFN-γ and TNF-α levels in maternal serum at 34th week; and with lower maternal IL-1β and higher maternal IL-10 at delivery.

Fractalkine (CX3CL1) is a chemokine with an important role in the fetal-placental vasculature development[11] and microglial cell migration-activation.[12] When considering its role as a myokine,[13] previous studies have suggested that physical exercise is not related neither to fractalkine mRNA nor to protein concentrations in the general population, which is in agreement with our results.[13-15] However, these findings cannot

be directly compared as there are no previous similar studies on fractalkine in pregnant women.

Besides its pro-inflammatory role,[16,17] muscle-derived IL-8 (CXCL8) can also act as an angiogenic factor during early and late gestation.[18] The slightly increased (non-significant statistical trend) maternal serum IL-8 concentrations observed in the exercise group (at the 34th week) might be suggestive of muscle IL-8-induced angiogenesis and placenta vascularization.[18,19] Moran, et al.[20] reported that their dietary-physical activity counselling intervention was not associated with either maternal (at 28th and 36th weeks) nor cord blood IL-8 concentrations.

One of the most anti-inflammatory immunosuppressive cytokines during pregnancy is IL-10 (e.g. inhibition of IL-1, IL-6, and TNF-α),[21] which plays a primordial role at the maternal-fetal interface.[17,21] Hence, the observed non-significant (statistical trend) positive associations in our study might suggest an exercise-induced IL-10 role. However, these results do not allow us to ascertain that conclusion. By comparison, Moran, et al.[20] did not observe significant changes in IL-10 concentrations with a physical activity counselling intervention.

Interferon gamma is a pleiotropic pro-inflammatory cytokine characterized by powerful immunomodulatory effects on immune responses,[17,22] and it plays a relevant role in the endometrial vasculature remodeling, angiogenesis, and maintenance of the decidua.[16,17,22] The higher decrease of IFN-γ observed at 34th week and delivery in the exercise group (although non-significant), might be indicative of a small and irrelevant effect of exercise on IFN-γ during pregnancy, as previously suggested in the general population.[23] Likewise, Moran, et al.[20] did not observe any significant change neither in maternal nor cord plasma IFN-γ between a physical activity counselling intervention and the control group.

Table S1. Inclusion and exclusion criteria in the GESTAFIT project.

Inclusion criteria
- Pregnant women aged 25-40 years old with a normal pregnancy course.
- Answering “no” to all questions on the PARmed-X for pregnancy.
- Being able to walk without assistance.
- Being able to read and write properly.
- Informed consent: Being capable and willing to provide written consent.
Exclusion criteria
- Having acute or terminal illness.
- Having malnutrition.
- Being unable to conduct tests for assessing physical fitness or exercise during pregnancy.
- Underweight
- Having pregnancy risk factors (such as hypertension, type 2 diabetes, etc.).
- Having a multiple pregnancy.
- Having chromosopathy or fetal malformations.
- Having uterine growth restriction.
- Having fetal death.
- Having upper or lower extremity fracture in the past 3 months.
- Suffering neuromuscular disease or presence of drugs affecting neuromuscular function.
- Being registered in another exercise program.
- Performing more than 300 minutes of at least moderate physical activity per week.
- Being unwilling either to complete the study requirements or to be randomized into the control or intervention group.

Study IV

Table S2. Intention to treat analyses showing the effect of the concurrent exercise-training program on maternal seru

	Changes in control group		Changes in exercise group		Model unadjusted				Model		
	Mean	SD	Mean	SD	B	SE	β	p-value	B	SE	
35th week-17th week (maternal serum, n=53)	(n=28)		(n=25)								
Fractalkine (pg/ml)	-0.35	101.10	16.70	83.54	17.05	25.66	0.09	0.51	14.06	18.71	0
Interleukin 1 beta (pg/ml)	0.67	3.13	0.16	1.99	-0.50	0.73	-0.10	0.50	-0.76	0.69	-0
Interleukin 6 (pg/ml)	0.74	3.27	-0.15	3.26	-0.89	0.90	-0.14	0.33	-0.84	0.71	-0
Interleukin 8 (pg/ml)	-1.68	9.48	3.57	7.11	5.25	2.33	0.30	0.03	3.63	2.06	0
Interleukin 10 (pg/ml)	0.55	13.74	8.15	10.47	7.60	3.39	0.30	0.03	5.58	2.56	0
Interferon gamma (pg/ml)	-0.55	9.97	-2.37	10.24	-1.81	2.78	-0.09	0.52	-2.71	2.45	-0
Tumor necrosis factor alpha (pg/ml)	1.51	2.29	1.02	2.32	-0.49	0.63	-0.11	0.44	-0.77	0.40	-0
Delivery-17th week (maternal serum, n=43)	(n=19)		(n=24)								
Fractalkine (pg/ml)	-3.22	69.74	13.63	97.14	16.84	26.47	0.10	0.53	29.95	19.40	0
Interleukin 1 beta (pg/ml)	3.24	2.86	1.35	3.54	-1.89	1.00	-0.28	0.07	-1.94	0.98	-0
Interleukin 6 (pg/ml)	26.91	9.86	29.22	18.82	2.31	4.77	0.08	0.63	2.15	4.80	0
Interleukin 8 (pg/ml)	14.53	14.57	19.80	14.85	5.27	4.52	0.18	0.25	2.98	3.59	0
Interleukin 10 (pg/ml)	18.65	13.98	28.52	15.20	9.87	4.51	0.32	0.03	9.69	4.00	0
Interferon gamma (pg/ml)	-2.50	9.08	-7.19	11.53	-4.69	3.23	-0.22	0.15	-3.75	1.96	-0
Tumor necrosis factor alpha (pg/ml)	4.66	2.72	3.86	2.87	-0.81	0.86	-0.14	0.36	-0.78	0.79	-0

SD, standard deviation; B, unstandardized regression coefficient; SE, standard error; β, standardized regression coeffi
method) were used to examine the differences on inflammatory markers between the control and exercise group. T
changes (from the exercise training group minus the control group) on cytokines concentrations were included in the l
variables, and the group (control=0 and exercise=1) as independent variable. When considering the "35th week-17th we
1 was adjusted for baseline values of the particular cytokine and adherence to the Mediterranean Diet score; and the
the relative percentage of daily total physical activity (total physical activity/accelerometer wearing time). When consid
point analyses, the model 1 was adjusted for baseline values of the particular cytokine; and the model 2 was add
gestational age at birth.

Table S3. Intention to treat analyses showing the effect of the concurrent exercise-training program on arterial and ve
(n=44).

	Model unadjusted				Model 1				
	B	**SE**	**β**	**p-value**	**B**	**SE**	**β**	**p-value**	**B**
Umbilical arterial serum (delivery)[a]									
Fractalkine (pg/ml)*	0.78	0.31	0.38	**0.02**	0.69	0.31	0.34	**0.03**	0.69
Interleukin 1 beta (pg/ml)*	0.54	0.27	0.31	0.06	0.55	0.28	0.32	0.06	0.57
Interleukin 6 (pg/ml)*	-0.81	0.31	-0.40	**0.02**	-0.76	0.32	-0.37	**0.02**	-0.78
Interleukin 8 (pg/ml)	5.80	9.03	0.11	0.53	6.80	9.30	0.13	0.47	7.63
Interleukin 10 (pg/ml)	2.41	1.11	0.34	**0.04**	2.23	1.14	0.32	0.06	2.21
Interferon gamma (pg/ml)	-0.69	0.42	-0.26	0.11	-0.71	0.43	-0.27	0.11	-0.71
Tumor necrosis factor alpha (pg/ml)	-1.52	1.01	-0.24	0.14	-1.62	1.05	-0.26	0.13	-1.65
Umbilical venous serum (delivery)									
Fractalkine (pg/ml)	5.80	35.22	0.03	0.87	0.94	35.18	0.00	0.98	-4.38
Interleukin 1 beta (pg/ml)*	-1.11	1.61	-0.11	**0.49**	-1.32	1.61	-0.13	0.42	-1.32
Interleukin 6 (pg/ml)	0.20	0.31	0.10	0.53	0.21	0.31	0.10	0.51	0.15
Interleukin 8 (pg/ml)*	0.62	1.22	0.08	0.61	0.64	1.24	0.08	0.61	0.42
Interleukin 10 (pg/ml)	0.17	0.30	0.08	0.59	0.16	0.31	0.08	0.61	0.12
Interferon gamma (pg/ml)	0.34	0.37	0.14	**0.37**	0.26	0.36	0.11	0.47	0.28
Tumor necrosis factor alpha (pg/ml)	-4.10	1.52	-0.38	**0.01**	-4.45	1.47	-0.42	**0.004**	-4.30

SD, standard deviation; B, unstandardized regression coefficient; SE, standard error; β, standardized regression coeffi
method) were used to examine the differences on inflammatory markers between the control and exercise group.
concentrations were included in the linear regression analyses as dependent variables, and the group (control=0 and e
model 1 was adjusted for adherence to the Mediterranean Diet score; and the model 2 was additionally adjusted for p
* Optimum Box-Cox transformations and a subtle variation of winsorizing (convert back from a z-score: replacing ext
±2.58 SDs from the mean) were performed on inflammatory markers. [a] indicate lower sample size of the contro
inflammatory markers.

Study IV

Table S4. Differences between arterial and venous cord serum inflammatory markers (n=34).

	All participants (n=34)					Control group (n=15)					
	Mean difference (Artery-Vein)					Mean difference (Artery-Vein)					
	Mean	SD	Mean Dif.	SE	p-value	Mean	SD	Mean Dif.	SE	p-value	Mean
Arterial fractalkine	346.9	101.3	61.6	18.5	**0.002**	314.6	91.0	48.4	26.9	0.09	372.3
Venous fractalkine	285.3	117.1				266.2	118.1				300.3
Arterial interleukin-6	16.8	4.9	3.8	0.8	**<0.001**	18.9	4.9	5.4	1.1	**<0.001**	15.0
Venous interleukin-6	13.0	5.3				13.4	5.9				12.5
Arterial interleukin-8	54.5	27.4	-7.4	4.9	0.14	51.7	31.6	-11.5	9.2	0.23	56.6
Venous interleukin-8	61.9	20.2				63.3	23.7				60.7
Arterial interleukin-10	11.5	3.6	-1.5	0.7	**0.04**	10.1	2.5	-2.4	0.8	**0.015**	12.4
Venous interleukin-10	13.0	3.6				12.6	3.5				13.3
Arterial interleukin-1beta	1.4	0.7	-0.2	0.2	0.32	1.1	0.9	-0.2	0.2	0.41	1.5
Venous interleukin-1beta	1.6	0.9				1.4	0.8				1.6
Arterial interferon gamma	2.9	1.3	0.2	0.2	0.31	3.2	1.4	.7	0.3	**0.05**	2.5
Venous interferon gamma	2.6	1.3				2.4	1.0				2.7
Arterial TNF-alpha	15.0	3.2	-1.0	0.8	0.22	15.8	3.5	-2.7	1.4	0.07	14.2
Venous TNF-alpha	16.0	4.8				18.5	4.5				13.9

Dif., difference; SD, standard deviation; SE, standard error; TNF, tumor necrosis factor alpha.

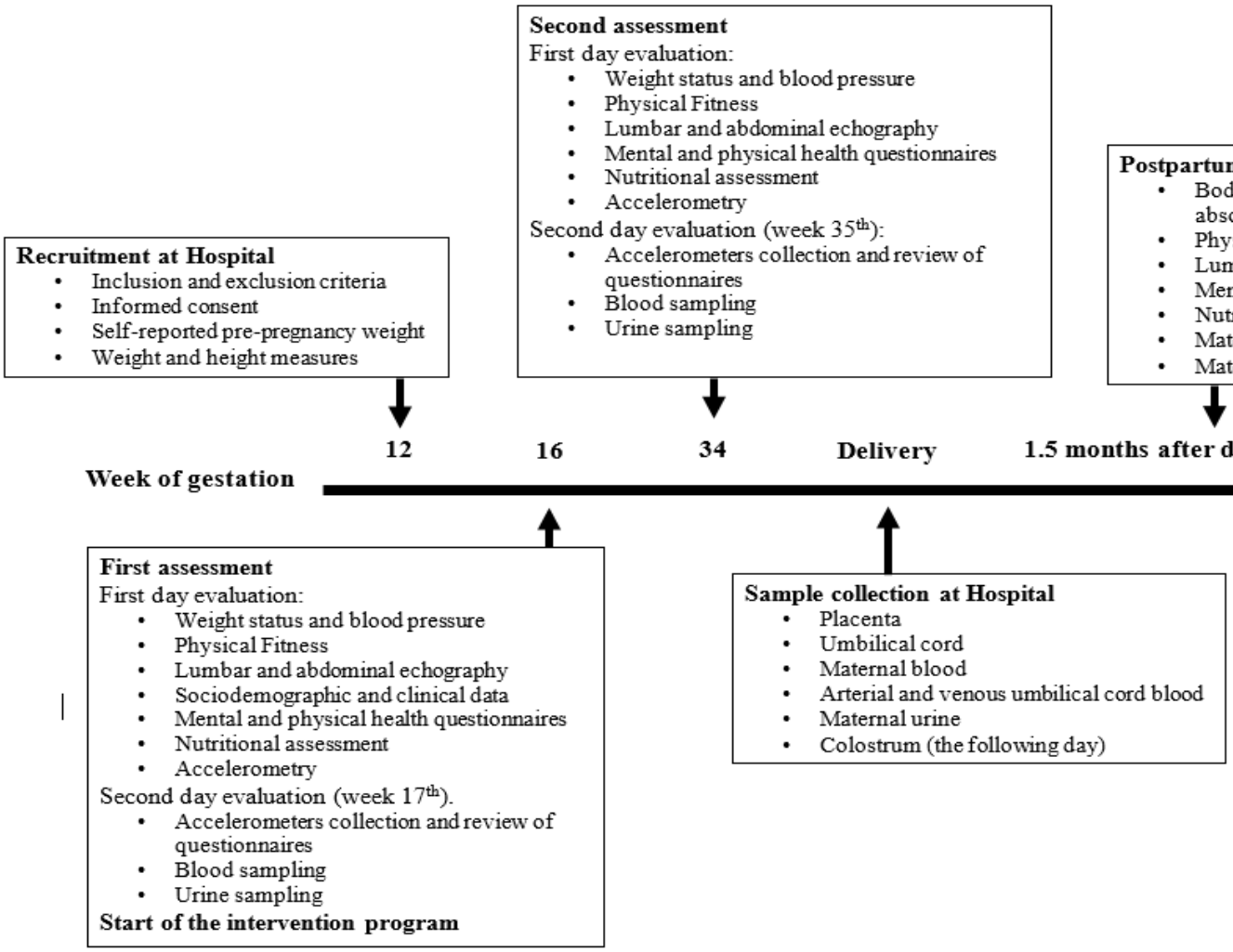

Figure S1. Assessments conducted along the GESTAFIT Project.

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STUDY V

The effects of prenatal exercise on maternal and foetal immunometabolism during pregnancy: The GESTAFIT project

ABSTRACT

Background: How exercise-induced stimuli affect and translate into immunometabolic adaptations during pregnancy is unclear. We previously found intervention effects on systematic cytokines (*IL-1β, IL-6, IL-8, IL-10, IFN-γ, and TNF-α).*

Objectives: To analyse the influence of a supervised concurrent exercise-training program from 17th gestational week until delivery on immunometabolic parameters – *glycaemic and lipid markers, cortisol and C-reactive protein*– in maternal, and arterial and venous cord serum. Secondary aims were to explore: i) whether the aforementioned cytokines during pregnancy are related to these immunometabolic markers, and if these associations are dependent on exercise; and ii) the role of these cytokines as mediators of the effects of exercise.

Methods: Eighty-eight pregnant women (age: 34±5years, pre-pregnancy body mass index: 22.5 (20.5, 25.9) kg/m^2), divided into exercise (n=44) and control (n=44) groups, participated in this quasi-experimental study –per-protocol basis–. The exercise group followed a 60-min 3 days/week concurrent (aerobic+resistance) exercise training. Maternal and arterial and venous cord serum glycaemic and lipid markers, cortisol and C-reactive protein, and cytokines, were measured with standard biochemical methods and Luminex xMAP technology, respectively.

Results: No significant differences between groups were found in maternal or cord serum glycaemic and lipid markers, cortisol and C-reactive protein (all, p>0.05); with the exception of arterial cord serum glucose levels which were slightly reduced in the exercise group (p=0.02). The increase in IL-8 mediated the effects of exercise on total cholesterol (indirect effect -9.1; 95%CI -24.6, -1.12) and low density lipoprotein-cholesterol (-8.9; -21.9, -1.1).

Conclusions: Exercise was not effective to directly induce meaningful changes in maternal and foetal immunometabolic markers during pregnancy. However, exercise indirectly reduced maternal total cholesterol and low density lipoprotein-cholesterol gains via an increase in IL-8.

INTRODUCTION

An adequate maternal-foetal homeostasis, which implies remarkable physiological adaptations, is necessary for a successful pregnancy[1-3]. Dysregulations of immunometabolic responses during pregnancy –*i.e. poor metabolism: high systemic glucose and lipid levels, excessive/insufficient pro-inflammatory status, etc.*– could lead to pregnancy complications[1,3-5], and thus to compromise the mother´s and offspring´s health at short and long-term[1,2,6].

Physical exercise could positively modulate maternal-foetal metabolism[7-18], and optimize pregnancy outcomes in normal-weight and overweight/obese women[16-22]. However, scientific evidence provides contradictory results, with some aerobic, concurrent (aerobic+resistance) and mixed (exercise+diet) exercise programs showing limited or no effects on maternal metabolism[23-29]. Additionally, the effects of exercise on foetal glucose and lipids have not been explored. In view of the weak and scarce evidence, mainly focused on preventing gestational diabetes mellitus (GDM) and weight-gain, further studies exploring the effects of concurrent exercise (which appears to be more effective[21]) on maternal-foetal metabolism are necessary. Moreover, these studies should consider non-diabetic pregnancies, since these women –characterized by less severe glucose intolerance than women with diabetes– might also manifest increased susceptibility to adverse outcomes[30].

Importantly, the underlying mechanisms by which maternal exercise-induced homeostatic perturbations could be translated into metabolic adaptations, remain unknown in pregnant women. Previous literature (few studies in pregnant rodents[7], and one in pregnant women[20]), have indirectly suggested that exercise-related factors –myokines– could play a noticeable role on the maternal-placental-foetal crosstalk, among other mechanisms. However, only we[8] and another study[31] have shown that exercise during pregnancy can modulate systemic concentrations of relevant cytokines (*IL-1β, IL-6, IL-8, IL-10, IFN-γ, and TNF-α*) from maternal origin –mostly-; although their specific origin remains undetermined. Whether these cytokines can drive some of the effects of exercise into maternal-foetal metabolism needs to be discovered.

Bearing all above in mind, the main aim of this study was to analyse the influence of a supervised concurrent exercise program from 17th gestational week until delivery on immunometabolic parameters -*glycaemic and lipid markers, cortisol and C-reactive*

protein (CRP)- in maternal, and arterial and venous cord serum. Secondary aims were to explore: i) whether changes in the aforementioned cytokines modulated by exercise (*IL-1β, IL-6, IL-8, IL-10, IFN-γ, and TNF-α*), are related to these maternal-foetal immunometabolic parameters during pregnancy, and if these associations are dependent on exercise; and ii) the role of these cytokines as mediators of the effects of exercise on immunometabolic parameters.

MATERIAL AND METHODS

Study design and population

The GESTAFIT project was initially a randomized controlled trial performed in the "San Cecilio and Virgen de las Nieves University Hospitals", and at the "Sport and Health University Research Institute" (Granada, Spain). The procedures, along with the inclusion-exclusion criteria (**Table S1**), are described elsewhere[11,32]. From the 384 pregnant women who were informed about the project during the first appointment with their gynaecologist at the 12th week of gestation, 159 women were finally recruited. All women signed a personal consent after being individually informed about the methodology. The GESTAFIT project was approved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15).

Sample size

The sample size was only determined for gestational weight gain (required sample, n=52)[32], but not for the other outcomes analysed in this study -given their exploratory background.

Randomization

Despite its initial randomized controlled trial design, this random component was finally broken because of some difficulties related to adherence of control women[8]; which represents a frequent methodological-ethical barrier in antenatal exercise research[33]. Thus, women were subsequently allocated to the exercise or control group depending on their personal preference and convenience to attend the intervention sessions. The only personnel not blinded to the allocation of women to the intervention groups were the responsible experts of the training sessions.

General procedure

During the study, women were evaluated by experienced researchers at 16-17th and 33rd-34th week (2 different days/assessment), and delivery (1 day/assessment). Socio-demographic characteristics, height and weight, sleep and dietary habits were assessed on the first day at 16th week. Before leaving, women were given accelerometers to wear until next appointment. One week later (17th week), accelerometers were returned in person, and maternal blood samples were collected by a nurse. At 33rd-34th week, the same assessments but the initial anamnesis were performed with identical timing to 16th-17th week. Minutes after delivery, obstetrics and gynaecological histories were gathered, and maternal and arterial and venous cord blood samples were collected. The general assessment procedures are detailed in **Figure S1**.

Intervention

The intervention program has been previously detailed elsewhere[8,11,32]. Overall, the exercise intervention was based on a concurrent training program from the 17th week until delivery (3 days/week, 60 minutes/session) consisting of a combination of aerobic-resistance exercises of moderate-to-vigorous (predominantly moderate with peaks of vigorous) intensity. This exercise protocol was designed by an expert multidisciplinary team, following the recommendations from the American College of Obstetricians and Gynaecologists[18]. The exercise progression was achieved by means of increasing volume and intensity/load as previously reported[32]. The rated perceived exertion (RPE: 0-10), along with other parameters (lumbar pain, urinary incontinence, etc.), were registered within a subgroup of women at the end of each session to control and adapt exercise progression. A device (Polar RCX-3) was also worn by three different participants in initial sessions to better control intensity. During the intervention, the research team gave 7 talks to the participants from the exercise group to provide them with basic advice for a healthier pregnancy (**Appendix A**).

In the control group, women were asked to continue with their usual activities. These participants were also invited to the 7 talks regarding basic advice for a healthier pregnancy because of ethical considerations, and to maintain their fidelity.

Outcomes

Healthcare experts from hospitals and physiologists responsible for the evaluations of immunometabolic biomarkers were blinded to the allocated treatment of participants.

Sociodemographic-clinical data, obstetric history and perinatal outcomes

Sociodemographic and clinical data (medications, suffering diseases or having pre-existing conditions, etc.), obstetric and reproductive history, maternal and offspring adverse events, and smoking and alcohol habits, were obtained from questionnaires and medical files. Data related to the type of delivery, number of abortions, foetal sex, etc. were collected from perinatal obstetric records (partogram).

Height and body mass

Pre-pregnancy weight was self-reported. Height and weight were measured (no shoes, light clothes) using a calibrated stadiometer (Seca 22, Hamburg) and electronic scale (InBody R20; Biospace, Seoul). Body mass index (BMI) was calculated [weight(Kg)/height(m^2)].

Dietary habits

The consumption and frequency of different foods were assessed using a food frequency questionnaire[34]. The grams consumed for each food were calculated by multiplying the food predetermined portion size by their respective frequency consumption. This information was used to estimate nutrition values and total energy intake (kcal/day) with the Evalfinut software. The Mediterranean Diet Score index[35] was created to assess the adherence to the Mediterranean dietary patterns.

Sedentary time and physical activity

Triaxial accelerometry located on the waist (ActiGraph GT3X+, Florida, US) was employed to objectively assess sedentary time (ST) and physical activity (PA) levels. Further information is provided elsewhere[36].

Sleep duration and quality

Sleep duration and efficiency were analysed with triaxial accelerometers (ActiSleep, ActiGraph GT3X+, Florida, US) placed on the wrist[36]. The Pittsburgh Sleep Quality Index Questionnaire was employed to assess sleep quality[37].

Laboratory methods

Blood collection

In standardized fasting conditions (8-9 a.m.) at our research centre (16^{th} and 33^{rd} weeks), maternal venous blood samples -5mL- were extracted from the antecubital vein, and collected in serum tubes. All exercisers performed the last bout of exercise 2 days previously to the 33^{rd} week extraction. Immediately after delivery, maternal and cord arterial and venous blood samples were extracted, and stored in serum tubes[19]. A 1mL

syringe was used for the blood extraction. Then, the samples were centrifuged at 1750 rpm for 10 minutes at 4°C (GS-6R Beckman Coulter, Brea, USA) to separate serum from formed elements. Subsequently, serum was aliquoted and frozen at -80ºC until analyses.

Immunometabolic biomarkers

Glucose, lipids and C-reactive protein

Maternal serum glucose, total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), phospholipids, and CRP concentrations were assessed using standard spectrophotometric enzyme assays (AU5822 Clinical Chemistry Analyser, Beckman Coulter, Brea, USA). At delivery, maternal and arterial and venous cord serum glucose, total cholesterol, triglycerides, HDL-C, LDL-C, and phospholipids were also assessed by spectrophotometric determination (BS-200 Chemistry Analyzer, Mindray Bio-medical Electronics, Shenzen, China).

Insulin and cortisol

Maternal insulin and cortisol were assessed via paramagnetic-particle-based chemiluminescence immunoassays (UniCel-Dxl800 Access Immunoassay analyser, Beckman Coulter, Brea, USA).

Inflammatory markers

Maternal, and arterial and venous serum cytokines (fractalkine, IL-1β, IL-6, IL-8, IL-10, IFN-γ, and TNF-α) were assessed with Luminex xMAP techonology (**Appendix B**)[8].

Insulin resistance and B-cell function

Standard formulas[38] were employed to calculate the homeostasis model assessment (HOMA)-IR (insulin resistance) and HOMA-B (B-cell function).

Statistical analysis

Only women who attended >75% of the exercise sessions and completed baseline and follow-up assessments, were included in the analyses (per-protocol rationale[11]). Descriptive statistics were conducted to show the sociodemographic-clinical characteristics (**Table 1**) and maternal and foetal immunometabolic biomarkers concentrations (**Table 2**). In linear regressions, simple models considering the interaction-term between the intervention and foetal sex with metabolic outcomes, were built to explore foetal sex dependency. Relevant confounders suggested by previous literature influencing the relation between the independent and dependent variable –meaningful change B≈15%–, were included in the analyses (see **Tables 3-4**):

baseline values of the respective outcome, specific gestational week at post-test assessments (at 33rd week and birth), baseline smoking habits and Mediterranean diet score, and type of delivery. Several extreme cases of specific outcomes were verified as influential outliers. These outliers were handled/adjusted (see **Appendix C**). Subsequently, optimum Box-Cox transformations were used for those models characterized by asymmetry of immunometabolic markers, and the violation of assumptions related to generalization of the results.

To address the first aim, linear regressions were used to explore differences on immunometabolic parameters –*glycaemic and lipid markers, cortisol and CRP*– between the control and exercise groups at the different time points. In multiple and single time point analyses (**Table 3**), maternal and cord serum parameters, and changes on these maternal immunometabolic markers from baseline to 33rd week, were included in the models as dependent variables, and the intervention group (control=0, exercise=1) as independent variable. Median regressions were employed for those models where the violation of assumptions was still appreciable after transformations. Regarding the secondary aims, linear regressions were used to analyse the association of cytokines, and changes in cytokines, with maternal and cord serum immunometabolic parameters (**Tables S2-S5**). Information about these cytokines concentrations and changes during pregnancy, and the influence of exercise on them, has been provided elsewhere[8].

Additionally, simple slope analyses were used to explore if these associations differed depending on foetal sex, physical exercise, and pre-pregnancy BMI. Lastly, simple mediation analyses (**Table 4, Figure S2**) were conducted to investigate the potential role of cytokines as mediators of the effect of exercise on maternal-foetal immunometabolic parameters. These mediation analyses were only conducted for those cytokines influenced by exercise (see our previous study[8], or **Tables S6-S7)**, and significantly associated with the aforementioned immunometabolic markers (**Tables S2-S5**). All the assumptions related to the generalization of the results were met. The analyses were conducted using SPSS 22.0 (IBM, NY, USA). The statistical significance was set at $p \leq 0.05$.

RESULTS

From all women willing to participate (n=384), the final study sample for the present study aims consisted of 88 Caucasian southern Spanish women (age 33.6±4.5 years, pre-pregnancy BMI 22.5 (20.5, 25.9) kg/m^2) without diagnosed cardiometabolic diseases. These participants were divided into control (n=44) and exercise (n=44) groups –see **Figure S3** and **Appendix E-**. No potential effect modification of exercise by foetal sex was found. Accordingly, foetal sexes were combined in all analyses. The sociodemographic and clinical characteristics of the participants are provided in **Table 1**. The mean exercise training attendance was 85%. The concentrations and differences on immunometabolic biomarkers are shown in **Table 2** and **Figures S4-S5**. At *baseline*, the exercise group was characterized by higher insulin and phospholipids levels and insulin resistance, and lower cortisol than controls (all $p<0.05$).

Effects of exercise on immunometabolic parameters

The effects of the exercise intervention on maternal-foetal immunometabolic markers are shown in **Table 3**. The exercise intervention did not induce significant changes in any maternal immunometabolic parameter compared to the control group ($p>0.05$). Regarding cord serum parameters, the exercise group showed similar concentrations of metabolic biomarkers compared to the control group ($p>0.05$); with the exception of arterial cord serum glucose levels, which were slightly reduced in the exercise group when adjusting for the Mediterranean diet score (B=-1.01, SE=0.41, $p=0.02$). The results remained similar after replicating the analyses grouped by pre-pregnancy BMI: normal-weight vs. overweight-obese women (data not shown).

When these analyses were additionally adjusted –stepwise manner– for maternal age, BMI pre-pregnancy, sleep duration/quality, energy intake, tobacco, relative percentage of ST and MVPA, foetal sex, parity, use of oxytocin, epidural (or other anaesthesia), caesarean section, type of delivery and cause related to start of the birth, the results remained similar. Only the associations of the exercise group with maternal glucose at delivery become significant ($p<0.05$), after adjusting for the use of oxytocin and epidural anaesthesia.

Table 1. Sociodemographic and clinical characteristics of pregnant women (n=88).

	Total (n=88)		Control (n=44)		Exercise (n=44)		p-value
Age (years)	33.6	4.5	33.9	4.9	33.4	4.2	0.55
Body mass index, pre-pregnancy (kg/m²)	22.5	(20.5, 25.9)	22.1	(20.5, 25.2)	23.4	(20.8, 27.0)	0.1
Gestational age							
1st assessment (baseline)	16.0	1.7	16.5	1.2	15.4	1.9	**0.003**
2nd assessment	33.0	(32, 34)	34.0	(33, 35)	32.0	(30, 33)	**<0.001**
Delivery	40.0	(39, 41)	39.0	(39, 40)	40.0	(39, 41)	0.08
Percentage of assistance					86.2	6.4	
Educational level, n (%)							
Non university degree	31	34.8	14	31.8	17	38.6	0.71
University degree	57	65.2	30	68.2	27	61.4	
Professional status, n (%)							
Work full/part time	61	68.5	31	70.5	30	68.2	0.88
Unemployed/Retired/Housekeeper	27	31.5	13	29.5	14	31.8	
Parity status (primiparious), n (%)	55	61.8	24	54.5	30	68.2	0.24
Offspring sex, n (%) ***(n=82)***							
Male	43	51.8	21	53.8	22	51.2	0.9
Female	39	48.2	18	46.2	21	48.8	
Use of oxytocin, n (%) ***(n=79)***	18	20.2	7	18.9	11	26.2	0.66
Use of epidural anaesthesia n (%) ***(n=80)***	54	60.7	23	59.0	31	75.6	0.24
Number of abortions	0.0	(0,1)	0.0	(0,1)	0.0	(0,1)	0.66
Type of delivery, n (%) ***(n=82)***							
Spontaneous	52	59.6	26	65.0	26	61.9	0.88
Vacuum extraction	13	14.6	5	11.4	8	19.0	
Forceps	2	2.2	1	2.3	1	2.4	
Caesarean Section	15	16.9	8	18.2	7	16.7	
Cardiovascular function, 16th week							
Systolic blood pressure (mmHg)	106.9	8.8	107.9	9.3	106.0	8.3	0.3
Diastolic blood pressure (mmHg)	63.9	7.7	63.1	8.5	64.6	6.9	0.35
Smoking during pregnancy (cigarettes/day)	0.3	1.4	0.5	1.9	0.1	0.6	0.19
Mediterranean diet adherence (0-50)	28.9	3.7	28.7	3.9	29.1	3.6	0.58
Sleep, 16th week ***(n=82)***							
Sleep time (accelerometry) min/day	430.1	46.2	435.8	50.8	425.3	42.0	0.31
Sleep quality (0-21)	6.0	(4, 9.0)	6.0	(4, 9)	5.0	(3, 7.0)	0.12
Sedentary lifestyle and PA, 16th week ***(n=82)***							
Sedentary time (min/day)	513.6	100.6	492.2	113.5	533.5	83.3	0.065
Moderate-vigorous PA (min/day)	39.1	(21.1, 51.6)	30.7	(19.0, 50.9)	42.7	(25.6, 52.4)	0.24
Average accelerometer wear time (min/day)	940.2	56.3	933.4	67.1	946.4	43.8	0.3
Relative % of daily sedentary time	54.6	10.1	52.7	11.2	56.4	8.7	0.1
Relative % of daily moderate-vigorous PA	4.6	5.3	5.0	7.4	4.3	2.0	0.57

PA, physical activity. Continuous variables are presented as mean -standard deviation- or median (interquartile range), unless otherwise indicated. P-values were calculated using independent sample Student's t-test (normal distribution, homoscedasticity), Welch´s test (normal distribution, heteroscedasticity), and Mann-Whitney U test (non-normal distribution) for continuous variables, and Chi-square test for categorical variables.

Study V

Table 2. Metabolic markers concentrations at three time points (n=88).

	16th week (n=88)					33th week (n=88)				
	Control (n=44)		Exercise (n=44)		p-value	Control (n=44)		Exercise (n=44)		p-value
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Maternal serum										
Glucose (mg/dL)	78.0	(72, 80)	77.0	(71, 81)	0.92	74.9	8.5	71.0	9.1	**0.05**
Insulin (microIU/dL)	3.9	(3.2, 4.9)	4.6	(3.5, 9.2)	**0.03**	6.1	2.4	8.1	7.8	0.13
Insulin resistance (HOMA-IR)	0.7	(0.5, 0.9)	0.9	(0.6, 1.8)	**0.03**	1.1	0.5	1.5	1.9	0.24
B cell function (HOMA-B)	90.9	(54.2, 149.1)	129.7	(70.9, 260.9)	0.06	166.0	(107.5, 331.9)	207.3	(96.4, 654.4)	0.34
Total cholesterol (mg/dL)	221.2	32.6	218.5	32.3	0.71	276.0	39.0	275.4	34.3	0.97
Triglycerides (mg/dL)	113.5	(84.3, 147.8)	100.0	(83, 137)	0.41	207.3	(165.5, 286.5)	185.0	(150, 236)	0.12
HDL-C (mg/dL)	66.6	9.9	69.6	10.6	0.14	66.5	12.4	67.9	10.1	0.57
LDL-C (mg/dL)	130.2	24.7	126.0	28.9	0.46	164.4	32.5	166.8	38.2	0.66
Phospholipids (mg/dL)[a]	186.5	(169.7, 202.4)	210.9	(198.2, 226.4)	**0.004**	222.2	(198.9, 235.7)	229.6	(199.5, 243.6)	0.44
Cortisol (mg/dL)	20.0	4.8	16.3	5.9	**0.03**	22.2	4.3	21.8	4.4	0.61
C-reactive protein (mg/dL)	0.37	(0.21, 0.84)	0.55	(0.26, 0.93)	0.41	0.32	(0.21, 0.66)	0.49	(0.25, 0.83)	0.19
Cord arterial serum										
Glucose (mg/dL)										
Cholesterol (mg/dL)										
Triglycerides (mg/dL)										
HDL-C (mg/dL)										
LDL-C (mg/dL)										
Phospholipids (mg/dL)[a]										
Cord venous serum										
Glucose (mg/dL)										
Cholesterol (mg/dL)										
Triglycerides (mg/dL)										
HDL-C (mg/dL)										
LDL-C (mg/dL)										
Phospholipids (mg/dL)[a]										

HDL-C, high density lipoprotein-cholesterol, LDL-C, low density lipoprotein cholesterol. SD, standard deviation. Data ar
(interquartile range=Q3, Q1). [a] n=45. P-values were calculated using independent sample Student's t-test, Welch´s tes

Table 3. Per-protocol analyses showing the effect of the concurrent exercise-training program on maternal and foetal serum meta

Biochemical markers	Maternal serum - Change 16th-33rdweek (Control n=44; Exercise n=44)										
	Within-group changes				Model 1				Model 2	Adjusted	
	Mean	SD	Mean	SD	B	SE	β	p-value	p-value	R2	B
Glucose (mg/dL)[ab]	-1.6	7.7	-5.2	14.1	-0.28	2.25	-0.01	0.90	0.79	0.44	-17.33
Insulin (microIU/dL)[a]	0.5	8.0	-1.2	15.0	2.16	1.37	0.13	0.12	0.12	0.69	
HOMA-IR[a]	0.0	1.9	-0.4	3.8	0.49	0.34	0.13	0.15	0.15	0.66	
HOMA-B[a]	160.7	725.0	176.4	961.5	0.55	95.10		0.99	0.99	0.20	
Cholesterol[a]	54.8	32.8	57.0	30.5	-0.17	8.47	0.00	0.98	0.53	0.08	-12.67
Triglycerides[ab]	99.8	42.0	88.7	54.6	5.62	11.85	0.06	0.64	0.86	0.10	14.79
HDL-C[a]	-0.1	7.8	-1.7	8.9	-1.11	2.37	-0.07	0.64	0.73	0.05	-12.17
LDL-C[a]	34.2	32.8	40.7	29.2	-3.07	8.17	-0.05	0.71	0.49	0.06	-2.10
Phospholipids[ae] (n=42)	34.3	56.5	20.6	50.8	20.10	15.80		0.21	0.33	0.34	-2.97
Cortisol	2.3	5.3	5.2	5.8	0.35	1.15	0.03	0.76	0.07	0.52	
C-reactive protein[ae]	-0.20	0.93	-0.05	0.41	0.13	0.08		0.88	0.72	0.20	

Biochemical markers	Arterial cord serum (Control n=15; Exercise n=10)						
	Model 1				Model 2	Adjusted	
	B	SE	β	p-value	p-value	R2	B
Glucose[f]	-0.58	0.41	-0.29	0.18	**0.02**	0.04	-8.61
Cholesterol[cdf]	-0.12	0.42	-0.06	0.78	0.89	0	5.76
Triglycerides[d]	-12.43	11.17	-0.23	0.28	0.11	0.09	-2.03
HDL-C[dg]	-1.96	3.62	-0.11	0.59	0.87	0.03	0.40
LDL-C[dfg]	-0.37	0.37	-0.20	0.33	0.42	0.01	-0.16
Phospholipids[d]	-9.51	10.19	-0.21	0.36	0.47	0	-9.07

SD, standard deviation; B, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient; HDL-C, H homeostasis model assessment; LDL-C, low density lipoprotein-cholesterol. A subtle variation of winsorizing was performed on arterial[c] and venous[d] cord serum biochemical markers. [e] Median regressions, and optimum Box-Cox transformations (for [f] arterial a after handling outliers. When considering the "33rd week-16th week" analyses, the model 1 was adjusted for baseline values of t post-test assessment (33rd week), and smoking habits at baseline; and the model 2 for baseline values of the respective outcon Mediterranean diet score at baseline. When considering "maternal markers at delivery" analyses, the model 1 was adjusted for and gestational week at birth; and the model 2 for the Mediterranean diet score at baseline, and type of delivery. When consideri adjusted for gestational week at birth; and the model 2 for the Mediterranean diet score at baseline. The adjusted R2 (pseudo R2 derived from the model 1. The results remained similar after exploring these associations by BMI at pre-pregnancy (normal-w controlling for the familywise error rate (Hochberg procedure), none significant associations remained significant.

Associations of cytokines with immunometabolic markers

The associations of cytokines concentrations with maternal and foetal immunometabolic markers are shown in **Tables S2-S5**. No potential effect modification by foetal sex was found. Changes in IL-8 (baseline-33rdweek) were inversely associated with changes in total cholesterol (B=-1.17, SE=0.48, p=0.02) and LDL-C (B=-1.17, SE=0.43, p=0.01). Changes in IL-10 (baseline-33rdweek) were associated with changes in triglycerides (B=-1.01, SE=0.45, p=0.03) and HDL-C (B=-0.28, SE=0.10, p=0.01). Increased IL-1β was associated with reduced HDL-C (baseline-33rdweek) and increased total cholesterol (baseline-birth) (both, p≤0.05). Arterial cord serum IL-6 was positively associated with arterial cord serum glucose (B=2.19, SE=0.96, p=0.03). The remaining, and the aforementioned associations after controlling for family wise error rate, were non-significant (p>0.05).

When exploring the dependence of these associations on physical exercise, changes in IL-6 (baseline-birth) were inversely related to maternal glucose at delivery (p=0.03) only in women from the exercise group. Additionally, arterial cord serum fractalkine and IFN-γ were inversely associated with arterial cord total cholesterol, LDL-C and HDL-C (all, p<0.05) in exercised women; and venous cord serum IL-6 was positively associated with venous cord triglycerides (p=0.002). In control women (not exercisers), increased IL-10 (baseline-33rdweek) was associated with reduced HDL-C (p=0.01).

Mediator role of cytokines

The role of cytokines as mediators of the exercise-induced effects on metabolic outcomes, and their kinetics during pregnancy, are addressed in **Table 4** and **Figures S6-S8**, respectively. The mediation analyses showed that exercise reduced maternal total cholesterol (B=-9.13; 95%CI -24.60, 32.52) and LDL-C (B=-8.91; 95%CI -21.90, -1.10) increases from baseline to 33rd week via an increase in IL-8 (p<0.05), and reduced HDL-C from baseline to 33rd week through an increase in IL-10 (B=-2.96; 95%CI -7.28, -0.23).

Sensitivity analyses

Because of the substantial percentage of missing data (authors do not recommend to perform imputations with >20% missing cases[39]), and the inaccuracy of imputed data, intention-to-treat analyses have not been considered to avoid erroneous conclusions (see **Appendix F**). Differences between arterial and venous cord serum metabolic parameters are shown in **Table S8**.

Table 4. Simple mediation analyses assessing the potential role of cytokines during pregnancy as mediators of the
(exercisers with >75% attendance) and metabolic parameters. Schematic diagram in **Figure S2.**

		Effect of the predictor on the mediator (a-path)			Effect of mediator on the outcome (b-path)			
Mediators (M)	**Outcomes** (Y)	**B**	**95% CI**		**B**	**95% CI**		
Change baseline to 33rdweek (n=44)	***Change baseline to 33rdweek (n=44)***							
Interleukin 8	Cholesterol[a]	**6.84**	**0.24**	**13.42**	**-1.34**	**-2.37**	**-0.31**	1
	LDL-C[a]	**7.23**	**0.77**	**13.69**	**-1.23**	**-2.16**	**-0.30**	4
Interleukin 10	Triglycerides	7.60	-0.80	15.99	0.93	-0.03	1.89	7
	HDL-C[a]	**9.49**	**0.31**	**18.67**	**-0.31**	**-0.52**	**-0.10**	2
Interleukin 1β	HDL-C	-0.99	-3.18	1.19	**-1.22**	**-2.11**	**-0.33**	-1
Change baseline to birth (n=34)	***Birth (n=34)***							
Interleukin 1β	Cholesterol	-1.92	-4.38	0.53	5.69	-0.11	11.49	5
Arterial cord serum (n=21)	***Arterial cord serum (n=21)***							
Interleukin 6[a]	Glucose	**-5.76**	**-10.04**	**-1.48**	1.27	-1.34	3.89	-1
Venous cord serum (n=27)	***Venous cord serum (n=27)***							
Tumour necrosis factor-α	Cholesterol[a]	**-4.68**	**-8.60**	**-0.76**	0.06	-0.03	0.95	0
Tumour necrosis factor-α	Phospholipids[a]	**-5.26**	**-9.11**	**-1.41**	1.68	-0.83	4.19	-4

B, unstandardized regression coefficient; CI, confidence interval; HDL-C, high density lipoprotein-cholesterol; LDL-
Confidence intervals are shown as 95% bias corrected and accelerated CI, and are based on 5000 bootstrap samples
performed on extreme outliers of predictors/outcomes. Multiple time point analyses were adjusted for baseline value
gestational week at post-test assessments (i.e. at 33rd week or birth), and maternal age. Single time point analyses were
and type of deliver. The results remained similar after additionally adjusting for the Mediterranean diet score (MDS
epidural anaesthesia. The indirect effect of exercise in cholesterol and LDL-C (via IL-8) was independent of pre-pregna

DISCUSSION

Evidence regarding the role of exercise on maternal and neonatal foetal health, and about how exercise-induced stimuli could be translated into metabolic adaptations, continues to be scarce and elusive. This is the first study exploring i) how a novel, well-designed, tightly-supervised, and tailored concurrent exercise intervention modulates not only maternal but also foetal metabolism, and ii) potential cytokines mediating its effects on metabolism.

Effects of exercise on metabolism

Despite appreciable within-group changes on few outcomes, our main findings indicate that exercise did not induce meaningful effects on maternal and cord serum immunometabolic biomarkers, except for a slight decrease in maternal glucose at delivery and arterial serum glucose. Some previous exercise programs have also failed -or shown limited success- to improve glycaemic control[23-28], while others were successful to reduce systemic glucose and insulin levels, and prevalence of GDM[10,12,14-17]. Importantly, these reductions were mainly driven by women with obesity and diabetes, as recently highlighted by a meta-analysis[9]. Regarding lipid metabolism[12,14,25,26], only Ramírez-Vélez, et al. [13] showed that concurrent exercise reduced systemic LDL-C and triglycerides in normal-weight pregnant women. Concerning foetal metabolites, no other studies are available to compare our results.

While the efficacy of exercise for GDM management is more evident, its effects on lipid metabolism -in women with/without impaired metabolic phenotype- appears to be limited. Experimental limitations along with discrepancies in procedures and exercise protocols, compliance, metabolic phenotype, etc. are very likely to explain this equivocal evidence. Why exercise was not effective in this context is the million-dollar question. We believe that pregnancy itself is an exceptional stimulus[1-3], which implies strong physiological responses that might mask some of the effects of exercise (rather than being non-effective). Additionally, parental environment influences powerfully intrauterine programming during early pregnancy and pre-conception[7,29]. Thus, implementing parental exercise before and in early pregnancy, that is when the key "modifiable" and most vulnerable biological processes take place[2,29], might be more effective than starting at the 17th week. Moreover, exercise has shown stronger effects

in more adverse phenotypes (e.g. obesity and GDM: greater room for change)[9,10,12,29], and most of our participants were healthy and normal-weight (70%). Lastly, mechanisms (e.g. epigenetics) by which the effects of exercise might be translated into maternal and foetal metabolic changes[7,20,40], or potential mediators (e.g. cytokines) which might indirectly drive its effects on metabolic phenotype[20,41], could be unperceived. This leaded us to further explore cytokines in pregnancy.

Cytokines as mediators of maternal-foetal metabolism

To better understand the role of cytokines in pregnancy, we firstly tested their associations with metabolic outcomes. Interestingly, most of the associations were similar in the exercise and control women, while some associations were exclusively for exercisers. For instance, an increase in IL-6 (baseline-birth) was related to lower maternal glucose at delivery only in exercisers (partially explained by labour-induced IL-6 increases for myometrium contractions[3]), and exercisers with higher venous cord serum fractalkine showed increased cord serum HDL-C and LDL-C. Only another study[41] in overweight-obese women addressed this topic, showing that TNF-α and IL-1β increases were related to impaired glucose-insulin axis in more inactive women. While the clinical interpretation of these associations is limited by the lacking evidence and warrants further research, these findings suggest that cytokines might play a regulator role –partly modulated by exercise/PA– in maternal-foetal metabolism.

To gain further insight into their capacity to drive metabolic changes in pregnancy, we explored the mediator role of those cytokines which were clearly influenced by exercise in a previous study[8], and were statistically related to immunometabolic outcomes in the aforementioned exploratory analyses. Noteworthily, our mediation analyses showed that exercise reduced maternal total cholesterol and LDL-C gains from baseline to 33rd week via an increase in IL-8. Considering that exacerbated dyslipidaemia in pregnancy can lead to preeclampsia, placental lesions and endothelial dysfunction[1,4], these findings suggest a potential mechanism (i.e. IL-8 increase) by which exercise might indirectly improve lipid metabolism regulation[13], and thus avoid related-adverse outcomes[1,4]. Whether reductions in systemic lipids via IL-8 could be related to the pro-angiogenic[20,42] and anti-atherosclerotic[43] effects of exercise at local (skeletal muscle and placenta) or systemic level[20], remains undetermined.

At first glance, the analyses also suggest that exercise reduced HDL-C from baseline to 33rd week through an increase in IL-10, which was unexpected since IL-10 acts as an anti-inflammatory vascular protector[5], and consequently, higher HDL-C concentrations might be expected. However, the IL-10 increase was not related to a decrease in HDL-C in the exercise group, but only in controls. Why IL-10 was related to lower HDL-C in controls, and whether the exercise-induced IL-10 might represent a protective mechanism to limit HDL-C decreases and related abnormalities[4], requires further research. Other indirect effects mediated by cytokines –such as changes in insulin sensitivity with increased PA via IL-6[41], could have been unperceived due to the limited statistical power in mediation analyses and/or confounding factors.

Overall, these findings postulate a role for these cytokines as potential messengers of the exercise-induced effects into metabolic changes during pregnancy, as suggested by previous reviews in pregnant women[20] and rodents[2,7,20]. Although it might be speculated that the origin of these exercise-induced cytokines –based in previous evidence[8,20,31,41] and our results– are the skeletal muscle cells, the specific source and the contribution of other organs remain uncertain. All this information, along with new studies addressing current knowledge gaps, is of basic and clinical interest to better understand how maternal exercise can influence maternal-foetal metabolism, and to develop/implement more tailored and effective exercise interventions during pregnancy.

LIMITATIONS

Selection bias might be present since the initial random component could not be finally kept (difficulties to maintain controls), as plausibly reflected by slight baseline differences between groups in insulin and insulin resistance. However, these differences did not appear to influence the results, and we accounted for them and potential confounders in all the analyses. Of note, the methodological quality of studies is likely to be more determinant than the absence of a randomized design itself[33]. Moreover, only interested women participated, and the sample size was “relatively” small. Other models/analyses (e.g. transcriptomics) from specific tissues (e.g. muscles and placenta) will have allowed us to better understand these findings. Despite the fact that most women are healthy and normal-weight, and we have deeply considered this issue in

sensitivity analyses, the heterogeneity of our sample (≈30% were overweight-obese women at pre-pregnancy) might hinder the interpretation of some results. Some strengths also deserve to be commented: i) this exercise intervention is a novel-tailored program designed by an expert multidisciplinary team following the latest guidelines in pregnancy[18], and based on last evidence[21,22]; ii) all sessions were strictly supervised, and the intensity, attendance and other parameters were monitored periodically; iii) this is the first time that the mediator role of cytokines on exercise is considered during pregnancy; iv) the immunometabolic parameters were assessed at multiple time points, and in both arterial and venous cord serum; and v) we have deeply considered important confounders such as objectively measured ST/PA (7 days, ≥10hours/day), sleep patterns and dietary habits, among others.

CONCLUSIONS

This exercise program was not effective to induce meaningful changes on maternal and foetal immunometabolic markers during pregnancy. However, exercise indirectly reduced maternal total cholesterol and LDL-C gains via an increase in IL-8. Future studies conducted earlier in pregnancy, targeting skeletal muscle and placenta, and exploring how exercise-induced stimuli affect and translate into metabolic adaptations, are necessary.

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SUPPLEMENTARY MATERIAL

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Figure S6. Kinetics during pregnancy of the immunometabolic markers concentrations implicated in the indirect effect of exercise on lipid markers.

Figure S7. Kinetics during pregnancy of the immunometabolic markers implicated in the indirect effect of exercise on HDL-C

Figure S8. Within-individual cytokines and C-reactive protein concentrations during pregnancy

Appendix A. *Talks provided to pregnant women*

During the duration of the intervention, the research team gave 7 lectures to pregnant women from both groups (exercise and control group) about: 1) the benefits of physical exercise for a better pregnancy, prevention and treatment of cardiovascular diseases and excessive weight gain; 2) ergonomic advises, exercises to perform at home and strategies to increase their daily physical activity levels; 3) the benefits of the Mediterranean Diet and adequate nutritional habits during pregnancy; 4) how to avoid toxics and chemicals during the pregnancy and breastfeeding; 5) pregnancy, postpartum and sex; 6) physical and mental preparation for the labour, what to expect; 7) nutritional education towards breastfeeding. We also used these conferences to maintain control group fidelity until the end of the program.

Appendix B. *Analyses of inflammatory markers*

We employed Luminex xMAP technology based on MILLIPLEX MAP kits to assess the cytokine profile from the collected serum in pregnant women. Luminex xMAP technology (Millipore, Darmstadt, Germany) is a mix of three existing and proved technologies: use of microspheres, flow cytometry, and laser technology, mixing digital signal processing and traditional chemistry immunoassay. Because of robust multiplexing, xMAP technology potentially delivers more data in less time than other bioassay products, with comparable results with enzyme linked immunosorbent assay and microarray. The technology offers several other distinct advantages over traditional methods such as speed and high throughput, versatility, flexibility, accuracy, and reproducibility. Particularly, for maternal pro-inflammatory and anti-inflammatory (fractalkine, interleukin-1β, interleukin-6, interleukin-8, interleukin-10, interferon-γ and tumour necrosis factor-α) determination, we used Human Sepsis Magnetic Bead Panel 3 Multiplex Assay (cat. No. HTH17MAG-14K). We prepared samples, reagents, and standards by following the manufacturer's instructions. Equipment settings: 50 events per bead, gate settings: 8,000-15,000, time out 60 seconds. Plate was read on LABScan 100 analyzer (Luminex Corporation, Texas, USA) with xPONENT software for data acquisition. The average values for each set of duplicate samples or standards were

within 15% of the mean. We determined cytokine concentrations by comparing the mean of duplicate samples with the standard curve for each assay.

Appendix C. *Outlier detection and management*

Nowadays, the presence of outliers is one of the most enduring and pervasive methodological changes in biomedical science research[2-4]. Worryingly, there is a lack of consensus about how addressing outliers (i.e. how defining, identifying and handling them). Since the decisions that researches make about this issue have important implications, we have included this section to promote transparency and the critical interpretation of the results, as previously recommended by several authors[2-4]. Although no specific guidelines exist about how addressing outliers, several studies[2-9] (especially that one from Aguinis, et al. [4]) have previously provided smart advices and recommendations to address them in the best possible way. Accordingly, the different steps to address outliers in the present study have been performed proceeding with the following recommendations. We have identified and handled outliers according to the basis for regressions, which are the main analyses involved in this study.

Error outliers

During the assessments at the different time points, questionnaires and tests (where errors related to data recording, coding, manipulation, etc. were likely and easily observed) were checked to identify clear error outliers, and correct them immediately by asking women, repeating the corresponding test, etc.

When lacking, misleading or inaccurate data, was identified posteriori (up to 2 weeks after the assessments), women were contacted to ensure the accuracy of these data points, or to correct these potential outliers (whenever appropriate for data) in the respective database. Singles construct techniques (box plots, descriptive statistics, percentage analyses, etc.) were performed to initially identify error outliers. Subsequently, we also employed multiple construct techniques to identify error outliers. Particularly, we identified error outliers based on the outlyingness of the observation in term of its residual score and scores of predictors (leverage values, Cook´s distance and standardized differences in beta, and standardized and studentized residuals). When it was not possible/appropriate to correct these data points, and we were sure that their

inaccuracy was related to human errors, device malfunction, miscalculations or similar circumstances (i.e. we had determined the cause of the identified outlying observation), these error outliers were removed from the respective database. Since these potential error outliers could have been caused by inherent variability in the data (in this case they would represent a legitimate part of the population), we were very prudent when identifying and handling them. We paid special attention to the reasoning behind the classification of data points as error outliers.

Interesting outliers

After the application of this first filter to the database, there were several remaining interesting outliers, which required additional analyses in depth. Thereby, we aimed at analysing these interesting outliers with quantitative approaches (e.g., we tried to analyse differences in how predictors were able to predict high and low outlier scores). However, the number of outliers was minimum, and only appreciable in few outcomes, which prevented us from performing these analyses properly. As consequence, we did not finally perform these analyses.

Influential outliers

Since it is not legitimate to simply drop the remaining potential outliers from the analyses (they tend to increase error variance, reduce the power of statistical test, etc.), nor plainly deleting them without any basis (they could be part of the inherent variability of the distribution of data), we analysed more in depth the influence of these outliers in the model. Aimed at checking their influence, we analysed how the deletion of specific outliers could affect the change of the model fit (e.g., changes in R^2; model fit outliers), parameters estimates (intercept, slope, regression coefficients, etc.; prediction outliers) and the assumptions of the model. If these remaining unusual cases were not finally identified as influential outliers, or they were identified but influenced the model slightly, these potential outliers were not handled (as observed in some outcomes the **Table 3**). In this case, these unusual data points were dropped in the analyses since they did not affect either the results or assumptions of the tests, and they could be caused by inherent variability in the data. By contrast, if these remaining unusual cases were confirmed as influential outliers which affected the model fit and parameter estimates (as appreciable in the **Table 3**), those influential outliers we handled. As general rule, no handling of outliers was performed in predictor variables since residual values from

models were small, and/or potential extreme values from predictors did not/scarcely influence the fit and coefficients of the model[4] (checked with changes in the coefficient of determinations, changes in the intercepts and slopes, Cook´s distances, centered leverage values, DFBETAS values, and the studentized residuals).

In order to handle the aforementioned influential outliers (when identified), a subtle variation of winsorizing [convert back from a z-score: replacing extreme scores (z>2.58; value equivalent to a probable outlier) with a score equivalent to ±2.58 standard deviations from the mean] was employed to handle these outliers. After handing these outliers, data distribution improved, and some of the problematic issues related to the assumptions of some models disappeared. Subsequently, data preparation was employed for those characterized by remaining asymmetry (skewness, kurtosis, etc.) of outcomes, and the violation of some assumptions related to the generalization of the results. Specifically, optimum Box-Cox transformations were used to reduce the impact of potential source of bias, and improve the goodness of fit of the data. After dealing with these "problematic" outcomes, the results remained similar (but with better and more symmetrical distribution of data) to the analyses without data preparation (i.e. without handling of outliers or/and applying Box-Cox transformations).

Appendix D. *Reasons for losses and exclusions during the enrolment and follow-up*

From the 159 women who participated in the study and were allocated to the control (n=87) or exercise (n=72) group, 10 controls dropped out of the study (lost to follow-up) because of: moving to another city (n=1), unwillingness to continue (n=7) or unknown reasons (n=2). In the control group, 33 women did not come to the evaluation (33rd week) because of personal reasons. Data loss (n=10) at delivery was related to women who did not contact us, attended private hospitals, or midwives who did not collect data/samples. In the exercise group, none woman dropped out the study. From the 72 women in the exercise group, 2 women did not come to the 33rd week, and only 48 women attended >75% of the sessions (58 women attended >66% of the sessions). In summary, 88 women (control n=44, exercise n=44) were included in the main analyses of the current study. Because of funding limitations, the glycaemic and lipid markers at delivery (n=39), and inflammatory markers during the whole pregnancy (n=48), could only be analysed in a subsample of pregnant women (n=66). This subsample of women

-1st wave of assessment-, was the only wave where participants were randomized (before breaking the random component). Intention-to-treat analyses were not performed due to their inappropriateness **(see Appendix F).**

Appendix E. *Intention-to-treat analyses*

In order to investigate more realistically the effectiveness of a concurrent exercise-training program on maternal and foetal immunometabolic markers when applied to the clinical practice, we aimed to replicate the aforementioned statistical analyses (Table 3) on an intention-to-treat basis (data not shown), as recommended by the CONSORT guidelines.

The number of missing cases in outcomes of interest at the different time points are shown below:

Maternal serum (16th week of gestation)

-Glucose,	missing cases (all women) n=20, **lost%=12.6**
-**Insulin,**	missing cases (all women) n=21, **lost%=13.2**
-**Insulin resistance (HOMA-IR),**	missing cases (all women) n=23, **lost%=14.5**
-**B-cell function (HOMA-B),**	missing cases (all women) n=23, **lost%=14.5**
-**Total cholesterol,**	missing cases (all women) n=19, **lost%=11.9**
-**Triglycerides,**	missing cases (all women) n=19, **lost%=11.9**
-**HDL-C,**	missing cases (all women) n=19, **lost%=11.9**
-**LDL-C,**	missing cases (all women) n=19, **lost%=11.9**
-**Phospholipids,**	missing cases (all women) n=99, **lost%=62.3**
-**Cortisol,**	missing cases (all women) n=21, **lost%=13.2**
-**C-reactive protein,**	missing cases (all women) n=20, **lost%=12.6**

Maternal serum (34th week of gestation)

-Glucose,	missing cases (all women) n=44, **lost%=27.7**
-**Insulin,**	missing cases (all women) n=45, **lost%=28.3**
-**Insulin resistance (HOMA-IR),**	missing cases (all women) n=46, **lost%=28.9**
-**B-cell function (HOMA-B),**	missing cases (all women) n=46, **lost%=28.9**
-**Total cholesterol,**	missing cases (all women) n=44, **lost%=27.7**
-**Triglycerides,**	missing cases (all women) n=44, **lost%=27.7**
-**HDL-C,**	missing cases (all women) n=44, **lost%=27.7**
-**LDL-C,**	missing cases (all women) n=44, **lost%=27.7**
-**Phospholipids,**	missing cases (all women) n=103, **lost%=64.8**
-**Cortisol,**	missing cases (all women) n=45, **lost%=28.3**
-**C-reactive protein,**	missing cases (all women) n=46, **lost%=28.9**

The lacking data in cases of immunometabolic outcomes measured at 16th and 34th week was "missing completely at random" (**MCAR**), except for cases from "phospholipids" which were "missing at random (**MAR**)" -*due to funding limitations, only a subsample of women (the 1st wave of women who were initially randomized) were analysed-.*

Maternal serum (delivery)

-Glucose, missing cases (all women) n=113, **lost%=71.1**
-**Total cholesterol,** missing cases (all women) n=113, **lost%=71.1**
-**Triglycerides,** missing cases (all women) n=113, **lost%=71.1**
-**HDL-C,** missing cases (all women) n=113, **lost%=71.1**
-**LDL-C,** missing cases (all women n=113, **lost%=71.1**
-**Phospholipids,** missing cases (all women) n=113, **lost%=71.1**

Arterial cord serum (delivery)

-Glucose, missing cases (all women) n=131, **lost%=82.4**
-**Total cholesterol,** missing cases (all women) n=131, **lost%=82.4**
-**Triglycerides,** missing cases (all women) n=134, **lost%=84.3**
-**HDL-C,** missing cases (all women) n=131, **lost%=82.4**
-**LDL-C,** missing cases (all women) n=131, **lost%=82.4**
-**Phospholipids,** missing cases (all women) n=134, **lost%=84.3**

Venous cord serum (delivery)

-Glucose, missing cases (all women) n=118, **lost%=74.2**
-**Total cholesterol,** missing cases (all women) n=118, **lost%=74.2**
-**Triglycerides,** missing cases (all women) n=121, **lost%=76.2**
-**HDL-C,** missing cases (all women) n=118, **lost%=74.2**
-**LDL-C,** missing cases (all women) n=118, **lost%=74.2**
-**Phospholipids,** missing cases (all women) n=121, **lost%=76.2**

The lacking data in most cases of immunometabolic outcomes measured at delivery was **MAR** -due to funding limitations, only a subsample of women (the 1st wave of women who were initially randomized) were analysed-. In order to analyse how "accurate" was the imputed data in those missing cases on immunometabolic markers outcomes, we compared the relative percentage of variation of the descriptive statistics (mean, and standard deviation) from the database before and after imputation. Overall, we observed that the variation in % of these parameters was:

Maternal serum (16th week of gestation)

-Glucose, %mean=0.9, %SD=35.7

-Insulin,	%mean=16, %SD=44.1
-Insulin resistance (HOMA-IR),	%mean=20.2, %SD=49.7
-B-cell function (HOMA-B),	%mean=40.3, %SD=65.6
-Total cholesterol,	%mean=0, %SD=6.1
-Triglycerides,	%mean=0.1, %SD=8.3
-HDL-C,	%mean=0.1, %SD=5.8
-LDL-C,	%mean=0.1, %SD=5.9
-Phospholipids,	%mean=19.2, %SD=96.8
-Cortisol,	%mean=0.4, %SD=0.8
-C-reactive protein,	%mean=4.7, %SD=25.9

Maternal serum (34th week of gestation)

-Glucose,	%mean=0.8, %SD=24.1
-Insulin,	%mean=28.5, %SD=81.7
-Insulin resistance (HOMA-IR),	%mean=40.3, %SD=88.6
-B-cell function (HOMA-B),	%mean=133.3, %SD=88.7
-Total cholesterol,	%mean=0.1, %SD=14.2
-Triglycerides,	%mean=2.4, %SD=13.3
-HDL-C,	%mean=0.2, %SD=6.2
-LDL-C,	%mean=1, %SD=13.3
-Phospholipids,	%mean=78.3, %SD=105.2
-Cortisol,	%mean=0.6, %SD=8
-C-reactive protein,	%mean=1.5, %SD=6.5

Maternal serum (delivery)

-Glucose,	%mean=10, %SD=56.9
-Total cholesterol,	%mean=0, %SD=46.4
-Triglycerides,	%mean=2.5, %SD=57.6
-HDL-C,	%mean=60.5, %SD=56.9
-LDL-C,	%mean=84.4, %SD=95.6
-Phospholipids,	%mean=0.1, %SD=46.3

Arterial cord serum (delivery)

-Glucose,	%mean=0.5, %SD=57.9
-Total cholesterol,	%mean=1, %SD=62.3
-Triglycerides,	%mean=0.5, %SD=61.9
-HDL-C,	%mean=0.7, %SD=56.9
-LDL-C,	%mean=1.3, %SD=53.3
-Phospholipids,	%mean=0, %SD=60.1

Venous cord serum (delivery)

-Glucose,	%mean=0.7, %SD=51.1
-Total cholesterol,	%mean=8.7, %SD=70.4
-Triglycerides,	%mean=0.6, %SD=52.9
-HDL-C,	%mean=0.2, %SD=51.3
-LDL-C,	%mean=12.5, %SD=79.2
-Phospholipids,	%mean=1.1, %SD=57.2

Considering that more than 20% of cases were missing in most outcomes (also in those outcomes not shown above *–i.e. changes from baseline to 33rd week and birth-),* and some authors do not recommend to perform imputations in this context[10], we decided not to use imputed data from these outcomes. This decision was supported by the fact that imputed parameters estimates (mean and standard deviation) were in general inaccurate, especially in those immunometabolic outcomes measured at delivery. Imputed data related to cytokines –employed for secondary analyses (**Tables S2-S5**)– was not either used (see Acosta-Manzano, et al.[11]). All in all, we decided not to show imputed data, either replicate these analyses on an intention-to-treat basis, to avoid potential erroneous conclusions and facilitate the interpretation of the results to readers.

Table S1. Inclusion and exclusion criteria in the GESTAFIT project

Inclusion criteria
- Pregnant women aged 25-40 years old with a normal pregnancy course.
- Answering "no" to all questions on the PARmed-X for pregnancy.
- Being able to walk without assistance.
- Being able to read and write properly.
- Informed consent: Being capable and willing to provide written consent.
Exclusion criteria
- Having acute or terminal illness.
- Having malnutrition.
- Being unable to conduct tests for assessing physical fitness or exercise during pregnancy.
- Having pregnancy risk factors (such as hypertension, type 2 diabetes, etc.).
- Having a multiple pregnancy.
- Having chromosopathy or foetal malformations.
- Having uterine growth restriction.
- Having foetal death.
- Having upper or lower extremity fracture in the past 3 months.
- Suffering neuromuscular disease or presence of drugs affecting neuromuscular function.
- Being registered in another exercise program.
- Performing more than 300 minutes of at least moderate physical activity per week.
-Being engaged in another physical exercise program
- Being unwilling either to complete the study requirements or to be randomized into the control or exercise group.

Table S2. Associations of cytokines changes (baseline - 33rd week) with changes in metabolic parameters (n=44 for all outcome

Predictors (changes from baseline to 33rd	Outcomes (changes from baseline to 33rd week)	Model 1				Model 2	Predictors (changes from baseline to 33rd week)	Outcomes (changes from baseline to 33rd week)
		B	SE	β	p-value	p-value		
Fractalkine	Glucose[a]	0.00	0.01	-0.06	0.70	0.79	Interleukin 1 B	Glucose[a]
	Insulin[ab]	0.00	0.00	0.15	0.31	0.31		Insulin[ab]
	Insulin resistance[ab]	0.00	0.00	0.13	0.39	0.40		Insulin resistance[ab]
	B cell function[ab]	0.00	0.00	-0.04	0.66	0.79		B cell function[ab]
	Cholesterol[a]	-0.03	0.05	-0.09	0.56	0.56		Cholesterol[a]
	Triglycerides[a]	-0.01	0.06	-0.02	0.88	0.89		Triglycerides[a]
	HDL-C[a]	-0.02	0.01	-0.21	0.19	0.16		HDL-C[a]
	LDL-C[a]	-0.01	0.04	-0.03	0.86	0.87		LDL-C[a]
	Phospholipids	-0.06	0.06	-0.10	0.37	0.34		Phospholipids
	Cortisol	-0.01	0.01	-0.18	0.14	0.13		Cortisol
Interleukin 6	Glucose[a]	-0.20	0.30	-0.10	0.51	0.31	Interferon-γ	Glucose[a]
	Insulin[ab]	-0.01	0.01	-0.06	0.69	0.71		Insulin[ab]
	Insulin resistance[ab]	-0.01	0.01	-0.07	0.66	0.70		Insulin resistance[ab]
	B cell function[ab]	0.02	0.03	0.06	0.51	0.66		B cell function[ab]
	Cholesterol[a]	-1.30	1.51	-0.14	0.39	0.40		Cholesterol[a]
	Triglycerides[a]	-3.07	1.78	-0.28	0.09	0.10		Triglycerides[a]
	HDL-C[a]	-0.72	0.40	-0.30	0.08	0.07		HDL-C[a]
	LDL-C[a]	0.36	1.37	0.04	0.79	0.80		LDL-C[a]
	Phospholipids	-4.32	1.99	-0.26	**0.04**	**0.04**		Phospholipids
	Cortisol	-0.24	0.17	-0.18	0.17	0.17		Cortisol
Interleukin 8	Glucose[a]	0.16	0.10	0.23	0.09	0.11	Tumour necrosis factor-α	Glucose[a]
	Insulin[ab]	0.00	0.00	0.15	0.31	0.33		Insulin[ab]
	Insulin resistance[ab]	0.00	0.00	0.19	0.20	0.24		Insulin resistance[ab]
	B cell function[ab]	0.00	0.01	-0.04	0.69	0.90		B cell function[ab]
	Cholesterol[a]	-1.17	0.48	-0.35	**0.02**	**0.02**		Cholesterol[a]
	Triglycerides[a]	0.87	0.62	0.21	0.17	0.17		Triglycerides[a]
	HDL-C[a]	-0.24	0.14	-0.27	0.10	0.09		HDL-C[a]
	LDL-C[a]	-1.17	0.43	-0.39	**0.01**	**0.01**		LDL-C[a]
	Phospholipids	-0.52	0.68	-0.09	0.45	0.38		Phospholipids
	Cortisol	0.06	0.06	0.13	0.31	0.36		Cortisol
Interleukin 10	Glucose[a]	-0.01	0.07	-0.01	0.92	0.70	C-reactive protein (n=88)	Glucose[a]
	Insulin[ab]	0.00	0.00	0.12	0.40	0.37		Insulin[ab]
	Insulin resistance[ab]	0.00	0.00	0.11	0.43	0.38		Insulin resistance[ab]
	B cell function[ab]	0.00	0.01	0.00	0.99	0.82		B cell function[ab]
	Cholesterol[a]	-0.52	0.38	-0.21	0.17	0.17		Cholesterol[a]
	Triglycerides[a]	1.01	0.45	0.33	**0.03**	**0.03**		Triglycerides[a]
	HDL-C[a]	-0.28	0.10	-0.42	**0.01**	**0.01**		HDL-C[a]
	LDL-C[a]	-0.45	0.34	-0.20	0.19	0.18		LDL-C[a]
	Phospholipids	-0.12	0.51	-0.03	0.81	0.93		Phospholipids
	Cortisol	-0.02	0.05	-0.07	0.60	0.67		Cortisol

Study V

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; HDL-C, high density lipo
lipoprotein-cholesterol. A subtle variation of winsorizing was performed on extreme outliers on metabolic parameters[a]. [b] C
conducted. Model 1 was adjusted for the baseline values of the respective cytokines and gestational week at 33rd week; and
maternal age. After controlling for the familywise error rate (Hochberg procedure), none significant associations remained
grouped by the intervention group (control vs. exercise group), the results remained similar, except for few cases. Particularly,
associated with changes in phospholipids and HDL-C in controls, respectively (all, p<0.05); whereas in those women from th
positively associated with changes in cortisol (p=0.05). When these analyses were grouped by the weight-status (normal-we
results remained similar, except for few cases. Particularly, only in overweight-obese women, changes in IL-6 were inversely as
changes in IL-8 were inversely associated cholesterol and LDL-C, and the changes in TNF-α were inversely associated with pho

Table S3. Associations of cytokines changes (baseline-birth) with serum metabolic parameters (birth) (n=34)

Predictors (changes from baseline to birth)	Outcomes (birth)	Model 1				Model 2
		B	SE	β	p-value	p-value
Fractalkine	Glucose[ab]	0.00	0.00	-0.01	0.98	0.77
	Cholesterol[ab]	0.06	0.12	0.09	0.60	0.62
	Triglycerides[a]	0.04	0.15	0.05	0.77	0.47
	HDL-C	-0.03	0.07	-0.06	0.71	0.43
	LDL-C[b]	0.00	0.00	-0.08	0.65	0.61
	Phospholipids	0.00	0.11	-0.01	0.97	0.90
Interleukin 6	Glucose[ab]	-0.01	0.01	-0.14	0.47	0.22
	Cholesterol[ab]	-0.16	0.62	-0.04	0.79	0.75
	Triglycerides[a]	-0.54	0.74	-0.12	0.47	0.75
	HDL-C	-0.12	0.34	-0.06	0.72	0.37
	LDL-C[b]	-0.01	0.01	-0.12	0.49	0.43
	Phospholipids	-0.09	0.55	-0.03	0.87	0.78
Interleukin 8	Glucose[ab]	-0.02	0.01	-0.32	0.08	**0.05**
	Cholesterol[ab]	-0.36	0.58	-0.10	0.54	0.54
	Triglycerides[a]	-0.38	0.70	-0.09	0.59	0.62
	HDL-C	0.18	0.34	0.10	0.60	0.66
	LDL-C[b]	0.02	0.01	0.24	0.16	0.17
	Phospholipids	0.03	0.53	0.01	0.96	0.97
Interleukin 10	Glucose[ab]	-0.01	0.01	-0.12	0.56	0.58
	Cholesterol[ab]	0.42	0.64	0.12	0.52	0.53
	Triglycerides[a]	0.15	0.77	0.04	0.85	0.86
	HDL-C	-0.04	0.35	-0.02	0.90	0.81
	LDL-C[b]	0.00	0.01	-0.05	0.80	0.79
	Phospholipids	0.28	0.55	0.09	0.62	0.62
Interleukin 1 B	Glucose[ab]	0.01	0.06	0.04	0.83	0.82
	Cholesterol[ab]	5.5	2.6	0.34	**0.05**	**0.05**
	Triglycerides[a]	1.26	3.33	0.07	0.71	0.64
	HDL-C	1.87	1.43	0.23	0.20	0.22
	LDL-C[b]	0.05	0.05	0.18	0.35	0.35
	Phospholipids	2.76	2.33	0.20	0.25	0.26
Interferon-γ	Glucose[ab]	0.00	0.02	-0.02	0.90	0.95
	Cholesterol[ab]	0.20	0.91	0.04	0.83	0.82
	Triglycerides[a]	-0.48	1.11	-0.08	0.67	0.59
	HDL-C	0.11	0.53	0.04	0.84	0.79
	LDL-C[b]	0.01	0.02	0.12	0.51	0.51
	Phospholipids	-0.47	0.82	-0.10	0.57	0.59
Tumour necrosis factor-α	Glucose[ab]	-0.11	0.07	-0.29	0.11	0.09
	Cholesterol[ab]	-0.76	3.6	-0.04	0.84	0.84
	Triglycerides[a]	-2.80	4.30	-0.12	0.52	0.56
	HDL-C	-0.14	1.88	-0.01	0.94	0.83
	LDL-C[b]	0.02	0.07	0.04	0.83	0.83
	Phospholipids	-3.52	2.97	-0.20	0.25	0.25

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol. A subtle variation of winsorizing was performed on extreme outliers on metabolic parameters[a]. [b] Optimum Box-Cox transformations were conducted. Model 1 was adjusted for the baseline values of the respective cytokines and gestational week at 33rd week; model 2 was additionally adjusted for maternal age. After controlling for the familywise error rate (Hochberg procedure), none significant associations remained significant. When these analyses were grouped by the intervention group, the results remained similar, except for changes in IL-6 which were inversely associated with changes in glucose in the exercise group only (p=0.03). When these analyses were grouped by weight-status (normal-weight vs. overweight-obese women), the results remained similar, except for changes in IFN-γ which were inversely associated with changes in glucose in overweight-obese women (p=0.006).

Table S4. Associations of arterial cord serum cytokines with arterial cord serum metabolic parameters (n=24).

Predictors (arterial cord serum)	Outcomes (arterial cord serum)	Model 1				Model 2
		B	SE	β	p-value	p-value
Fractalkine	Glucose	-0.02	0.04	-0.12	0.61	0.42
	Cholesterol[a]	0.04	0.04	0.20	0.36	0.40
	Triglycerides[b]	0.00	0.00	0.16	0.48	0.66
	HDL-C	0.00	0.02	-0.03	0.91	0.89
	LDL-C[ab]	0.00	0.00	0.31	0.15	0.24
	Phospholipids	0.01	0.05	0.04	0.86	0.90
Interleukin 6	Glucose	2.19	0.96	0.44	**0.03**	**0.04**
	Cholesterol[a]	-0.80	1.06	-0.16	0.46	0.45
	Triglycerides[b]	0.00	0.05	-0.01	0.96	0.85
	HDL-C	-0.45	0.40	-0.23	0.28	0.28
	LDL-C[ab]	0.02	0.04	0.09	0.66	0.75
	Phospholipids	-1.63	1.12	-0.33	0.16	0.09
Interleukin 8	Glucose	-0.18	0.17	-0.23	0.31	0.52
	Cholesterol[a]	-0.20	0.17	-0.25	0.26	0.29
	Triglycerides[b]	0.01	0.01	0.13	0.57	0.15
	HDL-C	-0.06	0.07	-0.18	0.39	0.39
	LDL-C[ab]	-0.01	0.01	-0.34	0.11	0.25
	Phospholipids	-0.14	0.21	-0.16	0.51	0.96
Interleukin 10	Glucose	-0.40	1.39	-0.07	0.78	0.91
	Cholesterol[a]	0.40	1.38	0.07	0.78	0.69
	Triglycerides[b]	0.05	0.07	0.17	0.48	0.11
	HDL-C	-0.33	0.53	-0.13	0.54	0.56
	LDL-C[ab]	0.05	0.05	0.19	0.40	0.14
	Phospholipids	-0.77	1.61	-0.11	0.64	0.88
Interleukin 1 B	Glucose	-4.77	7.10	-0.16	0.51	0.53
	Cholesterol[a]	-7.48	6.97	-0.25	0.30	0.31
	Triglycerides[b]	-0.65	0.43	-0.34	0.15	0.20
	HDL-C	-2.17	2.71	-0.18	0.43	0.45
	LDL-C[ab]	-0.33	0.28	-0.27	0.24	0.24
	Phospholipids	4.37	11.17	0.10	0.70	0.47
Interferon-γ	Glucose	0.86	3.50	0.06	0.81	0.89
	Cholesterol[a]	2.35	3.46	0.15	0.50	0.56
	Triglycerides[b]	0.37	0.18	0.42	0.05	0.23
	HDL-C	1.03	1.32	0.16	0.45	0.46
	LDL-C[ab]	0.19	0.13	0.29	0.18	0.35
	Phospholipids	11.45	3.97	0.56	**0.01**	0.06
Tumour necrosis factor-α	Glucose	1.28	1.49	0.19	0.40	0.56
	Cholesterol[a]	-0.15	1.51	-0.02	0.92	0.86
	Triglycerides[b]	0.03	0.07	0.10	0.66	0.79
	HDL-C	0.43	0.57	0.16	0.46	0.48
	LDL-C[ab]	0.00	0.06	-0.01	0.98	0.69
	Phospholipids	0.47	1.62	0.07	0.78	0.68

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol. A subtle variation of winsorizing was performed on extreme outliers on metabolic parameters[a]. [b] Optimum Box-Cox transformations were conducted. Model 1 was adjusted for the gestational week at birth; and model 2 was additionally adjusted for the type of delivery. After controlling for the familywise error rate (Hochberg procedure), none significant associations remained significant. When these analyses were grouped by the intervention group, the results remained similar, except for arterial cord fractalkine and IFN-γ which were inversely associated with arterial cord cholesterol, LDL-C and HDL-C in the exercise group only (all, $p<0.05$). When these analyses were grouped by weight-status (normal-weight vs. overweight-obese women), the results remained similar, except for arterial cord IL-6 which positively associated with arterial cord glucose in overweight-obese women ($p=0.002$).

Table S5. Associations of venous cord serum cytokines with venous cord serum metabolic parameters (n=33).

Predictors (venous cord serum)	Outcomes (venous cord serum)	Model 1				Model 2
		B	SE	β	p-value	p-value
Fractalkine	Glucose	-0.01	0.03	-0.06	0.73	0.75
	Cholesterol[ab]	0.00	0.00	0.14	0.44	0.56
	Triglycerides[a]	-0.02	0.03	-0.11	0.53	0.42
	HDL-C[a]	0.00	0.02	-0.04	0.84	0.99
	LDL-C[ab]	0.00	0.00	-0.06	0.74	0.56
	Phospholipids[a]	0.05	0.04	0.23	0.23	0.26
Interleukin 6	Glucose	0.72	0.69	0.19	0.30	0.28
	Cholesterol[ab]	0.01	0.03	0.03	0.85	0.94
	Triglycerides[a]	1.21	0.67	0.31	0.08	0.11
	HDL-C[a]	-0.52	0.36	-0.25	0.15	0.22
	LDL-C[ab]	-0.01	0.03	-0.04	0.84	0.61
	Phospholipids[a]	1.81	0.91	0.36	0.06	0.07
Interleukin 8	Glucose	-0.13	0.18	-0.13	0.47	0.50
	Cholesterol[ab]	0.01	0.01	0.15	0.39	0.49
	Triglycerides[a]	0.39	0.17	0.39	0.03	0.05
	HDL-C[a]	-0.02	0.09	-0.05	0.80	0.93
	LDL-C[ab]	0.00	0.01	0.07	0.71	0.86
	Phospholipids[a]	0.35	0.25	0.26	0.17	0.22
Interleukin 10	Glucose	0.26	1.03	0.05	0.80	0.78
	Cholesterol[ab]	0.10	0.05	0.37	**0.03**	**0.05**
	Triglycerides[a]	2.10	0.87	0.40	**0.02**	**0.04**
	HDL-C[a]	0.34	0.54	0.11	0.53	0.41
	LDL-C[ab]	0.07	0.05	0.25	0.16	0.21
	Phospholipids[a]	1.67	1.28	0.24	0.20	0.25
Interleukin 1 B	Glucose	-8.26	4.67	-0.31	0.09	0.10
	Cholesterol[ab]	0.20	0.23	0.15	0.39	0.52
	Triglycerides[a]	6.21	4.41	0.25	0.17	0.24
	HDL-C[a]	-1.88	2.54	-0.13	0.46	0.59
	LDL-C[ab]	0.29	0.23	0.22	0.21	0.30
	Phospholipids[a]	4.84	6.20	0.15	0.44	0.51
Interferon-γ	Glucose	-3.23	3.06	-0.19	0.30	0.32
	Cholesterol[ab]	-0.02	0.15	-0.02	0.90	0.61
	Triglycerides[a]	-3.19	2.89	-0.20	0.28	0.15
	HDL-C[a]	-0.64	1.62	-0.07	0.70	0.94
	LDL-C[ab]	0.04	0.15	0.04	0.82	0.87
	Phospholipids[a]	-3.86	3.98	-0.18	0.34	0.26
Tumour necrosis factor-α	Glucose	-0.10	0.66	-0.03	0.88	0.97
	Cholesterol[ab]	0.06	0.03	0.35	**0.04**	0.13
	Triglycerides[a]	0.07	0.64	0.02	0.91	0.56
	HDL-C[a]	0.17	0.34	0.09	0.63	0.20
	LDL-C[ab]	0.02	0.03	0.10	0.58	0.82
	Phospholipids[a]	1.57	0.83	0.34	0.07	0.10

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol. A subtle variation of winsorizing was performed on extreme outliers on metabolic parameters[a]. [b] Optimum Box-Cox transformations were conducted. After controlling for the familywise error rate (Hochberg procedure), none significant associations remained significant. Model 1 was adjusted for the gestational week at birth; and model 2 was additionally adjusted for the type of delivery. When these analyses were grouped by the intervention group, the results remained similar, except for venous cord IL-6 which was positively associated with venous cord triglycerides (p=0.002).

Study V

Table S6. Per-protocol analyses showing the effect of the concurrent exercise-training program on maternal serum inflammat

	Changes in control group		Changes in exercise group		Model unadjusted				Model 1			
	Mean	SD	Mean	SD	B	SE	β	p-value	B	SE	β	
33rd week-17th week (maternal serum, n=48)	**(n=28)**		**(n=20)**									
Fractalkine	-0.35	101.10	19.98	91.67	20.33	28.49	0.11	0.48	17.92	20.72	0.09	
Interleukin 1 beta	0.67	3.13	0.17	2.12	-0.50	0.81	-0.09	0.54	-0.79	0.76	-0.14	
Interleukin 6	0.74	3.27	-0.67	3.11	-1.41	0.94	-0.22	0.14	-1.19	0.73	-0.18	
Interleukin 8	-1.68	9.48	3.05	7.40	4.73	2.54	0.26	**0.07**	3.38	2.23	0.19	
Interleukin 10	0.55	13.74	6.80	8.88	6.25	3.50	0.25	**0.08**	4.66	2.47	0.19	
Interferon gamma	-0.55	9.97	-4.21	9.65	-3.65	2.88	-0.18	0.21	-4.11	2.50	-0.21	
Tumour necrosis factor-α	1.51	2.29	0.86	2.52	-0.66	0.70	-0.14	0.35	-1.03	0.43	-0.22	
Delivery-17th week (maternal serum, n=37)	**(n=19)**		**(n=18)**									
Fractalkine	-3.22	69.74	4.36	101.34	7.57	28.47	0.05	0.79	24.09	20.9	0.14	
Interleukin 1 beta	3.24	2.86	0.86	3.78	-2.38	2.10	-0.34	**0.04**	-2.38	1.06	-0.34	
Interleukin 6	26.91	9.86	27.06	18.93	0.15	4.92	0.01	0.98	0.23	4.97	0.01	
Interleukin 8	14.53	14.57	19.31	16.10	4.78	5.04	0.16	0.35	3.33	3.91	0.11	
Interleukin 10	18.65	13.98	27.30	16.93	8.65	0.10	0.28	**0.10**	9.40	4.55	0.30	
Interferon gamma	-2.50	9.08	-8.26	12.21	-5.76	3.53	-0.27	0.11	-3.97	2.03	-0.18	
Tumour necrosis factor-α	4.66	2.72	3.62	2.97	-1.04	0.94	-0.19	0.27	-1.03	085	-0.18	

SD, standard deviation; B, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient. including only women who attended ≥75% of the exercise sessions. Linear regression analyses (enter method) were used to ex markers between the control and exercise group. The within-group post-pre intervention changes (from the exercise train cytokines concentrations were included in the linear regression analyses as dependent variables, and the group (control=0 a When considering the "35th week-17th week" multiple point analyses, the model 1 was adjusted for baseline values of the pa Mediterranean Diet score; and the model 2 was additionally adjusted for the relative percentage of daily total physical activi wearing time). When considering the "delivery-17th week" multiple point analyses, the model 1 was adjusted for baseline va model 2 was additionally adjusted for parity status and gestational age at birth. * The adjusted R2 values shown are deriv assumptions related to the generalization of the results have been reasonably met, and non-transformations or data preparat

Table S7. Per-protocol analyses showing the effect of the concurrent exercise-training program on arterial and venous cord se
(n=38).

	Model unadjusted				Model 1				
	B	**SE**	**β**	**p-value**	**B**	**SE**	**β**	**p-value**	**B**
Umbilical arterial serum (delivery)[a]									
Fractalkine*	0.63	0.32	0.33	**0.06**	0.53	0.32	0.28	0.11	0.52
Interleukin 1 beta*	0.66	0.29	0.38	**0.03**	0.69	0.30	0.39	**0.03**	0.72
Interleukin 6*	-0.83	0.32	-0.42	**0.02**	-0.79	0.33	-0.40	**0.02**	-0.80
Interleukin 8	4.83	9.56	0.09	0.61	6.67	9.85	0.12	0.50	7.25
Interleukin 10	2.32	1.20	0.32	**0.06**	2.14	1.24	0.30	**0.10**	2.14
Interferon gamma	-0.65	0.44	-0.25	0.15	-0.70	0.46	-0.27	0.14	-0.68
Tumor necrosis factor alpha	-1.55	1.10	-0.24	0.17	-1.63	1.14	-0.25	0.17	-1.63
Umbilical venous serum (delivery)									
Fractalkine	34.58	37.38	0.15	0.36	27.29	36.93	0.12	0.47	23.68
Interleukin 1 beta*	0.21	0.32	0.11	0.53	0.18	0.33	0.10	0.58	0.17
Interleukin 6	-0.77	1.67	-0.08	0.65	-0.90	1.70	-0.09	0.60	-1.03
Interleukin 8*	0.20	0.32	0.11	0.53	0.22	0.33	0.11	0.50	0.16
Interleukin 10	0.37	1.28	0.05	0.78	0.31	1.31	0.04	0.82	0.23
Interferon gamma	0.34	0.41	0.14	0.41	0.24	0.39	0.10	0.54	0.29
Tumor necrosis factor alpha	-5.07	1.54	-0.48	**0.002**	-5.53	1.45	-0.53	**0.001**	-5.21

SD, standard deviation; B, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient.
including only women who attended ≥75% of the exercise sessions. Linear regression analyses (enter method) were used to ex
markers between the control and exercise group. The umbilical arterial serum cytokines concentrations were included in the
variables, and the group (control=0 and exercise=1) as independent variable. The model 1 was adjusted for adherence to the M
2 was additionally adjusted for parity status and gestational age at birth. * Optimum Box-Cox transformations and a subtle var
a z-score: replacing extreme scores with a score equivalent to ±2.58 SDs from the mean) were performed on inflammatory ma
control group (n=15) in all umbilical arterial serum inflammatory markers. [b] the adjusted R2 values shown are derived from th
individual influence of the exercise intervention without confounders). All the assumptions related to the generalization of the
dealing with the problematic outcomes, the results remained similar (but with better and more symmetrical distribution
preparation, excepting for the interleukin 1 beta which became statically significant.

Study V

Table S8. Differences between arterial and venous cord serum metabolic parameters (n=25).

	All participants (n=23)					Control group (n=14)				
	Mean difference (Artery-Vein)					Mean difference (Artery-Vein)				
	Mean	SD	Mean Dif.	SE	p-value	Mean	SD	Mean Dif.	SE	p-value
Arterial glucose	61.4	22.8	1.9	2.7	0.49	68.5	22.8	4.05	2.1	0.08
Venous glucose	59.5	21.0				64.4	22.4			
Arterial cholesterol	58.8	(45.8, 74.3)	7.6		**0.04**	59.5	(47.9, 72.7)	5.8		**0.04**
Venous cholesterol	50.3	(40.5, 62.7)				56.2	(49.9, 35.5)			
Arterial triglycerides	45.1	(36.5, 78.8)	7.3		0.26	49.3	(47.9, 72.7)	9.2	13.	0.07
Venous triglycerides	43.4	(32.8, 52.2)				43.7	(33.9, 58.7)			
Arterial HDL-C	27.8	9.4	3.80	1.9	0.06	28.6	10.0	4.34	2.5	0.11
Venous HDL-C	24.0	10.5				24.3	11.1			
Arterial LDL-C	7.9	(6.3, 11.6)	1.00		0.11	9.90	(6.5, 11.9)	1.0	4.3	**0.05**
Venous LDL-C	6.6	(5.6, 9.2)				7.2	(5.8, 9.5)			
Arterial phospholipids	96.2	24.7	0.57	7.3	0.94	100.8	25.6	-5.31	6.6	0.45
Venous phospholipids	95.8	26.8				105.1	19.2			

Dif., difference; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; SD, stand
values were calculated using paired sample Student's t-test (normal distribution), and Wilcoxon tests (non-normal d
outcomes correspond to those analyses where the Wilcoxon tests were conducted (non-parametric: estimates not

Table S9. Metabolic enzymes concentrations at three time points (n=44)

	16th week of gestation (n=88)					33th week of gestation (n=88)				
	Control (n=44)		Exercise (n=44)		p-value	Control (n=44)		Exercise (n=44)		p-value
Maternal serum	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
LDH (IU/L)	296.5	(221.4, 354.9)	293.8	(244.1, 314.1)	0.76	344.7	81.7	357.4	79.3	0.42
Alkaline phosphatase (IU/L)	47.6	18.3	47.6	18.3	0.91	155.7	46.9	141.9	55.2	0.26
Gamma GGT (IU/L)	10.2	7.1	10.4	4.3	0.94	10.2	4.5	10.3	4.8	0.96
ALTGPT (IU/L)	8.7	(5.5, 17.1)	6.5	(4.7, 10.2)	0.16	12.1	(7.8, 15.7)	9.8	(7.2, 18.7)	0.91
ASTGOT (IU/L)	13.1	6.3	13.8	11.8	0.85	19.4	11.7	21.2	13.1	0.67
Cord arterial serum										
LDH (IU/L)										
Alkaline phosphatase (IU/L)										
Gamma GGT (IU/L)										
ALTGPT (IU/L)										
ASTGOT (IU/L)										
Cord venous serum										
LDH (IU/L)										
Alkaline phosphatase (IU/L)										
Gamma GGT (IU/L)										
ALTGPT (IU/L)										
ASTGOT (IU/L)										

SD, standard deviation. Data are mean (standard deviation) or median (interquartile range=Q3, Q1). independent sample Student's t-test, Welch´s test, and Mann-Whitney U test.

Study V

Table S10. Per-protocol analyses showing the effect of the concurrent exercise-training program on maternal serum enzymes

	Within-group changes				Model 1				
	Mean	SD	Mean	SD	B	SE	β	p-value	B
Change (16th-33rd week)	Control (n=25)		Exercise (n=19)		(Control n=25; Exercise n=19)				(Contro
Lactate dehydrogenase	51.4	101.2	47.8	153.0	13.11	25.98	0.05	0.62	16.60
Alkaline phosphatase	108.1	49.8	94.3	56.5	-4.33	18.58	-0.04	0.82	-2.50
Gamma-Glutamyl transferase[c]	-0.1	8.0	-0.1	5.3	1.61	1.39		0.25	1.85
Alanine aminotransferase[ac]	-0.3	21.0	5.9	10.9	2.87	4.84		0.59	0.61
Aspartate aminotransferase[ac]	6.4	12.8	7.4	19.9.	0.08	5.27		0.99	2..28
Delivery					(Control n=19; Exercise n=20)				(Contro
Lactate dehydrogenase[a]					-0.07	0.36	-0.03	0.85	-0.10
Alkaline phosphatase[ac]					25.59	33.08		0.45	26.15
Gamma-Glutamyl transferase[ab]					0.18	0.36	0.09	0.61	0.02
Alanine aminotransferase[a]					1.23	2.69	0.08	0.65	-0.09
Aspartate aminotransferase[ab]					-0.18	0.36	-0.09	0.62	-0.05
Cord arterial serum (n=25)					(Control n=15; Exercise n=10)				(Contr
Lactate dehydrogenase[b]					-0.30	0.43	-0.15	0.50	-0.30
Alkaline phosphatase[b]					0.03	0.47	0.01	0.96	-0.21
Gamma-Glutamyl transferase					3.37	24.52	0.03	0.89	2.79
Alanine aminotransferase					3.19	2.67	0.23	0.25	3.96
Aspartate aminotransferase[b]					-0.15	0.43	-0.07	0.73	-0.17
Cord venous serum (n=36)					(Control n=17; Exercise n=19)				(Contro
Lactate dehydrogenase[b]					-0.05	0.35	-0.03	0.88	-0.04
Alkaline phosphatase[b]					0.13	0.38	0.06	0.73	0.13
Gamma-Glutamyl transferase[a]					-10.04	20.29	-0.09	0.62	-3.73
Alanine aminotransferase[ab]					-0.32	0.30	-0.18	0.30	-0.39
Aspartate aminotransferase					-9.10	8.82	-0.17	0.31	-11.24

SD, standard deviation; B, unstandardized regression coefficient; SE, standard error; β, standardized regression coefficient. performed on extreme outliers of metabolic parameters (only 1-2 extremes values within each outcome were treated). N predictor. [b] Optimum Box-Cox transformations were conducted. [c] Median regression analyses were employed for those mod assumptions (after handling data). Similar results were observed on these models when treated with median (non-parametric) considering the "33rd week-16th week" multiple point analyses, the model 1 was adjusted for baseline values of the particular o and smoking habits at baseline; and the model 2 for baseline values, gestational week at 33rd week, and the Mediterranean d "maternal enzymes at delivery" analyses, the model 1 was adjusted for baseline values of the particular outcome, and gestati the Mediterranean diet score at baseline, and type of delivery. Regarding cord serum enzymes, the model 1 was adjusted for g 2 for the Mediterranean diet score at baseline. d The adjusted R2 (pseudo R2 for median regressions) values shown are derive e model 1. The results remained similar after exploring these associations by BMI at pre-pregnancy (normal-weight vs. overwe

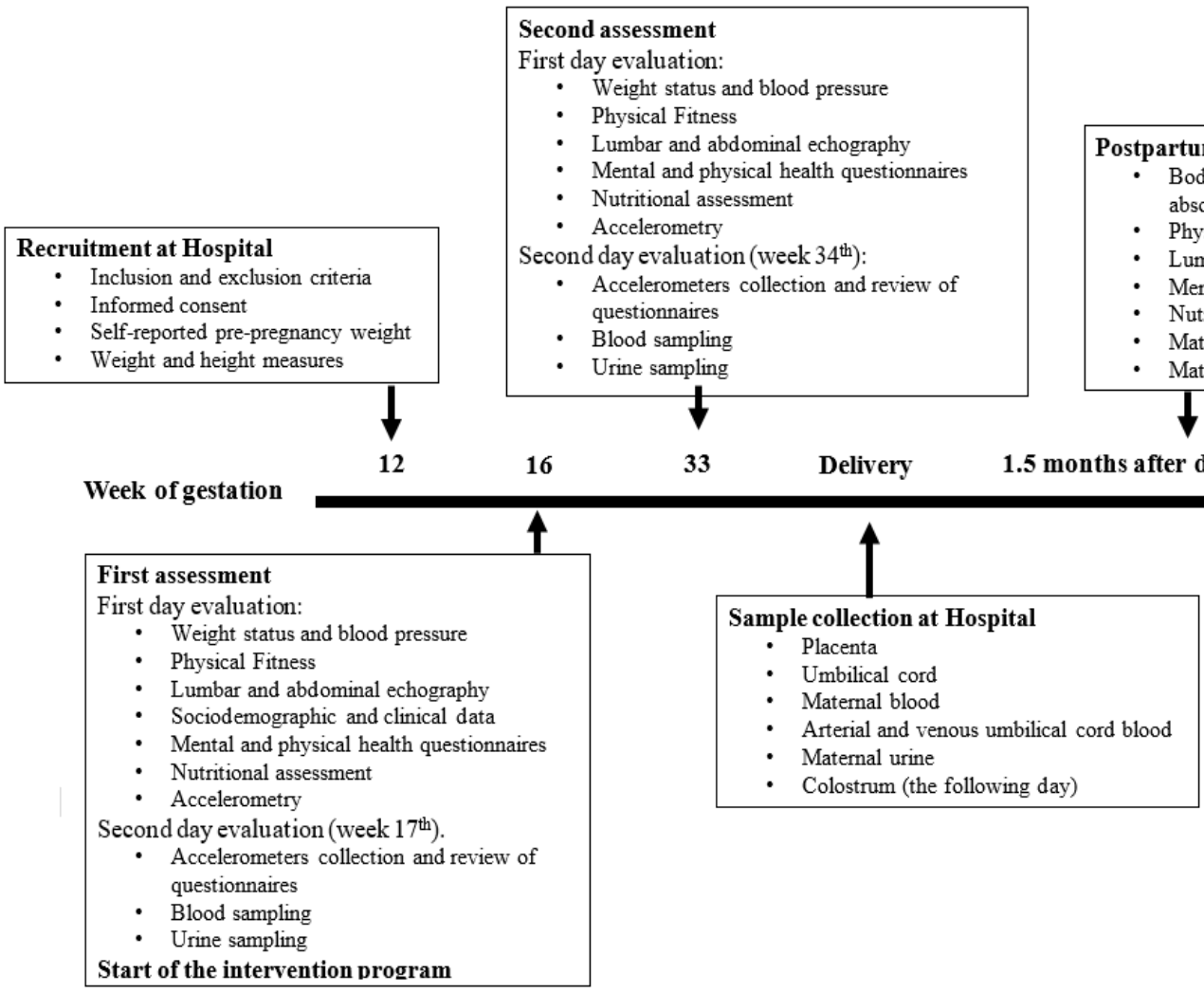
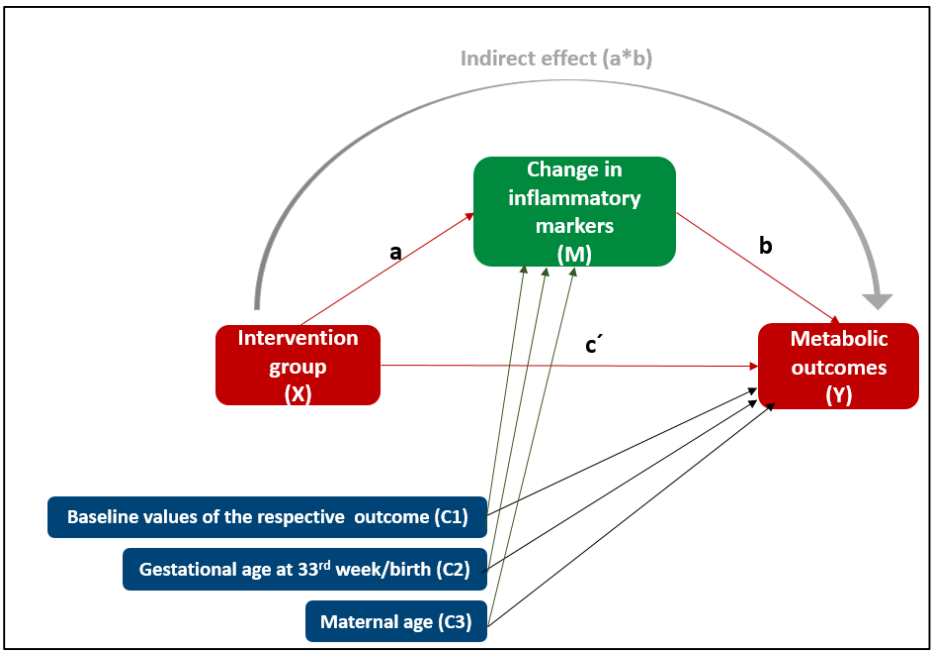
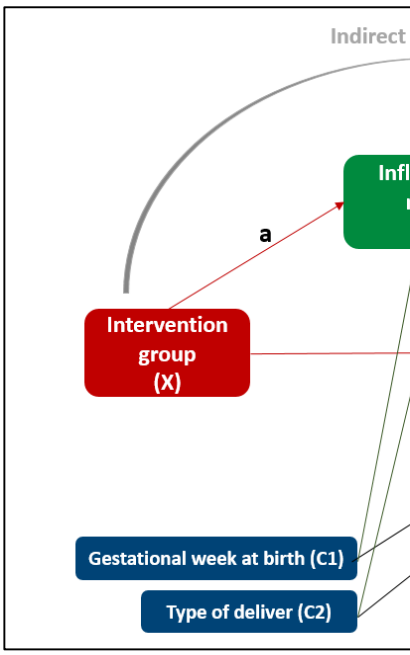


Figure S1. Assessments conducted along the GESTAFIT Project.

Study V





Multiple time point analyses *Singles time p*

Figure S2. Schematic diagram. The mediator role of cytokines in the association between physical exercis

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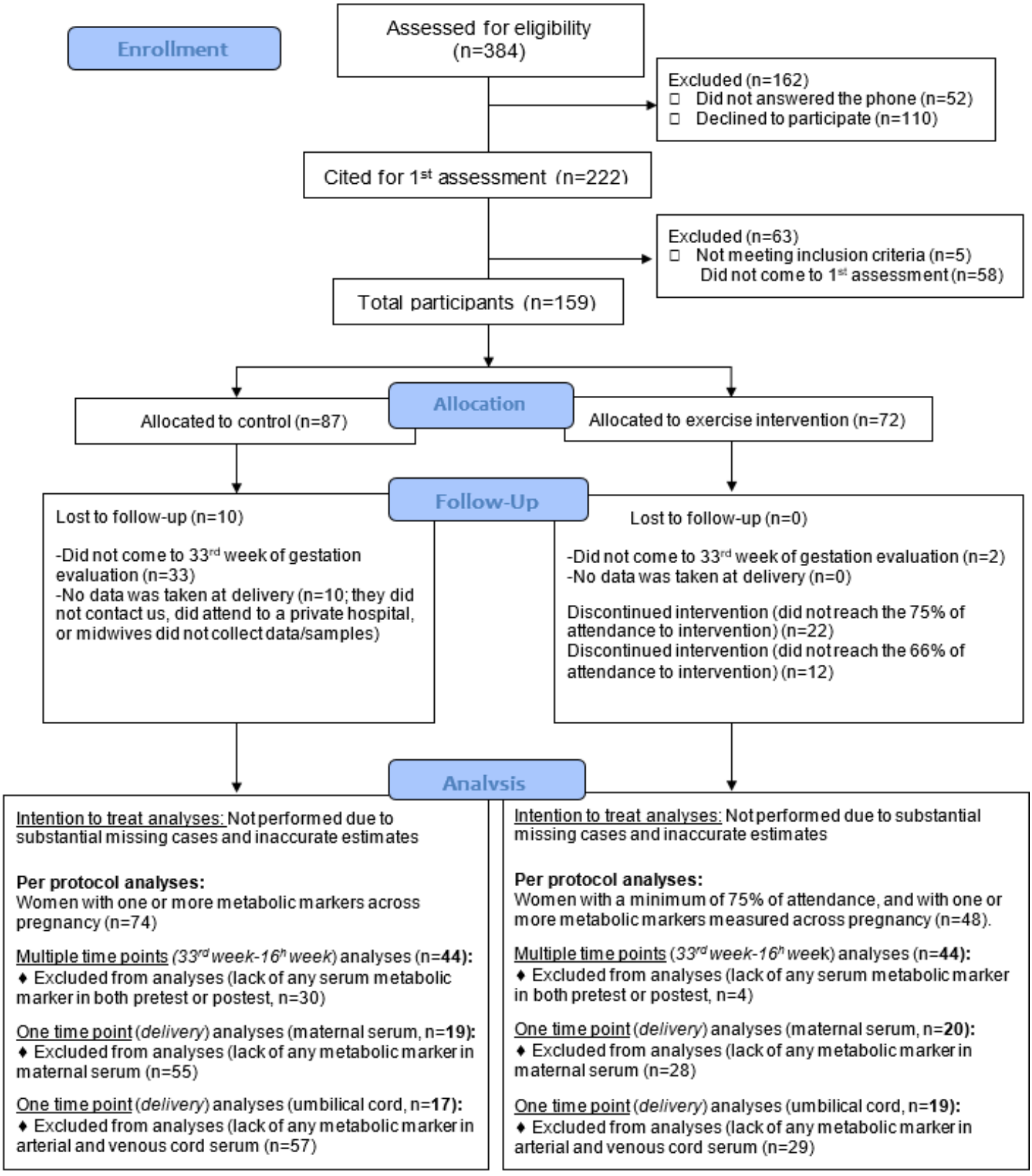


Figure S3. Flowchart: enrolment, allocation, follow-up and analyses processes.

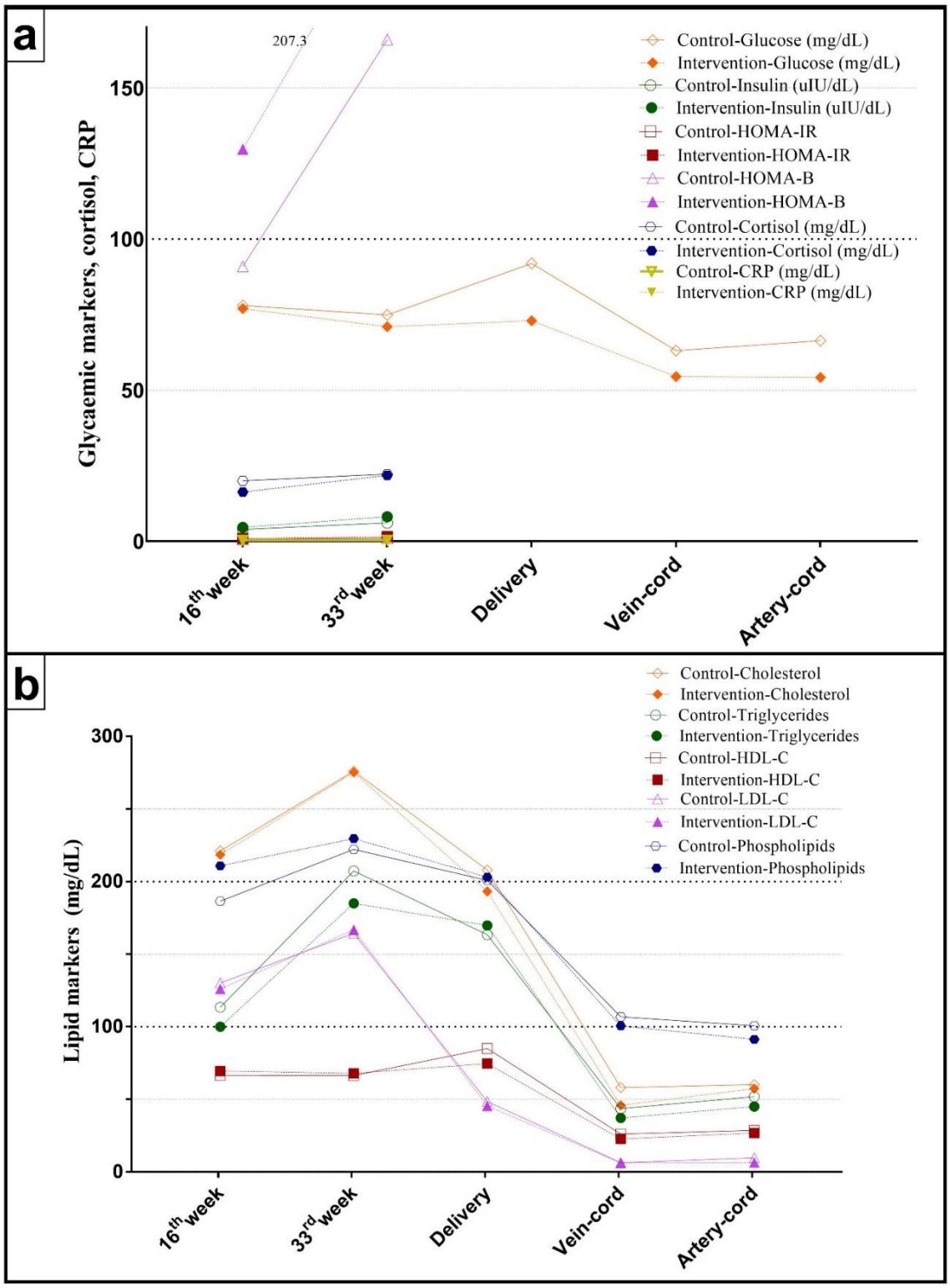


Figure S4. Immunometabolic markers levels during pregnancy (n=88). *CRP, C-reactive protein; HDL-C, high density lipoprotein-cholesterol; HOMA-B, homeostatic model assessment, B-cell function; IR, insulin resistance; LDL-C, low density lipoprotein-cholesterol.*

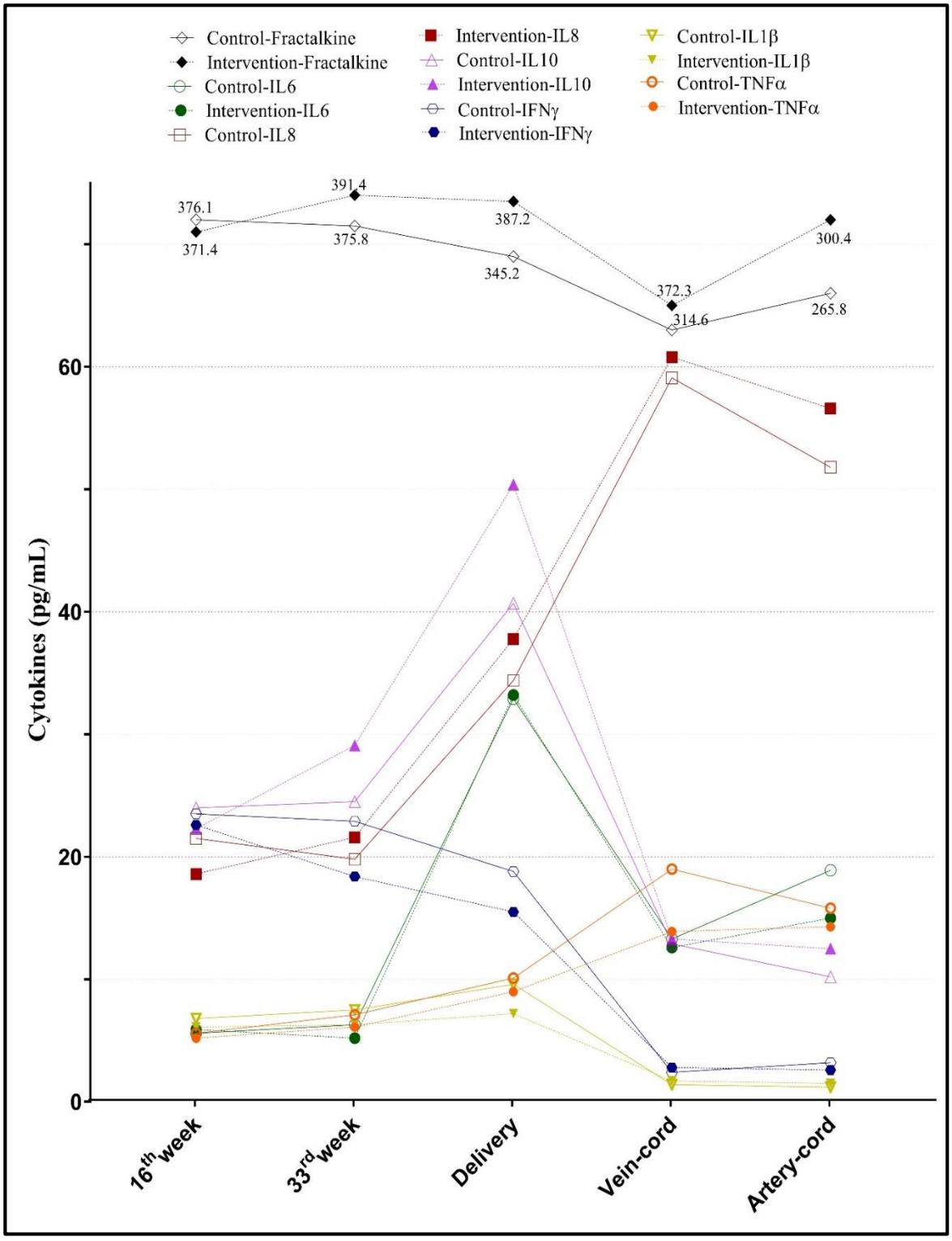


Figure S5. Cytokines levels during pregnancy (n=48). *IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IFN-γ, interferon gamma; IL-1β, interleukin-1β; TNF-α, tumour necrosis factor-alpha.*

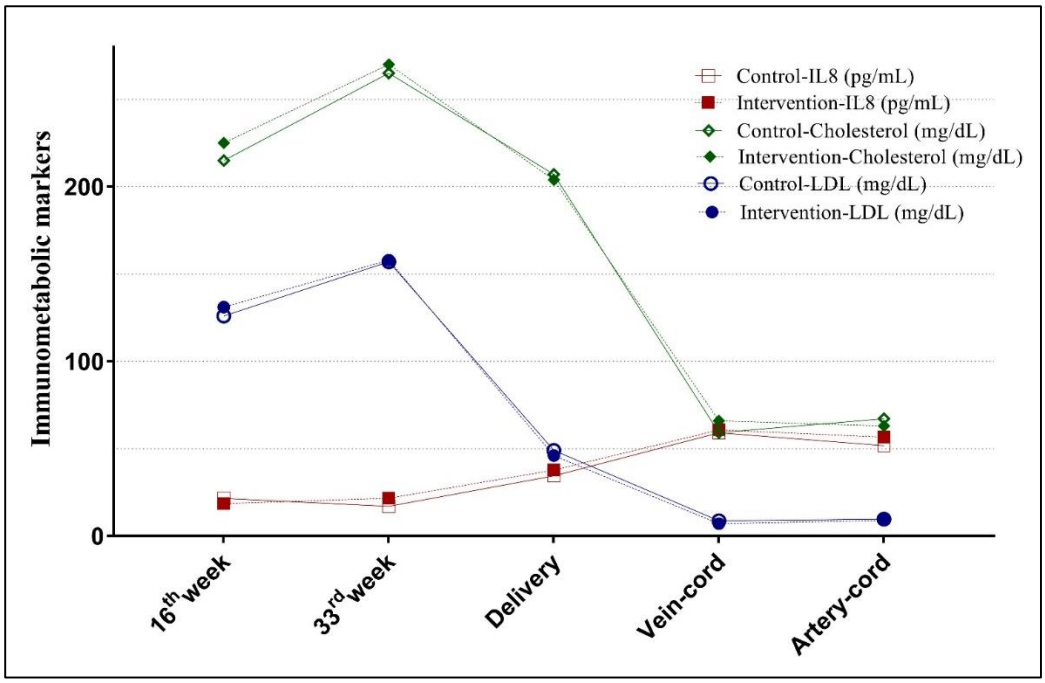


Figure S6. Kinetics during pregnancy of the immunometabolic markers concentrations implicated in the indirect effect of exercise (attendance>75%) on lipid markers (n=44): reduction in total cholesterol and LDL-*C* gains *–from baseline to 33rd week-* via an increase in IL8. *IL8, interleukin 8; LDL-C, low density lipoprotein-cholesterol.*

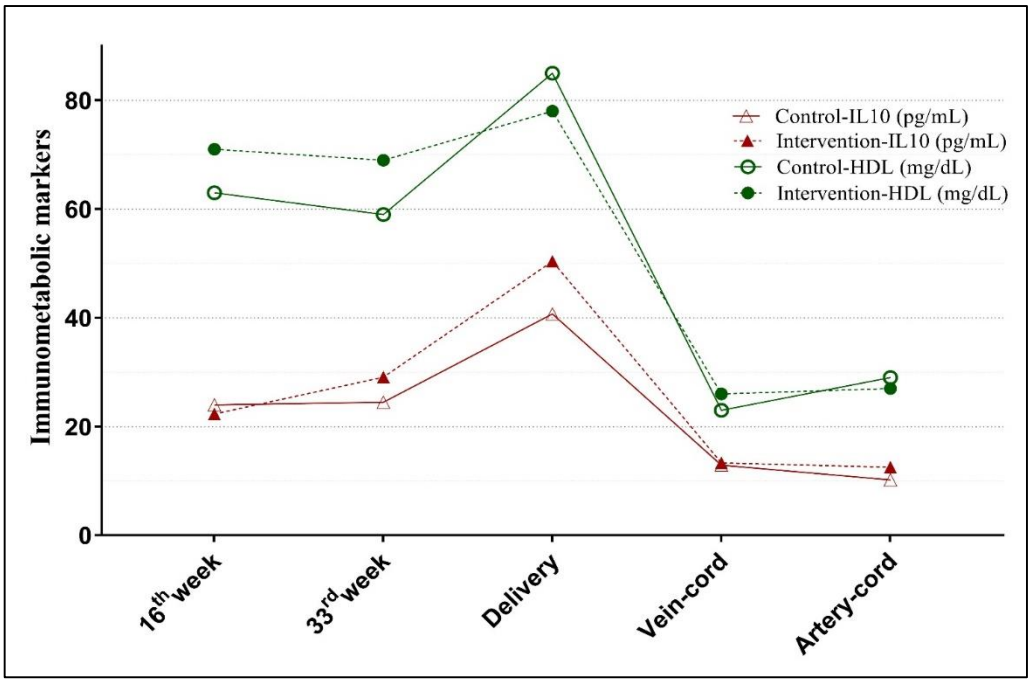


Figure S7. Kinetics during pregnancy of the immunometabolic markers implicated in the indirect effect of exercise (attendance>75%) on HDL-C (n=44): reduction in HDL-*C –from baseline to 33rd week-* via an increase in IL10. *HDL-C, high density lipoprotein-cholesterol; IL10, interleukin 10.*

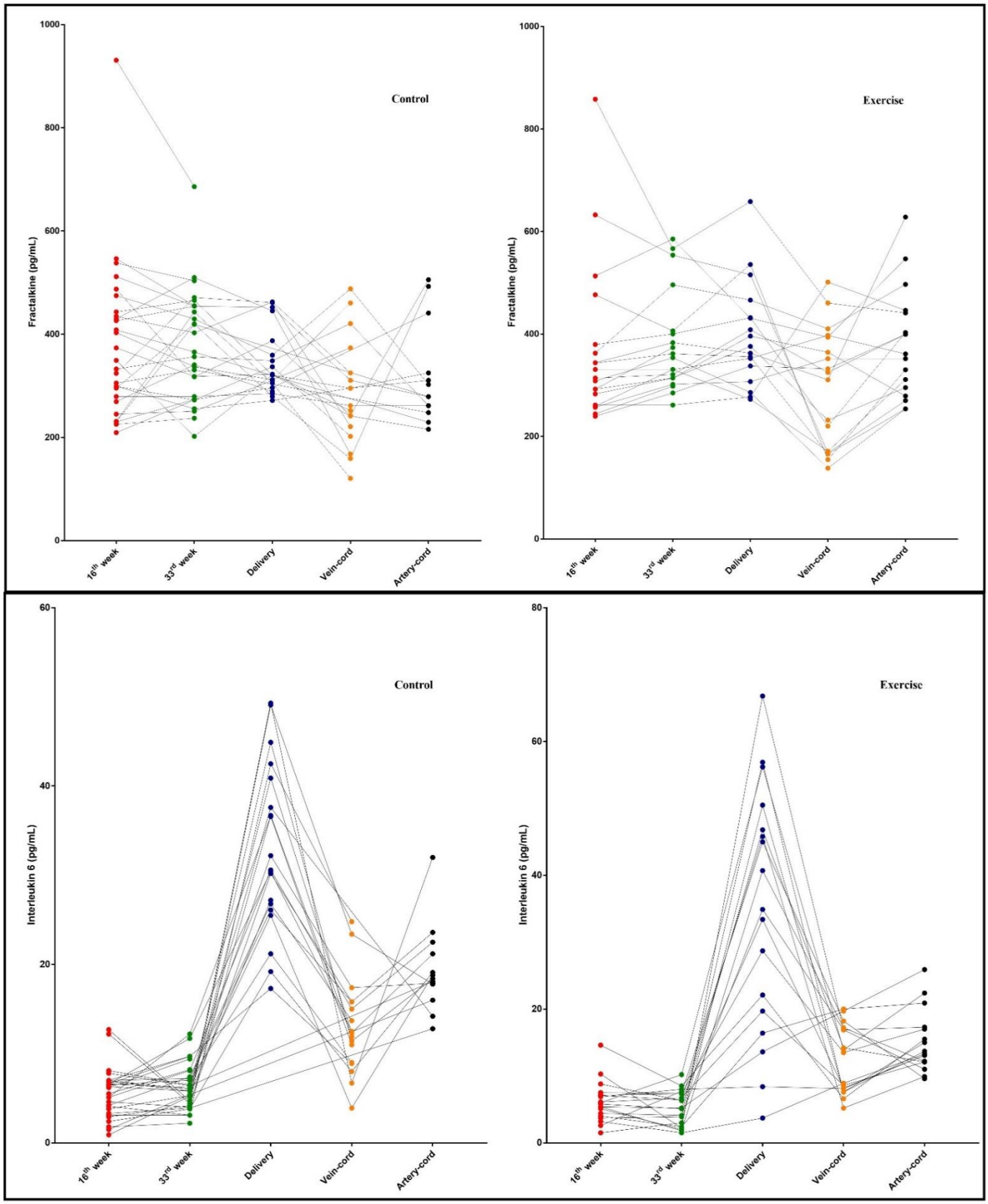


Figure S8. Within-individual cytokines and C-reactive protein concentrations during pregnancy (control n=28, exercise n=20; except for C-reactive protein: control n=44, exercise n=44).

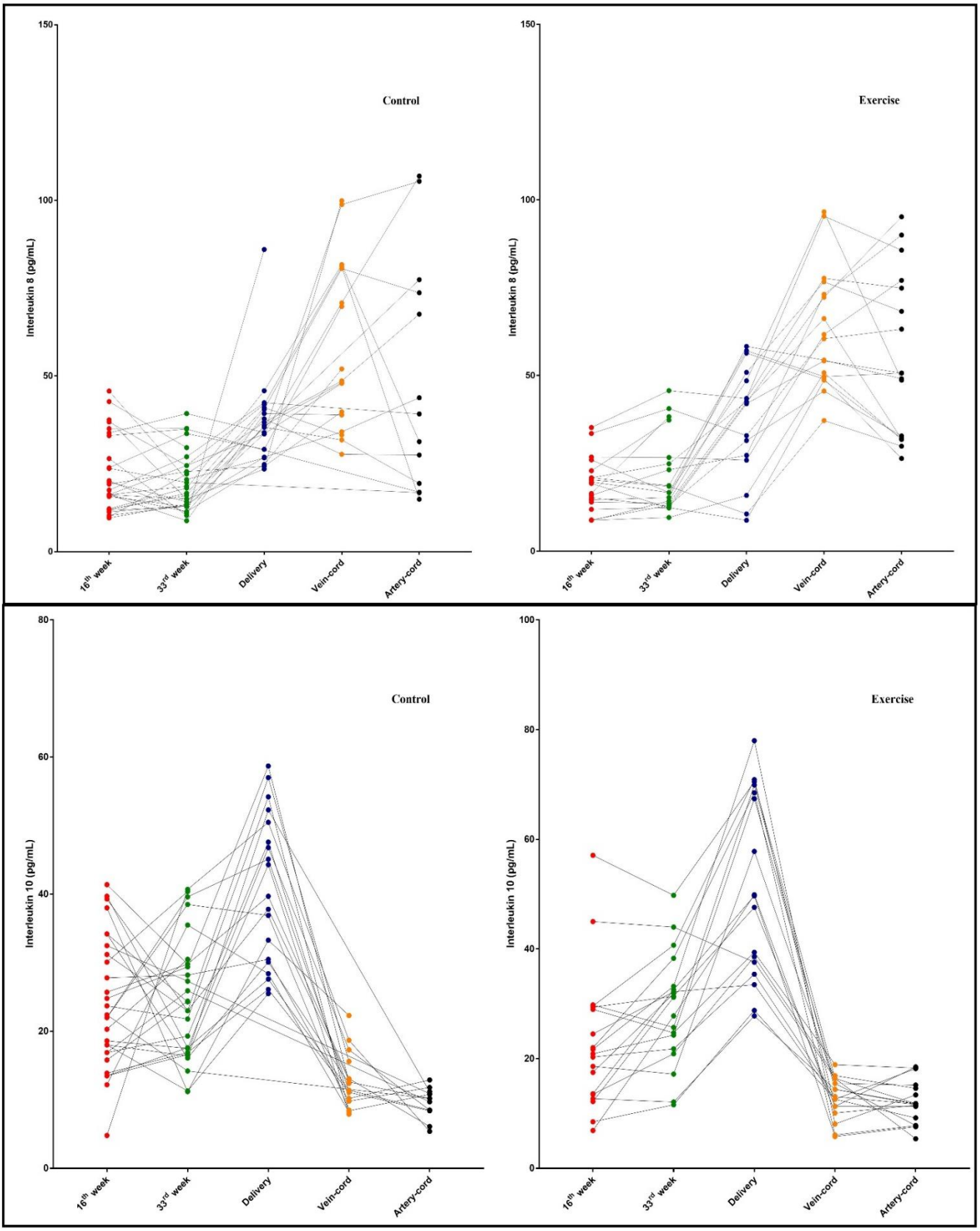
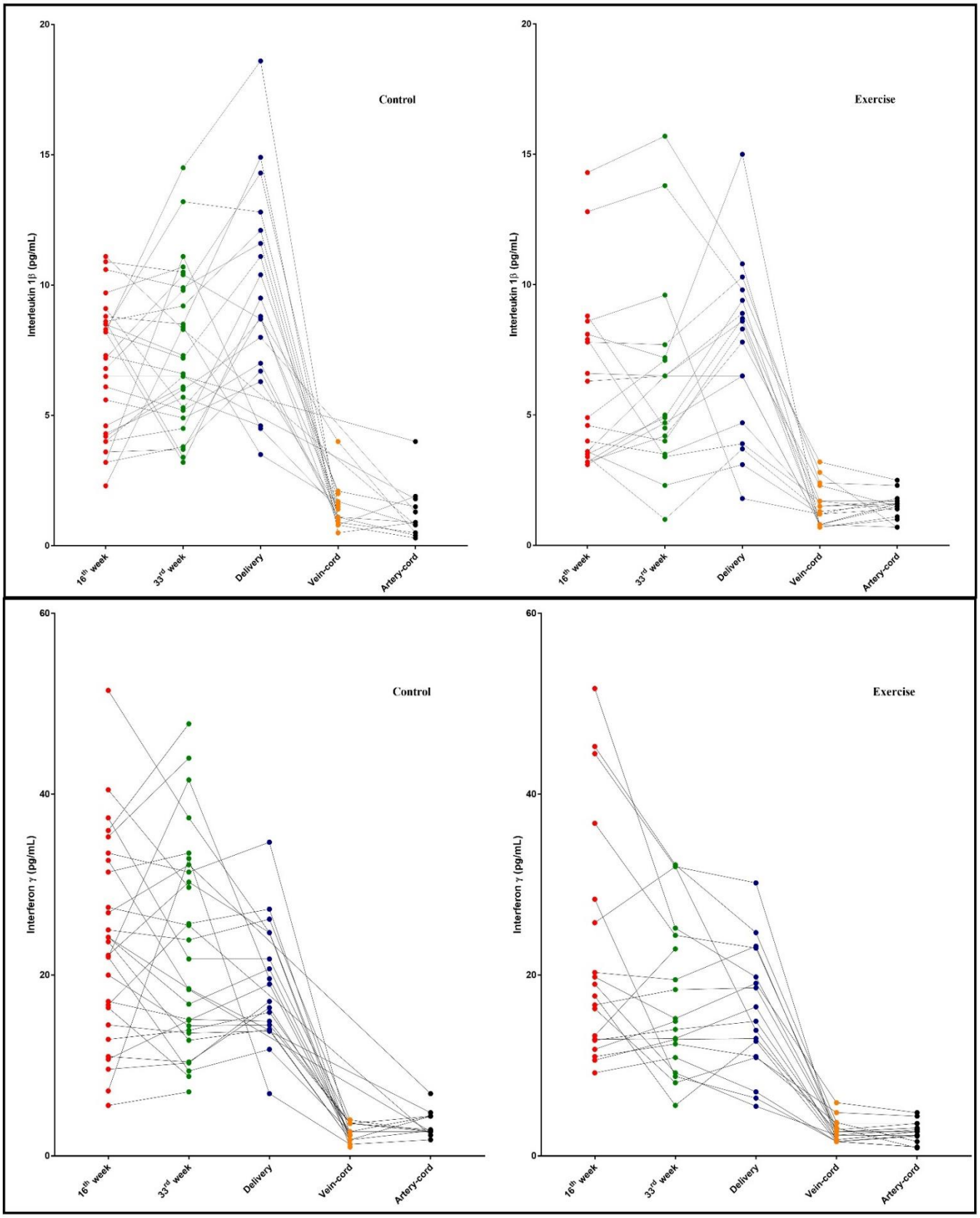


Figure S8 (continued).



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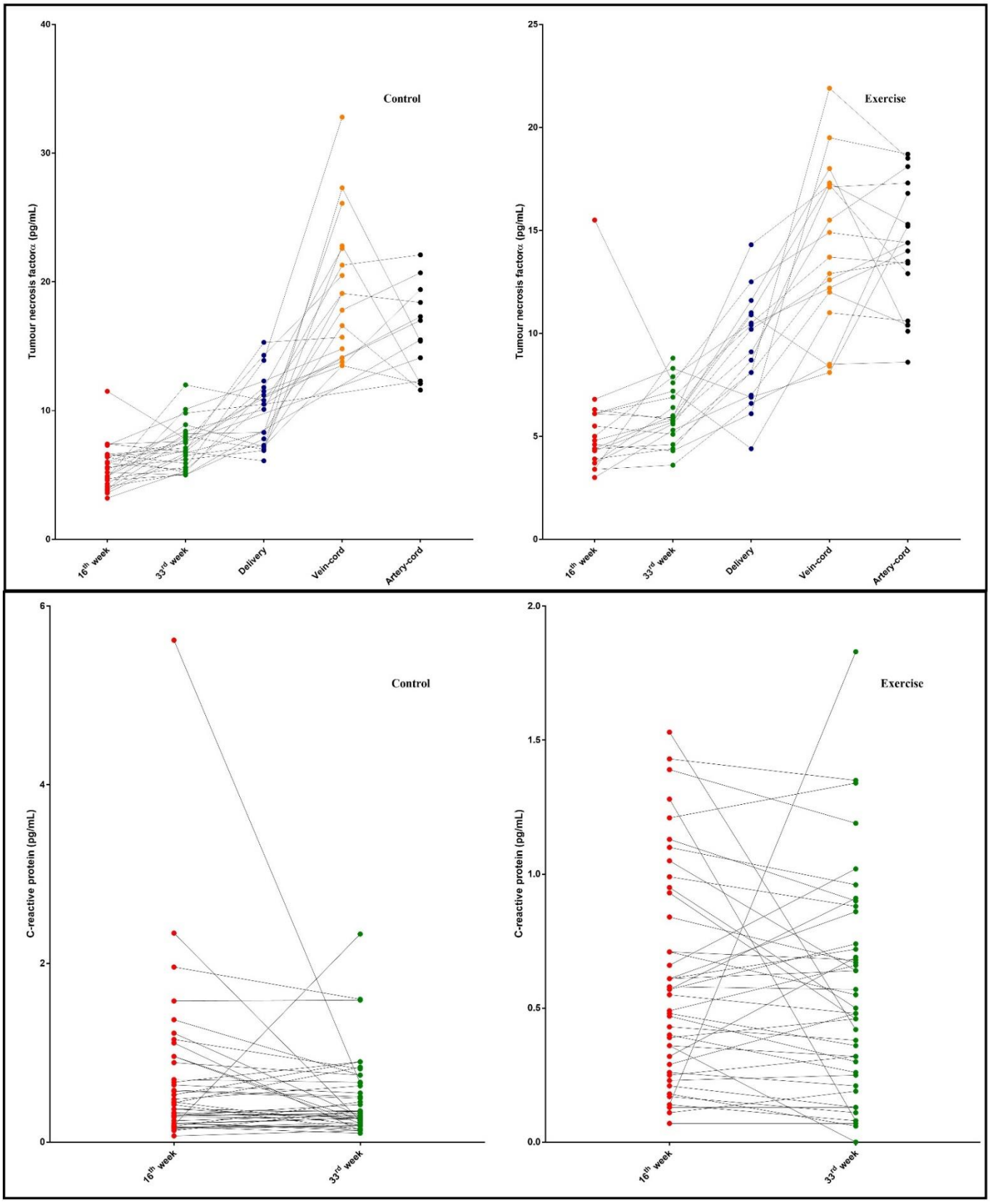

Figure S8 (continued).

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PART IV. Lifestyle and physical fitness: strategies to manage gestational weight-gain

STUDY VI

Influence of lifestyle and physical fitness on gestational weight-gain and postpartum weight retention: The GESTAFIT project

ABSTRACT

Objectives: To analyse i) the influence of lifestyle – *exercise intervention, sedentary time (ST), physical activity (PA), sleep and diet quality*– and physical fitness (PF) on gestational weight-gain and postpartum weight-retention, and their potential to prevent excessive weight-gain; ii) if exercise protects against the adverse outcomes related to excessive weight-gain.

Methods: From the 121 women included in this study, 101 participants were considered for the per-protocol intervention analyses (control n=54, exercise n=47). The exercise intervention consisted of a 3 days/week supervised concurrent (aerobic+resistance) exercise program. ST/PA and sleep, and diet quality (assessed by accelerometry and questionnaires), cardiorespiratory fitness (CRF; Bruce test) and muscle strength (handgrip), were assessed at 16th and 33rd week, and postpartum. Weight-gain and weight-retention were calculated with pre-pregnancy, early-middle, and late weights, and postpartum weight. Body composition, and maternal, arterial and venous cord serum, and colostrum and mature milk immunometabolic markers were secondary outcomes.

Results: The exercise intervention reduced late weight-gain (B=-2.73, SE=0.83, p=0.003) and weight-retention (B=-2.85, SE=1.3, p=0.03), independently of other lifestyle behaviours and PF, but did not prevent excessive weight-gain. Increasing CRF, muscle strength and sleep duration was slightly associated with lower mean (only CRF) and excessive weight-gain (p<0.05). Among the participants with excessive weight-gain, exercisers showed reduced systemic TNF-α, venous cord TNF-α and arterial cord IFN-γ, and greater arterial cord IL-10 and placenta weight than controls (p<0.05).

Conclusions: Exercise can optimise gestational weight-gain, and may attenuate the impaired phenotype related to excessive weight-gain. Increasing CRF, muscle strength and sleep duration might help to prevent excessive weight-gain.

INTRODUCTION

In pregnancy, not only adverse phenotypes such as obesity and gestational diabetes mellitus, but also exacerbated weight-gain and weight-retention, are strong determinants for birth complications[1] and future maternal and offspring diseases[1-3]. Thus, the spotlight of reference institutions is also on finding effective strategies to achieve recommended weight-gain during pregnancy[1,4].

Exercise has been proposed as a potential tool to control weight-gain and weight retention[5-8], and avoid excessive weight-gain during pregnancy (especially in overweight-obese women) [6,7,9]. However, evidence is equivocal[6,7,9-11], and has not considered whether the effects of exercise on weight-gain could be explained/confounded by other lifestyle behaviours –*such as sedentary time (ST), physical activity (PA), sleep and diet quality*– or physical fitness (PF). Actually, little is known about how these lifestyle behaviours and PF relate to gestational weight-gain. Hence, studies with an integrative approach could contribute to better understand their role. These studies should also focus on a priori lower-risk groups such as normal-weight women, since a considerable proportion of Western women are not overweight-obese.

Surprisingly, none previous study has either explored if exercise could protect THE maternal-foetal metabolism against the adverse alterations related to excessive weight-gain, which might represent another via to avoid metabolic disruptions and birth complications[12]. Whereas most studies have focused on whether exercise prevents excessive weight-gain and birth complications[6,8,13], only one study indirectly addressed the potential of exercise-induced weight-changes to avoid birth complications[14]. Therefore, the main aims of this study were to analyse the independent influence of lifestyle –*exercise intervention, ST, PA, sleep and dietary patterns*– and PF on maternal weight-gain and postpartum weight-retention, and their potential to prevent excessive weight-gain during pregnancy. A secondary aim was to explore if exercise could play a protector role attenuating the adverse metabolic outcomes related to exacerbated weight-gain.

MATERIAL AND METHODS

Study design and population

The GESTAFIT project was initially designed as a randomized controlled trial, and was conducted at the "Sport and Health University Research Institute", and at the "San Cecilio and Virgen de las Nieves University Hospitals" (Granada, Spain). This project was approved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15). The inclusion-exclusion criteria (**Table S1**) and procedures are described elsewhere[15,16]. From the 384 women who attended to the first gynaecological visit at 12th gestational week, and were informed about the aims and methodology of the project, 159 women were finally recruited and signed a written informed consent.

Sample size

The required sample size to have enough statistical power, and to determine the expected effect sizes on weight-gain, was of 52 women (26 per group)[16].

Randomization

The randomization was not feasible in all waves of participants because of difficulties related to adherence of control women; which represents a frequent methodological barrier in antenatal exercise research[17]. Thus, most women were allocated to the control/exercise group according to their personal preference and convenience to attend the exercise sessions. Most personnel were blinded to their allocation into the control/exercise group, excepting those responsible for the training sessions.

General procedure (Figure S1)

Women were evaluated at three temporal points during pregnancy, and one after delivery, by experienced researchers: at 16th and 33rd gestational weeks (2 days per assessment), delivery (2 days/assessment), and postpartum (1 day/assessment). Clinical characteristics (including weight and height), self-reported sleep and diet quality, and PF were assessed at 16th week. Before leaving, participants were provided with accelerometers to wear until the following appointment (to assess their ST and PA). At 17th week, accelerometers were personally returned, and maternal blood (fasting conditions) was extracted by a nurse. At 33rd week, the aforementioned assessments were performed with identical timing, except for the initial anamnesis. Immediately after delivery, arterial and venous umbilical cord blood samples were collected by

midwives, placenta was weighted, and perinatal obstetric records were gathered. One day after delivery, the colostrum was obtained at the hospital. One month after delivery (i.e. postpartum), mature milk was collected, maternal and neonatal buccal mucosa cells were extracted, and sleep, diet quality, anthropometrics, body composition, and PF were assessed.

Intervention

The exercise intervention consisted in a concurrent exercise program (from 17th week until delivery, 3 days/week, 60 minutes/session) of aerobic and resistance exercises of moderate-to-vigorous (mostly moderate with peaks of vigorous) intensity. This exercise protocol, which followed the recommendations from the ACOG[4], and was designed by a specialist multidisciplinary team, has been extensively detailed elsewhere[15,16,18]. During the exercise intervention, women were provided with 7 talks to promote healthier pregnancies (**Appendix A**).

Control women were also invited to these talks for ethical considerations, and to ensure their fidelity. They were also asked to continue with their daily lifestyle.

Outcomes

The personnel responsible for the obstetrics-perinatal records (hospital staff), and lab analyses (physiologists and biologists), were blinded to women's treatment allocation.

Clinical data, obstetric history and perinatal outcomes

Sociodemographic and clinical data, obstetric and reproductive history, adverse events, and alcohol and smoking habits, were acquired from medical files and questionnaires. Data related to number of abortions, type of delivery and duration, etc. were collected from birth records (partogram).

Cardiovascular function

Systolic and diastolic blood pressure (SBP; DBP) were assessed twice using a sphygmomanometer (M6 upper-arm Omron Health-Care Europe, the Netherlands), with women seated and relaxed[16].

Anthropometry

Pre-pregnancy body weight was self-reported, and weight at 16th and 33rd week and postpartum, were assessed (no shoes, light clothes) with an electronic scale (InBody-R20; Biospace, Seoul). Height was measured using a calibrated stadiometer (Seca 22,

Hamburg). Body mass index (BMI) was calculated as [weight (Kg)/height (m^2)]. Postpartum waist and hip circumference were also measured[16].

Weight-gain

Weight-gain and retention were defined according to the period of pregnancy: early weight-gain (weight difference from pre-pregnancy until 16th week), late weight-gain (from 16th to 33rd week), total weight-gain (from pre-pregnancy until 33rd week), and weight-retention (from pre-pregnancy until postpartum). Based on previous evidence[1,19], excessive early weight-gain was defined as early weight-gain greater than "*[2 kg +(X*(Gestational Week at 16th week - 13))]",* and excessive late weight-gain as late weight-gain greater than "*[(Gestational Week at 33rd week - 16th week)*X)]".* In these equations, 2 kg is the maximum weight-gain recommended for the first trimester, and X is the maximum weekly weight-gain recommended considering pre-pregnancy BMI (normal-weight: 0.50 kg/week, overweight: 0.33 kg/week, obese: 0.27 kg/week). Excessive total weight-gain was defined as weight-gain greater than *"excessive early weight-gain + excessive late weight-gain",* respectively.

Sedentary time and physical activity

During nine consecutive days (24 h/day, except for water activities), ST and PA were objectively measured using triaxial accelerometers (ActiGraph GT3X+, Florida, US) that women wore in their waist and non-dominant wrist. Accelerometer wear time and total vector magnitude counts (VMC) were estimated. ST (200 counts/min), and light (200-2690 counts/min) and moderate-to-vigorous (2690-6166 counts/min) PA were calculated for the waist accelerometer, based on recommended VMC cut-points[20]. Further information about filters, cut-offs and analyses is provided elsewhere[21].

Sleep behaviours

Sleep-related variables (sleep duration, in-bed time, time awaken after sleep onsets, and sleep efficiency) were objectively measured with triaxial accelerometers (ActiSleep, Florida, US) placed on the non-dominant wrist (Cole-Kripke algorithm)[20]. Procedures were similar to those described above. Sleep quality was assessed with the Pittsburgh Sleep Quality Index Questionnaire[16] (higher scores indicate worse sleep quality).

Dietary assessment

The Mediterranean Diet Score Index (MDS; higher scores indicate better adherence to Mediterranean dietary patterns, i.e., better diet quality) was calculated with a food

frequency questionnaire[16]. The estimated grams for each food were used to estimate total energy intake (kcal/day) using Evalfinut software.

Physical fitness

Upper-body muscle strength (a proxy of overall muscle strength in clinical studies) was measured with the handgrip strength test[16]. Participants were asked to squeeze the grip (adapted to their hand size[22]) of a digital dynamometer (TKK5101 Grip-D; Takey, Japan) to the maximum. For each hand, the best score of 2 trials was chosen, and the average was used for analyses.

Cardiorespiratory fitness (CRF) was assessed with the modified Bruce treadmill test[23,24], which consisted of increasing the speed and slope during progressive workload stages (see **Appendix B**). In this submaximal protocol, women were supported to attain the 85% of the age-predicted maximum heart rate ($85\%_{MHR}$) and target heart rate ($85\%_{THR}$). The test was stopped when the participants attained the $85\%_{THR}$ or reported volitional fatigue. Time to $85\%_{MHR}$ and $85\%_{THR}$ were considered proxies of CRF, as previously reported[23] ($CRF_{85\%MHR}$ and $CRF_{85\%THR,}$ respectively; see **Appendix B**). $CRF_{85\%THR}$ was only used for sensitivity analyses due to the reduced sample size (n=26).

Secondary/exploratory outcomes

Blood (maternal and cord) and milk were collected, and then, specific immunometabolic markers were analysed. Body composition (lean, fat and visceral adipose tissue mass, and body mineral density), and the maternal and neonatal genotype (several SNPs in FTO and MC4R genes) were also assessed. A detailed description about these procedures is provided in **Appendix C.**

Statistical analyses

Descriptive statistics were performed to show the characteristics of women (**Table 1**). The independent sample Student's, Welch´s and Mann-Whitney U tests (continuous variables), and the Chi-square test (categorical variables), were employed to detect differences between intervention groups. The interaction-term between lifestyle/PF and foetal sex with weight-gain and retention was checked by linear regressions. Relevant confounders according to previous evidence, and/or which were statistically related to the outcomes, were included in the models. Specifically, the following confounders were included: pre-pregnancy BMI, gestational week at post-test assessments (33rd week and birth) or weeks between birth and postpartum assessments,

energy intake, intervention group, and baseline values of the respective lifestyle behaviour. Several extreme cases confirmed as influential outliers were handled (**Appendix D**). In some cases, optimum Box-Cox transformations were employed.

To address the first aim, linear regressions were employed to analyse the influence of the exercise intervention (16^{th} week-delivery), and lifestyle and PF changes (16^{th}-33^{rd} week; 16^{th} week-postpartum) on weight-gain and weight-retention (**Table 2**). To test their independence, multiple linear regressions were conducted including all (or blocks of) lifestyle behaviours and PF together. Only women who attended >75% of the exercise sessions, and completed both 16^{th} and 33^{rd} week assessments, were considered for the per-protocol intervention analyses[15]. Additionally, logistic regressions were used to explore if lifestyle and PF changes prevented excessive late and total weight-gain (**Tables 3**). Subsequently, mediation analyses were employed to explore whether lifestyle and PF changes could explain the effects of exercise on weight-gain.

For the secondary aim, linear regressions were employed to explore the association of mean weight-gain and weight-retention with immunometabolic markers, maternal-neonatal birth outcomes, and postpartum body composition (**Table S2-S5**). Subsequently, simple slope analyses were used to explore if these associations differed depending on foetal sex, exercise, and weight status. Additionally, analyses of covariance were used to explore if immunometabolic markers were associated with late and total excessive weight-gain (**Tables S5-S6**), and the mean differences in these outcomes between exercisers and controls with excessive total weight-gain (**Table 4**).

Overall, the assumptions related to the generalization of the results were met. After multiple imputation for cases with missing data, the same analyses were conducted on an intention-to-treat basis. The analyses were conducted using SPSS-26.0 (IBM, NY, USA). The statistical significance was set at $p \leq 0.05$.

RESULTS

In total, 121 Caucasian southern Spanish women (age 33±5 years, pre-pregnancy BMI 23.7±3.9 kg/m^2) without diagnosed cardiometabolic disruptions, were included in the analyses. These women were divided into control (n=54) and exercise (n=67) groups (per-protocol: control n=54, exercise n=47). Additional information is provided in the **Figure S2** and **Appendix E**. The exercise group was characterized by higher energy intake at 16thweek (p<0.05), and had an average attendance to training sessions of 86% (**Table 1**). Graphical representations of maternal weight-gain by intervention and pre-pregnancy BMI groups, and distribution of women by weight-gain categories, are shown in **Figure 1** and **Figures S3-S4**.

Associations of lifestyle and physical fitness with weight-gain and retention

The associations of lifestyle and PF with gestational weight-gain and postpartum weight-retention are shown in **Table 2**. After adjusting for confounders, the exercise group increased less the mean weight-gain (*16th-33rd week*; B=-2.73, SE=0.83, p=0.001) and weight retention (model 2: B=-2.85, SE=1.3, p=0.03) than the control group. Additionally, an increase in CRF was significantly associated with lower weight-gain (model 2: *16th-33rd week*, p<0.001). The exercise group was associated with lower weight-gain independently of CRF changes (and vice-versa) or the other behaviours (p<0.05, data not shown). The other lifestyle behaviours and muscle strength (*16th-33rd week; 16th week-postpartum*) were not related to weight-gain or weight-retention. Mediation analyses showed that the effects of exercise on weight-gain were not explained by muscle strength or CRF changes (all, p>0.05). Generally, no effect modification of lifestyle behaviours or PF by foetal sex was found in weight-gain or weight-retention, except for upper-body muscle strength. Mothers who increased more upper-body muscle strength and had female foetuses, showed lower late weight-gain (p<0.05).

Table 1. Sociodemographic and clinical characteristics of pregnant women (n=121)

	Total (n=121)		Control (n=54)		Exercise (n=67)		p-value
Age (years)	33	5	34	5	33	4	0.22
Gestational age - 1st assessment	15.9	1.6	16.5	1.3	15.5	1.7	**<0.001**
Gestational age - 2nd assessment	33.0	1.9	34	(33, 35)	32	(31, 33)	**<0.001**
Gestational age - delivery	39.6	1.2	40	(39, 40.3)	40	(39, 41)	0.31
Percentage of attendance *					77.5	16.9	
Educational level, n (%)							
Non university degree	44	36.4	16	29.6	28	41.8	0.23
University degree	77	63.6	38	70.4	39	58.2	
Parity status (primarious), n (%)	76	62.8	32	59.3	44	65.7	0.59
Female offspring sex, n (%)	58	50.9	24	49	34	52.3	0.87
Use of oxytocin, n (%)	34	31.2	11	20.4	23	35.9	0.29
Use of epidural anesthesia, n (%)	82	73.9	30	62.5	52	82.5	**0.03**
Number of abortions	0	(0, 1)	0	(0, 1)	0	(0, 1)	0.25
Type of delivery, n (%)							
Spontaneous	69	59.5	30	60	39	59.1	0.51
Vacuum extraction	16	13.8	5	10	11	16.7	
Forceps	4	3.4	1	2	3	4.5	
Caesarean Section	27	23.3	14	28	13	19.7	
Smoking during pregnancy (cigarettes per day)	0	(0, 0)	0	(0, 0)	0	(0, 0)	0.15
Weight / Weight-gain							
Pre-pregnancy weight (kg)	63.9	11	61	8.8	66.3	12.1	**0.02**
Body mass index pre-pregnancy (kg/m²)	23.7	3.9	22.1	(20.5, 24.9)	23.4	(21.1, 27.4)	0.05
Weight at 16th week (kg)	66.0	10.9	63.4	9.2	68.1	11.8	**0.02**
Weight at 33rd week (kg)	74.7	10.9	73.6	10.2	75.5	11.5	**0.33**
Weight at postpartum (kg)[a]	68.4	11.6	67.1	11.0	69.2	11.9	**0.37**
Early weight gain - pre-pregnancy to 16th week (kg)	2.1	2.8	2.4	3.1	1.8	2.5	0.28
Late weight gain - 16th to 33rd week (kg)	8.7	3.4	10.2	3.1	7.5	3.2	**<0.001**
Total weight gain – pre-pregnancy to 33rdweek (kg)	10.8	4.9	12.6	4.9	9.1	4.4	**<0.001**
Weight retention - (kg)[a]	3.6	5.6	6	4.6	2.2	5.7	**0.001**
Sedentary lifestyle and PA, 16th week [b]							
Sedentary time (min/day)	510	100	495	109	521	91	0.18
Moderate-vigorous PA (min/day)	41	40	31	(20, 52)	35	(22, 48)	0.72
Vector magnitude counts	524449	233837	557374	315942	497104	128796	0.18
Average accelerometer wear time (min/day)	938	57	952	(883, 969)	953	(912, 979)	0.57
Relative percentage of daily sedentary time	54.3	10.1	53.1	10.8	55.3	9.4	0.25
Relative percentage of daily moderate-vigorous PA	4.4	4.8	4.9	6.7	4	1.9	0.31
Physical activity (wrist) 16th week [c]							
Vector magnitude counts	2322208	609579	2396871	669994	2261768	554022	0.24
Average accelerometer wear time (min/day)	934	65	926	79	941	50	0.22
Sleep parameters (accelerometry), 16th week [b]							
Sleep duration (min/day)	432	47	435	48	430	47	0.59
In-bed time (min/day)	491	49	493	54	490	46	0.73
Time awaken after sleep onsets (min/day)	54	21	50	(34, 65)	50	(40, 68)	0.46
Sleep efficiency (%)	88.1	4	89	5	88	4	0.87
Sleep quality (PSQI, 0-21)	5	(3, 9)	6	(4, 9)	5	(3, 9)	0.58
Dietary patterns, 16th week							
Adherence to the Mediterranean Diet Score (0-50)	29	4	29	4	30	4	0.25
Energy intake (kcal/day)	2546	88	2311	(1866, 2631)	2452	(2077, 3188)	**0.03**
Physical fitness, 16th week							
Upper-body muscle strength (kg)	27.5	3.9	27.8	4.3	27.2	3.6	0.45
$CRF_{85\%MHR}$ (s) [d]	380.4	103.9	357	101	394.4	104.4	0.08

$CRF_{85\%MHR}$, time to reach the 85% of the maximum heart rate in the submaximal Bruce treadmill test (a proxy of cardiorespiratory fitness); PA, physical activity; PSQI, Pittsburgh Sleep Quality Index. Continuous variables are presented as mean (standard deviation) when normally distributed, or median (interquartile range) when non-normally distributed, unless otherwise indicated. *When only considering those **women with >75% attendance, the average percentage of attendance was 86%.** [a] n=102, [b] n=108, [c] n=114, [d] n=99. P-values were calculated using independent sample Student's t-tests, Welch´s tests, and Mann-Whitney U tests for continuous variables, and Chi-square tests for categorical variables.

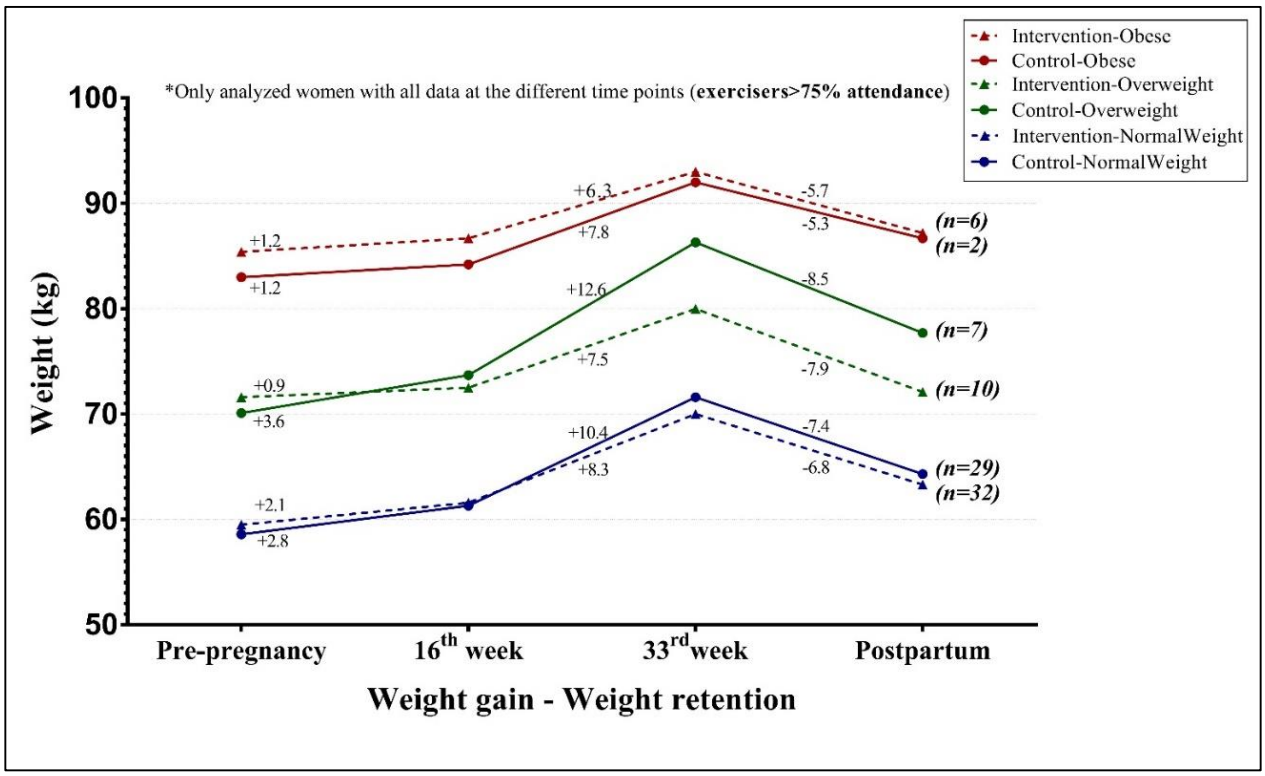

Figure 1. Schematic figure of the gestational weight-gain and postpartum weight retention during pregnancy according to the intervention group and pre-pregnancy weight-status (n=86). Only controls, and exercisers who attended more than 75% sessions, with weight data in all time points were included. *Similar weight-gain trajectories were observed when all exercisers - independently of the % attendance- were included in the exercise group (n for both groups=104).*

Additionally, we examined how lifestyle and PF changes were related to odds of having excessive late weight-gain (**Table 3)**. Exercise did not significantly prevent excessive weight-gain (p>0.05). Lower odds of showing excessive late weight-gain were observed in women who increased more their upper-body muscle strength (OR: 0.76, 95%CI: 0.60-0.96) and CRF (0.99, 0.98-0.99). Women who increased more their bed and sleep duration, and CRF, had lower odds of excessive total weight-gain (p<0.05).

Table 2. Associations of lifestyle and PF changes (from 16th to 33rd week, and from 16th week to postpartum) with maternal mean

	Weight gain (16th to 33rd week) (n=121)						Weight retention (p
	Model 1				Model 2	Adjusted	
	B	SE	β	p-value	p-value	R2 [b]	
Intervention *(n=101)*	-2.73	0.83	-0.42	**0.001**	**0.003**	0.14	**Intervention *(n=83)*[a]**
Lifestyle & Physical fitness changes *(16th-33rdweek)*							**Lifestyle & Physical fitness changes**
Sedentary time *(n=51)*	0.00	0.01	-0.10	0.48	0.76	0.07	Sedentary time *(n=47)*
Light PA *(n=51)*	0.02	0.01	0.31	**0.02**	**0.06**	0.16	Light PA *(n=47)*
Moderate-to-vigorous PA *(n=51)*	0.00	0.01	-0.01	0.94	1.00	0.06	Moderate-to-vigorous PA *(n=47)*
VMC (waist) *(n=51)*	0.00	0.00	0.05	0.70	0.83	0.06	VMC (waist) *(n=47)*
VMC (wrist) *(n=101)*	0.00	0.00	0.11	0.24	0.10	0.10	VMC (wrist) *(n=90)*
Bed time *(n=96)*	-0.01	0.01	-0.12	0.25	0.26	0.09	Bed time *(n=90)*
Sleep time *(n=96)*	-0.01	0.01	-0.07	0.51	0.41	0.08	Sleep time *(n=90)*
Awake time after sleep onset *(n=96)*	-0.01	0.01	-0.08	0.45	0.76	0.08	Awake time after sleep onset *(n=90)*
Sleep efficiency *(n=96)*	0.03	0.06	0.04	0.67	0.85	0.09	Sleep efficiency *(n=90)*
Sleep quality *(n=105)*	0.06	0.11	0.06	0.56	0.17	0.05	Sleep quality *(n=96)* ***(16th-Postpartum)***
Mediterranean Diet Score *(n=114)*	0.05	0.10	0.05	0.64	0.88	0.08	Mediterranean Diet Score *(n=80)* ***(16th-Postpartum)***
Upper-body muscle strength *(n=120)*	-0.01	0.10	-0.01	0.92	0.81	0.05	Upper-body muscle strength *(n=100)****(16th- Postpartu***
Cardiorespiratory fitness *(n=80)*[a]	-0.01	0.00	-0.25	**0.02**	**<0.001**	0.15	Cardiorespiratory fitness *(n=32)* ***(16th- Postpartum)***

B, unstandardized regression coefficient; β, standardized regression coefficient PA, physical activity; SE, standard error; VMC, vector magnitud

[a] A subtle variation of winsorizing was performed on extreme outliers of the predictor/outcome. Similar results were observed with outliers ha pregnancy BMI, and gestational week at 33rd week or weeks between birth-postpartum assessments (for weight gain and weight retention, were additionally adjusted for energy intake at 16thweek. Regarding the analyses of the other lifestyle changes with weight-gain analyses (16 the intervention group, and baseline values of the respective lifestyle behaviour; whereas in the analyses of the other lifestyle changes with we for the intervention group only. The results did not change after excluding pre-pregnancy BMI from previous models. [b] The adjusted R^2 values the familywise error rate (Hochberg procedure), only the associations of the intervention group and cardiorespiratory fitness remained associations by normal-weight (n=72) vs. overweight & obese women (n=29), the results remained similar for both groups; although, the effects in normal-weight women (p<0.05). In overweight & obese women, a non-significant trend was observed despite the greater reductions in lat (more inaccurate estimates). The effects of exercise on weight-retention were mainly driven by overweight-obese women (≈-7kg). When remained similar, but the association of CRF (16th week-postpartum) with weight retention became significant (model 2, n=26).

Table 3. Associations of lifestyle changes (from 16th to 33rd week) with excessive late and total weight-gain (adequate vs. excessive wei

	Adequate vs. Excessive late weight-gain								
	Model 1						Model 2		
	B	SE	OR	95%CI		p-value	p-value	B	SE
Intervention *(n=90)*	-0.45	0.50	0.64	0.24	1.69	0.36	0.67	-0.14	0.4
	Lifestyle & Physical fitness changes *(16th-33rd week)*								
Sedentary time *(n=43)*	0.00	0.00	1.00	1.00	1.01	0.44	0.73	0.00	0.0
Light PA *(n=43)*	-0.01	0.01	0.99	0.97	1.00	0.14	0.22	0.00	0.0
Moderate-to-vigorous PA *(n=43)*	0.00	0.01	1.00	0.99	1.02	0.62	0.60	0.01	0.0
VMC (waist) *(n=43)*	0.00	0.00	1.00	1.00	1.00	0.76	0.65	0.00	0.0
VMC (wrist) *(n=95)*	0.00	0.00	1.00	1.00	1.00	0.48	0.82	0.00	0.0
Bed time *(n=95)*	0.00	0.01	1.00	0.99	1.01	0.71	0.91	-0.02	0.0
Sleep duration *(n=95)*	0.00	0.01	1.00	0.98	1.01	0.59	0.88	-0.01	0.0
Awake time after sleep onset *(n=95)*	0.01	0.01	1.01	0.99	1.03	0.56	0.91	-0.01	0.0
Sleep efficiency *(n=95)*	-0.03	0.05	0.97	0.89	1.07	0.54	0.69	-0.02	0.0
Sleep quality *(n=97)*	0.08	0.08	1.09	0.92	1.28	0.31	0.15	0.13	0.0
Mediterranean Diet Score *(n=95)*	0.08	0.09	1.08	0.91	1.28	0.38	0.65	-0.09	0.0
Upper-body muscle strength *(n=104)*	-0.27	0.12	0.76	0.60	0.96	**0.02**	0.07	0.09	0.0
Cardiorespiratory fitness *(n=72)*[a]	-0.01	0.01	0.99	0.98	0.99	**0.05**	0.06	-0.01	0.0

B, unstandardized regression coefficient; β, standardized regression coefficient; OR, odds ratio; PA, physical activity; SE, standard error; VMC refers to $CRF_{85\%MHR}$. Late weight gain indicates the difference in weight from 16th to 33rd gestational week. Adequate weight gain is the category. The sample sizes for each subgroup were n=31 and n=74 (total n=105), respectively. The intervention group was introduced a winsorizing was performed on extreme outliers of the outcome. Similar results were observed with outliers handled and unhandled. In the maternal age. In the other lifestyle changes analyses, the models 1 were adjusted for maternal age and the baseline values of the respective energy intake at 16th week and occupational status. Cardiorespiratory fitness was associated with excessive late weight-gain independently Bed and sleep time was associated with excessive total weight-gain independently of cardiorespiratory fitness (and viceversa). After controllin no association remained statistically significant. When comparing **late** weight-gain analyses by normal-weight vs. overweight & obese wo although, the non-significant trend observed for the intervention group with lower excessive late weight-gain, was predominantly observe weight-gain analyses by pre-pregnancy BMI were not performed due to the reduced sample size within some categories. When the analyse significant (n=18).

Associations of weight-gain and retention with maternal-foetal outcomes

Late and total mean weight-gain were scarcely associated with maternal-foetal immunometabolic markers (**Tables S2-S3**), and were not associated with maternal-neonatal birth complications (p>0.05, data not shown). Postpartum weight-retention was positively associated with greater BMI, waist and hip circumference, fat and VAT mass, and lower lean and fat free mass at postpartum (all, $p \leq 0.05$; **Table S4**). Some associations differed according to foetal sex, intervention and pre-pregnancy BMI (**Tables S2-S4**: legends) -e.g. weight-retention was associated with waist/hip circumference, fat percentage and VAT only in controls ($p \leq 0.05$)-.

Excessive total weight-gain was inversely associated with IFN-γ (*16^{th}week-birth*), and positively related to postpartum BMI, diastolic blood pressure, hip circumference, relative fat free mass and fat mass (p<0.05; **Tables S5-S6**). When exploring these associations separately by the intervention group, exercisers with adequate total weight-gain showed a non-significant trend towards reduced maternal insulin levels and HOMA-IR compared to exercisers with excessive weight-gain (p=0.1; legend **Table S5**). Controls with adequate total weight-gain showed higher colostrum IL-10, and higher postpartum BMI and hip circumference than controls with excessive weight-gain (all, p<0.05; legend **Table S6**). Similar associations were found with excessive late weight-changes, except for a non-significant trend (p=0.06) towards having more non-spontaneous births in controls.

When only women with excessive total weight-gain were analysed (**Table 4**), exercisers were characterized by reduced systemic TNF-α (*16^{th}-33^{rd}week*), venous cord TNF-α and arterial cord IFN-γ, and greater arterial cord IL-10 and placenta weight, and postpartum BMI and hip circumference than controls (all, p<0.05).

Study VI

Table 4. Mean differences in maternal-foetal outcomes between exercise and control women with excessive total weight-gain (n=50)

	Control		Intervention		Control –Interv.		Control		Intervention		Control - Interv.	
	Mean	SE	Mean	SE	Mean D	SE	Mean	SE	Mean	SE	Mean D	SE
Biochemical markers	***Maternal serum (changes 16th-33rd week, n=50)***						***Maternal serum (birth, n=18)***					
Glucose (mg/dL)[ab]	-2.35	1.89	-1.78	2.05	-0.57	3.19	0.06	0.30	0.15	0.30	-0.09	0.43
Insulin (microIU/dL)[ab]	-0.46	1.21	3.58	1.32	-4.04	2.07						
HOMA-IR[a]	-0.07	0.18	0.42	0.19	-0.50	0.30						
HOMA-B[a]	99.31	156.6	390.59	161.7	-291.3	262.1						
Cholesterol (mg/dL)[a]	48.55	7.24	61.45	7.86	-12.90	12.34	212.1	13.96	200.88	13.96	11.22	21.11
Triglycerides (mg/dL)[ab]	87.78	9.89	101.21	10.72	-13.44	16.73	176.2	17.54	174.07	17.54	2.17	25.49
HDL-C (mg/dL)[a]	-2.11	2.04	-1.95	2.22	-0.16	3.56	92.32	10.56	74.09	10.56	18.23	17.73
LDL-C (mg/dL)[a]	34.88	6.81	40.32	7.38	-5.44	11.48	0.32	0.29	-0.21	0.29	0.53	0.43
Cortisol (mg/dL)	4.40	0.97	4.88	1.08	-0.48	1.66						
C-reactive protein (mg/dL)[a]	-0.15	0.09	0.06	0.10	-0.21	0.15						
Biochemical markers	***Arterial cord serum (birth, n=11)***						***Venous cord serum (birth, n=17)***					
Glucose (mg/dL)[c]	-0.44	0.35	0.18	0.28	-0.62	0.46	53.34	5.23	58.00	4.39	-4.67	6.84
Cholesterol (mg/dL)[c]	0.29	0.39	-0.42	0.31	0.71	0.51	-0.04	0.27	-0.21	0.23	0.17	0.35
Triglycerides (mg/dL)[cd]	0.19	0.36	-0.33	0.32	0.52	0.49	45.69	5.22	42.73	4.32	2.97	6.83
HDL-C (mg/dL)[c]	30.72	3.61	26.23	2.85	4.48	4.68	24.53	2.37	20.89	1.99	3.64	3.11
LDL-C (mg/dL)[cd]	0.60	0.36	-0.16	0.29	0.76	0.47	7.61	0.63	6.37	0.53	1.25	0.82
Phospholipids (mg/dL)[d]	98.46	9.47	94.25	8.32	4.21	12.79	93.73	8.05	99.60	6.66	-5.86	10.53
Cytokines	***Maternal serum (changes 16th-33rd week, n=22)***						***Maternal serum (changes 16th-birth, n=18)***					
Fractalkine (pg/ml)[e]	-13.96	23.58	-10.09	28.99	-3.87	39.58	-12.91	16.53	21.22	18.51	-34.13	25.03
Interleukin 1β (pg/ml)[e]	1.14	0.86	0.21	1.06	0.93	1.46	3.42	0.79	1.33	0.89	2.09	1.21
Interleukin 6 (pg/ml)[e]	0.45	0.87	-0.58	1.07	1.03	1.47	28.06	4.56	22.32	5.11	5.74	6.91
Interleukin 8 (pg/ml)	-1.72	2.40	4.59	2.95	-6.32	4.01	12.65	3.47	11.18	3.88	1.47	5.24
Interleukin 10 (pg/ml)	0.76	2.78	2.86	3.42	-2.09	4.67	17.65	4.21	24.27	4.72	-6.62	6.37
Interferon γ (pg/ml)	-3.61	2.68	-7.81	3.29	4.20	4.46	-8.11	1.41	-11.66	1.58	3.54	2.14
TNF-α (pg/ml)	1.62	0.53	-0.28	0.65	**1.90***	**0.89**	4.96	0.82	3.76	0.92	1.20	1.24
Cytokines	***Venous cord serum (birth, n=18)***						***Colostrum (postpartum, n=19)***					
Fractalkine (pg/ml)[g]	279.13	34.29	306.05	27.98	-26.92	44.34	-0.27	0.28	0.12	0.21	-0.39	0.35
Interleukin 1β (pg/ml)[fg]	1.26	0.23	1.58	0.19	-0.32	0.30	-0.22	0.28	0.44	0.21	-0.66	0.35
Interleukin 6 (pg/ml)[h]	12.84	1.59	13.43	1.29	-0.59	2.05	30.06	3.14	32.65	2.49	-2.60	4.02
Interleukin 8 (pg/ml)[f h]	60.02	6.10	60.55	4.97	-0.53	7.88	874.9	103.7	753.78	82.25	121.11	132.8
Interleukin 10 (pg/ml)	13.25	1.21	12.87	0.99	0.38	1.57	20.26	1.40	14.86	1.11	5.40	1.79
Interferon γ (pg/ml)	3.10	0.34	2.27	0.28	0.83	0.44	7.25	1.04	6.63	0.82	0.63	1.33
TNF-α (pg/ml)	17.42	1.41	15.79	1.15	**1.63***	**1.82**	13.66	1.86	16.09	1.46	-2.43	2.37
Body composition	***(postpartum, n=48)***											
Body mass index (kg/m^2)	24.78	0.41	25.91	0.29	**-1.13****	**0.51**						
Systolic blood pressure (mmHg)	105.52	1.87	108.70	1.27	-3.19	2.26						
Diastolic blood pressure (mmHg)	65.33	1.42	69.11	0.96	-3.78	1.72						
Bone mineral density (g/cm^2)	1.08	0.02	1.06	0.01	0.02	0.02						
Waist circumference (cm)	87.54	1.25	87.79	0.88	-0.26	1.53						
Hip circumference (cm)[i]	102.5	1.17	106.34	0.83	**-3.84***	**1.44**						
Relative lean mass	588.1	9.52	565.79	6.73	22.32	11.67						
Relative fat free mass	619.49	9.79	595.31	6.91	24.18	11.99						
Fat mass (g)	23634	782.7	27848	552.7	-4213	959.9						
Fat mass percentage	36.18	0.77	39.78	0.54	-3.61	0.94						
VAT mass (g)	351.93	22.94	373.18	16.20	-21.25	28.14						
Placenta weight (n=44)	541.10	19.30	621.8	19.60	**80.7*****	**28.6**						

B, Beta-cell function; GWG, gestational weight-gain; HDL-C, high density lipoprotein-cholesterol; HOMA, homeostasis model assessment; I
cholesterol; Mean D, mean difference; -, minus; SE, standard error; TNF-α , tumour necrosis factor alpha; VAT, visceral adipose tissue. Rel
fat free mass/weight. Handling of extreme outliers and/or Box-Cox transformations were applied on changes from 16th-33rd week[a] and birth[b], a
arterial[e] and venous[f] cord serum cytokines, on colostrum[g] and mature milk[h] cytokines, and on hip circumference[i]. All the models were ad
weeks after delivery, and baseline values of the respective outcome (only for maternal immunometabolic markers). Body composition variabl
mass index. After controlling for the familywise error rate (Hochberg procedure), only the association of the intervention group with placent

Sensitivity/exploratory analyses (data not shown)

When the analyses (Table 2) were additionally adjusted for maternal age, occupational status, parity, type o
of the start of the birth, baseline values of the lifestyle behaviours, smoking habits, sleep quality, MDS, ener
of ST/PA, the results remained similar. In the intention-to-treat analyses (**Appendix F**-data not shown), we
late and total weight-gain showed the same trend, but not significant (p<0.1); and its effects on weight-rete
The exercise group also showed a trend towards the prevention of excessive late weight-gain (p=0.058).
Additionally, since genetics is a relevant determinant for weight-gain[1], we explored whether women (and
were more susceptible to weight-gain and increased placental (and neonate) weight at birth (see **Appendix**

DISCUSSION

Despite the increasing literature regarding the role of lifestyle (including exercise) on maternal weight-gain, evidence is still equivocal and elusive, and none study has analysed which lifestyle behaviour or PF capacity is of greater utility to prevent excessive weight-gain during pregnancy. Moreover, little is known about how exercise-induced weight-changes are associated with maternal-foetal metabolism, and if exercise can attenuate the adverse alterations related to excessive weight-gain.

Effects of exercise on weight-gain and retention

The main finding of this study shows that our supervised-tailored exercise program notably reduced maternal weight-gain (2.71 kg) and weight-retention (2.25 kg), independently of other behaviours (ST/PA, sleep, etc.) and PF; which highlights the robustness of its effects. Noteworthily, none study has previously accounted for these relevant behaviours, which might have confounded the conclusions obtained until now on the effects of exercise on weight-gain. However, although more exercisers than controls avoided excessive weight-gain, exercise did not prevent excessive weight-gain (plausibly due to the reduced sample size within subgroups of women with adequate weight-gain).

These findings concur with those reported by previous effective exercise interventions[5,7-9,25,26], showing that exercise reduced mean weight-gain, although are contrary to others[10,11,27]. Intriguingly, in ours and previous studies[7,9], the preventive effect of exercise against weight-gain was observed in normal-weight but not in overweight-obese women, as expected given their adverse phenotype. This suggests that the effect of exercise on gestational weight-gain might be dependent on pre-pregnancy BMI levels. However, other exercise interventions clearly reduced weight-gain in overweight-obese women[5,8], which hinders the interpretation of the current evidence. These discrepancies are likely explained by differences in the exercise protocols, women's genotype and phenotype, statistical power, etc. Actually, meta-analytic evidence[6], which also outlines that exercise limits mean weight-gain (with smaller effects in overweight-obese/diabetic women) and prevents excessive weight-gain, is limited by the scarce studies and heterogeneity among study designs. Of note, contrary to this meta-analytic evidence[6], the current exercise intervention was also effective to limit weight-retention (mainly driven by overweight-obese women).

The role of lifestyle and PF on weight-gain and retention

Importantly, none of the other lifestyle behaviours or PF components was related to mean weight-gain in our study, except for greater CRF, which was independently associated with lower mean weight-gain. Additionally, we observed that increasing CRF and upper-body muscle strength was slightly related to lower excessive weight-gain. Although speculative, this might be explained because an increase in PF is related to an improvement in women´s metabolic profile[28-30], and an increase of their muscle mass[30] and resting energy expenditure[29,31] (the latter accounts ≈ for 60-70% of daily energy expenditure), leading to lower weight-gain[29,30,32]. The higher postpartum lean and fat free mass, and lower fat mass observed in our participants with reduced weight-gain, might support this speculation. Regarding sleep patterns, the low-quality and scarce evidence in pregnancy is contradictory[33,34]. Similarly to another study[34], we observed that increasing sleep duration was related to lower total excessive weight-gain, but not sleep quality –as usually reported[33,34]– or sleep disruptions. Dysregulations in the neuroendocrine control of appetite and satiety might underlie the link between short-duration and weight-gain[35].

Unfortunately, we cannot establish the causal direction of these associations. Actually, reverse-causality might be possible, being impaired CRF (indicative of worst cardiac function[36]) or sleep patterns a direct consequence of excessive weight-gain. Thus, whether increasing PF and sleep time could be useful tools for preventing excessive weight-gain, or rather represent impaired/physiological adaptations orchestrated by the course of pregnancy, is unknown. Why previous PA and dietary interventions limited exacerbated weight-gain[6], contrary to our study, is plausibly explained by the different study design (we analysed PA and MDS changes).

Although genetics can affect weight-gain in this context[1], our pregnant women´s genotype did not influence weight-gain susceptibility or placental weight. However, neonatal birth weight was greater in those women with CC genotype in rs6567160 and rs17782313 polymorphisms (MCR4 gene). The reduced sample size prevented us from interaction lifestyle-genotype analyses, and from further interpretation.

The protector role of exercise against excessive gestational weight-gain

Our main findings, along with the metabolic and body composition alterations usually observed with exacerbated weight-gain[1-3,19], raised the question of whether exercise

could play a protector role in maternal and foetal metabolism via counteracting the adverse alterations related to excessive weight-gain.

Firstly, we observed that exercisers with adequate weight-gain slightly reduced –but not significantly (p=0.1)– maternal insulin and insulin resistance compared to exercisers with excessive weight-gain. Secondly, controls with excessive weight-gain showed lower colostrum IL-10 and higher postpartum BMI and hip circumference than controls with adequate weight-gain; but this association was not observed in the exercised counterparts. Of note, we observed that exercisers with excessive weight-gain had lower systemic TNF-α, venous cord TNF-α, and arterial cord IFN-γ, and higher arterial cord IL-10 than controls with excessive weight-gain. Despite the exploratory ground, this suggest that exercise might have slightly attenuated the pro-inflammatory state[18] related to excessive weight-gain. Thirdly, exercise did not reduce the risk of birth complications associated with excessive weight-gain, which was probably due to the low number of complications reported. We only observed a non-significant trend (p=0.06) towards higher non-spontaneous births in controls with excessive weight-gain. Lastly, we observed that those women with excessive weight-gain who exercised *(vs. controls)* had increased placenta weight without changes in neonatal birth weight, which is indicative of improved placental development and function[8,37]. Taken together, these findings suggest that although exercise was not able to prevent excessive weight-gain, it may play an indirect protective role against exacerbated weight-gain by attenuating some of its associated adverse metabolic consequences. However, as previously postulated[14], exercise-induced weight changes were not either involved in the causal pathway between exercise and pregnancy complications in our study.

Given the robust effects of this exercise program on weight-gain and retention, which were independent of ST, PA, sleep, diet quality, and PF, its implementation in the clinical context is of considerable utility to better control weight-gain and weight-retention (i.e. weight management). This might help to avoid potential birth complications, impaired metabolism and future diseases[1]. Its application into the practice is further supported by: i) exercisers did not suffer any adverse event during the exercise program; and ii) exercise appears to modulate positively the anti-inflammatory environment and placental function/development potentially impaired by excessive

weight-gain. These plausible benefits are likely to be explained by both exercise-induced effects independent and dependent of reduced weight-gain[1,3,13,15,18,31,36,37].

Unfortunately, we cannot speculate about the clinical relevance of exercise in the associations between mean weight-gain and maternal-foetal outcomes, since lower/increased weight-gain in this context might reflect normal physiological alterations in pregnancy rather than improved/impaired metabolism. Studies with greater sample sizes, including several weight-gain ranges and considering pre-pregnancy BMI, are necessary before reaching any solid conclusion.

LIMITATIONS

Selection bias cannot be dismissed because roughly half of women were not randomized. Needless to say, a pure randomization in pregnancy studies is really difficult. However, we did our best to avoid other biases, since the overall methodological quality of the study is probably more decisive than the lack of a randomized design itself[38]. Pre-pregnancy weight was self-reported. However, considering that its association with clinical-measured pre-pregnancy weight is linear ($r=0.99$)[2], this is unlikely to affect our results. Moreover, the modest sample size for some analyses prevented us from "ideally" conducting the analyses by BMI categories. Thus, the heterogeneity of our sample (≈30% were overweight-obese) might hinder the interpretation of some results. However, we accounted for the pre-pregnancy BMI in all analyses –inclusive for weight-gain calculation as indicated by the IOM[1]. Some strengths need to be mentioned: i) an expert multidisciplinary team designed this novel tailored exercise intervention following the last ACOG guidelines[4] and previous evidence[39,40]; ii) all exercise sessions were strictly supervised, and the attendance, intensity, and other parameters were monitored regularly; iii) this is the first time that such a comprehensive insight of how lifestyle and PF influence weight-gain and weight-retention has been provided; iv) the immunometabolic markers were assessed at multiple time points, including arterial and venous cord serum, and breast milk; v) postpartum body composition were assessed with DXA; and vi) overcoming limitations from previous studies, we have considered relevant confounders such as objectively measured ST/PA (7 days, ≥10hours/day), sleep patterns, diet quality, and PF.

CONCLUSIONS

Overall, this concurrent exercise intervention was effective to reduce mean weight-gain and postpartum weight-retention, independently of other lifestyle behaviours and PF. Although exercise did not prevent excessive weight-gain, it appears to play a protector role in maternal-foetal metabolism against the impaired phenotype related to exacerbated weight-gain. Of note, higher CRF, muscle strength and sleep duration were related to lower late and/or excessive weight-gain. Further studies are warranted to verify whether exercise protects against adverse alterations related to excessive weight-gain, and to establish the causal link between lifestyle behaviours and PF with weight-gain.

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SUPPLEMENTARY MATERIAL

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Appendix A. *Talks provided to pregnant women*

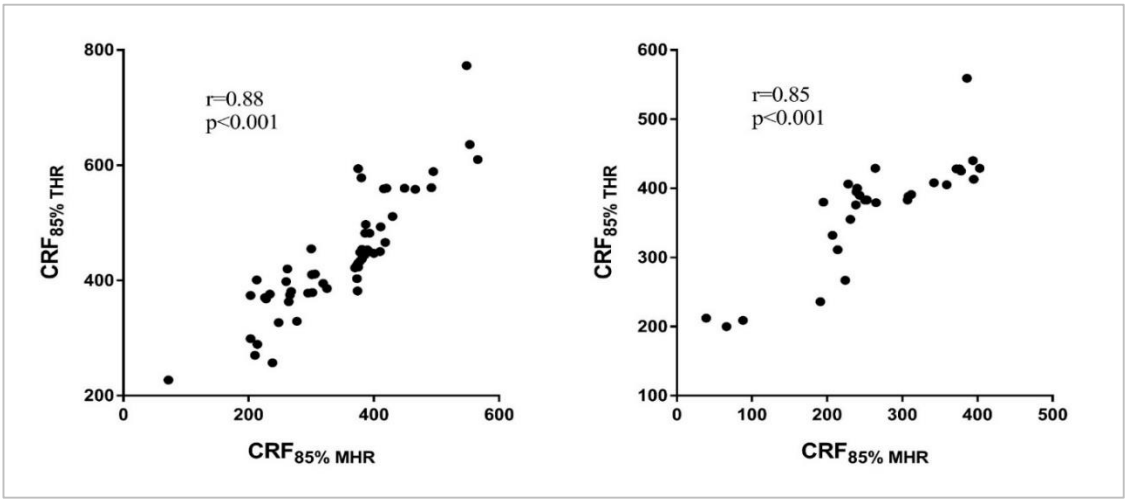
During the course of the intervention, the research team gave 7 lectures to pregnant women from both (exercise and control) groups about: i) the benefits of physical exercise for a better pregnancy, prevention and treatment of cardiovascular diseases and excessive weight-gain; ii) ergonomic advises, exercises to perform at home and strategies to increase their daily physical activity levels; iii) the benefits of the Mediterranean Diet and adequate nutritional habits during pregnancy; iv) how to avoid toxics and chemicals during the pregnancy and breastfeeding; v) pregnancy, postpartum and sex; vi) physical and mental preparation for the labour, what to expect; and vii) nutritional education towards breastfeeding. We also used these conferences to maintain the control group fidelity until the end of the program.

Appendix B. *Description of cardiorespiratory fitness*

Cardiorespiratory fitness (CRF) was assessed with the submaximal modified Bruce protocol[1,2]. This treadmill test consists in increasing the slope and speed of the treadmill during 5 progressive workload stages, each of 3 minutes (stage 1: 2.7 km/h, 10% inclination; stage 2: 4 km/h, 12%; stage 3: 5.5 km/h, 14%; stage 4: 6.8 km/h, 16%; stage 5: 8 km/h, 18%). During the trial, women were encouraged to first reach the 85% of their age-predicted maximum heart rate (85%MHR), and subsequently the 85% of their target heart rate ($85\%_{THR}$). The $85\%_{THR}$ was calculated according to the heart rate reserve (Karvonen formula)[3] to consider the within-individual basal heart rate. The test was finished when women reached the $85\%_{THR}$, or when they reported to reach volitional fatigue. If women did not reach at least the $85\%_{MHR}$, their data was not considered for the quantitative analyses. Previous authors have shown that not only time to exhaustion during the maximal Bruce treadmill test, but also time to $85\%_{THR}$ during the submaximal modified Bruce treadmill test are highly correlated with the direct measurement of the maximal volume of oxygen consumption (Vo2max) in women (r=0.92, r=0.82; respectively)[3]. Hence, and considering that exercising until volitional exhaustion might be an unsafe and unethical practice in pregnant women (potential burden to maternal/foetal health), time to $85\%_{MHR}$ and $85\%_{THR}$ were regarded as proxies of

cardiorespiratory fitness (since now on called as $CRF_{85\%MHR}$ and $CRF_{85\%THR}$). $CRF_{85\%MHR}$ and $CRF_{85\%THR}$ were highly correlated (r≈0.9; see in the figures below). Heart rate was continuously controlled with a monitor (Polar V800, Finland). Although cardiopulmonary submaximal exercise testing is usual and safe in pregnancy[4], a harness was employed to secure women (not for support) during the test to prevent any potential fall and the consequent risk. None complication or adverse consequence led us to stop the tests.

Correlations between $CRF_{85\%MHR}$ and $CRF_{85\%THR}$ at 16th week (left figure) and 33rd week (right figure). $CRF_{85\%MHR}$, time to 85% of the maximum heart rate; $CRF_{85\%THR}$, time to 85% of the target heart rate.



Appendix C. Secondary/exploratory outcomes

Blood (maternal and cord) and milk collection[5,6]

Maternal venous blood samples (5 mL) were extracted from the antecubital vein of women (16th and 33rd weeks) in standardized fasting conditions (8-9a.m.), and kept in serum tubes. Then, they were centrifuged (GS-6R Beckman Coulter, CA, USA), and serum was aliquoted, and frozen at -80ºC until analyses. Similarly, maternal, and arterial and venous cord blood samples were obtained immediately after delivery, centrifuged and aliquoted, and frozen at -80ºC until analyses.

Breast colostrum (3mL) and mature milk (5mL) were collected into Falcon tubes 24 hours and 6 weeks after birth, respectively[6]. After their processing, samples were frozen at -80ºC in Eppendorf tubes.

Immunometabolic biomarkers

Glucose and lipids markers, C-reactive protein, cortisol and insulin resistance (maternal/cord serum)

Spectrophotometric enzyme assays (AU5822 Clinical Chemistry Analyzer, Beckman-Coulter, CA, USA) were used to measure maternal serum glucose, total cholesterol, triglycerides, high and low density lipoprotein-cholesterol (HDL-C, LDL-C), phospholipids, and C-reactive protein (CRP) levels, at 16th and 33rd weeks. Chemiluminescence immunoassays (UniCel Dxl-800-Access Immunoassay analyser, Beckman-Coulter, CA, USA) were also used to assess maternal insulin and cortisol concentrations. The homeostasis model assessment (HOMA)-IR (insulin resistance) and HOMA-B (B-cell function) were estimated using standard formulas[7].

At delivery, spectrophotometric determination (BS-200 Chemistry Analyzer, Mindray Bio-medical Electronics CO.LTD, Shenzen, China) was employed to assess maternal and arterial and venous cord serum glucose, total cholesterol, triglycerides, HDL-C, LDL-C, and phospholipids.

Cytokines (maternal/cord serum and breast milk)

Luminex xMAP techonology based on MILLIPLEX MAP kits was employed to assess maternal, arterial and venous serum, and colostrum and milk cytokines (fractalkine, IL-1β, IL-6, IL-8, IL-10, IFN-γ, and TNF-α)[5,6]. Luminex xMAP technology (Millipore, Darmstadt, Germany) is a mix of three existing and proved technologies: use of microspheres, flow cytometry, and laser technology, mixing digital signal processing and traditional chemistry immunoassay. Because of robust multiplexing, xMAP technology potentially delivers more data in less time than other bioassay products, with comparable results with enzyme linked immunosorbent assay and microarray. The technology offers several other distinct advantages over traditional methods such as speed and high throughput, versatility, flexibility, accuracy, and reproducibility. Particularly, for maternal pro-inflammatory and anti-inflammatory (fractalkine, interleukin-1β, interleukin-6, interleukin-8, interleukin-10, interferon-γ and tumour necrosis factor-α) determination, we used Human Sepsis Magnetic Bead Panel 3 Multiplex Assay (cat. No. HTH17MAG-14K). We prepared samples, reagents, and standards by following the manufacturer's instructions. Equipment settings: 50 events per bead, gate settings: 8,000-15,000, time out 60 seconds. Plate was read on LABScan

100 analyser (Luminex Corporation, Texas, USA) with xPONENT software for data acquisition. The average values for each set of duplicate samples or standards were within 15% of the mean. We determined cytokine concentrations by comparing the mean of duplicate samples with the standard curve for each assay.

Body composition (postpartum)

Lean, fat and visceral adipose tissue (VAT) mass, as well as total body mineral density were measured with a whole-body dual-energy x-ray absorptiometry (DXA; Discovery Wi, Hologic, Bedford, MA) scanner[8], according to manufacturer´s recommendations. The software APEX-4.0.2 was employed for automatic delineation of anatomic regions.

Maternal and neonatal genotype

Mother and neonates were genotyped for several SNPs in FTO (rs1558902, rs8050136 and rs9939609) and MC4R (rs6567160 and 17782313) genes. Mucosa cells were collected with swabs, and DNA was isolated by a non-organic extraction (proteinase K and salting-out) previously described and validated[9,10], and later spectrophotometric quantification (NanoDrop-2000c, TermoFisher) was performed. Genotyping was performed using TaqMan® assays and Master Mix (Applied Biosystems, USA). Polymerase chain reaction and subsequently allelic discrimination were carried out in a QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems, USA). Plates included controls (with known genotype) and non-template control for each SNP. The data was analysed using the TaqMan® Genotyper Software.

Probes details: Gene ID, Chromosome, position, accession number (rs#) TaqMan™ assay ID (Thermo Fisher Scientific, MA, USA), reference and alternative alleles.

Gene	Chromosome	Position	SNP ID	Probe ID	Reference allele	Alternative allele
FTO	16	53769662	rs1558902	C___8917111_10	T	A
FTO	16	53782363	rs8050136	C___2031259_10	C	A
FTO	16	53786615	rs9939609	C__30090620_10	T	A
MCR4R	18	60161902	rs6567160	C___3058649_20	T	C
MCR4R	18	60183864	rs17782313	C__32667060_10	T	C

Appendix D. Outlier detection and management

Nowadays, the presence of outliers is one of the most enduring and pervasive methodological changes in biomedical science research[11-13]. Worryingly, there is a lack of consensus about how to address outliers (i.e. how defining, identifying and handling them). Since the decisions that researches make about this issue have important implications, we have included this section to promote transparency and the critical interpretation of the results, as previously recommended by several authors[11-13]. Although no specific guidelines exist about how addressing outliers, several studies[11-18] (especially that one from Aguinis, et al.[13]) have previously provided smart advices and recommendations to address them in the best possible way. Accordingly, the different steps to address outliers in the present study have been performed proceeding with the following recommendations. We have identified and handled outliers according to the basis for regressions, which are the main analyses involved in this study.

Error outliers

During the assessments at the different time points, questionnaires and tests (where errors related to data recording, coding, manipulation, etc. were likely and easily observed) were checked to identify clear error outliers, and correct them immediately by asking women, repeating the corresponding test, etc. When lacking, misleading or inaccurate data, was identified a posteriori (up to 2 weeks after the assessments), women were contacted to ensure the accuracy of these data points, or to correct these potential outliers (whenever appropriate for data) in the respective database. Singles construct techniques (box plots, descriptive statistics, percentage analyses, etc.) were performed to initially identify error outliers.

Subsequently, we also employed multiple construct techniques to identify error outliers. Particularly, we identified error outliers based on the outlyingness of the observation in term of its residual score. When it was not possible/appropriate to correct these data points, and we were sure that their inaccuracy was related to human errors, device malfunction, miscalculations or similar circumstances (i.e. we had determined the cause of the identified outlying observation), these error outliers were removed from the respective database. Since these potential error outliers could have been caused by inherent variability in the data (in this case they would represent a legitimate part of the population), we were very prudent when identifying and handling

them. We paid special attention to the reasoning behind the classification of data points as error outliers.

Interesting outliers

After the application of this first filter to the database, there were several remaining interesting outliers, which required additional analyses in depth. Thereby, we aimed at analysing these interesting outliers with quantitative approaches (e.g., we tried to analyse differences in how predictors were able to predict high and low outlier scores). However, the number of outliers was minimum, and only appreciable in few outcomes, which prevented us from performing these analyses properly. As consequence, we did not finally perform these analyses.

Influential outliers

Since it is not legitimate to simply drop the remaining potential outliers from the analyses (they tend to increase error variance, reduce the power of statistical test, etc.), nor plainly deleting them without any basis (they could be part of the inherent variability of the distribution of data), we analysed more in depth the influence of these outliers in the model. Aimed at checking their influence, we analysed how the deletion of specific outliers could affect the change of the model fit (e.g., changes in R^2; model fit outliers), parameters estimates (intercept, slope, regression coefficients, etc.; prediction outliers) and the assumptions of the model. If these remaining unusual cases were not finally identified as influential outliers, or they were identified but influenced the model slightly, these potential outliers were not handled (as observed in some outcomes the **Tables 2-3**). In this case, these unusual data points were dropped in the analyses since they did not affect either the results or assumptions of the tests, and they could be caused by inherent variability in the data. By contrast, if these remaining unusual cases were confirmed as influential outliers which affected the model fit and parameter estimates (as appreciable in the **Tables 2-3**), those influential outliers we handled.

In order to handle the aforementioned influential outliers (when identified), a subtle variation of winsorizing [convert back from a z-score: replacing extreme scores ($z>2.58$; value equivalent to a probable outlier) with a score equivalent to ±2.58 standard deviations from the mean] was employed to handle these outliers. After handing these outliers, data distribution improved, and some of the problematic issues related to the assumptions of some models disappeared. Subsequently, data preparation was

employed for those characterized by remaining asymmetry (skewness, kurtosis, etc.) of outcomes, and the violation of some assumptions related to the generalization of the results. Specifically, optimum Box-Cox transformations were used to reduce the impact of potential source of bias, and improve the goodness of fit of the data. After dealing with these "problematic" outcomes, the results remained similar (but with better and more symmetrical distribution of data) to the analyses without data preparation (i.e. without handling of outliers or/and applying Box-Cox transformations).

Appendix E. Reasons for losses and exclusions during the enrolment and follow-up

From the 159 women who participated in the study and were allocated to the control (n=87) or intervention (n=72) group, 10 controls dropped out of the study (lost to follow-up) because of: moving to another city (n=1), unwillingness to continue (n=7) or unknown reasons (n=2). In the control group, 33 women did not come to the evaluation (33rd week) because of personal reasons. Data loss (n=10) at delivery was related to women who did not contact us, attended private hospitals, or midwives who did/could not collect data/samples. In the exercise group, none woman dropped out the study. From the 72 women in the exercise group, 3 women did not come to the 33rd week, and only 47 women attended >75% of the sessions (57 women attended >66% of the sessions). In summary, 101 women (control n=54, exercise n=47) were included in the main analyses of the current study. Because of discontinued intervention and lack of data related to the specific time points, only 39 controls and 44 exercisers were considered for the weight-retention analyses (control n=39, exercise=44). Intention-to-treat analyses were performed with 87 and 72 women from the control and exercise group respectively. More information about these analyses are shown in **Appendix F**.

Appendix F. Intention-to-treat analyses

Aimed at investigating more realistically the effectiveness of a concurrent exercise-training program on gestational weight-gain (main outcome) when applied to the clinical practice, the aforementioned statistical analyses (**Tables 2-3**) were conducted on an intention-to-treat basis (data not shown), as recommended by the CONSORT guidelines.

Considering that more than 20% of cases were missing in some outcomes, and some authors do not recommend to perform imputations in this context[19], these analyses should be interpreted cautiously. Particularly, the number of missing cases in the different outcomes were:

-Weight at pre-pregnancy,	missing cases (all women) n=9, **lost%=5**
-**Weight at 16th week,**	missing cases (all women) n=0, **lost%=0**
-**Weight at 33rd week,**	missing cases (all women) n=36, **lost%=22.6**
-**Weight at postpartum,**	missing cases (all women) n=52, **lost%=32.7**
-**Weight-gain (pre-pregnancy to 16th week),**	missing cases (all women) n=11, **lost%=6.9**
-**Weight-gain (16th-33rd week),**	missing cases (all women) n=38, **lost%=23.9**
-**Weight-gain (pre-pregnancy to 33rd week),**	missing cases (all women) n=36, **lost%=22.6**
-**Weight-gain (pre-pregnancy to postpartum),**	missing cases (all women) n=57, **lost%=35.8**

The lacking data in most cases of these weight-gain outcomes was "missing completely at random" (**MCAR**). Overall, the number and percentage of missing cases were higher in the control group than in the exercise group.

In order to analyse how "accurate" was the imputed data in those missing cases on maternal weight outcomes, we compared the relative percentage of variation of the descriptive statistics (mean, standard deviation, and minimum and maximum values) from the database before and after imputation. Overall, we observed that the variation in % of these parameters was:

-Weight at pre-pregnancy,	%mean=0.6, %SD=8, %minimum=0, %maximum=18.5
-**Weight at 16th week,**	%mean=0, %SD=0, %minimum=0, %maximum=0
-**Weight at 33rd week,**	%mean=0.9, %SD=1.9, %minimum=0, %maximum=0.3
-**Weight at postpartum**,	%mean=0.3, %SD=5.3, %minimum=0, %maximum=3.9
-**Weight-gain (pre-pregnancy to 16thweek),**	%mean=0.3, %SD=1.7, %minimum=32, %maximum=19
-**Weight-gain (16th-33rd week),**	%mean=4.4, %SD=5.1, %minimum=68, %maximum=3.2
-**Weight-gain (pre-pregnancy to 33rd week),**	%mean=1.5, %SD=1.9, %minimum=76, %maximum=11
-**Weight-gain (pre-pregnancy to postpartum)**	%mean=5.2, %SD=17, %minimum=39, %maximum=15

The rest of statistical and procedures were done the same way as the per-protocol analyses.

Appendix G. Weight-gain susceptibility: FTO and MCR4 genes (n=81)

As exploratory analyses, analyses of covariance were employed to investigate whether women with a specific genotype could be more susceptible to weight-gain during pregnancy, and increased placental weight at birth. Additionally, we tested if newborns with a specific genotype were associated with increased birth weight.

These genes were chosen because of their relation with obesity and weight-gain in the general populations and pregnancy[20-22.]

-Non-significant reductions in late (*16th week-33rd week*), total weight-gain (*pre-pregnancy-33rd week*), and weight retention (*pre-pregnancy-postpartum*) were observed in women characterized by AA genotype in the rs1558902, rs8050136 and rs9939609 polymorphisms (FTO gene) (all, $p>0.10$). Non-significant reductions in weigh-retention were either observed in mothers from newborns with AA genotype in these SNPs ($p>0.05$). MCR4 SNPs were not related to weight-gain (all, $p>0.05$).

-Overall, no differences were observed in neonatal birth weight in women and newborns characterized by different genotypes, except for the polymorphisms rs6567160 and 17782313 of the MC4R gene in pregnant women. Specifically, women characterized by CC genotype in rs6567160 and rs17782313 polymorphisms showed increased neonatal birth weight ($p<0.05$, compared to CT and TT genotypes). However, these differences have to be interpreted cautiously given the reduced sample size (CC n=4, CT n=36, TT n=41).

-Similarly, no differences were observed in placental weight at birth between women and newborns characterized by different genotypes.

-The interaction between lifestyle behaviors and genotype were not considered due to limited sample size.

Future studies with considerable greater sample size are necessary to test these hypotheses.

Table S1. Inclusion and exclusion criteria in the GESTAFIT project

Inclusion criteria
- Pregnant women aged 25-40 years old with a normal pregnancy course.
- Answering “no” to all questions of the PARmed-X for pregnancy.
- Being able to walk without assistance.
- Being able to read and write properly.
- Informed consent: Being capable and willing to provide written consent.
Exclusion criteria
- Having acute or terminal illness.
- Having malnutrition.
- Being unable to conduct tests for assessing physical fitness or exercise during pregnancy.
- Having pre-pregnancy risk factors (such as hypertension, type 2 diabetes, etc.).
- Having a multiple pregnancy.
- Having chromosopathy or foetal malformations.
- Having uterine growth restriction.
- Having foetal death.
- Having upper or lower extremity fracture in the past 3 months.
-Suffering neuromuscular disease or presence of drugs affecting neuromuscular function.
- Being registered in another exercise program.
- Performing more than 300 minutes of at least moderate physical activity per week.
-Being engaged in another physical exercise program
- Being unwilling either to complete the study requirements or to be randomized into the control or intervention group.

Table S2. Association of **late** mean weight-gain (from 16th to 33rd week) with maternal and foetal immunometabolic markers (

	Model 1				Model 2	Model 1				Model 2	Model 1			
	B	**SE**	**β**	**p-value**	**p-value**	**B**	**SE**	**β**	**p-value**	**p-value**	**B**	**SE**	**β**	**p-val**
Biochemical markers			***Maternal serum Changes 16th-33rd week (n=86)***					***Maternal serum Birth (n=37)***					***Arterial cord serum (birth***	
Glucose (mg/dL)[abc]	0.15	0.42	0.05	0.72	0.30	0.05	0.06	0.14	0.44	0.24	-0.06	0.07	-0.21	0.3
Insulin (microIU/dL)[a]	0.05	0.03	0.16	0.19	0.17									
HOMA-IR[a]	0.04	0.03	0.15	0.25	0.18									
HOMA-B[a]	0.04	0.04	0.12	0.37	0.62									
Cholesterol (mg/dL)[abc]	1.21	1.18	0.13	0.31	0.53	3.64	3.40	0.21	0.29	0.40	-0.11	0.08	-0.31	0.1
Triglycerides (mg/dL)[abcd]	0.94	1.68	0.07	0.58	0.11	2.86	3.87	0.15	0.47	0.44	-0.23	0.07	-0.59	**0.0**
HDL-C (mg/dL)[ac]	-0.17	0.33	-0.06	0.62	0.80	0.66	1.70	0.08	0.70	0.97	0.53	0.80	0.15	0.5
LDL-C (mg/dL)[abcd]	1.21	1.13	0.13	0.29	0.49	0.07	0.06	0.20	0.31	0.42	0.01	0.08	0.03	0.8
Phospholipids (mg/dL)[d]	3.96	2.92	0.24	0.18	0.25	-2.34	2.93	-0.16	0.43	0.62	0.01	2.08	0.00	1.0
Cortisol (mg/dL)	0.19	0.22	0.10	0.41	0.93									
CRP (mg/dL)[a]	0.02	0.02	0.12	0.35	0.25									
Cytokines			***Maternal serum Changes 16th-33rd week (n=44)***					***Maternal serum Changes 16th-birth (n=36)***					***Arterial cord serum (birth***	
Fractalkine (pg/ml)[e]	-0.80	4.98	-0.03	0.87	0.90	-0.64	4.18	-0.03	0.88	0.72	-2.56	6.93	-0.08	0.7
Interleukin 1beta (pg/ml)[ef]	0.02	0.15	0.02	0.92	0.94	0.08	0.20	0.08	0.68	0.96	0.02	0.04	0.11	0.6
Interleukin 6 (pg/ml)[e]	-0.06	0.17	-0.06	0.73	0.94	-0.67	0.79	-0.17	0.41	0.56	-0.07	0.25	-0.05	0.7
Interleukin 8 (pg/ml)[f]	0.34	0.50	0.12	0.50	0.31	-1.71	0.96	-0.34	0.09	0.35	-3.27	1.82	-0.38	0.0
Interleukin 10 (pg/ml)	0.03	0.65	0.01	0.96	0.81	0.79	0.89	0.17	0.38	**0.05**	0.07	0.22	0.07	0.7
Interferon gamma (pg/ml)	-0.75	0.50	-0.25	0.14	0.14	-0.58	0.55	-0.20	0.30	0.09	-0.05	0.09	-0.11	0.6
TNF-α (pg/ml)	-0.10	0.12	-0.14	0.42	0.76	-0.15	0.15	-0.19	0.34	0.66	-0.13	0.22	-0.13	0.5
Cytokines (breast milk)			***Colostrum (postpartum, n=34)***					***Mature milk (postpartum, n=35)***						
Fractalkine (pg/ml)[g]	0.11	0.06	0.33	0.07	**0.05**	107.1	53.4	0.36	0.06	**0.04**				
Interleukin 1beta (pg/ml)[g]	0.10	0.06	0.31	0.11	0.13	-0.07	0.08	-0.18	0.40	0.87				
Interleukin 6 (pg/ml)[h]	-0.49	0.64	-0.14	0.45	0.28	0.02	0.07	0.05	0.81	0.68				
Interleukin 8 (pg/ml)[h]	-23.2	21.62	-0.18	0.29	0.18	-0.09	0.07	-0.29	0.17	0.22				
Interleukin 10 (pg/ml)	-0.74	0.31	-0.43	**0.02**	**0.03**	-0.40	0.23	-0.32	0.10	0.39				
Interferon gamma (pg/ml)	0.00	0.23	0.00	0.99	0.85	-0.08	0.12	-0.13	0.52	0.08				
TNF-α (pg/ml)	0.69	0.41	0.29	0.10	0.26	0.15	0.25	0.12	0.54	0.39				

B, unstandardized regression coefficient; β, standardized regression coefficient; CRP, C-reactive protein; HDL-C, high d
homeostasis model assessment; IR, insulin resistance; LDL-C, low density lipoprotein-cholesterol; SE, standard error; TNF-α, tu

extreme outliers *(only 1-4 extremes values within each outcome were treated)* and/or Box-Cox transformations were applie
birth[b], arterial[c] and venous[d] cord serum biochemical markers, arterial[e] and venous[f] cord serum cytokines, and on colostrum[g] a
1 were adjusted for pre-pregnancy body mass index and for the intervention group; and the **models 2** were additionally adjus
birth, energy intake at 16th week, and baseline values of the respective outcome (only for maternal serum markers). After c
(Hochberg procedure), no association remained statistically significant. When the analyses were grouped by foetal sex, the re
from those mothers with male foetuses were slightly associated with an increase in glucose levels (16th-33rdweek), and weigh
foetuses were associated with an increase in serum triglycerides (16th-33rdweek), and lower venous cord serum cholesterol.
pregnancy BMI was observed; some of the analyses by pre-pregnancy BMI were limited for several outcomes (e.g. arterial co
sample in the overweight-obese group. **When grouped by the intervention treatment**, higher weight-gain was associated w
lower colostrum IL-10 in controls (all, p<0.05); and with greater glucose at delivery, reduced maternal IL-8 at birth, and low
mature milk IFN-γ in exercisers (all, *p*<0.05).

Table S3. Association of **total** mean weight-gain (pre-pregnancy to 33rd week) with maternal and foetal immunometabolic mar

	Model 1				Model 1				Model 1		
	B	**SE**	**β**	**p-value**	**B**	**SE**	**β**	**p-value**	**B**	**SE**	**β**
Biochemical markers	***Maternal serum Changes 16th-33rd week (n=86)***				***Maternal serum Birth (n=37)***				***Arterial cord serum (bi***		
Glucose (mg/dL)[abc]	-0.01	0.27	-0.01	0.96	0.03	0.03	0.17	0.30	-0.02	0.04	-0.13
Insulin (microIU/dL)[a]	0.01	0.02	0.03	0.83							
HOMA-IR[a]	0.00	0.02	0.02	0.89							
HOMA-B[a]	0.04	0.03	0.16	0.21							
Cholesterol (mg/dL)[abc]	0.02	0.77	0.00	0.98	-0.91	2.05	-0.08	0.66	-0.08	0.05	-0.39
Triglycerides (mg/dL)[abcd]	2.02	1.04	0.22	0.06	0.80	2.39	0.06	0.74	-0.12	0.05	-0.47
HDL-C (mg/dL)[ac]	-0.24	0.21	-0.14	0.26	-0.38	1.00	-0.07	0.71	-0.06	0.46	-0.03
LDL-C (mg/dL)[abcd]	-0.21	0.73	-0.04	0.77	-0.02	0.04	-0.07	0.71	-0.02	0.05	-0.10
Phospholipids (mg/dL)[d]	3.04	1.79	0.29	0.10	-2.76	1.68	-0.29	0.11	-1.31	1.48	-0.24
Cortisol (mg/dL)	0.31	0.14	0.25	**0.05**							
CRP (mg/dL)[a]	0.01	0.01	0.06	0.62							
Cytokines	***Maternal serum Changes 16th-33rd week (n=44)***				***Maternal serum Changes 16th week-birth (n=36)***				***Arterial cord serum (bi***		
Fractalkine (pg/ml)[e]	-0.71	3.09	-0.04	0.82	-0.76	2.60	-0.06	0.77	-2.72	4.42	-0.12
Interleukin 1beta (pg/ml)[ef]	0.01	0.10	0.02	0.91	-0.01	0.12	-0.02	0.93	0.03	0.02	0.23
Interleukin 6 (pg/ml)[e]	-0.03	0.10	-0.05	0.78	-0.45	0.49	-0.18	0.36	0.04	0.16	0.04
Interleukin 8 (pg/ml)[f]	0.16	0.30	0.09	0.59	-1.02	0.59	-0.32	0.10	-0.21	1.24	-0.04
Interleukin 10 (pg/ml)	-0.09	0.39	-0.04	0.83	0.62	0.51	0.21	0.23	0.18	0.13	0.24
Interferon gamma (pg/ml)	-0.37	0.31	-0.20	0.24	-0.67	0.32	-0.36	**0.04**	0.00	0.06	0.01
TNF-α (pg/ml)	-0.13	0.07	-0.31	0.07	0.03	0.10	0.05	0.79	-0.01	0.14	-0.02
Cytokines (breast milk)	***Colostrum (postpartum, n=34)***				***Mature milk (postpartum, n=35)***						
Fractalkine (pg/ml)[g]	0.04	0.04	0.15	0.41	49.42	41.83	0.22	0.25			
Interleukin 1beta (pg/ml)[g]	0.08	0.04	0.35	0.06	0.00	0.06	0.01	0.96			
Interleukin 6 (pg/ml)[h]	0.07	0.46	0.03	0.88	-0.01	0.05	-0.02	0.92			
Interleukin 8 (pg/ml)[h]	-18.75	15.63	-0.21	0.24	-0.07	0.05	-0.27	0.19			
Interleukin 10 (pg/ml)	-0.46	0.23	-0.38	**0.05**	-0.22	0.17	-0.23	0.22			
Interferon gamma (pg/ml)	-0.06	0.16	-0.07	0.72	-0.17	0.09	-0.37	0.06			
TNF-α (pg/ml)	0.65	0.28	0.39	**0.03**	-0.01	0.18	-0.01	0.98			

B, unstandardized regression coefficient; β, standardized regression coefficient; CRP, C-reactive protein; HDL-C, high d homeostasis model assessment; IR, insulin resistance; LDL-C, low density lipoprotein-cholesterol; SE, standard error; TNF-α, tu extreme outliers *(only 1-4 extremes values within each outcome were treated)* and/or Box-Cox transformations were applie birth[b], arterial[c] and venous[d] cord serum biochemical markers, arterial[e] and venous[f] cord serum cytokines, and on colostrum[g] and were adjusted for pre-pregnancy body mass index, the intervention group, and for gestational week at 33rd week or birth. After (Hochberg procedure), none association remained statistically significant. When the analyses were grouped by **foetal sex**, th gain from those mothers with male foetuses were associated with an increase in glucose levels (16th-33rdweek), and a decrease dividing the analyses by pre-pregnancy body mass index, weight gains in normal-weight women was associated with an increa in IFN-γ levels (16th-birth), and lower venous cord serum LDL-C levels. **When the analyses were grouped by the intervention g** with greater glucose at delivery in exercisers; and with lower venous cord serum glucose, and colostrum and mature milk IL-1

Study VI

Table S4. Association of **weight-retention** (weight-gain from pre-pregnancy to postpartum) with maternal cytokines and body

	B	SE	β	p-v
Cytokines (mature milk)		***(Postpartum, n=28)***		
Fractalkine (pg/ml)	45.48	37.0	0.25	0
Interleukin 1 beta (pg/ml)	-0.02	0.06	-0.10	0
Interleukin 6 (pg/ml)[a]	0.00	0.05	0.01	0
Interleukin 8 (pg/ml)[a]	-0.08	0.04	-0.42	0
Interleukin 10 (pg/ml)	-0.12	0.17	-0.16	0
Interferon gamma (pg/ml)	-0.04	0.08	-0.10	0
Tumour necrosis factor alpha (pg/ml)	0.11	0.15	0.14	0
Body composition (postpartum)		***(Postpartum, n=82)***		
Body mass index (kg/m^2)	0.33	0.03	0.45	**<0**
Waist circumference (cm)	0.37	0.13	0.21	**0**
Hip circumference (cm)[a]	0.44	0.12	0.29	**<0**
Systolic blood pressure (mmHg)	0.16	0.19	0.10	0
Diastolic blood pressure (mmHg)	0.28	0.15	0.23	0
Bone mineral density (g/cm^2)	0.00	0.00	0.12	0
Lean mass (g)	156.96	74.73	0.19	**0**
Relative lean mass (lean mass/weight)	-5.05	0.80	-0.53	**<0**
Fat free mass (g)	158.68	77.09	0.19	**0**
Relative fat free mass (fat free mass/weight)	-5.39	0.81	-0.54	**<0**
Fat mass (g)	461.46	80.22	0.36	**<0**
Fat mass (%)	0.28	0.09	0.29	**<0**
Visceral adipose tissue mass (g)	5.82	2.45	0.21	**0**

B, unstandardized regression coefficient; β, standardized regression coefficient; SE, standard error. Extreme outli
each outcome were treated) were handle. All the analyses were adjusted for pre-pregnancy body mass index, the
delivery. After controlling for the familywise error rate (Hochberg procedure), most of the associations remained sta
circumference, body mineral density, fat free mass and visceral adipose tissue mass. When analyses were performe
similar; only weight-gain from those mothers with male foetuses were associated with an increase in waist circu
grouped by pre-pregnancy BMI, the results remained similar, except for the association of weight-gain with re
significant in obese women. **Only in controls**, weight retention was positively and clearly associated with waist and h
(p<0.01), and with visceral adipose tissue mass (p=0.058).

Table S5. Comparison of maternal immunometabolic markers (from 16th to 33rd week/birth, and at birth) betw
excessive **total** weight-gain (n=73)

	Adequate GWG		Excessive GWG		Adequate GWG – Excessive GWG			Adequate GWG		Exc
	Mean	SE	Mean	SE	Mean Diff.	SE	p-value	Mean	SE	Me
Biochemical markers				***Changes 16th-33rd week (n=73)***						
Glucose (mg/dL)[abd]	-5.73	1.48	-2.26	1.03	-3.47	1.81	0.06	0.13	0.28	0.1
Insulin (microIU/dL)[ac]	-1.16	0.98	0.61	0.69	-1.77	1.21	0.15			
HOMA-IR[ac]	-0.16	0.13	0.11	0.09	-0.27	0.16	0.09			
HOMA-B[ac]	239.51	125.1	182.39	92.88	57.12	157.4	0.72			
Cholesterol (mg/dL)[a]	63.43	6.27	53.13	4.36	10.30	7.69	0.19	209.80	15.52	207
Triglycerides (mg/dL)[ab]	96.64	8.62	97.10	5.96	-0.46	10.64	0.97	89.37	7.97	81.
HDL-C (mg/dL)[a]	1.81	1.70	-1.70	1.18	3.51	2.09	0.10	0.13	0.30	0.0
LDL-C (mg/dL)[ad]	39.02	6.25	36.45	4.35	2.57	7.68	0.74	209.05	13.09	198
Phospholipids (mg/dL)	39.23	9.65	26.16	8.04	13.07	12.59	0.31	174.32	18.89	177
Cortisol (mg/dL)	3.88	0.81	4.33	0.57	-0.46	1.00	0.65			
C-reactive protein (mg/dL)[a]	-0.15	0.07	-0.08	0.05	-0.07	0.09	0.44			
Cytokines (maternal serum)				***Changes 16th-33rd week (n=38)***						
Fractalkine (pg/ml)	16.62	14.71	-3.09	14.71	-19.70	20.97	0.35	-5.39	13.23	3.2
Interleukin 1 beta (pg/ml)	0.39	0.56	0.74	0.56	0.36	0.80	0.66	1.68	0.84	2.3
Interleukin 6 (pg/ml)	-0.45	0.53	0.39	0.53	0.84	0.76	0.28	27.67	4.18	25.
Interleukin 8 (pg/ml)	-1.20	1.73	1.99	1.73	3.18	2.48	0.21	22.11	4.00	12.
Interleukin 10 (pg/ml)	4.49	1.99	2.59	1.99	-1.90	2.83	0.51	26.81	3.75	20.
Interferon gamma (pg/ml)	-0.31	1.82	-3.85	1.82	-3.54	2.65	0.19	-2.80	1.58	-7.
Tumour necrosis factor	1.26	0.35	1.29	0.35	0.03	0.50	0.95	3.01	0.73	4.6

B, B-cell function; GWG, gestational weight-gain; HDL-C, high density lipoprotein-cholesterol; HOMA, homeos
resistance; LDL-C, low density lipoprotein-cholesterol; Mean Diff., mean difference; -, minus; SE, standard error. Out
(changes from 16th-33rd week[a], and birth[b]) were handled *(only 1-4 extreme values within each outcome).* Box-C
biochemical markers (changes from 16th-33rd week[c], and birth[d]**).** The models were adjusted for the intervention gro
birth, and baseline values of the respective outcome. The Post-Hoc Bonferroni test (single-step procedure) was em
between groups showed above. When replicating these analyses for **excessive late weight-gain,** all the results rer
total weight-gain analyses by the **intervention group**, exercisers with adequate weight-gain showed a trend towa
(Mean Dif. 3.8microIU/dL, SE 2.3, *p*-value=0.1) and HOMA-IR (Mean $Dif_{transformed.}$ 0.5, SE=0.3, *p*-value=0.1) from 16th
with excessive weight-gain. The rest of results remained similar. When replicating these analyses **(by interventio
gain**, all the results remained similar.

Table S6. Comparison of cord serum immunometabolic markers, postpartum breast milk cytokines, and maternal body c
adequate vs. excessive **<u>total</u>** weight-gain (n=73)

	Adequate GWG		Excessive GWG		Adequate GWG – Excessive GWG			Adequate GWG	
	Mean	SE	Mean	SE	Mean Diff	SE	p-value	Mean	SE
Biochemical markers (cord serum)				*Arterial cord serum (birth, n=18)*					
Glucose (mg/dL)[a]	-0.44	0.35	0.18	0.28	-0.62	0.46	0.20	53.34	5.23
Cholesterol (mg/dL)[ab]	0.29	0.39	-0.42	0.31	0.71	0.51	0.18	-0.04	0.27
Triglycerides (mg/dL)[ab]	0.19	0.36	-0.33	0.32	0.52	0.49	0.31	45.69	5.22
HDL-C (mg/dL)[b]	30.72	3.61	26.23	2.85	4.48	4.68	0.36	24.53	2.37
LDL-C (mg/dL)[ab]	0.60	0.36	-0.16	0.29	0.76	0.47	0.13	7.61	0.63
Phospholipids (mg/dL)[b]	98.46	9.47	94.25	8.32	4.21	12.79	0.75	93.73	8.05
Cytokines (cord serum)				*Arterial cord serum (birth, n=26)*					
Fractalkine (pg/ml)[c]	369.92	33.16	355.04	26.11	14.89	42.56	0.73	279.13	34.29
Interleukin 1 beta (pg/ml)[cd]	1.19	0.19	1.59	0.15	-0.39	0.25	0.12	1.26	0.23
Interleukin 6 (pg/ml)[c]	15.37	1.53	17.50	1.20	-2.14	1.96	0.29	12.84	1.59
Interleukin 8 (pg/ml)[d]	53.84	9.44	56.11	7.43	-2.27	12.12	0.85	60.02	6.10
Interleukin 10 (pg/ml)	10.11	1.10	12.69	0.86	-2.59	1.41	0.08	13.25	1.21
Interferon gamma (pg/ml)	3.14	0.41	2.95	0.32	0.19	0.53	0.72	3.10	0.34
Tumour necrosis factor alpha (pg/ml)	16.22	0.96	14.75	0.76	1.47	1.23	0.25	17.42	1.41
Cytokines (breast milk)				*Colostrum (postpartum, n=31)*					
Fractalkine (pg/ml)[e]	-0.27	0.28	0.12	0.21	-0.39	0.35	0.28	1133.74	239.69
Interleukin 1 beta (pg/ml)[e]	-0.22	0.28	0.44	0.21	-0.66	0.35	0.07	2.47	0.35
Interleukin 6 (pg/ml)[f]	30.06	3.14	32.65	2.49	-2.60	4.02	0.53	-0.37	0.27
Interleukin 8 (pg/ml)[f]	874.89	103.68	753.78	82.25	121.11	132.83	0.37	0.09	0.27
Interleukin 10 (pg/ml)	20.26	1.40	14.86	1.11	5.40	1.79	**0.01**	7.85	1.02
Interferon gamma (pg/ml)	7.25	1.04	6.63	0.82	0.63	1.33	0.64	3.63	0.47
Tumour necrosis factor alpha (pg/ml)	13.66	1.86	16.09	1.46	-2.43	2.37	0.32	7.91	1.05
Body composition				*(postpartum, n=72)*					
Body mass index (kg/m^2)	24.78	0.41	25.91	0.29	-1.13	0.51	**0.03**		
Systolic blood pressure (mmHg)	105.52	1.87	108.70	1.27	-3.19	2.26	0.16		
Diastolic blood pressure (mmHg)	65.33	1.42	69.11	0.96	-3.78	1.72	**0.03**		
Bone mineral density (g/cm^2)	1.08	0.02	1.06	0.01	0.02	0.02	0.42		
Z-score	-0.54	0.18	-0.58	0.13	0.04	0.22	0.85		
Waist circumference (cm)	87.54	1.25	87.79	0.88	-0.26	1.53	0.87		
Hip circumference[g] (cm)	102.51	1.17	106.34	0.83	-3.84	1.44	**0.01**		
Relative lean mass	588.11	9.52	565.79	6.73	22.32	11.67	0.06		
Relative fat free mass	619.49	9.79	595.31	6.91	24.18	11.99	**0.05**		
Fat mass (g)	23634.36	782.68	27848.05	552.67	-4213.69	959.91	**0.00**		
Fat mass percentage (%)	36.18	0.77	39.78	0.54	-3.61	0.94	**0.00**		
Visceral adipose tissue mass (g)	351.93	22.94	373.18	16.20	-21.25	28.14	0.45		

B, B-cell function; GWG, gestational weight-gain; HDL-C, high density lipoprotein-cholesterol; HOMA, homeostasis model asses
density lipoprotein-cholesterol; Mean Diff., mean difference; -, minus; SE, standard error. Handling of extreme outliers *(only 1*
were treated) and/or Box-Cox transformations were applied on arterial[a] and venous[b] cord serum biochemical markers, arte
colostrum[e] and mature milk[f] cytokines, and on hip circumference[g]. The models were adjusted for the intervention group and
delivery. Body composition variables were additionally adjusted for pre-pregnancy body mass index. The Post-Hoc Bonfe
employed for the pairwise comparisons between groups showed above. When organizing the analyses by the **intervention gr**
weight-gain was characterized by lower colostrum IL-10 (Mean Dif. 6.4pg/mL, SE 2.1, *p*=0.008), and higher postpartum BMI (M
hip circumference (Mean Dif. 4.5cm, SE 1.8, *p*=0.02) compared to women with adequate weight-gain. When replicating the
gain, all the results remained similar; except for mature milk IL-6 (lower in women with adequate weight-gain, *p*=0.002),
diastolic blood pressure (became non-significant), and body mineral density (lower in women with adequate weight-gain, *p*
weight-gain analyses **by the intervention group**, all the results remained similar; except for a non-significant trend toward
observed in control women (p=0.06).

Study VI

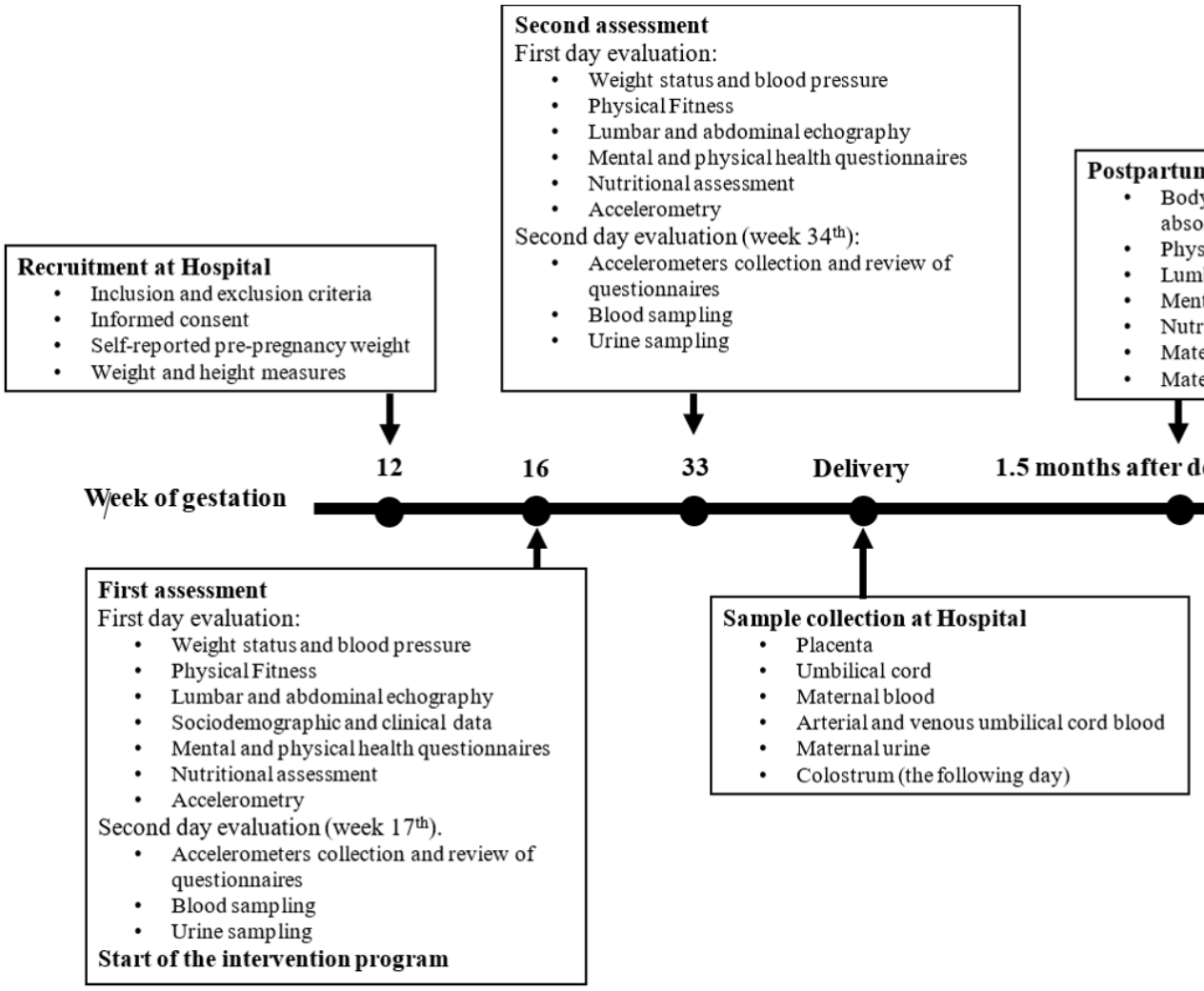


Figure S1. Assessments conducted along the GESTAFIT Project

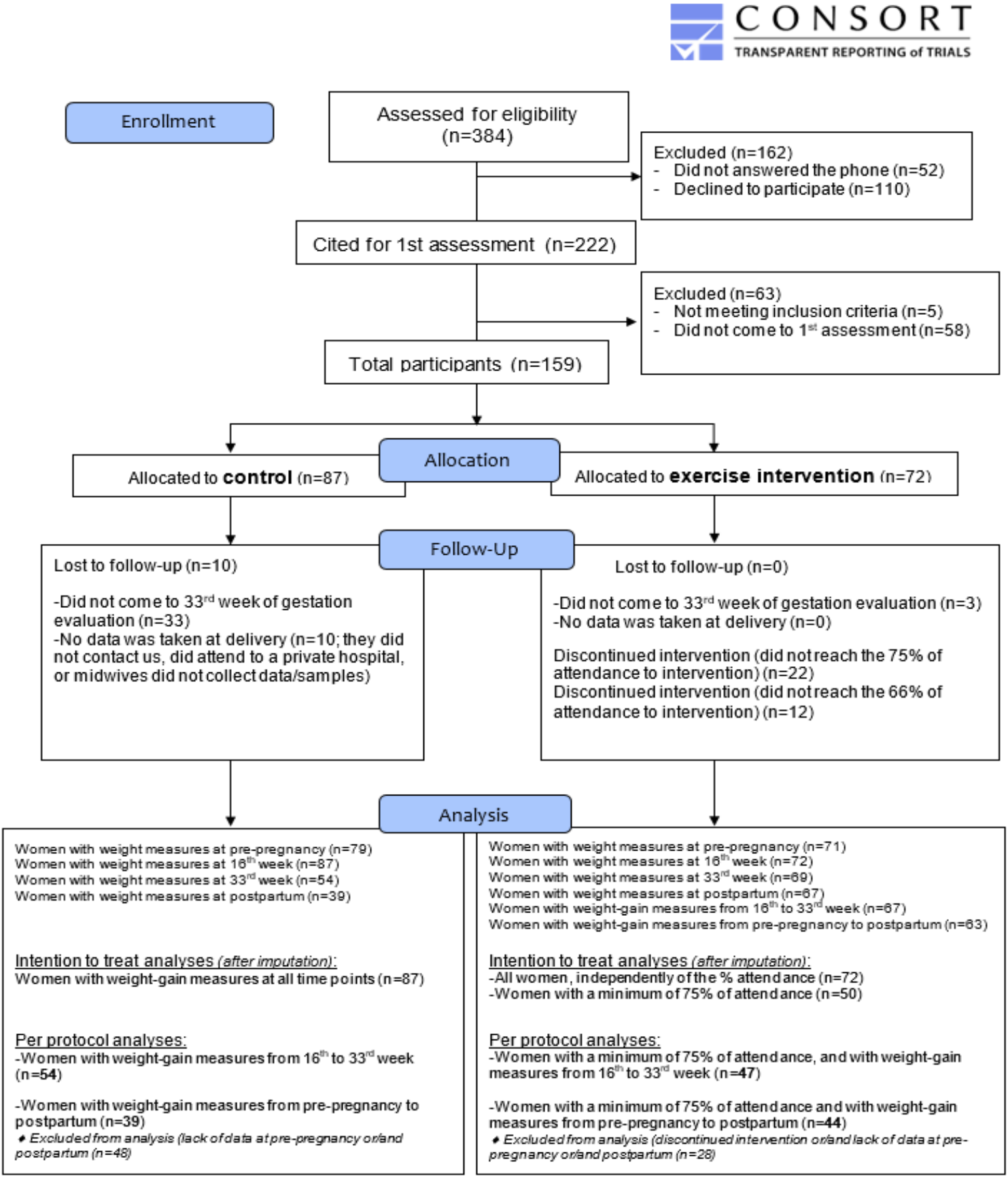


Figure S2. CONSORT flow chart diagram for the GESTAFIT study.

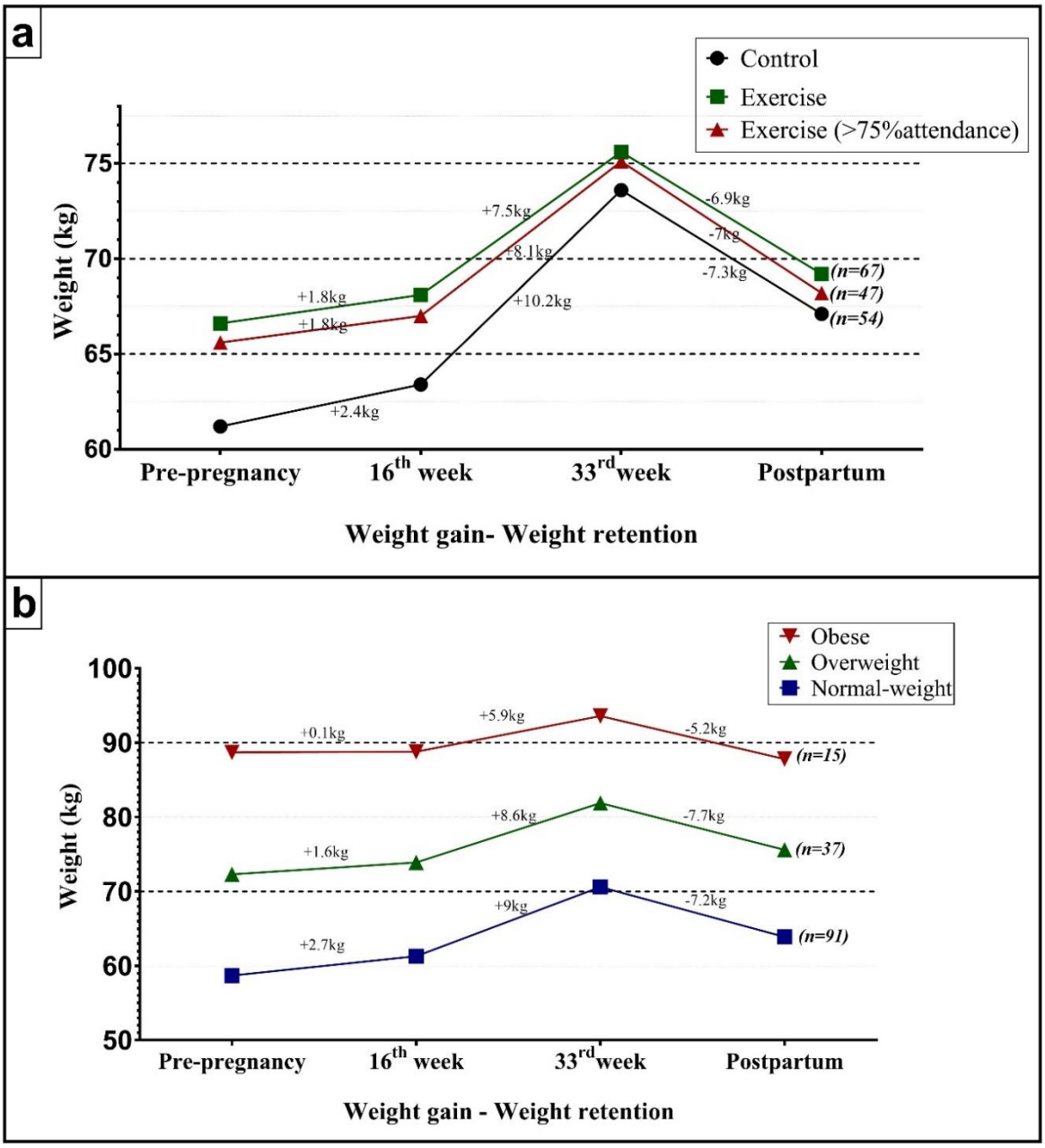


Figure S3. Gestational weight-gain and weight retention during pregnancy according to the intervention group (**2a**) or pre-pregnancy weight-status (**2b**).

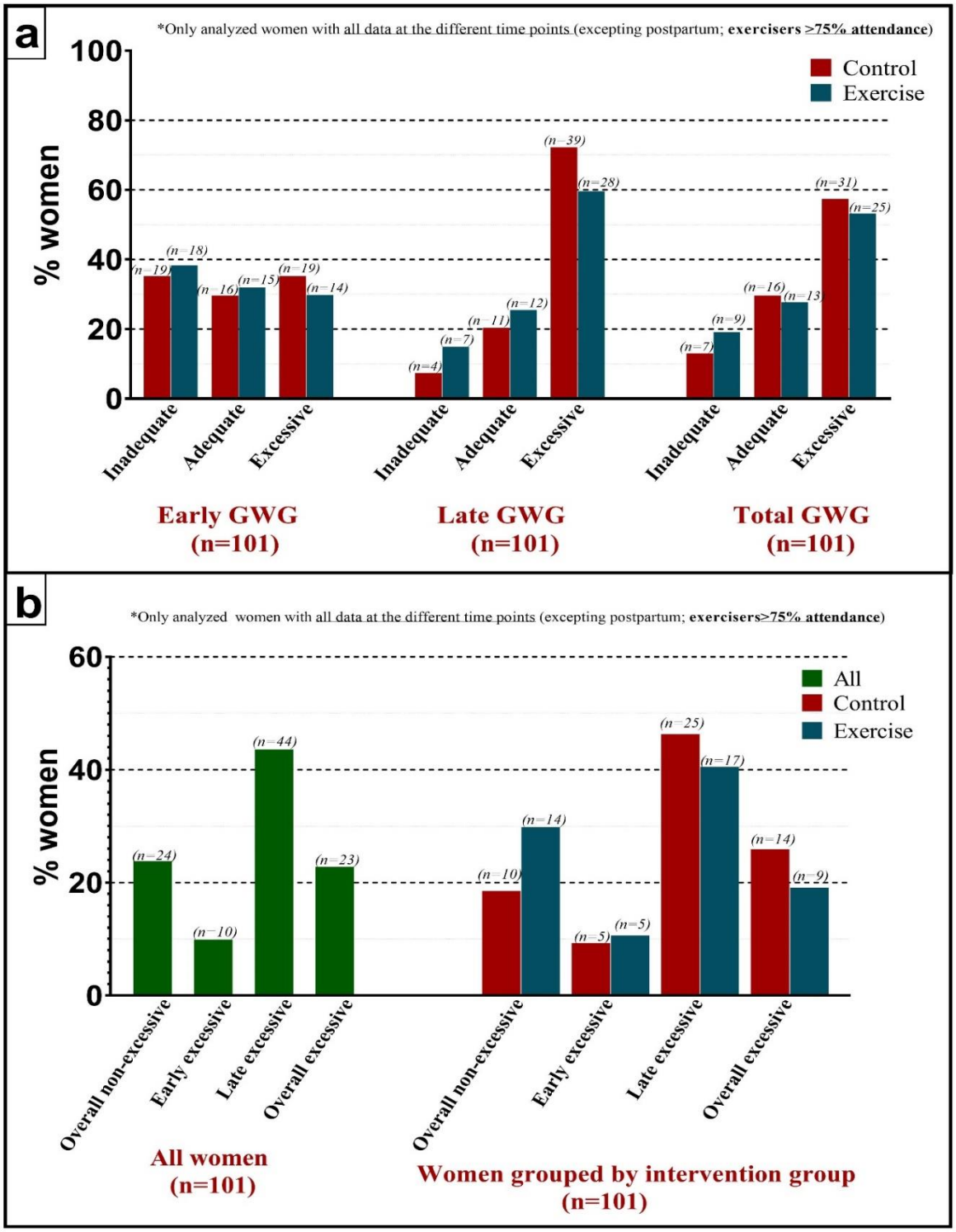


Figure S4. Distribution of women by gestational weight gain categories. GWG, gestational weight-gain. (**4a)** distribution of women by gestational weight-gain categories during the different periods of pregnancy; **(4b)** distribution of women by gestational weight-gain categories (aimed at evaluating the timing of excessive maternal weight-gain) and by the intervention group. *In the lower panel, the categories were defined as follows: overall non-excessive and excessive (those women who did not have excessive weight-gain during any period of pregnancy/ or those had excessive weight gain, at both, early and late pregnancy; respectively); early excessive (those women who had excessive weight-gain only during early pregnancy), and late excessive (those women who had excessive weight-gain only during late pregnancy).*

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GENERAL DISCUSSION

GENERAL DISCUSSION

In the present Doctoral Thesis, we address relevant gaps that have been unperceived so far in previous scientific literature in pregnancy. Additionally, we discuss the current methodology and findings with regard to state-of-the-art evidence, as well as the potential impact of our findings from a basic, clinical and/or practical perspective for pregnant women. Further, we stress the need of more studies in this novel field of research. Of note, we add the final touch by giving short personal opinions about the different matters in question.

THE IMPORTANCE OF SEDENTARY TIME AND PHYSICAL ACTIVITY IN PREGNANCY

A sedentary and inactive lifestyle is one of the most worrisome issues that our obesegenic society is facing nowadays. This is reflected by the increasing prevalence of sedentary behaviours and physical inactivity across all ages, and their harmful impact on people's health[1-5]. Unfortunately, in pregnancy, sedentary lifestyle and physical inactivity follow similar trends, and represent a negative influence for the mother and foetus. Previous evidence based on device-measures has shown that pregnant women spend approximately 57-78% of their time in sedentary behaviours[4,6], with a slight increase (or no change) from early pregnancy to late pregnancy[4,7,8]. Regarding PA levels, previous literature has also shown that pregnant women spend around 27.7-40% of their time in light PA, and <20% of their time in MVPA, with slight or no changes from early to late pregnancy[6,7]. In our whole cohort of the GESTAFIT study, pregnant women spent a third of the day in sedentary behaviours, and only 22% of the participants complied with the PA guidelines[9].

When comparing our results from **Study I** and **Study II** with previous research using similar approaches for measuring ST and PA (age range 18-55), pregnant women from our studies spent more time in sedentary behaviours than American and European non-pregnant women[8,10]. Moreover, we could observe that American and/or European women performed higher vigorous[10,11], bouted MVPA[12], total PA[13], and vector magnitude (VM) counts per minute[14] than our sample. The decrement in the adherence to PA guidelines might be explained by fear of harming the foetus, ignorance about PA recommendations, or pregnancy symptomatology[15]. However, other studies have

shown that American and/or European women are characterized by lower light[10], moderate[13,16], vigorous[10,13,16], MVPA[16], bouted MVPA[16], and total PA[12] than our pregnant women cohort (GESTAFIT project).

When we compared our participants from the GESTAFIT study (**Study I**) with other cohorts of early pregnant women, we observed that Dutch pregnant women[17] (31.5 years, BMI>25kg/m^2) performed lower sedentary time, light PA, and MVPA than our participants. However, if we compare our results with those from the European DALI study[18] (whole cohort; age=32.5, BMI=33.9; data not published), these early pregnant women are characterized by lower light PA, and higher ST, and moderate, vigorous, and MVPA. Discrepancies on ST and PA levels between studies of pregnant women are caused by different factors such as: gestational week, weight-status, marital status, educational level, ethnicity, the methodology employed for measuring PA levels, etc. We have actually shown that some of these factors might influence southern Spanish pregnant women regarding meeting PA recommendations[9]. As consequence of these discrepancies, and the scarce evidence, nowadays it is really complex to get adequate comparisons and conclusions from the role of ST and PA on immunometabolic responses during pregnancy. To understand these relationships is of particular importance in early pregnancy, that is when the maternal environment predominantly influences immunometabolic responses (including placental metabolism)[19].

In the **Study I**, we showed that higher levels of MVPA and/or meeting PA guidelines in early-middle pregnancy were related to lower systemic IL-1β and IFN-γ, and higher IL-8. Additionally, we observed that those women with higher levels of vigorous PA showed a non-significant trend towards increased circulating IL-6 levels. However, none association was observed with fractalkine, IL-10, or TNF-α. The clinical interpretation of these findings is complex given the bimodal function of these cytokines according to the source of origin, concentrations, time of exposure and inducing stimulus[20-23]. This interpretation is even more complex in pregnancy due to the high interaction between cytokines and other molecules in the mother-placenta-foetus crosstalk[24-26]. For instance, IL-1β and TNF-α are predominantly pro-inflammatory cytokines highly involved in the pathogenesis of metabolic-inflammatory abnormalities[27-29], but they also have a physiological-metabolic function in glucose homeostasis[20,30], and in implantation and parturition during pregnancy[31-33]. In contrast to

our results, van Poppel, et al.[25] observed that physically-active pregnant women presented greater IL-1β and TNF-α concentrations. They hypothesized that this was partially explained by the more pronounced pro-inflammatory status of women due to their overweight-obese status and impaired phenotype, differently to our subsample of pregnant women, which was predominantly normal-weight and without cardiometabolic diseases. Thus, in this context (i.e. healthier status during early-middle pregnancy: after implantation), the reduction in IL-1β via MVPA might be interpreted as an anti-inflammatory effect. Moreover, the higher IL-8, and the higher IL-6 and lower IFN-γ with PA, might be potentially related to a more vascularized placenta[34,35] and anti-inflammatory state[35,36], respectively. However, as previously explained, these cytokines can have bimodal effects in pregnancy[25,33,37], and thus these results should be interpreted cautiously. Future in vitro and in vivo studies on different metabolic phenotypes are indeed warranted to better understand this issue.

Moreover, in the **Study I**, we also observed that neither ST nor PA levels were associated with glucose or lipids. However, the few evidence available is contradictory. Whereas some studies are in agreement with our results[38,39], others have shown the opposite results (e.g. positive association between ST and LDL-C).[40-42] Why PA and ST were associated differently (or were not associated) with the immunometabolic markers, compared to other studies, is likely to be explained by: gestational age,[41,43] PA assessment with self-reported questionnaires[41,42], ethnicity, weight-status[25,38], and statistical power[41-43], among others.

Overall, these findings along with previous evidence[6,25,40] suggest that increasing MVPA could be useful to modulate immunometabolic responses; although it appears that the pre-conception metabolic phenotype might play an important role. This is of clinical relevance since any immunometabolic alteration (e.g. exacerbated IL-1β and TNF-α) in an unfavourable environment during early pregnancy, might prompt dysfunctional metabolism later in pregnancy (see introduction). Thereby, balancing immunometabolic responses via promoting higher MVPA levels from early pregnancy could be an alternative-complementary target to avoid potential metabolic disruptions and pregnancy complications. This is supported by recent literature showing that reducing ST and increasing PA from early or before pregnancy forward, is particularly

effective in terms of enhancing the glucose-insulin axis[6], and reducing the prevalence of GDM[40,44-46].

In this complex puzzle, lifestyle might also play an important function on placental development and metabolism[47-49], although its role has been usually unperceived. In fact, considering the inverse association between sedentary behaviours and neonatal adiposity observed in previous analysis of the DALI study[50], it is plausible that the placenta mediates some of the lifestyle effects on intrauterine programming and foetal development. Aimed at better understanding the underlying mechanistic pathways, in the **Study II**, we showed that ST earlier in pregnancy was inversely associated with term placenta FATP2 and FATP3 mRNA expression. However, MVPA during pregnancy had little if any effect on placental mRNAs. Only MVPA during early pregnancy was related to down-regulated placental GLUT1 expression; although this association was dependent on ST levels. In line with our results, previous research[49] at the Kristi Adamo lab has proposed that active women present down-regulated GLUT1 (when controlling for sugar intake) and FATP4 expression, which suggests a reduced potential for glucose and fatty acid transport to the foetus. By contrast, they observed in another study at protein level that MVPA was related to higher placental FATP4, and was not related to GLUT1, which might be explained by post-transcriptional changes[48]. They also suggested that active women were characterized by up-regulated SNAT2 expression (i.e. higher amino acid placental transport)[49]. Additionally, they suggested that PA might be useful to normalize foetal growth (adaptive response), given the inverse relationship of MVPA with insulin and mTOR signaling (e.g. IGF1, PRKAB1)[49], and its positive relationship with aquaporin family of genes expression[47]. It is necessary to state that differently to us (DALI study), they focused on normal-weight or overweight women, and used a different accelerometer brand. Taken together with our findings, it is clear that lifestyle in early-middle pregnancy do modulate placental development and function, independently of the weight-status. However, further evidence is necessary to better understand the scarce evidence.

Why the lifestyle-counselling intervention (**Study II**) was not effective to modulate these placental isoforms-*contrary to lifestyle at early pregnancy and changes in lifestyle*, might be explained by its low efficacy to improve lifestyle behaviours in this subsample of women. Another potential explanation is that reducing ST earlier in

pregnancy might have prompted different molecular and structural changes[47-49,51] in placental cells that persist throughout pregnancy and dictate placental phenotype at term[46-49,52]. However, other unknown drivers in late pregnancy and parturition might also have acutely influenced placental alterations[53]. Indeed, studies focused on different labour phenotypes, and in early active vs. inactive placental phenotypes (women finishing pregnancies before parturition *–e.g. abortion or elective delivery*), might be useful to understand how lifestyle regulate placental adaptations from early pregnancy.

Additionally, in the **Study II**, we explored which metabolic factors mediated changes in these placental mRNAs, and which cord blood metabolites related to these mRNAs mediated neonatal adiposity. Since maternal ST was highly related to insulin and insulin sensitivity in this cohort[6], we initially hypothesized that maternal metabolic parameters could drive the associations between ST and placental mRNAs. Although some associations between metabolic markers with placental gene expression were observed (e.g. insulin and insulin resistance with FATP2), none metabolic parameter mediated the relationship between lifestyle and placental mRNAs. Moreover, contrary to our initial expectations[54,55], FATP2 gene expression was inversely related to cord blood triglycerides and FFA, but not with neonatal adiposity. This might be explained by the interaction with other placental transporters and transcripts (e.g. lipoprotein and endothelial lipases, cytokines, location of FATPs, etc.). Another potential explanation is that FFA uptake into foetal tissues contributes to the steady-state levels in cord blood, which might also account for the inverse association of FATP2 mRNA with cord blood triglycerides and FFA. Of note, none cord blood metabolite mediated the association of placental mRNAs with neonatal adiposity, except for cord blood leptin, which partially explained the effects of PPAR-γ mRNA on neonatal adiposity. Unfortunately, no other evidence is available to compare or verify our findings. Further studies with greater sample size for mediation analyses are necessary before reaching any solid conclusion about the mechanistic insight.

Bearing in mind the **Study I** and **II**, and previous evidence, it appears that promoting a healthy lifestyle before or early in pregnancy might be more effective to modulate immunometabolic responses. However, evidence is scarce and contradictory. Thus, it is necessary to continue exploring the role of ST and PA from early pregnancy as tools to enhance/regulate intrauterine programming, and prevent short and long-term

adverse consequences. Ongoing studies from the GESTAFIT and DALI study, which could not be included in this thesis, will provide a greater mechanistic insight on this issue.

THE ROLE OF PHYSICAL FITNESS IN MATERNAL AND NEONATAL METABOLISM

From children to geriatric population, the potential of PF to confer a protector role in health is undeniable[2,56-63]. It is such its potential, that previous evidence has postulated that low PF (specifically CRF) represents the biggest public health problem of the 21st century[58]. Actually, it has been shown that CRF is the main risk factor for all-cause mortality, with higher impact even that obesity, smoking, hypertension, hypercholesterolemia or diabetes[57,58]. Similarly, both muscle strength[63,64] and flexibility[2,62] are also profoundly implicated in cardiometabolic health. Although these PF capacities are considerably influenced by genetics, they are predominantly dependent on modifiable components such as physical activity and exercise[65,66]. Thus, increasing PF via implementing targeted exercise programs (for instance) could represent an extraordinary strategy to modulate immunometabolism in pregnancy.

Unfortunately, and despite its clinical relevance, it has not been explored so far whether PF has a similar effect (as in the general population) in maternal and foetal metabolism during pregnancy. Only few studies have explored how PF relates to delivery outcomes, showing a beneficial role on new-born and birth outcomes[67-69]. Although some authors have shown that exercise improves metabolism in pregnancy[70-75], and accordingly, some of them might state that PF also improves metabolism *(because PF is mainly dependent on exercise and PA[66])*, this statement is inaccurate and has no scientific rigour. First, it is possible that some women participating in exercise programs do not improve PF (low responders to exercise), whereas others just following the recommendations from their healthcare professionals (as typically in RCT) might improve more PF due to higher susceptibility to lifestyle (high-responders to PA or daily activities)[51,76,77]. Moreover, other potential confounders such as diet, smoking, sleep, PF previous to the exercise program, diseases, etc. might condition/dictate PF capacities along with exercise[65]. Thus, it is not valid to conclude that PF modulates metabolism because exercise does. Although it might sound obvious, it is necessary to specifically assess PF to conclude this point. Additionally, although exercise appears to be effective to improve PF[78-81], or at least to maintain it during pregnancy, evidence is still scarce,

equivocal and heterogeneous[81]. Actually, there are two studies showing contradictory results regarding its effectiveness on CRF[82] and flexibility[78]. This lack of effectiveness might be related to the strong adaptations induced by pregnancy itself, which might lead to a decrease in PF during pregnancy[83]. This further supports the importance of assessing PF during pregnancy.

In the **study III**, we showed that PF was associated with several cardiometabolic markers during pregnancy, and might potentially confer a protector role in maternal metabolism –especially CRF and muscle strength in early pregnancy. Of note, these results were independent of relevant confounders such as ST, dietary habits and sleep duration. Regrettably, we could not verify our results due to the non-existence of previous studies in this topic. Although the mechanisms by which increased PF might be translated into lower cardiometabolic risk might be similar to those previously observed in the general population[36,51,59,63-65], no mechanistic insight has been provided yet either. However, the lower excessive weight-gain observed with increased CRF and upper-body muscle strength in the **Study VI**, might represent a potential mechanism by which increasing PF might lead to enhanced cardiometabolic profile. Specifically, an increase in PF, which is related to an improvement in women´s metabolic profile[2,84,85], and an increase of their muscle mass[64] and resting energy expenditure[64,84], might lead to lower weight-gain[61,84,85]; thereby contributing to a less impaired metabolic phenotype.

Additionally, in the **Study III**, we observed that normal-weight women who were fit (but not those unfit) had an improved metabolic phenotype compared to overweight-obese and unfit women, which emphasizes a prominent role for PF in pregnancy. This supports that a simple clinical message such as “Keep yourself fit and normal-weight before and during pregnancy” should be implemented into actions during pregnancy.

All in all, it appears that increasing PF, especially muscle strength and CRF in early pregnancy, might provide a cardio-protector effect in maternal metabolism; thereby potentially contributing to lower prevalence of pregnancy complications[61,86-92]. Hence, from a practical perspective, muscle strength and CRF are relevant targets to consider when designing concurrent exercise interventions to better regulate maternal metabolism. However, it is important to mention that our sample was relatively “healthy”, and thus the link between the PF-related protector effect and adverse

complications, need to be interpreted cautiously. Moreover, the PF tests employed have not been validated in pregnancy yet. Nowadays, in the GESTAFIT project, we are on phase of validating PF tests with criterion methods (except for CRF). Future studies using validated PF tests in different metabolic phenotypes during pregnancy, are indeed necessary before reaching any solid conclusion.

THE PROMISING BUT POORLY UNDERSTOOD ROLE OF PHYSICAL EXERCISE IN MATERNAL AND NEONATAL METABOLISM

Thanks to the remarkable work of researchers worldwide, and the cooperative effort[93,94] for reaching a common aim (i.e. improving women's and next generations' lives), nowadays the benefits of exercise in pregnancy are clearly evidenced; although, there is still a long way forward. Indeed, many mysteries in this novel field of research remain unrevealed, and are waiting to be discovered. Equally important, the healthcare personnel, who are in direct contact with pregnant women, are more aware of the importance of promoting exercise during pregnancy for the maternal-foetal health. Thus, as time goes on, it is becoming easier to link scientific evidence with clinical practice, thereby making more feasible the implementation of effective strategies (exercise programs), and importantly based on science evidence. Noteworthily, although exercise has been shown to be effective to reduce the prevalence of GDM, excessive weight-gain, pregnancy and birth complications, mental disorders, and lumbar pain, among others[15,93-99], exercise is not the absolute panacea in pregnancy. Despite its importance, other potential tools (not addressed in the current thesis) such as early screening for potential metabolic disruptions, healthy dietary and sleep habits, etc. are also important for an optimal course of pregnancy. Ideally, exercise should be targeted along with these components to maximise the effects of lifestyle during pregnancy.

Indeed, how to better control immunometabolism during pregnancy is one of the big gaps that need to be addressed as soon as possible; and exercise represents a promising option in this regard[48,70-75,94,100-104]. Unfortunately, experiments investigating the effects of exercise on maternal and foetal immunometabolism are scarce (inexistent regarding foetal metabolism), and show debatable effectiveness in some studies[85,105-111]. The importance of exploring this topic during pregnancy is further supported by the potential of some immunometabolic markers (e.g. cytokines) as mechanisms to transfer

the effects of exercise into metabolic adaptations[52,100]. However, only Clapp and Kiess [112], and Aparicio, et al.[103], have explored so far how exercise influences systemic and milk cytokines (differently to other studies exploring classical markers –*e.g. leptin*[106]*, CRP*[113]). Although, they did not explore the role of cytokines as potential messengers of the effects induced by exercise.

In the **Study IV**, we showed that exercise appeared to reduce maternal IL-1β and TNF-α (similarly to Clapp and Kiess [112]), arterial cord serum IL-6, and venous cord TNF-α levels, while increased arterial cord serum IL-1β. Some trends toward higher IL-8 and IL-10 were also observed with exercise. Although the exercise-induced changes on these cytokines appear to have a physiological-beneficial role[22,25,36,40,52], the interpretation in this context is difficult since their pro- or anti-inflammatory function depends on their concentrations, receptor distribution and duration of their stimulation[20-23]. In addition, the origin and clearance of these cytokines is also unclear in pregnancy[114,115], which initially prevented us from concluding if these changes were mainly driven by the mother, placenta or foetus. However, our findings suggested that IL-6 and fractalkine synthesis during parturition were mainly induced by the foetus, and cord serum IL-10 was likely of maternal-placental origin, which concurs with Mir et al.[114]. This leaded us to conclude that exercise might modulate foetal synthesis of IL-6 and/or placental clearance during parturition. However, more tissue/cell-specific analyses in women undergoing exercise programs during pregnancy are necessary to understand the contribution of the mother, placenta and foetus, and their crosstalk. Of note, despite the fact that we considered the most relevant confounders in parturition, other unnoticed factors related to this acute phase[53] might also have influenced the chronic immunometabolic responses modulated by exercise.

Thus, although we cannot reach any solid conclusion concerning the role of these cytokines in the maternal-placental-foetus crosstalk due to the scarce evidence, it appears that exercise could be useful to modulate some immunometabolic responses during pregnancy. This might help to prevent immunometabolic dysregulations and potential pregnancy complications. Hopefully, this study will prompt others to more specifically (in different tissues), and at different levels (i.e. transcriptomics and proteomics), investigate this issue during pregnancy.

In the **Study V**, we observed that exercise was not effective to modulate maternal and cord serum immunometabolic markers, except for a slight decrease in maternal and arterial cord serum glucose. The low effectiveness of this exercise intervention was unexpected, since a whole expert multidisciplinary team meticulously designed it based on the latest guidelines[115] and evidence[116] at that moment. Moreover, during its implementation and development, all the sessions were strictly supervised, and the intensity, attendance and other parameters were monitored periodically. However, these results concurred with some studies which also failed -or showed limited success- to improve glycaemic control[105-107,109,110,117] and lipid metabolism[71,72,107,108], contrary to other who did success[70-75,104]. This makes clear that evidence is not only scarce (or inexistent concerning foetal metabolism so far), but also contradictory. Experimental limitations along with discrepancies in methodology and exercise protocols, compliance, genotype, etc. are likely to explain this contradictory evidence. Of note, recent meta-analytic evidence[102] has emphasized that exercise only has a clear effect on reducing glucose in those women diagnosed with diabetes, which highlights its potential for GDM management. This is in line with previous evidence suggesting that increasing PA and reducing ST are effective strategies to prevent GDM[6,45].

Taken together, this leaded us to wonder the one million-dollar question: why exercise was not effective in this context if the exercise program was well-designed and strictly supervised? We reckon that pregnancy represents and extraordinary stimulus[31,53,118], with remarkable physiological responses that can mask some of the exercise-induced effects (rather than being non-effective). Moreover, the parental environment in pre-conception and early pregnancy substantially influences in-utero programming[19,100,111]. Hence, the implementation of exercise programs in these early stages (*i.e. when the key "modifiable" and most vulnerable biological processes occur[31,111]*) may be more effective than starting at the second trimester of gestation. Additionally, exercise has shown to induce stronger effects in severe adverse phenotypes such as GDM and obesity (*greater room for change*)[70,71,102,111], and most pregnant women were normal-weight (70%) and a priori healthy (in our Study V). Furthermore, we did not identify those women who were at higher risk of being non/low-responders to the exercise programme[77]. Indeed, considering women's

genotype and phenotype –*previously to starting the intervention*- to identify low-responders, and adapting the exercise program accordingly (e.g. increasing the intensity of exercise in this low-responders), might have improved the efficacy of our exercise program. Lastly, different mechanisms (e.g. epigenetics) by which the exercise-induced effects might result in maternal-foetal metabolic adaptations[24,52,100], or potential messengers (e.g. cytokines) that could indirectly drive its effects on metabolic phenotype[25,52], could have been undetected. The scarce evidence regarding the role of cytokines in the maternal-placental-foetus crosstalk leaded us to further explore cytokines in pregnancy, and their potential to drive the effects of exercise.

In the **Study V**, we additionally showed that cytokines could play a regulator role in maternal and foetal metabolism, which was partially modulated by exercise. This concurs with van Poppel et al.,[25] who showed that PA played a similar role in this story. Importantly, our findings demonstrated for the first time that few of these cytokines might act as potential mediators of the effects of exercise into metabolic changes during pregnancy (e.g. exercise reduced LDL-C via increasing IL-8 levels), as previously hypothesized in pregnant women[52], and demonstrated in rodents[31,52,100]. Unfortunately, as of today, we do not know the specific source of these maternal serum cytokines in pregnancy. However, based in previous evidence[25,52,101,112] and our findings, it is plausible that the origin of these exercise-induced cytokines are the skeletal muscle cells. Taken together, findings from the Study IV and V are relevant to better comprehend how prenatal exercise influences maternal and foetal metabolism, and design more effective and individual-adapted programs in pregnancy.

Of note, other exercise-induced mechanisms at tissue/cell-level might have partially dictated the observed and unperceived metabolic adaptations. However, we could not address these mechanisms in pregnant women due to ethical and feasibility reasons. More specifically, we could not carry out muscle, liver, or subcutaneous adipose tissue biopsies in pregnant women, which would have allowed us to better comprehend this story. Fortunately, at the Stanford lab[119,120], the candidate had the opportunity to explore how maternal exercise initiated in pre-conception (2 weeks before breeding) and performed during gestation (until 3 weeks after breeding), and stress stimuli, could influence mice's liver and skeletal muscle transcriptome. Specifically, five groups of wild type C57BL/6 mice were considered: i) *sedentary pre-*

*conception & sedentary gestation (**Sed**); ii) sedentary pre-conception & sedentary+stress gestation (**Sed-S**); iii) exercise (wheel) pre-conception & exercise gestation (**Ex-NS**); iv) exercise (wheel) pre-conception & exercise+stress gestation (**Ex-S**); and v) exercise (treadmill) pre-conception & exercise gestation (**Ex-Tr**).*

Overall, we could observe that maternal exercise and stress were stimuli able to induce some changes in glucose, fatty acid, and mitochondrial (not shown) metabolism, and acetylcarnitine transport in the liver and skeletal muscle (see **Figure 5**).

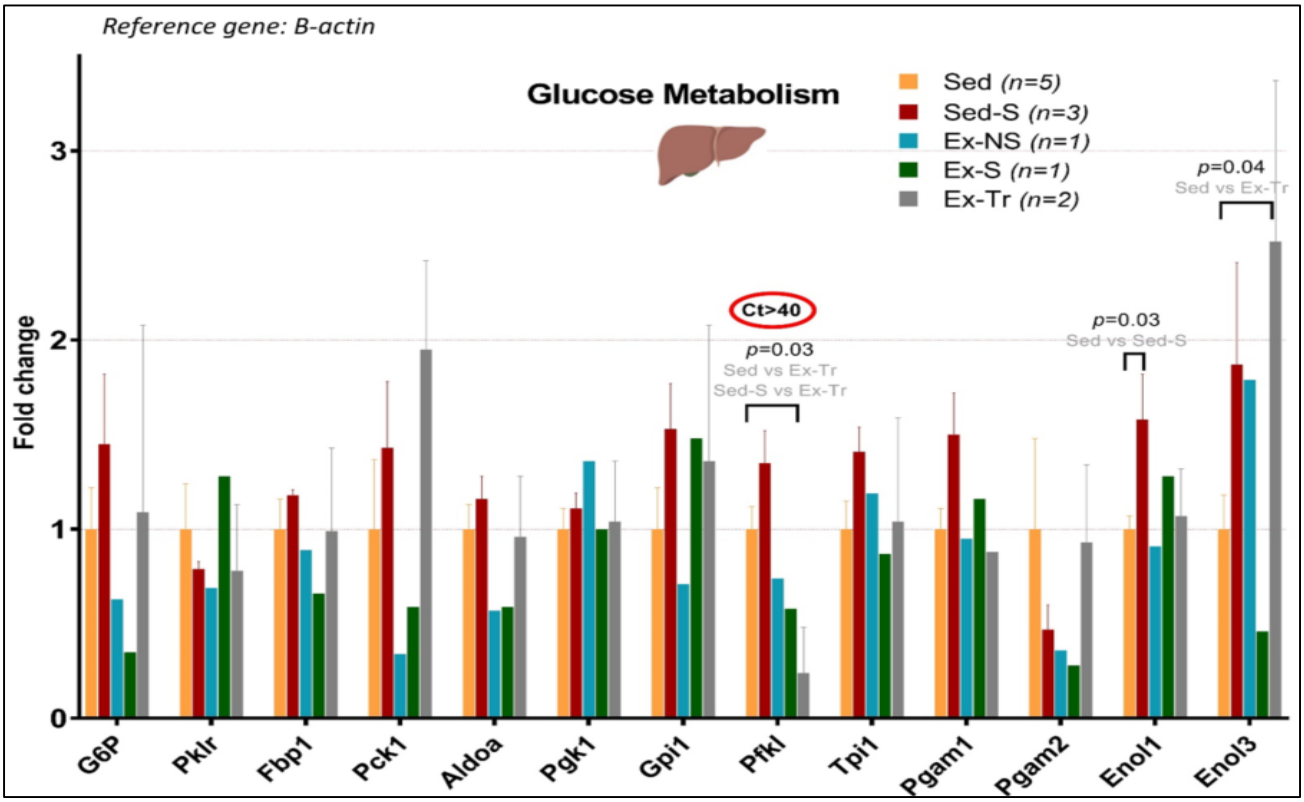

Figure 5. Influence of maternal exercise and stress on glucose and lipid metabolism and acetylcarnitine transport within liver and skeletal muscle. *Aldoa, aldolase, fructose-bisphosphate A; Enol, enoalse; Fbp, fructose-bisphosphatase; Gpi, glucose-6-phosphate isomerase; G6PB, glucose-6-phosphate dehydrogenase; PCK, phosphoenolpyruvate carboxykinase; Pfkl, phosphofructokinase; Pgam, phosphoglycerate mutase; Pgk, phosphoglycerate kinase; Pklr, pyruvate kinase; Tpi, triosephosphate Isomerase.*

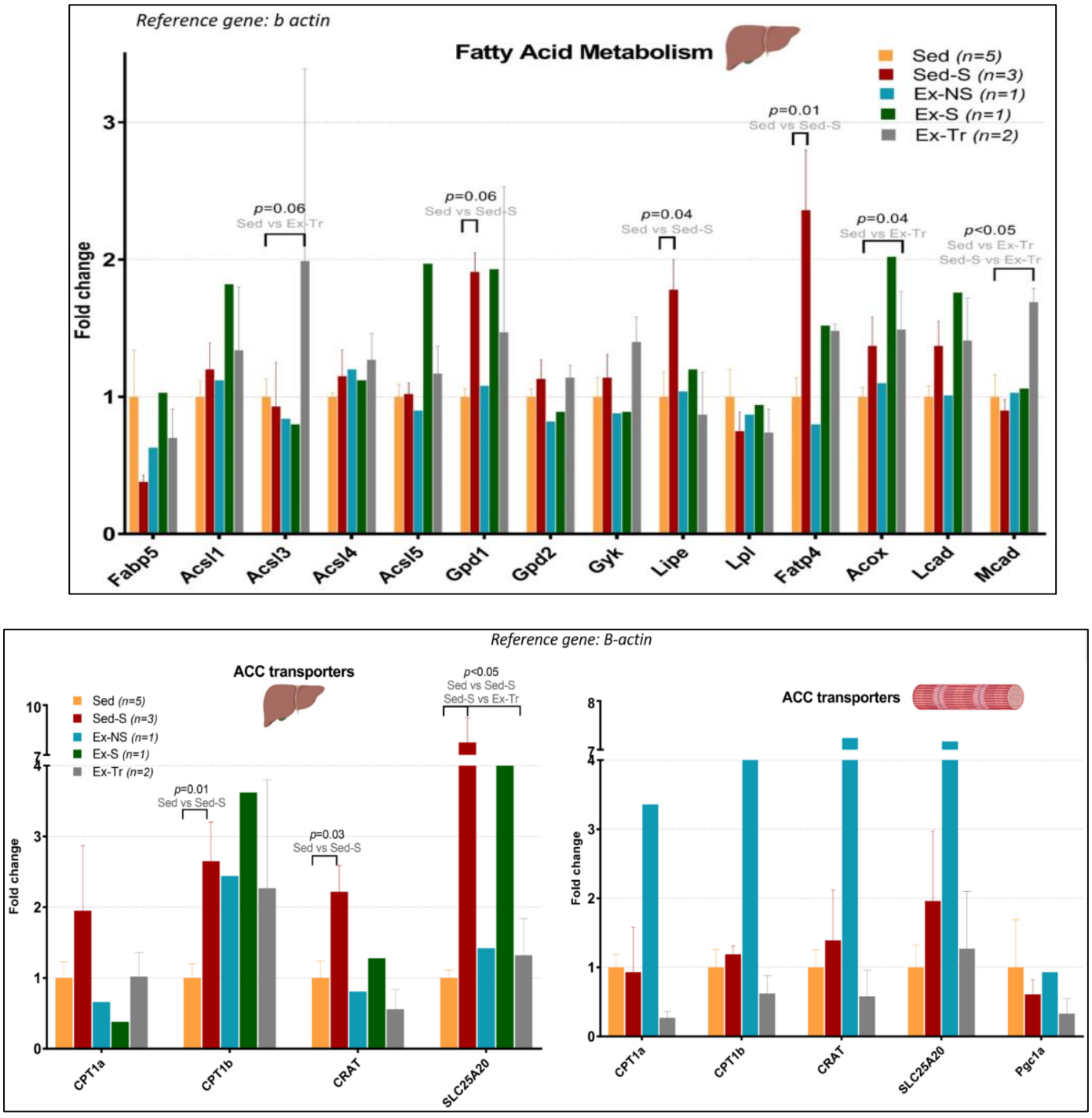

Figure 5 (continued). Influence of maternal exercise and stress on glucose and lipid metabolism and acetylcarnitine transport within liver and skeletal muscle. *Acox, peroxisomal acyl-coenzyme A oxidase 1; Acsl, acyl-coA synthetase long chain; Cpt1, carnitine palmitoyltransferase I; Crat, carnitine O-acetyltransferase; Fatbp, fatty acid binding protein; Fatp, fatty acid transport protein; Gpd, glycerol-3-phosphate dehydrogenase; Gyk, glycerol kinase; Lcad, long chain acly-CoA dehydrogenase; Lipe, lipase E, hormone sensitive type; Lpl, lipoprotein lipase; Mcad, medium-chain acyl-CoA dehydrogenase; Pgc1a, peroxisome proliferator-activated receptor-γ coactivator 1 alpha; SLC25A20, solute carrier family 25 member 20 (acylcarnitine carrier protein).*

These experiments, along with others at proteomic level, were not included in the current Thesis due to the reduced number of mice in some groups (e.g. mice in the Ex-NS group), and the lack of time to finish the whole experiment. However, they support the need to further analyse specific tissues such as liver and skeletal muscle. In line with these experiments, the Stanford research team have also previously shown that maternal exercise induces molecular changes not only in the liver or skeletal muscle, but also in adipose tissue[119]. Indeed, future studies at tissue level (e.g. muscle biopsies) and with different metabolic phenotypes in pregnant women, will shed more light on this issue.

Lastly, in the **Study VI**, we addressed another potential relevant mechanism for enhanced metabolic control via exercise: preventing and limiting excessive gestational weight-gain and postpartum weight retention. In agreement with previous literature[71,121-125], our findings showed that exercise notably reduced weight-gain and postpartum weight-retention. Of note, this was the first study showing the independence of its effects with regard to ST, PA, sleep, diet quality and PF. Our findings along with previous literature[77,95,123,124] also suggested that these effects of exercise on gestational weight-gain might be dependent on pre-pregnancy BMI levels. Although exercise did not prevent excessive weight-gain, it appeared to play an indirect protector role in maternal-foetal metabolism against the impaired metabolic phenotype related to excessive weight-gain. Unfortunately, we could not confirm our findings due to the non-existence of studies addressing this issue. Only one previous study has similarly shown that exercise might protect against some of the adverse effects of GDM (e.g. reduced risk of neonatal macrosomia)[126].

All in all, this study supports previous findings regarding the utility of implementing exercise as a weight management strategy, which might be useful to avoid impaired metabolism, birth complications and future diseases. However, further studies are warranted to verify the most effective exercise dose and type, and whether exercise protects against adverse alterations related to excessive weight-gain.

MAIN LIMITATIONS

Several strengths and limitations have been specifically noted for each study throughout the different parts of the doctoral Thesis. Nonetheless, there are general limitations which deserve more attention:

- Most of the studies and parts defining the current doctoral thesis are based on exploratory aims and secondary outcomes. Although we have accounted for multiplicity, and for the family wise error rate or false discovery rate, our findings are not derived from confirmatory trials, and thus should be interpreted according to their exploratory ground. Future exploratory and confirmatory trials are needed to verify our findings.

- We have used accelerometers to objectively assess ST and PA (waist), and sleep quality and quantity (wrist). Although we have employed a strict criterion for the processing and analyses (e.g. 7 days ≥10hours/day) in all studies (except for the Study II), the methodology employed has not been validated in pregnancy yet. Indeed, this represent an inherent limitation of the current evidence. We have applied the criteria that best suited to our population based on previous evidence and recommendations from the general population[127]. Future studies standardizing criteria for filters, processing, analyses, etc., and validating cut-points for ST and PA levels, are warranted.

- Selection biases might be present. In the studies IV, V and VI (GESTAFIT project), pregnant women were initially randomized into the intervention or control group according to the pre-specified protocol[128]. However, this random component could not be kept ultimately due to difficulties related to the complexity of maintaining women in the control group (avoiding high rates of withdrawal). Of note these methodological and ethical barriers are frequent in antenatal exercise research[129]. Thus, while roughly half of women were randomly allocated, the other participants were allocated to the exercise or control group according to their personal preference and convenience to attend the intervention sessions. This justifies the final quasi-experimental design of the GESTAFIT study. In the Study II (DALI study), we only analysed placental samples from one subgroup of women.

We preferably selected women for mRNA analyses from the intervention groups with PA counselling, since we expected the most relevant changes in ST and MVPA levels in these groups. Thus, women from the healthy eating group might be underrepresented in this study. Future studies investigating potential factors related to intervention drop-outs, and strategies to improve adherence of women, are warranted.

- In the study III and VI, the PF tests employed have not been validated in pregnant women yet, which represents an inherent limitation of pregnancy studies. Although we have used PF tests characterized by good psychometric properties[130-132], and adaptable, viable and safe for clinical populations[2,60,63,130-132], they might be inaccurate for pregnant women. This might be more worrisome in late pregnancy (i.e. when the physiological adaptations induced by pregnancy are more appreciable and restrictive). Of note, we are currently validating some of these PF tests at 16th and 33rd gestational week and postpartum with gold standard methods.

- The immunometabolic markers were measured in all studies at systemic level. Other models/analyses (e.g. transcriptomics) from specific tissues (e.g. muscles and placenta) should be addressed by future studies to better understand these findings. We could not perform tissue biopsies (e.g. muscle, liver) because of ethical and feasibility reasons. Of note, in the GESTAFIT project, we have just started to explore how ST, PA, PF and exercise influence placental markers (at genomic, transcriptomic and proteomic level).

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CONCLUDING REMARKS

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GENERAL CONCLUSION

The findings of the present Doctoral Thesis provide a greater insight on the role of sedentary time (ST), physical activity (PA), physical fitness (PF) and exercise in immunometabolism during pregnancy, and about the underlying mechanisms by which these stimuli might be translated into metabolic changes. We first highlight that reducing ST and increasing PA in early pregnancy are potential strategies to modulate immunometabolic responses. Subsequently, we provide evidence about the role of PF to confer a cardio-protector role in maternal metabolism; also indirectly via potentially reducing excessive weight-gain. Furthermore, we show that although exercise does not or scarcely influences maternal and foetal metabolism, it modulates maternal and foetal cytokines. In fact, we prove for the first time that few cytokines can mediate some of the effects of exercise into maternal metabolism. Finally, we evince that exercise can limit/control gestational weight-gain during pregnancy and postpartum weight-retention *–independently of other lifestyle behaviours and PF–* but not excessive gestational weight-gain. Exercise also appears to protect against the impaired metabolic phenotype related to exacerbated weight-gain.

SPECIFIC CONCLUSIONS

PART I. Role of sedentary time and physical activity on immunometabolism

- **Study I.** Increasing moderate-to-vigorous PA levels, or meeting PA recommendations, could be of utility to modulate the cytokine profile of women without metabolic disruptions in early to middle pregnancy. However, ST and PA do not appear to influence glucose or lipid levels *–at least directly*.
- **Study II.** Lifestyle-counselling interventions do not modulate the expression of placental molecules linked to glucose and lipid metabolism. However, lower ST in early to middle pregnancy is associated with higher expression of placental genes related to lipid transport (especially FATP2 and FATP3 mRNAs). Moderate-to-vigorous PA has little effect on placental mRNAs. Regarding the potential underlying mechanisms, placental FATP2 and FATP3 expression is regulated by the glucose-insulin axis. However, neither the glucose-insulin axis nor other

metabolic factors, mediate the associations of ST or PA with placental mRNAs. Moreover, up-regulated placental FATP2 mRNA is related to lower cord blood triglycerides and FFA, but not with neonatal adiposity.

PART II. Role of physical fitness on maternal and foetal metabolism

- **Study III.** Increased PF, particularly cardiorespiratory fitness and muscle strength in early to middle pregnancy, is related to an improved metabolic phenotype, and may confer a cardio-protector effect in maternal metabolism. “Keep yourself fit and normal-weight before and during pregnancy” is a major message for women. Cardiorespiratory fitness and muscle strength are important targets to consider when designing exercise programs aimed at better controlling maternal metabolism.

PART III. Role of physical exercise on immunometabolism

- **Study IV.** A concurrent exercise program during pregnancy might be a useful tool to modulate and control cytokines in pregnant women and their foetuses.
- **Study V.** Exercise does not have direct meaningful effects on maternal and foetal immunometabolic biomarkers, except for a decrease in maternal glucose at delivery and arterial cord serum glucose. Moreover, cytokines –*which are modulated by exercise*– appear to play a role in maternal and foetal metabolism. Of note, exercise indirectly reduces maternal total cholesterol and LDL-C gains via an increase in IL-8.

PART IV. Lifestyle and physical fitness: strategies to manage gestational weight-gain

- **Study VI.** Exercise robustly reduces maternal weight-gain during pregnancy and postpartum weight retention, independently of other lifestyle behaviours and PF. Although exercise is not able to avoid excessive gestational weight gain, it appears to protect against the impaired metabolic phenotype related to exacerbated weight gain. Moreover, greater CRF, muscle strength and sleep duration are associated with lower late and excessive weight-gain.

FUTURE PERSPECTIVES

Despite the great progress observed during the last years in some of the topics examined and discussed in the current Doctoral Thesis, there are still many related questions that remain incompletely understood. Future research should aim:

- To investigate how ST/PA and exercise programs characteristics [intensity, duration, bouts, frequency (days per week), recover, mode of exercise, etc.] influence maternal and foetal immunometabolism. Studies should discriminate properly ST, PA and exercise constructs to allow more easily comparisons between studies.

- To standardize procedures for accelerometer data collection and processing criteria to assess ST, PA and sleep parameters in pregnant women (at early, middle and late pregnancy). Additionally, to validate cut-offs for ST and PA levels in pregnancy should be also a priority for incoming accelerometry studies.

- To validate a PF test battery adapted and safe for pregnant women at early, middle, and late pregnancy, and at the postpartum period.

- To identify those factors responsible for the high rates of withdrawals in lifestyle interventions, and to implement interventions that consider/face these factors, thereby favouring successful adherence of women to interventions.

- To explore the influence of ST, PA, PF and exercise from early stages in pregnancy, and if possible from pre-conception. This will help us to understand if PA and exercise programs initiated early in pregnancy *(i.e. when the mother has a predominant noticeable influence on intrauterine programming, and the main biological processes take place)* could be more effective than those initiated at middle pregnancy. Ongoing projects such as the LIPP study and others (NCT02763150; NCT02346162) will shed some light on this issue.

- To explore the acute influence of exercise on molecular and metabolic responses during pregnancy. This will facilitate the understanding of how chronic adaptations (i.e. the consequence of repeated acute stimuli throughout time) are induced with exercise interventions.

- To distinguish clearly between normal and pathological conditions in pregnant women within the same study. This will allow researchers to better understand the different metabolic phenotypes (e.g. healthy vs. mild glucose tolerance vs. GDM woman), and thus to design more specific lifestyle interventions for pregnant women. Moreover, this will facilitate the analysis of those factors that predispose pregnant women at higher risk of being low-responders to exercise.

- To biologically characterize pre-term (e.g. abortion or elective deliveries) and in-term labor phenotypes; and collect placental tissues earlier in pregnancy in case of pre-term delivery. This will allow researchers to analyze early and late active vs. inactive placental phenotypes more in depth, which indeed will be useful to comprehend how lifestyle modulates placental phenotype from early pregnancy. Moreover, this will allow researchers to distinguish the effects induced by lifestyle (including exercise) on metabolism with regard to the acute effects of delivery.

- To investigate other models (e.g. metabolomics, proteomics) in specific tissues such as the skeletal muscle, adipose tissue and liver. This will allow researchers to better understand the origin and clearance of cytokines in rest conditions and in response to exercise, and the crosstalk between the mother (e.g. muscle, adipose tissue, and other organs), placenta and foetus. Moreover, this would allow to explore potential unperceived mechanisms (e.g. epigenetics, post-transcriptional modifications, etc.) which might translate the effects of exercise into metabolic adaptations.

- To understand the clinical relevance of changes in placental mRNA expression, cytokines concentrations, gestational weight-gain, etc. for intrauterine programming and foetal development.

APPENDICES

PAPERS DERIVED FROM THE DOCTORAL THESIS

PUBLISHED & INCLUDED IN THIS DOCTORAL THESIS

1. **Acosta-Manzano P**, Acosta FM, Femia P, Coll-Risco I, Segura-Jiménez V, Diaz-Castro J, Ochoa-Herrera J, Van Poppel MNM, Aparicio VA. Association of sedentary time and physical activity levels with glycaemic, lipid and inflammatory markers in early pregnancy. The GESTAFIT Project. *Scand J Med Sci Sports*. 2019. doi: 10.1111/sms.13547.

2. **Acosta-Manzano P**, Coll-Risco I, Van Poppel MNM, Segura-Jiménez V, Femia P, Romero-Gallardo L, Borges-Cosic M, Diaz-Castro J, Moreno-Fernández J, Ochoa-Herrera J, Aparicio VA. Influence of a concurrent exercise training intervention during pregnancy on maternal, and arterial and venous cord serum cytokines: the GESTAFIT Project. *Journal of Clinical Medicine.* 2019. doi: 10.3390/jcm8111862.

SUBMITTED & INCLUDED IN THIS DOCTORAL THESIS

1. **Acosta-Manzano P,** Leopold-Posh B, Simmons B, Devlieger R, Galjaard S, Corcoy R, Adelantado JM, Dunne F, Harreiter J, Kautzky-Willer A, Damm P, Mathiesen ER, Jensen DM, Andersen LL, Tanvig M, Lapolla A, Dalfra MG, Bertolotto A, Wender-Ozegowska E, Zawiejska A, Hill DJ, Snoek FJ, Jelsma JGM, Desoye G, van Poppel MNM. The unexplored role of sedentary time and physical activity in glucose and lipid metabolism related placental mRNAs in overweight and obese pregnant women: The DALI Lifestyle Randomized Controlled Trial. *Subbmited to Metabolism Clinical and Experimental.*

2. **Acosta-Manzano P*,** Acosta FM*, Flor-Alemany Marta, Gavilán-Carrera B, Delgado-Fernández M, Segura-Jiménez V, Aparicio VA. The protector role of physical fitness on maternal metabolism during pregnancy. The GESTAFIT project. *Subbmited to BJOG: an International Journal of Obstetrics and Gynaecology.*

3. **Acosta-Manzano P**, Flor-Alemany M, van Poppel MNM, Coll-Risco I, Segura-Jiménez V, Stanford KI, Aparicio VA. Influence of a concurrent exercise training during pregnancy on maternal and foetal immunometabolic biomarkers: the GESTAFIT project. *Subbmited to Diabetes, Obesity and Metabolism.*

4. **Acosta-Manzano P***, Acosta FM*, Coll-Risco I, Romero-Gallardo L, Flor-Alemany M, Martínez-González LJ, Álvarez-Cubero MJ, Segura-Jiménez V, Aparicio VA. Lifestyle, physical fitness, and maternal weight-gain and retention in pregnancy. The protective role of exercise against excessive weight-gain. The GESTAFIT project. *Subbmited to the American Journal of Obstetrics and Gynaecology.*

Curriculum Vitae - Research

1. Personal Data

Name:	**ACOSTA MANZANO, PEDRO PABLO**
Email:	pacostamanzano@gmail
Birth Date:	13/08/1992 (age: 28)
Birth Place:	Granada
Nationality:	Spanish
ORCID/Research ID:	0000-0002-4494-2353
Research Gate:	Pedro Acosta-Manzano

2. Current Affiliations

PhD candidate at the Sport and Health University Research Institute, University of Granada, Spain
Funded by the Spanish Ministry of Economy and Competitiveness
Department of Physical Education and Sports
Supervisor: Dr. Virginia A. Aparicio García-Molina
Co-supervisor: Dr. Victor Segura Jiménez
18/10/2015 - Present

3. Research experience

Principal Investigator **01/01/2019 – present**
Project Title & Design: The influence of sedentary time and physical activity intensity levels on placental inflammatory molecules in pregnant women.
Principal Investigator: ***Pedro Pablo Acosta Manzano***
Department of Physical Education and Sport, University of Granada, Spain
Funding: University of Granada €1000

Researcher **15/01/2017 – 01/05/2019**
Project Title & Design: Effects of a supervised physical exercise intervention during pregnancy on telomere length and gene expression markers related to adiposity in the mother and neonate. A randomized controlled trial.
Principal Investigator: Virginia A Aparicio
Department of Physical Education and Sport, University of Granada, Spain
Funding: Spanish Ministry & European Funding (*MSCA-COFUND* €56000)

Researcher **01/01/2017– 01/02/2019**
Project Title & Design: Comparison of genotype and phenotype in patients with fibromyalgia and patients with other related diseases (chronic pain, rheumatoid arthritis and depression). The role of physical activity and fitness
Principal Investigator: Manuel Delgado-Fernández
Department of Physical Education and Sport, University of Granada, Spain
Funding: Spanish Ministry €48753

Project Manager *(2 project managers in this project)* **01/03/2015 – 01/03/2017**
Project Title & Design: Influence of physical activity levels, physical fitness and nutritional habits of the pregnant women on diverse foetal and maternal health markers" Acronym: GESTAFIT
Principal Investigator: VirginiaA Aparicio
Department of Physical Education and Sport, University of Granada, Spain
Funding: Spanish Ministry & European Funding €100000

Collaborator **01/01/2015 – 31/12/2017**
Project Title & Design: Longitudinal follow-up and gene modulation in fibromyalgia. Effects of physical exercise and hydrotherapy on pain, health and quality of life
Principal Investigator: Manuel Delgado-Fernández

Department of Physical Education and Sport, University of Granada, Spain
Funding**:** Spanish Ministry €121000

Student assistant - Collaborator **01/01/2015 – 31/12/2015**
Project Title & Design: Effect of balneotherapy and physical exercise in hot water on the body temperature and pain in women with fibromyalgia.
Principal Investigator: Victor Segura Jiménez
Department of Physical Education and Sport, University of Granada, Spain
Funding: Spanish Ministry €4500

Student assistant - Collaborator **01/01/2015 – 07/07/2018**
Project Title & Design: Cost-effectiveness of an exercise intervention program in perimenopausal women. The Fitness League Against MENopause COst (FLAMENCO) randomized controlled trial
Principal Investigator: Virginia A Aparicio
Department of Physical Education and Sport, University of Granada, Spain
Funding: Spanish Ministry €38500

Student assistant **30/09/2014 – 05/04/2015**
Project Title & Design: Physical activity in women with fibromyalgia- effects on pain, health and quality of life
Principal Investigator: Manuel Delgado-Fernández
Department of Physical Education and Sport; University Hospital "Virgen de las Nieves", Granada, Spain
Funding: Spanish Ministry €120000

4. Education and training

Free attendance to Bachelor's degree in Medicine 20/04/2018 – 15/03/2020
University of Granada, Spain

PhD candidate in Biomedicine 18/10/2015 – 21/01/2021
Thesis title: The role of physical activity, physical fitness, and exercise on immunometabolism during pregnancy
Supervisor: Dr. Virginia A. Aparicio García Molina
Department of Physical Education and Sports, University of Granada, Spain

Master´s degree in Research on Physical activity and Health 01/10/2014 – 05/07/2015
Project Title: Do women with fibromyalgia present higher cardiovascular disease risk than healthy women?
Supervisor: Dr. Virginia A. Aparicio García Molina
University of Granada, Spain

Bachelor´s Degree in Sport Sciences 15/09/2011 – 27/07/2014
University of Granada, Spain
Best score in Bachelor´s Internship (exercise for health promotion, Sport and Health University Research Institute)
Erasmus Student Internship - 9 months at University of Bologna, Italy 05/09/2012 – 31/06/2013

5. Research internships

- 15/01/2021 – 15/02/2021: Institute of Human Movement Science, Sport & Health. University of Graz (Austria) with **Mireille MN van Poppel –** *(To be performed)*

- 15/10/2019 – 15/01/2020: Wexner Medical Center, The Ohio State University, Ohio (USA) with **Kristin Stanford**

- 25/08/2018 – 25/11/2018: Institute of Sport Science. University of Graz (Austria) with **Mireille MN van Poppel**

- 25/08/2017 – 25/11/2017: Institute of Molecular Sports and Rehabilitation Medicine, Paracelsus Medical Private University, (Austria) with **Josef Niebauer**

- 20/07/2015 – 20/09/2015: Faculty of Social & Behavioural Sciences, University of Utrecht - with **Rinee Geenen**

6. Funding, grants and personal awards

- 15/01/2021 – 15/02/2021. **ARQUS Alliance grant** *(Action Line 6- Research Support and Early Stage Researcher Development)*: 1-month research internship at the University of Graz. European Union (**€1200**).

- 20/01/2019 – present. **Research Support and Management contract**. European Union and Spanish Ministry of Economy and Competitiveness (2-years, **€35000**).

- 01/01/2019 – present. **Precompetitive Research Projects for early excellent investigators**: Principal Investigator. The influence of sedentary time and physical activity intensity levels on placental inflammatory molecules in pregnant women. Funded by the University of Granada **(€3000**).

- 05/09/2019 – 08/09/2019. **Young Investigator Travel Award** for the 51st annual DPSG Scientific meeting, Austria. Funded by the DPSG (**€500**).

- 03/07/2019 – 06/07/2019. **Personal grant**: attendance to an international congress as speaker: Funded by the EDCS Program, Czech Republic. University of Granada (**€400**).

- 25/08/2018 – 25/11/2018. **Personal grant**: 3 months research internship at the University of Graz, Austria. European Union (**€2200**).

- 25/08/2017 – 25/11/2017. **Personal grant**: 3-months research internship at the Paracelsus Medical Private University, Salzburg, Austria. University of Granada (**€2500**).

- 20/07/2015 – 20/09/2015. **Personal grant**: 2-months research internship at the University of Utrecht. European Union (€1200).

- 15/10/2014 – 15/07/15: **Initiation to research grant** (partial time). University of Granada (**€2000**).

7. Publication - bibliometric scores

h-Index Scholar/WOS:	9/5	Publications as Third Authorship:	8
Citations Scholar/WOS:	191/99	Publications with international collaborators:	15
Citations (Scholar -without self-citations):	111	Percentage as Q1:	82%
Publications:	**37**	Percentage as Q1 (first author):	**85%**
Publications as First Authorship:	**6**	Percentage as Open Access:	60%
Publications as Second Authorship:	5	Books (chapters):	1

As appreciable in my publication record, I have published in the **top journals** of different areas, with a very **multidisciplinary profile**. This is supported by the fact that all my publications as first author are in the first quartile (Q1, at its moments of publication) according to the Web of Sciences (WOS), and in several areas such as: *Sport Sciences, Obstetrics and Gynaecology; Endocrinology and Metabolism; Obesity; Menopause; Nutrition and Dietetics; Internal medicine; Biology; Physiology; and Rheumatology.* Link of Google Scholar Citations to access to the full list of publications: https://scholar.google.es/citations?user=UCNybBAAAAAJ&hl=es

Ten top publications (equally contributed*)**

- **Acosta-Manzano P**, Coll-Risco I, Van Poppel MNM, Segura-Jiménez V, Femia P, Romero-Gallardo L, Borges-Cosic L, Diaz-Castro Javier, Moreno-Fernández J, Ochoa-Herrera J, Aparicio VA. Influence of a concurrent exercise training intervention during pregnancy on maternal, and arterial and venous cord serum inflammatory markers: the GESTAFIT Project. Journal of Clinical Medicine. 2019. *IF: 5.7, Q1.*

- **Acosta-Manzano P**, Acosta FM, Femia P, Coll-Risco I, Segura-Jiménez V, Diaz-Castro Javier, Ochoa-Herrera J, Van Poppel MNM, Aparicio VA. Association of sedentary time and physical activity levels with glycaemic, lipid and inflammatory markers in early pregnancy. The GESTAFIT Project. Scand J Med Sci Sports. 2019. *IF=3.63, Q1, Rank: 11/83.*

- **Acosta-Manzano P**, Rodriguez-Ayllon M, Acosta FM, Niederseer D, Niebauer J. Beyond general resistance training. Hypertrophy versus muscular endurance training as therapeutic interventions in adults with type 2 diabetes mellitus: A systematic review and meta-analysis. Obesity Reviews. 2020. *IF: 8.2, Q1, Rank: 8/145.*

- **Acosta-Manzano P,** Segura-Jiménez V, Coll-Risco I, Borges-Cosic M, Castro-Piñero J, Delgado-Fernández M, Aparicio VA. Association of sedentary time and physical fitness with ideal cardiovascular health in perimenopausal women: The FLAMENCO project. Maturitas. 2019. *IF: 3.7, Q1, Rank: 9/83.*

- Gavilán-Carrera B*, **Acosta-Manzano P*,** Segura-Jiménez V, Soriano-Maldonado A, Borges-Cosic M, Aparicio VA, Delgado-Fernandez M. Understanding the association of sedentary time, physical activity, and sleep duration with body composition in women with fibromyalgia. The al-Ándalus Project. 2019. *IF:5.7, Q1 Rank:15/160.*

- **Acosta-Manzano P**, Segura-Jiménez V, Estévez-López F, Álvarez-Gallardo IC, Soriano-Maldonado A, Borges-Cosic M, Aparicio VA. Do women with fibromyalgia present higher cardiovascular disease risk profile than healthy women? The al-ándalus project. Clinical and Experimental Rheumatology. 2017. *IF: 3.3, Q2, Rank: 15/30.*

- Baena-García L, Ocón O, **Acosta-Manzano P,** Coll-Risco I, Borges-Cosic M, Romero-Gallardo L, De la Flor-Alemany M, Aparicio VA. Association of sedentary time and physical activity during pregnancy with maternal and neonatal birth outcomes. The GESTAFIT Project. Scand J Med Sci Sports. 2018. *IF=3.63, Q1, Rank: 11/83.*

- Rodriguez-Ayllon M, **Acosta-Manzano P**, Coll-Risco I, Romero-Gallardo L, Borges-Cosic M, Estévez-López F, Aparicio VA. Associations of physical activity, sedentary time and physical fitness with mental health during pregnancy: The GESTAFIT project. J Sport Health Sci. 2019. *IF: 5.14, Q1, Rank: 5/85.*

- Marín-Jiménez N, **Acosta-Manzano P**, Borges-Cosic M, Baena-García L, Coll-Risco I, Romero-Gallardo L, Aparicio VA. Association of self-reported physical fitness with pain during pregnancy: The GESTAFIT Project. Scand J Med Sci Sports. *IF=3.63, Q1, Rank: 11/83.*

- Aparicio VA, Ocón O, Diaz-Castro Javier, **Acosta-Manzano P**, Coll-Risco I, Borges-Cosic M, Romero-Gallardo L, Moreno-Fernández J, Ochoa-Herrera J. Influence of a concurrent exercise training program during pregnancy on colostrum and mature human milk inflammatory markers: Findings from the GESTAFIT Project. Journal of Human Lactation. 2018. *IF: 2.2, Q1, Rank: 14/123.*

8. International Conferences

Nº Oral communications/poster presentations: 4/7 Nº international conferences: 11

Top 5 International Conferences

05/09/2020	**52nd Diabetic Pregnancy Study Group Annual Meeting**, Virtual. Poster presentation: "Potential mechanisms and pregnancy implications related to lifestyle-induced mRNA changes. The DALI Lifestyle Randomized Controlled Trial"
05/09/2019 - 08/09/2019	**51st Diabetic Pregnancy Study Group Annual Meeting**. Graz, Austria. Oral presentation: "The role of sedentary time and physical activity during pregnancy on placental lipid, inflammatory and hormone mRNAs in overweight-obese pregnant women. The DALI Randomized Controlled Trial"
03/07/2019 - 06/07/2019	**24thAnnual European College of Sports Sciences** Congress, Prague, Czech Republic Oral presentation: "Resistance training as potential therapeutic intervention in type 2 diabetes mellitus: a meta-analysis of randomized control trials"
20/06/2018 - 22/06/2018	**III International JIFFI congress**, Granada, Spain. Oral presentation: "Association of sedentary time with glycaemic, lipid, and inflammatory markers in early pregnancy The GESTAFIT Project".
21/06/2017 - 22/06/2017	**I Biomedical Scientific Conference**, Granada, Spain. Oral presentation: "Association of sedentary time and physical fitness with ideal cardiovascular health in perimenopausal women. The FLAMENCO project"

9. Organization of international conferences

03/07/2019 - 06/07/2019	**24th Annual Congress of the European College of Sports Sciences**, Prague, Czech Republic. Organization coordinator (volunteer) and mediator

10. Reviewer articles

I have reviewed several manuscripts within this field of research for international collaborators *(not acknowledged),* and one top journals: *BJOG.*

11. Memberships

20/01/2020 - present	**ACSM** Special Interest Group-Pregnancy and Postpartum
15/01/2020 - present	**UCEENS, Scientific Unit of Excellence** on Exercise, Nutrition and Health (UGR)
10/16/2019 - present	**EFSD**, European Foundation of the Study of Diabetes
09/12/2018 - present	**EXERNET:** Research Network on Physical Activity and Health for Special Population

12. Management activities

As experienced researcher, I am the responsible person -along with the Dr. Virginia A Aparicio- of **administering the inventory** (e.g. handling perishable products), **economic resources** (e.g. expenses), and the **communication with external services/companies**. I have also contributed to organize different academic courses:

15/09/2020 – Present	Master´s degree "Physical activity, exercise and health for women". **Participation-Promotion**
03/09/2018 – 09/09/2018	International Doctoral Summer School in Nutrition and Exercise for a healthier pregnancy. **Organization and workshop**
01/06/2016	Scientific course: Exercise training and Nutrition during pregnancy, Granada, Spain. **Organization and lecture**

13. Teaching and supervising activities

- **Lectures** on the Master´s degree: "Research on Physical activity, Health, and Disease", University of Granada
- **Lectures** on multiple health topics at the Faculty of Sports Science, University of Granada, Spain
- **Academic training supervisor**: Physical Activity&Health internships. University of Granada, Spain (2015-2016)
- **Co-supervisor** (non-formally) of 2 Master Thesis and 1 Bachelor student during my PhD studies. I have also supervised (**non-formally)** young researchers who joined to projects in which I was the project manager and principal investigator
- **Orientation seminars in secondary education (**for students interested on the Sports Sciences Bachelor´s degree)
- **Internal research seminars** to share scientific knowledge (e.g. methodological issues and statistical analyses)

14. Courses attended (Transferable skills)

14/10/2019 – 16/10/2019	Course: CITI program. Lab animal research and working with the IACUC
04/02/2019 – 06/02/2019	Course: Analyses of bioactive compounds in pre-clinical experimental models
10/04/2018 – 11/05/2018	Course: Mediation analyses in intervention studies in humans
05/02/2018	Course: Epigenetics
08/01/2018 – 12/01/2018	Course: Statistical analyses in clinical trials
22/05/2017 – 25/05/2017	PhD course: Early programming in pregnancy
01/05/2017 – 04/05/2017	PhD course: Basic histology techniques in biomedicine
12/04/2017	Course: Physical activity metrics-accelerometry
28/11/2016 – 02/12/2016	Course: Research, innovation, intellectual property and knowledge transference
11/10/2016 – 14/10/2016	PhD course Scientific writing: How to write a paper
01/08/2016 – 04/08/2016	PhD course: R statistic software
29/07/2016 – 31/07/2016	Summer course: From nutritional genomic to personalized nutrition
18/04/2016 – 22/04/2016	PhD course: Systematic review of studies. Meta-analyses
04/04/2016 – 08/04/2016	PhD course: Modelling of categorical data with SPSS
22/02/2016 – 24/02/2016	PhD course: Strategies for effective research publication
12/09/2015 – 13/09/2015	Course: Physical exercise in pregnancy and postpartum
16/03/2015 – 20/03/2015	Course: Physical exercise and nutrition in special populations

15. Outreach activities

- 05/04/19 – present. **Diffusion** of relevant studies related to my field of search (including those published by me) via my academic Twitter page: https://twitter.com/PedroAM_ugr, and websites such as Research Gate.

- 10/09/2018 - 20/04/2020. Five studies where I was involved -2 of them as first author (*Obesity Reviews and Scand J Med Sci Sports*)-, were disseminated to the Spanish media with support of the university press office; leading to interviews for **national and international** radio programmes, newspapers and blogs, etc.

- 06/04/2018- 01/10/19. Several interviews by local and **national media** to make public our project and results.

- 19/10/2018 - 20/10/18. Workshop on the ethics of scientific publication: "Scientific Publication; quantity vs. quality".

- 20/11/2013. Open-doors workshop for children at the Science park "Diet and healthy lifestyle" Inprofood.

ACKNOWLEDGEMENTS/AGRADECIMIENTOS

Después de unos años, por fin llega el día en el que uno escribe las últimas frases de su tesis y reflexiona sobre todo lo vivido. Aunque la tesis sea un mérito trámite, este libro es importante para mí porque refleja en parte lo que he vivido, disfrutado, aprendido y sufrido durante estos últimos 5 años de vida, tanto en lo personal como profesional. Aunque es cierto que hay muchos momentos de estrés y de agobio debido a las presiones y competencias que tenemos, a veces nos olvidamos de que el doctorado es una de las mejores etapas, sino la mejor, para curiosear y seguir aprendiendo sobre algo que nos interesa, sentar nuestras bases, volver a ser críos y pensar desde cero, darnos cuenta de lo poco que sabemos, etc. Es por ello, que esta etapa nos brinda la oportunidad de acercarnos al lado más bonita de la ciencia: curiosear, experimentar y ayudar a los demás. Y por supuesto, las increíbles ventajas en lo que respecta a viajar y conocer nuevas personas alrededor de todo el mundo, vivir experiencias internacionales, flexibilidad horaria, en definitiva, cosas que no cambiaría por nada. Sin embargo, es una pena que a veces nos olvidamos de este lado maravilloso de la ciencia, siendo yo el primero al que le ha ocurrido en alguna ocasión.

Aquí es donde puedo decir que he sido afortunado. He tenido la gran suerte de rodearme de gente que me lo recordaba continuamente, y que han conseguido mantener viva esa llama (pasión) por la ciencia, incluso cuando las peores lluvias caían. Sin duda alguna, a día de hoy, toda la gente que he conocido, y en especial mis amigos y familia, me han permitido valorar donde estoy hoy y lo afortunado que soy, el por qué me dedico a esto, y porqué es importante para mí. Todos ellos han hecho que este proceso de aprendizaje sea maravilloso, un periodo de disfrute, de goce, de celebraciones. Es por ello que me gustaría agradecerles a todos ellos (incluidos a los que me olvido) el haberme acompañado todoS estos años.

Familia académica

Me gustaría empezar los agradecimientos hacia una persona especial y que considero que es el gran padre de la Facultad y de la familia de investigación. Lo gracioso de esta historia es que tú todavía no lo sabes, pero si a día de hoy me dedico a la investigación, es gracias a ti. Tú fuiste la persona que me empujaste a empezar. Desde la primera clase de salud contigo, me transmitiste con humildad esa pasión por ayudar a la gente y

aprender. Sin embargo, fue gracias al trabajo de fin de grado (que fui como un crio corriendo a pedírtelo el primero), que tuve la oportunidad de trabajar más cerca de ti y darme cuenta de lo mucho que me gustaba este mundo. No olvidaré ese día que pensé: quiero seguir aprendiendo más, y lo quiero hacer con él. Y por supuesto el día que Blanquis y yo nos presentamos en tu oficina y te lo comentamos, y nos acogiste con los brazos abiertos. **Manolo** tu forma de ser es increíble. Solamente te diré que me ha llevado 4 años convencer a mis padres de que los jefes AMIGOS como tú existen, que siempre estabas preocupado por nosotros porque tuviésemos contrato, porque no estuviésemos burned-out, porque estuviésemos a gusto...incluso nos has ayudado a evaluar! (un catedrático rompiendo esquemas jajaj).

Todo esto me llevo al trabajo de fin de máster, donde le dije que quería estar con él también. Pero él con su humildad, nos dijo que era mejor que estuviésemos con gente más joven, y quizás, preparada para lo que venía. Y fue cuando nos introdujo a 2 personas de las que me gustaría hablar. La primera de ellas es mi directora/AMIGA **Virginia Aparicio**, que suena muy formal pero tras los primeros 5 minutos con ella, te das cuenta que muy muy muy formal….no es que sea. Ella fue la persona que alimentó mi motivación en el mundo de la fisiología: a indagar, a aprender, a descubrir, a pensar siempre porqué podían ocurrir las cosas, esa has sido tú!!! Y esto es una cosa que valoro muchísimo. Todos los geni@s tienen un poco de locura, y tú tienes mucha locura acumulada (a ver si sale la regla de 3 ;). Quien realmente conozca a Vir sabe que su espontaneidad va de la mano con lo poco que escucha, pero también de su generosidad, de sus ganas de ayudarte, de que estés cómodo, y muchas más cosas que la definen como una persona increíble. Siempre me ha dado consejos personales y laborales, y lo más importante, anteponiendo mi bienestar personal a aspectos de trabajo que le podían perjudicar a ella directamente o indirectamente como IP. Fijaros si es generosa, que en todas las reuniones, comidas, etc. ella siempre intenta invitar a todos los suyos, y es que ya es hasta incómodo y no sabemos cómo adelantarnos. Es una experta en ir al cuarto de baño y dejarlo pagado. El colmo es que invita incluso a copas…pues una buena supervisora de tesis, claro que sí! (me enseñó la "Sal" y todo). A día hoy, muchos alumnos que empiezan con nosotros vienen y me dicen "es que me da un poco de miedo como es, no sabemos por dónde va a aparecer" jajjaa; y ahí es dónde me doy cuenta de todo lo que he aprendido y disfrutado con ella, y de que en mis comienzos yo también

pensaba igual, y ahora estoy súper cómodo hablando de ciencia o de temas banales con ella. Estoy convencido de que seguiré trabajando mucho tiempo a tu lado porque aunque tengas tus defectos (que son unos cuantos), me encanta! Aunque sé que nunca me perdonarás la broma a las 4 de la mañana del paritorio con tu madre despierta yendo al hospital jaja

La otra es mi director/AMIGO **Víctor**. Todavía me acuerdo cuando lo conocí, que él estaba agobiado haciendo una cosa y no levantaba la cabeza del ordenador, y dije uii que tío más friki. Yo no quiero ser como él. Prejuicioso por mi parte se quedaba corto. Es más, me acuerdo en la reunión para buscar un co-direct@r para la tesis, que Blanca y yo entramos en el despacho de Manolo, y él nos recomendó a Víctor. Lo primero que le dije a Blanquis cuando salimos de la reunión fue "nos quieren colar al matado este"….ajja Fijaros en lo que hace la ignorancia, y la suerte que tuve/tuvimos de que esta persona tan humana e increíble sea a día de hoy mi director de tesis. Yo siempre digo que a mí me toco el combo perfecto. Me toco Virginia, la "loca" de las ideas pero desastre en metodología, y Víctor el crítico y perfeccionista. Él encendió esa llama de querer hacerlo siempre todo lo mejor posible. A día de hoy, me pongo a reflexionar sobre mi modo de trabajar, y creo sin duda, que estas dos personas han hecho que saque lo mejor de mí en estos aspectos. Fuera del trabajo, qué decir que Víctor no sepa. Lo considero un gran amigo para las buenas y malas, con él que he podido disfrutar de muchos paseos, eventos, celebraciones, fiestas, tesis, fines de semana, y experiencias increíbles, y lo que nos queda todavía. Como siempre me dice…yo soy tu dire de tesis, pero también de la VIDA. La verdad es que me ha enseñado muchas cosas y siempre se preocupa por mí. Si tuviese que decir algo con lo que me quedo de él, sería con todas las veces que ha pasado por debajo del futbolín, perdido en levantar peso en el gimnasio, jugando al pádel, fútbol, corriendo, y un sinfín de cosas que espero que sigas entrenando para poder alcanzarme algún día. Como doctorando te digo que esto has sido lo único en lo que me has defraudado: eres un poco matadillo, pero tranquilo yo seguiré enseñándote ;). Gracias a MANOLO, VIR Y VICTOR, por siempre tratarme por igual y anteponiendo el ámbito personal al laboral.

Por supuesto hay mucha otros amigos que me han acompañado y a los que me gustaría agradecerles que hayan hecho esta etapa tan bonita, independientemente del

apellido que lleven (FIBRO, GESTAFIT, PROFITH, biomecánica, etc.), puesto que esas fronteras son tonterías. **Albertico** (y sus chistes malos en pedro antonio), **Inmilla** rubia (siempre con su sonrisa), **Manu** Herrador (siempre con sus coñas), **Dani** Camiletti (el que nunca para), y **Farranduco** (cuantas fiestas compartidas en diferentes partes del mundo) nunca olvidaré esos pasos iniciales que dimos con vosotros, y lo que aprendimos y disfrutamos al mismo tiempo. Mi **milki** que es más dulzona que el chocolate milka (y eso es difícil), y vale oro como persona. Quien tenga la suerte de pasar más tiempo con ella, como yo la tuve, lo descubrirá sin duda. Mil gracias por todo y por esas conversaciones privadas a las 7 de la tarde donde se permitía discutir de todo. Mi compi de los 3 proyectos (¿por qué hicimos eso? Jaja): que grandes momentos que hemos pasado de evaluaciones, excursiones, cruces-corpus, fiesta... Oleee esa canaria que nos explica las diferencias entre canarias, las islas…y ya me he liado. **Inmolita**, que suerte tuvimos de que fichases por FIBRO aquel año que todos nos preguntábamos quien eras. Pues una persona genial con la que tuve la suerte de compartir al final casi 3 años, y espero que mucho más. Cuantas conversaciones de perros y de pan de pipas jajja. De verdad, tú nos queráis engordar. Gracias por haber hecho tan amenas las evaluaciones y tan divertidas. Te mereces conseguir ese puesto de educación que tanto deseas y lo conseguirás, el mundo necesita gente como tú en las aulas. **Irenufla**, mi catalana favorita…que penica me dio que te fueses para el norte….para matarte ☺. Una de las cosas que más me gustaron de ti es que tenías claras tus prioridades en la vida, y querías disfrutar de la vida. Eres increíble y tenemos una visita pendiente a Girona para verte (y a Alex también que no me olvido de él jajaj)…maldito covid. Espero que practiques tu listening que me tenías cansado con el qué dices Pedro…de verdad chica, el nivel listening mal ehh jajjajaja. Cuando quieras montamos otra chirigota y la liamos parda!! **Martica**, el futuro de nuestro GESTAFIT, y nuestra joven promesa, que de tiempo hemos pasado discutiendo este año que si estadística, que si genética…y qué de risas con nuestro LUMINEX y placenta. Hazlo tú!, no hazlo tu! No tú jajaja que no nos fiábamos ninguno. Eres genial y espero que sigas con esa actitud de aprender! Rezo porque vir no te lleve por su camino de locura como ya le pasó a **Nuria**, nuestra otra promesa que nos abandonó, desertora! Vete con los gaditanos. Que ya vemos tus prioridades jaja. Que grande eres Nuria, y qué loca estás. Espero que sigas así durante mucho tiempo y que nadie te cambie. Que valéis muchos las dos!! A **Lidia** nuestra trainer y **Laurica** nuestra

midwife, mil gracias por todo (sobre por esas conversaciones de tampones, copas, y sexo en el embarazo jajjja). Aunque no nos hayamos visto mucho este año, qué de risas nos echamos en las evaluaciones, en las reuniones, tesis, etc. El dúo inglés, sois grandísimas personas y profesionales (aunque esto no hace falta decirlo). A **Olga, Pilar** y **Lara**, que aunque hayamos pasado menos tiempo juntos, sois geniales y he aprendido mucho de vosotras.

A todos los de la sala de becarios, que durante tantos años nos hemos reído (a los que se fueron y a los que llegaron), mil gracias por esos momentos. **Rominica** (la vecina chilena – ahí lleva el donut 3 años, cuando quieras jaja), **M. Jesus** (que eso no es frio, vete a las 7 de la mañana a coger aceitunas), **Amador**, **Alex** y **Javi** futbol (os hacía lo que quería jugando al fútbol), **Pablico C**., **Patri**, **Manu Ávila**, **Rafilla**, **Artachico**…

Al equipo IMUDS, mil gracias por todo: **Javi Steinburg** y su **eddi** feroz (siempre estaré enamorado de vosotros, pese a vuestra locura, ansiedad constante, sois de lo mejorcito que hay…lo digo de corasónnn), **Irenilla Canoas** y **Pepilla** (que grandes que sois como amigas y como personas…ojalá hubiese más gente así, valéis mucho), **Abelín** (que gran persona eres, y además night hunter; con el que he disfrutado muchísimo estos años, y los que vendrán), **Pablilo** Maluma, **Wendy Daniela, Juanmilla, Hui, Lourdes, Borjita** rabo de toro, **Guille** cabesilla, **Pepillo** Justin, **Jairo, Lucia, Pato, Luis, Ester, Juan José** (por seguir la tradición de mi bro jjaa), **Manu D**, **Luquillas**, **Cristina M**, **Anayara** (nuestra cinéfila incomprendida), **Eli**, **Juan Pablo**, **Alex** Biomecanica, **Gabri**, **Unai**, **Cristina C**, **Alex** de la O, **Vicky** la rubia, y mucha otra gente. Y por supuesto otras personas que no son del IMUDS, pero como si lo fuesen: nuestras queridas vascas (**María**, **Lide** y **Maddi**) y nuestra castellonense loca favorita **Mireilla**.

No se me puede olvidar mencionar a **Pablo Tercedor, Jonatan, Fran, Palma, Miguelón, Patro, Toté, Pepe Castro,** y **Pedro F.,** porque sois grandes personas que me habéis enseñado mucho durante este tiempo, ya sea en el trabajo o con una cerveza en la mano, y que siempre os habéis esforzado porque los jóvenes sigamos avanzando (aunque muchas veces esto no se vea). También a **Jesús Huertas**, que junto con Manolo, considero que sois los mejores profesores que tuve en la carrera. Ojalá te quedes siempre en nuestra facultad!

También me gustaría a agradecer a todas esas personas que estuvieron ahí durante la carrera y máster, y que sin duda alguna formaron parte de esta aventura, pero en particular a mi **RAUL** gordaco (y nuestras cañitas de chocolate en los descansos) **Salvita**, **Sergio**, **Samu** y **Anita.**

Indeed, I would like to mention some international friends who influenced me during this learning phase. I will never forget my first research internship with **Rinie** in Utrecht. It was such an amazing experience. He did not expect anything from us, but learning and enjoying our first research stay outside. I remember when I met him and I thought wowww, this amazing researcher looks so kind and beaming (as my grandfather). Thanks for being so nice with us. Also to **Farranduco**, **Michelle** and **Vera** who were incredible nice with us there (dancing salsa, sailing…). During my second research internship in Salzburg, I realised that this is a small world. I was lucky enough to meet my dear **Goksel**, one of that guys that you meet, and you want in your life forever. Thanks for being so incredible. Also my petit **Lucile**, **Matteo**, **Pablo**, **Macarena**, and **Carlos**, you did from this experience an amazing one. Hopefully, we will meet all again soon. I did not believe when I had to come back to Austria (Graz) for my third internship. I did not want. However, there, I discovered that my passion in research was pregnancy. This was thanks to the inspiring conversations with **Mireille** and **Anna** (and Virginia as well), who made me wonder about different points I have never thought about. Mireille and Anna, you know I really admire the way you work, and the international environment that you have created at your office. Hopefully, I will spend next two years with you in Austria. I cannot think about a better place to carry on learning, working and enjoying. Thanks for everything and for being so incredible. But most importantly, you along with **Matteo**, made me feel at home. I had such a fun time with you...I will not forget about the famous phrase from Mireille: it is what it is…and your open-mind for everything, the funny Spanish words from Anna haha, and of course the beers and tiramisus with Matteo (always willing to this ☺). I would also like to thanks other amazing people I met: **Gernot**, **Julia**, **Wolfgang**, **Francesco**, **Annika**, **Bernhard**, **Johannes**, **Sylvie** and **Philips**. Of course, I cannot forget about my International Spanish-Chilean family in Graz: **Xisca**, **Ale**, **Luis**, **Rosa**, **Ivan**, **Lucia**, **Hugo** and **Maria**. My last internship takes me to Ohio, USA. Despite the fact that it is in the middle of nowhere, I had the opportunity to work there with amazing researchers and better people. Thanks

Kristin for being so welcoming from the beginning, for inviting me to your house in Christmas, and for allowing me to experiment so much at the lab. Thanks to you I discovered that I really want to go deeper in molecular biology. Now, my perfect triangle has been shaped: exercise, metabolism and pregnancy from a molecular perspective. This research lab is composed of so nice and amazing people: **Kelsey** (and her unforgettable "buckeye" dessert), **Shinsuke** (thanks so much for helping me in the lab and for our amazing trips; I really respect you), **Revati** (the human heart of the lab), **Pablito** (the beast), **Lisa** (always so helpful), **Diego** and **Kate**. Also thanks to my amazing Chilean and International friends (**Matias**, **Gloria**, **Pia**, **Alicia**, **Joan**, **Debrus**, **Son**…).

LA FAMILIA QUE SE ELIGE

Sin embargo, el principio de esta aventura se remonta mucho tiempo atrás (al instituto), cuando tuve la suerte de conocer y afortunadamente elegir (sin ser consciente) a la familia que aunque no es de sangre, es como si lo fuera. Nunca se me olvidarán las famosas clases de Física (sin zapatillas después de sudar), Estadística (durante poco tiempo para los universitarios), Inglés (siempre castigados por pertenecer a la última fila), Ciencias de la Tierra (fin del mundo), Proyecto tecnológico (copiando los videos de "como conocí a vuestra madre"), dibujo lineal (todo por la arquitectura), filosofía (Raquel llorando por el 10), lengua (los malditos helados en los exámenes)…y un sinfín de historias que consiguieron unirnos. De ahí que pese a todas nuestras peleas, las famosas intervenciones, linceadas, reconciliaciones, bombas, sigamos a pie del cañón a día de hoy, y podamos contar entre nosotros siempre que haga falta, sea en la cercanía o a la distancia. A todos vosotros, siempre me siento agradecido de teneros en mi vida: **Jose** (filipino-lince, el creador de la AMAZING PORTADA), **Nachis** (mi ovejilla peluda), **Serafín** (mi delfín hasta el fín), **Manuel** (el pescador- my partner in crime), **Elia** (el palomino que se pica), **Cristina** (la hater), **Elena** (la pequeña zamorana), **Aidis** (la wilona), **Maria** (la carchunera), **Juanis** (mansanito, monolo), **Reichel** (record mundial salto de altura), **Romerico** (el trifásico lacost) **Lauris** (perris alfa), **Fabi** (nuestra rubia), **Julio** (el gemelo elegante), **Javi** (el gemelo perdedor), **Jaimito** (la tortuga italiana) **Joaquin** (el loco irlandés), **Carlos f** (el desertor francés), **Carlos g** (el nuevo vigoréxico-up)**, Danisito** (nuestro amor ciclista) y **Mavi** (cinéfila que no tiene ni idea). Vosotros VALÉIS ORO PARA MÍ, y ESTAS PALABRAS NO OS HACEN JUSTICIA. Pero necesitaría

MUCHAS TESIS para hablar de vosotros, y de lo que significáis para mí; *por eso he preferido simplificarlo*. Si me preguntasen de lo que más orgulloso me siento a día de hoy, sin duda, una de ellas sería VOSOTROS, **LOS AMIGOS QUE SE ELIGEN**. Aunque algunas de las personas de las que he hablado anteriormente, también me hubiese gustado meterlas en esta sección, no lo hice para que fuese más fácil ordenarlo todo.

Por supuesto, esta familia motrileña-granadina también está formada de muchas otras personas que no estuvieron con nosotros en el instituto, pero si a lo largo de todo esta etapa: **Barbarica** (no motrileña técnicamente), **Adrii**, **Lusia**-tailandesa, **Luquitas** mouse, **Víctor**-dire, **Emilico**, **Nick**-ruso, **Angelico-**cordobés, **Macarena**, **María**-onubense, **Irene Bloqueo** y algunas otras que se me habrán pasado con las prisas (Sorry ajaja).

Por supuesto no me puedo olvidar de mi **Pablico Raya**, mi niño ciclista, la bestia imparable, el capullo que florece, el pájaro libre, al amigo que se le conoce como el filósofo, para mí has sido uno de los grandes descubrimientos de estos años y vales mucho como persona, en serio. Aunque más chulo que un ocho, tienes un corazón muy grande. Nos queda mucho por delante juntos!! También mi **Niquillo Pico Pala**, ¿quién no ha escuchado esta historia alguna vez?. Todo hijo de Dios debería conocerla jaja. Niquillo que de momentos juntos...Tú fuiste el que me quístate la vergüenza a ligar!!! Y ahora con un churumbel…quién lo diría! Como amigo tuyo, no me pude alegrar más cuando dejaste la investigación porque sé que era lo que te iba a hacer feliz. Eres muy grande, te queremos telita, y en Granada siempre tendrás una casa cuando lo necesites. Y mi **carlanguita**, que es como un hermano para nosotros. Nos veamos más o menos, siempre, siempre, rezando por nuestro bienestar. Este hombre es un puro corazón. Como le digo a mi hermano siempre, que suerte tuvimos de que te cruzases con él durante la carrera. Una bendizione!! jaja A **Sandrufliña**, gracias por enseñarme el norte y abrirme los ojos a un mundo que desconocía. Me enseñaste mucho sobre la vida.

Mi Bernadica…que decir de ti!! Que me tienes contento ¬¬ vaya últimas semanitas de tesis que me has dado jajajja Todavía me acuerdo el día que te conocí y me contaste acerca de la asociación de Nepal…no podía creer que hubiese gente tan buena y especial. Pensaba que esa humanidad se estaba perdiendo. Gracias por lo corto pero intenso que ha sido el conocerte. Por todas las risas, por alegrarnos la cuarentena

y engordarnos, por ser cómo eres! Eres increíble y todavía nos queda mucho por disfrutar juntos.

Y bueno que decir de **Elis** palomino, que es la que siempre se pica conmigo y que las palabras sobran entre nosotros. Hace poco pasé por tu primer piso de plaza de toros donde pasamos los primeros años de la carrera, y me acordé de todo lo que hemos vivido juntos. Todas nuestras peleas, reconciliaciones con pipas, noches de biomecánica, exámenes de habilidades motrices, copeas, todos los países dónde hemos estado juntos…Eres una grandísima, repito grandísima amiga, que siempre cuida y se preocupa de los suyos. Mil gracias por ser como eres y por estar ahí para las buenas y las malas. Siempre podrás contar conmigo en la cercanía o la lejanía para lo que sea. Por muchos viajes y aventuras más!! Por esa compi de vida que me ha acompañado desde el insti (15 años ya…!!!).

También me gustaría hablar de una persona muy especial que he querido dejar para el final, mi fake girlfriend **Blanquita**, que tantas oportunidades de ligar me ha quitado ¬¬. My White snow con la que he vivido muchas cosas, y a la que tanto valoro (L). Durante 2 años, literalmente, he pasado más tiempo con ella que con mis hermanos o familia...me ha llegado a gritar y todo… 2 veces!! Esto es un mérito pues creo que poca gente lo ha conseguido jajaja Todavía me acuerdo el primer dia de carrera, cuando no me respondiste y pensé que borde, y al cabo del tiempo descubrimos que ibas con auriculares…jaja Y fíjate, desde ahí todo lo que hemos vivido juntos: carrera, fiestas, máster, Holanda, Marruecos, farinato, mouse de chocolate, doctorado…Eres una amiga a la que aprecio muchísimo y a la que hay conocer bien para darse cuenta de lo que vale. Poca gente he conocido así!!! Mil gracias por cuidarme y por anteponer nuestra amistad al trabajo y tonterías relacionadas. Cuando me necesites, mejor dicho SIEMPRE que me necesites, PODRÁS CONTAR CONMIGO para lo que haga falta!!!!

Y por supuesto también he dejado para el final a mi **EQUIPO BIGOTUDO**. Todas las personas hasta ahora me han marcado de alguna forma y han dejado huella. Pero el equipo bigotudo (formado por el tucancillo, el muffin y la morsilla) ha tenido un rol muy importante en esta etapa. Ellos han estado en los momentos más difíciles, y sino fuera por ellos no sé cómo podría haber seguido adelante. Me ayudaron a tomar las decisiones correctas, a no dejarme influenciar por nadie, a ser ético a mis principios, a seguir adelante en los malos momentos, a que no hacen falta 10 personas para celebrar un

cumple en el extranjero si estás con esa gente especial (nunca olvidaré nuestros domingos de pizza en Utrecht). Aunque también hemos pasado parte de los mejores momentos juntos (carrera, máster, Holanda, Polonia, País Vasco, Turquía, Rotterdam, Granada, fiestas y mil aventuras que nos quedan juntas). **Marifly** (que orgulloso estoy de este nombre jaja), sabes lo que te valoro, te odio, te adoro…Eres una persona pretty important para mí y eso es indiscutible. Ninguna otra persona me ha ayudado a superar dos rupturas amorosas como tú hiciste en Holanda y antes de Salzburgo. He vivido muchas cosas contigo y espero que esto siga por mucho tiempo!!! Pequeña diablillo eres increíble y vales mucho! Y por supuesto **Francisquillo**, que ahora hablaré de ti.

Si alguien tiene mérito de que a día de hoy este aquí, y sea quién soy como persona, humano, investigador…ESA ES **MI <u>FAMILIA DE SANGRE</u>** Y QUE VOLVERÍA A ELEGIR UNA Y OTRA VEZ. Creo que hoy en día es especialmente duro el hecho de no tener una beca o un contrato fijo, y trabajar tan duro. Pero esto es incluso más duro para nuestros padres, que no entienden porque sus hijos se han metido en una cosa así, no entienden que no hagamos unas oposiciones y malgastemos nuestro tiempo en algo inseguro, no entienden que trabajemos 12 horas al día por una miseria y sacrificando nuestro tiempo libre, vacaciones…no entienden por qué optimizamos el tiempo al máximo y lo valoramos tantísimo… nadie va a entenderlo, no hay una explicación lógica. Pero esto es muy simple papi chulo y mamita…. AMAMOS LO QUE HACEMOS Y AMAMOS LA GENTE CON LO QUE LO HACEMOS! Y sé que por fin lo habéis entendido, y os agradecemos mil que nos hayáis apoyado tantísimo. Sabéis lo duro que es que vuestro padre y madre os digan ¿cómo te podemos ayudar para que vaya más rápido con ese trabajo (que era urgente)? Me decían "No queremos que estés más tiempo con esto"…Me acuerdo que mi madre cocinaba y me ayudaba a ahorrar tiempo con la comida, mientras que mi padre me instalaba y desinstalaba programas continuamente para no perder tiempo. Incluso mi padre me llegó a decir que si me podía ayudar con el Excels…¿Sabéis la cara que se te queda cuando te dicen esto? Todas estas cosas y muchas otras me han ayudado a día de hoy a dejar claras mis prioridades. Jamás tendré palabras para agradeceros todo lo que habéis hecho por mí y los valores que me habéis enseñado: *el ayudar a la gente, respetar a los demás, querer, tener pasión por la vida y por disfrutar, por jugártela en muchos momentos, por pelear las cosas, por enseñarme*

a luchar por lo justo, por no dejar las cosas a medias y no rendirme, pero también por saber qué cosas son las que merecen la pena y cuáles no. A mi **Mamuchi**, gracias por esa sonrisa infinita. Habré estudiado cientos de proteínas, genes, e historias relacionadas, pero jamás entenderé como un abrazo tuyo puede darme esas fuerzas para seguir adelante. Por nuestros cafés mañaneros en la playita, por las compricas, por el interrogatorio a nuestras chicas, por que hagas paella cuando sabes que la odio, por soportar las gilipolleces que decimos y las tonterías que hacemos, por apoyarnos aunque te duela que nos vayamos al extranjero, por querer viajar por nosotros, por presumir de tus hijos con tus amigas, por saber por fin de que va mi tesis e incluso explicármelo, por habernos transmitido y ayudado tantísimo. MIL GRACIAS por todo!!. A mi **PAPI chulo**, que se pone pesadito por las noches cuando estamos viendo las pelis, pero que lo ha dado todo por sus niños. Tú nos has hecho uno de los mejores regalos que nunca nos han hecho: el amor hacia el deporte. Gracias por habernos llevado sin cansancio de un lado a otro (a Almería, Galicia, Cartagena, Cádiz…) y por haber fomentado que hiciésemos todos los deportes desde pequeños. Gracias por ayudarnos tantísimo día tras día, por estar ahí cuando lo necesitamos, por ALEGRARTE MÁS QUE NOSOTROS cuando conseguimos algo, por esas cosquillas que rompen costillas, y por mil cosas más. Como siempre decimos, tenemos LA **SUERTE DE TENER A LOS MEJORES PADRES DEL MUNDO**!!

Por supuesto a mis hermanitos. Empezaré hablando de mi sister favorita, la rebelde de la familia, la que te mete directas como puñales, pero la que tanto queremos y daríamos todo por ella. Si con alguien he compartido mi amor no por viajar, sino por SUMERGIRSE en una cultura, esa es ella, mi hermanita **Irene**. A ella le ha tocado la lotería y la desgracia, dos mellizos que le enseñaban lo que es la vida y la ponían en su lugar (pues es la hermana pequeña y tiene que tenerlo claro jaja), pero que nadie más se meta con ella, or the gemeliers will come (y antes estábamos gordicos como para dar miedo). Mil gracias por ser como eres y por habernos cuidado tantísimo (que me pagaste y todo mi estancia en USA jajja). Somos un desastre y eso es incambiable. Es nuestra forma de ser, pasotas por naturaleza. Pero tú siempre estás ahí para insistir en hacer cosas juntas y vivir aventuras juntos, para que te visitemos a dónde haga falta (Londres, Málaga, Lucena -*nunca estuve jaja*-, USA...), para sacarnos de quicio por supuesto, para traernos

helado y palmera kínder, por volverme loco con la estadística, por tus clases de best English teacher ever, por copiarnos el doctorado jaja…Contigo hemos vivido mil historias desde pequeños, todavía nos queda mucho por vivir. Eres genial y no me puedo imaginar tener una hermana mejor (L).

A mi hermanico **Francisquillo**; la joya de la corona en esta tesis se la lleva este hombre. Las palabras se quedan cortas para agradecerte todo lo que has hecho en esta etapa para que yo esté aquí. Y digo esto no sea porque sea el mejor investigador que conozco (*por su pasión, sus ganas de descubrir, de ayudar a la gente, su amor a la ciencia, su rigurosidad, su forma de pensar...*), sino porque ante todo es humilde y humano. Creo que una cosa de la que nos olvidamos en este mundo es del aspecto emotivo. Esto es un aspecto fundamental en todos los grupos de investigación. Siempre veo como gente de su grupo de investigación, amigos o de nuestro alrededor se apoya en él para todo esto. Él siempre, encantado, se ofrece a ayudar y apoyar a la gente no solamente en el trabajo, sino también en lo personal. Todo esto, junto con lo anterior, es lo que define no sólo a un buen investigador, sino a una PERSONA INCREIBLE. Eres la persona, junto con mi sister, que más admiro en este mundo. Si tuviese que nombrar un gran maestro que me haya enseñado cosas durante esta etapa, ESE ERES TÚ!! Si el mundo fuese justo, tú serías uno de mis directores de tesis. Gracias por ser el pilar para apoyarme cuando las cosas no iban bien, cuando me daban palos, en esos momentos de susceptibilidad, por ponerme y quitarme presión cuando lo necesitaba, y por supuesto por todo lo vivido juntos, que no es poco.
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A todos los que me habéis ayudado a llegar hasta aquí, y estuvisteis o seguís en mi vida, sois increíbles. Sois la mejor parte de esto. En esta tesis, hay infinidad de cosas, experiencias, risas y trabajo que no aparecen físicamente, pero que están reflejadas y me han definido como persona. Gracias a todos por haberme ayudado a descubrir mi pasión por la ciencia y mantener la llama encendida. Gracias por animarme a dar lo mejor día tras día. Gracias simplemente por seguir ahí. Quiero acabar con dos frases que espero siempre me definan como persona-investigador:

"Be less curious about people, and more curious about ideas"

"Lo difícil se hace, lo imposible se intenta"

Pregnancy induces extraordinary metabolic changes in women's physiology to ensure a successful pregnancy. However, in some women predisposed to an unfavourable genotype/lifestyle, these alterations can lead to short and long-term adverse outcomes. Indeed, a dysfunctional metabolic machinery in pregnancy has the potential to negatively affect not only one life, but two (mother and offspring), and possibly next generations.

Physical activity, physical fitness and exercise are promising tools to optimise metabolic control in pregnancy, and thus avoiding potential complications and future diseases. Unfortunately, evidence on this topic is very scarce and elusive, and many questions remain unrevealed.

In the current Doctoral Thesis, we provide a greater and novel insight on the role of physical activity, physical fitness and exercise in immunometabolism, and the underlying mechanisms during pregnancy.

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