Tesis Doctoral Internacional / International Doctoral Thesis

INFLUENCE OF MEDITERRANEAN DIET ADHERENCE AND DIETARY HABITS DURING GESTATION ON SEVERAL MATERNAL AND NEONATAL BIOCHEMICAL, GENETIC AND ANTHROPOMETRIC MARKERS

INFLUENCIA DE LA ADHERENCIA A LA DIETA MEDITERRÁNEA Y LOS HÁBITOS DIETÉTICOS DURANTE EL EMBARAZO SOBRE DIVERSOS MARCADORES BIOQUÍMICOS, GENÉTICOS Y ANTROPOMÉTRICOS DE LA MADRE Y EL NEONATO

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MARTA DE LA FLOR ALEMANY

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Autor: Marta de la Flor Alemany
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A mis padres, Inmaculada y Brígido, y a mi hermana, María, por su comprensión y paciencia, a veces, casi infinita. Espero recuperar el tiempo que he estado ausente.

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1. RESEARCH PROJECTS AND FUNDING

The present International Doctoral Thesis was performed as a result of the following research projects:

1. GESTAFIT Project 1: Effects of supervised aerobic and strength training in overweight and grade I obese pregnant women on maternal and fetal health markers: The GESTAFIT Project. Andalucía Talent Hub Program launched by the Andalusian Knowledge Agency, co-funded by the European Union's Seventh Framework Program, Marie Skłodowska-Curie actions (COFUND – Grant Agreement nº 291780) and the Ministry of Economy, Innovation, Science and Employment of the Junta de Andalucía (156.763 €). 01/03/2015 to 28/02/2017. I.P.: Virginia A. Aparicio García-Molina.

2. GESTAFIT Project 2: Efectos de un programa de ejercicio físico supervisado durante el embarazo sobre la longitud de los telómeros y marcadores de expresión génica relacionados con la adiposidad en la madre y el neonato. Ensayo controlado aleatorizado (PI-0395-2016). Consejería de Salud de la Junta de Andalucía (56.178€). 01/01/2017 al 31/12/2019. I.P.: Virginia A. Aparicio García-Molina.

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ABBREVIATIONS

4. ABBREVIATIONS

ANCOVA, One-way analysis of covariance

BMI, Body mass index

BP, Blood pressure

CD-RISC, Connor-Davidson Resilience Scale

CES-D, Center for Epidemiological Studies Depression Scale

CI, Confidence intervals

DBP, Diastolic blood pressure

EPDS, The Edinburgh Postnatal Depression Scale

FTO, Fat mass and obesity-associated protein

G.W., Gestational week

GESTAFIT, GESTAtion and FITness

GWG, Gestational weight gain

HDL, High-density lipoprotein

HOMA-IR, Homeostatic model assessment-insulin resistance

HRQoL, Health-related quality of life

IL, Interleukin 6

LDL, Low-density lipoprotein

MD, Mediterranean diet

MDScale, Mediterranean Diet Scale

MDS-P, Mediterranean Diet scale for pregnant women

MedDietScore, Mediterranean Diet Score

MFP, Mediterranean Food Pattern

OR, Odds ratio

PANAS-S, State Positive Affect Schedule

PPWR, Postpartum weight retention

PSQI, Pittsburgh Sleep Quality Index

SBP, Systolic blood pressure

SF-36, Short-Form Health Survey 36

SMDQ, Short Mediterranean Diet questionnaire

SNPs, Single-nucleotide polymorphisms

STAI-S, State Trait Anxiety Index

TMMS, The Trait Meta-Mood Scale

TNF-*a*, Tumour necrosis factor-*a*

VLDL, Very-low-density lipoprotein

ABSTRACT/RESUMEN

5. ABSTRACT/RESUMEN

Pregnancy is a physiological stage in which fetal development takes place. The maternal nutritional status during pregnancy will affect health outcomes in the mother and the fetus-newborn. Therefore, the study of the effect of the mother's lifestyle, including exercise and diet, on certain maternal and fetal health markers becomes essential. It seems that a high Mediterranean diet (MD) adherence and exercise during pregnancy can confer a protective effect against different diseases that may affect the mother and the newborn. Unfortunately, evidence is very scarce and many questions remain still unrevealed. In this present International Doctoral Thesis, we address knowledge gaps and provide a novel insight on: I) the assessment of the MD adherence during pregnancy and its relationship with sociodemographic, lifestyle and pregnancy-related determinants; II) the role of MD adherence during pregnancy on materno-fetal cardiometabolic health; III) the role of MD adherence during pregnancy on maternal sleep quality and mental health and; IV) the influence of MD during pregnancy on materno-fetal genetics.

Our results suggest that two out of five MD indices that were investigated (i.e., the Mediterranean Diet Score and the Mediterranean Food Pattern) could be recommended to assess the diet's positive or negative cardiometabolic impact during pregnancy **(Study I)**. Moreover, older age, lower body mass index, greater overall physical fitness, greater cardiorespiratory fitness, muscle strength, and elements of a healthy lifestyle such as avoiding tobacco and meeting physical activity recommendations were associated with higher adherence to the MD during gestation **(Study II)**. A greater MD adherence during pregnancy was associated with better values of maternal lipid and inflammatory markers **(Study III)** and a cardioprotective effect during gestation **(Study IV)**. However, we could not find an association between maternal MD adherence and neonatal lipid, glycemic and inflammatory markers. Furthermore, we observed that the effects of an exercise intervention during pregnancy on postpartum body composition might be enhanced by following a MD during pregnancy **(Study V)**. Regarding maternal mental health, a greater MD adherence was associated with better sleep quality, lower negative affect, depressive symptoms, and anxiety, and with higher emotional regulation, resilience, positive affect, and health-related quality of life along the pregnancy course **(Studies VI, VII and VIII)**. A greater MD adherence was also associated with lower depressive symptoms and postpartum depression risk **(Study IX)**. Moreover, a concurrent exercise program during pregnancy appeared to be effective to reduce gestational weight gain. Interestingly, the exercise program also proved an effect, albeit lower, on gestational weight gain in those women with a genetic predisposition to obesity **(Study X)**.

Additionally, we found that placental telomere length might be positively influenced by lifestyle factors such as MD adherence combined with exercise during gestation **(Study XI)**. Overall, the findings from the present International Doctoral Thesis highlight the positive role of lifestyle factors, such as MD adherence and exercise during pregnancy, on several materno-fetal health outcomes such as cardiometabolic health, postpartum body composition, sleep quality, mental health, and placental telomere length.

RESUMEN

El embarazo es una etapa fisiológica en la que se produce el desarrollo del feto. El estado nutricional de la madre durante el embarazo afectará a los resultados de salud de la madre y del feto-recién nacido. Por lo tanto, el estudio del efecto del estilo de vida de la madre, incluyendo el ejercicio y la dieta, sobre ciertos marcadores de salud materna y fetal se vuelve esencial. Parece que una alta adherencia a la dieta mediterránea (DM) y el ejercicio durante el embarazo pueden conferir un efecto protector contra diferentes enfermedades que pueden afectar tanto a la madre como al recién nacido. Desafortunadamente, la evidencia científica al respecto es escasa y muchas preguntas permanecen sin respuesta. En la presente Tesis Doctoral Internacional, abordamos las lagunas de conocimiento, y proporcionamos una mayor visión sobre I) la evaluación de la adherencia a la DM durante el embarazo y su relación con determinantes sociodemográficos, de estilo de vida y relacionados con el embarazo; II) el papel de la adherencia a la DM durante el embarazo sobre la salud cardiometabólica materno-fetal; III) el papel de la adherencia a la DM durante el embarazo sobre la calidad del sueño materno y la salud mental y IV) la influencia de la DM durante el embarazo sobre marcadores genéticos materno-fetales.

Nuestros resultados sugieren que dos de los cinco índices de DM estudiados (i.e., the Mediterranean Diet Score y the Mediterranean Food Pattern) podrían recomendarse para evaluar el impacto cardiometabólico positivo o negativo de la dieta durante el embarazo **(Estudio I)**. Además, mayor edad, menor índice de masa corporal, mayor condición física general, mayor capacidad cardiorrespiratoria, fuerza muscular y elementos de un estilo de vida saludable, como evitar el tabaco y cumplir las recomendaciones de actividad física, se asociaron con una mayor adherencia a la DM durante la gestación **(Estudio II)**. Una mayor adherencia a la DM durante el embarazo se asoció con mejores valores de marcadores lipídicos e inflamatorios maternos **(Estudio III)** y con un efecto cardioprotector durante la gestación **(Estudio IV)**. Sin embargo, no pudimos encontrar una asociación entre la adherencia materna a la DM y los marcadores lipídicos, glucémicos e inflamatorios neonatales. Además, observamos que los efectos de una intervención de ejercicio durante el embarazo sobre la composición corporal posparto podrían verse potenciados por el seguimiento de una DM durante el embarazo **(Estudio V)**. En cuanto a la salud mental materna, una mayor adherencia a la DM se asoció con una mejor calidad del sueño, un menor afecto negativo, síntomas depresivos y ansiedad, y con una mayor regulación emocional, resiliencia, afecto positivo y calidad de vida relacionada con la salud a lo largo del embarazo **(Estudios VI, VII y VIII)**. Una mayor adherencia a la DM se asoció con menores síntomas depresivos y riesgo de

depresión postparto **(Estudio IX)**. Además, un programa de ejercicio concurrente durante el embarazo pareció ser eficaz para reducir el aumento de peso gestacional. El programa de ejercicio también demostró un efecto, aunque menor, sobre el aumento de peso gestacional en aquellas mujeres con predisposición genética a la obesidad **(Estudio X)**. Adicionalmente, descubrimos que la longitud de los telómeros de la placenta podría estar positivamente influenciada por factores de estilo de vida como la adherencia a la DM combinada con el ejercicio durante la gestación **(Estudio XI)**.

En conjunto, los hallazgos de la presente Tesis Doctoral Internacional ponen de manifiesto el papel positivo del estilo de vida, incluyendo una alta adherencia a la DM y el ejercicio durante el embarazo sobre varios resultados de salud materno-fetal, como la salud cardiometabólica, la composición corporal postparto, la calidad del sueño, la salud mental y la longitud de los telómeros en placenta.

GENERAL INTRODUCTION

6. GENERAL INTRODUCTION

6.1. The impact of diet during pregnancy

Significant changes in dietary habits and physical activity levels have occurred worldwide as a result of industrialization, urbanization, economic development and food market globalization (1–3). There has been an increase in the intake of trans and saturated fats whereas the intake of fiber, complex carbohydrates, fruits and vegetables (main sources of vitamins and antioxidants) has decreased (2,4). This could promote nutritional imbalances and, consequently, increase the risk of several diseases (5). These nutritional behaviour changes are even more dangerous during pregnancy, a very complex physiological period where fetal development takes place (6,7).

6.1.1. General nutrition remarks during pregnancy

Pregnancy is a time of rapid and profound physiological changes from the time of conception until birth (8). Nutritional requirements increase during pregnancy to maintain maternal metabolism and tissue accretion while supporting fetal growth and development (9). Nutrition during pregnancy should be varied, adequate and balanced (7,10). Each meal must contain dishes supporting carbohydrates, proteins, lipids, micronutrients and bioactive compounds, assuring complementation and synergies of nutrients to meet the increased needs of the mother and the fetus (7,10).

6.1.2. Energy expenditure during pregnancy

Although during pregnancy, maternal energy requirements are substantially increased (11), an excessive macronutrients intake (fat, carbohydrates or protein) may also alter the homeostatic mechanisms in the fetus, predisposing the offspring to serious chronic diseases, including cardiovascular disease, diabetes mellitus and cancer later in life (11–13).

Caloric intake should increase by approximately 300 kcal/day during pregnancy (14). This value is derived from an estimate of 80,000 kcal needed to support a full-term pregnancy and accounts not only for increased maternal and fetal metabolism but for fetal and placental growth. Dividing the gross energy cost by the mean pregnancy duration (250 days after the first month) yields the 300 kcal/day estimate for the entire pregnancy (14). However, energy requirements are generally the same as non-pregnant women in the first trimester and then increase in the second trimester, estimated at 340 kcal and 452 kcal per day in the second and third trimesters, respectively (14). Furthermore, energy requirements vary significantly depending on a woman's age, body mass index (BMI), and physical activity levels. Caloric

intake should therefore be individualized based on these factors (15,16). Of note, the requirement for some nutrients increases in greater proportion even from the first trimester, which creates the need to improve the quality of the diet to ensure that the small extra energy intake covers the increased nutrient requirement. This extra energy intake should be at the expense of nutrient-rich foods such as wholegrain or fortified cereals, legumes, fruits, vegetables, dairy products and lean meats (15).

6.1.3. Macronutrients

Carbohydrates

Carbohydrates are essential nutrients, providing energy to both the mother and the growing fetus (17). The recommended dietary intake of carbohydrates to meet energy, fiber, and micronutrient needs ranges from 45 to 65% of daily energy intake (15). Since carbohydrates constitute the main source of energy for the fetus, it is advisable to ensure an intake of at least 175 g/day to prevent ketosis, meet fetal glucose requirements and maintain adequate glycemia during pregnancy (18). In order to ingest adequate amounts of carbohydrates, a correct choice of carbohydrate-containing foods should be made, opting for complex carbohydrates such as cereals and legumes. Priority should be given to choosing whole-grain products to help cover, together with fruit and vegetable intake, the daily dietary fiber recommendations of 28 g/day during pregnancy in order to avoid constipation, a common pathology in this physiological situation (18–20).

Protein

Protein is an essential component of a healthy diet to ensure growth and maintenance. During pregnancy, a stage of life defined by rapid growth and development, adequate protein intake is crucial to ensure a healthy pregnancy (17). Protein deposition in maternal and fetal tissues increases throughout pregnancy occurring mostly during the third trimester. The recommended protein intake during pregnancy is 60g/day which represents an increase from 46g/day in non-pregnant women. In other words, this increase reflects a shift from 0.8g/kg body weight/day in non-pregnant women to a recommendation of 1.1 g/kg body weight/day in pregnant women (17,20–22).

Lipids

Dietary fat is an important source of energy (17). Total fat intake should comprise between 20% to 35% of daily energy intake, similar to non-pregnant women (15). The amount of fat in the diet depends on energy needs for adequate weight gain. It is important to include fatty acids of the "omega-6" and "omega-3" family as they are essential for the proper functioning of the utero-placental system, the development of the nervous system and the retina of the fetus during pregnancy and of the child during lactation (10,19,23). Essential fatty acid requirements are usually met with one or two servings of fish per week (19,23). Moreover, the consumption of saturated fatty acids during pregnancy should be limited, as excessive intake has been associated with adverse events, both for the pregnant population and their offspring (affecting neurobehavioral development of the infants) (24).

Table 1. Energy and macronutrient recommended intake during pregnancy

Nutrient/energy	Recommendation
Energy	1st Trimester +0 kcal/day 2nd Trimester +340 kcal/day 3rd Trimester +452 kcal/day
Protein	60 g/day (1.1g/kg body weight/day)
Carbohydrates	45-64% of daily energy intake (>175g/day)
Fat	20-35% of daily energy intake (similar to non-pregnant adult women)

Adapted from (10,15,18).

6.1.4. Micronutrients

The recommendations for daily micronutrient intake for adult woman are determined by the "Recommended Dietary Allowances." In general, these Recommended Dietary Allowances refer to the levels of intake of essential nutrients that are judged by the Food and Nutrition Board of the Institute of Medicine to be adequate to meet the known nutrient needs of practically all healthy persons (25). Recommended Dietary Allowances of energy and nutrients and/or adequate intakes during gestation are available, although there is not consensus among experts (20,25,26). **Table 2** shows the dietary allowances for most vitamins and minerals during pregnancy.

Table 2. Recommendations of energy and nutrient intakes for healthy women aged 20-49 years and the increments proposed in pregnancy

Micronutrient	SENC		IOM	
	Adult women	Pregnant women	Adult women	Pregnant women
Vitamin C (mg)	60	80	75	85
Thiamine (mg)	0.9	1.0	1.1	1.4
Riboflavin (mg)	20-39 years: 1.4 40-49 years: 1.3	20-39 years: 1.6 40-49 years: 1.5	1.1	1.4
Niacin equivalents (mg)	20-39 years: 15 40-49 years: 14	20-39 years: 17 40-49 years: 16	14	18
Vitamin B_6 (mg)	1.6	1.9	1.3	1.9
Folate (µg)	400	600	400	600
Vitamin B_{12} (µg)	2	2.2	2.4	2.6
Vitamin A (µg)	800	800	700	770
Vitamin D (µg)	15	15	5	5
Vitamin E (mg)	12	15	15	15
Vitamin K (µg)	90	90	90	90
Calcium (mg)	1000	1300	1000	1000
Phosphorus (mg)	700	700	700	700
Magnesium (mg)	330	450	310	350
Iron (mg)	18	18	18	27
Zinc (mg)	15	20	8	11
Iodine (µg)	110	135	150	220
Selenium (µg)	55	65	55	60

IOM, Institute of Medicine; SENC, Spanish Society for Community Nutrition.

Adapted from (20,25,26).

6.2. Mediterranean dietary pattern

As a modifiable factor, diet is a key area for intervention in pregnant women (13). Therefore, identifying what foods, in what quantities, and even how often these foods should be eaten, remain important questions (27). Nutrition status during pregnancy plays a decisive role in the well-being of the mother and the fetus (17,28). Many analyses in this field are based on a single or a few food items or nutrients (29). However, foods are not consumed in isolation and people eat foods containing a mix of nutrients and non-nutrients (30). Dietary patterns study the whole diet and provide a simple but helpful, comprehensive and complementary approach way to deliver meaningful results to the respective population (13). As a result, dietary patterns have the potential to be used as a valid tool in assessing the relationship between diet and pregnancy outcomes.

For European countries, the MD is known to be one of the healthiest dietary patterns (31). The traditional MD is characterized by a high intake of vegetables, legumes, fruits, nuts, cereals and olive oil, a low intake of saturated lipids, a moderately high intake of fish, a low-to-moderate intake of dairy products, a low intake of meat and poultry, and a regular but moderate intake of wine and generally during meals (31). Apart from the characteristic of foods intake and specific culinary forms, this dietary pattern also includes a psychosocial aspect, giving vital importance to the context in which each meal is taken, usually accompanied by other people (32).

6.2.1. Mediterranean diet and health

Diseases and conditions linked to an unhealthy diet include cardiovascular disease (29.2% of global deaths), diabetes (171 million people worldwide) and cancer (12.5% of global deaths) (2). Obesity has reached alarming proportions, and it is estimated that at least 300 million adults are clinically obese (33–35). Consequently, the growing impact of chronic degenerative pathologies (such as cardiovascular and respiratory disease, type 2 diabetes and Alzheimer's disease, hypertension, dyslipidaemia and cancers) in high income countries requires and pushes towards the development of new preventive strategies to reduce the incidence and prevalence of these diseases (33). In this context, the adoption of healthy eating patterns has been at the center of interest in many studies (36,37). Among these dietary patterns, a greater MD adherence is associated with a significant improvement in health status, as seen by a significant reduction in overall mortality (9%), mortality from cardiovascular disease (9%), incidence of or mortality from cancer (6%), and incidence of Parkinson's disease and Alzheimer's disease (13%) (33,36,38–41). These results seem to be clinically relevant for public

health, in particular for encouraging a Mediterranean-like dietary pattern in terms of both primary and secondary prevention of chronic diseases (33,36,38–41).

6.3. Mediterranean diet recommendations during pregnancy

Mediterranean-style diets have been associated with lower gestational weight gain (GWG) and lower risk of gestational diabetes (42), preterm delivery (43), lower blood pressure (BP) (44), and lower cardiometabolic risk in the adult population (45). Although recent evidence suggests that the MD is an optimal diet to consume during pregnancy (46), few studies have examined the potential benefits of the MD adherence on maternal and fetal outcomes (considering the MD as a whole rather than focusing on the effect of its components) (47). Regarding fetal outcomes, recent studies have shown the protective role of MD during pregnancy against excessive or insufficient fetal growth (48), preterm birth (49), neural tube defects (50), asthma and allergy (51), excessive adiposity and other adverse metabolic markers in the offspring (52).

The Mediterranean dietary pattern has become customary to be represented in the form of a pyramid for general population (30). The base of which refers to foods which are suggested to be consumed most frequently and the top of the pyramid to those foods that should be consumed rarely. The remaining food occupy intermediate positions**.** In particular, this dietary pattern consists of (6,30):

- Daily consumption of whole cereals such as bread, rice, pasta, or others (8 servings/day), vegetables (2–3 servings/day), fresh fruits (4–6 servings/day), olive oil (culinary fat for addressing and cooking) and non-fat or low-fat dairy products mainly as cheese and yogurt (1–2 servings/day).
- Weekly consumption of potatoes (4–5 servings/week), fish (4–5 servings/week), olives, beans, pulses, and nuts (>4 servings/week) and more rare poultry (1–3 servings/week), eggs and sweets (1–3 servings/week).
- Monthly consumption of red meat and meat products mainly cured luncheon meat (4–5 servings/month).
- High consumption of garlic, parsley, onion, basil, oregano, curcuma, anis, sesame, cinnamon, and other species.

This pattern is also characterized by moderate consumption of wine (1–2 wineglasses/day) which is not recommended during pregnancy.

Although a weekly consumption of fish is included in the MD studies, the risks and benefits of fish during pregnancy can often seem contradictory (53). This is in part due to that most fish

contain competing benefits and risks in the forms of omega-3 fatty acids and mercury (54,55). Omega-3 fatty acids are critical for fetal brain development and have been associated with improved vision in preterm infants, as well as better cardiovascular health later in life (56). Ideally, pregnant women would consume those fish that are low in mercury and high in omega-3 fatty acids such as salmon, sardines, and anchovies (15). High mercury fish such as shark, swordfish, tilefish, and king mackerel should be avoided.

The Spanish Society of Community Nutrition has modified the pyramid adapting it to a healthy nutrition considering the nutritional requirements and needs for the mother and the fetus during pregnancy **(Figure 1)** (57–59).

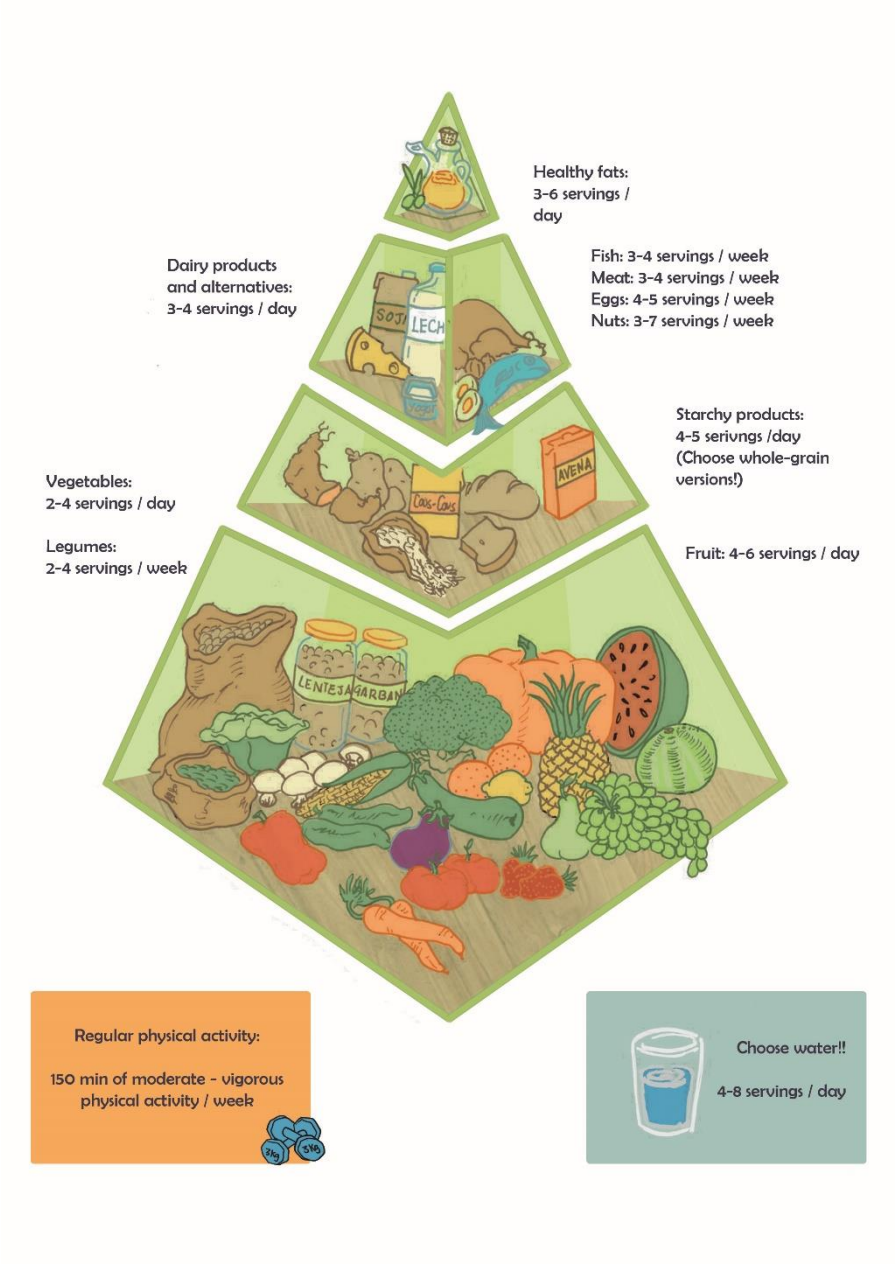

Figure 1. The healthy eating pyramid for pregnant women. Adapted from (57,58). (Source: Own elaboration).

As the Spanish Society of Community Nutrition describes (57–59), pregnant women are encouraged to consume a larger number of servings per day in each food group in comparison with non-pregnant women. It is recommended to consume 4–5 starchy products per day, 4–6 fruit pieces plus vegetables servings, 3–4 milk glasses or dairy products, and 2 protein food servings daily (30,57–61) **(Table 3)**. Food distribution should be made according to the number of meals. An example of distribution for a total of 6 meals regarding to energy contribution could be: breakfast, 20%, midday snack, 10%, lunch, 30–35%, afternoon snack, 10%, dinner, 20–25%, post-dinner, 5% (6). Last meal or "post-dinner" snack would contribute to the avoidance of possible hypoglycemia induced between dinner and breakfast, assuring adequate fetal glucose use and maternal stores of proteins and fats (6,62).

Table 3. Recommended servings per day for pregnant and non-pregnant women

Food groups	Recommended servings		Source of food and portion size
	Adult women	Pregnant women	
Whole-grain cereals and potatoes	4-5/day	4-5/day	Bread 40-60 g Rice/pasta 60-80 g Potatoes 150-200 g
Vegetables	2-3/day	2-4/day	150-200 g
Fruits	3-4/day	4-6/day	120-200 g
Dairy products	2-3/day	3-4/day	200-250 ml milk 200-250 g yogurt 40-60 g hard cheese 80-125 g fresh cheese
Legumes	2-4/week	2-4/week	60-80g
Poultry	3/week	3-4/week	100-125g
Fish	3-4/week	3-4/week	125-150g
Eggs	3-5/week	4-5/week	53-63 g
Nuts	3-4/week	3-7/week	20-30g
Olive oil	3-6/day	3-6/day	10 ml

Adapted from (30,58–61).

6.4. Mediterranean diet adherence assessment during pregnancy

There is a lack of uniformity in the tools employed to assess MD adherence during pregnancy (29,63–65) such as the number of components included, classification categories for each item, measurement scales, statistical parameters (mean, median, or quintiles of daily intake), and the contribution of each component (positive or negative) to the total score (66,67). Usually, the indices used in pregnancy to assess MD adherence are not specifically validated in pregnant women but in the general population (68). The dietary habits are affected during pregnancy (e.g., pregnant women must not drink alcohol) and require specific assessment tools. Only one adapted index for pregnant women has been previously proposed (69), yet the interpretation of its score is not adapted from the cut-off points used in the general population (which includes different items). As a result, it remains unknown which dietary index might be more appropriate to assess the MD adherence during pregnancy.

6.5. Mediterranean diet during pregnancy, gestational weight gain and maternal body composition

Pregnancy is a biological and natural process in women's life which can trigger physiological changes in body composition and cause significant weight gain in childbearing women (70). Gestation has traditionally been considered a time for weight gain, not weight loss (15). The "mandatory" weight gain during pregnancy is approximately 8 kg which accounts for the fetus, the placenta, amniotic fluid volume, and adaptations to maternal tissues (e.g., uterus, breast, blood volume) (15). A lower weight gain less than this amount implies that existing maternal adipose and protein stores would be mobilized in order to support the pregnancy (15).

In reality, In reality, the GWG is usually higher than this mandatory weight gain, reaching 10 kg on average (71). In Europe, excessive GWG is frequently observed with a prevalence of 51%(72), which often is long-term retained after birth (73–75), contributing to maternal obesity (74,76).

Obesity is a high-risk factor for several pregnancy and delivery complications such as gestational diabetes, gestational hypertension, and preeclampsia (28,77,78). The risk of giving birth to a macrosomic neonate increases proportionally with increasing BMI (77). The risk of macrosomia as well as the risk of pregnancy complications are related to GWG and high GWG is a predictor of obesity in infancy and adulthood (71).

Recommendations for GWG should be individualized according to pre-pregnancy BMI (calculated as kg/m^2) to improve pregnancy outcomes, avoid excessive maternal postpartum

weight retention (PPWR), and reduce the risk of later chronic disease for the child (79). The Institute of Medicine developed guidelines for adequate GWG dependent on maternal pre-pregnancy BMI **(Table 4)**. There is evidence suggesting that weight gains within Institute of Medicine recommendations are potentially associated with healthy fetal and maternal outcomes (80). Both low and high GWG have been associated with adverse maternal and infant outcomes (81). According to recent evidence, the absolute risk for any adverse outcome increased across the full range of maternal pre-pregnancy BMI and was largely independent of GWG (81). The lowest absolute risks were observed among women with low to normal pre-pregnancy BMI and a moderate to high total GWG. The highest risk was for women with a BMI of 40.0 kg/m^2 or greater and GWG of 20.0 kg to 21.9 kg.

Table 4. Appropriate weight gains during pregnancy

Pre-pregnancy BMI (kg/m^2)	**Total weight gain (kg)**	**Weekly weight gain range in the second and third trimester (kg)**
Underweight (<18.49 kg/m2)	12.5 - 18	0.51 (0.44-0.58)
Normal weight (18.5-24.99 kg/m^2)	11.5 – 15.9	0.42 (0.35-0.50)
Overweight (25.0-29.99 kg/m2)	6.8 - 11.4	0.28 (0.23-0.33)
Obese (>30.0 or more kg/m2)	5.0 – 9.0	0.22 (0.17-0.27)

BMI, Body mass index; GWG, Gestational weight gain. Adapted from (80,82).

Compared with weight gain during other periods of life, excessive weight gain retained after childbirth, otherwise known as PPWR, seems to be more harmful as a result of its accumulation in central rather than in peripheral deposits (70,83,84). Altogether, overweight and a less favourable distribution of body fat (i.e., increases in visceral and abdominal fat and the ratio of abdominal to gynecoid fat mass) are well-known risk factors for greater cardiometabolic risk, hypertension, impaired glucose tolerance and elevated triglycerides (85,86). Recent evidence (87) suggests that maternal body composition might predict perinatal outcomes more accurately than maternal weight. Hence, it is imperative to find potential strategies to improve maternal body composition in order to avoid future materno-fetal complications.

In this context, the MD has been related to normal-weight status and protection against the risk of overweight, obesity and metabolic syndrome (88). Moreover, a high MD adherence has been associated with greater fat-free mass and the prevention of muscle loss (89), lower BMI and weight gains (90,91) in non-pregnant adult population. During gestation, the adherence

to the MD could prevent excessive GWG, modulating fat mass increase (92). However, to the best of our knowledge, no evidence has explored the relationship between MD adherence during pregnancy and postpartum body composition. Therefore, further studies are needed to confirm these results and to promote educational lifestyle interventions aimed to indicate MD as a healthy dietary pattern to prevent excessive GWG and adverse pregnancy outcomes. Genetics plays an important role in the development of obesity (93). Several single-nucleotide polymorphisms (SNPs) of obesogenic genes have been related to an increased risk of overweight or obesity (94). Specifically, the risk allele ("A") of the fat mass and obesity-associated protein (*FTO*) rs9939609 polymorphism has been well-documented as a major contributor to childhood and adult obesity (95,96). Studies on pregnant women suggest a positive association of the presence of rs9939609 A variants with high pre-pregnancy weight (93), pre-pregnancy BMI (93), and excessive GWG (97). In particular, women carrying the obesity risk allele (TA + AA) were up to 3 kg heavier, and had a 1 kg/m^2 higher pre-pregnancy BMI compared to women carrying the non-risk genotype (TT) (98). Physical activity and exercise attenuate the harmful effect of the *FTO* rs9939609 adverse genotype on weight status and BMI in the non-pregnant adult population (99–101). However, there is no evidence of genotype–exercise interactions on GWG.

6.6. Mediterranean diet during pregnancy, biochemical markers and cardiometabolic risk

6.6.1. Mediterranean diet during pregnancy and glycemic profile

Two clear periods can be distinguished during pregnancy (102). The first one lasts two thirds of the whole pregnancy. It is an anabolic period which consists of formation of energy deposits (102). It is followed by a second period where maternal stores are mobilized, and the fetus exponentially grows, preparing for delivery (102).

During the first period a marked increase of insulin level and sensitivity occurs in the mother, with parallel increases of placenta size, amniotic volume and fat stores (102). However, fetal weight gain is small in comparison to that of the mother. During the store distribution period, a physiological increase of insulin resistance and insulin degradation takes place in the mother, in parallel to the exponential fetal growth that partially or totally blocks the gain rhythm of maternal stores (102,103). This metabolic situation assures the availability of glucose for the maternal and fetal brains and addresses glucose to the mammary gland, reducing the uptake of glucose by other maternal tissues (102,103).

When glucose homeostasis is not physiologically balanced, changes and adaptation take place during pregnancy, predisposing the individual to degenerative diseases later in life (6,104). In some non-diabetic women, an alteration of carbohydrate metabolism occurs during pregnancy; thus, although fasting glycemia is normal, after a carbohydrate load, the glycemia increases over normal values (6). This situation is rather more frequent at the end of pregnancy and is known as gestational diabetes (104). Gestational diabetes is frequently diagnosed in the second trimester of gestation, when increased levels of insulin-antagonic hormones (e.g., chorionic somatotropin) are present (103). These hormones modify the sensitivity of peripheral receptors of insulin, addressing glucose to the fetus and increasing mobility of maternal adipose tissue lipids (6).

The prevalence of gestational diabetes is increasing in parallel with higher rates of obesity and older age at pregnancy becoming a major public health problem (105). The MD, strengthened by the use of extra virgin olive oil and nuts, has been beneficial in preventing gestational diabetes, type 2 diabetes and cardiovascular disease (105–107).

6.6.2. Mediterranean diet during pregnancy and lipid profile

Metabolic adaptations of the mother are essential to ensure appropriate growth and development of the fetus. Consequently, higher concentrations of circulating lipids are a normal physiological adaptive response throughout gestation (108,109).

Maternal fat accumulation takes place during the first half of pregnancy, mainly due to maternal hyperinsulinemia and enhanced adipose tissue insulin sensitivity (10,103). During late pregnancy, lipoprotein lipase activity is decreased (102). This metabolic situation gives rise to lower fat uptake by maternal adipose tissue, that together with the increases in lipolytic activity of adipose tissue (102), results in an increased breakdown of the fat depot originated during the first term of pregnancy. Free fatty acids and glycerol are respectively converted in the liver into acyl-CoA in glycerol-3-phosphate originating triglycerides which are packed as very-low-density lipoprotein (VLDL) cholesterol (102). The insulin-resistance in late gestation contributes to both increased fat lipolysis and VLDL production and secretion (102).

These metabolic fates occurring at late pregnancy condition the increase of VLDL-cholesterol levels and, thus, of VLDL-triglycerides and triglycerides (102). In some pregnant women the serum cholesterol is also increased at the end of gestation, mainly due to cholesterol is also being carried by VLDL-cholesterol. Thus, the increased number of VLDL particles during late gestation explains the raise in cholesterol although other metabolic modifications would be at work (102).

It can also be suggested that during gestation dietary mechanisms to control low density lipoproteins (LDL) must have less significance than at the pre-gestational stage; thus, pregnancy cholesterolemia should have poor diagnostic value, as increased serum cholesterol seems to be consequence of the transitory metabolic situation generated during gestation (6). In fact, it has been observed that the cholesterolemia of pregnant women consuming or not a MD did not significantly differ (6).

6.6.3. Mediterranean diet during pregnancy and vascular function

Important maternal cardiovascular changes occur during normal pregnancy including an increase in maternal blood volume that is preceded by vasodilatation, which results in a drop in BP during the first half of gestation, before returning to pre-pregnancy values toward term (44). In mothers who develop an elevated BP or preeclampsia, abnormal cardiovascular adaptation occurs reflected by a different pattern of BP change (44).

At the earliest stage, vascular inflammation is activated by proinflammatory stimuli such as saturated fat intake, hypercholesterolemia, obesity, hyperglycemia, and hypertension, stimulating the secretion of inflammatory cytokines that promote the formation of endothelial adhesion molecules. These molecules are subsequently released into the circulation, where they mediate the adhesion of circulating monocytes and lymphocytes to the vascular endothelium (110).

Various dietary components, such as fatty acids, arginine, vitamins C and E, and folate, have been hypothesized to influence cardiovascular adaptation to pregnancy partly due to their potential effects on endothelial function (111), possibly interfering with normal vascular adjustments (vasodilatation) to pregnancy (112). However, the single nutrient approach does not take biological complexity resulting from interactions between nutrients into account. For this reason, recently a shift toward dietary pattern analysis has emerged as a constructive method to explore the relation between diet and disease. In this respect, dietary patterns have been demonstrated to reduce markers of inflammation and endothelial dysfunction (113). This may imply that the first trimester is a critical period for cardiovascular adaptations related to maternal nutrition and subsequent BP development (44).

The first step in the management of hypertension and other cardiovascular risk factors is to follow a healthy diet, such as the traditional MD (114) and to improve additional lifestyle measures, such as reducing body weight and increasing physical activity. The MD is rich in fruit and vegetables, which have been considered to be rich sources of phytochemicals, and are inversely associated with a high BP and incidence of hypercholesterolemia, among other

effects (115). It has been demonstrated, that systolic blood pressure (SBP) and diastolic blood pressure (DBP) were higher among women with low adherence to the MD compared with women with high MD adherence (44).

6.6.4. Mediterranean diet during pregnancy and inflammatory profile

The idea that pregnancy is associated with immune suppression has created a myth of pregnancy as a state of immunological weakness and, therefore, of increased susceptibility to infectious diseases. However, it is a time of life where there is a modulation of the immune system that does not always implies an inmunological hypofunctioning. It has three distinct immunological phases that are characterized by distinct biological processes and can be symbolized by how the pregnant woman feels (116–118).

Implantation, placentation, and the first and early second trimester of pregnancy resemble "an open wound" that requires a strong inflammatory response (119). Meanwhile, the mother's well-being is clinically affected: she feels sick because her whole body is struggling to adapt to the presence of the fetus (in addition to hormone changes and other factors, this inflammatory response is responsible for "morning sickness"). Thus, the first trimester of pregnancy could be defined as a proinflammatory phase (116).

The second immunological phase of pregnancy is the optimal time for the mother well-being. This is a period of rapid fetal growth and development. The woman no longer suffers from nausea and fever as she did in the first stage, in part, because the immune response is no longer the predominant endocrine feature (118).

Finally, during the last immunological phase of pregnancy, the fetus has completed its development, all the organs are functional and ready to deal with the external world. Now the mother needs to deliver the baby, and this can only be achieved through renewed inflammation (120). Proinflammatory environment promotes the contraction of the uterus, expulsion of the baby and rejection of the placenta. In conclusion, pregnancy is a proinflammatory and anti-inflammatory condition, depending upon the stage of gestation (118). The source of inflammation is uncertain; various inflammatory factors have been proposed as stimulators of this inflammatory process, such as infectious agents and any factor leading to endothelial injury. It has been suggested that higher concentrations of serum interleukin 6 (IL-6) and tumour necrosis factor-α (TNF-α) could cause low-grade systemic inflammation, which might increase coronary risk (11). In addition, chemokines such as fractalkine, which is expressed on various proinflammatory cells like monocytes, T-cells, and natural killer cells has a dual function as it exists both as a transmembrane protein implicated

in cell adhesion and migration and in a cleaved form acting as a chemoattractant, both contributing to vascular inflammation and injury (121).

The main nutrients of the MD are fiber, monounsaturated fatty acids, n-3 polyunsaturated fatty acids, vitamin C, vitamin E, and carotenoids, and all of which are associated with lower inflammation. Saturated fatty acids or trans fatty acids and high glucose and high-fat meals may induce postprandial inflammation and, hence, are considered as proinflammatory factors (122). It has been concluded that the MD exerts its anti-inflammatory and immunomodulating effect through downregulation of the expression of leukocyte adhesion molecules, decreasing proinflammatory IL (IL-1, IL-6, IL-7, IL-8, and IL-18), TNF-*a* and its receptors (122,123).

It has been shown that subjects with high MD adherence had greater serum antioxidant capacity than those with low MD adherence indicating that the quality of the diet could have a different effect using a single food matrix (124). Even if pigments and other phytochemicals contribute to total antioxidant activity, after consuming a high-quality diet, subjects exhibited an increase in antioxidant capacity and a decrease in TNF-α and an increase in IL-10 (125). The increased circulating levels of endogenous and exogenous antioxidants and the synergistic effects of bioactive food constituents, improving the immune system, seem to explain the overall quality of the MD, as well as the increased consumption of fruits and vegetables as a focal point (124,125).

6.6.5. Mediterranean diet during pregnancy and cardiometabolic risk during pregnancy

Mediterranean dietary pattern has been previously associated with better cardiometabolic markers and lower cardiometabolic risk in adult population (30,45,126–129). However, studies regarding dietary patterns and cardiometabolic risk markers during pregnancy are scarce and mostly focused on the relationship between maternal dietary patterns and isolated metabolic risk factors (42,130). Importantly, pregnant women with several cardiometabolic risk factors are more likely to have adverse maternal (preterm delivery, preeclampsia, gestational diabetes mellitus) and fetal (small/large for gestational age, neonatal asphyxia, fetal demise) outcomes (131). Therefore, further studies are required to explore the association of healthy dietary patterns during gestation such as MD with cardiometabolic risk during pregnancy.

6.7. Mediterranean diet during pregnancy and mental health

6.7.1. Mediterranean diet during pregnancy and maternal sleep quality

Among others factors, changes in hormone levels, physical discomfort, and fertility-related anxiety during pregnancy, might lead to sleep disturbances such as insufficient sleep time,

poor sleep quality, insomnia, restless legs syndrome, subjective sleep-disordered breathing and diagnosed obstructive sleep apnea (132,133). Disordered sleep is a widespread issue during pregnancy, with studies suggesting that up to 75% of expectant mothers experience poor sleep quality throughout gestation (134–137).

Sleep disturbances are associated with an array of maternal complications and adverse fetal outcomes including pre-eclampsia, gestational hypertension, gestational diabetes, cesarean section, preterm birth, large for gestational age and stillbirth (138). Therefore, investigate and identify potential factor that could exert a beneficial effect on sleep quality during pregnancy is warranted. In this regard, healthy dietary patterns characterised by a high intake of fruits, vegetables and a low intake of saturated fatty acids and sugar, like the MD, might positively influence sleep quality in non-pregnant adult population (139–141). However, little is known about how the MD adherence and its components may be linked to measures of sleep quality in pregnant women.

6.7.2. Mediterranean diet during pregnancy, well-being and ill-being

Depression and anxiety symptoms have been reported as the most common mental disorders during pregnancy and postpartum (142–144). In fact, current data shows that between 7% and 15% of pregnant women are affected by antenatal depression (145), between 14% and 54% by global antenatal anxiety (146), and around 10% by postnatal depression (147). Pregnancy complications such as higher risk for preeclampsia, spontaneous preterm delivery and low birth weight seems to be more common among women with mental disorders or poor mental health (145,146,148). Considering the impact of maternal mental health on adverse materno-fetal health outcomes, it is particularly important to identify potential protective factors for mental health in pregnant women (149–151). An optimal mental health is defined by low levels of psychological ill-being and high levels of well-being (152). Among the many factors studied that might exert an impact of mental health, maternal dietary intake has emerged as a possible area of intervention for both reducing psychological ill-being and improving psychological well-being in pregnant women and their offspring (153,154). In this sense, the assessment of dietary exposures that might be related to maternal mental health has traditionally focused on individual foods and nutrients (155). However, there is a trend in nutrition research toward the assessment of the whole diet, including measures of diet quality, when examining the diet-disease relationship to account for the potential interactions between the different food groups (153). Indeed, mental health and diet are strongly interrelated, with studies suggesting a bi-directional association. A woman's psychological condition (e.g., stress and anxiety) might

exert a negative influence on dietary habits by increasing energy-dense and nutrient-poor food intake, decreasing intakes of key micronutrients during pregnancy (156), while at the same time food choices might influence mental health (157). In this context, poorer diet quality and unhealthy dietary patterns including refined grains, sweets, energy drinks and fast foods compared with healthy dietary patterns based on fruits, vegetables, fish and whole grains have been positively associated with antenatal depressive symptoms (154,158,159). In addition, observational (160,161) and intervention (162) studies have demonstrated that the adherence to the MD was associated with decreased prevalence of depression and depressive symptoms in non-pregnant adult population. This relationship remains to be corroborated in pregnant women.

6.8. Mediterranean diet during pregnancy and its influence on the new-born

During pregnancy, the fetus receives all required nutrients through maternal-fetal blood circulation and is dependent on maternal nutrient stores and dietary intake. However, in the context of normal food availability, the effect of maternal diet on fetal growth is much more complex to determine, namely because of several determinants of fetal growth and a plausible interaction between them (i.e., maternal nutritional status and maternal diet) (163).

6.8.1. Maternal diet and fetal/neonatal insulin sensitivity

Maternal diet, and particularly its carbohydrate type and content, influences maternal blood glucose concentrations (164). However, different carbohydrate foods promote different glycemic responses. It is postulated that eating primarily high glycemic foods results in fetal-placental overgrowth and excessive maternal weight gain, which leads to a predisposition to fetal macrosomia, while intake of low glycemic foods predispose to normal infant birthweight and normal GWG (165).

Diets with a dysbalanced macronutrient energy contribution negatively affect the insulin resistance of neonates, inducing a pre-diabetic profile at birth or at the fetal stage (6). Increments in uterine saturated fatty acids decrease insulin sensitivity and raise permanently the glucose/insulin ratio in the offspring, suggesting poor glucose utilization. The increase intake in omega-3 fatty acids is related to greater insulin sensitivity (166). Mothers following diets with high adherence to the MD during pregnancy deliver infants with low insulinemia and homeostatic model assessment-insulin resistance (HOMA-IR) at birth (63).

6.8.2. Mediterranean diet during pregnancy and neonatal lipid markers

During gestation, fetal endogenous cholesterol production is enhanced to assure cholesterol transfer to endocrine system and other organs. Unfortunately, the effects of diet on lipoproteins in the fetus and at birth have been scarcely studied. To the best of our knowledge, the scarcity could be related to the fact that the average cholesterol level in different neonate populations is similar (6). According to maternal diet during pregnancy, it has been investigated that mothers with low adherence to the MD delivered neonates with higher LDL-cholesterol. The transference of placental lipids has been largely refereed, contributing the lipid profile of maternal diet to the plasma lipid profile of the neonate (167). The existence of active LDL-cholesterol receptors controlling circulating LDL particles has been reported in the fetus (168). As each LDL particle contains just a molecule of Apo B, it can be calculated that neonates whose mothers consumed a MD presented less LDL particles in comparison with women who did not follow that dietary pattern (6).

6.8.3. Mediterranean diet during pregnancy and placental telomere length

Telomeres are nucleoprotein structures located at the ends of chromosomes that protect cells from chromosomal instability and shorten naturally with every cellular division in normal cells (169). Telomeres are like the clocks of the cell, as they mark the number of cell divisions until the cell dies (170). Thus, telomere attrition may mediate aging of placenta and fetal membranes, which takes place toward the end of pregnancy (170). The placenta controls intrauterine development by supplying oxygen, nutrients, and regulating the bioavailability of specific hormones involved in fetal and postnatal growth and development, thus playing a key role in programming neonatal development (171). Previous evidence has implicated shortened placental telomere length in the pathogenesis of pregnancy complications including preeclampsia (172), uncontrolled diabetes (173), intrauterine growth retardation (174), spontaneous preterm birth (175) and unexplained stillbirths (176). In this context, lifestyle factors including healthy diets such as the MD and physical activity might exert a beneficial effect on telomere length in blood cells (177–181). However, the extrapolation of these benefits to placental telomere length is not straightforward since this association has not been previously investigated.

6.9. Gaps addressed in this International Doctoral Thesis

Table 5. Overall view of the gaps identified and the contribution of this Doctoral Thesis

	Gap	Contribution
SECTION I	There are no clear recommendations on the choice of tools to assess MD adherence during pregnancy with no available cut-off points to evaluate MD adherence in pregnant women	Practical recommendations on what MD indices are useful to assess MD adherence during gestation and development of cut-off points for the assessment of MD in pregnant women **(Study I)**
	The identification of factors that may influence the MD adherence during pregnancy is limited	Identification of sociodemographic factors, lifestyle behaviors and pregnancy-related determinants associated with MD adherence during pregnancy **(Study II)**
SECTION II	The MD benefits on cardiometabolic markers have been widely investigated in non-pregnant adult population. However, there is still the need to further investigate the association of MD on materno-fetal cardiometabolic markers and maternal cardiometabolic risk	Cross-sectional and longitudinal studies on MD adherence during gestation, materno-fetal cardiometabolic markers and maternal cardiometabolic risk **(Studies III and IV)**
	It has not been investigated whether an exercise intervention during pregnancy should be combined with an optimal MD adherence to exert a beneficial effect on postpartum body composition	Investigation of the effects of an exercise intervention delivered to pregnant women on postpartum body composition and, to what extent these effects are moderated by an optimal MD adherence during pregnancy **(Study V)**
SECTION III	Information on the association of MD adherence during pregnancy and mental health and sleep is scarce	Cross-sectional and longitudinal studies on MD adherence sleep quality, psychological ill-being, well-being and health-related quality of life throughout gestation **(Studies VI, VII and VIII)**
	There has not been investigated whether the effects of an exercise intervention delivered to pregnant women plus an optimal MD adherence could exert a beneficial effect on postpartum depression	Investigation of the effects of an exercise intervention delivered to pregnant women on postpartum depression and, whether an optimal MD adherence during pregnancy modulates these effects **(Study IX)**
SECTION IV	There is no evidence of women's susceptibility to obesity and exercise interactions on GWG, neither of differences in dietary intake and MD adherence among different genotype	Study to explore whether the effects of an exercise program during pregnancy on GWG are influenced by genetic susceptibility to obesity and to explore differences in MD adherence according to women's genotype **(Study X)**
	The benefits of lifestyle factors including diet and physical activity on telomere length measured in blood cells have been widely reported. However, the extrapolation of these benefits to placental telomere length has not been investigated to date	Investigation of the effects of an exercise intervention delivered during pregnancy on placental telomere length and, whether MD adherence during pregnancy moderates the effects of the exercise intervention on placental telomere length **(Study XI)**

GWG, gestational weight gain; MD, Mediterranean diet.

AIMS/OBJETIVOS

7. AIMS/OBJETIVOS

The overall aim of this International Doctoral Thesis was to explore the association of dietary habits and MD adherence during pregnancy with maternal and neonatal health-related outcomes. This overall aim is addressed in nine specific aims which correspond to eleven different studies.

Section I. Mediterranean diet assessment during gestation and its relationship with sociodemographic, lifestyle and pregnancy-related determinants.

- **Specific aim I:** to provide practical considerations on what MD indices are useful to assess MD adherence during pregnancy. To develop cut-off points for the assessment of MD based on their association with a clustered cardiometabolic risk throughout gestation **(Study I)**.
- **Specific aim II:** to evaluate the influence of sociodemographic factors (age, education, marital and working status), lifestyle behaviors (smoking habit, physical activity levels, physical fitness components), and pregnancy-related determinants (pre-pregnancy BMI, parity, number of miscarriages and number of children) on MD adherence during pregnancy **(Study II)**.

Section II. Influence of Mediterranean diet during pregnancy on materno-fetal cardiometabolic health.

- **Specific aim III:** to analyse the associations of MD adherence and MD components during pregnancy with maternal and cord arterial and venous glycemic, lipid, and inflammatory serum markers **(Study III),** and maternal cardiometabolic risk **(Study IV)**.
- **Specific aim IV:** to study whether the effects of an exercise program during pregnancy on postpartum body composition are moderated by following a MD **(Study V)**.

Section III. Influence of Mediterranean diet during pregnancy on maternal sleep and mental health.

- **Specific aim V:** to analyse the associations of MD adherence and MD components during pregnancy with maternal sleep quality during pregnancy **(Study VI)**.
- **Specific aim VI:** to analyse the association of MD adherence and MD components with maternal psychological ill-being and well-being **(Study VII)** and health-related quality of life during pregnancy **(Study VIII)** and postpartum depression **(Study IX)**.

- **Specific aim VII:** to study whether the effects of an exercise program during pregnancy on maternal mental health are moderated by following a MD **(Study IX)**.

Section IV. Influence of Mediterranean diet during pregnancy on materno-fetal genetics.

- **Specific aim VIII:** to investigate whether the effect of an exercise intervention delivered to pregnant women are moderated by women's susceptibility to obesity, as it is indicated with the presence of the SNP rs9939609 of the *FTO* gene, and to study differences in energy intake and MD adherence according to *FTO* genotype **(Study X)**.
- **Specific aim IX:** to investigate whether the effects of an exercise intervention delivered to pregnant women on placental telomere length are modulated by MD adherence during pregnancy **(Study XI)**.

OBJETIVOS

El objetivo general de esta Tesis Doctoral Internacional fue explorar la asociación de los hábitos dietéticos y la adherencia a la dieta Mediterránea (DM) durante el embarazo con los resultados relacionados con la salud materna y neonatal. Este objetivo general se aborda en nueve objetivos específicos que se corresponden con un total de once estudios diferentes.

Sección I. Evaluación de la dieta mediterránea durante la gestación y su relación con determinantes sociodemográficos, de estilo de vida y relacionados con el embarazo.

- **Objetivo específico I:** aportar consideraciones prácticas sobre qué índices son útiles para evaluar la adherencia a la DM durante el embarazo. Desarrollar puntos de corte para la evaluación de la DM en función de su asociación con el riesgo cardiometabólico a lo largo de la gestación **(Estudio I)**.
- **Objetivo específico II:** evaluar la influencia de los factores sociodemográficos (edad, educación, estado civil y laboral), de estilo de vida (hábito tabáquico, niveles de actividad física, condición física) y los determinantes relacionados con el embarazo (índice de masa corporal previo al embarazo, paridad, número de abortos y número de hijos) en la adherencia a la DM durante el embarazo **(Estudio II)**.

Sección II. Influencia de la dieta Mediterránea durante el embarazo en la salud cardiometabólica materno-fetal.

- **Objetivo específico III**: analizar las asociaciones de la adherencia a la DM y los componentes de la DM durante el embarazo con los marcadores glucémicos, lipídicos y séricos inflamatorios maternos y del cordón umbilical **(Estudio III)** y el riesgo cardiometabólico materno **(Estudio IV)**.
- **Objetivo específico IV**: estudiar si los efectos de un programa de ejercicio durante el embarazo sobre la composición corporal posparto están modulados por seguir una óptima adherencia a la DM **(Estudio V)**.

Sección III. Influencia de la dieta Mediterránea durante el embarazo sobre el sueño y la salud mental materna.

- **Objetivo específico V**: analizar las asociaciones de la adherencia a la DM y los componentes de la DM durante el embarazo con la calidad del sueño de la madre **(Estudio VI)**.
- **Objetivo específico VI:** analizar la asociación de la adherencia a la DM y los componentes de la DM durante el embarazo con el malestar y el bienestar psicológico de la madre **(Estudio VII)** y la calidad de vida relacionada con la salud durante la gestación materna **(Estudio VIII)** y la depresión posparto **(Estudio IX)**.
- **Objetivo específico VII:** estudiar si los efectos de un programa de ejercicio durante el embarazo sobre la salud mental materna están modulados por seguir una óptima adherencia a la DM **(Estudio IX)**.

Sección IV. Influencia de la dieta Mediterránea durante el embarazo en la genética materno-fetal.

- **Objetivo específico VIII**: investigar si el efecto de una intervención de ejercicio administrada a mujeres embarazadas está modulado por la susceptibilidad de las mujeres a la obesidad, como se indica con la presencia del SNP rs9939609 del gen *FTO* y estudiar las diferencias en la ingesta energética y la adherencia a la DM según el genotipo *FTO* **(Estudio X)**.
- **Objetivo específico IX**: investigar si los efectos de una intervención de ejercicio administrada a mujeres embarazadas sobre la longitud de los telómeros de la placenta después del parto están modulados por la adherencia a la DM durante el embarazo **(Estudio XI)**.

METHODS

8. METHODS

The present International Doctoral Thesis is composed of eleven studies classified within four different sections: **Section I** focuses on MD assessment during gestation and its relationship with sociodemographic, lifestyle and pregnancy-related determinants; **Section II** focuses on the influence of MD during pregnancy on materno-fetal cardiometabolic health and postpartum body composition; **Section III** focuses on the influence of MD during pregnancy on sleep and mental health during gestation and depression during postpartum, and **Section IV** focuses on the influence of MD during pregnancy on materno-fetal genetics. All these parts address knowledge gaps under the framework of the GESTAtion and FITness (GESTAFIT) project conducted in pregnant women.

8.1. Study design and population

The GESTAFIT project was initially designed as a randomized controlled trial which was carried out in Granada (southern Spain) between November 2015 and April 2018 (Identifier: NCT02582567) (182). The main aim of the GESTAFIT project was to evaluate the effects of a supervised concurrent exercise intervention (aerobic + resistance) on maternal and fetal health. The study was carried out 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 and exclusion criteria are detailed in **Table 6**. Briefly, we included women aged 20-40 years with a normal pregnancy course, and giving birth (singleton) at 37-42th gestational week (g.w.) via spontaneous/vaginal delivery, or cesarean section without severe materno-fetal pathology.

Table 6. 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 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 engaged in another physical exercise program
- Being unwilling either to complete the study requirements or to be randomized into the control or intervention group.

Three hundred and eighty-four pregnant women attended their first gynecological visit at the hospital at the 12th g.w. and were informed about the study's aims and procedures. Among them, a total of 159 women were recruited after showing interest in joining the study **(Figure 2)**. All participants signed a written personal informed consent.

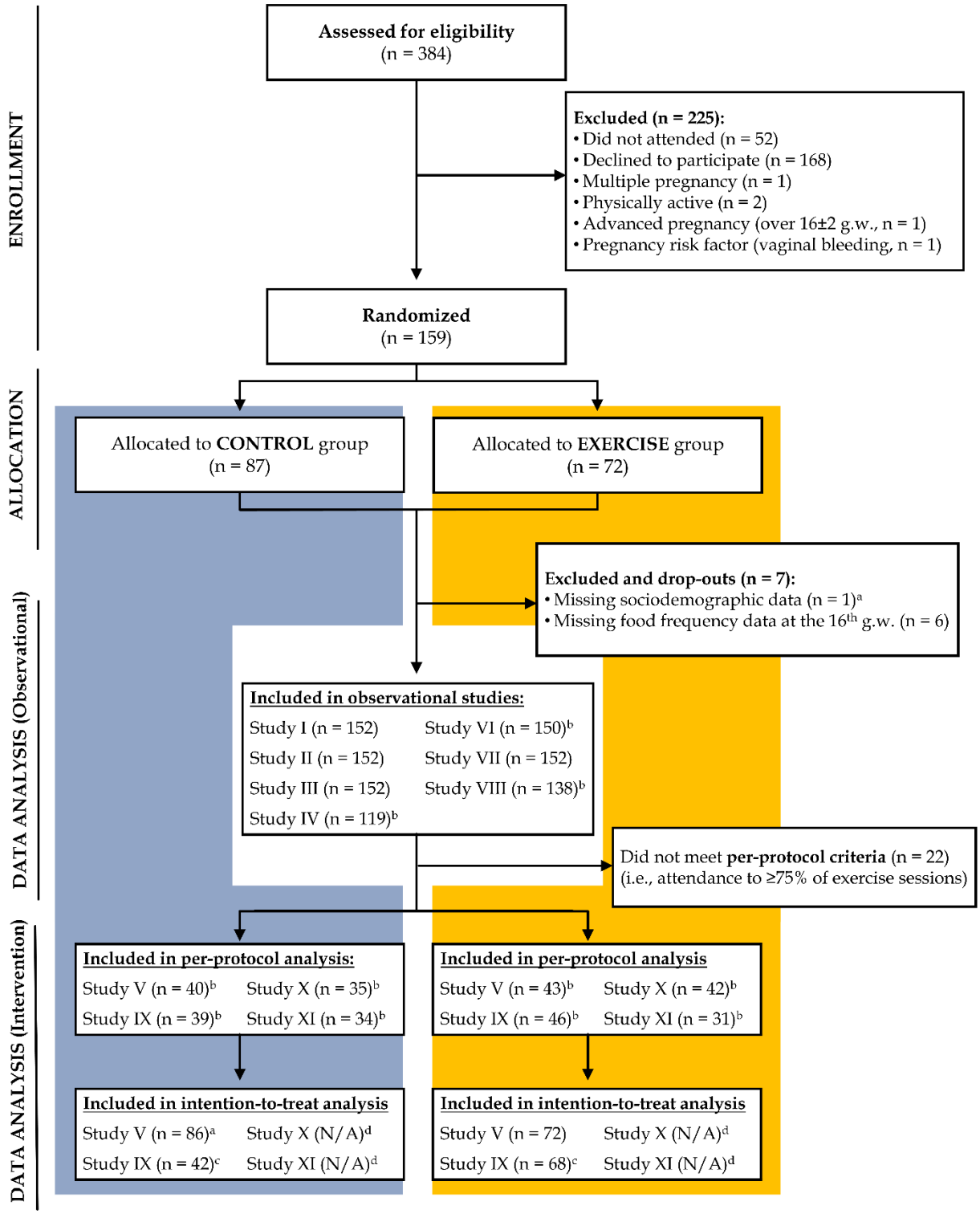

Figure 2. Flow diagram of the participants. [a]Excluded in observational, per-protocol and intention-to-treat analyses; [b]Less sample size because missing data in any of the outcomes of this study; [c]Only participants with baseline data were imputed; [d]Secondary exploratory outcomes performed in a subsample, only per-protocol analyses were conducted.

8.2. Sample size calculation

The sample size for this 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 al. (183) 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) were necessary. We planned to recruit 60 women assuming a 15% of potential withdrawals.

8.3. Randomization and blinding

The study was conducted in three waves for feasibility reasons. 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). Nonetheless, the randomized component was broken in the second and third waves to ensure enough adherence to the program; which represents a frequent methodological barrier in antenatal exercise research (184). Thus, half the women were not randomized but allocated to the control/exercise group according to their personal convenience. As a result, the GESTAFIT project was finally characterized by a quasi-experimental design. Most personnel were blinded to their allocation into the control/exercise group, excepting those responsible for the training sessions.

8.4. Exercise intervention

The exercise intervention consisted of a concurrent supervised-tailored exercise program (from 17th g.w. until delivery, 3 days/week, 60 minutes/session) of aerobic and resistance exercises of moderate-to-vigorous (mostly moderate with peaks of vigorous) intensity. Sessions consisted of a 10-minute warm-up, a 40-minute muscular (circuits of resistance exercises and short aerobic blocks) or aerobic block (dance or functional circuits), and a 10-minute cooldown. Resistance exercises involved anterior and posterior chain dominant, pull, push, and core exercises. The exercise training program was designed following the standards by the American College of Obstetricians and Gynecologists (185), and the latest scientific evidence (186,187). The intensity of exercises was set at moderate according to the women's perceived effort within the range of 12 to 16 on the Borg scale of perceived exertion ranging from 6 (light effort) to 20 (heavy effort) (188). The attendance of the participants to the exercise sessions was recorded to measure adherence to the exercise programme.

8.5. Control group

Pregnant women allocated into the control group did not participate in the training sessions and were asked to continue with their usual activities. For ethical reasons, the research team held 7 lectures to pregnant women from both groups (exercise and control group) during the duration of the intervention 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 MD 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 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.

8.6. Measurements

A brief description of the measures from the GESTAFIT project which are used in this Thesis is presented below. More details on all the evaluations conducted can be found elsewhere (182). Likewise, a more detailed definition of the data collection for each specific study is presented in the results section of this International Doctoral Thesis.

8.6.1. General procedures

Women were evaluated at several time points during pregnancy and postpartum by trained researchers: at the 16th and 34th g.w. (2 days/assessment), delivery (2 days/assessment), and at the early postpartum (i.e., 6 weeks after giving birth) (1 day/assessment). The general procedures of the GESTAFIT project are presented in **Figure 3**. At the 16th g.w. sociodemographic and clinical characteristics were assessed with an initial anamnesis. Other questionnaires were also employed to collect health information related to sleep and diet quality, among others. Additionally, anthropometrics and physical fitness 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 the 17th g.w., the accelerometers along with the diaries were returned, and maternal blood was extracted by a trained nurse. After the baseline assessment, the exercise intervention was initiated and performed until delivery. At the 33rd-34th g.w., the same assessments were performed with identical timing. 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. At the 6th week after giving birth, the mature milk from mothers was collected, maternal and neonatal buccal mucosa cells were extracted, and anthropometrics, body composition, sleep, diet quality, and physical fitness were evaluated.

8.6.2. Sociodemographic factors

An initial survey (anamnesis) was performed to compile information on the sociodemographic and clinical characteristics at the 16th g.w.

8.6.3. Dietary assessment and Mediterranean diet adherence

Dietary habits were assessed with a food frequency questionnaire previously evaluated for validity in Spanish non-pregnant adult population (189). Women were asked about the frequency of consumption of the different food groups (*never* or *number of times per day, week, month,* or *year*). Information about meal and snack patterning was assessed with two questions included in the food frequency questionnaire. Energy (Kcal), macro, and micronutrient intake were calculated using the Evalfinut software, which includes the Spanish Food Composition Database (25).

The following MD indices were employed to assess MD adherence: the Mediterranean Food Pattern (MFP) (190), the Mediterranean Diet Scale (MDScale) (191), the Short Mediterranean Diet questionnaire (SMDQ) (127), the Mediterranean Diet Score (MedDietScore) (30) and the Mediterranean Diet scale for pregnant women (MDS-P) (64). We calculated all dietary indices by using dietary data obtained from the food frequency questionnaire (189) and following the recommendations for each corresponding index.

8.6.4. Blood collection

Maternal blood samples (5mL) were extracted from the antecubital vein in standardized fasting conditions (at the 16th and 34th g.w.), arterial and vein cord blood samples were extracted immediately after delivery. All blood samples were collected in serum tubes and were allowed to clot (coagulation) at room temperature for 30 minutes. Subsequently, samples were centrifuged at 1750 rpm during 10 min at 4ºC in a refrigerated centrifuge (GS-6R Beckman Coulter, Brea, CA, USA) to obtain serum which was aliquoted and frozen at -80ºC until analysed.

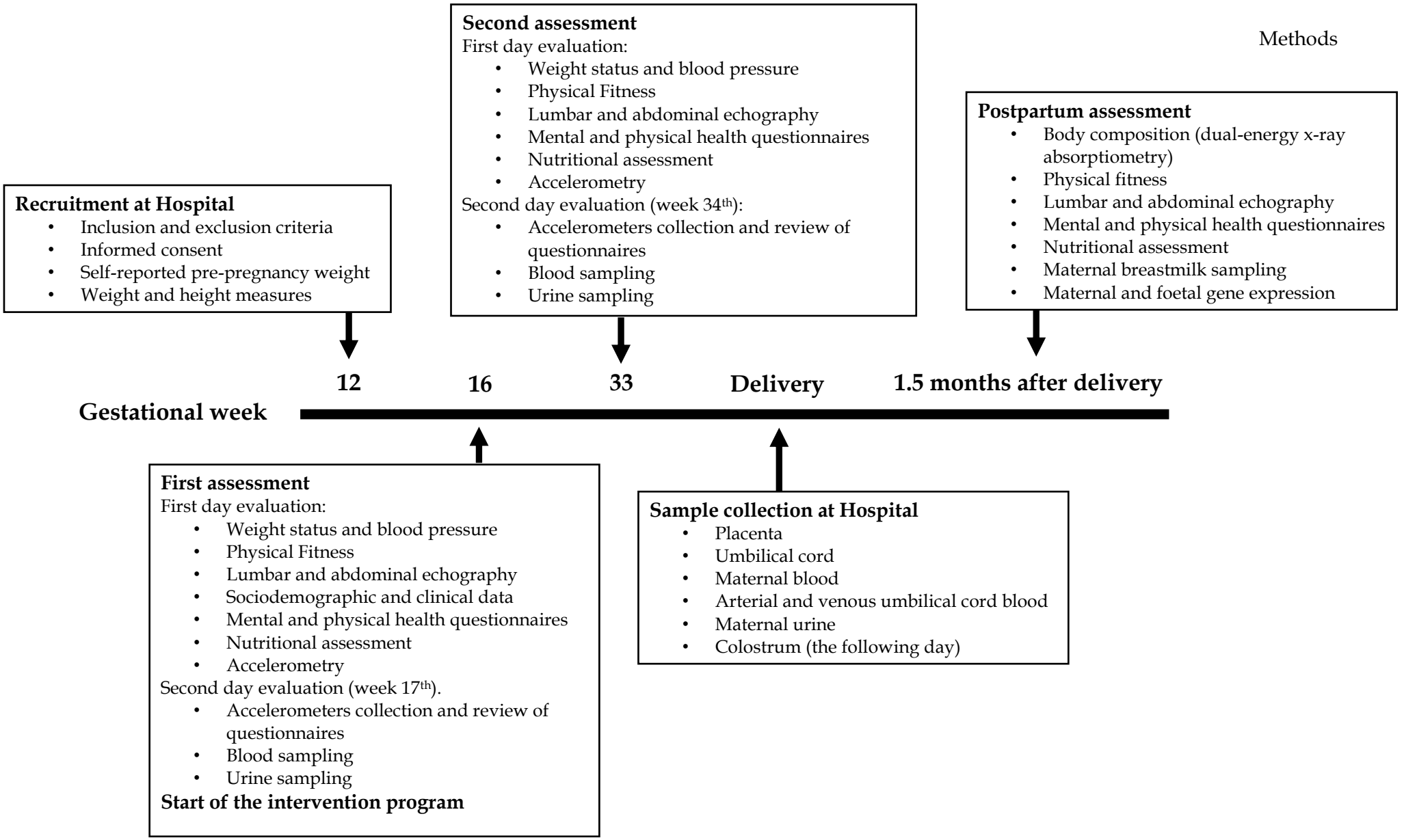

Figure 3. Assessments conducted along the GESTAFIT project.

8.6.5. Glycemic and lipid profiles and C-reactive protein

Maternal concentrations of serum total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL-cholesterol, glucose, phospholipids, triglycerides and C-reactive protein were measured with standard spectrophotometric enzyme assays (AU5822 Clinical Chemistry Analyser, Beckman Coulter, Brea, USA). Cord arterial and venous serum total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, triglycerides and C-reactive protein were measured with spectrophotometric determination (BS-200 Chemistry Analyzer, Mindray Bio-medical Electronics, Shenzen, China).

8.6.6. Insulin

Maternal insulin was measured with paramagnetic-particle-based chemiluminescence immunoassays (UniCel-Dxl800 Access Immunoassay analyser, Beckman Coulter, Brea, USA).

8.6.7. Inflammatory markers

Some maternal and cord arterial and venous pro-inflammatory and anti-inflammatory cytokines (IL-6, IL-8, IL-10, IL-1beta, Interferon gamma and TNF-α) were assessed with Luminex xMAP technology. Plate was read on LABScan 100 analyser (Luminex Corporation, Texas, USA) with xPONENT software for data acquisition. Average values for each set of duplicate standards were within 15% of the mean.

8.6.8. Maternal anthropometry, postpartum weight retention and body composition

Pre-pregnancy body weight was self-reported at the recruitment (12^{th} g.w.). Weight was measured at the 16^{th} and 34^{th} g.w. and at the 6^{th} week after giving birth (no shoes, light clothes) with an electronic scale (InBody-R20; Biospace, Seoul). Height was also measured using a stadiometer (Seca 22, Hamburg, Germany). BMI was calculated as kg/m^2.

Postpartum body composition measurements were assessed using dual-energy X-ray absorptiometry (Discovery DXA system; Hologic, Marlborough MA, USA). A whole-body scan was performed considering the manufacturer´s guidelines to ensure the quality of the data. The APEX 4.0.2. software (Hologic Series Discovery) was used to draw an automatic delineation of anatomic regions.

8.6.9. Vascular function

A BP monitor (M6 upper arm blood pressure monitor Omron. Omron Health Care Europe B.V. Hoolddorp, The Netherlands) was employed to assess SBP and DBP, which were

measured after 5 minutes of rest on two separate occasions (with 2 minutes between trials) with the person seated.

8.6.10. Physical activity levels

Physical activity was monitored for 9 days (24 h/day, except for water activities) with non-dominant-wrist-worn accelerometers (ActiGraph GT3X+, Florida, US) (192). Sedentary time (min/week), moderate-vigorous physical activity (min/week), total physical activity levels (min/week), and percentage of participants who met the international physical activity recommendations of at least 150 min of moderate-vigorous physical activity per week were calculated (193).

8.6.11. Physical fitness assessment

The complete physical fitness battery employed has been previously described (182). Briefly, the back-scratch test (as a measure of overall shoulder range of motion) was employed to assess flexibility (194). Cardiorespiratory fitness was assessed with the 6-min walk test, along a 45.7-m rectangular course (194). Muscle strength was evaluated by handgrip (as a measure of overall body strength) with a digital dynamometer (TKK 5101 Grip-D; Takey, Tokyo, Japan) (195).

8.6.12. Sleep Quality

The Spanish version of the Pittsburgh Sleep Quality Index (PSQI) was employed to assess sleep quality (196).

8.6.13. Psychological well-being

Positive affect was assessed with the State Positive Affect Schedule (PANAS-S) (197,198).
The 3 subscales of The Trait Meta-Mood Scale (TMMS) (199) were employed to assess emotional attention, emotional clarity and emotional regulation. The Connor-Davidson Resilience Scale (CD-RISC) was employed to assess resilience to stress (200). Health-related quality of life (HRQoL) of the participants was assessed with the Spanish version of the Short-Form Health Survey 36 (SF-36) (201).

8.6.14. Psychological ill-being

Negative affect was assessed with the PANAS-S negative subscale (197,198). The State Trait Anxiety Index (STAI-S) questionnaire was employed to evaluate state-anxiety levels in

pregnant women at the moment of the evaluation (202,203). The validated 20-item Spanish version of the Center for Epidemiological Studies Depression Scale (CES-D), was employed to assess pregnant antenatal depression (204). The Edinburgh Postnatal Depression Scale (EPDS) (205) was employed to detect postpartum depression.

8.6.15. Genotype

Participants were genotyped for 1 SNP (*FTO* gene). Mucosa cells were collected with swabs, and DNA organic extraction and spectrophotometric quantification (NanoDrop-2000c, TermoFisher) procedures were performed. The TaqMan® Genotyping Assay was conducted according to the manufacturer's protocol. Data were analysed using the QuantStudio Real-Time PCR Software v1.3.

8.6.16. Relative placental telomere length

Placental samples were collected immediately after delivery. Relative placental telomere length (T/S ratio) was assessed by a quantitative real time polymerase chain reaction-based method developed by Cawthon et al. (206) with some modifications (207).

8.7. Methodological overview of the studies included in the International Doctoral Thesis

Table 7 shows the methodological overview of all studies in the present international Doctoral Thesis.

Table 7. Methodological overview of all studies in the present International Doctoral Thesis

Study	Design	Participants	Predictor/independent variables and moderator if applicable (instruments)	Outcomes/dependent variables (instruments)
Study I *Assessing the Mediterranean diet adherence during pregnancy: practical considerations based on the associations with cardiometabolic risk*	Cross-sectional and longitudinal	152 Caucasian pregnant women (age: 32.9±4.6 years)	Dietary habits (food frequency questionnaire) and MD adherence assessed by five MD indices (MDScale, MedDietScore, MDS-P, MFP, SMDQ).	Immunometabolic markers: glycemic and lipid markers (standard methods). Pre-pregnancy body weight (kg) (self-reported), height (cm) (stadiometer), BP (BP monitor).
Study II *Associations between sociodemographic factors, lifestyle behaviors, pregnancy-related determinants, and Mediterranean diet adherence among pregnant women: The GESTAFIT project*	Cross-sectional	152 Caucasian pregnant women (age: 32.9±4.6 years)	Time spent in sedentary behaviours, MVPA, and total PA (triaxial accelerometry). CRF (6-min walk test), muscle strength (handgrip test), flexibility (back scratch test). Number of miscarriages, parity, marital status, educational level, working status, and smoking habit (self-reported questionnaires).	Dietary habits (food frequency questionnaire) and MD adherence (MFP [i.e., a MD index]).
Study III *Association of Mediterranean diet adherence during pregnancy with maternal and neonatal lipid, glycemic and inflammatory markers. The GESTAFIT project*	Cross-sectional and longitudinal	152 Caucasian pregnant women (age: 32.9±4.6 years)	Dietary habits (food frequency questionnaire) and MD adherence (MedDietScore [i.e., a MD index]).	Immunometabolic markers: glycemic and lipid markers, C-reactive protein (standard methods), insulin (chemiluminescence immunoassays) and pro- and anti-inflammatory cytokines (LUMINEX xMAP technology).
Study IV *Influence of the degree of adherence to the Mediterranean Diet and its components on cardiometabolic risk during pregnancy. The GESTAFIT project*	Cross-sectional and longitudinal	119 Caucasian pregnant women (age: 33.2±4.4 years)	Dietary habits (food frequency questionnaire) and MD adherence (MedDietScore [i.e., a MD index]).	Anthropometry (scale, measuring tape), immunometabolic markers: glycemic and lipid markers (standard methods).
Study V *Influence of an exercise intervention plus an optimal Mediterranean diet adherence during pregnancy on postpartum body composition. The GESTAFIT project*	Quasi-experimental	83 Caucasian pregnant women (age: 33.5±4.3 years)	Independent: Supervised exercise intervention Exercise group (n=43): concurrent (aerobic+resistance) training program from the 17th g.w. until delivery (3 days/week, 60 minutes/session) of moderate-to-vigorous intensity. Control group (n=40): usual care Moderator: MD adherence (MedDietScore [i.e., a MD index]).	Anthropometry (scale, measuring tape), body composition (DXA).
Study VI *Influence of dietary habits and Mediterranean diet adherence on sleep quality during pregnancy. The GESTAFIT project*	Longitudinal	150 Caucasian pregnant women (age: 32.9±4.6 years)	Dietary habits (food frequency questionnaire) and MD adherence (MFP [i.e., a MD index]).	Sleep Quality (PSQI).

Table 7. Methodological overview of all studies in the present International Doctoral Thesis

Study	Design	Participants	Predictor/independent variables and moderator if applicable (instruments)	Outcomes/dependent variables (instruments)
Study VII *Associations of Mediterranean diet with psychological ill-being and well-being throughout the pregnancy course: The GESTAFIT project*	Longitudinal	152 Caucasian pregnant women (age: 32.9±4.6 years)	Dietary habits (food frequency questionnaire) and MD adherence (MFP [i.e., a MD index]).	Psychological well-being (PANAS-S, TMMS, CD-RIC). Psychological ill-being (PANAS-S, STAI-S, CES-D).
Study VIII *A greater Mediterranean diet adherence is associated with better health-related quality of life during pregnancy. The GESTAFIT project*	Longitudinal	138 Caucasian pregnant women (age: 32.9±4.6 years)	Dietary habits (food frequency questionnaire) and MD adherence (MFP [i.e., a MD index]).	Health-related quality of life (SF-36).
Study IX *Exercise, Mediterranean diet adherence or both during pregnancy to prevent postpartum depression – GESTAFIT Trial secondary analyses*	Quasi-experimental, and longitudinal[a]	85 Caucasian pregnant women (age: 33.4±4.2 years)	Independent: Supervised exercise intervention Exercise group (n=46): concurrent (aerobic+resistance) training program from the 17th g.w. until delivery (3 days/week, 60 minutes/session) of moderate-to-vigorous intensity Control group (n=39): usual care Moderator: MD adherence (MFP [i.e., a MD index]).	Postpartum depression (EPDS).
Study X *Are the effects of exercise on gestational weight gain moderated by the FTO gene polymorphism rs9939609? The GESTAFIT project*	Quasi-experimental	77 Caucasian pregnant women (age: 33.4±4.1 years)	Independent: Supervised exercise intervention Exercise group (n=42): concurrent (aerobic+resistance) training program from the 17th g.w. until delivery (3 days/week, 60 minutes/session) of moderate-to-vigorous intensity Control group (n=35): usual care Moderator: MD adherence (MedDietScore [i.e., a MD index]).	Anthropometry (scale, measuring tape), genotype (TaqMan® assays)
Study XI *Influence of an exercise intervention plus an optimal Mediterranean diet adherence during pregnancy on the telomere length of the placenta. The GESTAFIT project*	Quasi-experimental	65 Caucasian pregnant women (age:33.3±4.2 years)	Independent: Supervised exercise intervention Exercise group (n=31): concurrent (aerobic+resistance) training program from the 17th g.w. until delivery (3 days/week, 60 minutes/session) of moderate-to-vigorous intensity Control group (n=34): usual care Moderator: MD adherence (MedDietScore [i.e., a MD index]).	Placental telomere length (quantitative real time polymerase chain reaction based method).

BMI, Body mass index; BP, Blood pressure; CD-RISC, Connor-Davidson Resilience Scale; CES-D, Center for Epidemiological Studies Depression Scale; CRF, Cardiorespiratory; fitness; DXA, Dual-energy X-ray absorptiometry; EPDS, The Edinburgh Postnatal Depression Scale; FTO, Fat mass and obesity-associated protein; G.W., Gestational week; HRQoL, Health-related quality of life; MD, Mediterranean diet; MDScale, Mediterranean Diet Scale; MDS-P, Mediterranean Diet scale for pregnant women; MedDietScore, Mediterranean Diet Score; MFP, Mediterranean Food Pattern; MVPA, Moderate-vigorous physical activity; PA, Physical activity; PANAS-S, State Positive Affect Schedule; PSQI, Pittsburgh Sleep Quality Index; SF-36, Short-Form Health Survey 36; SMDQ, Short Mediterranean Diet questionnaire; STAI-S, State Trait Anxiety Index; TMMS, The Trait Meta-Mood Scale.

RESULTS AND DISCUSSION

SECTION I. Mediterranean diet assessment during gestation and its relationship with sociodemographic, lifestyle and pregnancy-related determinants

STUDY I: Assessing the Mediterranean diet adherence during pregnancy: practical considerations based on the associations with cardiometabolic risk

Flor-Alemany M, Migueles JH, Acosta-Manzano P, Marín-Jiménez N, Baena-García L, Aparicio VA.

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ABSTRACT

Aim: The aim of the present study was to provide practical considerations for assessing MD adherence during pregnancy based on the association with cardiometabolic risk.

Methods: A total of 152 Caucasian pregnant women were included in this longitudinal study. A food frequency questionnaire was fulfilled by pregnant women at the 16th g.w. We calculated five MD indices: the MFP, the MDScale, the SMDQ, the MedDietScore, and the MDS-P. The cardiometabolic risk score consisted of pre-pregnancy BMI, BP, glucose, triglycerides, and HDL-cholesterol (at the 16th and 34th g.w.).

Results: Multiple linear regression models showed that the MFP, the MedDietScore, and the SMDQ were associated with lower cardiometabolic risk at the 16th and 34th g.w. (β's: -0.193 to -0.415, all $p<0.05$); and the MDS-P at the 34th g.w. (β=-0.349, $p<0.01$). A comparison of these models with the *J* test showed that the MFP and the MedDietScore outperformed the SMDQ at the 16th g.w. (p's<0.05); while the MedDietScore outperformed the SMDQ, MFP, and MDS-P (p's<0.05) at the 34th g.w. Receiver-Operating-Characteristic-derived thresholds for the MFP, MedDietScore and MDS-P indices were 21, 30, and 6 points, respectively, to identify women with high cardiometabolic risk.

Conclusion: The MFP and MedDietScore are recommended to assess MD adherence during pregnancy, as these showed the strongest associations with cardiometabolic risk. Our validated thresholds might assist in the detection of poor dietary patterns during pregnancy.

INTRODUCTION

The gestational period is a crucial time of growth, development, and physiological changes for the mother and child (208). Diet during pregnancy is key for the health status of both the mother and the new-born (13,29). Although previous evidence is based on a single or a few food items or nutrients (29), the diet should be considered as the combination of the nutritive and non-nutritive components (209). Dietary patterns can comprehensively and meaningfully assess the relation between the diet quality and pregnancy outcomes (13).

The MD is one of the healthiest dietary patterns recognized in Europe (31). MD in pregnancy lowers the maternal cardiometabolic risk (210), the risk for excessive GWG, gestational diabetes (42), and preterm delivery (43), and the offspring insulin resistance (63) and adiposity (52). However, most of the MD indices used in pregnancy are not specifically validated in pregnant women (68). The dietary habits are affected during pregnancy (e.g., avoid alcohol) and require specific assessment tools. Thus far, there is only a MD index that has been adapted for pregnant women (69), yet the cut-off points to interpret its score were not adapted. As a result, it is challenging to assess the MD adherence during pregnancy (29,52).

Assessing MD adherence in pregnancy is especially relevant since recent studies suggest that pregnant women are drifting away from the MD-like pattern (211,212). This study aimed to provide practical considerations on what MD indices are useful to assess MD adherence during gestation based on their association with a clustered cardiometabolic risk at the 16th and 34th g.w.

METHODS

Study design and participants

This longitudinal study forms part of the GESTAFIT project (182). The GESTAFIT delivered an exercise intervention to pregnant women (more details elsewhere (182)). From the 384 pregnant women assessed for eligibility, 159 met the eligibility criteria **(Table 6)** and signed a written informed consent. Among them, 152 had valid data in sociodemographic and clinical characteristics and MD adherence **(Figure 2)**. A total of 33 pregnant women (at the 16th g.w.) and 45 pregnant women (at the 34th g.w.) had missing data in biochemical markers, BP and/or body composition. Therefore, 119 (at the 16th g.w.) and 107 (at the 34th g.w.) pregnant women were included in the analyses. All procedures were approved by the Ethics Committee on Clinical Research of Granada, Regional Government of Andalusia, Spain (code: GESFIT-0448-N-15).

Maternal anthropometry and body composition

Pre-pregnancy body weight was self-reported as the women were enrolled in this study at the 12th g.w. Although measured weight is preferable, self-report is a cost-effective and practical measurement approach that shows very good concordance with measured body weight (213). Height was measured using a stadiometer (Seca 22, Hamburg, Germany) at the 16th g.w. Pre-pregnancy BMI was calculated as pre-pregnancy weight (kg) divided by squared height (m^2).

Cardiometabolic health markers

Blood pressure was assessed twice (with 2 minutes between trials) with an upper-arm BP monitor (Omron M6, Omron Health Care Europe B.V. Hoolddorp, The Netherlands) with the person seated. The lowest value was selected for the analysis.

Blood samples were collected after all-night fasting. Venous blood samples (5mL) were collected in serum tubes. After 30 minutes at room temperature (to allow the sample to clot), samples were centrifuged at 1750 rpm for 10 min at 4ºC in a refrigerated centrifuge (GS-6R Beckman Coulter, Brea, CA, USA) to obtain serum. Glucose, triglycerides, and HDL-cholesterol were assessed with standard procedures using an auto-analyzer (AU5822 Clinical Chemistry Analyzer, Beckman Coulter, Brea, CA, USA).

Cardiometabolic risk

A clustered cardiometabolic risk score was created as previously described by Lei et al. (131) from the Z-scores for pre-pregnancy BMI, mean BP [defined as (SBP+DBP)/2], serum fasting glucose, triglycerides and HDL-cholesterol at the 16th and 34th g.w. The HDL-cholesterol z-score was multiplied by -1 prior to be averaged with the rest of factors. Higher clustered cardiometabolic status indicates greater cardiometabolic risk.

Dietary assessment

A food frequency questionnaire validated in Spanish non-pregnant adults was employed (189). The food frequency questionnaire (189) consisted of a list of foods, portion sizes, and food groups adapted to the Spanish population (25). Participants were asked about the frequency of consumption of the different foods, and additionally about meal and snack patterning with two extra questions. Energy (Kcal), macro, and micronutrient intake were calculated using the Evalfinut software (25). We calculated five MD adherence indices from the food frequency questionnaire data (189): The MFP (190), the MDScale (191), the SMDQ (127), the MedDietScore (30), and the MDS-P (64). The indices were calculated following the

recommendations in the validation studies (further detailed in **Table 8**). Higher scores indicate higher diet quality. A moderate alcohol intake, also typical of the MD, was not considered for calculating these indices in this group of women These dietary indices have been previously associated with better cardiometabolic health in pregnant (29,64,214–217) or non-pregnant populations (30,45,126–129). The components included in each index are detailed in **Table 9**.

Table 8. Definition and references for the Mediterranean diet indices calculated for the specific Study I aims

Index	Definition	Original Range	Range adapted to pregnant women	Reference
MFP	Quintile-based sum score of olive oil, fiber, fruits, vegetables, fish, cereals, meat and subproducts. Intake of each of the indicated groups in grams per day was calculated. The distribution was calculated within the study sample and each participant was assigned a score from 1 to 5 depending on the quintile of intake. The elements were combined by adding the quintile values.	8-40	7-35	(190)
MDScale	It consists of eight components in g/day (cereals, legumes, fruits and nuts, fish, high-fat dairy, meat, alcohol, and ratio of monounsaturated lipids to saturated lipids). Participants whose consumption of meat and dairy was below the median were assigned a value of 1, and participants whose consumption was at or above the median were assigned a value of 0. The other components were assumed to be beneficial and were scored on a reverse scale.	0-9	0-8	(191)
SMDQ	A specific frequency score was assigned to each food component (1 attributable point) when food consumption met the established criteria for nine elements including olive oil, fruit, vegetables or salad, fruits and vegetables, legumes, fish, wine, meat, and cereals (wholegrain bread, white bread, and white rice.	0-9	0-8	(127)
MedDietScore	It consists of eleven variables (wholegrain cereals, potatoes, fruits, vegetables, pulses, fish, olive oil, red wine, red meat and subproducts, poultry and whole dairy products) ranging from 0 to 5 according to their position in the Mediterranean diet pyramid.	0-55	0-50	(30)
MDS-P	It consists of eleven components including vegetables, fruits and nuts, legumes, cereals, fish, dairy, meat, the ratio of monosaturated to saturated fatty acids, folic acid intake, calcium intake, and iron intake. A high intake (above participants' median intake) of vegetables, fruits and nuts, pulses, cereals, fish, high monounsaturated to saturated fatty acids, and low intake (below the participants' median intake) of meat and dairy products scoring 1 point per component. The intake of folic acid, iron, and calcium was scored in relation to the Spanish recommended daily intake (25), scoring 1 point if the intake of the micronutrients was greater or equal to two-thirds of the recommended daily intake or if the women were taking nutritional supplements.	0-11	0-11	(64)

MDScale, Mediterranean Diet Scale; MedDietScore, Mediterranean Diet Score; MDS-P, Mediterranean diet scale for pregnant women; MFP, Mediterranean Food Pattern**;** SMDQ, Short Mediterranean Diet questionnaire.

Table 9. Components included in the Mediterranean diet adherence indices

	Food groups included in the Mediterranean diet adherence indices				
Category	**MFP**	**MDScale**	**SMDQ**	**MedDietScore**	**MDS-P**
Cereals	Cereals (g/day) High glycemic foods (g/day)	Cereals (g/day)	White bread (s/day) White rice (s/week) Whole grain-bread (s/week)	Whole-grain cereals (s/month)	Cereals (g/day)
Dairy products		High fat dairy (g/day)		Whole dairy products (s/month)	High fat dairy (g/day)
Fat	Olive oil (g/day)	Monosaturated/ saturated lipid ratio	Olive oil (s/day)	Olive oil (s/week)	Monosaturated/ saturated lipid ratio
Fiber	Fiber (g/day)				
Fish	Fish (g/day)	Fish (g/day)	Fish (s/week)	Fish (s/month)	Fish (g/day)
Fruits, vegetables and nuts	Fruits (g/day) Vegetables (g/day)	Fruits and nuts (g/day) Vegetables (g/day)	Fruits (s/day) Fruits and vegetables (s/day) Vegetables (s/day)	Fruits (s/month) Vegetables (s/month)	Fruits and nuts (g/day) Vegetables (g/day)
Legumes		Legumes (g/day)	Legumes (s/week)	Legumes (s/month)	
Meat	Meat and subproducts (g/day)	Meat and subproducts (g/day)	Meat (s/day)	Poultry (s/month) Red meat and subproducts (s/month)	Meat and subproducts (g/day)
Micronutrients					Calcium (mg/day) Folic acid (µg/day) Iron (mg/day)
Potatoes				Potatoes (s/month)	

MDScale, Mediterranean Diet Scale; MedDietScore, Mediterranean Diet Score; MDS-P, Mediterranean diet scale for pregnant women; MFP, Mediterranean Food Pattern; S, servings; SMDQ, Short Mediterranean Diet questionnaire.

Statistical analysis

Descriptive data were summarized as mean (standard deviation) or frequency (%) as appropriate. Linear regression models adjusted for maternal age, smoking habit, and number of children, were used to explore the cross-sectional associations between MD adherence and clustered cardiometabolic risk at the 16th g.w. (n=119). The longitudinal associations including the clustered cardiometabolic risk at the 34th g.w. (n=107) were additionally adjusted for the exercise intervention. We used the *J* test (218) to compare the regression models including

different MD indices. Likewise, multiple linear regression analysis was performed for the association of MD indices with the individual cardiometabolic markers (i.e., pre-pregnancy BMI, SBP, DBP, glucose, triglycerides, and HDL- cholesterol) adjusting for the aforementioned confounders. We explored the interaction between age (0= below 33 years old and 1= above 33 years old) and the MD adherence (with all MD indices) on clustered cardiometabolic risk during pregnancy. Since the clustered cardiometabolic risk*MD adherence interaction term was not significant (all *p*'s>0.2) we decided not to conduct separate models for women according to age categories.

We investigated the classification capacity to detect high cardiometabolic risk with receiver Operating Characteristic curves at the 16th and 34th g.w. As such, women in the Tertile 3 of the clustered cardiometabolic risk score were considered to be at high risk. The area under the curve was obtained from the sensitivity versus specificity curves as a measure of diagnostic accuracy for each index, and 95% confidence intervals (95% CI) for the areas under the curve were derived (219). Area under the curve values of 0.90 were considered excellent; 0.80–0.89, good; 0.70–0.79, fair; and <0.70, poor (220). Then, thresholds were developed, seeking to maximize both sensitivity (i.e., true positives) and specificity (i.e., true negatives). As such, the closest threshold to the perfect sensitivity and specificity was identified, i.e., the minimum value in equation (221): $(1- \text{sensitivities})^2 + (1 - \text{specificities})^2$. The percentage of agreement and the relationship in the classification across indices was assessed with Kappa coefficients over the receiver Operating Characteristic curves -derived thresholds (222).

Linear regression models were conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY), the *J* test and the the receiver Operating Characteristic analyses were conducted with the lmtest and the pROC R packages (v.4.1.1) (223). The level of significance was set at $p \leq 0.05$.

RESULTS

Of the 152 pregnant women (32.9±4.6 years) included, 119 had valid cardiometabolic data at the 16th g.w., and 107 at the 34th g.w. **(Figure 2)**. The sociodemographic and clinical characteristics are shown in **Table 10**.

Table 10. Sociodemographic and clinical characteristics of the Study I participants

Variable	Mean (SD)
Age (years)	32.9 (4.6)
Pre-pregnancy weight (kg) (n=140)	65.0 (12.3)
Height (cm) (n=150)	163.9 (6.2)
Pre-pregnancy BMI (kg/m²)	24.2 (4.2)
Dietary indices	
MFP (7-35)	20.6 (5.0)
MDScale (0-8)	4.0 (1.5)
SMDQ (0-8)	5.8 (1.2)
MedDietScore (0-50)	28.9 (3.9)
MDS-P (0-11)	5.7 (2.0)
Educational Status	**n (%)**
Low educational status	35 (23.0)
Medium educational status	27 (17.8)
High educational status	90 (59.2)
Smoking habit	
Current smoker	13 (8.6)
Former smoker	58 (37.0)
Never smoker	81 (38.2)
Number of children	
0	91 (59.9)
1	52 (34.2)
2	9 (5.9)
Taking nutritional supplements (yes)	128 (84.2)
Heart Disease Diagnosis (yes)	0
High cholesterol diagnosis (yes)	3 (2.0)

Values shown a mean (standard deviation) unless otherwise is indicated. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein**;** MDScale, Mediterranean Diet Scale; MDS-P, Mediterranean diet scale for pregnant women; MedDietScore, Mediterranean Diet Score; MFP, Mediterranean Food Pattern**;** SD, Standard deviation; SMDQ, Short Mediterranean Diet questionnaire.

The cross-sectional and longitudinal associations of the MD indices with the clustered cardiometabolic risk (Z-score) are shown in **Figure 4**. Greater adherence to the MFP, SMDQ, and MedDietScore were significantly associated with lower clustered cardiometabolic risk at the 16th g.w. and 34th g.w. (β's ranging from -0.193 to -0.415, *p's*<0.05). In addition, a greater MDS-P score was associated with lower clustered cardiometabolic risk at the 34th g.w. (β=-0.349, *p*<0.01). The *J* test analyses showed no significant differences in the models including the MFP and the MedDietScore at the 16th g.w. (*p*=0.256), while the SMDQ showed a lower performance than the MFP (*p*=0.05) and the MedDietScore (*p*=0.027). At the 34th g.w., the models including the MFP and the MDS-P were not statistically different (*p*=0.138), while the MedDietScore outperformed the MFP (*p*=0.003), the SMDQ (*p*<0.001) and the MDS-P (*p*<0.001).

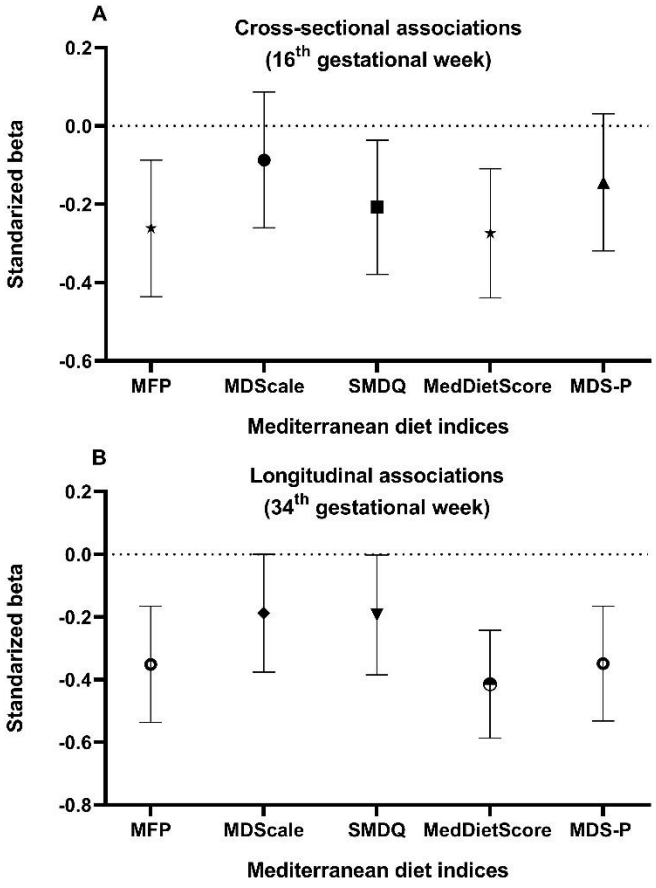

Figure 4. Linear regression analysis assessing the association of the Mediterranean diet indices and the clustered cardiometabolic risk at the 16th and 34th gestational weeks. Dots represent β values and bars represent 95% confidence interval. (A) Cross-sectional associations of the Mediterranean diet indices and the clustered cardiometabolic risk at the 16th gestational weeks. Model adjusted for age, smoking habit and number of children. (B) Longitudinal associations of the Mediterranean diet indices and the clustered cardiometabolic risk at the 34th gestational weeks. Model adjusted for age, smoking habit, number of children and exercise intervention. MDScale, Mediterranean Diet Scale; MedDietScore, Mediterranean Diet Score; MDS-P, Mediterranean Diet scale for pregnant women; MFP, Mediterranean Food Pattern; SMDQ, Short Mediterranean Diet questionnaire. Dietary indices with the same symbol did not differ significantly when compared with the J-test (p>0.256).

The cross-sectional and longitudinal associations between MD indices with the individual cardiometabolic markers (at the 16th and 34th g.w.) are shown in **Table 11**. Higher MFP was associated with lower pre-pregnancy BMI (p=0.039). Greater MFP, SMDQ, MedDietScore, and MDS-P were associated with lower SBP at the 16th and 34th g.w. (all, p<0.05). Higher MedDietScore and MDS-P were associated with lower DBP at the 34th g.w. (both, p<0.05). Greater MFP and MDS-P were associated with lower glucose at the 34th g.w. (both, p<0.05). In addition, MFP, MDScale, and MedDietScore were associated with higher HDL-cholesterol (all, p<0.05).

Table 11. Association of Mediterranean diet indices with individual cardiometabolic markers during pregnancy

Dietary Index	Pre-pregnancy BMI		SBP		DBP		Glucose		Triglycerides		HDL-C	
Cardiometabolic risk markers at the 16th gestational week (n=119)[a]												
	β*	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
MFP (7-35)	-0.194	0.039	-0.213	0.029	-0.109	0.264	-0.079	0.413	-0.102	0.289	0.188	0.040
MDScale (0-8)	-0.020	0.830	-0.142	0.135	-0.013	0.890	0.001	0.995	0.022	0.817	0.160	0.069
SMDQ (0-8)	-0.134	0.144	-0.210	0.027	-0.154	0.105	0.000	1.000	-0.060	0.520	0.196	0.027
MedDietScore (0-50)	-0.168	0.061	-0.224	0.016	-0.160	0.084	-0.009	0.920	-0.129	0.159	0.263	0.002
MDS-P (0-11)	-0.124	0.180	0.208	0.029	-0.094	0.326	-0.005	0.956	-0.011	0.905	0.100	0.266
Cardiometabolic risk markers at the 34th gestational week (n=107)[b]												
	β[c]	*p*[c]	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
MFP (7-35)	-	-	-0.215	0.033	-0.167	0.106	-0.210	0.033	-0.124	0.227	0.254	0.010
MDScale (0-8)	-	-	-0.201	0.039	-0.092	0.360	-0.177	0.064	-0.037	0.707	0.160	0.028
SMDQ (0-8)	-	-	-0.132	0.190	-0.137	0.181	-0.001	0.989	-0.101	0.320	0.158	0.106
MedDietScore (0-50)	-	-	-0.243	0.012	-0.194	0.050	-0.154	0.105	-0.183	0.062	0.348	<0.001
MDS-P (0-11)	-	-	-0.309	0.002	-0.230	0.024	-0.254	0.009	-0.136	0.178	0.146	0.137

[a]Model adjusted for age, smoking habit and number of children. [b]Model additionally adjusted for exercise intervention. Higher clustered cardiometabolic status entails higher cardiometabolic risk. [c]Pre-pregnancy BMI is not shown for gestational week 34th since it was measured prior to pregnancy period. HDL, high-density lipoprotein; MDScale, Mediterranean Diet Scale; MedDietScore, Mediterranean Diet Score; MDS-P, Mediterranean diet scale for pregnant women; MFP, Mediterranean Food Pattern; SMDQ, Short Mediterranean Diet questionnaire. *Since pre-pregnancy BMI is not affected by the intervention the model was not adjusted.

Table 12 and **Figure 5** show the receiver Operating Characteristic curves-derived thresholds together with their sensitivity, specificity, and area under the curve values. The MFP showed an area under the curve of 0.69 (95% CI: 0.59–0.79) at the 16th g.w., and of 0.67 (95% CI: 0.56–0.77) at the 34th g.w. The MedDietScore showed an area under the curve of 0.70 (95% CI: 0.60–0.81) at the 16th g.w., and of 0.71 (95% CI: 0.61–0.81) at the 34th g.w. The MDS-P showed an area under the curve of 0.62 (95% CI: 0.51–0.72) at the 16th g.w., and of 0.70 (95% CI: 0.60–0.80) at the 34th g.w. The cut-off points that maximized the sensitivity and specificity in each index were 21 for the MFP, 30 for the MedDietScore, and 6 for the MDS-P at both 16th and 34th g.w.

Table 12. Derived thresholds to identify pregnant women who adhere to the Mediterranean diet at the 16th and 34th gestational weeks

MD indices	Threshold	Sensitivity	Specificity	AUC (95% CI)
Clustered cardiometabolic risk at the 16th g.w.				
MFP (7-35)	21	0.62	0.68	0.69 (0.59-0.79)
MedDietScore (0-50)	30	0.59	0.75	0.70 (0.60-0.81)
MDS-P (0-11)	6	0.57	0.58	0.62 (0.51-0.72)
Clustered cardiometabolic risk at the 34th g.w.				
MFP (7-35)	21	0.65	0.64	0.67 (0.56-0.77)
MedDietScore (0-50)	30	0.59	0.75	0.71 (0.61-0.81)
MDS-P (0-11)	6	0.62	0.72	0.70 (0.60-0.80)

AUC: area under the curve; CI, confidence interval; G.W.: gestational week; MD: Mediterranean diet; MDS-P, Mediterranean diet scale for pregnant women; MedDietScore, Mediterranean Diet Score; MFP, Mediterranean Food Pattern.

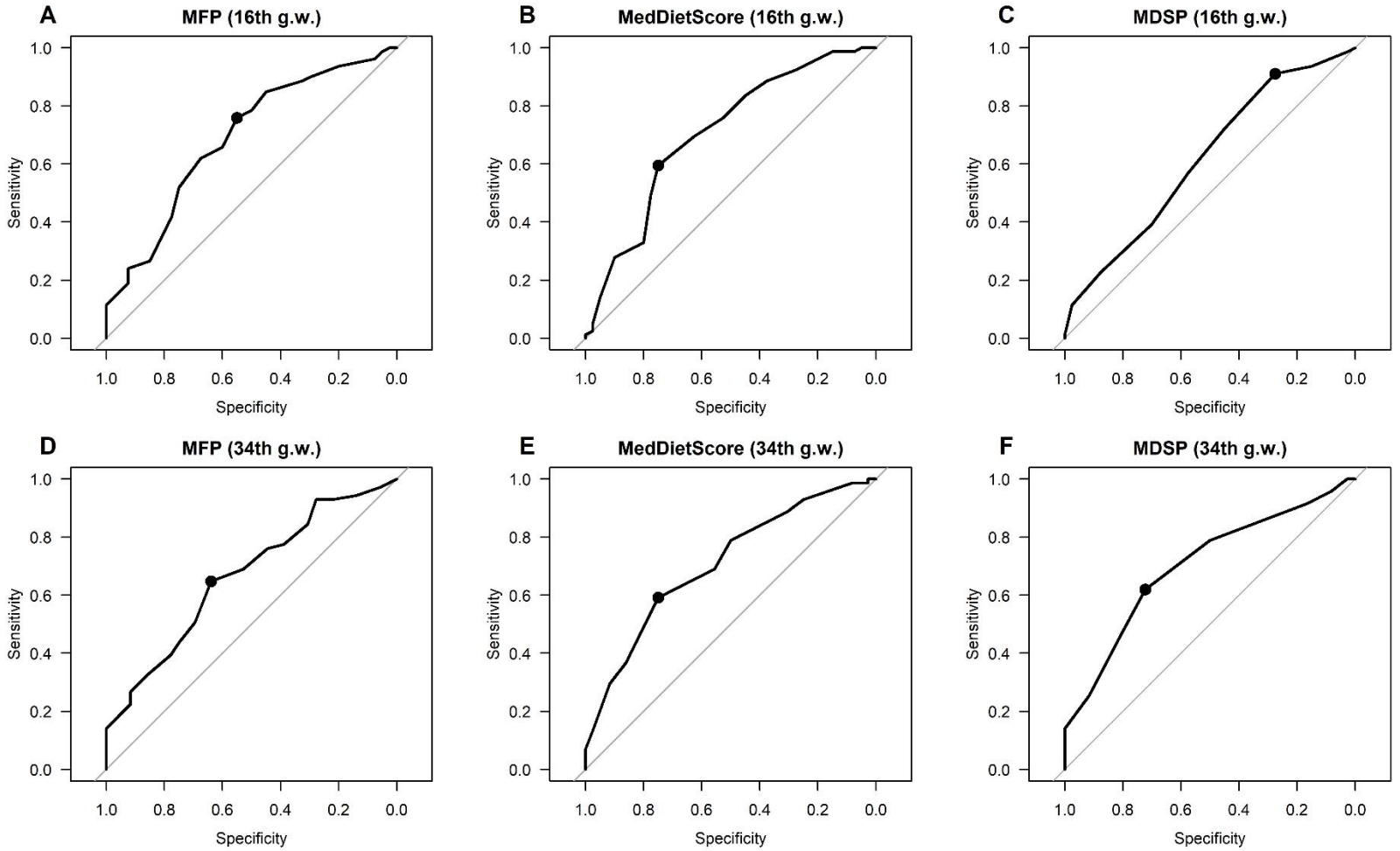

Figure 5. Discriminative power of the Mediterranean dietary indices recommended (ROC curves) to detect high cardiometabolic risk at the 16th and 34th gestational week. MDS-P, Mediterranean diet scale for pregnant women; MedDietScore, Mediterranean Diet Score; MFP, Mediterranean Food Pattern; ROC, receiver operating characteristic.

Figure 6 shows the percentage of pregnant women who adhered to the MD according to the thresholds derived for the different indices recommended. Small differences (<3%) were found in the percentage of women adhering to MD across indices.

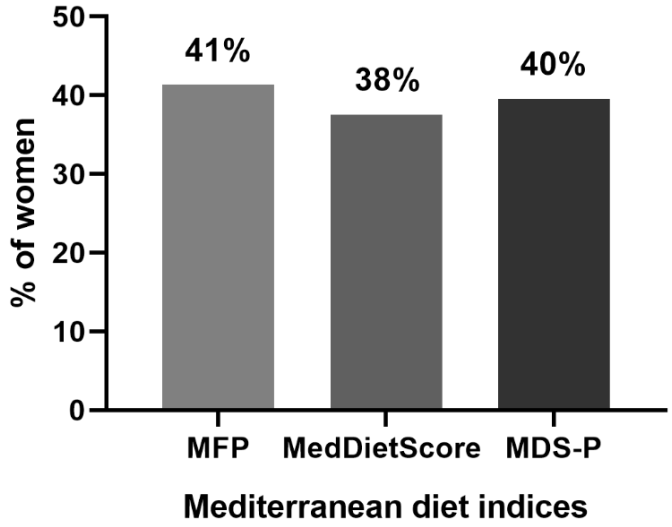

Figure 6. Percentage of participants who adhere to the Mediterranean diet according to the thresholds derived for the different recommended indices.

The percentage of agreement and the relationship between the recommended MD indices are shown in **Figure 7**. The lowest agreement was found between MDS-P and MedDietScore (% agreement= 67.8; kappa coefficient= 0.319), the highest was found between MFP and MedDietScore (% agreement= 76.3; kappa coefficient= 0.505).

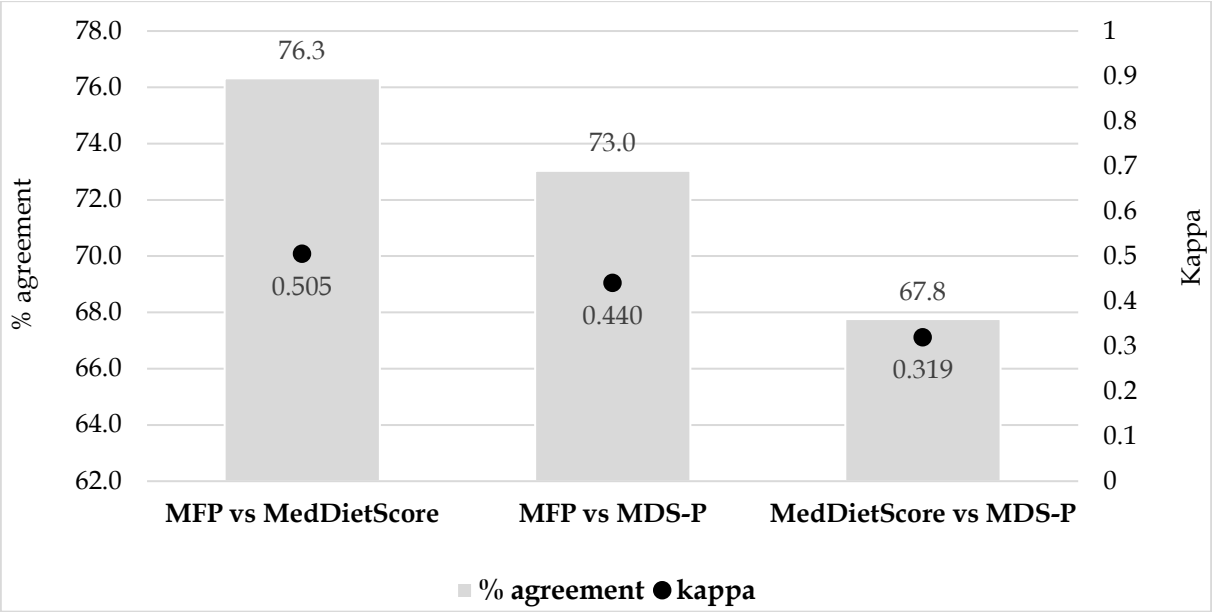

Figure 7. Percentage of agreement between the recommended Mediterranean diet indices using the receiver operating characteristic-derived thresholds. Kappa coefficients are classified as < 0, no agreement; 0-0.19, poor agreement; 0.20-0.39, fair agreement; 0.40-0.59, moderate agreement; 0.60-0.79, substantial agreement; 0.80-0.99, almost perfect agreement; 1, perfect agreement. MDScale, Mediterranean Diet Scale; MedDietScore, Mediterranean Diet Score; MDS-P, Mediterranean diet scale for pregnant women; MFP, Mediterranean Food Pattern; SMDQ, Short Mediterranean Diet questionnaire.

DISCUSSION

Our results suggest that the MedDietScore, the MFP, and the MDS-P could be recommended to assess MD adherence during pregnancy. The MedDietScore outperformed the other indices in the association with cardiometabolic risk at the 16th g.w. and 34th g.w. Furthermore, the MFP was associated with cardiometabolic risk at the 16th and 34th g.w., and the MDS-P at the 34th g.w. Among the recommended indices, the cut-off points proposed to detect high cardiometabolic risk were 21 for the MFP, 30 for the MedDietScore, and 6 for the MDS-P at both 16th and 34th g.w.

There is a lack of uniformity between indices to assess MD adherence during pregnancy (29,63,64), specifically in the number of components included, the classification categories, measurement scales, statistical parameters (mean, median, or quintiles of daily intake), and the positive/negative contribution of each component to the total score (66,67). This complicates the comparability and reproducibility of the MD adherence measurement during pregnancy (29). Previous studies have shown that maternal dietary patterns (including the MD) are associated with better cardiometabolic markers (29,44,130,224,225). We confirmed this finding with 4 out of the 5 MD indices tested with differing association sizes and

prediction capacity, suggesting that using different indices could lead to inconsistent results on the association of MD with cardiometabolic health. The MedDietScore, the MFP, and the SMDQ indices were associated with lower cardiometabolic risk at the 16th g.w., and we observed that the MedDietScore and the MFP indices were superior to the SMDQ. This suggests that these two indices (i.e., MFP and MedDietScore) could be indistinctly recommended to understand the relationship between MD and the cardiometabolic risk at the 16th g.w. At the 34th g.w., the MDS-P was also associated with lower cardiometabolic risk. In late pregnancy (i.e., 34th g.w.), the MedDietScore was superior to the MDS-P, MFP, and SMDQ indices in the association with cardiometabolic risk. Therefore, the MFP and the MedDietScore could be appropriate to assess the association between MD adherence and cardiometabolic risk at the 16th and 34th g.w., whereas the MDS-P could also be appropriate at the 34th g.w. This recommendation is considered to be feasible since all indices could be calculated with the data obtained from the same food frequency questionnaire. Discrepancies between SMDQ, MDScale and MDS-P indices regarding their association with cardiometabolic risk might be partially attributable to the components and categorization of the food components included in each index. The SMDQ index in the present study is comprised of eight elements (in servings/day or servings/week) while the MDScale includes 8 elements (in g/day) and the MDS-P additionally includes 3 elements (in mg/day or µg/day). Moreover, the MDScale relies on the median-split of the specified items and the establishment of a cut-off, in each food group, and then to the attribution of scores of 1 or 0. Otherwise, the SMDQ uses predefined cut-off portions. Regarding cereals, the MDScale includes only one cereal component (i.e., cereals) while the SMDQ index distinguishes between 3 groups of cereals such as white bread, rice, and whole grains. These components might partially explain differences between SMDQ, MDScale, and MDS-P indices and their relationship with cardiometabolic risk.

The MFP and the MedDietScore attribute a positive value when the consumption of ethanol met the established criteria. However, alcohol consumption was not considered in the scores because women must not drink alcohol during pregnancy, and data of our participants showed no consumption at all. It is worth mentioning that previous publications did not adapt the general population cut-offs points to pregnant women, which is needed given that (for example) pregnant women are not expected to drink alcohol (29,63,68). As a result, there are no adapted cut-off points for MD adherence in pregnant women. In this study, we used the receiver Operating Characteristic curves to establish such cut-off points, based on the detection of high cardiometabolic risk during pregnancy. The cut-off points that maximized the sensitivity and specificity were 21 for the MFP, 30 for the MedDietScore, and 6 for the MDS-P

at both the 16th and 34th g.w. These cut-off points are slightly different from the previously-advised for the general population. In the original validation of the MFP index (190), it was found a negative association between the MFP score and the risk for myocardial infarction. The authors observed that the risk for myocardial infarction ceased decreasing for scores ≥20. In agreement, we found that a cut-off point of 21 in the MFP maximized both the sensitivity and specificity to detect high cardiometabolic risk. Women with more than 30 points on the MedDietScore were considered adherent to the MD. In the original questionnaire, this score corresponds to medium adherence (MedDietScore of 30-33.99) but not high adherence (MedDietScore≥34) (30,226). Since we did not account for the alcohol intake, the maximum score considered for these analyses in the MedDietScore was 50 points instead of 55. Therefore, it seems plausible that women who are classified as adherent to the MD will have a lower absolute score compared to the one proposed in the original validation study (i.e., MedDietScore≥34). Participants with a MDS-P ≥6 were defined as being adherent to the MD in the present study, a cut-off that is considered adequate compliance (MDS-P of 5-8) but not high compliance (MDS-P≥9) to the MD according to the original questionnaire. The cut-off points in the original questionnaire were developed based on tertiles of the score distribution in the study participants and not based on a clinical outcome (64). In order to increase the MD adherence to achieve scores greater than or equal to the ones proposed, it is necessary to increase those components that improve the MD adherence score (e.g., whole-grain cereals, fruits, vegetables, pulses or fish) and decrease those that reduce it (e.g., red meat or high glycemic foods). For instance, in the MedDietScore case increasing intake of fruit from 1-4 times/month to 13-18 times/month would increase the MedDietScore by 3 points. Similarly, decreasing intake of red meat and subproducts from 9-12 times/months to 1-4/month would increase the MedDietScore by another 2 points.

Another interesting finding was that only 40% of participants were adherent to the MD accordingly to the receiver Operating Characteristic curves-derived cut-off points. This concurs with recent evidence suggesting that pregnant women are drifting away from the MD (29). Small differences (<3%) were found between the MFP, MedDietScore, and MDS-P in the percentage of individuals adherent to the MD, which increases the robustness of the finding. This could be explained because dietary indices employed in the present study were highly correlated to each other and we used consistent methods to classify the cardiometabolic risk and to derive the cut-off points for each index. This could be attributable to the fact that the same food frequency questionnaire (189) was employed and the same trained nutritionist administered the food frequency questionnaires, increasing the internal consistency.

However, the classification agreement is far from perfect (68-76%). This indicates that around 1/3 of the women in this study are being differently classified by the indices (either in the favourable or unfavourable diet). This is also supported by the kappa coefficients, which showed poor to moderate agreement across indices. Furthermore, different indices seem to associate with different cardiometabolic risk factors, suggesting that each index might associate with a different risk profile, which might have important clinical implications. However, establishing these patterns of association is not within our study objectives, and the sample size and our study design do not allow reaching conclusive findings on this. Future studies might seek for association patterns between the indices recommended in this study and each one of the cardiometabolic risk factors.

A recent systematic review (227) where five MD assessment indices were compared, showed that the MedDietScore provides the best evidence of MD adherence. Similarly, in the present study, the MedDietScore was superior to the rest of the studied MD indices in the association with cardiometabolic risk along the pregnancy course. The MedDietScore is particularly useful because, for each of the assessed food groups, five classifications are possible; thus, it has been suggested to be more representative of the consumption of MD food items (66). In the present study, the MedDietScore showed a high percentage of agreement with the MFP (74%) but a smaller-but still moderate- percentage of agreement with the MDS-P (68%). This concurs with previous studies (29,66) where the MedDietScore and the MDScale showed a similar percentage of agreement (i.e., 65%). MDS-P is based on the MDScale adding three components (i.e., intake of Fe, folic acid and Ca, since these micronutrients are required in optimal amounts during pregnancy). In the MDS-P one point is assigned if the participant consumes up to two-thirds of the recommended intake of Fe, folic acid, or calcium and/or takes supplements. Most participants (85%) were taking supplements which means that most of the pregnant women could have scored 3 points in those items. Therefore, not many differences may be expected between the MDScale concerning the MDS-P, explaining why a similar percentage of agreement was found between MedDietScore and MDS-P.

Limitation and strengths

Firstly, results should be interpreted cautiously given the relatively small sample size for this specific purpose. Secondly, as this is a cross-sectional study, causality cannot be determined. Regarding strengths, we assessed dietary habits and cardiometabolic status by employing widely used MD indices which are often used as a reference in adult population studies to study cardiometabolic risk. Moreover, we included a wide range of cardiometabolic factors

within the overall risk score created, which strengthens the usefulness of these proposed indices.

CONCLUSION

We recommend the potential use of the MFP and the MedDietScore indices at the 16th g.w. and the MedDietScore at the 34th g.w. to assess the positive or negative cardiometabolic impact of the diet during pregnancy. The receiver Operating Characteristic curves-derived threshold for the MFP and MedDietScore were 21 and 30 points, respectively. This suggests that these two indices could be recommended to understand the relationship between the MD and the cardiometabolic risk during pregnancy above the rest of the MD indices included in this study. This screening would help to identify pregnant women with a higher risk of complications since worse cardiovascular risk has been associated with adverse pregnancy outcomes.

STUDY II: Associations between sociodemographic factors, lifestyle behaviors, pregnancy-related determinants, and Mediterranean diet adherence among pregnant women: The GESTAFIT project

Flor-Alemany M, Nestares T, Marín-Jiménez N, Baena-García L, Aparicio VA.

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ABSTRACT

Aim: To examine sociodemographic factors, lifestyle behaviors and pregnancy-related determinants associated with MD adherence during pregnancy.

Methods: A total of 152 Caucasian pregnant women were included in this cross-sectional study. Dietary habits and MD adherence were assessed with a food frequency questionnaire. Physical activity levels and physical fitness components (cardiorespiratory fitness, relative muscle strength, and flexibility) were objectively measured. A clustered overall physical fitness index was calculated.

Results: Participants with a high MD adherence were older, had a lower BMI, spent more time in moderate-vigorous physical activity, had a greater overall physical fitness, cardiorespiratory fitness, and relative muscle strength compared to participants with low MD adherence (all, $p<0.05$). When we explored factors associated with improved MD adherence with logistic regression analysis, we found that the following factors: lower pre-pregnancy BMI (OR=2.337; $p=0.026$), meeting physical activity recommendations (OR=2.377; $p=0.045$), higher relative muscle strength (OR=2.265; $p=0.016$) and higher overall physical fitness (OR=5.202; $p=0.004$), increased the chances to adhere to the MD.

Conclusion: Older age, lower BMI, greater physical fitness, and meeting physical activity recommendations were associated with higher MD adherence. These factors should be considered to better design educational programs and guidelines focused on improving materno-fetal health status during pregnancy.

INTRODUCTION

Inadequate maternal nutrition can adversely affect both, the mother and the growing fetus (228). Many analyses in this field have been based on a single or a few food items or nutrients (229). Notwithstanding, epidemiological studies have underlined the importance of assessing the impact of the overall diet quality on health, emphasizing the concept of dietary patterns (13,36). The analysis of dietary patterns has proven to be a simple and effective way to improve different health outcomes (13). In this context, the MD is known to be one of the healthiest dietary patterns which protect against the development of many diseases in all age groups (36,45,47). Mediterranean-style diet has been associated with lower GWG and lower risk of gestational diabetes (42), lower BP (44), and lower cardiometabolic risk in the adult population (45). With this in mind, few studies have examined the potential benefits that the MD adherence could exert on maternal and fetal outcomes (considering the MD as a whole rather than focusing on the effect of its components) (47). Regarding fetal outcomes, recent studies have shown the protective role of MD during pregnancy against excessive or insufficient fetal growth (48), preterm birth (49), neural tube defects (50), asthma and allergy (51), excessive adiposity and other adverse metabolic markers in the offspring (52). The identification of factors that may influence the MD adherence would be key in programs aimed at improving the level of adherence to this particular dietary pattern. This fact deserves special attention taking into account studies suggesting that pregnant women are drifting away from the Mediterranean diet-like pattern (211). Therefore, this study aimed to evaluate the influence of sociodemographic factors (age, education, marital and working status), lifestyle behaviors (smoking habit, physical activity levels, physical fitness components), and pregnancy-related determinants (pre-pregnancy BMI, parity, number of miscarriages and number of children) on MD adherence during pregnancy.

METHODS

Study design and participants

The complete methodology of GESTAFIT project has been described elsewhere (182). Briefly, from the 159 pregnant women who met the inclusion-exclusion criteria **(Table 6)**, this cross-section study included 152 pregnant women (mean age 32.9±4.6 years) who had valid data in sociodemographic characteristics, lifestyle behaviors and pregnancy-related determinants, and food frequency questionnaires **(Figure 2)**. Written informed consent was signed by all participants.

Sociodemographic factors

The evaluation procedures were carried out at the 16th g.w. when an initial survey (anamnesis) was performed to compile information on the sociodemographic characteristics and pregnancy-related determinants (i.e., age, number of miscarriages, parity, smoking habit, educational level, and marital and educational status).

Maternal anthropometry and body composition

Pre-pregnancy body weight was self-reported. At the 16th g.w. height was measured using a scale (InBody R20; Biospace, Seoul, Korea) and a stadiometer (Seca 22, Hamburg, Germany), respectively. Those measurements were employed to calculate pre-pregnancy BMI as weight (kg) divided by squared height (m^2).

Physical activity levels

To objectively measure physical activity levels at the 16th g.w., accelerometry was employed. Women were asked to wear a tri-axial accelerometer attached to their non-dominant waist (Actigraph GT3X+, Pensacola, Florida, US) for nine consecutive days. Sedentary time (min/week), moderate-vigorous physical activity (min/week), total physical activity levels (min/week), and percentage of participants who met the international physical activity recommendations of at least 150 min of moderate-vigorous physical activity per week were calculated (193).

Physical fitness tests

The complete physical fitness battery employed has been previously described (182). Briefly, the back-scratch test (as a measure of overall shoulder range of motion) was employed to assess flexibility (194). Cardiorespiratory fitness was assessed with the 6-min walk test, along a 45.7-m rectangular course (194). Muscle strength was evaluated by handgrip (as a measure of overall body strength), with a digital dynamometer (TKK 5101 Grip-D; Takey, Tokyo, Japan) (195). Relative muscle strength was calculated as absolute handgrip strength divided by maternal weight at the 16th g.w., and used in the analyses as recommended to address the confounding of strength by weight status (230).

Clustered physical fitness

A clustered physical fitness index (overall physical fitness) was created as the mean of the z-scores [(value-mean)/standard deviation)] of flexibility, relative muscle strength, and cardiorespiratory fitness. Higher scores indicate better physical fitness levels.

Dietary assessment

A food frequency questionnaire validated in Spanish non-pregnant adult population was employed to assess dietary habits (189). Although questionnaires were administered to participants at the 16th g.w. and 34th g.w. by a trained nutritionist, the present study only targeted women in the second trimester of pregnancy (13th to 27th g.w.). The first trimester of pregnancy is characterized in most women by morning sickness, whereas dietary habits during the second trimester of pregnancy are relatively more constant, being more representative of dietary behavior across the whole gestational period (231). Moreover, we explored differences in MD adherence between the early second trimester of pregnancy (i.e., 16th g.w.) and the third trimester of pregnancy (i.e., 34th g.w.) and overall MD adherence remained unchanged (232). Consequently, the dietary pattern registered at the 16th g.w. was taken as representative in this study sample. The MFP was employed to assess MD adherence as previously done in this study sample (232). This dietary index was constructed with the data obtained from the food frequency questionnaire considering the intake of olive oil, fiber, fruits, vegetables, fish, cereals, meat, and alcohol. The score ranges from 8 to 40 points. However, to adapt the score to pregnant women we did not consider alcohol consumption, thus, the maximum score for pregnant women in the present study sample ranges from 7 to 35 points. Women were defined as adherent to the MD if they had a MD adherence above 21 points as previously stated in this study sample (233). Therefore, participants were classified as having a high MD adherence if they had a score of ≥21 points in the MFP index.

Statistical analysis

Sociodemographic factors, lifestyle behaviors, pregnancy-related determinants, physical activity levels, overall physical fitness, and individual physical fitness components were compared between women with high MD adherence versus women with low MD adherence by Student's t-test. To determine the differences among qualitative variables a chi-square test was performed. Univariate and multivariate logistic regression analyses were performed to explore potential sociodemographic factors, lifestyle behaviors and pregnancy-related determinants that could be associated with MD adherence. The odds ratio (OR) with a 95%

CI was estimated, the level of significance was set at $p<0.05$. Linear regression analyses were performed to explore the association of dietary habits with overall physical fitness and individual physical fitness components. Differences in dietary habits by MD adherence (low MD adherence vs. high MD adherence) were compared by Student's t-test. Differences in dietary habits by physical activity recommendations (not-meeting physical activity recommendations vs. meeting physical activity recommendations) were compared by Student's t-test. Differences in dietary habits by smoking habit (current smoker vs. no smoker) were compared by Student's t-test. To accomplish this, the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY) was employed.

RESULTS

Sociodemographic factors, lifestyle behaviors and pregnancy-related determinants of the study participants by the degree of MD adherence are shown in **Table 13**. Participants with a high MD adherence (i.e., ≥21 points in the MFP index) were older ($p=0.022$) and had a lower pre-pregnancy BMI ($p=0.020$) compared to participants with a low MD adherence (i.e., <21 points in the MFP index). In addition, pregnant women with high MD adherence spent more time in moderate-vigorous physical activity compared to the group with low adherence ($p=0.054$). No differences were found regarding sedentary time and total physical activity (min/week) (both, $p>0.05$). Furthermore, the group with high MD adherence had greater cardiorespiratory fitness ($p=0.002$), relative muscle strength ($p=0.021$), flexibility ($p=0.032$) and overall physical fitness ($p<0.001$), compared to the group with low MD adherence.

Table 13. Sociodemographic and clinical characteristics of the Study II participants

	All women (n=152)	Low MD adherence (n=89)	High MD adherence (n=63)	*p*
Age (years)	32.9 (4.6)	32.2 (4.4)	33.9 (4.7)	0.022
Pre-pregnancy BMI (kg/m²) (n=81 vs 57)	24.9 (4.2)	24.9 (4.5)	23.2 (3.7)	0.020
PA (n=72 vs 60)				
Sedentary time (min/day)	514.0 (91.5)	509.1 (93.0)	519.8 (90.0)	0.509
Moderate-to-vigorous PA (min/day)	36.4 (20.8)	33.2 (20.7)	40.3 (20.5)	0.054
Total PA (min/day)	423.7 (88.9)	411.8 (90.5)	437.9 (85.4)	0.092
Physical fitness				
Cardiorespiratory fitness (m) (n=37 vs 25)	605.7 (48.1)	590.8 (44.2)	627.7 (45.8)	0.002
Relative muscle strength (kg/body weight) (n=89 vs 60)	0.414 (0.08)	0.402 (0.08)	0.431 (0.07)	0.021
Flexibility (cm) (n=89 vs 61)	3.9 (6.0)	3.1 (6.2)	5.2 (5.3)	0.032
Overall physical fitness (Z-score) (n=37 vs 25)	0.1 (0.7)	-0.2 (0.6)	0.5 (0.5)	<0.001
Miscarriages n(%)				
No	63 (41.4)	39 (43.8)	24 (38.1)	0.480
Yes	89 (58.6)	50 (56.2)	39 (61.9)	
Parity n(%)				
Nullipara	91 (59.9)	52 (58.4)	39 (61.9)	0.667
Primipara	61 (40.1)	37 (41.6)	24 (38.1)	
Marital status n(%)				
Non-married	62 (40.8)	34 (22.4)	28 (18.4)	0.440
Married	90 (59.2)	55 (61.8)	35 (55.6)	
Educational level n(%)				
No university studies	62 (40.8)	41 (46.1)	21 (33.3)	0.116
University studies	90 (59.2)	48 (53.9)	42 (66.7)	
Working status n(%)				
Unemployed	48 (31.6)	31 (34.8)	17 (27.0)	0.305
Employed	104 (68.4)	58 (65.2)	46 (73.0)	
Smoking habit n(%)				
No smoking	139 (91.4)	80 (89.9)	59 (93.7)	0.414
Smoking	13 (8.6)	9 (10.1)	4 (6.3)	

Column totals not equalling the total sample size are due to missing data. P values for categorical variables were based on $\chi 2$ tests. BMI, body mass index; G.W., gestational week; PA, physical activity.

Differences in dietary habits by MD diet adherence (low MD adherence vs. high MD adherence) are shown in **Table 14**. We found that women with a high MD adherence had a greater intake of whole-grain cereals (p<0.001), fruits (p<0.001), vegetables (p<0.001), pulses (p=0.002), fish (p<0.001), olive oil (p<0.001) and nuts (p<0.001) and a lower intake of red meat and subproducts (p=0.032) and sweets (p<0.001).

Table 14. Differences in dietary habits by Mediterranean diet adherence (low Mediterranean diet adherence vs. high Mediterranean diet adherence)

	Low Mediterranean diet adherence (n=89)	**High Mediterranean diet adherence (n=63)**	***p***
Whole-grain cereals (s/week)	2.8 (4.3)	6.6 (5.4)	<0.001
Potatoes (s/week)	2.4 (1.3)	2.3 (1.2)	0.518
Fruits (s/week)	11.5 (5.3)	18.7 (6.5)	<0.001
Vegetables (s/week)	19.5 (9.1)	34.1 (11.1)	<0.001
Pulses (s/week)	2.5 (1.1)	3.1 (1.3)	0.002
Fish (s/week)	4.2 (1.9)	5.8 (2.4)	<0.001
Red meat and subproducts (s/week)	6.0 (3.2)	4.8 (3.6)	0.032
Poultry (s/week)	2.8 (1.2)	2.5 (4.5)	0.113
Dairy products (s/week)	12.9 (8.3)	12.9 (8.8)	0.958
Olive oil (s/week)	11.2 (6.6)	15.7 (6.5)	<0.001
Nuts (s/week)	3.3 (7.8)	7.9 (5.7)	<0.001
Sweets (s/week)	12.3 (7.6)	7.5 (4.1)	<0.001

Values are shown as mean (Standard deviation). S, servings.

When we explored factors associated with improved MD adherence with logistic regression analysis **(Figure 8)**, we found that the following factors: having pre-pregnancy normal weight (OR=2.337; p=0.026), meeting physical activity recommendations (OR=2.377; p=0.045), higher relative muscle strength (OR=2.265; p=0.016) and higher overall physical fitness (OR=5.202; p=0.004), increased the chances to adhere to the MD.

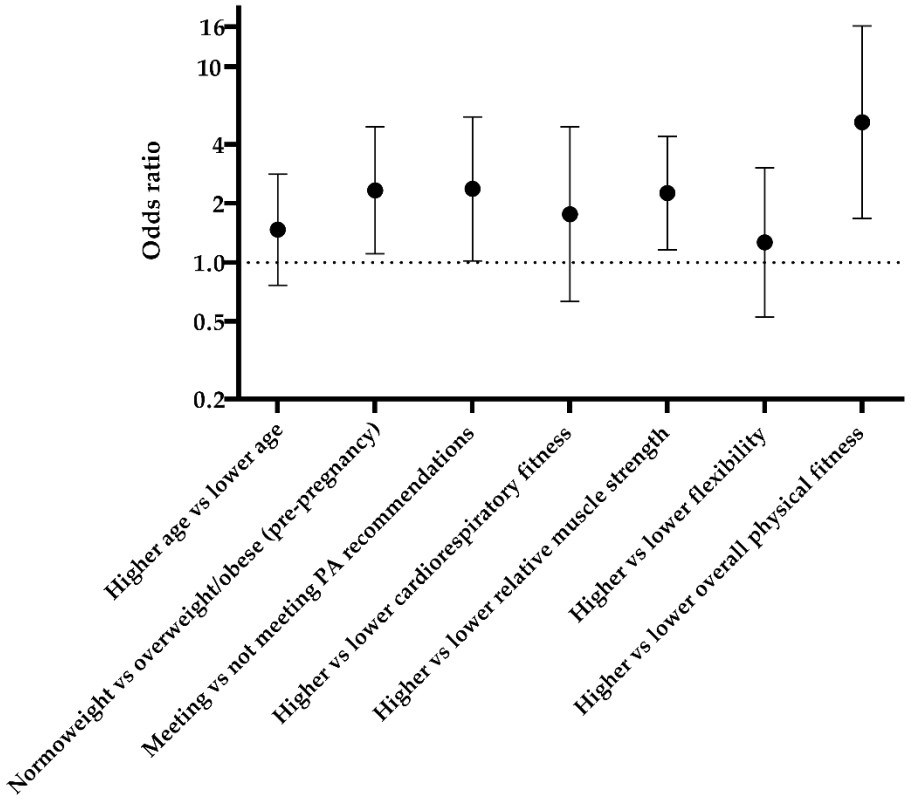

Figure 8. Determinants of adherence to the Mediterranean diet. PA, physical activity.

The linear regression analysis assessing the association of dietary habits with individual physical fitness components and overall physical fitness is shown in **Table 15**. A greater consumption of whole-grain cereals (p=0.012), fruits (p=0.003), and fish (p=0.031) were associated with greater cardiorespiratory fitness. A higher intake of red meat and subproducts was associated with lower cardiorespiratory fitness (p=0.032). Regarding relative muscle strength, a higher intake of poultry was associated with lower relative muscle strength (p=0.014). No associations were found between dietary habits and flexibility (all, p>0.05). In addition, a higher intake of fruits (p=0.015) and vegetables (p=0.004) and a lower intake of poultry (p=0.041) were associated with greater overall physical fitness.

Table 15. Associations of dietary habits with overall physical fitness and physical fitness components

	B	β	Confidence interval 95% (B)		*p*
			Lower	Upper	
Cardiorespiratory fitness (m) (n=62)					
Whole-grain cereals (s/week)	2.862	0.316	0.639	5.084	0.012
Potatoes (s/week)	-9.129	-0.222	-19.459	1.201	0.082
Fruits (s/week)	2.190	0.366	0.752	3.629	0.003
Vegetables (s/week)	0.811	0.207	-0.177	1.800	0.106
Pulses (s/week)	0.215	0.006	-8.768	9.198	0.962
Fish (s/week)	5.711	0.275	0.552	10.870	0.031
Red meat and subproducts (s/week)	-4.111	-0.272	-7.868	-0.355	0.032
Poultry (s/week)	3.001	0.084	-6.214	12.217	0.517
Dairy products (s/week)	-1.313	-0.226	-2.772	0.147	0.077
Olive oil (s/week)	-0.014	-0.002	-1.746	1.719	0.987
Nuts (s/week)	1.654	0.198	-0.462	3.771	0.123
Relative muscle strength (kg/body weight) (n=149)					
Whole-grain cereals (s/week)	0.002	0.035	-0.006	0.009	0.671
Potatoes (s/week)	0.024	0.134	-0.005	0.052	0.102
Fruits (s/week)	0.003	0.114	-0.001	0.008	0.168
Vegetables (s/week)	0.002	0.090	-0.001	0.005	0.275
Pulses (s/week)	-0.001	-0.008	-0.031	0.028	0.922
Fish (s/week)	0.005	0.048	-0.011	0.021	0.559
Red meat and subproducts (s/week)	-0.005	-0.077	-0.016	0.006	0.350
Poultry (s/week)	-0.034	-0.200	-0.061	-0.007	0.014
Dairy products (s/week)	0.002	0.095	-0.002	0.007	0.251
Olive oil (s/week)	0.001	0.036	-0.004	0.006	0.667
Nuts (s/week)	0.005	0.112	-0.002	0.012	0.174
Flexibility (cm) (n=150)					
Whole-grain cereals (s/week)	0.071	0.062	-0.116	0.258	0.454
Potatoes (s/week)	0.121	0.026	-0.638	0.880	0.753
Fruits (s/week)	0.059	0.072	-0.074	0.192	0.384
Vegetables (s/week)	0.040	0.083	-0.038	0.117	0.312
Pulses (s/week)	-0.280	-0.058	-1.059	0.499	0.479
Fish (s/week)	0.340	0.129	-0.086	0.765	0.117
Red meat and subproducts (s/week)	-0.250	0.142	-0.534	0.034	0.084
Poultry (s/week)	-0.847	-0.176	-1.724	0.030	0.058
Dairy products (s/week)	-0.046	-0.065	-0.160	0.068	0.426
Olive oil (s/week)	0.050	0.058	-0.090	0.189	0.481
Nuts (s/week)	0.078	0.066	-0.114	0.270	0.422
Overall physical fitness (z-score) (n=62)					
Whole-grain cereals (s/week)	1.482	0.201	-0.384	3.348	0.117
Potatoes (s/week)	0.043	0.027	-0.378	0.464	0.838
Fruits (s/week)	3.426	0.306	0.678	6.174	0.015
Vegetables (s/week)	6.227	0.364	2.114	10.339	0.004
Pulses (s/week)	0.072	0.038	-0.424	0.569	0.772
Fish (s/week)	0.586	0.182	-0.232	1.403	0.157
Red meat and subproducts (s/week)	-0.922	-0.208	-2.040	0.196	0.104
Poultry (s/week)	-0.486	-0.260	-0.952	-0.021	0.041
Dairy products (s/week)	0.473	0.041	-2.502	3.448	0.752
Olive oil (s/week)	0.094	0.009	-2.481	2.670	0.942
Nuts (s/week)	1.634	0.204	-0.390	3.657	0.112

β, standardized beta; S, serving.

Differences in dietary habits by physical activity recommendations (not-meeting physical activity guidelines vs. meeting physical activity guidelines) are shown in **Table 16**. Women meeting physical activity recommendations had a higher intake of whole-grain cereals (p=0.012), lower intake of potatoes (p=0.017), greater intake of fish with evidence of statistical significance (p=0.053), and nuts (p=0.021) compared to their counterparts. Differences in dietary habits by smoking habit (current smoker vs. no smoker) are shown in **Table 17**. Current smokers had a lower intake of whole-grain cereals (p=0.012), lower intake of fruits (p=0.019), higher intake of pulses (p=0.007), and higher intake of sweetened beverages (p=0.024) compared to non-smokers.

Table 16. Differences in dietary habits by meeting physical activity recommendations (not-meeting physical activity guidelines vs. meeting physical activity guidelines)

	Not meeting PA recommendations (n=103)	**Meeting PA recommendations (n=29)**	***p***
Whole-grain cereals (s/week)	3.9 (4.6)	7.2 (6.2)	0.012
Potatoes (s/week)	2.5 (1.2)	1.9 (1.1)	0.017
Fruits (s/week)	14.2 (7.5)	16.7 (6.6)	0.109
Vegetables (s/week)	26.6 (11.9)	29.9 (11.2)	0.186
Pulses (s/week)	2.8 (1.3)	2.8 (1.2)	0.810
Fish (s/week)	4.8 (2.2)	5.7 (2.5)	0.053
Red meat and subproducts (s/week)	5.6 (3.5)	6.0 (3.8)	0.561
Poultry (s/week)	2.8 (1.3)	2.8 (1.8)	0.910
Dairy products (s/week)	12.4 (7.8)	13.8 (9.9)	0.409
Olive oil (s/week)	12.9 (7.1)	15.3 (6.0)	0.100
Nuts (s/week)	4.6 (4.7)	7.7 (6.3)	0.021

Values shown as mean (Standard deviation). PA, physical activity; S, servings.

Table 17. Differences in dietary habits by smoking status (current smoker vs. no smoker)

	Non-smoker (n=139)	**Current smoker (n=13)**	***p***
Whole-grain cereals (s/week)	4.6 (5.3)	1.2 (3.0)	0.012
Potatoes (s/week)	2.3 (1.3)	3.0 (1.3)	0.100
Fruits (s/week)	14.9 (7.2)	10.0 (6.9)	0.019
Vegetables (s/week)	25.5 (12.2)	29.4 (14.9)	0.273
Pulses (s/week)	2.7 (1.3)	3.3 (0.6)	0.007
Fish (s/week)	4.9 (2.3)	4.4 (2.0)	0.421
Red meat and subproducts (s/week)	5.5 (3.5)	6.7 (2.7)	0.159
Poultry (s/week)	2.7 (1.4)	2.9 (1.1)	0.632
Dairy products (s/week)	12.7 (8.4)	14.7 (8.9)	0.398
Olive oil (s/week)	13.2 (6.9)	11.3 (7.2)	0.338
Nuts (s/week)	5.2 (5.1)	4.4 (6.1)	0.587
Sweetened beverages (s/week)	1.6 (2.1)	3.7 (2.9)	0.024

Values shown as mean (Standard deviation). S, servings.

DISCUSSION

Our results suggest that a higher MD adherence was more frequent in older pregnant women and those with lower pre-pregnancy BMI. This higher adherence was also associated with healthy behaviors such as spending more time in moderate-vigorous physical activity and meeting physical activity recommendations, avoiding tobacco, as well as with other possible determinants of health such as greater overall physical fitness during pregnancy.

Previous evidence (211,212) suggests that pregnant women are drifting away from the Mediterranean dietary pattern. We found that less than half of the participants (41%) had a high MD adherence which concurs with the prevalence of 33% reported by a study conducted in the same geographical area in Spanish pregnant women (216). A recent systematic review conducted by Doyle et al. (234) has shown that dietary habits during pregnancy also depend on other health-related behaviors (with older, better educated, affluent, non-smoking, and physically active women being more likely to follow healthier dietary patterns). Therefore, this review (234) highlights the need for more studies to assess sociodemographic and pregnancy-related factors that might affect the diet during this stage. As a result, it is clinically relevant to determine factors that might be associated with this low MD adherence in this population and to identify those that might increase MD adherence.

In our study, women with high MD adherence presented lower BMI before pregnancy which is in agreement with previous evidence suggesting that pre-pregnancy BMI (235) was inversely associated with diet quality during pregnancy. It is important to highlight that a poorer diet quality before pregnancy may contribute to a greater pre-pregnancy BMI. As a result, it would be possible that overweight and obese pregnant women had lower diet quality and therefore, lower MD adherence during the first trimester of pregnancy. Notwithstanding, since we do not have available data regarding dietary habits before pregnancy, we cannot verify this hypothesis. In this context, it has been suggested that the intake of individual food groups such as fruits, vegetables and fish (236–239) remained similar during pregnancy compared to the pre-pregnancy period. In contrast, intake of red meat, bread, rice, pasta and potatoes significantly decreased between preconception and pregnancy (236). Usually, the overall dietary pattern does not substantially change from preconception to pregnancy periods, apart from minor changes in individual food groups (237,240).

Regarding social determinants, educational level and working status are widely employed as indicators of socio-economic status (241). In our study, no differences in MD adherence were found between women who has university studies and those who were working compared to their counterparts. This is in line with a previous study conducted by Maugeri et al. (242) in

pregnant women where no associations of socio-demographic characteristics with the adherence to a "prudent" dietary pattern (characterized by high intake of potatoes, raw and cooked vegetables, legumes, rice, and soup) were found. The fact that most of the participants of the present study sample had University studies and were working might explain the absence of greater associations possibly due to our small and homogenous sample (small room for change). Notwithstanding, it has been stated that healthy dietary habits are more common among older and high-educated individuals (242). Our results partially confirm these findings since women with a high MD adherence were older than those who had a low MD adherence. This is in agreement with previous evidence (44,52) suggesting that older women are more likely to have a better diet quality compared to younger women. The authors (44,52) attributed this difference to the fact that older women were likely to have planned pregnancies, better nutritional knowledge and, consequently, were more likely to eat more healthily to prepare for the pregnancy and to have better adherence to national guidelines, and, therefore higher diet quality.

Among the lifestyle behaviors that may exert an influence on dietary habits, smoking habit has been named as a determinant of unhealthy dietary patterns in adult pregnant and non-pregnant populations (243). However, we did not observe a statistically significant impact of smoking habit on MD adherence during pregnancy in our study sample. This might be partially explained by the fact that the majority of pregnant women (91%) were not smokers which might have prevented us to find statistical differences between groups regarding MD adherence. We found that current smokers had a lower intake of whole-grain cereals and fruits and a higher intake of pulses and sweetened beverages compared to non-smokers. Previous evidence (211) suggested that women who smoke during the first trimester of pregnancy consume less fruit and more sweetened beverages throughout the pregnancy and post-partum periods, and a greater amount of red and processed meat, sweet cereals, and legumes in the second trimester (211) which is in agreement with our findings.

There is a consensus that people with higher physical activity levels tend to present also other healthy lifestyle behaviors than their sedentary counterparts (244). Savard et al. (245) showed that the best predictor of poorer diet quality (during the second trimester of pregnancy) was lower physical activity levels (assessed with the Pregnancy Physical Activity Questionnaire). In our study, participants with a high MD adherence spent more time in moderate-vigorous physical activity compared to those with a low MD adherence. This might be partially explained by the more varied diet consumed by physically active people, as previously suggested (246). We confirmed that women meeting physical activity recommendations

showed a greater intake of whole-grain cereals, potatoes, fish, and nuts (components of the MD) compared to their counterparts. The positive association between physical activity and MD adherence is noteworthy in this context, suggesting that health interventions should address adequate diet and physical activity levels in conjunction (216). In addition, participants with a high MD adherence presented greater cardiorespiratory fitness, muscle strength, flexibility and greater overall physical fitness compared to those with low MD adherence. There is little evidence to support the association between MD adherence and/or isolated food groups and overall physical fitness during pregnancy, but it requires special attention due to its potential maternal-fetal benefits on the prevention of adverse perinatal outcomes (247). Cardiorespiratory fitness is one of the most relevant components of physical fitness since it is especially identified as an important marker of cardiovascular health (248). Recent evidence suggests a strong relationship between dietary patterns and cardiorespiratory fitness (248). In addition, this physical fitness component is positively related to diet quality, fruit and vegetable intake and negatively associated with a meat dietary pattern (reflecting a dietary pattern with relatively high loadings of meat) (249). Similarly, we observed that women with a high MD adherence performed better in the cardiorespiratory fitness test (walking around 40 m more) compared with women with a low MD adherence. In addition, a higher consumption of whole-grain cereals, fruits, and fish were associated with greater cardiorespiratory fitness whereas a higher intake of red meat and subproducts was associated with lower cardiorespiratory fitness. The Mediterranean dietary pattern is rich in cardio-protective nutrients, fiber, antioxidants compounds such as β-carotene, vitamin C, and vitamin E, and bioactive compounds including monosaturated and polyunsaturated fatty acids, present in plan food, fish, nuts, and extra-virgin olive oil (248,250,251). Therefore, it seems plausible that higher adherence to this dietary pattern might be associated with greater cardiorespiratory fitness. In this sense, previous evidence has shown its effects on improving aerobic capacity (252) which might be partially explained through decreasing vasoconstriction and BP (253), modulating the immune response, and reducing inflammation and oxidative stress (254).

In the same line, relative muscle strength was associated with improved MD adherence during pregnancy. This is in agreement with previous evidence (255) where an association of a healthy dietary pattern characterized by high consumption of fruits and vegetables with greater levels of muscle strength and balance was found in women during the first stage of adult life (255). However, given the present cross-sectional design is not possible to determine the direction of the relationship between individual physical fitness components, overall physical fitness, and

MD adherence. As a result, it is not clear whether a higher MD adherence is accompanied by a better physical fitness, or those that present an optimal physical fitness comply with a greater MD adherence.

Limitations and strengths

Limitations of the present study need to be mentioned. Firstly, a major limitation of our study is that physical fitness tests have not been validated in pregnancy. Notwithstanding, this represents an inherent limitation of pregnancy studies, and the employed physical fitness tests are characterized by good psychometric properties and are adaptable, viable, and safe for clinical populations (256–258). Secondly, dietary patterns differ between places, populations, and cultural contexts, so a direct comparison with other non-Spanish populations and other healthy dietary patterns cannot be warranted. Regarding strengths, we include a wide range of sociodemographic, lifestyle/health-related behaviors, and pregnancy-related determinants. In addition, physical activity was objectively estimated through accelerometry which is considered the "gold standard" method.

CONCLUSION

The current study presents evidence on specific factors influencing MD adherence during gestation. Older age, lower BMI, greater overall physical fitness, greater cardiorespiratory fitness, muscle strength, and elements of a healthy lifestyle such as avoiding tobacco and meeting physical activity recommendations were associated with higher MD adherence. All these factors should be considered for a better design of specific educational programs and guidelines focused on improving health status during pregnancy.

SECTION II. Influence of Mediterranean diet during pregnancy on materno-fetal cardiometabolic health

STUDY III: Association of Mediterranean diet adherence during pregnancy with maternal and neonatal lipid, glycemic and inflammatory markers. The GESTAFIT project

Flor-Alemany M, Acosta-Manzano P, Migueles JH, Baena-García L, Aranda P, Aparicio VA.

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ABSTRACT

Aim: To examine the association of MD adherence during pregnancy with maternal and neonatal lipid, glycemic, and inflammatory markers.

Methods: This study utilizes data from 152 women who took part in the GESTAFIT trial, and from the umbilical cord arterial and venous serum in a subsample of 35 newborns. The MedDietScore, derived from food frequency questionnaires, was employed to assess MD adherence at the 16th g.w. Total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and glucose were assessed in the mother (at the 16th and 34th g.w.) and in cord arterial and venous serum with standard procedures using an auto-analyzer. Pro-inflammatory and anti-inflammatory cytokines (IL-6, IL-8, IL-10, IL-1beta, interferon-gamma, TNF-α) were measured with Luminex xMAP technology.

Results: A greater MD adherence was associated with higher HDL-cholesterol and lower LDL-cholesterol, LDL-cholesterol/HDL-cholesterol ratio, triglycerides, triglycerides/HDL-cholesterol ratio, and lower TNF-α in the mother at the 16th and the 34th g.w. ($|\beta|$: 0.191 to 0.388, *p's*<0.05). A higher intake of whole grain cereals, fruits, vegetables and fish, and a lower intake of sweets were associated with higher HDL-cholesterol and lower LDL-cholesterol, LDL-cholesterol /HDL-cholesterol ratio, triglycerides, triglycerides/HDL-cholesterol ratio, and TNF-α at the 16th and 34th g.w. ($|\beta|$: 0.188 to 0.334, *p's*<0.05). No associations were found with the cord arterial and venous serum markers (*p's*>0.05).

Conclusion: A greater MD adherence during pregnancy, driven by a higher intake of wholegrain cereals, fruits, vegetables and fish, and a lower intake of sweets, positively associated with the maternal lipid and inflammatory serum markers throughout the pregnancy. MD adherence during pregnancy was not associated with cord serum markers.

INTRODUCTION

During pregnancy, essential immunometabolic adaptations occur to sustain pregnancy and promote fetal growth and development (259,260). Nonetheless, an exacerbation or dysregulation of inflammatory and cardiometabolic markers might lead to a higher risk of developing pregnancy-related complications such as gestational hypertension, preeclampsia, gestational diabetes, preterm labour, and spontaneous abortion (130,260–263). These complications have adverse outcomes for both the mother and infant, which may appear during pregnancy and birth, or even suppose a lifelong risk for cardiovascular disease, type-2 diabetes, and other chronic metabolic conditions for the offspring (260).

Maternal dietary intake is one potentially modifiable behaviour that might positively impact materno-fetal immunometabolic markers (130). However, evidence on whole diet that considers complex nutrient and food interaction when examining materno-fetal cardiometabolic health is rare and conflicting (264–266). In this context, the MD might be beneficial for materno-fetal health (47). For the mother, achieving a healthier dietary pattern, such as the MD, seems to lower the risk for gestational diabetes (107,267), gestational hypertension (225), and is associated with lower triglycerides (130) and fasting blood glucose (267) concentrations. As for the fetus, a greater maternal MD adherence has been associated with a reduced occurrence of preterm birth, better lipoprotein concentration, and insulin sensitivity measured in the umbilical cord (47,63,69) .To our knowledge, only one previous trial (the CARRDIP, Cardiovascular Risk Reduction Diet in Pregnancy) has investigated the effect of a cholesterol-lowering diet based on fish, low-fat meats and dairy products, oils, whole grains, fruits, and legumes during pregnancy on materno-fetal lipid and inflammatory markers (265,266). They found that this diet lowered total cholesterol and LDL-cholesterol concentrations in the mothers throughout the pregnancy (266), yet this did not lead to improvements in the maternal or the fetal lipid and inflammatory profiles (265).

Further studies considering not only dietary habits but also diet quality during pregnancy, and their relation with both maternal and fetal immunometabolic serum markers, are required to provide more robust evidence in this relevant topic. Therefore, the aim of the present study was to analyse the associations of MD adherence during pregnancy and MD components, with maternal and cord arterial and venous glycemic, lipid, and inflammatory serum markers.

METHODS

Study design and participants

These are secondary analyses of the GESTAFIT project where a concurrent exercise program (60 min/session, 3 days/week of combined aerobic and strength training) from the 17th g.w. until delivery was conducted (182). The participants were recruited between the 11th to 13th g.w. at the "San Cecilio" University Hospital (Granada, Spain) during their first gynecologist checkup. The study was carried out at the "Sport and Health University Research Institute" (Granada, Spain), and at the "San Cecilio and Virgen de las Nieves University Hospitals" from November 2015 to April 2018. This study was approved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15). Of 384 pregnant women assessed for eligibility, 159 women who met the inclusion-exclusion criteria and accepted to participate **(Table 6)** were recruited for the GESTAFIT project. All participants provided a written informed consent. Among them, a total of 152 had valid data in sociodemographic characteristics and MD adherence at the 16th g.w., and were included in the present analyses **(Figure 2)**. Due to finding limitations, thirty-five out of these 152 women provided cord serum samples for the glycemic, lipid, and inflammatory markers assessment.

Sample size calculation

The sample size for this study depended on the 'a priori' analyses of the statistical power performed in the GESTAFIT project (182). Based on the primary outcome (i.e., maternal weight gains), we planned to recruit 60 women assuming a statistical power of 90%, $\alpha = 0.05$, and a 15% of potential withdrawals. Given the exploratory basis of the present study (secondary outcomes) we did not calculate the sample size

Maternal anthropometry and body composition

Mothers reported their pre-pregnancy weight at the recruitment (i.e., 12th g.w.). Although measured weight is preferable, self-report is a cost-effective and practical measurement approach that shows very good concordance with measured body weight (213). Weight and height were measured at the first and the second contact with the project team (16th g.w. and 34th g.w., respectively). Body weight and height were assessed using a scale (InBody R20; Biospace, Seoul, Korea) and a stadiometer (Seca 22, Hamburg, Germany), respectively. Height and pre-pregnancy weight were used to calculate pre-pregnancy BMI, as weight (kg) divided by squared height (m^2).

Blood collection

Maternal blood samples (5mL) were extracted from the antecubital vein in standardized fasting conditions (at the 16th and 34th g.w.), arterial and vein cord blood samples were extracted immediately after delivery. All blood samples were collected in serum tubes and were allowed to clot (coagulation) at room temperature for 30 minutes. Subsequently, samples were centrifuged at 1750 rpm during 10 min at 4ºC in a refrigerated centrifuge (GS-6R Beckman Coulter, Brea, CA, USA) to obtain serum which was aliquoted and frozen at -80ºC until analysed

Biochemical markers

Glycemic profile, lipids, and C-reactive protein

Maternal concentrations of serum total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, phospholipids, triglycerides and C-reactive protein were measured with standard spectrophotometric enzyme assays (AU5822 Clinical Chemistry Analyser, Beckman Coulter, Brea, USA). Cord arterial and venous serum total cholesterol, HDL-cholesterol, LDL-cholesterol, glucose, triglycerides and C-reactive protein were measured with spectrophotometric determination (BS-200 Chemistry Analyzer, Mindray Bio-medical Electronics, Shenzen, China). In addition, triglycerides/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios were calculated (268,269).

Insulin

Maternal insulin (at the 16th and 34th g.w.) was measured with paramagnetic-particle-based chemiluminescence immunoassays (UniCel-Dxl800 Access Immunoassay analyser, Beckman Coulter, Brea, USA).

Inflammatory markers

Some maternal and cord arterial and venous pro-inflammatory and anti-inflammatory cytokines (IL-6, IL-8, IL-10, IL-1beta, Interferon gamma, TNF-α) were assessed with Luminex xMAP technology. Plate was read on LABScan 100 analyser (Luminex Corporation, Texas, USA) with xPONENT software for data acquisition. Average values for each set of duplicate samples or standards were within 15% of the mean.

Maternal insulin resistance

To assess maternal insulin resistance the HOMA-IR was employed as previously described (HOMA-IR= insulin*glucose/22.5)(270).

Dietary assessment and Mediterranean diet adherence

Dietary habits were assessed at the 16th and 34th g.w. with a food frequency questionnaire previously evaluated for validity in Spanish non-pregnant adult population (189). By means of the data obtained from the food frequency questionnaire the MedDietScore (30) was employed to assess MD adherence as previously done in this study sample (210). The MedDietScore (30) consists of eleven variables (i.e., wholegrain cereals, potatoes, fruits, vegetables, pulses, fish, olive oil, red wine, red meat and subproducts, poultry and whole dairy products) ranging from 0 to 5 according to their position in the MD pyramid (271). Therefore, the total score ranges from 0 to 55. A moderate alcohol intake, also typical of the MD, was not considered for calculating the index in this group of women, since they must not drink alcohol during this period. As a result, the maximum score considered for these analyses in pregnant women was 50 points. Higher scores indicate higher MD adherence and, therefore, higher diet quality. The dietary pattern registered at the 16th g.w. was taken as representative of the pregnancy diet habits as previously done in this study sample (272).

Statistical analysis

Descriptive characteristics are presented as means and standard deviations for continuous variables and as frequency and percentages for categorical variables.

As initially designed (182) statistical analysis was conducted on a per-protocol basis. We only included women who attended more than 75% of exercise sessions and had valid data in both baseline and follow-up assessments. The changes (postest [34th g.w.]-pretest [16th g.w.]) of MD adherence and MD components were calculated. Changes (postest-pretest) in dietary variables were included as dependent variables, whereas the exercise intervention (control or exercise group) as independent variable.

Hierarchical linear regression analyses were performed to examine the associations of MD adherence (i.e., explanatory variable) with maternal and neonatal lipid, glycemic and inflammatory serum markers (i.e., glucose, HOMA-IR, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, phospholipids, IL-6, IL-8, IL-10, IFN-γ, TNF-α and C-reactive protein). The stepwise method was used, entering potential confounders (i.e., age, GWG, and smoking habit) in step 1 to test their association with each outcome (lipid, glycemic and

inflammatory serum markers in separate models). In the step 2 of the hierarchical regressions, we entered the MD adherence as a predictor and each lipid, glycemic, and inflammatory serum marker as outcomes in separate regression analyses after the inclusion of the relevant confounders (i.e., those which explained a significant amount of the variance in lipid, glycemic, and inflammatory serum markers) found in the step 1. The confounders for each model are specified in the footnotes of **Table 20**, **Table 21**, and **Table 22**. These steps were the same in the cross-sectional association models (i.e., maternal outcomes measured at the 16th g.w.) and in the longitudinal association models (i.e., maternal outcomes measured at the 34th g.w. and neonatal outcomes). The longitudinal models were additionally adjusted for the exercise intervention in the step 2 to account for the possible effect of the intervention conducted within the GESTAFIT project on these outcomes. As an exploratory analysis, additional linear regressions were performed using the MD components as explanatory variables and maternal (at the 16th and 34th g.w.) and neonatal serum markers after adjusting for the confounders previously found relevant in the hierarchal linear regression. The Benjamini-Hochberg procedure (273) was applied to account for the random effect in multiple comparisons for all the tests included in the primary analysis (i.e., MD adherence associations with materno-fetal immunometabolic serum markers at the 16th and 34th g.w.) and separately for all the tests included in the MD components analysis (i.e., MD components associations with maternal immunometabolic serum markers at the 16th and 34th g.w.) with q=0.05 (false discovery rate). All analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY) and the level of significance was set at $p \leq 0.05$.

RESULTS

The total sample size for the present analyses comprised 152 Caucasian pregnant women (32.9±4.6 years, pre-pregnancy BMI 24.2±4.2 kg/m^2) who had valid data in sociodemographic characteristics and MD adherence at the 16th g.w. **(Figure 10).** The sociodemographic characteristics of the study sample are shown in **Table 18**.

Table 18. Sociodemographic and clinical characteristics of the Study III participants

Variable	N	Mean (SD)
Age (years)	152	32.9 (4.6)
Pre-pregnancy weight (kg)	140	65.0 (12.3)
Weight at the 16th g.w. (kg)	150	66.9 (11.9)
Weight at the 34th g.w. (kg)	121	74.6 (10.9)
Gestational weight gain (pre-pregnancy to 16th g.w.) (kg)	138	2.1 (2.8)
Gestational weight gain (pre-pregnancy to 34th g.w.) (kg)	116	10.6 (5.0)
Pre-pregnancy body mass index (kg/m²)	138	24.2 (4.2)
Mediterranean diet score (0-50)	152	28.9 (3.9)
Number of children	152	**n (%)**
0		91 (59.9)
1		53 (34.9)
2		8 (5.3)
Educational status	152	
University studies		90 (59.2)
No University studies		62 (40.8)
Marital status	152	
Married		90 (59.2)
Single/divorced/widow		62 (40.8)
Working status	152	
Working		104 (68.4)
Not working		48 (31.6)
Sex of the baby	137	
Male		66 (48.2)
Female		71 (51.8)
Type of delivery	135	
Spontaneous		79 (58.5)
Instrumental vacuum/forceps		22 (16.3)
Cesarean		34 (25.2)
Smoking habit	152	
Current smoker		81 (53.3)
Former smoker		13 (8.6)
Never smoker		58 (38.2)

Values shown as mean (standard deviation) unless otherwise is indicated. G.w., gestational week.

Differences in MD adherence and MD components between pre- and post-intervention for the control and exercise groups (per protocol basis) are shown in **Table 19**. No changes were observed regarding MD adherence between the control and exercise groups.

Table 19. Influence of the exercise intervention on Mediterranean diet adherence and Mediterranean diet components

	Within-group changes (Post-Pre)		B	Confindence Interval (95%) for B		β	*P*
	Control group (n=51)	Exercise group (n=46)		Lower	Upper		
Food groups (servings/week)							
Whole-grain cereals	1.9 (3.4)	-0.1 (6.1)	-1.521	-3.541	0.499	-0.154	0.138
White bread and rice	1.6 (5.2)	0.3 (6.6)	-1.194	-3.444	1.056	-0.101	0.295
Potatoes	-0.1 (1.6)	0.2 (1.3)	0.174	-0.377	0.724	0.059	0.533
Fruits	2.9 (6.9)	0.7 (7.8)	-1.057	-3.769	1.654	-0.072	0.441
Vegetables	2.2 (8.4)	2.1 (7.8)	0.203	-2.890	3.297	0.013	0.897
Pulses	0.2 (1.0)	-0.2 (0.9)	-0.380	-0.735	-0.026	-0.193	0.036
Fish	0.2 (2.0)	0.6 (2.0)	0.719	-0.024	1.462	0.178	0.058
Red meat and sb.	-0.03 (2.76)	0.2 (2.8)	0.256	-0.716	1.229	0.047	0.602
Poultry	-0.1 (2.0)	-0.03 (2.4)	0.354	-0.459	1.167	0.080	0.390
Dairy products	1.1 (3.5)	0.8 (4.1)	-0.350	-1.787	1.087	-0.046	0.630
Olive oil	0.8 (10.3)	0.1 (6.4)	-0.397	-3.553	2.759	-0.023	0.803
Nuts	2.6 (4.8)	-0.9 (6.5)	-1.812	-4.031	0.407	-0.153	0.108
Sweets	0.5 (5.9)	0.3 (4.1)	-0.208	-2.250	1.834	-0.020	0.840
Mediterranean Diet Score (0-50)	0.5 (2.8)	-0.2 (3.0)	-0.437	-1.532	0.658	-0.074	0.430

Values shown as mean (standard error). Model adjusted for baseline values (i.e.,16th gestational week). Within-group changes show the differences between post-pre intervention results for each variable with negative values indicating a reduction in the post evaluation compared to pre-evaluation; β. standardized beta. CI, confidence interval; S, servings.

Hierarchical linear regression analyses for the association of MD adherence with maternal glycemic, lipid, and inflammatory markers at the 16th g.w. are presented in **Table 20**. A greater MD adherence was associated with higher HDL-cholesterol (β=0.289, *p*=0.001), lower LDL-cholesterol (β=-0.264, *p*=0.003), LDL-cholesterol/HDL-cholesterol ratio (β=-0.388, *p*<0.001) and lower triglycerides/HDL-cholesterol ratio (β=-0.235, *p*=0.009). There was evidence of statistical significance in the association of MD adherence with total cholesterol level (β=-0.171, *p*=0.058). After correcting for multiplicity, we observed that all the cross-sectional associations between MD adherence and maternal immunometabolic serum markers remained significant.

Table 20. Cross-sectional associations of the Mediterranean diet adherence with maternal glycemic, lipid and inflammatory serum markers at the 16th gestational week

Cross-sectional associations				
	Standardized Coefficients (β)	**Confidence interval (95%)**		***p***
		Lower	**Upper**	
Lipid and glycemic profile				
Glucose (mg/dL) (n=120)†	-0.023	-0.250	0.803	0.803
HOMA-IR (n=117)†	0.056	-0.129	0.240	0.550
Total cholesterol (mg/dL) (n=121)‡	-0.171	-0.347	0.006	0.058
HDL-cholesterol (mg/dL) (n=121)††	0.289	0.126	0.453	**0.001**
LDL-cholesterol (mg/dL) (n=121)†	-0.264	-0.439	-0.089	**0.003**
LDL-cholesterol/HDL-cholesterol (n=121)†	-0.388	-0.555	-0.221	**<0.001**
Triglycerides (mg/dL) (n=121)†	-0.142	-0.321	0.038	0.121
Triglycerides/HDL-cholesterol (n=121)†	-0.235	-0.412	-0.059	**0.009**
Inflammatory profile				
Interleukin 6 (pg/mL) (n=46)†	-0.069	-0.372	0.234	0.647
Interleukin 8 (pg/mL) (n=46)†	0.261	-0.032	0.554	0.080
Interleukin 10 (pg/mL) (n=46)†	0.214	-0.083	0.510	0.154
Interleukin 1 beta (pg/mL) (n=46)†	0.038	-0.266	0.341	0.803
Interferon gamma (pg/mL) (n=46)†	0.206	-0.091	0.504	0.178
Tumor necrosis factor alpha (pg/mL) (n=46)†	0.117	-0.185	0.418	0.690
C-reactive protein (n=120)†	-0.028	-0.210	0.761	0.761

†Unadjusted. ‡Adjusted by age. ††Adjusted by age, gestational weight gain (body weight at the 16th gestational week-pre-pregnancy body weight) and smoking habit. Boldface indicates those outcomes which surpassed the multiple comparison test. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Hierarchical linear regression analyses for the association of MD adherence with maternal glycemic, lipid and inflammatory markers at the 34th g.w. are presented in **Table 21**. A greater MD adherence was associated with higher HDL-cholesterol (β=0.348, p<0.001), lower LDL-cholesterol/HDL-cholesterol ratio (β=-0.191, p=0.049), triglycerides (β=-0.195, p=0.044), triglycerides/HDL-cholesterol ratio (β=-0.293, p=0.002) and lower TNF-α (β=-0.299, p=0.034). After the multiple test correction, the associations between MD adherence, HDL-cholesterol and triglycerides/HDL-cholesterol ratio remained significant.

Table 21. Longitudinal associations of the Mediterranean Diet Score with maternal glycemic, lipid and inflammatory serum markers at the 34th gestational week

Prospective associations				
	Standardized Coefficients (β)	**Confidence interval (95%) Upper**	**Upper**	***p***
Lipid and glycemic profile				
Glucose (mg/dL) (n=108)†	-0.164	-0.356	0.028	0.093
HOMA-IR (n=107)‡	-0.097	-0.287	0.092	0.312
Total cholesterol (mg/dL) (n=108)†	0.080	-0.114	0.273	0.414
HDL-cholesterol (mg/dL) (n=108)††	0.348	0.173	0.522	**<0.001**
LDL-cholesterol (mg/dL) (n=108)†	0.065	-0.129	0.258	0.509
LDL-cholesterol/HDL-cholesterol (n=108)†	-0.191	-0.381	-0.001	0.049
Triglycerides (mg/dL) (n=108)†	-0.195	-0.384	-0.005	0.044
Triglycerides/HDL-cholesterol (n=108)†	-0.293	-0.476	-0.109	**0.002**
Inflammatory profile				
Interleukin 6 (pg/mL) (n=49)†	-0.113	-0.408	0.181	0.443
Interleukin 8 (pg/mL) (n=49)†	-0.129	-0.423	0.165	0.382
Interleukin 10 (pg/mL) (n=49)††	-0.190	-0.459	0.079	0.162
Interleukin 1 beta (pg/mL) (n=49)†	-0.047	-0.340	0.245	0.747
Interferon gamma (pg/mL) (n=49)†	0.121	-0.172	0.414	0.411
Tumor necrosis factor alpha (pg/mL) (n=49)†	-0.299	-0.573	-0.024	0.034
C-reactive protein (n=107)†	-0.020	-0.215	0.174	0.836

†Adjusted by exercise intervention. ‡Adjusted by exercise intervention and gestational weight gain (weight at the 34th gestational week-pre-pregnancy body weight). ††Adjusted by exercise intervention and age. Boldface indicates those outcomes which surpassed the multiple comparison test. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

No associations were found between MD adherence and cord arterial and venous serum glycemic, lipid and inflammatory markers **(Table 22)** (all, $p>0.05$).

Table 23 shows the associations between the MD components and the maternal serum markers that were associated with the MD adherence at the 16th and 34th g.w. At the 16th g.w. a greater intake of whole grain cereals, fruits and fish and a lower intake of sweets was associated with greater HDL-cholesterol, lower LDL-cholesterol, LDL-cholesterol/HDL-cholesterol ratio and lower triglycerides/HDL-cholesterol ratio (|β| ranging from 0.188 to 0.281, all $p<0.05$). At the 34th g.w. a greater intake of whole grain cereals, vegetables and fish was associated with greater HDL-cholesterol, lower LDL-cholesterol/HDL-cholesterol ratio, triglycerides, lower triglycerides-HDL-cholesterol ratio and lower TNF-α (|β| ranging from 0.193 to 0.334, all $p<0.05$). After correcting for multiplicity, we observed that the cross-sectional association between fish, HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratio remained significant. Additionally, the longitudinal associations between vegetables, fish and

HDL-cholesterol and the association between fish and triglycerides/HDL-cholesterol ratio remained significant.

Table 22. Longitudinal associations of maternal Mediterranean diet adherence with cord arterial and venous serum markers

Artery umbilical cord blood	Standardized Coefficients (β)	Confidence interval (95%)		*p*
		Lower	Upper	
Umbilical arterial serum				
Lipid and glycemic profile				
Glucose (mg/dL) (n=24)†	0.094	-0.296	0.485	0.619
Total Cholesterol (mg/dL) (n=24)‡	-0.076	-0.483	0.331	0.702
HDL-cholesterol (mg/dL) (n=24)‡	-0.363	-0.768	0.042	0.077
LDL-cholesterol (mg/dL) (n=24)‡	0.096	-0.318	0.510	0.634
LDL-cholesterol/HDL-cholesterol (n=24)‡	0.311	-0.118	0.740	0.146
Triglycerides (mg/dL) (n=22)††	0.243	-0.178	0.663	0.241
Triglycerides/HDL-cholesterol (n=22)††††	0.211	-0.185	0.607	0.276
Inflammatory profile				
Interleukin 6 (pg/mL) (n=31)‡‡	0.009	-0.318	0.335	0.956
Interleukin 8 (pg/mL) (n=31)‡‡	0.188	-0.206	0.582	0.338
Interleukin 10 (pg/mL) (n=31)‡‡	0.207	-0.168	0.583	0.267
Interleukin 1 beta (pg/mL) (n=31)‡‡	-0.101	-0.496	0.293	0.603
Interferon gamma (pg/mL) (n=31)‡‡	0.077	-0.302	0.456	0.680
Tumor necrosis factor alpha (pg/ml) (n=31)‡‡	0.032	-0.350	0.413	0.866
Umbilical venous serum				
Lipid and glycemic profile				
Glucose (mg/dL) (n=32)‡‡	-0.075	-0.438	0.289	0.677
Total cholesterol (mg/dL) (n=32)‡‡	0.107	-0.273	0.486	0.570
HDL-cholesterol (mg/dL) (n=32)‡‡‡	0.094	-0.253	0.440	0.584
LDL-cholesterol (mg/dL)(n=32)‡‡	0.043	-0.339	0.424	0.821
LDL-cholesterol/HDL-cholesterol‡‡	-0.052	-0.411	0.307	0.769
Triglycerides (mg/dL) (n=29)††††	0.065	-0.294	0.424	0.712
Triglycerides/HDL-cholesterol (n=29)††††	0.096	-0.287	0.478	0.611
Inflammatory profile				
Interleukin 6 (pg/mL) (n=35)†	0.011	-0.344	0.366	0.949
Interleukin 8 (pg/mL) (n=35)‡‡	-0.021	-0.388	0.345	0.906
Interleukin 10 (pg/mL) (n=35)†††	0.147	-0.196	0.489	0.389
Interleukin 1 beta (pg/mL) (n=35)‡‡	0.307	-0.044	0.657	0.084
Interferon gamma (pg/mL) (n=35)††	0.143	-0.194	0.481	0.393
Tumor necrosis factor alpha (pg/mL) (n=35)‡‡	0.234	-0.099	0.568	0.162

†Adjusted by exercise intervention and age. ‡Adjusted by exercise intervention and sex of the baby. ††Adjusted by exercise intervention and type of deliver. ‡‡ Adjusted by exercise intervention. †††Adjusted by exercise intervention and smoking habit. ‡‡‡ Adjusted by exercise intervention and gestational weight gain. ††††Adjusted by exercise intervention, age and type of deliver. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 23. Association of Mediterranean diet components with maternal glycemic, lipid and inflammatory serum markers at the 16th and 34th gestational weeks

	Cross-sectional associations								Longitudinal associations									
Food groups (servings/week)	**HDL-cholesterol (n=121)**		**LDL-cholesterol (n=121)**		**LDL-cholesterol /HDL-cholesterol (n=121)**		**Triglycerides/ HDL-cholesterol (n=121)**		**HDL-cholesterol (n=108)**		**LDL-cholesterol /HDL-cholesterol (n=108)**		**Triglycerides (n=108)**		**Triglycerides/ HDL-cholesterol (n=108)**		**Tumor necrosis factor alpha (n=49)**	
	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*	β	*p*
Whole-grain cereals	0.015	0.862	-0.092	0.315	-0.051	0.576	-0.188	0.039	0.029	0.761	0.019	0.845	-0.180	0.064	-0.138	0.156	-0.292	0.038
White bread and rice	0.008	0.928	-0.031	0.736	-0.029	0.749	0.172	0.060	-0.051	0.591	-0.083	0.393	0.092	0.341	0.075	0.439	0.247	0.083
Potatoes	-0.062	0.483	-0.127	0.166	-0.070	0.446	0.013	0.886	0.105	0.271	-0.101	0.301	-0.060	0.539	-0.075	0.437	-0.202	0.156
Fruits	0.139	0.113	-0.136	0.137	-0.215	0.018	-0.122	0.181	0.139	0.145	-0.129	0.185	-0.031	0.752	-0.121	0.208	-0.188	0.191
Vegetables	0.039	0.654	-0.025	0.782	-0.046	0.617	0.011	0.903	0.263	**0.004**	-0.154	0.112	-0.127	0.190	-0.230	0.016	-0.334	0.017
Pulses	0.049	0.576	-0.129	0.160	-0.141	0.123	0.057	0.534	0.154	0.101	-0.188	0.052	-0.031	0.753	-0.084	0.385	-0.062	0.665
Fish	0.272	**0.002**	-0.107	0.242	-0.281	**0.002**	-0.202	0.027	0.305	**0.001**	-0.193	0.050	-0.156	0.111	-0.277	**0.004**	-0.239	0.117
Red meat and sb.	-0.068	0.443	0.173	0.058	0.159	0.082	0.085	0.355	0.038	0.686	0.101	0.303	0.025	0.795	0.005	0.955	0.023	0.874
Poultry	-0.060	0.490	0.013	0.890	0.040	0.666	-0.008	0.926	0.029	0.760	-0.063	0.519	-0.077	0.429	-0.069	0.478	-0.212	0.147
Dairy products	0.024	0.783	-0.089	0.334	-0.084	0.357	0.011	0.901	-0.020	0.832	-0.119	0.223	-0.009	0.930	-0.009	0.925	0.062	0.670
Olive oil	0.019	0.836	-0.017	0.851	-0.010	0.914	0.164	0.072	0.037	0.696	-0.149	0.124	0.166	0.086	0.114	0.239	-0.106	0.466
Nuts	0.144	0.096	-0.055	0.547	-0.120	0.190	-0.029	0.754	0.104	0.282	-0.125	0.213	0.131	0.187	0.042	0.676	-0.092	0.533
Sweets	0.078	0.385	0.216	0.017	0.135	0.140	0.031	0.736	-0.046	0.634	0.099	0.312	0.128	0.187	0.162	0.091	-0.039	0.788

Model adjusted for the cofounders previously found significantly associated with the outcomes in the hierarchical linear regression. Boldface indicates those outcomes which surpassed the multiple comparison test. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Sb, subproducts.

DISCUSSION

Our results suggest that a greater MD adherence during pregnancy, which seems to be driven by a higher intake of fish, whole grain cereals, fruits, vegetables, and a lower intake of sweets, was associated with better maternal lipid serum markers (i.e., lower total cholesterol, higher HDL-cholesterol and lower LDL-cholesterol, LDL-cholesterol/HDL-cholesterol ratio and triglycerides/HDL-cholesterol ratio) throughout the gestation. Notwithstanding, a greater MD adherence during pregnancy was not associated with cord arterial and venous serum markers.

Our findings of an association between MD adherence and maternal cardiometabolic markers throughout gestation were generally consistent with results from other studies (130,224,266). Previous randomized controlled trials (224,266,274) and observational studies (130,269) found an association between healthier maternal dietary patterns and lower total cholesterol, higher HDL-cholesterol, lower LDL-cholesterol, LDL-cholesterol/HDL-cholesterol ratio and triglycerides. Regarding MD adherence, pregnant women in the second trimester of pregnancy (22-26th g.w.) who had a high MD adherence showed lower triglycerides, LDL and total cholesterol compared with pregnant women with a low MD adherence (1). In line with previous studies which showed the beneficial effect of MD adherence regarding blood lipid control (275,276) we verified that a greater MD adherence during gestation was associated with lower LDL-cholesterol concentrations, higher HDL-cholesterol, as well as lower LDL-cholesterol/HDL-cholesterol ratio, triglycerides, cholesterol and triglycerides/HDL-cholesterol ratio values at the 16th and 34th g.w., thus indicating a favourable lipid profile. Moreover, we examined which MD components explained the associations between MD adherence and maternal lipid serum markers. We found that a greater intake of fish (mainly driven by white fish [1.3 servings/week], blue fish [1.0 servings/week], canned fish [1.0 servings/week] and shellfish [0.6 servings/week]), whole-grain cereals, fruits and vegetables, and a lower intake of sweets was associated with higher HDL-cholesterol, lower LDL-cholesterol, LDL-cholesterol/HDL-cholesterol ratio, triglycerides and triglycerides/HDL-cholesterol ratio. Although our cross-sectional design does not allow inferring causality, these significant associations in pregnant women, without cardiometabolic diseases, are suggestive of a role of diet in the lipid profile. In agreement, a multi-ethnic cohort study showed that a "healthy" dietary pattern, characterized by high intakes of whole grains, fruit, dairy, vegetables and unsaturated cooking oil, was inversely associated with total cholesterol, LDL-cholesterol, and triglycerides concentrations, and positively associated with HDL- cholesterol concentration (277). Furthermore, women with a dietary pattern characterized by high intake

of fruits, yogurt, nuts, vegetables, whole-grain cereals and legumes, among other components, was associated inversely associated with LDL-cholesterol, LDL-cholesterol/HDL-cholesterol ratio and triglycerides (269). On the contrary, a dietary pattern characterized by high intake of snacks, processed meat, fast-food, alcoholic beverages and sweets was directly associated with LDL- cholesterol in adult women (269). The higher intake of fruits, vegetables, nuts, and whole grains in these patterns should have contributed to greater dietary fiber and phytochemicals contents, known to be beneficial to risk factors such as LDL- cholesterol (278). On the contrary, a combination of unhealthier dietary habits in the "Processed" dietary pattern could explain the deleterious association found (269,279).

Besides, in the present study a greater MD adherence was associated with lower concentrations of TNF-α. These results are in line with the conclusion that the MD exerts its anti-inflammatory and immunomodulatory effect by decreasing proinflammatory interleukins such as TNF-α and its receptors (122,123). Moreover, it was proven that green vegetables and fruits rich in folate, flavonoids and antioxidants, can significantly reduce the concentrations of serum inflammatory markers, such as TNF-α (280). Similarly, we found that a greater intake of wholegrain cereals and vegetables were associated with lower concentrations of TNF-α at the 34th g.w. To the authors knowledge, this study is the first reporting such an association, which might be relevant to attenuate the pregnancy-induced inflammation processes. However, this finding should be taken with caution since this association was disregarded by the multiple comparison test, and future studies with higher sample sizes should therefore confirm or contrast this finding.

The lack of association between MD adherence and serum glucose and HOMA-IR in our study agrees with previous studies (281,282), but differs from others (130,224,283,284). In this context, a systematic review and meta-analysis has not shown the effect of the Dietary Approaches to Stop Hypertension on fasting blood glucose and insulin resistance in non-pregnant adult population (282). Among pregnant women, adherence to a dietary pattern characterized by an intake of poultry, nuts, cheese, and whole grains was not associated with maternal fasting glucose (281). Notwithstanding, other studies in pregnant women (130,224) at the 24-29th g.w. showed that the Dietary Approaches to Stop Hypertension score-based method and a dietary pattern characterized by high consumption of fruits, vegetables, whole grains, low fat dairy, breakfast bars, and water have been associated with lower maternal glucose, insulin and HOMA-IR (130). However, different g.w. in the measurements may partially account with the differing findings. Furthermore, there is a lack of uniformity between indices to assess diet quality during pregnancy (29), specifically in the number of

components included, classification categories for each item; measurement scales; statistical parameters (mean, median, or quintiles of daily intake); and the contribution of each component (positive or negative) to the total score (66,67). The Dietary Approaches to Stop Hypertension diet index is a sample quintile-based sum score of 7 components (fruits, vegetables, dairy, meat, poultry, fish, and eggs; nuts, seeds, and legumes; fats and oils; and sodium) (285) whereas the MedDietScore is based on 11 food groups (non-refined cereals, fruits, vegetables, potatoes, legumes, olive oil, fish, red meat, poultry, full fat dairy products and alcohol) that includes predefined portions and absolute cut-offs for each food group, and assesses the frequency of consumption per month (30). This complicates the comparability between these two indices and could explain, at least in part, the heterogeneity of findings in dietary patterns research during pregnancy. We did not observe an association between MD adherence, MD components and HOMA-IR throughout pregnancy. Although the mechanisms explaining associations between maternal dietary patterns, insulin resistance and triglyceride concentrations are still partially unclear, the results are biologically plausible. According to previous evidence (130), healthier dietary patterns (e.g., Tertile 3 in Dietary Approaches to Stop Hypertension diet) have higher intakes of fruits, vegetables, and whole grains. These food groups are rich sources of antioxidants, phytochemicals, vitamin C, and dietary fiber which may contribute to the protective associations seen in their study. Discrepancies between the association of dietary patterns with maternal cardiometabolic markers might be partially explained by differences in the ethnicity of the sample population given the fact that the Dietary Approaches to Stop Hypertension dietary pattern and its association with cardiometabolic profiles differed by country, with significant within-country variations by socio-demographic characteristics (286). However, it is likely due to large methodological differences, such as reverse causation given the cross-sectional design, the method of assessing diet quality, the choice of covariates and variation in sample sizes and sample characteristics, and resulting statistical power. Little information is available on the effects of maternal diet during pregnancy on the offspring's glycemic, lipid and inflammatory serum markers with previous studies reporting mixed findings (63,69,266). Gesteiro et al. (63,69) found that pregnant women with higher MD adherence during the first trimester of pregnancy (12-15th g.w.) had infants with lower glycemia, insulinemia, HOMA-IR and LDL-cholesterol compared with women with low MD adherence. The transference of placental lipids has been largely referred, suggesting that a maternal diet with low MD score negatively affects the neonatal LDL-cholesterol (63). However, we did not find differences between neonatal LDL-cholesterol and the degree of adherence to MD diet during gestation. In agreement with our results,

Killeen et al. (274) found no associations between maternal inflammatory potential of the diet and triglycerides, HDL-cholesterol, LDL-cholesterol, total cholesterol and glucose in the cord blood. Furthermore, a maternal diet which promoted fish, low-fat meats and dairy products, oils, whole grains, fruits, vegetables, and legumes decreased maternal cholesterol concentrations, but not cord and neonatal lipids neither inflammatory markers (265,266) which is in agreement with our findings.

Limitations and strengths

Some limitations need to be highlighted. First, the observational design does not allow a clear cause–effect identification. Second, the results should be interpreted cautiously, as we could be limited to detect small association sizes. Larger studies should further explore these associations in order to corroborate our results. Third, the participants were enrolled in an exercise intervention that might affect our findings. However, we included the group allocation as confounder in our longitudinal analyses. Furthermore, we observed that the GESTAFIT exercise intervention did not have any effect on the MD adherence, neither on any of its components (except for legumes). Thus, the primary exposure (i.e., exercise) is not likely to have affected our findings. Fourth, the limited data (n=35) in our study regarding cord blood could bias our findings. However, we found no differences in the baseline characteristics of the drop-outs and the completers. Additionally, although our findings were corrected for multiple comparison testing, the likelihood of making a type I error might not be completely disregarded and future studies should confirm our findings. Several strengths of this study are worth considering. A detailed definition of the dietary habits and a valid assessment of the MD diet adherence was employed. Furthermore, lipid, glycemic and inflammatory serum markers were assessed during the second and the third trimester of pregnancy and in the arterial and vein cord blood which provides a more comprehensive understanding of materno-fetal immunometabolic serum markers along the pregnancy course.

Conclusion

Our results suggest a positive role of MD adherence during pregnancy on maternal lipid serum markers. Likewise, MD adherence might be positively associated to the inflammatory marker TNF-α, although this finding should be corroborated in future studies. However, a greater MD adherence during pregnancy was not associated with neonatal lipid, glycemic and inflammatory markers. Future studies are warranted to confirm these associations and determine the underlying mechanisms.

STUDY IV: Influence of the degree of adherence to the Mediterranean diet and its components on cardiometabolic risk during pregnancy. The GESTAFIT project

Flor-Alemany M, Acosta-Manzano P, Marín-Jiménez N, Baena-García L, Aranda P, Aparicio VA.

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ABSTRACT

Aim: Studies regarding dietary patterns and cardiometabolic risk markers during pregnancy are scarce. The aim of the present study was to analyse whether different degrees of adherence to the MD and the MD components were associated with cardiometabolic markers and a clustered cardiometabolic risk during pregnancy.

Methods: This study comprised 119 pregnant women from the GESTAFIT project. Dietary habits were assessed with a food frequency questionnaire at the 16th and 34th g.w. The MedDietScore was employed to assess MD adherence. The following cardiometabolic markers were assessed: pre-pregnancy BMI, SBP, DBP, fasting glucose, triglycerides and HDL-cholesterol.

Results: A greater MD adherence was associated with a better cardiometabolic status in cross-sectional (16th g.w.) and prospective analyses (MD adherence at the 16th g.w. and cardiometabolic markers at the 34th g.w.; SBP, DBP and HDL-cholesterol; all, p<0.05). Participants with a high MD adherence had a lower clustered cardiometabolic risk compared to participants with a low MD adherence at the 16th and 34th g.w. (both, p<0.05). A higher intake of fruits, vegetables and fish and a lower intake of refined cereals and red meat and subproducts were associated with a lower cardiometabolic risk at the 16th and 34th g.w. (all, p<0.05).

Conclusion: A higher MD adherence, a greater intake of fruits, vegetables and fish and a lower intake of refined cereals and red meat and subproducts showed a cardioprotective effect throughout gestation.

INTRODUCTION

During pregnancy, essential cardiometabolic changes take place in order to allow fetal growth (130). Nevertheless, an inadequate adaptation to these changes (i.e., adverse cardiometabolic markers, including high level of triglycerides, glucose, high BP, maternal obesity and low levels of HDL-cholesterol), may result in an increased risk of cardiometabolic diseases, such as gestational hypertension, preeclampsia and gestational diabetes mellitus (29,130,131).

Dietary patterns during gestation are potential modifiable behaviours that may exert a positive influence on pregnancy cardiometabolic markers (130). In this regard, the MD has been associated with a better cardiometabolic status and a lower cardiometabolic risk in adult population (30,45,126–129). Notwithstanding, studies regarding dietary patterns and cardiometabolic risk markers during pregnancy are scarce (42,130). For instance, a Mediterranean-style diet in pregnancy has been associated with a lower risk of gestational diabetes (42); a dietary pattern characterised by a high intake of fruits, vegetables, whole grains, low fat dairy, breakfast bars and water has been associated with lower maternal glucose levels and insulin resistance (130); the Dietary Approaches to Stop Hypertension score-based method has been associated with lower levels of maternal triglycerides (130), better glucose tolerance, BP and overall lipid profile (224); and a dietary pattern comprising a high intake of vegetables, vegetable oils, pasta, rice, fish, and legumes, a moderate intake of alcohol and a low intake of sweets has been associated with lower maternal BP (44). Importantly, pregnant women with a worse metabolic profile are more likely to have adverse maternal (preterm delivery, preeclampsia or gestational diabetes mellitus) and fetal (small/large for gestational age, neonatal asphyxia or fetal demise) outcomes (131). Therefore, the main aim of the present study was to explore whether different degrees of adherence to the MD were associated with cardiometabolic markers and a clustered cardiometabolic risk in pregnant women. A secondary, exploratory aim was to study the association of specific MD components with this clustered cardiometabolic risk.

METHODS

Study design and participants

The present longitudinal study takes part in the GESTAFIT project, where a novel exercise intervention was conducted (182). The complete methodology of this project (including the eligibility criteria and the sample size calculation) have been published elsewhere (182). The sample size was only determined for the primary outcome of the GESTAFIT project (i.e., maternal weight gains) resulting in 52 pregnant women (26 per group) (182). This study was

approved by the Ethics Committee on Clinical Research of Granada, Regional Government of Andalusia, Spain (code: GESFIT-0448-N-15). From the 384 pregnant women assessed for eligibility, 159 met the eligibility criteria **(Table 6)** and signed a written informed consent. Among them, 152 had valid data in sociodemographic and clinical characteristics and MD adherence **(Figure 2)**. A total of 33 pregnant women (at the 16th g.w.) and 45 pregnant women (at the 34th g.w.) had missing data in biochemical markers, BP and/or body composition. Therefore, 119 (at the 16th g.w.) and 107 (at the 34th g.w.) pregnant women were included in the analyses.

The evaluation procedures were performed on 2 non-consecutive days. At the 16th g.w., data on nutritional and clinical information (e.g., smoking habit and alcohol intake) was gathered through face-to-face interviews by trained staff. In addition, BP and body composition were assessed (in the same order as mentioned). On the second appointment, participants attended our research center for blood sample extractions. At the 34th g.w., a second assessment was carried out where trained staff gathered the same information as they previously did.

Exercise Intervention

The exercise program which was carried out in the GESTAFIT project was designed following the standards of the American College of Obstetricians and Gynecologists (185) and has been previously detailed (287,288). Briefly, women randomized into the exercise group followed a concurrent exercise program (60 min/session, 3 days/week of combined aerobic and strength training) from the 17th g.w. until delivery.

Control Group

Pregnant women allocated into the control group did not participate in the training sessions and were asked to continue with their usual activities. For ethical reasons, the research team held a series of conferences to address the importance of physical activity and healthy dietary habits during pregnancy. Both the control and exercise groups attended theses conferences.

Maternal anthropometry and body composition

At the first evaluation, height was measured using a stadiometer (Seca 22, Hamburg, Germany). Pre-pregnancy body weight (kg) was self-reported. These values were used to calculate pre-pregnancy BMI, as weight (kg) divided by squared height (m^2). Pre-pregnancy weight status classified based on self-reported pre-pregnancy weight and height has been previously validated (289).

Vascular function

A BP monitor (M6 upper arm blood pressure monitor Omron. Omron Health Care Europe B.V. Hoolddorp, The Netherlands) was employed to assess SBP and DBP, which were measured after 5 minutes of rest on two separate occasions (with 2 minutes between trials) with the person seated. Both values were recorded, although only the lowest value of the two trials was selected for the analysis.

Biochemical markers

Venous blood samples (5mL) after all night fasting were collected in serum tubes. The blood sample was allowed to clot (coagulation) at room temperature for 30 minutes. Then, samples were centrifuged at 1750 rpm during 10 min at 4ºC in a refrigerated centrifuge (GS-6R Beckman Coulter, Brea, CA, USA) to obtain serum. Glucose, triglycerides and HDL-cholesterol were assessed with standard procedures using an auto-analyser (AU5822 Clinical Chemistry Analyzer, Beckman Coulter, Brea, CA, USA).

Clustered cardiometabolic risk

A clustered cardiometabolic risk (Z-score) was created as the mean of the standardized scores [(value - mean)/standard deviation] of pre-pregnancy BMI, mean BP [defined as (SBP+DBP)/2], serum fasting glucose, triglycerides and HDL-cholesterol, since these factors have been previously employed as a cluster of metabolic risk factors during pregnancy (131). For variables characterized by a lower metabolic risk with increasing values (HDL-cholesterol), z-scores were multiplied by −1. A higher clustered cardiometabolic status indicates a higher cardiometabolic risk

Physical activity levels

Participants were asked to wear a tri-axial accelerometer attached to their waist (Actigraph GT3X+, Pensacola, Florida, US) for nine consecutive days, 24 hours/day (192). The total physical activity (min/day) was calculated.

Dietary assessment and Mediterranean diet adherence

A validated food frequency questionnaire designed by Mataix et al. (189) was administered to participants by a trained nutritionist at the 16th and 34th g.w. asking about their dietary habits. The MedDietScore was employed to assess MD adherence (31, 212). It was calculated by using dietary data obtained from a food frequency questionnaire (189). We previously observed that

the MD adherence and dietary habits remained unchanged in our participants between the 16th g.w. and the 34th g.w. (232). Consequently, the dietary pattern at the 16th g.w. was considered representative of the pregnancy period. The MedDietScore consists of eleven variables (whole-grain cereals, potatoes, fruits, vegetables, pulses, fish, olive oil, red wine, red meat and subproducts, poultry and whole dairy products) ranging from 0 to 5 according to their position in the MD pyramid (271). The total score ranges from 0 to 55, with higher values indicating a greater adherence to the MD, and therefore, higher diet quality. A moderate alcohol intake, also typical of the MD, was not considered for calculating the index in this group of women, since they must not drink alcohol during this period. Therefore, the maximum score considered for these analyses in pregnant women was 50 points. Women were defined as adherent to the MD if they had a MD adherence above 30 points as previously stated in this study sample (233).

Statistical analysis

Descriptive statistics (mean ± standard deviation for quantitative variables and number of women [%] for categorical variables) were employed to describe sociodemographic and clinical characteristics of the study participants at baseline (16th g.w.). Nominal variables were analysed by using the Chi-squared test.

Linear regression analyses after adjusting for maternal age, smoking habit (at the 16th and 34th g.w.) and the exercise intervention (at the 34th g.w.) were used to explore cross-sectional associations of MD adherence with cardiometabolic risk biomarkers and clustered cardiometabolic risk at the 16th g.w. (n=119). In addition, longitudinal analyses (n=107) were performed to analyse the association of the MD adherence at the 16th g.w. with cardiometabolic risk biomarkers and the clustered cardiometabolic risk at the 34th g.w. The statistical power analyses showed statistical power of 95% to detect small-to-medium association sizes at the 16th g.w. (n=119, minimum detectable f^2=0.11) and at the 34th g.w. (n=107, minimum detectable f^2=0.12) (290).

Participants were classified as having a high MD adherence if they had a score of ≥30 points in the Mediterranean Diet Score index (233). Subsequently, the clustered cardiometabolic risk was compared between these groups by a one-way analysis of covariance (ANCOVA) after adjusting for maternal age, smoking habit (at the 16th and 34th g.w.) and the exercise intervention (at the 34th g.w.) for both, cross-sectional and longitudinal associations.

Linear regression analyses after adjusting for maternal age, smoking habit (at the 16th and 34th g.w.) and the exercise intervention (at the 34th g.w.) were used to explore cross-sectional and

longitudinal associations of the MD adherence with the clustered cardiometabolic risk at the 16th and 34th g.w.

A paired student's T-test was performed to explore differences in total physical activity levels, body weight, MD components and MD adherence of pregnant women by g.w. (16th versus 34th g.w.).

The Benjamini-Hochberg procedure (273) was applied to account for the random effect in multiple comparisons for all the tests included in the primary analysis (i.e., MD adherence associations with cardiometabolic risk biomarkers and clustered cardiometabolic risk), and separately for all the tests included in the dietary habits analysis (i.e., dietary habits associations with clustered cardiometabolic risk) with q=0.05. All analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY) and the level of significance was set at $p \leq 0.05$

RESULTS

The total sample size for the present analyses comprised 119 Caucasian pregnant women (33.2±4.4 years, pre-pregnancy BMI 24.1±4.1 kg/m^2) who had valid data in the food frequency questionnaire (189), vascular function, biochemical markers and body composition measurements **(Figure 2)**. The descriptive characteristics of the study sample are shown in **Table 24**.

Table 24. Sociodemographic and clinical characteristics of the Study IV participants

Variable	Mean (SD)
Age (years)	33.2 (4.4)
Pre-pregnancy weight (kg)	65.1 (12.2)
Pre-pregnancy BMI (kg/m²)	24.1 (4.1)
Underweight	3 (2.5)
Normal weight	74 (62.2)
Overweight	30 (25.2)
Obese	12 (10.1)
Mediterranean diet adherence (0-50)	29.0 (4.0)
Vascular function	
Systolic blood pressure (mmHg)	107.8 (9.3)
Diastolic blood pressure (mmHg)	64.3 (7.9)
Glycemic and lipid profile	
Fasting glucose (mg/dL)	77.4 (15.9)
HDL-cholesterol (mg/dL)	68.2 (11.4)
Triglycerides (mg/dL)	121.3 (48.9)
Educational Status	**n (%)**
Low educational status	13 (10.9)
Medium educational status	36 (30.3)
High educational status	70 (58.8)
Smoking habit	
Current smoker	10 (8.4)
Former smoker	44 (37.0)
Never smoker	65 (54.6)
Alcohol consumption (no, n [%])	119 (100)
Taking nutritional supplements ((yes, n [%])	102 (85.7)

Values shown as mean (SD) unless otherwise is indicated. SD, Standard deviation.

Differences in total physical activity, body weight, dietary habits and MD adherence of pregnant women by g.w. (16th versus 34th g.w.) are shown in **Table 25**. At the 34th g.w. pregnant women had lower levels of total physical activity (p=0.001), higher body weight (p=0.001), a higher intake of fruits (p=0.007) and vegetables with evidence of statistical significance (p=0.057) compared to the 16th g.w.

Table 25. Differences in total physical activity, Mediterranean diet components and Mediterranean diet adherence of pregnant women by gestational week (16th versus 34th gestational weeks)

Variables	16th gestational week	34th gestational week	*p*
Total physical activity (min/day) (n=46)	415.9 (87.0)	369.2 (86.7)	0.001
Body weight (kg) (n=100)	66.4 (10.2)	75.0 (10.2)	0.001
Whole-grain cereals (servings/week)	5.6 (6.5)	5.9 (6.7)	0.672
White bread and rice (servings/week)	8.9 (6.6)	9.1 (6.6)	0.768
Potatoes (servings/week)	2.3 (1.3)	2.4 (2.1)	0.698
Fruits (servings/week)	14.8 (7.5)	17.0 (8.9)	0.007
Vegetables (servings/week)	24.7 (12.0)	26.8 (12.8)	0.057
Pulses (servings/week)	2.7 (1.5)	2.6 (1.1)	0.304
Fish (servings/week)	4.9 (2.5)	5.3 (2.5)	0.243
Red meat and subproducts (servings/week)	5.6 (4.4)	5.4 (3.6)	0.615
Poultry (servings/week)	2.6 (1.2)	3.1 (2.8)	0.113
Dairy products (servings/week)	13.0 (10.6)	12.6 (8.8)	0.696
Olive oil (servings/week)	13.8 (12.4)	13.2 (7.6)	0.666
Mediterranean diet adherence (0-50)	29.1 (3.9)	29.4 (3.8)	0.516

Values shown as mean (standard deviation).

The association of MD adherence, cardiometabolic risk biomarkers and clustered cardiometabolic risk are shown in **Table 26.** The cross-sectional analyses showed that a greater adherence to the MD at the 16th g.w. was associated with lower pre-pregnancy BMI, SBP, greater HDL-cholesterol and a lower clustered cardiometabolic risk (all, $p<0.05$). Regarding the longitudinal analyses, a greater MD adherence at the 16th g.w. was associated with lower pre-pregnancy BMI, SBP, DBP, triglycerides, higher HDL-cholesterol and a lower clustered cardiometabolic risk at the 34th g.w. (all, $p<0.05$). We performed an additional analysis adjusting for objectively measured total physical activity (min/day) and educational status but results remained the same (data not shown). After correcting for multiplicity, we observed that the cross-sectional associations between MD adherence (at the 16th g.w.) and BMI and the longitudinal associations between MD adherence (at the 16th g.w.) and BMI, DBP and triglycerides (at the 34th g.w.) became non-significant.

Table 26. Association of Mediterranean diet adherence, cardiometabolic risk biomarkers and clustered cardiometabolic risk during pregnancy

	Cross-sectional associations 16th gestational week (n=119)		Prospective associations Diet in week 16th and outcomes in week 34th (n=107)	
Cardiometabolic risk biomarkers	**Standardized Coefficients (β)**	p^a	**Standardized Coefficients (β)**	p^b
Pre-pregnancy BMI	-0.196	0.034	-0.199	0.041
SBP	-0.225	**0.015**	-0.260	**0.007**
DBP	-0.166	0.073	-0.198	0.044
Glucose	-0.022	0.813	-0.171	0.079
Triglycerides	-0.149	0.109	-0.194	0.047
HDL-C	0.299	**0.001**	0.348	**<0.001**
Cardiometabolic risk				
*Clustered cardiometabolic risk**	-0.309	**0.001**	-0.428	**<0.001**

[a]Model adjusted for maternal age and smoking habit. [b]Model additionally adjusted for exercise intervention. Bold values indicate those outcomes which surpassed the multiple comparison test. BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure. *A higher clustered cardiometabolic status entails higher cardiometabolic risk.

The clustered cardiometabolic risk profile (Z-score) by degree of MD adherence (i.e., low MD adherence vs high MD adherence) is shown in **Figure 9**. In the cross-sectional and longitudinal analyses, the group with a high MD adherence had a lower cardiometabolic risk than the group with a low MD adherence (p=0.001 and p=0.018, respectively).

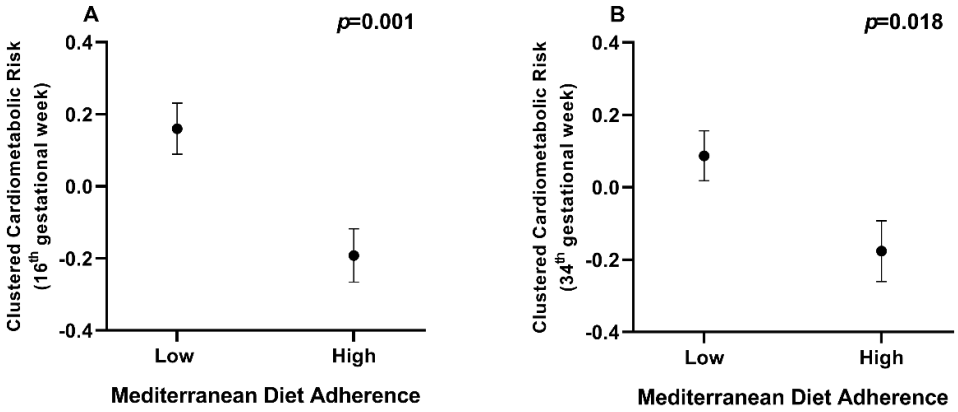

Figure 9. Clustered cardiometabolic risk (z-score) by Mediterranean diet adherence. Model was adjusted for age and smoking habit at the 16th g.w. Model was additionally adjusted for exercise intervention at the 34th g.w. Dots represent mean and bars standard error. (A) Clustered cardiometabolic risk by low vs. high Mediterranean Diet Score at the 16th gestational week. (B) Clustered cardiometabolic risk by low vs. high Mediterranean Diet Score at the 34th gestational week. Higher clustered cardiometabolic status, higher cardiometabolic risk.

The associations of MD components with the clustered cardiometabolic risk (Z-score) at the 16th and the 34th g.w. are shown in **Table 27**. In the cross-sectional analyses, a higher intake of fruits and fish and a lower intake of red meat and subproducts were associated with a lower clustered cardiometabolic risk at the 16th g.w. (all, $p<0.05$). At the 34th g.w., a greater intake of fruits, vegetables, fish and pulses was associated with a lower cardiometabolic risk at the 34th g.w. (all, $p<0.05$). Of note, after correcting for multiplicity, we could observe that the associations between fruits and the clustered cardiometabolic risk throughout gestation remained significant. In addition, the cross-sectional association between red meat and subproducts and cardiometabolic risk (at the 34th g.w.) remained significant.

Table 27. Association of Mediterranean diet components with the clustered cardiometabolic risk (Z-score) during gestation.

	Cross-sectional associations		**Longitudinal associations**	
Food groups	**16th gestational week (n=119)**		**Diet at the 16th gestational week and cardiometabolic risk at the 34th gestational week (n=107)**	
	β	***P*[a]**	**β**	***p*[b]**
Whole-grain cereals (s/week)	-0.152	0.106	-0.186	0.065
White bread and rice (s/week)	0.136	0.153	0.134	0.176
Potatoes (s/week)	-0.004	0.963	-0.004	0.997
Fruits (s/week)	-0.255	**0.006**	-0.318	**0.001**
Vegetables (s/week)	-0.022	0.814	-0.255	0.009
Pulses (s/week)	0.054	0.563	-0.208	0.034
Fish (s/week)	-0.209	0.032	-0.189	0.064
Red meat and subproducts (s/week)	0.208	0.025	0.149	0.131
Poultry (s/week)	0.159	0.088	0.047	0.639
Dairy products (s/week)	-0.014	0.884	0.064	0.526
Olive oil (s/week)	0.027	0.785	0.074	0.473

[a]Model adjusted for maternal age and smoking habit. [b]Model additionally adjusted for exercise intervention. Bold values indicate those outcomes which surpassed the multiple comparison test. BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure. A higher clustered cardiometabolic status entails higher cardiometabolic risk.

DISCUSSION

Our results suggest that the MD adherence was associated with most of the studied cardiometabolic markers throughout pregnancy. Moreover, a clear cardioprotective effect was observed across gestation when a high MD adherence was reached regardless of potential confounders such as age, smoking habit and exercise. In addition, a higher intake of fruits, vegetables and fish and a lower intake of refined cereals and red meat and subproducts were

associated with a lower cardiometabolic risk during gestation. Of note, individual associations between food groups and cardiometabolic risk were less significant than the association between the MD adherence and the cardiometabolic risk.

The lifestyle habits, sociodemographic and clinical characteristics of the study sample were similar to those observed in previous epidemiological studies conducted in Spanish (211,216,291) and non-Spanish (130) pregnant populations in the same age range. Regarding pre-pregnancy body composition of the study sample (pre-pregnancy BMI [23.2 kg/m^2]), it was similar to the one observed by Martin et al. (130) in which pregnant women with the highest adherence (Tertile 3) to the Dietary Approaches to Stop Hypertension score-based method had a pre-pregnancy BMI of 22.8 kg/m^2 (130). In addition, a recent study (291) conducted in Spain reported that 37% of pregnant women were overweight/obese prior to pregnancy, similar to our results (35%). Comparing the early second trimester (16th g.w.) with the third trimester (34th g.w.) we observed a higher intake of fruits and vegetables as previously reported (232). However, no differences were found regarding MD adherence, suggesting that food behaviours remained unchanged during pregnancy in accordance with previous evidence (211). Additionally, we found that women had lower total physical activity levels at the third trimester of pregnancy compared to the early second trimester of pregnancy. This is in line with previous evidence that suggested that physical activity levels decrease along gestation (292). In particular, it has been shown (291) that between the second and the third trimester of pregnancy there was a decrease in physical activity (assessed with the International Physical Activity Questionnaire) which is highly in agreement with our findings. In this regard, the high prevalence of overweight and obesity prior to pregnancy and the decreasing time spent in total physical activity might imply a higher cardiometabolic risk in this population (130,293,294).

In this context, previous studies (29,44,130,224) have suggested that specific maternal dietary patterns (comprising a high intake of vegetables, fruits, whole grains, fish and nuts and a low consumption of meat, saturated fats and refined grains) are associated with better results in cardiometabolic markers, including triglycerides, glucose, BP and cholesterol. Similarly, we found that the MD adherence was associated with a lower BP and higher HDL-cholesterol at the 16th and 34th g.w.

Compelling evidence (44,295) indicates that some dietary strategies were effective for preventing cardiovascular disease in a non-pregnant population, including an increasing intake of omega-3 fatty acids from fish and a high consumption of fruits, vegetables, nuts and whole-grain cereals and with a low intake of refined cereals. A Mediterranean dietary pattern

has long been associated with a lower incidence of cardiovascular disease in the general population (30). Notwithstanding, a recent systematic review and meta-analysis (296) concluded that there is still some uncertainty regarding the effects of a Mediterranean-style diet on cardiovascular disease and cardiometabolic risk factors for both primary and secondary cardiovascular disease prevention. As a result, it is clinically relevant to determine the appropriate degree of adherence in which the aforementioned cardiovascular protection could be reached in pregnant women. This is especially important since recent studies suggest that pregnant women are drifting away from the Mediterranean diet-like pattern (211,212).

It has been recently proposed that women with several cardiometabolic risk factors during pregnancy have a higher risk of adverse pregnancy outcomes (131). When we further explored the association between MD and the clustered cardiometabolic risk (Z-score) we found that a higher MD adherence was associated with a better clustered cardiometabolic status throughout the pregnancy course. This could be explained because this traditional dietary pattern (typical among Mediterranean regions, including Spain) has been suggested as an ideal model for cardiovascular disease prevention (297,298). Interestingly, a clear cardioprotective effect throughout pregnancy was observed when a high MD adherence was reached regardless of potential confounders such as age, smoking habit or exercise. This is in agreement with previous evidence in non-pregnant adult population (299,300), suggesting that a higher adherence to the MD promoted cardioprotective effects.

A recent systematic review (227), where five MD assessment indices were compared, showed that the MedDietScore provides the best evidence of MD adherence. As a result, we decided to employ this dietary index to assess MD adherence in this study sample. Notwithstanding, to accomplish the second objective of the present study, we analyzed the association between individual components of the Mediterranean dietary pattern and a clustered cardiometabolic risk previously employed in pregnant women (131). We confirmed that components which are suggested to be protective against cardiovascular disease (295), including fruits, vegetables and fish were associated with a lower clustered cardiometabolic risk, and those supposed to be detrimental for cardiovascular disease (301), including red meat and subproducts and refined cereals, were associated with a greater clustered cardiometabolic risk in this specific population.

Limitation and strengths

There are some limitations that should be underlined. Firstly, as this is a longitudinal study, causality cannot be concluded. Secondly, the results should be interpreted cautiously, as we could be limited to detect small association sizes. Larger studies should further explore these associations in order to corroborate our results. Regarding strengths, we assessed dietary habits and cardiometabolic status in both, the 16th and 34th g.w., and we employed a widely used MedDietScore. In addition, we included a wide range of cardiometabolic factors within the overall risk score created, which strengthen the usefulness of our results.

CONCLUSION

Overall, our results indicate that a higher MD adherence could be associated with better cardiometabolic markers and a cardioprotective effect throughout gestation. Regarding MD components, a higher intake of fruits, vegetables and fish and a lower intake of refined cereals and red meat and subproducts might be associated with lower cardiometabolic risk during pregnancy.

STUDY V: Influence of an exercise intervention plus an optimal Mediterranean diet adherence during pregnancy on postpartum body composition. The GESTAFIT project

Flor-Alemany M, Acosta-Manzano P, Migueles JH, Henriksson P, Löf M, Aparicio VA.

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ABSTRACT

Aim: To study whether the effects of an exercise program during pregnancy on postpartum body composition are moderated by following a healthy dietary pattern (i.e., MD).

Methods: Of the 102 pregnant women (control n=40, exercise=62) included in this quasi-experimental trial, 83 participants met the per-protocol criteria (control n=40, exercise n=43). The exercise intervention consisted of a 60-min, 3 days/week throughout pregnancy from 17th g.w., supervised concurrent (aerobic+resistance) exercise program. A food frequency questionnaire and the MedDietScore (min-max: 0-50) were employed to assess dietary habits and MD adherence during pregnancy, respectively. Postpartum body composition was measured with dual-energy X-ray absorptiometry at the 6th week after birth.

Results: After adjustments for pre-pregnancy BMI and age, the BMI and the gynecoid fat mass at postpartum were lower in the exercise compared to the control group (p=0.022 and p=0.048, respectively). There was an interaction showing that the MD adherence during pregnancy positively moderated the effects of the exercise intervention on postpartum lean mass (p=0.024), fat mass percentage (p=0.092), android fat mass (p=0.076), and android-to-gynecoid fat mass (p=0.019). The Johnson-Neyman technique revealed that the effects of exercise were enhanced at a MedDietScore of ~31 for lean mass, ~25 for fat mass, ~23 for android fat mass and ~29 for android-to-gynecoid fat mass.

Conclusion: Our results suggest that a concurrent-exercise training plus an optimal MD adherence during pregnancy might be a useful strategy to promote a healthier body composition at the postpartum period.

INTRODUCTION

Pregnancy can trigger physiological permanent changes in body composition and weight gain (70). In Europe, 51% of women experience excessive GWG (72), which is often retained long term after birth (73–75), contributing to the obesity rates in adult women (74,76). PPWR is particularly harmful as it promotes central rather than peripheral fat accumulation (70,83,84). Overall and central/visceral excess of fat are well-known risk factors for greater cardiometabolic risk (e.g., hypertension, impaired glucose tolerance, and elevated triglycerides) (85,86). Hence, it is imperative to find effective strategies to improve maternal body composition at postpartum to avoid future complications.

Exercise interventions during pregnancy can potentially control and avoid excessive GWG and PPWR (183,302,303). The current scientific literature is limited on the effects of different types of exercise programs on GWG, and specially on PPWR and postpartum body composition. Some studies have shown improvements in GWG and body composition as a result of aerobic training alone (303), or in combination with resistance training during pregnancy (183,304) or postpartum (305), while another study did not find any effect on GWG, PPWR, or fat measurements (306).

Regarding the diet, two previous studies have examined the relationship between dietary energy intake and PPWR (74,307). This relationship might be further investigated by examining the dietary patterns beyond the energy intake. Dietary patterns can capture interactions between individual dietary components (308). The MD, characterised by high intake of vegetables, fruits, pulses, fish, olive oil, cereals, nuts, and seeds, and a low consumption of processed food, red meat and dairy products (30), promotes healthy weight in adults (88), and lowers cardiometabolic risk in pregnant women (210). Moreover, a high MD adherence has been associated with greater fat-free mass (89), lower BMI, and weight gains (90,91) in non-pregnant adults. However, to the best of our knowledge, no previous study has explored the relationship between MD adherence during pregnancy and postpartum body composition.

Overall, interventions including diet and exercise components appear to be more effective in promoting postpartum weight and fat mass loss (309), and preserving lean body mass, compared to diet (310) or exercise alone (306). In fact, according to a systematic review (310), exercise should be complemented with a healthy diet to promote weight loss at postpartum. Thus, it is necessary to study the interaction effect of an exercise intervention throughout gestation with a healthy nutritional pattern. Therefore, this study aimed: 1) to investigate the effects of an exercise intervention delivered to pregnant women on postpartum body

composition and, 2) to examine whether an optimal MD adherence during pregnancy modulates these effects.

METHODS

Study design and participants

We employed data from the GESTAFIT project (Identifier: NCT02582567) (182). The study was carried out at the "Sport and Health University Research Institute" (Granada, Spain), and at the "San Cecilio and Virgen de las Nieves University Hospitals" from November 2015 to April 2018. This project was approved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15). The complete methodology of this project, and the inclusion-exclusion criteria **(Table 6)** are available elsewhere (182). We included women aged 20-40 years with a normal pregnancy course, and giving birth (singleton) at 37-42 weeks via spontaneous/vaginal delivery, or cesarean section without severe materno-fetal pathology. Of 384 pregnant women assessed for eligibility, 159 women were randomized. All women provided a written informed consent. A total of 102 pregnant women who had valid data in postpartum body composition measurements and dietary habits were included in this quasi-experimental study. Among them, 83 participants were considered for the per-protocol intervention analyses (exercise n=40, control n=43) **(Figure 2)**. These are secondary analyses from the GESTAFIT Project (182). Power calculations were determined based on the primary outcomes (GWG and maternal/neonatal glycemic profile), and it was 52 women (26 per group) (182). Additionally, a posteriori power calculation analyses showed that this study has a power of 80% to detect medium-sized effect sizes with the 83 women included in the analyses ($f^2 \geq 0.09$) (311).

Randomization and blinding

The study was conducted in three waves. The GESTAFIT project was initially designed as a randomized control trial (computer-generated simple randomisation). Nonetheless, the randomized component was broken in the second and third waves to ensure enough adherence to the program; which represents a frequent methodological barrier in antenatal exercise research (184). Thus, half the women were not randomized but allocated to the control/exercise group according to their personal convenience. Most personnel were blinded to their allocation into the control/exercise group, excepting those responsible for the training sessions.

Exercise Intervention

The exercise intervention consisted of a concurrent supervised-tailored exercise program (from the 17th g.w. until birth, 3 days/week, 60 minutes/session) of aerobic and resistance exercises of moderate-to-vigorous (mostly moderate with peaks of vigorous) intensity. Sessions consisted of a 10-minute warm-up, a 40-minute muscular (circuits of resistance exercises and short aerobic blocks) or aerobic block (dance or functional circuits), and a 10-minute cooldown. Resistance exercises involved anterior and posterior chain dominant, pull, push, and core exercises. The exercise training program was designed following the standards by the American College of Obstetricians and Gynecologists (185), and the latest scientific evidence (186,187). During the intervention, women were provided with 7 seminars to promote healthier pregnancies.

Control Group

Pregnant women in the control group did not attend the exercise sessions and were asked to keep their usual activities, yet they were invited to the seminars.

Measurements

Assessments were performed in 2 non-consecutive days. At the 16th g.w. participants' sociodemographic, body weight, height, and dietary habits were assessed (in that specific order). On the second appointment (6 weeks after giving birth), postpartum body composition was measured.

Sociodemographics

Sociodemographic characteristics of the study sample were gathered through medical files and questionnaires (i.e., age, educational and marital status, and smoking).

Maternal anthropometry, postpartum weight retention and body composition

Pre-pregnancy body weight was self-reported at the recruitment (12th g.w.). Weight was measured 6 weeks after giving birth (no shoes, light clothes) with an electronic scale (InBody-R20; Biospace, Seoul). Height was measured using a stadiometer (Seca 22, Hamburg, Germany). BMI was calculated as kg/m^2.

Postpartum body composition measurements were assessed using dual-energy X-ray absorptiometry (Discovery DXA system; Hologic, Marlborough MA, USA). Body composition

outcomes included total lean mass and fat mass, fat mass percentage, android fat mass (truncal adiposity), gynecoid fat mass (peripheral fat), visceral fat, ratio of gynecoid to total fat mass, and ratio of android to gynoid fat mass. A whole-body scan was performed considering the manufacturer´s guidelines to ensure the quality of the data. The APEX 4.0.2. software (Hologic Series Discovery) was used to draw an automatic delineation of anatomic regions.

Physical activity

Physical activity was monitored for 9 days (24 h/day, except for water activities) with non-dominant-wrist-worn accelerometers (ActiGraph GT3X+, Florida, US). Total and moderate-vigorous physical activity (min/day) were estimated.

Dietary assessment and Mediterranean diet adherence

A food frequency questionnaire validated in Spanish adults (189) was administered by a trained nutritionist at the 16th g.w. and 34th g.w. to assess dietary habits. Women were asked about the frequency of consumption of the different food groups (*never* or *number of times per day, week, month,* or *year*). This study targeted women in the second trimester of pregnancy (13th to 27th g.w.). The first trimester is characterised by morning sickness, whereas dietary habits during the second trimester are more constant and representative of diet across the entire gestation (231). Moreover, we observed similar MD adherence between the 16th g.w. and the 34th g.w. in our sample (232). Consequently, the dietary habits of the 16th g.w. were considered for analyses.

The MedDietScore developed by Panagiotakos et al. (30) was derived from the food frequencies reports (189) to assess MD adherence as previously done in this study sample (210). The MedDietScore consists of eleven variables (wholegrain cereals, potatoes, fruits, vegetables, pulses, fish, olive oil, red wine, red meat and subproducts, poultry, and whole dairy products) ranging from 0 to 5 according to their position in the MD pyramid (271). The total score ranges from 0 to 55, with higher values indicating greater adherence to the MD. A moderate alcohol intake, also typical of the MD, was not considered in this group of women since they are recommended not to drink alcohol during gestation. There were no women consuming alcohol during pregnancy. Therefore, the score considered for these analyses ranged from 0 to 50 points.

Statistical analysis

Descriptive characteristics were summarized as mean (standard deviation) or frequencies (%) as appropriate. As initially designed (182), the primary statistical analysis was conducted using the per-protocol procedure. We only included women who attended more than 75% of exercise sessions and had valid postpartum body composition data (288). We performed an ANCOVA adjusted for age and pre-pregnancy BMI to explore differences in postpartum body composition between the control and exercise groups. Multiple imputations were performed for the intention-to-treat analyses, and the ANCOVA models were replicated including all the women recruited for the project according to the Consolidated Standards of Reporting Trials (CONSORT) guidelines.

The choice of covariates was based *a priori* on what was previously reported in the literature. Age and pre-pregnancy BMI have been previously associated with body composition in pregnant women (307). We performed an additional analysis adjusting for weeks between birth-postpartum assessments, and parity but results remained unchanged (data not shown).

We explored the exercise*MD adherence interaction effects on the postpartum body composition. Potential moderators were further investigated when the exercise*MD interaction term showed a $p<0.2$ and graphically represented using the median-split of the MD adherence score. Additionally, moderation analyses were conducted using the PROCESS macro 3.1 (312) to provide greater resolution for clarifying interactions. PROCESS utilizes ordinary least squares regression analysis when predicting continuous variables (i.e., postpartum body composition outcomes) and a bias-corrected bootstrap method (i.e., 5000 bootstrapped samples) to estimate the conditional (moderated) effects. The Johnson-Neyman technique was used to test for significance along a continuum of moderator values (i.e., MD score) and delineates the slope of the relationship. In this regard, the technique highlights specific MD adherence score cut-points in which the direction of the effect between the exercise intervention on the postpartum body composition changed (i.e., passed from negative to positive effect). The moderation analyses were adjusted for age and pre-pregnancy BMI. All analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY) and the level of significance was set at $p\leq0.05$

RESULTS

Of the 83 pregnant who met the per protocol criteria **(Table 28)**, the mean (standard deviation) age was 33.5 (4.3) years, and the BMI at the postpartum was 25.4 (4.2) kg/m^2. Most of the women had University studies (n=55, 66%) and were married (n=49, 59%). At the postpartum,

mean (standard deviation) weight retention was 4.1 (5.6) kg with 20% (n=16) of women having returned to their pre-pregnancy body weight **(Table 28)**. Women attended to 86% of the sessions on average. No between-groups differences were found at baseline (all, $p>0.05$).

Table 28. Sociodemographic and clinical characteristics of the Study V participants

Variable	Total women (n=83)	Control (n=40)	Exercise (n=43)
Age (years)	33.5 (4.3)	33.8 (4.3)	33.3 (4.3)
Pre-pregnancy body mass index (kg/m²)	23.7 (3.9)	23.2 (3.3)	24.4 (4.0)
Total physical activity (min/day) (n=36 vs 41)	427.0 (94.2)	437.6 (96.8)	417.7 (92.0)
Moderate-vigorous physical activity (min/day) (n=36 vs 41)	39.2 (21.7)	37.8 (23.4)	40.5 (20.3)
Percentage of attendance*			86.3 (6.5)
Postpartum body composition			
Body weight (kg) (n=37 vs 42)	68.2 (10.7)	68.0 (70.8)	68.4 (10.7)
Height (cm) (n=37 vs 42)	163.6 (5.6)	163.5 (5.5)	163.7 (5.7)
Postpartum weight retention (kg) (pp-pre) (n=37 vs 42)	4.1 (5.6)	5.8 (4.6)	2.6 (5.9)
Body mass index (kg/m²) (n=37 vs 42)	25.4 (4.2)	25.5 (3.99)	24.4 (4.0)
Total lean mass (g)	38665.1 (4514.6)	38544.8 (4780.2)	38776.9 (4306.8)
Total fat mass (g)	26138.6 (7257.6)	25993.0 (6916.4)	26273.9 (7640.5)
Total fat mass (%)	38.5 (5.5)	38.5 (5.3)	38.5 (5.8)
Total android fat mass (g)	1855.1 (701.0)	1834.4 (637.8)	1874.5 (762.1)
Total gynecoid fat mass (g)	5218.7 (1280.9)	5287.8 (1215.6)	5154.3 (1350.0)
Visceral fat (g)	365.3 (152.7)	363.3 (152.3)	367.1 (154.8)
Gynecoid to total fat mass ratio	0.202 (0.02)	0.206 (0.02)	0.198 (0.02)
Android to gynecoid fat mass	0.350 (0.08)	0.343 (0.08)	0.357 (0.08)
Mediterranean diet adherence (0-50)	29.1 (3.9)	28.4 (4.0)	29.7 (3.7)
Educational status			
University studies	55 (66.3)	29 (72.5)	26 (60.5)
No university studies	28 (33.7)	11 (27.5)	17 (39.5)
Marital status			
Married	49 (59.0)	24 (60.0)	25 (58.1)
Single/divorced/widow	34 (41.0)	16 (40.0)	18 (41.9)
Type of breastfeeding (n=81)			
Exclusive (only breast)	55 (67.9)	23 (60.5)	32 (74.4)
Mixed (breast and formula milk)	16 (19.8)	10 (26.3)	6 (14.0)
Artificial (only formula milk)	10 (12.3)	5 (13.2)	5 (11.6)
Smoking habit (yes, n [%])	6 (7.2)	5 (12.5)	1 (2.3)
Alcohol intake (yes, n [%])	0 (0)	0 (0)	0 (0)
Women returning to pre-pregnancy weight (yes, n [%])[a] *(n=37 vs 42)*	16 (20.3)	1 (2.7)	15 (35.7)

Values shown as mean (standard deviation) unless otherwise is indicated. [a]Women who weighted the same or less compared to their weight prior to pregnancy. *When considering women on an intention to treat basis, the average percentage of attendance was 75.3%. SD, standard deviation. PPWR, postpartum weight retention.

The effects of exercise on postpartum body composition are shown in **Table 29**. Per-protocol analyses showed that participants in the exercise group had lower postpartum BMI (between-group difference [95% CI]: 1.18 [0.17-2.18] kg/m²; p=0.022) and gynecoid fat mass (between-

group difference [95% CI]: 431.2 [3.52-858.89] g; *p*=0.048). Participants in the exercise group had lower but non-statistically significant total fat mass (between-group difference [95% CI]: 1723.2 [-236.9 to 3683.2] g; *p*=0.084). These differences were no longer significant in the intention-to-treat analyses **(Table 30)**.

Table 29. Influence of the exercise program on postpartum body composition (per protocol)

Variable	Control (n=40)	Exercise (n=43)	Between-Group Difference (B) (95% CI)	*P*
Per-protocol basis*				
Body weight (kg) (n=37 vs 42)	69.59 (1.05)	67.33 (0.99)	2.26 (-0.63 to 5.15)	0.124
Body mass index (kg/m²) (n=37 vs 42)	26.07 (0.36)	24.89 (0.35)	1.18 (0.17-2.18)	0.022
Total lean mass (g)	39054.49 (568.19)	38302.81 (547.72)	751.68 (-830.81 to 2334.17)	0.347
Total fat mass (g)	27031.30 (703.76)	25308.14 (678.40)	1723.16 (-236.91 to 3683.22)	0.084
Total fat mass (%)	39.14 (0.70)	37.95 (0.67)	1.19 (0-0.75 to 3.14)	0.226
Total Android fat mass (g)	1931.42 (70.77)	1784.20 (68.22)	147.23 (-49.89 to 344.34)	0.141
Total gynecoid fat mass (g)	5442.07 (153.56)	5010.86 (148.03)	431.21 (3.52-858.89)	0.048
Visceral fat (g)	381.16 (18.1)	350.5 (17.5)	30.65 (-19.87 to 81.17)	0.231
Gynecoid to total fat mass	0.204 (0.002)	0.200 (0.002)	0.004 (-0.002 to 0.010)	0.196
Android to gynecoid fat mass	0.351 (0.010)	0.349 (0.010)	0.002 (-0.027 to 0.030)	0.897

Values shown as mean (standard error). Model adjusted by age and pre-pregnancy body mass index (kg/m²). *The average percentage of attendance was 86%. SE, standard error.

Table 30. Influence of the exercise program on postpartum body composition (intention to treat)

Variable	Control (n = 86)	Exercise (n = 72)	Between-Group Difference (B) (95% CI)	*P*
Per-protocol basis*				
Body weight (kg)	68.7 (0.7)	67.8 (0.7)	0.845 (-1.078 to 2.768)	0.387
Body mass index (kg/m²)	25.9 (0.2)	25.4 (0.3)	0.516 (-0.194 to 1.225)	0.153
Total lean mass (g)	38779.5 (365.8)	39071.6 (400.0)	-292.078 (-1365.919 to 781.763)	0.592
Total fat mass (g)	24438.4 (1782.8)	25193.2 (1949.5)	-754.845 (-5988.664 to 4478.974)	0.776
Total fat mass (%)	35.8 (2.0)	37.8 (2.2)	-2.093 (-8.112 to 3.926)	0.493
Total Android fat mass (g)	1693.3 (260.7)	1762.2 (285.1)	-68.968 (-834.416 to 696.480)	0.859
Total gynecoid fat mass (g)	5603.7 (196.4)	5220.6 (214.7)	383.149 (-193.279 to 959.577)	0.191
Visceral fat (g)	371.6 (12.5)	374.7 (13.6)	-3.082 (-39.678 to 33.514)	0.868
Gynecoid to total fat mass	0.216 (0.058)	0.185 (0.063)	0.031 (-0.139 to 0.201)	0.718
Android to gynecoid fat mass	0.143 (0.090)	0.323 (1.0)	-0.180 (-0.445 to 0.085)	0.182

Values shown as mean (standard error). Model adjusted for age and pre-pregnancy body mass index (kg/m²). * The average percentage of attendance was 75%. SE, standard error.

The MD adherence during pregnancy was found to potentially moderate the effects of the exercise intervention on postpartum lean mass, fat mass percentage, android fat mass, and android-to-gynecoid fat mass (all *p*'s<0.2). Those participants who received the exercise

intervention and had a greater MD adherence showed higher lean mass, lower fat mass percentage, android fat mass, and android-to-gynecoid fat mass compared to participants who received the intervention and had a lower MD adherence **(Figure 10)**.

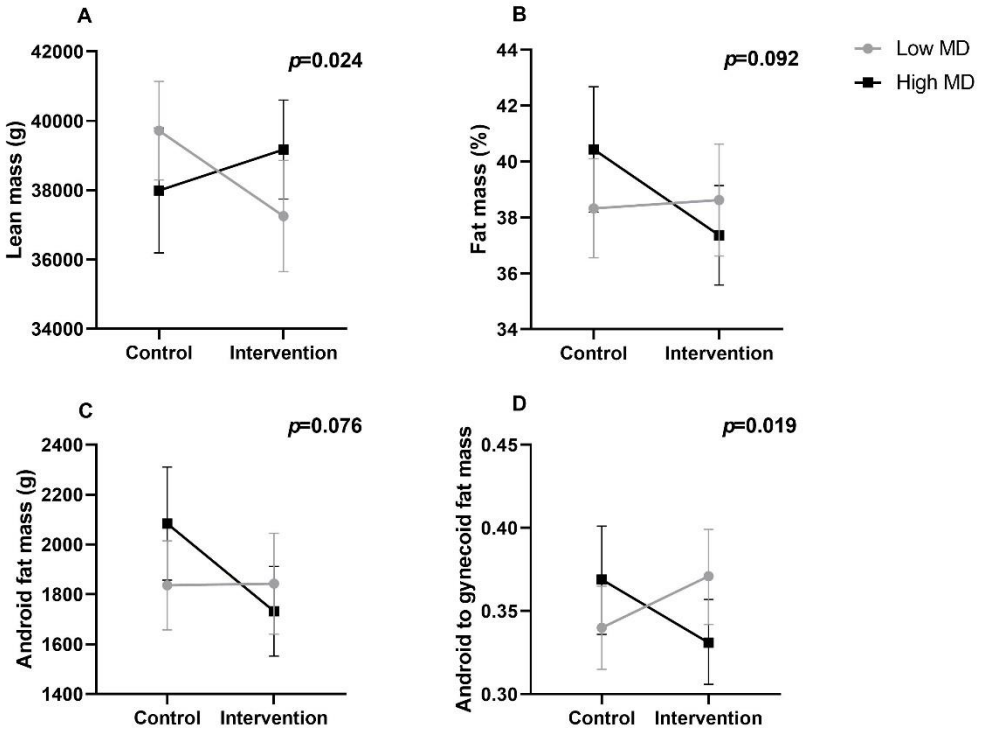

Figure 10. Differences in postpartum body composition outcomes according to exercise intervention and the degree of adherence to the Mediterranean diet (below median vs above median). (A) Interaction between the exercise intervention and the Mediterranean diet adherence on the lean mass. (B) Interaction between the exercise intervention and the Mediterranean diet adherence over the fat mass percentage. (C) Interaction between the exercise intervention and the Mediterranean diet adherence over the android fat mass. (D) Interaction between the exercise intervention and the Mediterranean diet adherence over the android to gynecoid fat mass. MD, Mediterranean diet.

The effects of exercise on postpartum body composition outcomes as a function of the MD adherence score are shown in **Figure 11**. The Johnson-Neyman technique revealed that the direction of the effects changed at a MedDietScore of ~31 for lean mass, MD ~25 for fat mass, MD ~23 for android fat mass, and MD ~29 for android-to-gynecoid fat mass (percentiles 76, 20, 5, and 52 in the study sample, respectively).

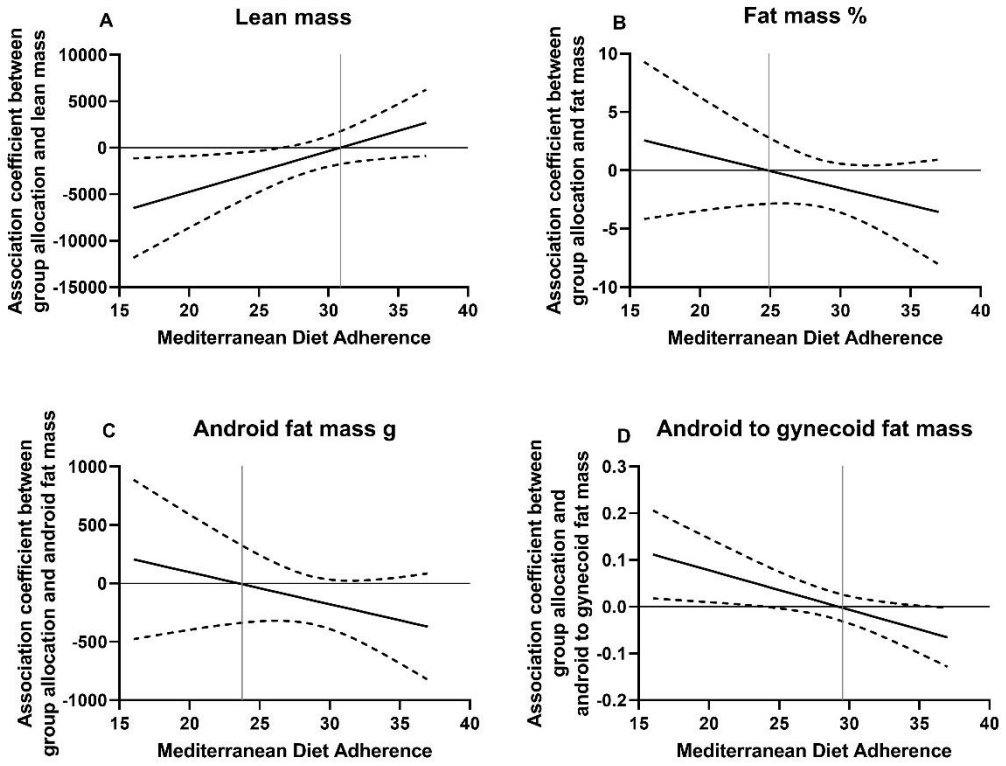

Figure 11. Regression slope estimate and 95% confidence interval for the effects of exercise on postpartum body composition outcomes as a function of the Mediterranean diet adherence. (A) Regression slope estimate and 95% confidence interval for the effects of exercise on lean mass as a function of the Mediterranean diet adherence. (B) Regression slope estimate and 95% confidence interval for the effects of exercise on fat mass as a function of the Mediterranean diet adherence. (C) Regression slope estimate and 95% confidence interval for the effects of exercise on android fat mass as a function of the Mediterranean diet adherence. (D) Regression slope estimate and 95% confidence interval for the effects of exercise on android to gynecoid fat mass as a function of the Mediterranean diet adherence.

DISCUSSION

Our results suggest that the effects of a concurrent exercise (aerobic+strength) program on postpartum body composition could be amplified by the adherence to a healthy dietary pattern (i.e., MD) during pregnancy. At postpartum, those women exercising and following an optimal MD pattern during pregnancy presented greater lean mass, lower percentage of fat mass, lower android fat mass and lower android-to-gynecoid fat mass compared to women exercising with a low MD adherence. Furthermore, we observed that the exercise effects were boosted with MedDietScore of >31 for lean mass, >25 for fat mass, >23 for android fat mass, and >29 for android-to-gynecoid fat mass.

There is evidence suggesting that excessive PPWR contributes to long-term female overweight and obesity rates (73,310,313), which substantially increases the risk of diet-related chronic

disorders (314). PPWR at 6 weeks postpartum ranges from 3 to 7 kg (315) with an average weight retention of 3.7 kg at 6 weeks postpartum among European pregnant women (76), similar to the average weight retention that we found in the present study (i.e., 4.1 kg). Notwithstanding, only 20% of pregnant women at 6 weeks postpartum reached their baseline weight measured prior to pregnancy which is in agreement with previous evidence reporting a similar percentage (22%) (316).

In this context, it is well-known that diet and exercise are major components of most weight loss programs for the general population (313). Concerning exercise, previous studies showed that neither physical activity (317) nor exercise (318) during pregnancy influenced BMI or body fat percentage during gestation, and postpartum body weight. Although Ruchat et al. (319) and Haakstad and Bo et al. (320) found that pregnant women who were randomized to an exercise intervention group during gestation retained less postpartum weight, no other postpartum body composition outcomes were assessed. On a per protocol basis (when only considering those women with >75% attendance), we found a significant reduction in BMI and gynecoid fat mass in the exercise compared with the control group. These associations were no longer significant in the intention-to-treat analyses (after multiple imputation of the data). This may be partially explained by differences in the sample size but also by the fact that women included in the intention-to-treat analyses did not attend 75% of the exercise intervention programme, or even dropped out of the study. In fact, the average attendance was 86% in the per-protocol sample and 75% when including all women (intention-to-treat). Therefore, it seems plausible that a mitigation of the effects of the exercise intervention on postpartum body composition might be observed on an intention-to-treat basis analysis.

We did not find between-groups differences on postpartum body weight without accounting for the moderation effect of diet. However, according to a systematic review (310), exercise alone is no sufficient to promote weight loss in women after childbirth. Interventions including diet and exercise appear to be more effective in promoting postpartum body weight and fat mass loss (309) than exercise alone (306). Interestingly, those participants who received the exercise intervention and had an optimal MD adherence (above median) did not show differences in postpartum body weight but showed a higher lean mass, a lower percentage of fat mass, and a healthier distribution of fat mass (i.e., lower android fat mass and lower android-to-gynecoid fat mass). Because of the increase in one body composition component (i.e., lean mass) and the decrease in another (i.e., fat mass), the total weight might have remained stable between exercise and control groups. However, there were positive changes in postpartum body composition outcomes, which might be clinically meaningful as

previously stated (321). In addition, we observed that adhering to a MD pattern might be crucial to either observe or boost these effects of the exercise on the postpartum body composition. This MD adherence ought to be above 23 (percentile 14.5 in the study sample) for android fat mass, above 25 (percentile 20.5) for fat mass percentage, above 29 (percentile 51.8) for android-to-gynecoid fat mass, and above 31 (percentile 75.9) for lean mass. Therefore, our findings show the potential role of the diet on the effects of exercise on postpartum body composition. However, this should be further tested by combining diet and exercise interventions in randomized controlled trials.

Limitations and strengths

The results of the present study should be considered considering some limitations. Firstly, this is a quasi-experimental study which is a limitation since women were not purely randomized. Secondly, our sample size might be limited and larger trials should contrast our findings. Thirdly, body composition was only assessed after 6 weeks postpartum, thus, longer follow-ups could provide additional useful information. Regarding strengths, the measurement tool employed to assess body composition (i.e., dual-energy X-ray absorptiometry) is widely valid and reliable, which guarantees the quality of the data. Furthermore, a detailed definition of the dietary habits and a valid assessment of the MD adherence was employed. In addition, the exercise group followed a novel concurrent exercise that combines aerobic+resistance training, which has been proved as the most useful protocol to improve cardiometabolic status during pregnancy (322).

CONCLUSION

Overall, our results suggest that the exercise effects on postpartum body composition might be enhanced by following a healthy dietary pattern (i.e., MD) during pregnancy. Specifically, those participants who received the exercise intervention and had an optimal MD adherence showed greater lean mass, lower fat mass and a healthier distribution of body fat. Further randomized controlled trials are needed to examine the benefits of a combined exercise and diet intervention during pregnancy (i.e., ensuring a MD adherence of at least 31 points in the MedDietScore) to reduce adiposity and preserve lean mass beyond pregnancy. The inclusion of dietary advice within exercise training programs should be considered in future projects.

SECTION III. Influence of Mediterranean diet during pregnancy on sleep and mental health

STUDY VI: Influence of dietary habits and Mediterranean diet adherence on sleep quality during pregnancy. The GESTAFIT project

Flor-Alemany M, Nestares T, Alemany-Arrebola I, Marín-Jiménez N, Borges-Cosic M, Aparicio VA.

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ABSTRACT

Aim: To examine the association of dietary habits and MD adherence with sleep quality during pregnancy.

Methods: This study comprised 150 pregnant women from the GESTAFIT project (mean age 32.9 ± 4.6 years). Dietary habits were assessed with a food frequency questionnaire at the 16th and 34th g.w. The MFP was employed to assess MD adherence. Sleep quality was assessed with the PSQI global score at the 16th and 34th g.w.

Results: A higher consumption of fruits was associated with better sleep quality at the 16th g.w. (p<0.05). A greater olive oil consumption and a higher MD adherence were associated with better sleep quality at the 16th and 34th g.w. (all, p<0.05). Contrarily, a higher red meat and subproducts consumption was associated with worse sleep quality at the 34th g.w. (p<0.05). The group with a high MD adherence showed better sleep quality than the group with a low MD adherence at the 16th and 34th g.w. (both, p<0.05).

Conclusion: A higher adherence to the MD, a greater intake of fruits and olive oil and a lower intake of red meat and subproducts were associated with better sleep quality along the pregnancy course.

INTRODUCTION

Sleep disturbances are common complaints during pregnancy, with recent studies suggesting that almost 50% of expectant mothers experience poor sleep quality, with rates close to 75% by the third trimester of pregnancy (135–137). Assessments of sleep quality during pregnancy might be clinically relevant given the evidence that poor sleep quality is linked with an array of adverse health outcomes including inflammation, metabolic syndrome and type 2 diabetes (135,323–325). Moreover, recent hypotheses suggest that poor sleep quality is associated with negative birth outcomes such as increased odds of preterm birth, cesarean section, shorter length of gestation and longer labour (135,325,326), whereas good sleep quality is associated with a better Apgar score among neonates and birth weight (327).

Considering the impact of sleep-related habits on adverse health outcomes, it is crucial to investigate and identify potential dietary determinants of sleep quality during pregnancy (140). Among the many factors studied that could exert an influence on sleep quality, diet seems to have an impact on both, sleep quality and its related health outcomes (323). Indeed, sleep and diet are in fact strongly interrelated, with recent studies (139,323,328,329) suggesting a bi-directional association: poor sleep quality may negatively affect dietary habits by reducing overall diet quality and increasing appetite and caloric intake (139), while at the same time food choices might influence sleep quality (329). With this in mind, poorer dietary patterns, such as those characterized by a high fat and sugar content, have been linked to worse sleep quality in all age groups (330–332). On the contrary, cross-sectional studies (139–141) have shown that diets with a high intake of fruits, vegetables and a lower intake of saturated fatty acids, such as the MD, might be beneficial for sleep quality in adult population. Although these observations helped to establish a sleep-diet relation, little is known about how the MD adherence and its components may be linked to measures of sleep quality in pregnant women. Therefore, the aim of the present study was to explore the association of dietary habits and the MD adherence with sleep quality during pregnancy.

METHODS

Study design and participants

The present cross-sectional study forms part of the GESTAFIT project, where a novel exercise intervention was conducted (182). The entire methodology of the project, the inclusion-exclusion criteria **(Table 6)** and the sample size calculation to detect clinically meaningful changes in the intervention program have been published elsewhere (182). The required sample size was only determined for the primary outcome (i.e., maternal weight gains) of the

GESTAFIT project. A total of 159 Spanish pregnant women (32.9±4.6 years old) enrolled in this study in three waves for feasibility reasons. The participants were recruited between the 11-13th g.w. at the "San Cecilio" University Hospital (Granada, Spain) during their first gynecologist checkup. This study was approved by the Ethics Committee on Clinical Research of Granada, Regional Government of Andalusia, Spain (code: GESFIT-0448-N-15). The procedures described in the manuscript have been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). From the 159 pregnant women recruited, this cross-sectional study included 150 women (mean age 32.9±4.6 years) at the 16th g.w. who had valid data in the food frequency questionnaire and the PSQI (**Figure 2**). From the 150 pregnant women, thirty-two had missing data in the food frequency questionnaire and/or the PSQI global score at the 34th g.w. As a result, a total of 118 pregnant women were included for the present analyses at the 34th g.w.

Sociodemographic characteristics

The evaluation procedures were carried out at the 16th and 34th g.w. at the Sport and Health University Research Institute. At the 16th g.w. data regarding sociodemographic and lifestyle characteristics (i.e., age, educational, marital, and working status, number of children, smoking habit and physical or psychological disease diagnosis) were collected through an initial survey (anamnesis).

Maternal anthropometry and body composition

Pre-pregnancy body weight was self-reported. Body weight and height were measured using a scale (InBody R20; Biospace, Seoul, Korea) and a stadiometer (Seca 22, Hamburg, Germany), respectively. Those measurements were employed to calculate pre-gestational BMI and BMI at the 16th g.w. as weight (kg) divided by squared height (m^2).

Dietary assessment and Mediterranean diet adherence

Dietary habits were collected by using the food frequency questionnaire designed by Mataix et al. (189). The same trained nutritionist administered the questionnaires to pregnant women at the 16th and 34th g.w. The MFP (a MD index) was constructed with the data obtained from the food frequency questionnaire (189). We employed this dietary index because it was previously associated with lower cardiometabolic risk along the pregnancy course in this study sample (233). The MFP was calculated based on previously published literature (190). It consists of eight elements (olive oil, fiber, fruits, vegetables, fish, cereals, meat and alcohol)

ranging from 8 to 40. Notwithstanding, alcohol consumption was not considered when calculating the total score. Thus, the maximum score ranges from 7 to 35, where higher scores indicate greater MD adherence. Women were defined as adherent to the MD if they had a MD adherence above 21 points as previously stated in this study sample (233). Therefore, participants were classified as having a high MD adherence if they had a score of ≥21 points in the MFP index.

Sleep quality

The Spanish version of the PSQI (196) was employed to assess sleep quality at the 16th and 34th g.w. since the PSQI has been shown to have a good construct validity among pregnant women (333). The PSQI is a self-rated questionnaire that measures sleep quality from the previous month, comprised of 19 questions divided into seven categories: subjective sleep quality, sleep latency, sleep duration, sleep disturbances, sleep efficiency, use of sleep medication and daytime dysfunction. Each component scores from 0 to 3, with a total score that ranges from 0 to 21 with lower values indicating better sleep quality (196). The suggested cut off is 5 points differentiating "good" from "bad" sleepers (196).

Statistical analyses

Descriptive statistics (mean [standard deviation] for quantitative variables, and number of women (%) for categorical variables) were employed to describe participants' sociodemographic characteristics. The distribution of the data was examined for all the study variables, and the PSQI global score showed a skewed distribution that could not be normalized after several transformations (e.g., logarithmic transformations). Subsequently, we performed the Spearman's correlation analysis between the dietary habits, the MFP score and the PSQI global score at the 16th and 34th g.w. Differences between dietary habits, MFP and PSQI global score by the g.w. (16th g.w. versus 34th g.w.) were tested using the Wilcoxon nonparametric test. Differences in PSQI global score by MD adherence (low MD adherence vs. high MD adherence) were compared by using the Mann–Whitney U nonparametric test. We performed additional analyses to further explore whether the concurrent physical exercise program, which was carried out in the GESTATIT project (182), exerted an influence on the studied associations. To account for the exercise intervention delivered in the GESTAFIT project Spearman's correlations were employed to assess the association between the dietary habits, the MD adherence and the PSQI global score at the 34th g.w. only in the control group (n=52). All analyses were performed using the Statistical Package for Social Sciences (IBM

SPSS Statistics for Windows, version 22.0, Armonk, NY); the level of significance was set at $p<0.05$.

RESULTS

Sociodemographic characteristics of the participants are shown in **Table 31.**

Table 31. Sociodemographic and clinical characteristics of the Study VI participants

Variable	Mean (SD)
Age (years)	32.9 (4.6)
Pre-gestational body mass index categorization (n=136)	
Normal weight (n %)	87 (64.0)
Overweight (n%)	34 (25.0)
Obese (n%)	11 (11.0)
16th gestational week	
Body mass index (kg/m²) (n=148)	24.9 (4.1)
Pittsburgh Sleep Quality Index global score (0-21)	6.01 (3.2)
Poor sleep quality (n, %)	72 (48.0)
Mediterranean Food Pattern (7-35)	20.6 (5.1)
34th gestational week (n=118)	
Pittsburgh Sleep Quality Index global score (0-21)	8.83 (3.76)
Poor sleep quality (n %)	89 (75.4)
Mediterranean Food Pattern (7-35)	21.1 (5.4)
Educational Status	n (%)
Non-University studies	62 (41.3)
University studies	88 (58.7)
Marital status	
Single/divorced	62 (41.3)
Married	88 (58.7)
Working status	
Not working (unemployed/homework/student/sick leave)	48 (32.0)
Partial-time employed/ Full-time employed	102 (68.0)
Number of children	
0	90 (60.0)
1 or more	60 (40.0)
Smoking status ((yes, n (%))	13 (8.7)
Physical or psychological disease diagnosis ((yes, n (%))	61 (40.7)

Values shown as mean (SD) unless otherwise is indicated. SD, Standard deviation.

The Spearman's correlation analysis assessing the association of dietary habits and the MD adherence with the PSQI global score at the 16th and 34th g.w. is shown in **Table 32**. At the 16th g.w., a higher consumption of fruits, olive oil and a higher MD adherence were associated with a lower PSQI global score (i.e., better sleep quality) (p=0.008, p=0.048 and p=0.039, respectively). In addition, a higher red meat and subproducts consumption was associated

with a higher PSQI global score with borderline significance (i.e., worse sleep quality) (p=0.078). At the 34th g.w., a higher consumption of olive oil and a higher MD adherence were associated with a lower PSQI global score (p=0.038 and p=0.001, respectively). A higher red meat and subproducts consumption was associated with a greater PSQI global score (p=0.032). Additional sensitivity analyses (i.e., associations of MD adherence with PSQI global score at the 34th g.w.) showed similar results when exclusively including the control group participants in the analyses **(Table 33)**.

Table 32. Association between the Mediterranean diet adherence and the Mediterranean diet components with the Pittsburgh Sleep Quality Index global score at the 16th and 34th gestational weeks

Food groups	**PSQI global score[a] (16th gestational week)**	**PSQI global score[a] (34th gestational week)**
Whole-grain cereals (s/week)	-0.056	-0.158
Potatoes (s/week)	-0.012	0.099
Fruits (s/day)	-0.216**	-0.126
Vegetables (s/day)	-0.025	-0.089
Pulses (s/week)	0.112	0.043
Fish (s/week)	0.032	-0.087
Red meat and subproducts (s/week)	0.144	0.198*
Poultry (s/week)	0.064	0.101
Whole dairy products (s/week)	0.012	-0.094
Olive oil (s/week)	-0.162*	-0.192*
Nuts (s/week)	-0.096	-0.160
Sweets (s/week)	0.048	0.138
Mediterranean Food Pattern (7-35)	-0.169*	-0.301**

[a]A higher score means worse sleep quality. PSQI, Pittsburgh Sleep Quality Index; S, servings. *p<0.05; **p<0.01.

Table 33. Association between dietary habits, Mediterranean diet adherence and Pittsburgh Sleep Quality Index global score at the 34th gestational week in the control group

	Control group
Food groups	**PSQI global score[a] (34th gestational week) (n=52)**
Whole-grain cereals (s/week)	-0.107
Potatoes (s/week)	0.234
Fruits (s/day)	-0.214
Vegetables (s/day)	-0.157
Pulses (s/week)	-0.027
Fish (s/week)	-0.175
Red meat and subproducts (s/week)	0.169
Poultry (s/week)	0.139
Whole dairy products (s/week)	-0.099
Skimmed dairy products (s/week)	-0.035
Olive oil (s/week)	-0.367**
Nuts (s/week)	-0.225
Sweets (s/week)	0.165
Mediterranean Food Pattern (7-35)	-0.451**

[a]A higher score means worse sleep quality. PSQI, Pittsburgh Sleep Quality Index; S, servings. *p<0.05; **p<0.01.

The PSQI global score at the 16th and 34th g.w. by degree of MD adherence is shown in **Figure 12**. Pairwise comparisons showed that the group with a high MD adherence had a lower PSQI global score than the group with a low MD adherence at the 16th g.w. and 34th g.w. (p=0.008 and p=0.005, respectively).

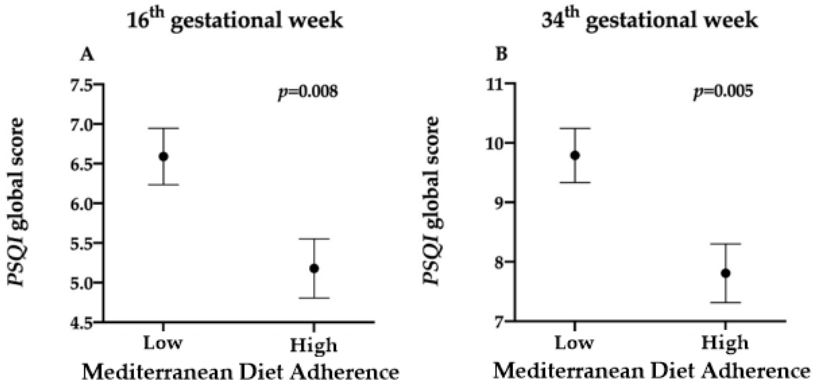

Figure 12. Pittsburgh Sleep Quality Index global score by Mediterranean diet adherence. Dots represent mean and bars standard error. (A) Pittsburgh Sleep Quality Index global score by low vs. high Mediterranean diet adherence at the 16th gestational week. (B) Pittsburgh Sleep Quality Index global score by low vs. high Mediterranean diet adherence at the 34th gestational week. PSQI, Pittsburgh Sleep Quality Index.

Differences between dietary habits, MD adherence and the PSQI global score by g.w. (16th g.w. versus 34th g.w.) are shown in **Table 34**. Regarding dietary habits, pregnant women at the 34th g.w. had higher intake of fruits, vegetables and whole dairy products (p=0.010, p=0.014 and p=0.044, respectively). No differences were found regarding MD adherence (p>0.05). In addition, pregnant women at the 34th g.w. had a higher PSQI global score (p<0.001).

Table 34. Differences in dietary habits, Mediterranean diet adherence and Pittsburgh Sleep Quality Index global score of pregnant women by gestational week

	16th gestational week	**34th gestational week**	**p**
Food groups			
Whole-grain cereals (s/week)	4.9 (5.3)	5.6 (6.3)	0.101
Potatoes (s/week)	2.3 (1.2)	2.3 (1.5)	1.000
Fruits (s/day)	2.1 (1.1)	2.4 (1.2)	0.010
Vegetables (s/day)	3.6 (1.7)	3.8 (1.7)	0.014
Pulses (s/week)	2.6 (1.1)	2.6 (1.1)	0.394
Fish (s/week)	4.9 (2.3)	5.3 (2.4)	0.112
Red meat and subproducts (s/week)	5.4 (3.4)	5.7 (3.8)	0.340
Poultry (s/week)	2.6 (1.2)	2.6 (1.3)	0.862
Whole dairy products (s/week)	2.9 (3.9)	4.0 (1.8)	0.044
Olive oil (s/week)	13.9 (11.6)	13.3 (7.9)	0.888
Sweets (s/week)	8.9 (6.4)	9.5 (6.9)	0.195
Nuts (s/week)	5.2 (5.3)	5.5 (5.4)	0.487
Mediterranean Food Pattern (7-35)	21.0 (5.0)	21.1 (5.4)	0.847
Pittsburgh Sleep Quality Index global score (0-21)[a] (n=116)	6.20 (3.3)	8.76 (3.8)	<0.001

Data shown as mean (standard deviation). [a]A higher score means worse sleep quality. S, servings.

DISCUSSION

The main finding of the present study is that a greater MD adherence was associated with better sleep quality during both, the 16th and 34th g.w. In addition, a greater consumption of fruits, olive oil and a lower intake of red meat and subproducts (i.e., beef, pork, viscera and cold meat products) were associated with better sleep quality along gestation. Moreover, pregnant women with a high MD adherence showed better sleep quality than the groups with low MD adherence at the 16th and 34th g.w.

Sleep quality is often compromised in pregnant women and aggravated over the course of pregnancy (334). A recent study (335) reported that 47% of pregnant women had poor sleep quality (as measured by the PSQI) between the 12th and 20th g.w., the same percentage that we found at the 16th g.w. (48%). Moreover, sleep quality significantly decreased from second to

third trimester, with 75% of pregnant women reporting a poor sleep quality at the 34th g.w. which is in agreement with a previous study that showed that 75-83% of pregnant women had poor sleep quality in the 3rd trimester of pregnancy (7-8 months) (137).

Comparing the early second trimester with the third trimester, we observed a significantly higher intake of fruits, vegetables and dairy products in the third trimester, as previously reported (336). It is possible that participants might have increased their fruits, vegetables and dairy products intakes due to nutritional advice which usually promotes fruit, vegetables and dairy consumption in order to meet the nutritional requirements of pregnancy (337). However, our results showed that adherence to the MD remained unchanged across pregnancy. This finding suggests that food behaviour of our sample did not change during gestation, which concurs with previous studies (63,211). Moreover, during early gestation, food intake can be often affected by nausea and vomiting, physiological phenomena linked to hormonal changes during this period (92). However, women recruited in this study were all after the 13th week of gestation, which could partially explain the lack of differences between food habits between different gestational stages.

It has been established that poor sleep quality negatively affects dietary habits by reducing overall diet quality and increasing appetite and caloric intake (139). Notwithstanding, recent data also suggest a bi-directional association by which food choices might positively influence sleep quality (329). Recent studies (139,140) showed an association between the adherence to the Mediterranean dietary pattern and sleep quality, suggesting that plant-rich diets might be beneficial for sleep in adult population. However, evidence in pregnant women is scarce. A study conducted by Chang et al. (338) in overweight and obese pregnant women showed direct associations between sleep disturbances and dietary fat intake, and also between shorter time to fall asleep and a higher fruit and vegetables intake. Nonetheless, neither diet quality or dietary patterns were included in these studies. In agreement with our findings, a more recent study (334) showed that better sleep quality was associated with greater diet quality and a greater adherence to a dietary pattern based on fruits, vegetables and rice. In fact, in the present study sample, a higher MD adherence (which is a diet high in fruits, vegetables, fiber, and low in saturated fatty acids) was associated with better sleep quality along pregnancy.

Further, participants with a high MD adherence had a better sleep quality during the pregnancy course than the group with a low MD adherence. This concurs with a previous study in non-pregnant adult population where those individuals with a greater adherence to the Mediterranean dietary pattern presented overall better sleep quality compared to those with less adherence (140). Moreover, if such an eating pattern influences sleep during

pregnancy, it is not clear which specific component or components of the Mediterranean dietary pattern would exert a stronger influence. To further explore this issue, we also studied the different food groups that compose the Mediterranean dietary pattern, finding that a higher intake of fruits and olive oil and a lower intake of red meat and subproducts were associated with better sleep quality during pregnancy. A previous study (140) checked if any of the MD components alone could explain the association of the MD score with better sleep quality, suggesting that olive oil consumption itself might play an independent role in sleep quality, which is highly in agreement with our results. Regarding fruits, it has been suggested that the odds of meeting or exceeding the sleep recommendations (i.e., 7–9 h per day for adults aged 18–64 years old) increase by 12% in pregnant women for every additional fruit serving consumed (339). In the same line, a study conducted in women within 5 years of childbirth found that women with longer sleep duration (≥9 hours) had poorer overall diet quality, a lower intake of fruits and a higher intake of calories from solid fats and added sugar, compared to women with an adequate sleep duration (7-8 hours) (340). Evidence also suggests that diets rich in fats and carbohydrates, with a tendency to snack between foods, are associated with poorer sleep quality and fewer sleeping hours in the general population (330,331,341). The MFP which was employed to calculate the MD adherence does not directly assess sugary food intake. For this reason, the sweets variable (including soft drinks, preserved juices, biscuits, baked goods and chocolate) was additionally calculated, as it represents an important component of unhealthy dietary habits. Sweets intake in this study sample was slightly greater than one serving per day, an amount that is within the recommended intake of sugary foods (<3 servings/day) accordingly to the final nutritional objectives for Spanish population (342). This result could be due to the limitation of the items of the food frequency questionnaire itself or to underreporting but could also be derived from the wish of the mothers to follow healthier dietary patterns during pregnancy, avoiding highly processed foods. Moreover, the observed lower sugary food intake in this group could also explain why no correlations were found between them and the PSQI global score.

In this study sample, a higher intake of red meat and subproducts were associated with poorer sleep quality along gestation. Similarly, a study conducted by Lana et al. (343) suggested that a high protein intake derived from meat (white or red meat) was associated with poor sleep quality in non-pregnant adult population. The detrimental effect of red meat and subproducts on sleep quality might be exerted through the protein content of meat as previously stated (343). The effects of protein on sleep quality could be related to two amino acids (tryptophan and tyrosine) and their capacity to synthesize melatonin, serotonin and dopamine (involved

in the sleep-wake cycle) (343). It has been suggested that a high consumption of protein could reduce the blood circulation ratio of tryptophan/tyrosin, which could result in a lower synthesis of brain sleep inductors and a consequently deterioration of sleep parameters, which is in agreement with our findings (343–345). Other food groups, that are naturally rich in protein and were tested in this study (e.g., dairy, poultry), did not show significant results between them and the PSQI global score. Since red meat and subproducts intake was the most consumed group of meat, this finding might overlap the potential influence of other sources of animal protein.

It has been suggested that the high isoflavone and tryptophan content of plant-based diets (i.e. MD) may be the mechanism by which plant foods may enhance sleep quality (139). Interestingly, in a sub-sample of participants from the PREDIMED study, participants in the two MD groups showed an increment in tryptophan concentrations and this was related to lower non-stroke outcomes (346). The authors suggested that changes in tryptophan may be involved in the cardio-protective effects of the MD (346). Sleep and sleep-related metabolite derivatives of tryptophan, melatonin and serotonin, were not measured in this study. Nevertheless, given our understanding of tryptophan metabolism, sleep improvements may have further played a role in this result (139).

Limitations and strengths

When considering the results of the present study, some limitation ought to be kept in mind. Firstly, the cross-sectional design of the study provides information without a clear cause-effect identification. As a result, we cannot determine whether a healthier diet affects sleep quality or, on the contrary, sleep features lead to unhealthy dietary behaviours. Secondly, since we employed a food frequency questionnaire in order to assess dietary habits, we are aware of its recall bias and its lower accuracy when compared to 24h food diary. Nonetheless, the food frequency questionnaire (which is widely employed in nutritional epidemiology) was conducted by the same trained nutritionist along the pregnancy course. Importantly, it is necessary to acknowledge that both the sleep quality and the dietary adherence were self-reported. While PSQI is a widely employed tool validated in pregnant population (333), it is not as valid as an objective measure of sleep such as polysomnography.

CONCLUSIONS

The present study provides some evidence linking the MD to better sleep quality during pregnancy, especially among sedentary women. Specifically, a higher intake of fruits and olive oil, a lower intake of red meat and subproducts and a greater adherence to the MD are associated with better sleep quality along the pregnancy course. Given the limited number of studies available, further research is warranted to explore the impact of maternal healthy dietary habits on sleep quality during pregnancy allowing to investigate on causality and its mechanisms. Intervention studies are warranted to explore whether plant-based diets (i.e., the MD) might positively influence sleep quality during gestation.

STUDY VII: Associations of Mediterranean diet with psychological ill-being and well-being throughout the pregnancy course: The GESTAFIT project

Flor-Alemany M, Baena-García L, Migueles JH, Henriksson P, Löf M, Aparicio VA.

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ABSTRACT

Aim: The relation between diet and maternal mental health during pregnancy might be relevant to prevent adverse materno-fetal outcomes. This study examined the association of MD adherence and MD components with mental health during pregnancy.

Methods: These secondary analysis of the GESTAFIT trial included longitudinal data from 152 pregnant women. Dietary habits were assessed with a food frequency questionnaire, and MD adherence was derived from it using the MFP (i.e., a MD index). Psychological ill-being (i.e., negative affect, anxiety, and depression) and well-being (i.e., emotional intelligence, resilience, positive affect) were assessed with the Spanish version of well-established self-reported questionnaires. Cross-sectional (16^{th} g.w.) and longitudinal associations (34^{th} g.w.) between MD and mental health were studied using linear regression models.

Results: A greater MD adherence was inversely associated with negative affect and anxiety; and positively associated with emotional regulation, resilience and positive affect at the 16^{th} and 34^{th} g.w. ($|\beta|$ ranging from 0.179 to 0.325, all $p<0.05$). Additionally, a higher intake of whole grain cereals, fruits, vegetables, fish, olive oil and nuts, and a lower intake of red meat and subproducts and sweets were associated with lower negative affect, anxiety, depression and higher emotional regulation, resilience and positive affect throughout gestation ($|\beta|$ ranging from 0.168 to 0.415, all $p<0.05$).

Conclusion: A higher intake of whole grain cereals, fruits, vegetables, fish, olive oil and nuts, together with a lower intake of red meat and sweets, resulted in a higher MD adherence, which was associated with a better mental health during pregnancy.

INTRODUCTION

Pregnancy is a major life event that entails biological, psychological, and social changes in the women's mental health (347). Depression and anxiety are the most prevalent mental health disorders during pregnancy (348). Current data indicates that 26-31% of pregnant women are at risk of depression in the second trimester (349), 7-15% suffer from antenatal depression (145), and 14-54% from antenatal anxiety (146). These mental health disorders seem to increase the risk for pregnancy-related complications (e.g., preeclampsia, spontaneous preterm delivery or low birth weight) (145,146,348).

Therefore, identifying protective factors for mental health in pregnant women is warranted (350). Both, low levels of psychological ill-being and high levels of well-being should be considered to reach an optimal metal health status (152). The dietary intake during pregnancy might affect the psychological ill-being and well-being in pregnant women (153,154). Previous research found that the intake of certain food groups and nutrients (i.e., refined grains, sweets, energy drinks, and fast foods) increases the risk for antenatal depressive symptoms compared with alternative healthy choices (i.e., fruits, vegetables, fish and whole grains) (154,158,159). Notwithstanding, there is a shift in the nutrition field towards assessing the whole diet and its quality to investigate the diet-disease relationship (153). As an example, the MD (characterized by a high intake of fruits, vegetables, whole grains, fiber, olive oil, and low intake of red meat, dairy, and processed foods) is associated with a lower risk of depression in the general population (351), yet information in pregnant women is scarce.

Thus, research investigating not only single food groups, but also the diet quality during pregnancy (e.g., MD), is required to provide robust evidence on the association of diet with psychological ill-being and well-being. The aim of this study was to analyze the association of dietary habits and MD adherence at the 16th g.w. with psychological ill-being and well-being at the 16th and the 34th g.w.

METHODS

Study design and participants

This longitudinal study was conducted in Granada (Spain), within the GESTAFIT project framework, where a concurrent training program from the 17th g.w. until delivery (3 days/week, 60 minutes/session) was conducted (182). It consisted in a combination of aerobic-resistance exercises of moderate-to-vigorous intensity. 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 study was carried out at the "Sport and Health University Research Institute" (Granada, Spain), and at the "San Cecilio and Virgen de las Nieves University Hospitals" from November 2015 to April 2018. A total of 159 women met the inclusion-exclusion criteria **(Table 6)**. Among them, 152 participants were included upon providing data in sociodemographic characteristics and MD adherence at the 16th g.w. **(Figure 2)**. Women provided a written informed consent. The study was approved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15).

Sociodemographic characteristics

Sociodemographic characteristics (i.e., age, number of miscarriages and educational and working status) were compiled with a self-reported questionnaire (anamnesis) at the 16th g.w.

Sample size calculation

The sample size for this study depended on the 'a priori' analyses of the statistical power performed in the GESTAFIT project (182). Based on the primary outcome (i.e., maternal weight gains), we planned to recruit 60 women assuming a statistical power of 90%, $\alpha = 0.05$, and a 15% of potential withdrawals. Given the exploratory basis of the present study (secondary outcomes) we did not calculate the sample size. Notwithstanding, we performed an "a posteriori" analysis to investigate the effect sizes detectable in this study. Assuming a statistical power of 80% and 5% type I error, we have enough sample to detect small-to-medium standardized association sizes at the 16th g.w. ($n \geq 117$, minimum detectable $f^2=0.12$) and at the 34th g.w. ($n \geq 109$, minimum detectable $f^2=0.13$) (290).

Psychological well-being

Positive affect (the extent to which a person feels enthusiastic, active, and alert) was assessed with the positive subscale of the PANAS-S (197,198). This questionnaire is comprised of 10 positive and 10 negative emotional states that are answered on a 5-point Likert scale (1-5). The total score ranges from 10 to 50 with higher scores reflecting greater affective well-being (PANAS-S positive subscale). The PANAS is one of the most widely used measures of affectivity and has been reported to have excellent psychometric properties in the adult population (197,198,352).

The 3 subscales of TMMS (199) were employed to assess emotional attention (i.e., to what extent individuals tend to observe and think about their feelings and moods), emotional clarity (i.e., the understanding of one's emotional states) and emotional regulation (i.e., individuals' beliefs about ability to regulate their feelings). The Spanish modified version of the TMMS (199) had appropriate reliability and validity. Each subscale is comprised of 8 items which are rated on a 5-point Likert scale (1-5). Therefore, the total scores range from 8 to 40, with higher scores reflecting greater attention, clarity, and regulation.

The CD-RISC was employed to assess resilience to stress (i.e., individual's ability to thrive despite adversity) (200). The CD-RISC consists of 10 items which are score from 0 to 4. Therefore, the total score ranges from 0 to 40, with higher scores indicating a greater resilience. In regard to reliability, the CD-RISC showed good psychometric properties in young adults; with a Cronbach's α of 0.85 (200).

Psychological ill-being

Negative affect (a variety of aversive mood states, including anger, contempt, disgust, guilt, fear, and nervousness) was assessed with the PANAS-S negative subscale (197,198). The total score ranges from 10 to 50 with higher scores reflecting greater emotional distress.

The STAI-S questionnaire was employed to evaluate state-anxiety levels in pregnant women at the moment of the evaluation (202,203). It consists of 20 items on a four-point scale (0–3). The total score ranges from 0 to 60 with higher values indicating greater levels of anxiety.

The validated 20-item Spanish version of the CES-D, the most accepted calculation method across the literature, was employed to assess pregnant antenatal depression (204). The CES-D is composed of 20 items; each one scored in a scale from ''0'' to ''3'', thus the total score varies from 0 to 60. In regard to reliability, it has very good internal consistency (Cronbach's α=0.91) with similar coefficients by groups of age and sex and by interviewer (204). In this study, we applied the cut-off point of 16 to dichotomize the group into having or not risk of clinical depression as previously done in pregnant women (350).

Low back pain

Low back pain was assessed with the Pain Visual Analogue Scale (353). The score is determined by measuring the distance on the 100-mm line between the "no pain" anchor and the participant's mark.

Dietary assessment and Mediterranean diet adherence

A food frequency questionnaire validated in Spanish non-pregnant adult population (189) was used to assess dietary habits at the 16th and 34th g.w. in face-to-face interviews by trained staff. The MFP (190) was derived from the food frequency questionnaire following previously-defined standards. The MFP consists in a quintile-based sum score of eight food groups (olive oil, fiber, fruits, vegetables, fish, cereals, meat, and alcohol) and it ranges from 5 to 40 (higher scores indicate higher MD adherence). Alcohol consumption was not considered since pregnant women must not drink alcohol. Thus, our score consisted of seven elements and it ranged from 4 to 35. The present study only targeted women in the second trimester of pregnancy (13th to 27th g.w.), where dietary habits are relatively more constant, being more representative of dietary behaviour across the whole gestational period (231). Moreover, we previously observed that the MD adherence and dietary habits remained unchanged in our participants between the 16th g.w. and the 34th g.w. (232). Consequently, the dietary pattern at the 16th g.w. was considered representative of the pregnancy period.

Statistical analysis

Descriptive statistics were summarized as mean (standard deviation) or frequency (%) as appropriate. Linear regression analyses were employed to study the associations of MD adherence (at the 16th g.w.) with psychological ill-being and well-being (at the 16th and the 34th g.w.). Covariates were selected based on exploratory analyses performed in a previously-published study involving this study sample (354). Two models were conducted, Model I was unadjusted and Model II was adjusted for age, educational status, number of miscarriages and low back pain. The number of miscarriages have been previously associated with anxiety and depressive symptoms during pregnancy (355). Thus, we investigated the influence of this covariate in our models by (1) comparing the psychological ill-being and well-being outcomes between women with at least one miscarriage and women without any by an ANCOVA after adjusting for age, educational status and low back pain (at the 16th g.w.) and the exercise intervention (at the 34th g.w.); and (2) exploring the interaction between number of miscarriages (0= no miscarriages and 1= one or more miscarriages) and the MD adherence on psychological ill-being and well-being during pregnancy. Since the number of miscarriages*MD adherence interaction term was not significant (all *p*'s>0.2) we decided not to conduct separate models for women with no miscarriages or one or more miscarriages. The Model II in the longitudinal analysis (i.e., MD at 16th g.w. and mental health at 34th g.w.) was additionally adjusted for the group allocation to account for the exercise intervention

delivered in the GESTAFIT project. Moreover, since the associations between MD adherence, ill-being and well-being might be affected by baselines values the Model III in the longitudinal analyses was additionally adjusted by baseline values (i.e., ill-being and well-being indicators at the 16th g.w.). Furthermore, we investigated the group allocation*MD adherence interaction term, which was not significant (all *p's*>0.2), and thus we decided not to conduct separate models for women in the control and intervention groups. Additional sensitivity analyses were conducted only in the control group (n: 46-53 depending on the outcome) and results remained the same.

Finally, linear regression models were employed to explore the associations between single food groups and those mental health indicators associated with the MD adherence. Cross-sectional and longitudinal associations with the abovementioned covariates were performed.

The Benjamini-Hochberg procedure (273) was applied to account for the random effect in multiple comparisons for all the tests included in the primary analysis (i.e., MD adherence associations with mental health indicators at the 16th and 34th g.w.) and separately for all the tests included in the MD components analysis (i.e., MD components associations with mental health indicators at the 16th and 34th g.w.) with q=0.05 (false discovery rate).

All analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY) and the level of significance was set at $p \leq 0.05$.

RESULTS

Among the 159 pregnant women participating in the GESTAFIT project, 152 provided valid data on MD adherence and sociodemographic characteristics **(Figure 2)**. Psychological ill-being and well-being, and clinical and sociodemographic characteristics of study participants are shown in **Table 35**. Briefly, most participants (59%) presented a high educational status (i.e., university), were married or with partner (59%), were working (68%), and did not have any miscarriage in the past (59%). Around 26% of women were at risk of clinical depression at the 16th g.w. and 38% at the 34th g.w.

Table 35. Sociodemographic and clinical characteristics of the Study VII participants

Variable	n	Mean (SD) or n (%)
16th gestational week		
Age (years)	152	32.9 (4.6)
Low back pain (VAS)	152	22.2 (24.5)
Educational Status, n (%)	152	
Low educational status		17 (11.2)
Medium educational status		45 (29.6)
High educational status		90 (59.2)
Marital status, n (%)	152	
Married/with partner		90 (59.2)
Divorced/Single/widow		62 (40.8)
Working status, n (%)	152	
Working		104 (68.4)
Not working		48 (31.6)
Number of miscarriages, n (%)	152	
0		89 (58.6)
1		44 (28.9)
2		16 (10.5)
3 or more		3 (2.0)
Mediterranean diet adherence (7-35)	152	20.6 (5.0)
Psychological ill-being		
Negative Affect (PANAS-S, 10-50)[a]	141	17.3 (6.7)
Anxiety (STAI-S, 20-80)[b]	140	14.2 (9.0)
Depression risk score (CES-D, 0-60)[c]	117	11.2 (8.1)
Depression (yes)[d] (n%)	117	30 (25.6)
Psychological well-being		
Emotional Attention (TMMS-A, 8-40)[e]	142	25.39 (6.2)
Emotional Clarity (TMMS-C, 8-40)[f]	142	30.56 (4.9)
Emotional Regulation (TMMS-R, 8-40)[g]	142	30.02 (5.2)
Resilience (CD-RISC, 0-40)[h]	138	30.21 (5.2)
Positive Affect (PANAS-S, 10-50)[i]	141	34.33 (6.6)
34th gestational week		
Psychological ill-being		
Negative Affect (PANAS-S, 10-50)[a]	115	18.62(7.0)
Anxiety (STAI-S, 20-80)[b]	109	17.0 (10.9)
Depression risk score (CES-D, 0-60)[c]	117	13.27 (7.7)
Depression (yes)[d] (n%)	117	44 (37.6)
Psychological well-being		
Emotional Attention (TMMS-A, 8-40)[e]	119	25.60 (5.9)
Emotional Clarity (TMMS-C, 8-40)[f]	119	30.38 (5.3)
Emotional Regulation (TMMS-R, 8-40)[g]	119	30.11 (5.1)
Resilience (CD-RISC, 0-40)[h]	112	30.08 (5.1)
Positive Affect (PANAS-S, 10-50)[i]	115	33.0 (7.6)

Values shown as mean (standard deviation) unless otherwise is indicated [a], higher scores reflect greater emotional distress. [b], higher values reflect greater levels of anxiety.[c], higher scores indicate the presence of more depressive symptomatology. [d], *cut-off score* of *16* is indicative of potential depression. [e], higher scores reflect greater attention. [f], higher scores reflect greater clarity. [g], higher scores reflect greater regulation. [h], higher scores indicate a greater resilience. [i], higher scores reflect greater affective emotional health/experience. . SD, standard deviation; VAS, visual analogue scale.

The cross-sectional associations between MD adherence and psychological ill-being and well-being indicators (at the 16th g.w.) are shown in **Table 36**. In model II (adjusted model), MD adherence was inversely associated with anxiety (β=-0.200, SE=0.086, *p*=0.022) and we observed a borderline non-significant association with depression (β=-0.181, SE=0.098, *p*=0.066). Regarding well-being, MD adherence was positively associated with emotional regulation (β= 0.179, SE=0.088, *p*=0.043), resilience (β= 0.206, SE=0.089, *p*=0.022) and positive affect (β= 0.182, SE=0.082, *p*=0.029).

Longitudinal associations of MD adherence with mental health indicators (at the 34th g.w.) are presented in **Table 36**. The adjusted model (Model II) showed that MD adherence was inversely associated with negative affect (β=-0.241, SE=0.096, *p*=0.014) and anxiety (β=-0.325, SE=0.098, *p*=0.001), and we observed a borderline non-significant association with depression (β=-0.171, SE=0.092, *p*=0.066). Furthermore, MD adherence was positively associated with emotional regulation (β=0.295, SE=0.089 *p*=0.001), resilience (β= 0.259, SE=0.101, *p*=0.012), and positive affect (β=0.185, SE=0.092, *p*=0.048). The associations between MD, negative affect (β=-0.183; SE=0.081, *p*=0.026), anxiety (β= -0.172; SE=0.083, *p*=0.040) and emotional regulation (β= 0.171; SE=0.083, *p*=0.041) remained significant after adjusting by baseline values (Model III). After correcting for multiplicity, we observed that the cross-sectional and longitudinal associations between MD adherence and mental health indicators remained significant.

Table 36. Cross sectional and longitudinal associations of Mediterranean diet adherence with psychological ill-being and psychological well-being

	Cross-sectional (16th g.w.)					**Longitudinal (16th vs 34th g.w.)**							
		Model I		Model II			Model I		Model II[a]			Model III	
Mental health indicators	n	β	p	β	p	n	β	β	β	p	n	β	p
Psychological ill-being													
Negative Affect	141	-0.149	0.077	-0.130	0.150	115	-0.224	**0.016**	-0.241	**0.014**	111	-0.183	**0.026**
Anxiety	140	-0.205	**0.015**	-0.200	**0.022**	109	-0.295	**0.002**	-0.325	**0.001**	105	-0.172	**0.040**
Depression	117	-0.229	**0.013**	-0.181	0.066	117	-0.184	**0.048**	-0.171	0.066	91	0.078	0.403
Psychological well-being													
Emotional Attention	142	-0.023	0.790	-0.016	0.860	119	-0.162	0.078	-0.106	0.263	114	-0.055	0.450
Emotional Clarity	142	0.129	0.125	0.114	0.203	119	0.167	0.073	0.121	0.212	114	0.087	0.319
Emotional Regulation	142	0.202	**0.016**	0.179	**0.043**	119	0.306	**0.001**	0.295	**0.001**	114	0.171	**0.041**
Resilience	138	0.191	**0.025**	0.206	**0.022**	112	0.275	**0.003**	0.259	**0.012**	107	0.120	0.145
Positive Affect	141	0.144	0.089	0.182	**0.029**	115	0.202	**0.030**	0.185	**0.048**	111	0.070	0.369

Model I was unadjusted. Model II was adjusted for age, educational status, number of miscarriages, low back pain.

[a]Model II in the longitudinal analysis was additionally adjusted for exercise intervention. Model III in the longitudinal analysis was additionally adjusted for baseline values (i.e., mental health indicator at the 16th gestational week). Boldface indicates those outcomes which surpassed the multiple comparison test.

The cross-sectional associations of single food groups with psychological ill-being and well-being (at the 16th g.w.) after adjusting for the above-mentioned covariates are shown in **Table 37.** A higher intake of whole-grain cereals, fruits, vegetables, fish and nuts, and a lower intake of red meat and subproducts and sweets was associated with lower negative affect, anxiety and depression and greater emotional regulation, resilience and positive affect (|β| ranging from 0.168 to 0.268, all p<0.05). After correcting for multiplicity, we observed that the associations between vegetables, resilience and positive affect and the associations between fish, nuts and positive affect remained significant.

The longitudinal associations of single food groups with psychological ill-being and well-being (at the 34th g.w.) after adjusting for the above-mentioned covariates are shown in **Table 38**. A higher intake of fruits, olive oil and nuts together with a lower intake of red meat and subproducts was associated with lower negative affect, anxiety and depression and greater emotional regulation, resilience and positive affect (|β| ranging from 0.205 to 0.415, all p<0.05). After correcting for multiplicity, we observed that the associations fruits, negative affect, anxiety, depression and emotional regulation remained significant. Additionally, the associations between red meat, anxiety and resilience and the associations between olive oil, nuts and resilience remained significant.

Table 37. Cross-sectional association of single food groups, psychological ill-being and psychological well-being at the 16th gestational week

	Psychological ill-being						Psychological well-being					
	Negative Affect		Anxiety		Depression		Emotional Regulation		Resilience		Positive Affect	
N	141		140		117		142		138		141	
Single food groups	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Whole grain cereals (s/wk)	-0.183	0.035	-0.164	0.052	-0.172	0.065	0.041	0.634	-0.041	0.645	-0.038	0.640
Potatoes (s/wk)	0.081	0.366	0.055	0.527	0.021	0.823	-0.036	0.675	-0.042	0.637	-0.065	0.427
Fruits (s/wk)	-0.194	0.039	-0.182	0.036	-0.228	0.022	0.185	0.035	0.060	0.507	0.130	0.112
Vegetables (s/wk)	-0.008	0.928	-0.097	0.252	-0.059	0.540	0.168	0.048	0.268	**0.002**	0.244	**0.002**
Pulses (s/wk)	0.082	0.350	-0.019	0.828	0.026	0.786	0.109	0.202	0.044	0.622	0.109	0.179
Fish (s/wk)	0.025	0.777	0.064	0.459	0.032	0.738	0.053	0.543	0.052	0.561	0.213	**0.008**
Red Meat and subproducts (s/wk)	0.141	0.103	0.140	0.098	0.238	0.009	0.002	0.983	-0.084	0.337	0.004	0.963
Poultry (s/m)	0.032	0.718	-0.007	0.930	-0.113	0.226	-0.001	0.988	-0.055	0.535	0.097	0.227
Dairy products(s/wk)	0.033	0.708	-0.038	0.655	0.006	0.946	-0.010	0.909	0.036	0.683	-0.038	0.634
Olive Oil (s/wk)	0.005	0.950	-0.030	0.724	-0.090	0.328	-0.017	0.837	0.061	0.481	-0.033	0.678
Nuts (s/wk)	0.032	0.715	-0.007	0.931	0.037	0.693	0.190	0.025	0.170	0.055	0.220	**0.006**
Sweets (s/wk)	0.183	0.045	0.220	0.012	0.140	0.150	-0.032	0.722	-0.066	0.471	0.035	0.679

Analyses were adjusted for age, educational status, number of miscarriages and low back pain. Boldface indicates those outcomes which surpassed the multiple comparison test. S, servings; wk, week.

Table 38. Longitudinal association of single food groups, psychological ill-being and psychological well-being at the 34th gestational week

	Psychological ill-being						Psychological well-being					
	Negative Affect		Anxiety		Depression		Emotional Regulation		Resilience		Positive Affect	
N	115		109		117		119		112		115	
Single food groups	β	p	β	p	β	p	β	p	β	p	β	p
Whole grain cereals (s/wk)	-0.165	0.093	-0.129	0.229	-0.099	0.288	0.070	0.470	-0.051	0.611	-0.064	0.497
Potatoes (s/wk)	-0.023	0.815	0.083	0.410	0.170	0.067	-0.082	0.402	0.018	0.865	-0.007	0.942
Fruits (s/wk)	-0.357	**<0.001**	-0.415	**<0.001**	-0.365	**<0.001**	0.278	**0.004**	0.205	0.045	0.243	0.010
Vegetables (s/wk)	0.059	0.538	-0.003	0.977	-0.040	0.663	0.027	0.778	0.140	0.162	0.135	0.139
Pulses (s/wk)	0.084	0.390	0.005	0.957	0.111	0.234	0.027	0.780	0.025	0.801	0.126	0.174
Fish (s/wk)	-0.053	0.588	-0.051	0.622	0.025	0.787	0.107	0.289	0.034	0.735	0.102	0.276
Red Meat and subproducts (s/wk)	0.086	0.373	0.237	**0.016**	0.143	0.116	0.021	0.828	-0.234	**0.015**	-0.163	0.075
Poultry (s/m)	0.099	0.309	0.043	0.670	-0.050	0.591	-0.007	0.936	-0.104	0.292	0.089	0.336
Dairy products(s/wk)	0.074	0.450	0.049	0.631	0.031	0.737	-0.056	0.548	-0.016	0.875	0.106	0.260
Olive Oil (s/wk)	-0.108	0.253	-0.142	0.153	-0.006	0.944	0.108	0.229	0.304	**0.001**	0.014	0.874
Nuts (s/wk)	-0.206	0.043	-0.231	0.026	0.040	0.681	0.210	0.027	0.247	**0.017**	0.173	0.076
Sweets (s/wk)	0.101	0.326	0.009	0.932	0.151	0.112	-0.019	0.846	-0.032	0.758	-0.016	0.867

Analyses were adjusted for age, educational status, number of miscarriages, low back pain and exercise intervention. Boldface indicates those outcomes which surpassed the multiple comparison test. S, servings; wk, week.

Additional sensitivity analyses (i.e., longitudinal associations of MD adherence at the 16th g.w. with mental health indicators at the 34th g.w.) showed similar results when exclusively including the control group participants in the analyses **(Table 40)**.

Table 39. Longitudinal associations of Mediterranean diet adherence at the 16th gestational week with psychological ill-being and psychological well-being at the 34th gestational week in the control group

	Longitudinal (16th vs 34th g.w.)				
		Model I		**Model II**	
Mental health indicators	n	β	p	β	p
Psychological ill-being					
Negative Affect	51	-0.417	**0.002**	-0.391	**0.007**
Anxiety	46	-0.573	**<0.001**	-0.583	**<0.001**
Depression	51	-0.305	**0.030**	-0.309	**0.018**
Psychological well-being					
Emotional Attention	53	-0.544	0.589	0.005	0.969
Emotional Clarity	53	0.195	0.162	0.210	0.150
Emotional Regulation	53	0.272	**0.049**	0.287	**0.016**
Resilience	48	0.352	**0.014**	0.334	**0.034**
Positive Affect	51	0.212	0.136	0.252	0.071

Model I was unadjusted. Model II was adjusted for age, educational status, number of miscarriages, low back pain. Boldface indicates statistical significance (p<0.05)

Differences in psychological ill-being and psychological well-being of pregnant women at the 16th and 34th g.w. by number of miscarriages are shown in **Table 40**. No differences were found in psychological ill-being and psychological well-being in women with no miscarriages or one or more miscarriages (all, p`s>0.05).

Table 40. Differences in psychological ill-being and psychological well-being of pregnant women at the 16th and 34th gestational weeks by number of miscarriages

Mental health indicators	n	No previous miscarriages	Previous miscarriages	p
Psychological ill-being (16th g.w.) [a]				
Negative Affect	142 (82 vs 60)	16.64 (0.75)	18.40 (0.88)	0.137
Anxiety	141 (81 vs 60)	13.54 (0.99)	15.13 (1.16)	0.306
Depression	118 (71 vs 47)	10.99 (0.98)	11.99 (1.21)	0.526
Psychological ill-being (34th g.w.) [b]				
Negative Affect	115 (69 vs 46)	17.99 (0.85)	19.56 (1.05)	0.253
Anxiety	109 (65 vs 44)	17.74 (1.38)	15.87 (1.69)	0.402
Depression	117 (70 vs 47)	13.86 (0.90)	12.4 (1.10)	0.311
Psychological well-being (16th g.w.) [a]				
Emotional Regulation	143 (85 vs 58)	29.98 (0.57)	30.0 (0.69)	0.984
Resilience	138 (82 vs 56)	30.41 (0.59)	29.91 (0.71)	0.587
Positive Affect	142 (82 vs 60)	34.23 (0.69)	34.29 (0.81)	0.955
Psychological well-being (34th g.w.) [b]				
Emotional Regulation	119 (70 vs 49)	29.54 (0.59)	30.93 (0.71)	0.136
Resilience	112 (67 vs 45)	30.22 (0.64)	29.89 (0.78)	0.752
Positive Affect	115 (69 vs 46)	32.67 (0.89)	33.43 (1.09)	0.598

Values shown as mean (standard error). [a]Analyses were adjusted for age, educational status, number of miscarriages and low back pain. [b]Analyses were additionally adjusted for exercise intervention. Boldface indicates statistical significance (p<0.05).

DISCUSSION

Our results suggest that MD adherence during gestation is associated with lower negative affect, anxiety, and depression; and with greater emotional regulation, resilience, and positive affect during pregnancy. These associations seem to be driven by a higher intake of whole grain cereals, fruits, vegetables, fish, olive oil and nuts, and a lower intake of red and processed meat and sweets.

Women are at increased risk of experiencing mental health problems during pregnancy that can impact theirs' and the infant's health (145,146). The number of previous miscarriages have been shown to exert a negative influence on anxiety and depressive symptoms during pregnancy (355). Recent evidence showed that the risk of miscarriages ranges from 12% in women aged 20-29 years increasing to 65% in women aged 45 years and older. The average population prevalence of women who have had one or more previous miscarriages was 41% which is within the range of estimated miscarriage risk given that pregnant women in the present study aged 25-40 years (356). This percentage of miscarriages might be considered high when only comparing with women within the same mean age (i.e., 30-34 years; miscarriage risk = 14%) (356). In this sense, our analyses were adjusted for this covariate to

account for the possible effect on mental health indicators, yet we did not observe any associations between number of miscarriages and mental health throughout the pregnancy course. Additionally, we did not observe any differences in psychological ill-being and well-being based on the number of miscarriages, neither did the miscarriages moderated the association of MD adherence with psychological health. Thus, we strongly believe that the number of miscarriages is not affecting the results reported in this study. We observed that 26% of our sample were at risk of depression at the 16th g.w., and this proportion increased to 38% at the 34th g.w., which agrees with previous estimations (349,357). MD adherence may exert a beneficial effect on mental health outcomes in adults, such as depressive symptoms, cognitive status and quality of life, altogether improving the brain health (351). However, research regarding MD adherence and mental health during pregnancy is limited. Previous studies in pregnancy mainly focus on the associations of diet quality with depressive symptoms (153,156,358–360), and with other psychological ill-being indicators to a lesser extent (e.g., stress and negative affect (159,361,362)). For instance, maternal dietary patterns similar to the MD (i.e., rich in vegetables, fruits, pulses, fish and nuts, among other components) were associated with lower depression during pregnancy (358,359). Likewise, Paskulin et al. (360) found that pregnant women with a low intake of fruits, beans and with high "common-Brazilian" dietary pattern composed of foods popular in Brazilian culture, such as rice or noodles, French rolls, beans, boneless beef/chicken or eggs, coffee with sugar, margarine, and artificial juices had higher prevalence of mental disorders (including depression and anxiety). Fowles et al. (159). found that women with diet quality below the median (i.e., Diet quality index) had higher depressive symptoms and stress than women above the median. Additionally, levels of depression tend to increase throughout pregnancy (363), and a recent study (358) suggested that the diet-mental health association might exist along the pregnancy course.

By virtue of the repeated measurements, our findings add evidence to the literature showing that MD adherence was associated with lower anxiety at the 16th and 34th g.w., and with less negative affect at the 34th g.w. Therefore, according to our results, adherence to a MD may attenuate experience of negative affect especially in the third trimester of pregnancy when women generally suffer more stress and anxiety. Lindsay et al. (362) found no associations between MD adherence and negative affect during early-mid pregnancy (12th-20th g.w.). Given that psychological ill-being fluctuates during pregnancy (348), the lack of association between MD and negative affect found by Lindsay et al. (362) is not generalizable to the third trimester of pregnancy when we did find such association. A systematic review (361) showed an inverse

association between dietary patterns comprised of whole foods, fruits, vegetables, fish and seafood (which happens to be characteristic of MD) and perinatal anxiety and depression, which agrees with our findings. Moreover, we found that a greater intake of whole grain cereals and fruits, and a lower intake of red meat and subproducts and sweets was associated with lower negative affect, anxiety, and depression during gestation. These findings are in agreement with previous studies in pregnant (156) and non-pregnant women (364), and in the general population (365,366); and could be explained by the fact that these predominant nutrients in these food groups (i.e., saturated fats and sugar) have pro-inflammatory effects when consumed in excess (367).

Pregnancy is a period during which psychological well-being often declines (368). Current evidence supports the importance of the MD for the well-being in the non-pregnant adult population (369). Micronutrient deficiencies including iron, zinc, folate, vitamin D and, particularly, essential fatty acids seem to affect the well-being in pregnancy (370), yet the evidence on the MD adherence and well-being during pregnancy is scarce (368). In this regard, we found that MD adherence was related to well-being indicators at the 16th and 34th g.w., suggesting that MD may improve well-being throughout the pregnancy course. This relation could be explained by the synergistic combination of single nutrients that are positively linked to mental health. These nutrients include those which are protective against oxidative stress such as the monounsaturated fatty acids present in the olive oil, the polyunsaturated fatty acids in fish, the folate and B vitamins in fruits, vegetables, nuts and legumes (371). Ferrer-Casales et al. (372) found that omega-3 fatty acids, present in fish, nuts, and grains, and the B vitamins found in fruits and vegetables, are the most important nutrients for the central nervous system functioning (e.g., neurotransmission, gene expression, and mood). This may explain our results on the association of higher intakes of fruits, vegetables, nuts and olive oil with lower psychological ill-being and higher resilience, emotional regulation, and positive affect. Of note, after adjusting for baseline values (i.e., mental health indicator at the 16th g.w.) the associations between MD, resilience and positive affect became non-significant. This means that the potential effect of MD on resilience and positive affect is not observable when considering the baseline levels of these indicators. Future studies with larger sample sizes should further explore this association to elucidate whether MD might be associated in pregnant women with certain levels on these variables.

The potential biochemical and physiological mechanisms underlying the association between diet and mental health are poorly understood. The literature has suggested the role of dietary factors in the monoamine synthesis, inflammation processes, hypothalamic–pituitary–adrenal

axis regulation, and neurogenesis (373). Additionally, diet can promote the production and secretion of brain-derived neurotrophic factor , a peptide implicated in synaptic plasticity and neuronal survival, whose levels are decreased in pregnant women with depression (374). Previous evidence shows that MD adherence is associated with lower levels of pro-inflammatory cytokines that inhibit the brain-derived neurotrophic factor expression (375). Furthermore, recent evidence has focused on the influence of gut microbiota on emotional behaviour, neurological processes and symptoms of both depression and anxiety (376,377). The gut microbiota is strongly affected by diet (377); thus, specific dietary patterns (or even single food groups) might prevent mental disorders by changes in the microbiota composition and function (376). A "healthy" dietary pattern (such as the MD) contains a larger amount of fruits, vegetables, and wholegrains, a rich source of prebiotics such as fermentable carbohydrates, polyols, and phytochemicals which promoted the growth and activity of beneficial bacteria (378). MD during pregnancy has been associated with increased maternal gastrointestinal tract microbial diversity (379). Increased consumption of fruits, vegetables and legumes with low red meat consumption were the key components driving this association (379). In this line, we found that a greater MD adherence was associated with lower negative affect, anxiety, and depression during gestation. Similarly, the dietary factors associated with lower negative affect, anxiety, and depression in our study sample (i.e., wholegrain cereals, fruits and nuts), are protective of the microbiota and the mucous layer, leading to an anti-inflammatory environment (380). Contrarily, red meat and sugar were associated with higher anxiety and depression, which seems plausible since they are likely to interrupt the normal function of the gut-brain, induce mucus loss and microbiota disturbance, leading to a pro-inflammatory environment (380).

Limitations and strengths

The findings and implications of this study should be interpreted with a number of limitations in mind. First, the observational design does not allow a clear cause–effect identification. Second, the results should be interpreted cautiously, as we could be limited to detect small association sizes. Larger studies should further explore these associations in order to corroborate our results. Third, the participating women were enrolled in an exercise intervention that might affect our findings. However, we investigated the potential interaction between exercise and diet, we included the group allocation as confounder in our longitudinal analyses, and we further performed sensitivity analyses exclusively in the control group, all analyses suggesting no effect of the intervention on our findings. Fourth, the missing data in

our study could bias our findings. Similar dropout rates have occurred in previous studies in pregnant women (381), and we found no differences in the baseline characteristics of the drop-outs and the completers. Fifth, although the questionnaires used to assess mental health were valid and reliable for the general population, their psychometric properties have not been extensively tested during pregnancy, except for the STAI which is validated in pregnant women (202). However, all the questionnaires employed in the present study to assess psychological ill-being and well-being have been previously employed in pregnant women (350,354). Future studies should investigate the validity of the mental health questionnaire in pregnant women. Additionally, although our findings were corrected for multiple comparison testing, the likelihood of making a type I error might not be completely disregarded and future studies should confirm our findings.

Several strengths of this study are worth considering. A detailed definition of the dietary habits and a valid assessment of the MD diet adherence was employed. Furthermore, psychological ill-being and well-being indicators were assessed during the second and the third trimester of pregnancy which provides a more comprehensive understanding of mental health along the pregnancy course.

CONCLUSION

A greater MD adherence during gestation was associated with lower negative affect, depressive symptoms, and anxiety, and with higher emotional regulation, resilience, and positive affect along the pregnancy course. A higher intake of wholegrain cereals, fruits, vegetables, fish, olive oil and nuts, together with a lower intake of red meat and sweets seemed to explain the observed associations of MD adherence with mental health indicators. Therefore, our findings suggest that the promotion of a higher diet quality during pregnancy might be effective to prevent mental health issues in pregnant women, yet this should be further tested by diet interventions in well-designed randomized controlled trials.

STUDY VIII: A greater Mediterranean diet adherence is associated with better health-related quality of life during pregnancy. The GESTAFIT project

Flor-Alemany M, Migueles JH, Marín-Jiménez N, Baena-García L, Löf M, Aparicio VA.

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ABSTRACT

Aim: To examine the association of dietary habits and MD adherence during pregnancy with HRQoL measured at the 16th and 34th g.w.

Methods: A total of 138 pregnant women (age: 32.9±4.6 years old) were included in this longitudinal study. At the 16th g.w., dietary habits were assessed with a validated food frequency questionnaire, and the MD adherence was derived from it using the MFP. HRQoL was assessed with the Spanish version of the SF-36 at the 16th and 34th g.w.

Results: A greater MD adherence was associated with better SF-36 physical functioning, bodily pain, vitality, social functioning, emotional role, mental health, and *physical /mental component summary* in cross-sectional (16th g.w.) and longitudinal analyses (34th g.w.) (β ranging from 0.168 to 0.273, all $p<0.05$). The associations of the MD adherence with the HRQoL domains seem to be explained by higher intake of whole grain cereals, fruits, vegetables, fish and nuts, and a lower intake of potatoes, red meat and sweets ($|\beta|$ ranging from 0.171 to 0.385, all $p<0.05$).

Conclusion: A greater MD adherence, driven by a higher intake of whole grain cereals, fruits, vegetables, fish and nuts and a lower intake of potatoes, red meat and sweets, was associated with better HRQoL throughout pregnancy.

INTRODUCTION

HRQoL is a concept determining the self-perception of health-related physiological, psychological, and social constructs (382). Previous evidence showed that pregnant women experience a decrease in their HRQoL throughout gestation (383). In fact, evaluating the HRQoL during gestation is clinically relevant since HRQoL is associated both maternal and infant health (pregnancy monitoring, pregnancy outcomes, maternal postpartum health, and the psychomotor development of the infant) (384,385).

MD adherence has been found to be associated with improved HRQoL in non-pregnant adult population, both by observational (386–389) and intervention studies (390). However, the relationship between MD adherence and MD components (i.e., food groups that are characteristic of the MD such as fruits, vegetables, wholegrain cereal and fish) with HRQoL throughout gestation has not been previously investigated. This is especially important given the fact that pregnant women are drifting away from the Mediterranean dietary pattern (211,212). Overall, previous evidence showed a moderate MD adherence that remained unchanged throughout pregnancy (211), with only 22% to 40% of pregnant women having a high MD adherence (391).

Therefore, the aim of this study was to examine the association of MD adherence and MD components with HRQoL during gestation. Such data may be relevant in future community and population-based programs or policies for improved mental and physical HRQoL.

METHODS

Study design and participants

These are secondary analyses from the GESTAFIT project where a concurrent exercise intervention (i.e., aerobic plus strength training) from the 17th g.w. until birth was conducted (182). A total of 159 pregnant women met the inclusion-exclusion criteria **(Table 6)** and signed a written informed consent. Among them, a total of 138 women who had valid data in sociodemographic characteristics, MD adherence and SF-36 at the 16th g.w. were included in the present study **(Figure 2)**. The study was approved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15).

Sociodemographic characteristics

A self-reported questionnaire (anamnesis) was employed to assess sociodemographic and clinical characteristics of pregnant women (i.e., age, number of miscarriages, having a diagnose of depression or anxiety and educational and working status).

Sample size calculation

The sample size for this study depended on the 'a priori' analyses of the statistical power performed in the GESTAFIT project (182). According to the primary outcome (i.e., maternal weight gains), we planned to recruit 60 women assuming a statistical power of 90%, $\alpha = 0.05$, and a 15% of potential withdrawals. Since the present analyses are secondary outcomes from the GESTAFIT Project we did not calculate the sample size.

Low-back pain

A Visual Analogue Scale was employed to assess low-back pain (353). The score was determined by measuring the distance (mm) on a 10-cm line between the "no pain" anchor and the participant's mark. Total score ranges from 0 to 100 with higher values indicating higher pain.

Health-related quality of life

HRQoL of the participants was assessed with the Spanish version of the SF-36 (201). The SF-36 has been widely employed in pregnant women (382–384,392,393), and is comprised of 36 items that are grouped into eight domains: physical functioning, physical role, bodily pain, general health, vitality, social functioning, emotional role, and mental health. The number of items varies from two to ten between dimensions. The items are of multiple-choice, with two to six answer options, that are subsequently recategorized to 0 to 100 scores, and averaged to obtain each dimension score. To determine the score for one dimension, at least half of the questions in the dimension need to be answered (201). These domains are grouped into two global constructs: *physical component summary* which includes physical functioning, physical role, bodily pain and general health, and the *mental component summary* which includes vitality, social functioning, emotional role, and mental health. The total score ranges from 0 to 100 in all domains with higher scores indicating better health. Both the original and the Spanish versions of the SF-36 are reliable and valid instruments for evaluating HRQoL in pregnant

women (394). The two summary measures (i.e., *physical / mental component summary*) have demonstrated good internal reliability (Cronbach's α=0.92 and 0.91) (395).

Dietary assessment and Mediterranean diet adherence

Dietary habits were assessed at the 16th and 34th g.w. with a food frequency questionnaire validated in Spanish non-pregnant adult population (189). With the data obtained from the food frequency questionnaire, the MFP (190) (i.e., a MD adherence index) was constructed as previously done in this study sample (232). The MFP score consists in a quintile-based sum score of eight food groups (olive oil, fiber, fruits, vegetables, fish, cereals, meat, and alcohol). The total score ranges from 8 to 40. In the present study alcohol consumption was not considered. Therefore, the maximum score ranges from 7 to 35, where higher scores indicate greater MD adherence. Dietary habits at the 16th g.w. were considered representative of the pregnancy period in this study sample as previously done in this study sample (272).

Statistical analysis

Descriptive statistics were summarized as mean (standard deviation) for quantitative variables, and frequency (%) for categorical variables as appropriate. Differences in HRQoL throughout pregnancy (i.e., 16th vs. 34th g.w.) were compared by paired Student's t-test.

Hierarchical linear regression analyses were performed to examine the associations of MD adherence (i.e., explanatory variable) with SF-36 domains and SF-36 physical and mental component summaries (at the 16th and 34th g.w.). The stepwise method was used, entering potential confounders (i.e., age, educational status, number of miscarriages and low back pain) in step 1 to test their association with each outcome (SF-36 domains and SF-36 *physical / mental component summary*). The choice of covariates was based on a previous study conducted in the same sample (90) where we explored the association of potential confounders with mental health outcomes. In the step 2 of the hierarchical regressions, we entered the MD adherence as a predictor and each SF-36 domains and SF-36 *physical / mental component summary* as outcomes in separate regression analyses after the inclusion of confounders previously found significantly associated with the outcomes in step 1. The relevant confounders (those which explained a significant amount of the variance in SF-36 domains and SF-36 *physical / mental component summary*) in each model are specified in the footnotes of **Table 43** and **Table 44**. These steps were the same in the cross-sectional association models (i.e., SF-36 measured at the 16th g.w.) and in the longitudinal association models (i.e., SF-36 measured at the 34th g.w.). Of note, the models including SF-36 bodily pain were not adjusted by lumbar pain. The

longitudinal models were additionally adjusted for the exercise intervention in the step 2 to account for the possible effect of the intervention conducted within the GESTAFIT project (182) on these outcomes. As an exploratory analysis, additional linear regressions were performed using the MD components as explanatory variables and SF-36 domains and SF-36 *physical / mental component summary* (at the 16th and 34th g.w.) after adjusting for the confounders previously found relevant in the hierarchal linear regression.

Sensitivity analyses were performed by investigating the longitudinal associations (i.e., associations of MD adherence at the 16th g.w. with HRQoL at the 34th g.w.) exclusively in the control group. This sensitivity analysis was conducted to completely remove the potential effect of the intervention in our findings. These analyses were adjusted for the above-mentioned confounders. The Benjamini-Hochberg procedure (273) was applied to account for the random effect in multiple comparisons for all the tests included in the primary analysis (i.e., MD adherence HRQoL at the 16th and 34th g.w.) and separately for all the tests included in the MD components analysis (i.e., MD components associations with HRQoL at the 16th and 34th g.w.) with q=0.05 (false discovery rate).

All analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY) and the level of significance was set at $p \leq 0.05$.

RESULTS

HRQoL, clinical and sociodemographic characteristics of study participants are presented in **Table 41**.

Table 41. Sociodemographic and clinical characteristics of the Study VIII participants

Variable	n	Mean (SD) or n (%)
16th gestational week		
Age (years)	138	32.9 (4.6)
Low-back pain (mm)	138	23.5 (24.7)
Educational Status, n (%)	138	
Low educational status		17 (12.3)
Medium educational status		42 (30.4)
High educational status		79 (57.2)
Marital status, n (%)	138	
Married/with partner		81 (58.7)
Divorced/Single/widow		56 (40.6)
Working status, n (%)	138	
Working		91 (65.9)
Not working		47 (34.1)
Number of miscarriages, n (%)	138	
0		81 (58.7)
1		39 (28.3)
2		15 (10.9)
>3		3 (2.1)
Disease diagnosis (physical/psychological) (yes, n[%])	138	56 (40.6)
Current smoker (yes, n[%])	138	13 (9.4)
Mediterranean diet adherence (7-35)	138	20.6 (5.0)
36-Item Short Form Health Survey [a]		
Physical functioning (0-100)	138	82.3 (14.3)
Physical role (0-100)	138	67.1 (24.8)
Bodily pain (0-100)	138	60.9 (25.4)
General health (0-100)	138	77.4 (15.0)
Vitality (0-100)	138	53.9 (17.2)
Social functioning (0-100)	138	78.4 (21.8)
Emotional role (0-100)	138	90.4 (15.0)
Mental health (0-100)	138	75.6 (13.8)
Standardized physical component scale	138	46.7 (7.6)
Standardized mental component scale	138	50.8 (7.5)
34th gestational week		
36-Item Short Form Health Survey [a]		
Physical functioning (0-100)	114	67.9 (18.9)
Physical role (0-100)	114	55.5 (23.4)
Bodily pain (0-100)	114	52.9 (25.8)
General health (0-100)	114	78.5 (17.6)
Vitality (0-100)	114	51.9 (17.0)
Social functioning (0-100)	114	76.4 (21.7)
Emotional role (0-100)	114	90.2 (15.2)
Mental health (0-100)	114	74.5 (15.6)
Standardized physical component scale	114	41.7 (8.8)
Standardized mental component scale	114	52.3 (7.9)

Values shown as mean (standard deviation) or n (%). unless otherwise is indicated. [a], higher scores reflect greater health-related quality of live. G.W., gestational week; SD, standard deviation; VAS, visual analog scale.

Table 42. Differences in health-related quality of life by gestational week

	16th gestational week	**34th gestational week**	***p***
Physical functioning (0-100)	83.0 (13.7)	67.7 (19.2)	<0.001
Physical role (0-100)	67.7 (24.1)	55.1 (23.7)	<0.001
Bodily pain (0-100)	61.5 (25.2)	53.4 (26.3)	0.001
General health (0-100)	77.3 (15.7)	78.1 (18.1)	0.532
Vitality (0-100)	53.7 (17.2)	52.4 (17.5)	0.408
Social functioning (0-100)	78.8 (21.9)	76.5 (21.8)	0.327
Emotional role (0-100)	91.0 (14.6)	91.1 (15.5)	0.569
Mental health (0-100)	75.7 (14.0)	74.5 (15.8)	0.393
Standardized physical component scale	47.0 (7.7)	41.6 (9.0)	<0.001
Standardized mental component scale	50.8 (7.4)	52.3 (8.1)	0.035

Values shown as mean (Standard deviation).

Lower scores in physical functioning, physical role, bodily pain and *physical/ mental component summary* were observed in 34th g.w. compared to 16th g.w. (all, $p<0.05$) **(Table 42)**.

Cross-sectional associations of MD adherence with HRQoL (at the 16th g.w.) are shown in **Table 43**. A greater MD adherence was associated with better SF-36 physical functioning, bodily pain, vitality, emotional role, mental health, and physical and mental component summaries (β ranging from 0.168 to 0.219, all $p<0.05$). After correcting for multiplicity, we observed that the cross-sectional association between MD adherence and SF-36 Bodily pain became non-significant.

Table 43. Cross-sectional associations of Mediterranean diet adherence with health-related quality of life at the 16th gestational week

Diet at the 16th gestational week and quality of life at the 16th gestational week				
	β	**95% Confidence Interval**		***p***
		Lower	**Upper**	
Physical functioning (0-100) †	0.219	0.054	0.385	**0.010**
Physical role (0-100) †	0.110	-0.058	0.279	0.199
Bodily pain (0-100) †*	0.190	0.024	0.357	**0.025**
General health (0-100) ††	0.129	-0.036	0.295	0.125
Vitality (0-100) ‡	0.191	0.029	0.352	**0.021**
Social functioning (0-100) †	0.121	-0.048	0.289	0.158
Emotional role (0-100) †	0.190	0.024	0.357	**0.026**
Mental health (0-100) ‡	0.202	0.040	0.365	**0.015**
Standardized physical component scale‡	0.168	0.004	0.333	0.045
Standardized mental component scale‡	0.185	0.022	0.349	**0.027**

†Unadjusted. ‡Adjusted by lumbar pain. ††Adjusted by low-back pain and educational status. *Lumbar pain was not included in this model. Boldface indicates those outcomes which surpassed the multiple comparison test.

Longitudinal associations of MD adherence with HRQoL (at the 34th g.w.) are shown in **Table 44**. A greater MD adherence was associated with better SF-36 bodily pain, social functioning, emotional role and SF-36 *physical component summary* (β ranging from 0.190 to 0.273, all $p<0.05$). After correcting for multiplicity, we observed that the longitudinal association between MD adherence and SF-36 Bodily pain remained significant.

Table 44. Longitudinal associations of Mediterranean diet adherence with health-related quality of life at the 34th gestational week

Diet at the 16th gestational week and quality of life at the 34th gestational week				
	β	95% Confidence Interval		*p*
		Lower	Upper	
Physical functioning (0-100) †	0.137	-0.025	0.300	0.096
Physical role (0-100) ‡	0.120	-0.064	0.304	0.199
Bodily pain (0-100) ‡*	0.273	0.093	0.454	**0.003**
General health (0-100) ††	0.164	-0.018	0.346	0.076
Vitality (0-100) ‡	0.119	-0.070	0.308	0.215
Social functioning (0-100) ‡‡	0.231	0.046	0.417	0.015
Emotional role (0-100) †	0.203	0.022	0.383	0.028
Mental health (0-100) ‡‡	0.115	-0.077	0.306	0.237
Standardized physical component scale‡	0.190	0.017	0.364	0.032
Standardized mental component scale †††	0.155	-0.030	0.339	0.099

†Adjusted by exercise intervention and number of abortions. ‡ Adjusted by exercise intervention. ††Adjusted by exercise intervention, low-back pain and educational status. ‡‡ Adjusted by exercise intervention and educational status. ††† Adjusted by exercise intervention, number of abortions and educational status. *Lumbar pain was not included in this model. Boldface indicates those outcomes which surpassed the multiple comparison test.

Cross-sectional associations of individual food groups with HRQoL after adjusting for covariates previously found significantly associated with the outcomes in the hierarchical linear regression are shown in **Table 45**. A greater intake of whole-grain cereals, fruits, vegetables, fish and a lower intake of potatoes, red meat and sweets was associated with better physical functioning, bodily pain, vitality, emotional role, mental health, and *physical / mental component summary* (|β| ranging from 0.166 to 0.242, all $p<0.05$).

Table 45. Cross-sectional association of food groups and health-related quality of live at the 16th gestational week

	Physical function		Bodily pain		Vitality		Emotional role		Mental health		Standardized physical component scale		Standardized mental component scale	
Food groups	***β***	***p***	***β***	***p***	***β***	***p***	***β***	***p***	***β***	***p***	***β***	***p***	***β***	***p***
Whole grain cereals (s/wk)	0.178	0.037	0.171	0.045	0.082	0.327	0.139	0.105	0.070	0.404	0.184	0.028	0.076	0.370
Potatoes (s/wk)	-0.126	0.139	-0.163	0.056	-0.002	0.981	0.007	0.931	0.032	0.706	-0.237	0.005	0.097	0.248
Fruits (s/wk)	0.242	**0.004**	0.188	0.027	0.192	0.020	0.210	0.014	0.239	**0.004**	0.151	0.073	0.206	0.014
Vegetables (s/wk)	0.196	0.021	-0.020	0.814	0.077	0.359	0.157	0.065	0.073	0.383	0.065	0.442	0.066	0.432
Pulses (s/wk)	-0.087	0.310	-0.107	0.212	0.050	0.547	-0.073	0.397	-0.069	0.410	-0.097	0.250	-0.050	0.555
Fish (s/wk)	-0.021	0.807	0.211	0.013	0.101	0.229	0.185	0.030	0.177	0.034	0.055	0.520	0.221	0.008
Red Meat and sb. (s/wk)	-0.086	0.317	-0.087	0.310	-0.048	0.570	0.022	0.798	-0.175	0.038	-0.097	0.254	-0.092	0.281
Poultry (s/m)	-0.031	0.719	0.065	0.448	-0.018	0.833	0.090	0.293	0.072	0.393	-0.001	0.999	0.082	0.334
Dairy products(s/wk)	-0.094	0.274	-0.116	0.175	0.014	0.867	-0.068	0.430	0.027	0.747	-0.091	0.279	0.032	0.708
Olive Oil (s/wk)	0.031	0.717	0.066	0.441	0.019	0.817	0.126	0.141	0.086	0.307	0.020	0.810	0.126	0.135
Nuts (s/wk)	-0.025	0.773	0.060	0.477	0.058	0.490	0.079	0.355	0.054	0.519	0.014	0.868	0.076	0.369
Sweets (s/wk)	-0.210	0.013	-0.097	0.259	-0.100	0.229	-0.039	0.653	-0.087	0.301	-0.141	0.094	-0.070	0.405

Model adjusted for the cofounders previously found significantly associated with the outcomes in the hierarchical linear regression. Boldface indicates those outcomes which surpassed the multiple comparison test. S, servings; wk, week.

Longitudinal associations of the individual food groups with HRQoL after adjusting for covariates previously found significantly associated with the outcomes in the hierarchical linear regression and the exercise intervention are shown in **Table 46**. A greater intake of whole-grain cereals, fruits, nuts and a lower intake of red meat and sweets was associated with better bodily pain, social functioning, emotional role and *mental component summary* (|β| ranging from 0.182 to 0.385, all $p<0.05$).

Table 46. Longitudinal association of food groups at the 16th gestational week and health-related quality of live at the 34th gestational week

	Bodily pain		**Social functioning**		**Emotional role**		**Standardized physical component scale**	
Food groups	***β***	***p***	***β***	***p***	***β***	***p***	***β***	***p***
Whole grain cereals (s/wk)	0.163	0.090	0.201	0.036	0.021	0.829	0.200	0.026
Potatoes (s/wk)	-0.118	0.208	-0.175	0.061	-0.108	0.243	-0.145	0.098
Fruits (s/wk)	0.330	**<0.001**	0.385	**<0.001**	0.233	**0.011**	0.226	0.010
Vegetables (s/wk)	0.044	0.642	0.040	0.672	0.086	0.356	0.091	0.305
Pulses (s/wk)	-0.059	0.530	-0.141	0.131	-0.047	0.611	-0.074	0.400
Fish (s/wk)	0.112	0.243	0.073	0.445	0.119	0.208	0.062	0.488
Red Meat and sb. (s/wk)	-0.191	0.040	-0.182	0.052	-0.006	0.949	-0.119	0.176
Poultry (s/wk)	0.130	0.167	-0.029	0.760	0.007	0.937	0.137	0.121
Dairy products(s/wk)	0.054	0.568	0.014	0.881	0.088	0.346	-0.032	0.717
Olive Oil (s/wk)	0.013	0.886	0.108	0.249	0.125	0.172	0.092	0.292
Nuts (s/wk)	0.157	0.108	0.078	0.425	0.246	**0.010**	0.016	0.862
Sweets (s/wk)	-0.098	0.296	-0.259	**0.005**	0.070	0.448	-0.167	0.055

Model adjusted for the cofounders previously found significantly associated with the outcomes in the hierarchical linear regression. Boldface indicates those outcomes which surpassed the multiple comparison test. S, servings; wk, week.

Additional sensitivity analyses showed similar results when only including participants in the control group **(Table 47)**.

Table 47. Longitudinal association of Mediterranean diet adherence at the 16th gestational week and health-related quality of life at the 34th gestational week in the control group (n=49)

	Diet at the 16th gestational week and quality of life at the 34th gestational week			
	β	95% Confidence interval		*p*
		Lower	Upper	
Physical functioning (0-100) †	0.264	-0.019	0.546	0.066
Physical role (0-100) ‡	0.279	-0.003	0.561	0.052
Bodily pain (0-100) ‡	0.382	0.111	0.654	0.007
General health (0-100) ††	0.284	0.006	0.564	0.045
Vitality (0-100) ‡	0.288	0.006	0.569	0.045
Social functioning (0-100) ‡‡	0.366	0.091	0.642	0.010
Emotional role (0-100) †	0.266	0.010	0.522	0.042
Mental health (0-100) ‡‡	0.395	0.129	0.661	0.005
Standardized physical component scale‡	0.314	0.036	0.593	0.028
Standardized mental component scale †††	0.403	0.148	0.658	0.003

†Adjusted by number of abortions. ‡ Unadjusted. ††Adjusted by low-back pain and educational status. ‡‡ Adjusted by educational status. ††† Adjusted by number of abortions and educational status. Boldface indicates statistical significance ($p<0.05$).

DISCUSSION

Our results suggest that a greater MD adherence during pregnancy was associated with better SF-36 physical functioning, bodily pain, general health, vitality, emotional role, social functioning, mental health and SF-36 *physical / mental component summary*. These associations seem to be driven by a higher intake of whole grain cereals, fruits, vegetables, fish and nuts, and a lower intake of potatoes, red and processed meat, and sweets.

HRQoL is a conceptualisation reflecting an individual's physical and mental well-being and has emerged as an essential consideration in the prevention and treatment of diseases (396). HRQoL deteriorates in women over the course of pregnancy (383,392,397). This study found that SF-36 physical functioning, physical role, bodily pain and SF-36 *physical component summary* decreased throughout gestation whereas SF-36 *mental component summary* improved, which is highly in agreement with previous evidence reporting that physical domains decline (383,392) and mental domains improve as pregnancy progresses (392).

Previous studies (398) showed an association between MD adherence and HRQoL suggesting that this dietary pattern may be beneficial for HRQoL in the non-pregnant adult population, whereas others did not observe such association (399). Notwithstanding, evidence in pregnant women is scarce. In this regard, a study conducted by Miura et al. (400) showed that following a "Japanese" dietary pattern, characterized by the intake of foods such as rice, miso soup, Japanese wheat noodles, cereals, beans, vegetables, fruits, meats, Japanese tea, vegetable juice,

and 100% fruit juice, and also by low intake of milk, was associated with poor mental and physical HRQoL in pregnant women in the first trimester of gestation. Moreover, a medium-high intake of an "unbalanced" dietary pattern, characterised by a high intake of grain cereals, bottled tea, bottled coffee, and carbonated drinks, and low intake of green and yellow vegetables, fruits, and green tea was associated with poorer physical and mental HRQoL. Furthermore, a study which included 144 pregnant women in the first trimester of gestation showed that proper nutritional habits that take into consideration the type of food consumed (wholegrain bread, vegetables, or fruit) were strongly correlated with all aspects of HRQoL in pregnant women with high General Index of Intensity of Health Behaviours (401). Nonetheless, neither diet quality nor dietary patterns were included in this study (401). Besides, in these previous studies (400,401), HRQoL was only assessed during the first trimester of pregnancy. In the present study, we explored the associations between MD adherence and HRQoL at the 16th and 34th g.w. adding evidence to the literature showing that a greater MD adherence (a diet high in fruits, vegetables, and fiber, and low in saturated fatty acids) was associated with better HRQoL along the pregnancy course. Moreover, we also studied the different food groups that comprise the Mediterranean dietary pattern, finding that a higher intake of whole grain cereals, fruits, vegetables, fish and nuts, and a lower intake of potatoes, red and processed meat and sweets were associated with better HRQoL during pregnancy. Similarly, a previous study in women in fertile age conducted by Azupogo et al. (396) found a potential beneficial role of a high vegetable intake on HRQoL. Similarly, Södergren et al. (402) found a positive association between fruit and vegetable intake and self-rated health among older Australian adults. Furthermore, the present study suggests that fish and nuts consumption is associated with better HRQoL during pregnancy which concurs with previous evidence in non-pregnant adult population (403,404). Regarding meat, a previous study conducted in type 2 diabetes patients showed that a diet with more meat was a negative factor for the HRQoL (405). In the same context, a low intake of red meat and carbonated beverages were associated with better scores for several HRQoL subscales in Breast Cancer Survivors (399).

One of the most accredited hypotheses is that MD is positively associated with better overall health status and reduced risk of major chronic diseases because of its high content of different beneficial compounds, such as antioxidants (largely present in leafy vegetables and fruits), dietary fiber, polyunsatured (mainly from fish and nuts) and monounsatured fatty acids (olive oil) (398,406). In fact, a recent study has offered interesting indications on this issue by highlighting a major role played by dietary antioxidants in explaining the relationship

between MD and HRQL (398). Epidemiological studies have confirmed a correlation between diet and depression, pointing out the importance of a diet rich in antioxidants and other essential compounds typical of MD in reducing the risk of depression, which may explain the positive effects in improving mental health (407). Regarding physical health, there are several biological and physiological mechanisms that could explain the beneficial effects of an MD pattern, such as reduced vascular inflammation and coagulation markers or improved endothelial function (408,409). In this regard, HRQoL has been associated with high adherence to Mediterranean-like eating patterns and inversely related to unhealthy dietary habits (398) which is highly in agreement with our findings.

Furthermore, recent evidence has focused on the influence of gut microbiota on emotional behaviour, neurological processes and HRQoL indicators as well as depression and anxiety status (376,377,410). A disruption in the microbial composition of the gut has been associated with many neurological disorders with inflammatory components (411). As a result, diet can have direct or indirect (via the microbiota) effects on neurological disorders (411). In fact, the dietary factors associated with better HRQoL (i.e., higher intake of anti-inflammatory components like fruits, vegetables, whole grain cereals, fish and nuts and a lower intake of pro-inflammatory components such as red meat and sweets) were are protective of the microbiota and the mucous layer, leading to an anti-inflammatory environment (380).

Limitation and strengths

Some limitations need to be highlighted. Firstly, given the observational design of the present study a clear cause–effect identification is not possible. As a result, we cannot determine whether a healthier diet affects HRQoL or, on the contrary, a poorer HRQoL lead to unhealthy dietary behaviours. Secondly, the participants were enrolled in an exercise intervention that might affect our findings regarding the 34th g.w. However, we included the group allocation as confounder in our longitudinal analyses to account for the possible effect of the intervention conducted within the GESTAFIT project on these outcomes. Additionally, although our findings were corrected for multiple comparison testing, the likelihood of making a type I error might not be completely disregarded and future studies should confirm our findings. Several strengths of this study are worth considering. A detailed definition of the dietary habits and a valid assessment of the MD diet adherence was employed. Furthermore, HRQoL was assessed at multiple time points (i.e., 16th and 34th g.w.) is widely valid and reliable, which guarantees the quality of this data.

CONCLUSION

A greater MD adherence was associated with better scores in all the mental and physical components of HRQoL along the pregnancy course. Specifically, a greater intake of whole grain cereals, fruits, vegetables, fish and nuts and a lower intake of potatoes, red meat and sweets seemed to explain these associations. Our findings may be emphasising the importance of improving the MD adherence during pregnancy in future community and population-based programs or policies to improve HRQoL throughout gestation. Given the limited number of studies available, further research is warranted to explore the impact of maternal healthy dietary habits on HRQoL during pregnancy and investigate causality and its mechanisms.

STUDY IX: Exercise, Mediterranean diet adherence or both during pregnancy to prevent postpartum depression—GESTAFIT Trial secondary analyses

Flor-Alemany M, Migueles JH, Alemany-Arrebola I, Aparicio VA, Baena-García L.

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ABSTRACT

Aim: To investigate the effects of a concurrent exercise training on postpartum depression, and secondarily, the potential role of MD adherence on the exercise effects.

Methods: Out of the 100 women included, 85 were considered in the per-protocol analyses (exercise n=46, control n=39). The exercise program was delivered in 60-min sessions, 3 days/week, from the 17th g.w. until birth. Dietary habits and MD adherence were assessed with a food frequency questionnaire and the MFP (i.e., a MD index). Postpartum depression was assessed with the EPDS at 6 weeks postpartum.

Results: The postpartum depression score was not statistically different between control and exercise groups ($p>0.05$). A higher consumption of fruits ($\beta=-0.242$, $p=0.022$), and lower intake of red meat and subproducts ($\beta=0.244$, $p=0.020$), and a greater MD adherence ($\beta=-0.236$, $p=0.027$) were associated with lower postpartum depression.

Conclusion: Greater MD during pregnancy was associated with lower depressive symptoms, and risk for postpartum depression. Postnatal depression was not reduced by prenatal exercise. Promoting fruit consumption while controlling the intake of red meat during pregnancy might prevent postnatal depression.

INTRODUCTION

Globally, between 10 and 15% of women are affected by depression during the postpartum period (412). Postpartum depression typically onsets between the 4th-6th week after giving birth, and it is identified as a critical public health problem (413,414). In fact, postnatal depression might chronify after giving birth, potentially exerting harms on the mother and the offspring later in life (413,414). For the mother, it can lead to intense sadness, anxiety, and failure to initiate and/or maintain breastfeeding (415), which may affect the mother-to-child relationship (416). Furthermore, children of mothers with depression are at higher risk for underweight and stunted growth in the first year (417), and to suffer from an array of physical and psychological conditions (416,418).

Interventions before childbirth have a huge importance in the prevention of the highly-prevalent postpartum depression and its potential consequences. In this context, exercise and healthy diets might partially offset the depressive symptoms in the non-pregnant adult population (419–421). Exercise during pregnancy seems to decrease the prevalence of depression in the postpartum (422) and the depressive symptoms (423); although this is inconclusive since previous exercise programs of both aerobic and strengthening exercises did not find a protective effect on postpartum depression (147,424,425). Regarding diet, the MD is certainly the most frequently studied dietary pattern with several beneficial effects on depression in the non-pregnant adult population (421). However, the extrapolation of these benefits to pregnant women is not straightforward and previous literature reports mixed findings (158,426).

Thus, it is warranted to study the combined effect of a healthy nutritional pattern (i.e., MD adherence) together with exercise adapted to pregnancy throughout gestation, to explore potential positive moderation role of MD adherence on the exercise effects. Therefore, the purpose of the present study was threefold: 1) To explore the effects of an exercise intervention delivered during pregnancy on postpartum depression; and secondarily, 2) to investigate the association of MD adherence during pregnancy with postpartum depression, and 3) to check whether Mediterranean diet during pregnancy moderates the effects of exercise on postpartum depression.

METHODS

Study Design and Participants

These are secondary analyses from the GESTAFIT project where an exercise intervention was conducted. The entire methodology of the project has been previously published (182). The

study was carried out at the "Sport and Health University Research Institute" (Granada, Spain), and at the "San Cecilio and Virgen de las Nieves University Hospitals". Of 384 pregnant women assessed for eligibility, 159 women met the inclusion-exclusion criteria **(Table 6)**. Among them, a total of 85 women who had valid data in sociodemographic characteristics, MD adherence at the 16th gestational week (g.w.), and postpartum depression assessed at the 6th week after giving birth were included for this analysis (Supplementary Figure S1). All participants provided a signed informed consent and the study was ap-proved by the Clinical Research Ethics Committee of Granada, Government of Andalusia, Spain (code: GESFIT-0448-N-15).

Sample size calculation

A priori sample size calculations were only performed for the primary outcome of GESTAFIT (i.e., gestational weight gain), which resulted in 52 pregnant women (26 per study arm). Notwithstanding, a posteriori sample size calculation considering the sample size of this study (n=85) showed a statistical power of 80% to detect small-to-medium as-sociation sizes ($f2 \geq 0.12$). Statistical power analyses were performed using G*Power 3.1 (290).

Procedures

Randomization and blinding

The GESTAFIT project was conducted in three waves for feasibility reasons. Upon an observed high dropout rate in the control group at the beginning of the study, the initial study design (randomized control trial) was partially broken in the second and third waves of participants, which has been frequently reported in research exercise during pregnancy (184). As a result, half the women were not randomised but allocated according to their convenience. Nor the gynaecologists, midwives from hospitals nor the research team who was responsible for the assessment were aware of participant`s group allocation.

Exercise group

The exercise program training program has been extensively detailed elsewhere (182). In brief, it followed the standards of the American College of Obstetricians and Gynecologists (185) and the latest scientific evidence in this field of research (187). From the 17th g.w. until birth, the exercise program consisted of both aerobic and strength exercises (60 minutes each session, three days per week). The exercises were of moderate intensity, according to the rating of the perceived effort scale reported by the participants (i.e., 12-16 points out of a range from 6 to

20, Borg scale)(188). To determine compliance to the exercise program, the participants' attendance at the exercise sessions was tracked.

Control group

Pregnant women in the control group were asked to keep their regular activities and were not invited to the training sessions. Nevertheless, the research team organized a number of workshops on physical exercise and healthy eating practices during pregnancy for ethical considerations. These workshops were delivered to the control and exercise groups.

Assessments

Postpartum depression

The EPDS (205) was employed to detect postpartum depression. The present study employed the validated Spanish version scale of EPDS (427). This scale consists of 10 Likert-style items that ranges from 0 to 3., which results in a final score of 0 to 30 (higher scores reflects higher severity of depressive symptoms). An EPDS score of 10/11 out of 30 has been suggested to detect major and minor depression, with a sensitivity of 79%, and specificity of 95.5% (the EDPS Cronbach's α was 0.87). The EPDS was considered a continuous indicator of depressive symptoms, and concomitantly cate-gorized to detect postpartum depression as EPDS score ≥ 10.

Dietary assessment and Mediterranean diet adherence

To assess dietary habits, we employed a food frequency questionnaire validated in the Spanish adults from the Andalusian region (189). The Mediterranean Food Pattern (a MD adherence index) was then calculated using the food frequency questionnaire, as it has previously been done in this study population (232). The MFP (190) is comprised of eight elements including olive oil, fiber, fruits, vegetables, fish, cereals, meat and alcohol ranging from 1 to 5. Therefore, the total score ranges from 8-40, notwithstanding, alcohol consumption was not considered when calculating the total score. As a result, the maximum score in the present study ranges from 7 to 35, where higher scores indicate greater MD adherence. Women were defined as adherent to the MD if they had a MD adherence above 21 points as previously stated in this study sample (233). Therefore, participants were classified as having a high MD adherence if they had a score of ≥21 points in the MFP index.

Covariates

Sociodemographic characteristics

At the 16th g.w. participant's sociodemographic and clinical characteristics (i.e., age, educational, marital, and working status, number of miscarriages, smoking habit and diagnosis of depression/anxiety) were assessed with a self-reported questionnaire (anamnesis).

Low back pain

Low-back pain was assessed with the Pain Visual Analogue Scale as the measured distance (mm) in a 100-mm line going from 0 (i.e., "no pain") to the participants mark (353).

Statistical analyses

Participants' characteristics at baseline were summarized as mean (standard deviation) or frequency (%) as appropriate. We used one-way analysis of covariance (ANCOVA) to explore the between-groups differences in postpartum depression. As it was defined in the study protocol, the analyses were conducted under the per-protocol principles (182). We only included women who attended more than 75% of exercise sessions. Multiple imputations were performed to estimate missing data in specific outcomes. Subsequently, differences in postpartum depression between the control and exercise groups were conducted on an intention-to-treat basis to evaluate more realistically the effectiveness of this concurrent exercise-training program when applied to the clinical practice according to the CONSORT guidelines.

Hierarchical linear regression was used to investigate the associations dietary habits and MD adherence during gestation with postpartum depression. The stepwise method was used, entering potential confounders in step 1 to test their association with postpartum depression (outcome). The choice of potential confounders was based on a previous study which was conducted in the same sample (354) where we explored the association of potential confounders with the mental health outcomes (i.e., age, having a diagnosis of depression or anxiety, number of abortions, smoking habit, GWG, low-back pain, marital status, and educational level). This step 1 was performed to select the relevant confounders that explain a significant amount of variance of postpartum depression (see **Table 50** footnotes). Next, dietary habits and MD adherence during pregnancy and postpartum were included in step 2, which retained the confounders previously found significantly associated with the outcomes in step 1.

We explored the OR of having postpartum depression (EPDS≥10) as a function of the experimental group (exercise vs control), MD adherence (low vs high MD adherence), and the interaction between the experimental group and the MD adherence. The association between MD adherence and postpartum depression was assessed by logistic regression obtaining OR and their 95% CI. All analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY) and the level of significance was set at $p \leq 0.05$

RESULTS

Of the 159 women who met the inclusion-exclusion criteria **(Table 6)** and were recruited for the GESTAFIT project, a total of 85 pregnant women had available data on dietary habits, MD adherence, sociodemographic and clinical characteristics and postpartum depression were included for the present analysis (**Figure 2)**. Descriptive characteristics of study participants are shown in **Table 48**. Briefly, 42% of the pregnant women had 1 or more miscarriages in the past. In addition, more than half of the participants (69%) had a high educational status (i.e., University studies), were married or with a partner (61%) and were working (72%). The mean EPDS score was 5.9 (standard deviation=4.2) at early postpartum. The prevalence of significant depressive symptoms was 17% (defined as EPDS≥10).

Table 48. Sociodemographic and clinical characteristics of the Study IX participants

Variable	Total cohort (n=85)	Counselling (n=39)	Exercise (n=46)
Age (years)	33.4 (4.2)	33.6 (4.2)	33.22 (4.2)
Mediterranean diet adherence	21.2 (4.9)	19.8 (5.2)	22.4 (4.2)
Percentage of attendance[a]			85.5 (7.5)
Pregestational body mass index (n=83)	23.7 (3.7)	23.0 (3.1)	24.2 (4.1)
Gestational weight gain[b]	11.3 (4.9)	13.4 (4.5)	9.4 (4.4)
Low back pain (VAS)	19.6 (22.9)	17.8 (23.8)	21.3 (22.3)
Postpartum depression			
Edinburgh (0-30)	5.9 (4.2)	-	-
Edinburgh ≥10 (yes, n [%])	14 (16.5)	6 (15.4)	8 (17.4)
Educational Status		**n (%)**	
Low educational status	7 (8.2)	3 (7.7)	4 (8.7)
Medium educational status	19 (22.4)	6 (15.4)	13 (28.3)
High educational status	59 (69.4)	30 (76.9)	29 (63.0)
Marital status			
Married/with partner	52 (61.2)	24 (61.5)	28 (60.9)
Divorced/Single/widow	33 (38.8)	15 (38.5)	18 (39.1)
Working status			
Working	61 (71.8)	29 (74.4)	32 (69.6)
Not working	24 (28.2)	10 (25.6)	14 (30.4)
Number of miscarriages			
0	49 (57.6)	21(53.8)	28 (60.9)
1	26 (30.6)	10 (25.6)	16 (34.8)
2	8 (9.4)	7 (17.9)	1 (2.2)
3	2 (2.4)	1 (2.6)	1 (2.2)
Smoking (yes, n [%])	6 (7.1)	5 (12.8)	1 (2.2)
Diagnosis of depression/anxiety (yes, n [%])	2 (2.4)	1 (2.6)	1 (2.2)

Values shown as mean (standard deviation) unless otherwise is indicated. [a]When considering women on an intention to treat basis, the average percentage of attendance was 77.5%. [b]Weight at the 34th gestational week -pre-pregnancy weight. SD, standard deviation. SD, standard deviation; VAS, visual analogue scale.

Postpartum depression according to exercise intervention (control vs exercise groups) (in per-protocol and intention to treat basis) are shown in **Table 49**. Although non-significant, in a per-protocol basis analysis the exercise group showed a reduction in postpartum depression scores (EPDS score difference= −1.327 [95% CI: −3.099 to 0.446], p=0.140). Similar results were obtained on an intention-to-treat basis analysis.

Table 49. Influence of the exercise intervention on postpartum depression

	Control group	Exercise group	P[a]	P[b]
Per-protocol basis (≥75% attendance)				
Edinburgh (n=39 vs 46)	6.6 (0.7)	5.3 (0.6)	0.299	0.140
Intention to treat				
Edinburgh (n=42 vs 68)	6.8 (0.7)	5.6 (0.5)	0.337	0.149

Values shown as mean (standard error). [a]Model unadjusted. [b]Model adjusted for number of miscarriages.

Hierarchical linear regression analyses for the association of dietary habits and MD adherence during pregnancy and postpartum depression are presented in **Table 50**. A higher intake of fruits (β=-0.242, *p*=0.022), a lower intake of red meat and subproducts (β=0.244, *p*=0.020) and a greater MD adherence (β=-0.236, *p*=0.027) were associated with lower postpartum depression.

Table 50. Associations of dietary habits and Mediterranean diet adherence during pregnancy with postpartum depression severity (n=85).

	Longitudinal associations	
	β	***p***
Food groups		
Whole-grain cereals (servings/week)†	0.023	0.830
Potatoes (servings/week)†	0.138	0.195
Fruits (servings/week)†	-0.242	0.022
Vegetables (servings/day)†	-0.058	0.599
Pulses (servings/week)†	-0.031	0.772
Fish (servings/week)†	-0.114	0.284
Red meat and subproducts (servings/week)†	0.244	0.020
Poultry (servings/week)†	-0.105	0.327
Dairy products (servings/week)†	-0.053	0.627
Olive oil (servings/week)†	-0.160	0.133
Nuts (servings/week)†	-0.054	0.616
Sweets (servings/week)†	0.026	0.806
Mediterranean diet adherence (7-35)†	-0.236	0.027

The predictor variable was introduced in separate hierarchical regression models. Potential confounders (i.e., age, having a diagnosis of depression or anxiety, number of abortions, smoking habit, gestational weight gain, lumbar pain, marital status and educational level) were included into step 1 of the stepwise regression to test their association to the outcomes, so that for each outcome only the relevant confounders were retained in the final models. In step 2, the exposure variable of interest, dietary habits and Mediterranean diet adherence was entered using the method ENTER to force to be in the model. †Adjusted by number of abortions.

The OR for presenting postpartum depression (EPDS≥10) as a function of the exercise intervention (exercise vs control group), the MD adherence (below vs above 21), and the interaction of exercise and MD adherence are graphically shown in **Figure 13**. The exercise intervention had a null effect on postpartum depression (OR=1.005, 95% CI:0.310 to 3.294, *p*=0.993). An optimal MD adherence during pregnancy (MFP≥21) was associated with a 72% lower risk of postpartum depression (OR=0.278, 95% CI:0.077 to 1.009; *p*=0.052). Regarding the interaction between exercise and diet, those participants who received the exercise intervention and had a greater MD adherence (i.e., higher median split) had a lower non-significant risk of postpartum depression relative to participants in the control group and below the median MD adherence (OR=0.302, 95% CI:0.048 to 1.892, *p*=0.201).

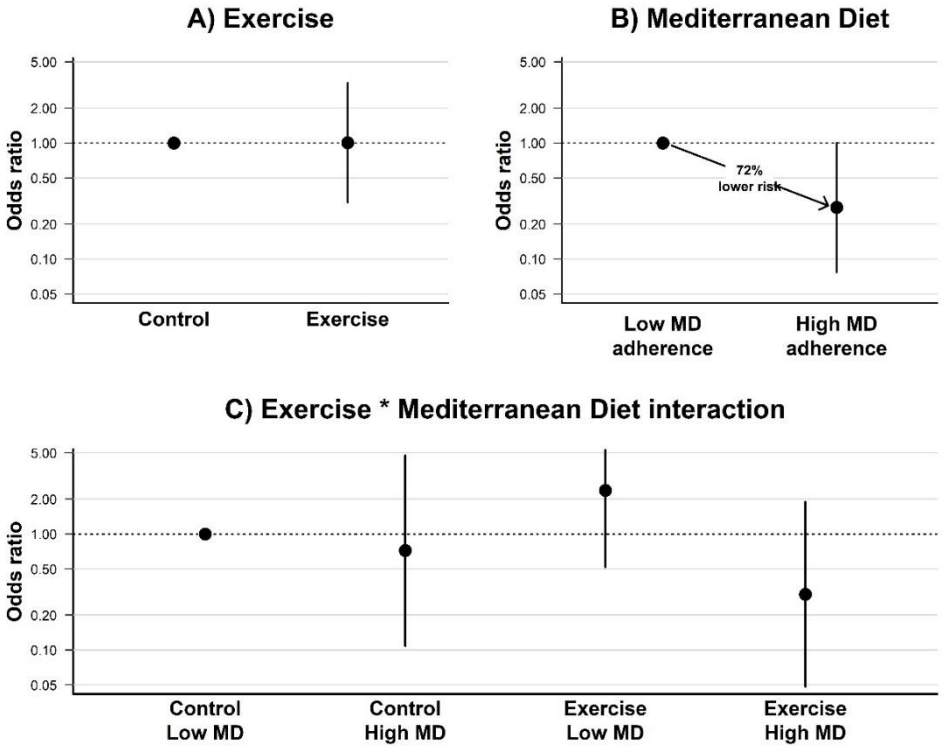

Figure 13. Odds ratio for postpartum depression relative to A) experimental group (exercise vs control), B) Mediterranean diet adherence (above vs below median), and C) the interaction between exercise and Mediterranean diet adherence.

DISCUSSION

The findings of this two-arm counselling vs exercising groups suggest that exercising during pregnancy did not significantly affect the risk for postpartum depression. However, a greater MD adherence, including a higher consumption of fruits and lower of red meat and subproducts during pregnancy, was associated with lower depressive symptoms and a lower likelihood of depression in the early postpartum.

The prevalence of postpartum depression in our sample was of 17%, which matches the estimated in the Spanish population (i.e., 15% to 22%) (427,428). Postpartum depression can affect maternal and child health (412). Thus, establishing effective, safe, inexpensive, and well-accepted interventions to prevent and treat postpartum depression is paramount (420).

Exercise may prove to be an effective non-pharmacological way for women to regulate mood states following pregnancy (429). Aerobic and strength training are effective in decreasing depression symptoms and enhancing positive mood in people with a diagnosis of depression (419). However, research examining exercise during pregnancy to prevent postpartum depression is inconclusive (430,431). According to a systematic review, exercise reduced postpartum depression assessed with the EPDS by 4 points (430). These findings were contrary to another systematic review that did not find exercise to reduce postnatal depressive symptoms (431). In contrast, our findings showed no effect of exercise on postpartum depression, which might be explained by the lack of statistical power, since our findings go in the same direction that the observed in the previous meta-analysis, yet findings in the GESTAFIT trial did not reach statistical significance.

Previous studies (147,422,425) have focused on the effects of an exercise intervention program during gestation on the risk of developing postpartum depression. Similar to our results, two previous trials (147,425) observed a non-statistically significant reduction in the EDPS score in the exercise group compared to controls (from 16-20th g.w. to 32-36th g.w.). Similarly, Vargas-Terrones et al. (422) observed a significant reduction in the risk for postpartum depression in the exercise group compared with controls. These discrepancies could be attributable to differences in the exercise program conducted and the tools employed to assess postpartum depression. First, we monitored the exercise intensity according to participants' perceived exertion which is considered valid during pregnancy (432). However, common concerns regarding exercise during pregnancy might influence women's perceived exertion (433). For this reason, the exercise intensity achieved by some participants might not reflect the intensity that was intended for improvements in this outcome. Nonetheless, Vargas-Terrones et al. (422) monitored the intensity of the sessions with heart rate monitor, targeting a 55–60% of heart rate reserve during the sessions. Moreover, the prevalence of postpartum depression reported by Vargas-Terrones et al. (422) was 30% in the control group and 15% in the exercise group. Notwithstanding, the prevalence of postpartum depression in the studies conducted by Coll et al. (425) and Songøygard et al. (147) ranged from 4% to 9% while we found a prevalence that ranged from 15% to 17%. It might be that exercise is particularly effective in those populations with higher rates of depressive symptoms. Vargas-Terrones et al. (422) employed

the CES-D while Coll et al. (425) and Songøygard et al. (147) employed the same questionnaire that we employed in our study (i.e., EPDS). The CES-D has been showed to overestimate postpartum depression compared with the EPDS (434) which could explain why Vargas-Terrones et al. (422) reported a higher prevalence of postpartum depression. Moreover, the proportion lost to follow-up was higher in the control (55%) than in the intervention group (29%), which may have affected the prevalence of higher EPDS scores in the control compared to the intervention group. This lower prevalence might have resulted in lower statistical power to detect differences. Therefore, the present findings must be interpreted with caution.

Maternal nutrition might influence the development and course of postpartum depression (158,426,435). Previous evidence has focused on the association of nutrients or individual food groups and postpartum depression with contradictory results (435–438). Hamazaki et al. (436) found that higher intake of fish and/or n-3 polyunsaturated fatty acids during pregnancy was associated with a reduced risk of postpartum depression; while Nathanson et al. (435) did not observe such association. Otherwise, supplementation with B vitamins (such as riboflavin) (437), yet not with folic acid (438), seems beneficial for postpartum depression. This inconsistency between studies could be explained by the traditional single nutrient- or food-based approach which may provide an incomplete picture of the relationship between diet and mental health failing to account for the interaction between nutrients, and how they could contribute to depressive symptoms (435,439). Dietary patterns, however, better reflect food and nutrient consumption and may therefore be suitable for analysis in postpartum depression epidemiology (426). Better diet quality has been associated with a lower likelihood of depressive disorders among non-pregnant adult population (421). However, according to a recent systematic review studies examining the association between diet quality and post-natal depressive symptoms are urgently required (158).

We found that a greater MD adherence during pregnancy was associated with lower postpartum depression. In addition, women with a high MD adherence (i.e., MFP≥21) were about 72% less likely to present high levels of postpartum depression. Similarly, Chatzi et al. (440) found that women who followed a 'healthy' diet during pregnancy (14-18th g.w.) comprising vegetables, fruit, nuts, pulses, fish and seafood, olive oil, and dairy products had a 50% reduced risk of depressive symptoms at 8-10 weeks after birth. Besides, we investigated the single components of the MD index and observed that a greater intake of fruits and lower of red meat and subproducts was associated with lower postpartum depression. Similarly, previous studies support our findings on nuts and fruits (441), as well as on red meat and subproducts (442).

Several factors such as oxidative stress, inflammation, and changes in vascularization, have been proposed to cause diet-induced damage to the brain and have been associated with the occurrence of depression (443). A healthy diet has the potential to regulate these factors. It has been hypothesized that inflammatory processes might be behind of the onset and maintenance of depressive disorders (444). Interestingly, red meat has been associated with inflammatory biomarkers (445) and an increased risk of depression in the Spanish non-pregnant adult population (160). On the contrary, fruit antioxidants might potentially suppress the inflammation and neuronal cell damage, and, subsequently cognition (446). Moreover, diet influences oxidative processes, which may also be implicated in the pathophysiology of depressive illnesses (447,448). This is because depression increases the production of proinflammatory cytokines, which induce reactive oxygen species, which then trigger the lipid peroxidation process, compromising the brain (447). Therefore, a lower intake of pro-oxidant rich foods (e.g., red meat and subproducts) and a greater intake of antioxidant-rich foods (e.g., fruits) could be recommended in the perinatal period (449). In this sense, the MD could be particularly useful since it has been showed to neutralize oxidative stress and reduce the risk of depression (450). This could explain why a MD characterized by a high number of dietary constituents such as antioxidant nutrients, dietary fiber and fat composition could be associated with lower postpartum depression.

According to previous evidence (431), exercise-only interventions during pregnancy did not clearly affect the severity of depressive symptoms at postpartum. Therefore, interventions including diet and exercise components might be more effective in preventing postpartum depression compared to exercise interventions alone. However, evidence about the interaction between diet and exercise on postpartum depression is limited. Although non-significant, participants in the intervention group with high MD adherence scored 3 points less in postpartum depression than the control group with low MD adherence. Our limited sample size could explain the lack of statistical significance in a relatively large effect size (70% lower risk). Therefore, our exploratory findings might suggest the potential role of diet on the effects of exercise in postpartum women's depression, yet this should be further tested by combining diet and exercise interventions in randomized controlled trials sufficiently powered.

Limitations and strengths

This study is not free from limitations. Firstly, this is quasi-experimental study which is a limitation since women were not purely randomized. Notwithstanding, the randomised component was partially broken because of difficulties related to the adherence of control

women to their intervention regime; which represents a frequent methodological barrier in antenatal exercise research (184). Secondly, postpartum depression was assessed with the self-administered EPDS, which does not relate to a clinical diagnosis of depression. Notwithstanding, EPDS is a well-established and widely-used screening tool for postpartum depression which has been validated and with high specificity and sensitivity (427). Even though we have investigated the potential moderation role of diet on the effects of exercise on postpartum depression, this study only considered diet as an observed factor; thus, the interaction effects of exercise x diet could not be determined. Further research using a 2x2 factorial design based on exercise and diet interventions is warranted to gain insight into this question. Regarding strengths, participating women were followed-up from the 16th g.w. until the postpartum measurement, allowing us to consider a variety of potential confounding exposures during pregnancy and early life. Additionally, we included several potential confounders, such as sociodemographic factors (age, education and marital status), life-style behaviours (smoking), and other risk factors (having a diagnosis of depression or anxiety, number of abortions, gestational weight gain and lumbar pain).

CONCLUSION

Overall, we found that exercise did not significantly reduce the depressive symptoms at postpartum. However, a greater MD adherence, and specifically the consumption of more fruits and less red meat and subproducts, was associated with lower depressive symptoms and postpartum depression. The interaction between exercise and MD could lead to lower risk for postpartum depression, although larger randomized controlled trials should formally test this hypothesis.

SECTION IV. Influence of Mediterranean diet during pregnancy on materno-fetal genetics

STUDY X: Are the effects of exercise on gestational weight gain moderated by the FTO gene polymorphism rs9939609? The GESTAFIT project

Flor-Alemany M, Migueles JH, Martínez González LJ, Álvarez Cubero MJ, Alcántara-Domínguez C, Aparicio VA.

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ABSTRACT

Aim: To investigate whether the effects of an exercise program during pregnancy on GWG are influenced by genetic susceptibility to obesity.

Methods: A subsample of the GESTAFIT trial of pregnant women who were genotyped for the *FTO* gene polymorphism rs9939609 were analysed (n=77, control n=35, exercise=42). Women non-susceptible to obesity (TT carriers) were compared to A risk allele carriers (TA+AA carriers).

Results: GWG was lower in the exercise compared to the control group (GWG difference: -3.592 [95% CI: -5.591 to -1.266] kg, p=0.003). The interaction term of the exercise*genetic susceptibility to obesity was significant (p=0.030). Among the TT carriers, women in the exercise group showed a 6 kg GWG lower than controls (95% CI: -11.590 to -0.423 kg, p=0.036). Among those women with susceptibility to obesity (TA+AA carriers), the exercise group also had a lower GWG than the control group, yet of lower magnitude (GWG difference: -2.576 [95% CI: -5.030 to -0.122] kg, p=0.040).

Conclusion: The exercise intervention was effective in the reduction of GWG, independently of genotype. These effects might be of greater magnitude in those women who are not genetically susceptible to obesity (*FTO* rs9939609 TT carriers).

INTRODUCTION

Excessive GWG affects half of the pregnancies worldwide (451). This increases the risk of perinatal complications such as hypertensive disorders of pregnancy, gestational diabetes, fetal macrosomia, and cesarean birth rates (77,451); and long-term metabolic consequences for mothers and children (77).

Exercise interventions have been proposed to control and avoid excessive GWG (302), yet it remains unknown whether the exercise effects on GWG are influenced by the genetic background. Several SNPs of obesogenic genes have been related to an increased risk of overweight or obesity (93,452). Specifically, the risk allele ("A") of *FTO* rs9939609 polymorphism is a major contributor to obesity (95,96). Studies on pregnant women suggest a positive association of the presence of rs9939609 A variants with high pre-pregnancy weight (93), pre-pregnancy BMI (93), and excessive GWG (97). Women carrying the obesity risk allele (TA + AA) were up to 3 kg heavier, and had a 1 kg/m^2 higher pre-pregnancy BMI compared to women carrying the non-risk genotype (TT) (98).

Exercise attenuates the harmful effect of the FTO rs9939609 adverse genotype on weight status and BMI in the adult population (99–101,453). However, there is no evidence of genotype–exercise interactions on GWG. Addressing this question is clinically relevant for the treatment of obesity and will help to develop personalized, genotype-based exercise recommendations to prevent excessive GWG for those at high genetic risk. In this regard, the GESTAFIT trial found a GWG reduction or around 3 kg induced by an exercise program during pregnancy, from the 17th to 34th g.w. (454). In this study, we aimed to investigate whether the effect of exercise was moderated by women's susceptibility to obesity, as it is indicated with the presence of the SNP rs9939609 of the *FTO* gene.

METHODS

A subsample of 77 pregnant women recruited for the GESTAFIT project (control n=35; intervention n=42) who had valid data in GWG (body weight measured at the 17th and 33th g.w.± 2 weeks) and were successfully genotyped were included in these secondary analyses **(Figure 2)**. Upon difficulties in the adherence of the control subjects, half of the women were allocated to their group according to their personal convenience. The detailed exercise intervention has been previously described elsewhere (454). Pregnant women in the control group did not take part in the exercise training program and were asked to continue with their usual activities.

Gestational weight gain

Pre-pregnancy body weight was self-reported at the 12th g.w. Weight at the 33th g.w. (±2 weeks) was measured (no shoes, light clothes) with an electronic scale (InBody-R20; Biospace, Seoul). Total GWG (kg) was calculated as weight difference from pre-pregnancy until 34th g.w.

Genotype

Participants were genotyped for rs9939609 SNP (*FTO* gene). Mucosa cells were collected with swabs, and DNA was isolated by an organic extraction (proteinase K and salting-out) previously described and validated (455,456), and later spectrophotometric quantification (NanoDrop-2000c, TermoFisher) was performed. Genotyping was performed using TaqMan® assays and TaqMan® Genotyping Master Mix 2X (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 QuantStudio Real-Time PCR Software v1.3.

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

Dietary assessment

Dietary habits were assessed with an adult-validated food frequency questionnaire (189). MD adherence was derived from it using the MedDietScore (30). The Evalfinut software was employed to estimate total energy intake (kcal/day).

Statistical analysis

Differences in GWG between the control and exercise groups was assessed with an ANCOVA. GWG was included as dependent variable, exercise group as fixed factor, and age and gestational age at the 34th g.w. as covariates. As for the main analysis, the interaction term between group (i.e., exercise versus control) and *FTO* rs9939609 genotype (wild-type genotype: TT vs genotypes with at least 1 risk allele: TA + AA) was investigated. As initially

designed (182), statistical analysis was conducted on a per-protocol basis including women who attended more than 75% of exercise sessions. All analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY) and the level of significance was set at $p \leq 0.05$.

RESULTS

Sociodemographic and clinical characteristics of the participants are shown in **Table 51**.

Table 51. Sociodemographic and clinical characteristics of the Study X participants

Variable	Total women (n=77)	Control (n=35)	Exercise (n=42)
Age (years)	33.5 (4.1)	34.1 (3.9)	32.9 (4.3)
Pre-pregnancy body mass index (kg/m²) (n=75)	23.6 (3.7)	22.9 (3.0)	24.2 (4.2)
Gestational age at birth	39.6 (1.2)	39.4 (1.4)	39.7 (1.0)
Percentage of attendance	-	-	85.7 (7.6)
Mediterranean diet adherence	29.3 (3.9)	28.6 (4.0)	29.9 (3.7)
Educational status			
University studies	53 (68.8)	27 (77.1)	26 (61.9)
No university studies	24 (31.2)	8 (22.9)	16 (38.1)
Working status			
Working	54 (70.1)	26 (74.3)	28 (66.7)
Not working	23 (29.9)	9 (25.7)	14 (33.3)
Marital status			
Married	47 (61.0)	22 (62.9)	25 (59.5)
Single/divorced/widow	30 (39.0)	13 (37.1)	17 (40.5)
Smoking habit (yes, n [%])	4 (5.2)	3 (8.6)	1 (2.4)

Values shown as mean (standard deviation) unless otherwise is indicated.

In this subsample (n=77, control: TT n=13, TA+AA n=22; exercise: TT n=13, TA+AA n=29), the women in the exercise group showed a smaller GWG (GWG difference: -3.592 [95% CI: -5.591 to -1.266] kg, p=0.003) than those in the control group **(Figure 14)**. The interaction term of the exercise**FTO* gene polymorphism rs9939609 was statistically significant (p=0.030). Women in the exercise group characterized by TT genotype in the *FTO* gene polymorphism rs9939609 showed a smaller GWG (GWG difference: -6.007 [95% CI: -11.590 to -0.423] kg, p=0.036) than A allele carriers (GWG difference: -2.576 [95% CI: -5.030 to -0.122] kg, p=0.040) **(Figure 14)**.

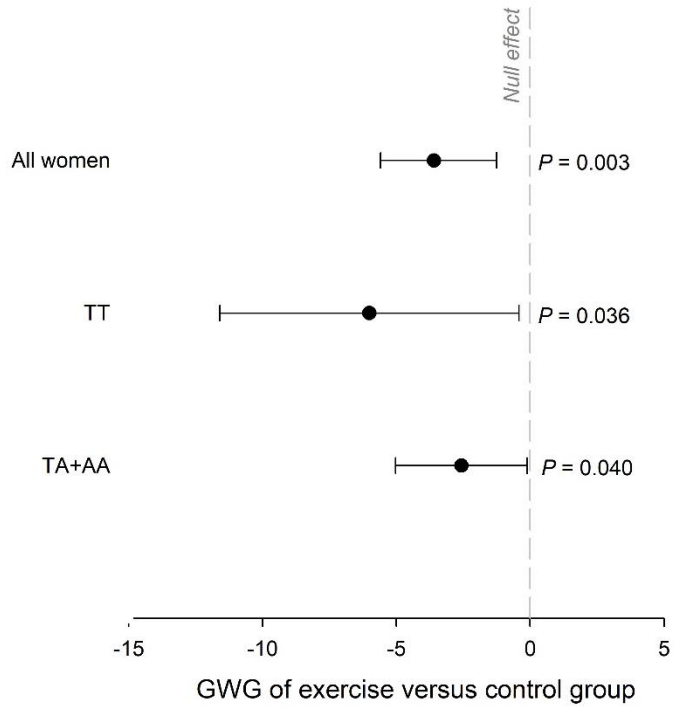

Figure 14. Differences in gestational weight gain according to exercise intervention and *FTO* rs9939609 genotype (TT versus AT+AA). GWG, gestational weight gains; Model adjusted for maternal age and gestational age at the 34th gestational week. AA, homozygote for risk allele; AT, heterozygote; TT, wild type.

No differences between energy intake, dietary habits nor MD adherence were found between women characterized by TT genotype in the *FTO* gene polymorphism rs9939609 vs. A allele carriers (all, p>0.05) **(Table 52)**.

Table 52. Differences in energy intake, dietary habits and Mediterranean diet adherence by FTO rs9939609 genotype (TT vs TA+AA)

	FTO rs9939609		
	TT (n=25)	**TA+AA (n=49)**	***p***
Energy (kcal)	2435.9 (796.7)	2358.3 (601.3)	0.630
Food groups			
Whole-grain cereals (servings/week)	7.1 (1.4)	5.5 (1.0)	0.356
Potatoes (servings/week)	2.4 (0.2)	2.1 (0.2)	0.307
Fruits (servings/week)	15.5 (1.4)	15.6 (1.0)	0.933
Vegetables (servings/week)	24.4 (2.4)	25.3 (1.7)	0.776
Pulses (servings/week)	3.0 (0.3)	2.5 (0.2)	0.175
Fish (servings/week)	5.5 (0.5)	5.3 (0.4)	0.710
Red meat and subproducts (servings/week)	6.0 (0.9)	5.6(0.6)	0.717
Poultry (servings/week)	2.4 (0.2)	2.7 (0.2)	0.307
Dairy products (servings/week)	11.3 (1.8)	12.3 (1.2)	0.658
Olive oil (servings/week)	14.7 (2.6)	14.3 (1.9)	0.899
Nuts (servings/week)	4.8 (1.0)	4.8 (0.7)	0.975
Sweets (servings/week)	7.9 (5.6)	8.5 (5.6)	0.289
Mediterranean diet adherence (0-50)	30.1 (0.8)	28.8 (0.6)	0.205
Pre-pregnancy body mass index (kg/m²)	24.0 (4.4)	23.4 (3.4)	0.554

Values shown as mean (Standard deviation). AA, homozygote for risk allele; TA, heterozygote; TT, Wild type.

DISCUSSION

Our results indicate that pregnant women in the exercise group showed a smaller GWG than those in the control group regardless of their genotype. Notwithstanding, women homozygous for the non-risk allele of *FTO* rs9939609 (TT) showed a lower GWG compared with risk-allele carriers (TA+AA). However, exercise was effective even in those women susceptible to obesity as classified with the *FTO* rs9939609 gene. This suggests that: (i) all women could benefit from exercise in terms of GWG, and (ii) genetic predisposition to obesity may explain the effect size of the intervention. The exercise intervention might attenuate the risk associated with the genetic predisposition to obesity during pregnancy.

We have previously reported that our supervised-tailored exercise program notably reduced maternal weight-gains (2.71 kg) in 101 women recruited from the GESTAFIT Project (454). Of note, the effect on GWG is larger in this study (0.5 kg more) because we have employed total GWG (i.e., weight gain from pre-pregnancy to 34th g.w.), and investigated the effects in a subsample of the total women investigated in our previous study (i.e., those who were successfully genotyped for the *FTO* gene). Notably, no differences were found in baseline characteristics (e.g., age, pre-pregnancy BMI) between the 77 women genotyped and the rest of the GESTAFIT participants. The results have been replicated with late weigh gains (i.e.,

weight gain from 17th to 34th g.w.) and are consistent. Women in the exercise group presented lower GWG, consistently with previous trials (302). A novel finding is the interaction between the exercise intervention and the FTO rs9939609 genotype. Although the genotype-lifestyle interaction effect on adiposity outcomes is confirmed in non-pregnant adult population (99,100,453), the evidence of this effect in pregnancy is limited. Previous evidence (457,458) showed a relationship between *FTO* and obesity phenotype with behavioural factors such as energy intake and physical activity as important modulators in the association. In our study, TT carriers showed a 3.4 kg lower GWG than A allele carriers of rs9939609. This suggests that the exercise intervention might be more beneficial in women who are not genetically predisposed to obesity. We also found a beneficial effect, albeit lower, in *FTO* risk allele carriers, suggesting that risk-allele carriers (TA+AA) also respond to exercise intervention although to a lower extent. Previous evidence showed that *FTO* obesity-susceptibility genotype influences the body fat responses to regular exercise (101). Resistance to exercise-induced reduction in total adiposity may represent one mechanism by which the FTO A allele promotes overweight and obesity(101). Nonetheless, currently no other studies have reported interactions between FTO rs9939609 and exercise interventions in pregnant women on GWG, and this deserves further investigation.

Diet is other environmental factor that potentially contribute to interindividual differences in body fat mass (93). We did not find differences in energy intake, food groups consumption nor Mediterranean diet adherence between TT carriers and TA+AA carriers. Thus, obesity-associated SNP in *FTO* did not seem to influence dietary intake nor diet quality among pregnant women. This in in line with previous studies(93,96) but inconsistent with others (459). Martins et al. (459) reported that the A allele for the FTO rs9939609 was associated with an increase in total energy intake and in the percentage of energy from ultra-processed foods during pregnancy. However, Hasselbalch et al. (96) suggested that polymorphisms in the *FTO* gene do not have a role in regulating food intake and preference for specific food items which is highly in agreement with our findings.

However, our findings should be interpreted cautiously given the relatively-small sample size and the non-pure-randomized design of the trial. Future research should confirm or contrast our findings.

CONCLUSION

Pregnant women in the exercise group showed a smaller GWG than those in the control group regardless of their genotype. This effect was amplified in those women who were not genetically susceptible to obesity (*FTO* rs9939609 TT carriers). Notwithstanding, the exercise program also proved an effect, albeit lower, on the GWG in those women with genetic predisposition to obesity (*FTO* rs9939609 AT and AA carriers). This may have important public health implications because genetic susceptibility to excessive GWG in the presence of *FTO* variants may be reduced by adopting a physically active lifestyle during pregnancy.

STUDY XI: Influence of an exercise intervention plus an optimal Mediterranean diet adherence during pregnancy on the telomere length of the placenta. The GESTAFIT project

Flor-Alemany M, Acosta-Manzano P, Migueles JH, Varela A, Baena-García L, Quiles JL, Aparicio VA.

Submitted to Placenta

ABSTRACT

Aim: To study whether the effects of an exercise program during pregnancy on placental relative telomere length after delivery are moderated by following a healthy dietary pattern (i.e., MD).

Methods: A total of 65 participants met the per-protocol criteria (control n=34, exercise n=31). The exercise intervention consisted of a 60-min, 3 days/week throughout pregnancy from 17th g.w., supervised concurrent (aerobic+resistance) exercise program. A food frequency questionnaire and the MedDietScore (min-max: 0-50) were employed to assess dietary habits and MD adherence during pregnancy, respectively. Placental relative telomere length was assessed by a quantitative real time polymerase chain reaction-based method.

Results: No differences were found in placental relative telomere length between the exercise and the control groups (p=0.557). The interaction-term between exercise and MD adherence with placental placental relative telomere length was significant (p=0.001). Specifically, those women with high MD adherence showed longer placental relative telomere length after birth in the exercise group compared to controls (mean difference=0.467, p=0.010).

Conclusion: A concurrent-exercise training plus an optimal MD adherence during pregnancy might prevent the placental relative telomere length shortening.

INTRODUCTION

Telomeres are nucleoprotein structures that serve as guardians of genome stability by ensuring protection against both cell death and senescence (460). Previous evidence has implicated shortened placental relative telomere length in the pathogenesis of pregnancy complications including preeclampsia (172), gestational diabetes (173), intrauterine growth restriction (174), spontaneous preterm birth (175), and unexplained stillbirths (176). In this context, exercise and healthy diets such as the MD might partially offset telomere shortening measured in blood cells (178–181). However, the extrapolation of these benefits to placental relative telomere length has not been investigated to date. Therefore, the purposes of the present study were: (1) to explore the effects of an exercise intervention delivered during pregnancy on placental telomere length; and, 2) to investigate whether MD adherence during pregnancy moderates the effects of the exercise intervention on placental relative telomere length.

METHODS

Study design and participants

These are secondary analyses from the GESTAFIT project where a concurrent exercise program consisting of aerobic and resistance exercises from the 17th g.w. until birth was conducted (182) (Identifier: NCT02582567). Pregnant women in the control group did not take part in the exercise training program and were asked to continue with their usual activities (182). A total of 65 pregnant women (exercise n=31, control n=34) who had valid data in placental relative telomere length and dietary habits during pregnancy were included in this study **(Figure 2)**.

Randomization and blinding

The study was conducted in three waves. The GESTAFIT project was initially designed as a randomized control trial (computer-generated simple randomisation). Nonetheless, the randomized component was broken in the second and third waves to ensure enough adherence to the program; which represents a frequent methodological barrier in antenatal exercise research (184). Thus, half the women were not randomized but allocated to the control/exercise group according to their personal convenience. Most personnel were blinded to their allocation into the control/exercise group, excepting those responsible for the training sessions.

Exercise Intervention

The exercise intervention consisted of a concurrent supervised-tailored exercise program (from 17th g.w. until delivery, 3 days/week, 60 minutes/session) of aerobic and resistance exercises of moderate-to-vigorous (mostly moderate with peaks of vigorous) intensity. Sessions consisted of a 10-minute warm-up, a 40-minute muscular (circuits of resistance exercises and short aerobic blocks) or aerobic block (dance or functional circuits), and a 10-minute cooldown. Resistance exercises involved anterior and posterior chain dominant, pull, push, and core exercises. The exercise training program was designed following the standards by the American College of Obstetricians and Gynecologists (185), and the latest scientific evidence (186,187). During the intervention, women were provided with 7 seminars to promote healthier pregnancies.

Control Group

Pregnant women in the control group did not attend the exercise sessions and were asked to continue with their usual activities, yet they were invited to the seminars.

Sociodemographics

Sociodemographic characteristics of the study sample were gathered through medical files and questionnaires (i.e., age, educational and marital status, and smoking).

Dietary assessment and Mediterranean diet adherence

A food frequency questionnaire validated in Spanish adults (189) was administered by a trained nutritionist at the 16th g.w. and 34th g.w. to assess dietary habits. This study targeted women in the second trimester of pregnancy (13th to 27th g.w.). The first trimester is characterised by morning sickness, whereas dietary habits during the second trimester are more constant and representative of diet across the entire gestation (231). Moreover, we observed similar MD adherence between the 16th g.w. and the 34th g.w. in our sample (232). Consequently, the dietary habits of the 16th g.w. were considered for analyses. The MedDietScore developed by Panagiotakos et al. (30) was derived from the food frequencies reports (189) to assess MD adherence as previously done in this study sample (210). The MD Score consists of eleven variables (wholegrain cereals, potatoes, fruits, vegetables, pulses, fish, olive oil, red wine, red meat and subproducts, poultry, and whole dairy products) ranging from 0 to 5 according to their position in the MD pyramid (271). The total score ranges from 0

to 55, with higher values indicating greater adherence to the MD. A moderate alcohol intake, also typical of the MD, was not considered in this group of women since they are recommended not to drink alcohol during gestation. There were no women consuming alcohol during pregnancy. Therefore, the score considered for these analyses ranged from 0 to 50 points. Participants were classified as having a high MD adherence if they had a score of ≥30 points in the MedDietScore as previously done in this study sample (233).

Relative placental telomere length

Placental samples were collected immediately after delivery. Placentas were visually examined ensuring completeness, consistency, absence of accessory lobes, placental infarction, tumours, and nodules and samples of 2x2 cm were obtained. The sample was taken from the central placental region, excluding areas with necrosis, signs of ischemia or calcification. The samples were washed with saline solution (0.9% and 0.1% butylhydroxytoluene). All the samples, once prepared, were aliquoted and kept in a -80ºC freezer until posterior analysis.

DNA was isolated by a NucleoSpin Tissue kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Relative telomere length was assessed by a quantitative real-time polymerase chain reaction (QRT-PCR) method developed by Cawthon et al. (206) with some modifications(207). In this relative quantification approach, the amount of telomere hexameric repeat (T) is measured and compared to the amount of a single-copy gene (S) that is assumed to be constant for the same sample to establish the ratio of telomere repeat copy number to the single gene copy number (T:S ratio). Here, we chose the Rplp0 gene, as Cawthon et al. This is a well-conserved gene located on chromosome 7 and has been used for gene dosage studies. Amplification of telomeric DNA (T), together with that of the single-copy genomic Rplp0 gene (S) was performed on a Micro Amp Optical 384-well Reaction Plate (Applied Biosystems, Foster City, CA, USA) using Applied Biosystem's 7900HT Fast Real-Time PCR system. All samples were run in duplicate to account for possible technical variation. For this, 5 ng of placental-derived genomic DNA was dried overnight at room temperature in a 384-well plate placing two samples from each subject in adjacent wells and re-suspending them in 10µl of either the telomere or Rplp0 PCR reaction mixture. The telomere reaction mixture consisted of 5µl of Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 270nM Tel-1b primer (5′-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3′), 900 nM Tel-2b primer (5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3′) and double-distilled H2O. The specific reaction mixture for Rplp0 was similar except that it included 300 nM forward primer (5′-CAGCAAGTGGGAAGGTGTAATCC-3′) and 500 nM

reverse primer (5′-CCCATTATATCATCAACGGGTACAC-3′). The reaction conditions were set at 95°C for 10 min followed by 40 cycles of data collection consisting of a denaturation step at 95°C for 15 s and an annealing/extension at the 54°C step for 4 min in the case of the telomere assay or at 58°C for 2 min in the case of the Rplp0 assay. An additional melting curve analysis consisting of 95°C for 15 s, 60°C for 15 s and 95°C for 15 s was performed at the end of each reaction to verify specific amplification. To assess and compensate for interplate variations in PCR efficiency, each 384-well plate contained a 9-point standard curve from 0.23 to 30 ng in addition to the samples, using genomic human DNA pool derived from our samples. For this, DNA was serially diluted using double-distilled H2O, and each dilution was placed in 384-well plates per triplicate for both the Rplp0 gene and telomere repeats. Threshold values were set to 0.2 on Sequence Detector Systems version 2.4 software, and real-time PCR results were exported to an Excel (Microsoft, Redmond, WA, USA) spreadsheet for analysis. The standard curve was plotted from the mean Cq vs. the log of serial dilution concentrations, excluding points beyond the linear range, and only samples with quantification cycle (Cq) values inside curve were used in subsequent analyses. Cq values, mean and standard deviation were calculated for each sample, and those with a standard deviation greater than 0.5 were disregarded in further analyses. Then the absolute concentration of the telomere hexameric repeat (T) was divided by the absolute concentration of the Rplp0 gene (S) according to efficiency values calculated from their standard curves. The resulting value (T:S ratio) was divided by the T:S ratio determined from the calibrator DNA (one of our samples). The resulting ratio expresses the amount of telomere hexameric repeats, called RTL.

Statistical analyses

Differences in placental relative telomere length between the control and exercise groups were explored by an ANCOVA. Placental relative telomere length was included as dependent variable, exercise group as fixed factor, and maternal age, gestational age at birth, sex of the baby, and smoking habits as covariates. Additionally, the interaction term between group (i.e., exercise versus control) and MD adherence (i.e., low versus high) was investigated. As initially designed (182), statistical analysis was conducted on a per-protocol basis including women who attended more than 75% of exercise sessions. All analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 22.0, Armonk, NY) and the level of significance was set at $p \leq 0.05$.

RESULTS

Descriptive characteristics of study participants are shown in **Table 53**.

Table 53. Sociodemographic and clinical characteristics of the Study XI participants

Variable	Total women (n=65)	Control (n=34)	Exercise (n=31)
Age (years)	33.3 (4.2)	33.3 (4.4)	33.3 (4.1)
Pre-pregnancy body mass index (kg/m²)	23.6 (3.7)	23.4 (3.2)	23.9 (4.2)
Gestational age at birth	39.7 (1.1)	39.6 (1.2)	39.7 (1.0)
Percentage of attendance	-	-	86.3 (7.2)
Mediterranean diet adherence	28.4 (4.1)	27.6 (4.2)	29.3 (3.8)
Educational status			
University studies	41 (63.1)	24 (70.6)	17 (54.8)
No university studies	24 (36.9)	10 (29.4)	14 (45.2)
Working status			
Working	43 (66.2)	23 (67.6)	20 (64.5)
Not working	22 (33.8)	11 (32.4)	11 (35.5)
Marital status			
Married	36 (55.4)	19 (55.9)	17 (54.8)
Single/divorced/widow	29 (44.6)	15 (44.1)	14 (45.2)
Sex of the baby			
Male	34 (47.7)	16 (47.1)	15 (48.4)
Female	34 (52.3)	18 (52.9)	16 (51.6)
Smoking habit (yes, n [%])	6 (9.2)	5 (14.7)	1 (3.2)

Values shown as mean (standard deviation) unless otherwise is indicated.

No differences were found in placental relative telomere length after adjusting for maternal age, gestational age at birth, sex of the baby and smoking habit between control and exercise groups (p=0.557) **(Figure 15)**. The maternal MD adherence during gestation was found to be a moderator on the effects of the exercise intervention on placental relative telomere length (p=0.001). In particular, those participants who received the exercise intervention and had a high MD adherence showed a longer placental relative telomere length after birth (relative telomere length difference= 0.467 [95% CI: 0.113-0.802], p=0.010).

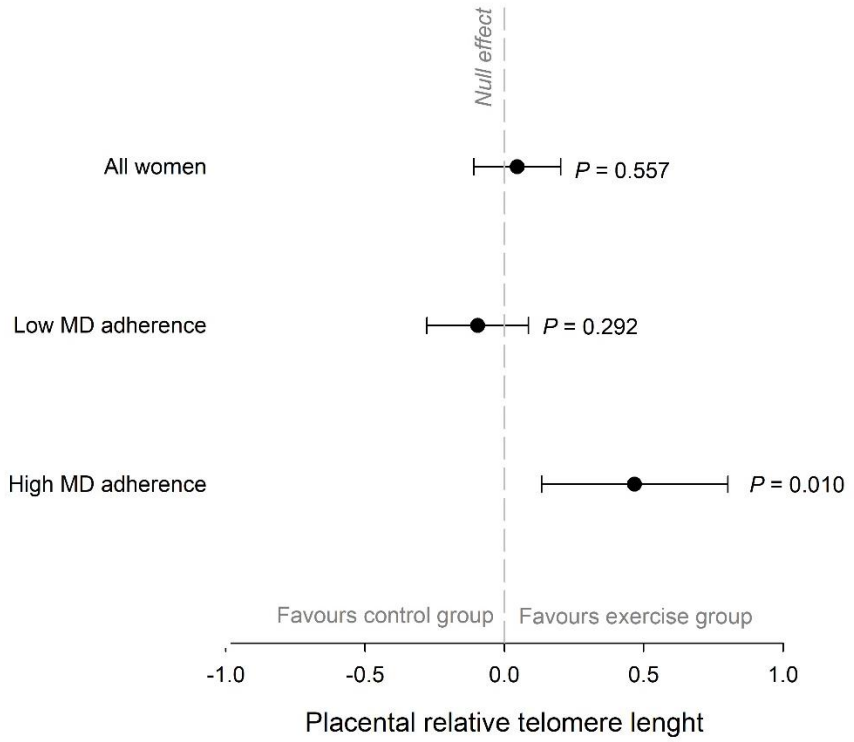

Figure 15. Differences in placental relative telomere length according to exercise intervention and the degree of adherence to the Mediterranean diet (low Mediterranean diet adherence vs high Mediterranean diet). Model adjusted for maternal age, gestational week at birth, sex of the baby and smoking habit. MD, Mediterranean diet.

DISCUSSION

Our results showed that a concurrent-exercise training combined with an optimal MD adherence during pregnancy might be a useful strategy to promote longer placental relative telomere length.

It has been reported that placental relative telomere length progressively shorten throughout the gestation (461). The rate of telomere shortening can be accelerated by factors that affect the mother and result in oxidative stress (461,462). Lifestyle factors including exercise and diet have shown to positively influence the oxidation-inflammation pathway (463,464). Telomeres are particularly sensitive to oxidative stress (462). Exercise training seems to have an antioxidant effect (464), it seems plausible that diet and exercise are related to telomere length. However, we did not observe effects of the exercise intervention on placental relative telomere lenght. Regarding diet, dietary antioxidants and consumption of antioxidant-rich, plant-derived foods help maintain telomere length (465). The MD is considered to be one of the most recognized diets for disease prevention and healthy aging, partially due to its demonstrated

anti-inflammatory and antioxidative properties which may exert a positive impact on telomere length (178). This may, at least in part, explain our findings that participants who received the exercise intervention and had a greater MD adherence (i.e., MedDietScore≥30) showed longer placental relative telomere length compared to participants in the control group and with a low MD adherence. This is in agreement with previous evidence suggesting that MD adherence is associated with longer telomeres measured in blood cells in the non-pregnant population (465). Regarding placental relative telomere length, only one previous observational study conducted by Vahter et al. (466) suggested that placental telomere length is associated with variations in maternal nutrition during pregnancy. Notwithstanding, they did not assess dietary habits nor dietary patterns but concentrations of essential trace elements in maternal serum (466). This approach based on blood, serum, plasma, and urine nutritional biomarkers can be complemented by the assessment of the actual dietary patterns used in the GESTAFIT project. The dietary approach better reflect food and nutrient consumption, and may therefore be suitable for assessing the relation between the diet quality and telomere length and for providing practical recommendations directly on the dietary habits of pregnant women (13).

Altogether, it seems that the diet quality, in combination with exercise, might prevent the placental relative telomere length shortening in pregnant women, which might have implications for clinical pregnancy outcomes (172–176). However, our findings should be considered with caution given the non-pure-randomized design of the trial and our limited sample size. Future research is needed to confirm or contrast our findings.

CONCLUSION

This study indicates that placental telomere length might be positively influenced by lifestyle factors such as diet combined with exercise during gestation. Future studies should elucidate the concerning potential role of placental relative telomere length for child health. The interaction between exercise and MD could lead to longer placental relative telomere length, although larger randomized controlled trials should formally test this hypothesis.

LIMITATIONS AND STRENGHTS

10. LIMITATIONS AND STRENGHTS

10.1. General limitations

The findings of the present International Doctoral Thesis should be interpreted with caution due to a number of limitations. An overall view of the main limitations is presented here. For more details, please see the specific limitations section of each study in Results and Discussion.

Section I includes a longitudinal and a cross-sectional study. The longitudinal study **(Study I)** set the basis for the rest of the International Doctoral Thesis, describing the recommended MD indices to assess MD adherence during pregnancy, and identifying some research gaps that were approached in this Thesis. Notably, this study was limited by the relatively small sample size and the observational design of the study. **Study II** describes sociodemographic factors, lifestyle behaviors and pregnancy-related determinants associated with MD adherence during pregnancy. This study was limited by the inclusion of physical fitness tests that are not validated in pregnancy. Notwithstanding, this represents an inherent limitation of pregnancy studies, and the employed physical fitness tests are characterized by good psychometric properties and are adaptable, viable, and safe for clinical populations (256–258). Of note, we are currently validating some of the physical fitness tests employed against gold standard methods. Furthermore, dietary patterns differ between places, populations, and cultural contexts, so a direct comparison with other non-Spanish populations and other healthy dietary patterns cannot be warranted. Of note, we are currently studying the feasibility and usefulness of the MD in a totally different context (pregnant women in Sweden).

Section II includes two cross-sectional analyses **(Studies III and IV)** and a quasi-experimental study **(Study V)**. The cross-sectional design of **Studies III and IV** limits the causation interpretation. This limitation may be also considered in **Studies VI**, **VII** and **VIII**. Thus, the associations observed between MD adherence, materno-fetal cardiometabolic markers and maternal cardiometabolic risk, sleep quality and mental health in this International Doctoral Thesis should be considered with caution and further confirmed in well-designed randomized controlled-trials. Additionally, the participants were enrolled in an exercise intervention that might affect our findings. However, we included the group allocation as confounder in our longitudinal analyses. We observed that the GESTAFIT exercise intervention did not have any effect on the MD adherence, neither on any of its components (except for legumes). Thus, the primary exposure (i.e., exercise) is not likely to have affected our findings. However, is it still

needed to study the feasibility of intervening on MD adherence during gestation. We found observational evidence on the possible effects of MD. This should be additionally tested in dietary intervention studies. Fourth, the limited data (n=35) in our study regarding cord blood could bias our findings. However, we found no differences in the baseline characteristics between women who provided cord serum samples and those who did not. Additionally, although our findings were corrected for multiple comparison testing, the likelihood of making a type I error might not be completely disregarded and future studies should confirm our findings. In **Study V**, we examined whether the effects of an exercise program during pregnancy on postpartum body composition are moderated by following a healthy dietary pattern (i.e., MD). Body composition was only assessed after 6 weeks postpartum, thus, longer follow ups could provide additional useful information. Furthermore, the quasi-experimental study design is a limitation since women were not purely randomized. This limitation may be also considered in **Section III (Study IX)** and in the studies of **Section IV (Studies X and XI)**. Our sample size might be limited, especially in these studies **(Studies V, IX, X and XI)**, where the stratification of sample on secondary outcomes might be underpowered and larger trials should contrast our findings.

Similarly, **Section III** has limitations to be considered. Some of the analyses performed may lack enough statistical power given the limited sample size. In this regard, similar drop-out rates have occurred in previous studies in pregnant women (381), and we found no differences in the baseline characteristics of the drop-outs and the completers. Furthermore, participants were enrolled in an exercise intervention that might affect our findings regarding the 34th g.w. However, we included the group allocation as confounder in our longitudinal analyses to account for the possible effect of the intervention conducted within the GESTAFIT project on these outcomes. Although the questionnaires used to assess sleep quality, mental health and HRQoL were valid and reliable for the general population, their psychometric properties have not been extensively tested during pregnancy, except for the STAI and the EPDS which are validated in pregnant and postpartum women (202,427). Additionally, although our findings were corrected for multiple comparison testing, the likelihood of making a type I error might not be completely disregarded and future studies should confirm our findings.

10.2. General strengths

An overall strength of this International Doctoral Thesis which affects all the sections is the measurement of MD adherence twice during the pregnancy course, with validated tools, and the thorough investigation of the MD scores and all the MD components performed in all the studies included. Similarly, the consideration of the MD adherence in combination with other lifestyle and environmental factors (e.g., physical activity or exercise) make this research translational to the clinical practice. And finally, the consideration of the various health dimensions (body composition, cardiovascular, sleep quality, mental health) provide a wide overview of the effects and potential benefits of following a healthy diet during this important stage in the women's life.

Specifically, in **Section I**, a major strength arises from the wide range of cardiometabolic factors within the overall risk score created, which strengthens the usefulness of these proposed indices **(Study I)**. Likewise, the wide investigation of the correlates of MD adherence might provide insights on environmental and modifiable factors that might be consider to improve the dietary patterns in pregnant women **(Study II)**.

In **Section II**, lipid, glycemic and inflammatory serum markers **(Studies III and IV)** were assessed during the second and the third trimester of pregnancy and in the arterial and vein cord blood which provides a more comprehensive understanding of materno-fetal immunometabolic serum markers along the pregnancy course. Furthermore, the measurement tool employed to assess body composition (i.e., dual-energy X-ray absorptiometry) is widely valid and reliable, which guarantees the quality of the data **(Study V)**.

Similarly, in **Section III**, sleep quality, mental health and HRQoL were assessed during the second and the third trimester of pregnancy which provides a more comprehensive understanding of mental health along the pregnancy course **(Studies VI-VIII)**. Moreover, **Study IX** included women from the follow-up of a birth cohort, allowing us to account for the effect of exposures during pregnancy and early life measured prospectively within the cohort. This may be also considered in **Section IV (Studies X and XI)**.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

11.1. Conclusions

11.1.1. General conclusion

The findings of the present International Doctoral Thesis provide greater insight into the role of MD adherence during pregnancy with maternal and neonatal health-related outcomes. We first highlight which MD indices are more appropriate to assess MD adherence during gestation, along with the factors that are associated with a greater MD adherence during pregnancy. Subsequently, we provide evidence about the role of the MD adherence and its components to confer a cardioprotective effect on maternal metabolism. Furthermore, we show the beneficial role of MD adherence during pregnancy on maternal sleep quality and mental health. Finally, we evidence the role of exercise, together with an optimal MD adherence on materno-fetal health outcomes (i.e., postpartum body composition, postpartum depression and placental telomere length).

11.1.2. Specific conclusions

Section I. Mediterranean diet assessment during gestation and its relationship with sociodemographic, lifestyle and pregnancy-related determinants.

- **Specific conclusion I:** The MedDietScore and the MFP could be recommended to assess MD adherence during pregnancy. The cut-off points proposed to detect high cardiometabolic risk were 30 for the MedDietScore and 21 for the MFP throughout gestation **(Study I)**.
- **Specific conclusion II:** Older age, lower BMI, greater overall physical fitness, greater cardiorespiratory fitness, muscle strength, and elements of a healthy lifestyle such as avoiding tobacco and meeting physical activity recommendations were associated with higher MD adherence **(Study II)**.

Section II. Influence of Mediterranean diet during pregnancy on materno-fetal cardiometabolic health.

- **Specific conclusion III:** A greater MD adherence during pregnancy, which seems to be driven by a higher intake of fish, whole grain cereals, fruits, vegetables, and a lower intake of refined cereals, sweets and red meat, was associated with better maternal lipid serum markers and lower cardiometabolic risk throughout gestation **(Study III and IV)**. Notwithstanding, a greater MD adherence during pregnancy was not associated with cord arterial and venous serum markers **(Study III).**

- **Specific conclusion IV:** At postpartum, those women exercising and following an optimal MD adherence during pregnancy presented greater lean mass, lower percentage of fat mass, lower android fat mass and lower android-to-gynecoid fat mass compared to women exercising with a low MD adherence **(Study V)**.

Section III. Influence of Mediterranean diet during pregnancy on maternal sleep and mental health.

- **Specific conclusion V:** A greater MD adherence, driven by higher consumption of fruits, and olive oil and a lower intake of red meat and subproducts was associated with better sleep quality along gestation **(Study VI)**.
- **Specific conclusion VI:** A greater MD adherence during gestation was associated with lower negative affect, anxiety, and depression; and with greater emotional regulation, resilience, positive affect and HRQoL during pregnancy and postpartum depression. These associations seemed to be driven by a higher intake of whole grain cereals, fruits, vegetables, fish, olive oil and nuts, and a lower intake of red and processed meat and sweets **(Studies VII-IX)**.
- **Specific conclusion VII:** The interaction between exercise and MD could lead to lower risk for postpartum depression, yet this should further test in larger randomized controlled trials **(Study IX)**.

Section IV. Influence of Mediterranean diet during pregnancy on materno-fetal genetics.

- **Specific conclusion VIII:** Our results indicate that pregnant women in the exercise group showed a smaller GWG than those in the control group regardless of their genotype. Notwithstanding, women homozygous for the non-risk allele of *FTO* rs9939609 showed a lower GWG compared with risk-allele carriers **(Study X)**.
- **Specific conclusion IX:** Our results showed that a concurrent-exercise training combined with an optimal MD adherence during pregnancy might be a useful strategy to promote longer placental telomere length **(Study XI)**.

11.2. Future perspectives

Despite the great progress observed during the last years in some of the topics examined and discussed in the current International Doctoral Thesis, there are still many related questions that remain incompletely understood. Future research should aim:

- To investigate how MD adherence influence maternal and fetal immunometabolism, confirming the associations found in this Thesis and determine the underlying mechanisms.

- To standardize procedures for MD assessment during pregnancy to avoid methodological discrepancies between studies. Additionally, to validate cut-offs points to assess the degree of MD adherence in pregnancy should be also a priority for incoming studies.

- To study the effectiveness of MD in other contexts and study populations.

- To explore whether MD adherence during pregnancy from early stages in pregnancy, and if possible, from pre-conception influence sleep quality, mental health, HRQoL by diet interventions in larger well-designed randomized controlled trials.

- To explore the impact of exercise plus and optimal MD adherence during pregnancy and prior to pregnancy, if possible, on postpartum body composition, postpartum depression and placental telomere length in larger well-designed randomized controlled trials. This will help us to understand if dietary 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.

- To study the feasibility of intervening on MD adherence during gestation. We found observational evidence on the possible effects of MD. However, it is still needed to test it in dietary intervention studies.

- 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 biologically characterize pre-term (e.g., abortion or elective deliveries) and in- term labor phenotypes. 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 (diet and exercise) modulates placental phenotype from early pregnancy.

- To understand the clinical relevance of changes in placental telomere length for intrauterine programming and fetal development.

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ANNEXES

13. ANNEXES

13.1. Papers derived from the Doctoral Thesis

13.1.1. Published and included in this Doctoral Thesis

1. **Flor-Alemany M,** Nestares T, Marín-Jiménez N, Baena-García L, Aparicio VA. Associations between Sociodemographic Factors, Lifestyle Behaviors, Pregnancy-Related Determinants, and Mediterranean diet Adherence among Pregnant Women: The GESTAFIT Project. Nutrients. 2022;14(7):1348.

2. **Flor-Alemany M**, Acosta-Manzano P, Migueles JH, Baena-García L, Aranda P, Aparicio VA. Association of Mediterranean diet adherence during pregnancy with maternal and neonatal lipid, glycemic and inflammatory markers. The GESTAFIT project. Maternal and Child Nutrition. 2022, e13454.

3. **Flor-Alemany M,** Acosta P, Marín-Jiménez N, Baena-García L, Aranda P, Aparicio VA. Influence of the degree of adherence to the Mediterranean diet and its components on cardiometabolic risk during pregnancy. The GESTAFIT project. Nutr Metab Cardiovasc Dis. 2021;31(8), 2311–2318.

4. **Flor-Alemany M**, Nestares T, Alemany-Arrebola I, Marín-Jiménez N, Borges-Cosic M, Aparicio VA. Influence of Dietary Habits and Mediterranean Diet Adherence on Sleep Quality during Pregnancy. The GESTAFIT Project. Nutrients. 2020;12:3569.

5. **Flor-Alemany M,** Baena-García L, Migueles JH, Henriksson, P, Löf M, Aparicio VA. Associations of Mediterranean diet with psychological ill-being and well-being throughout the pregnancy course: The GESTAFIT project. Qual Life Res. 2022;31(9):2705-2716.

6. **Flor-Alemany M**, Migueles JH, Alemany-Arrebola I, Baena-García L, Aparicio VA. Exercise, Mediterranean Diet Adherence or Both during Pregnancy to Prevent Postpartum Depression—GESTAFIT Trial Secondary Analyses. International Journal of Environmental Research and Public Health. 19(21), 14450.

7. **Flor Alemany M**, Migueles JH, Acosta-Manzano P, Marin Jimenez N, Baena Garcia L, Aparicio VA. Assessing the Mediterranean diet adherence during pregnancy: practical considerations based on the associations with cardiometabolic risk. Pregnancy Hypertension. 2023;31:17-24.

13.1.2. Submitted and included in this Doctoral Thesis

8. **Flor-Alemany M**, Acosta-Manzano P, Migueles JH, Henriksson P, Löf M, Aparicio VA. Influence of an exercise intervention plus an optimal Mediterranean diet adherence during pregnancy on postpartum body composition. The GESTAFIT Project. *Submitted to Food & Function.*

9. **Flor-Alemany M**, Migueles JH, Marín-Jiménez N, Baena-García L, Löf M, Aparicio VA. A greater Mediterranean diet adherence is associated with better health-related quality of life during pregnancy. The GESTAFIT project. *Submitted to Women's Health.*

10. **Flor-Alemany M**, Migueles JH, Martínez González LJ, Álvarez Cubero MJ, Alcántara-Domínguez C, Aparicio VA. Are the effects of exercise on gestational weight gain moderated by the FTO gene polymorphism rs9939609? The GESTAFIT project. *Submitted to Acta Obstetricia et Gynecologica Scandinavica.*

11. **Flor-Alemany M**, Acosta-Manzano P, Migueles JH, Varela A, Baena-García L, Quiles JL, Aparicio VA. Influence of an exercise intervention plus an optimal Mediterranean diet adherence during pregnancy on the telomere length of the placenta. The GESTAFIT project. *Submitted to Placenta*

13.2. Curriculum Vitae

MARTA DE LA FLOR ALEMANY

CV AT A GLANCE

Born | 12/04/1994 (28 years old)

e-mail | floralemany@ugr.es

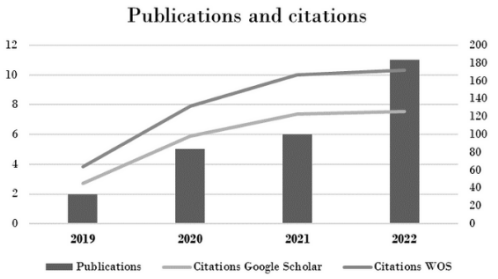
RESEARCH

ROLE IN PROJECTS

GESTAFIT Project:
- Data collection
- Lab analyses
- Dietary data processing
- Databases
- Data analyses
- Manuscript writing

GESTAFITOS Project:
- Management and organization
- Data collection
- Dietary data processing
- Databases
- Data analyses
- Manuscript writing

Additionally, I have been in charge of the dietary data processing of 2 national projects



INTERNATIONAL STAYS AND GRANTS

2016/2017
Initiation to research grant
Department of Biochemistry and Molecular Biology II.
Faculty of Pharmacy.
University of Granada

2017/2018
Collaboration research grant
Department of Physiology
University of Granada

Master's degree in Human Nutrition
Master's degree in food, exercise and sports for health

2018/2019
31/10/2018
Start PhD Studies
Funding: Spanish Ministry of Education, Culture, and Sports

2020/2021
02/02-02/05
Research
Linköping University
(Sweden)
Pontus Henriksson

2021/2022
07/09-06/12
Research
Karolinska Intitute
(Sweden)
Marie Löf

10/05-08/08
Research
Karolinska Intitute
(Sweden)
Jairo H. Migueles

OUTREACH

- **Best score in Bachelor's degree in Human Nutrition**
- **Twenty five communications in national and international conferences**
- **Awarded as best poster communication at the Simposium VI Simposium EXERNET. "Exercise is Medicine"**

TEACHING

I have had the opportunity to teach in the Faculty of Pharmacy in the University of Granada. I have been involved in:

- ***Cellular and Human Physiology I***
- ***Cellular and Human Physiology II***
- ***Human Physiology***
- ***Clinical Physiology and Biochemistry***
- ***Physiopathology***
- ***Functional Tests: Applications for Nutrition***

13.2.1. Personal Data

Name: **DE LA FLOR ALEMANY, MARTA**
Email: floralemany@ugr.es
Birth Date: 12/04/1994 (age: 28)
Birthplace: Melilla
Nationality: Spanish
ORCID/Research ID: 0000-0001-8256-5053

13.2.2. Current affiliations

PhD candidate at the Doctoral program in Nutrition and Food Science of University of Granada, Spain.

- Department of Physiology. University of Granada, Spain.
- Institute of Nutrition and Food Technology, University of Granada, Spain.
- Sport and Health University Research Institute (iMUDS), Granada, Spain University of Granada, Spain
- Funded by the Spanish Ministry of Education, Culture, and Sports (Grant number FPU17/03715)
- Supervisor: Dr. Virginia A. Aparicio García-Molina **31/10/2018 - Present**

13.3.3. Research experience

- **Researcher** **01/12/2018-31/12/2019**
  Project Title & Design: The influence of sedentary time and physical activity intensity levels on inflammatory profile 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** **01/01/2017-31/12/2019**
  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)
- **Project Manager** **01/01/2020-30/06/2022**
  Project Title & Design: Effects of a supervised physical exercise intervention during pregnancy on body composition, physical fitness and motor, cognitive and language development of the offspring.
  Principal Investigator: Virginia A Aparicio. Department of Physical Education and Sport, University of Granada, Spain.
  Funding: Ministry of economy innovation and science (B-CTS-162-UGR18 €6400).

13.3.4. Education and training

- **PhD candidate** **2018-2023**
  Doctoral Programme in Nutrition and Food Sciences of the University of Granada.

Thesis title: Influence of dietary habits and Mediterranean diet adherence during gestation on several maternal and neonatal biochemical, genetic, psychosocial and anthropometric markers.
Supervisor: Dr. Virginia A. Aparicio García Molina. Department of Physiology, University of Granada, Spain

- **Master´s degree** **2017-2018**
  Master's degree in Human Nutrition (Grade 9.79/10), University of Granada, Spain.
- **Master's degree** **2017-2018**
  Master's degree in food, exercise and sports for health. University of Granada, Spain (Food & fit).
- **Bachelor's degree** **2013-2017**
  Bachelor's degree in Human Nutrition (Grade 9.413/10), University of Granada, Spain. 2013-2017. Best score in Bachelor's degree in Human Nutrition.

13.3.5. Research internships

- **Research internship** **02/02/2021-02/05/2021**
  Department of Medical and Health Sciences, Faculty of Medicine. Linköping University (Sweden) under the supervision of Pontus Henriksson.
- **Research internship** **07/09/2021-06/12/2021**
  Department of Biosciences and Nutrition. Karolinska Institutet (Sweden) under the supervision of Marie Löf.
- **Research internship** **10/05/2022-08/08/2022**
  Department of Biosciences and Nutrition. Karolinska Institutet (Sweden) under the supervision of Jairo Hidalgo Migueles.

13.3.6. Funding, grants and personal awards

- **Grant** **2018-2023**
  Spanish Ministry of Education, Culture, and Sports (4 years and 5 months, Grant number FPU17/03715).
- **Grant** **02/02/2021-02/05/2021**
  Personal grant: 3-months research internship Department of Medical and Health Sciences, Faculty of Medicine. Linköping University (Sweden). University of Granada (€2160).
- **Grant** **07/09/2021-06/12/2021**
  Personal grant: 3-months research internship Department of Biosciences and Nutrition. Karolinska Institutet (Sweden). Spanish Ministry of Education, Culture, and Sports (€4100).
- **Grant** **10/05/2022-08/02/2022**
  Personal grant: 3-months research internship Department of Biosciences and Nutrition. Karolinska Institutet (Sweden). Spanish Ministry of Education, Culture, and Sports (€4100).
- **Grant** **2017-2018**
  Collaboration research grant (partial time). Department of Physiology. Faculty of Pharmacy. University of Granada (8 months, €2000).

- **Grant** **01/09/2016-30/06/2017**
  Initiation to research grant (partial time). Department of Biochemistry and Molecular Biology II. Faculty of Pharmacy. University of Granada (€500).

13.3.7. Publication list

Published and included in this International Doctoral Thesis

- **Flor-Alemany M,** Nestares T, Marín-Jiménez N, Baena-García L, Aparicio VA. Associations between Sociodemographic Factors, Lifestyle Behaviors, Pregnancy-Related Determinants, and Mediterranean diet Adherence among Pregnant Women: The GESTAFIT Project. Nutrients. 2022;14(7):1348.

- **Flor-Alemany M**, Acosta-Manzano P, Migueles JH, Baena-García L, Aranda P, Aparicio VA. Association of Mediterranean diet adherence during pregnancy with maternal and neonatal lipid, glycemic and inflammatory markers. The GESTAFIT project. *Accepted in Maternal and Child Nutrition.*

- **Flor-Alemany M,** Acosta P, Marín-Jiménez N, Baena-García L, Aranda P, Aparicio VA. Influence of the degree of adherence to the Mediterranean diet and its components on cardiometabolic risk during pregnancy. The GESTAFIT project. Nutr Metab Cardiovasc Dis. 2021;31(8), 2311–2318.

- **Flor-Alemany M**, Nestares T, Alemany-Arrebola I, Marín-Jiménez N, Aparicio VA. Influence of Dietary Habits and Mediterranean Diet Adherence on Sleep Quality during Pregnancy. The GESTAFIT Project. Nutrients. 2020;12:3569.

- **Flor-Alemany M,** Baena-García L, Migueles JH, Henriksson, P, Löf M, Aparicio VA. Associations of Mediterranean diet with psychological ill-being and well-being throughout the pregnancy course: The GESTAFIT project. Qual Life Res. 2022;31(9):2705-2716.

- **Flor-Alemany M**, Migueles JH, Alemany-Arrebola I, Baena-García L, Aparicio VA. Exercise, Mediterranean Diet Adherence or Both during Pregnancy to Prevent Postpartum Depression—GESTAFIT Trial Secondary Analyses. International Journal of Environmental Research and Public Health. 19(21), 14450.

Other journal publications as first author not included in this International Doctoral Thesis

- **Flor-Alemany M**, Marín-Jiménez N, Nestares T, Borges-Cosic M, Aranda P, Aparicio, VA. Mediterranean diet, tobacco consumption and body composition during perimenopause. The FLAMENCO project. Maturitas. 2020;137:30–36.

- **Flor-Alemany M**, Marín-Jiménez N, Coll-Risco I, Aranda P, Aparicio VA. Influence of dietary habits and Mediterranean diet adherence on menopausal symptoms. The FLAMENCO project. Menopause. 2020;27(9):1015-1021.

Co-authored journal publications

- Marín-Jiménez N, Borges-Cosic M, Ocón-Hernández O, Coll-Risco I, **Flor-Alemany M**, Baena-García L, Castro-Piñero J, Aparicio VA. Association of Self-Reported Physical Fitness with Pregnancy Related Symptoms the GESTAFIT Project. Int J Environ Res Public Health. 2021;18(7):3345.

- Nestares T, Martín-Masot R, **Flor-Alemany M**, Bonavita A, Maldonado J, Aparicio VA. Influence of Ultra-Processed Foods Consumption on Redox Status and Inflammatory Signaling in Young Celiac Patients. Nutrients. 2021;13(1):156.

- Baena-García L, Marín-Jiménez N, Romero-Gallardo L, Borges-Cosic M, Ocón-Hernández O, **Flor-Alemany M,** Aparicio VA. Association of Self-Reported Physical Fitness during Late Pregnancy with Birth Outcomes and Oxytocin Administration during Labour-The GESTAFIT Project. Int J Environ Res Public Health. 2021;18(15):8201.

- Aparicio VA, **Flor-Alemany M**, Marín-Jiménez N, Coll-Risco I, Aranda P. A 16-week concurrent exercise program improves emotional well-being and emotional distress in middle-aged women: the FLAMENCO project randomized controlled trial. Menopause. 2021;28(7):764-771.

- Nestares T, Martín-Masot R, de Teresa C, Bonillo R, Maldonado J, **Flor-Alemany M**, Aparicio VA. Influence of Mediterranean diet Adherence and Physical Activity on Bone Health in Celiac Children on a Gluten-Free Diet. Nutrients. 2021;13(5):1636.

- Nestares T, Martín-Masot R, Labella A, Aparicio VA, **Flor-Alemany M**, López-Frías M, Maldonado J. Is a Gluten-Free Diet Enough to Maintain Correct Micronutrients Status in Young Patients with Celiac Disease?. 2020;12(3):844.

- Marín-Jiménez N, Ruiz-Montero PJ, **De la Flor-Alemany M**, Aranda P, Aparicio VA. Association of objectively measured sedentary behavior and physical activity levels with health-related quality of life in middle-aged women: The FLAMENCO project. Menopause. 2020;27(4):437-443.

- Aparicio VA, Marín-Jiménez N, Coll-Risco I, **de la Flor-Alemany M**, Baena-García L, Acosta-Manzano P, Aranda P. Doctor, ask your perimenopausal patient about her physical fitness; association of self-reported physical fitness with cardiometabolic and mental health in perimenopausal women: the FLAMENCO project. Menopause. 2019;26(10):1146-1153.

- Baena-García L, Ocón-Hernández 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. 2019;29(3):407-414.

- Marín-Jiménez N, **Flor-Alemany M**, Baena-García L, Coll-Risco I, Castro-Piñero J, Aparicio VA. Physical fitness and maternal body composition indices during pregnancy and postpartum: the GESTAFIT project. Eur J Sport Sci. 2022;1-11.

- Acosta-Manzano P, Acosta FM, **Flor-Alemany M**, Gavilán-Carrera B, Delgado-Fernández M, Baena-García L, Segura-Jiménez V, Aparicio VA. The Protective Role of Physical Fitness on Cardiometabolic Risk During Pregnancy: The GESTAtion and FITness Project. Int J Sport Nutr Exerc Metab. 2022;32(3):163-176.

- Rubini A, Vilaplana-Prieto C, **Flor-Alemany M**, Yeguas-Rosa L, Hernández-González M, Félix-García FJ, Félix-Redondo FJ, Fernández-Bergés D. Assessment of the Cost of the Mediterranean diet in a Low-Income Region: Adherence and Relationship with Available Incomes. BMC public health. 2022; 22(1):58.

- Aparicio VA, Baena-García L, **Flor-Alemany M**, Martínez-González LJ, Varela-López A, Sánchez C, Quiles JL. Differences in maternal and neonatal cardiometabolic markers and placenta status by foetal sex. The GESTAFIT project. Womens Health (Lond). 2022;18:17455057221117976.

- Baena-García L, **Flor-Alemany M**, Marín-Jiménez N, Aranda P, Aparicio VA. A 16-week multicomponent exercise training program improves menopause-related symptoms in middle-aged women. The FLAMENCO project randomized control trial. Menopause. 2022;29(5):537-544.

- Acosta-Manzano P, Acosta FM, Coll-Risco I, Romero-Gallardo L, **Flor-Alemany M**, Martínez-González LJ, Alvarez-Cubero MJ, Segura-Jiménez V, Aparicio VA. The Influence of Exercise, Lifestyle Behavior Components, and Physical Fitness on Maternal Weight Gain, Postpartum Weight Retention, and Excessive Gestational Weight Gain. Int J Sport Nutr Exerc Metab. 2022;1-14.

- Coll-Risco I, **de la Flor Alemany M**, Acosta-Manzano P, Borges-Cosic M, Camiletti-Moirón D, Baena-García L, Aparicio VA. The influence of an exercise program in middle-aged women on dietary habits. The FLAMENCO project. Menopause. 2022. Online ahead of print.

13.3.8. Books and book chapter

- Lidia Romero Gallardo, Irene Coll Risco, Olga Ocón Hernández, Milkana Borges Cosic, Pedro Acosta Manzano, **Marta de la Flor Alemany**, Laura Baena García, Virginia A. Aparicio García-Molina. Entrenamiento durante el embarazo. Guía ilustrada y desarrollada para profesionales.

- Teresa Nestares, Rafael Martín-Masot, **Marta Flor-Alemany**, Antonela Bonavita, Jose´ Maldonado and Virginia A. Aparicio. Nutritional Deficiency in Celiac Disease Current Perspective.

13.3.9. Distinguished communications in conferences (total = 25)

- **III Conference of Researchers of the Faculty of Pharmacy 17/11/2022-18/11/2022**
  Oral communication: Inflammatory potential of the diet on inflammatory and cardiometabolic markers and cardiometabolic risk during pregnancy. The GESTAFIT project.

- **V International JIFFI congress 22/06/2022-24/06/2022**
  Poster presentation: Association of Mediterranean diet adherence during pregnancy with body composition of the offspring at 4 years of age. The GESTAFITOS project.

- **II Research Congress of the PTS Granada 09/02/2022-11/02/2022**
  Poster presentation: Influence of dietary habits and Mediterranean diet adherence on physical fitness during pregnancy.

- **IV International JIFFI congress 26/06/2019-28/06/2019**
  Poster presentation: Association of dietary habits and Mediterranean diet adherence during pregnancy with materno-fetal inflammatory profile. The GESTAFIT project.

- **VI Simposium EXERNET. "Exercise is Medicine" 19/10/2018-20/10/2018**
  Association between physical activity levels and the adherence to Mediterranean diet during early pregnancy. Findings from the GESTAFIT Project.
  Awarded as best poster communication at the Simposium.

13.3.10. Organization of international conferences

- **Organizing committee 22/06/2022-24/06/2022**
  IV International JIFFI congress

- **Organizing committee 24/11/2021-25/11/2021**
  Jornadas de Investigadores en Formación: Fomentando la interdisciplinariedad (JIFFI) – Special Issue II

13.3.11. Teaching and supervising activities

Teaching

- **Teaching 2021/2022**
  Cellular and Human Physiology II. Bachelor's Degree in Human Nutrition and Dietetics (15 hours)
  Clinical Physiology and Biochemistry. Bachelor's Degree in Pharmacy (15 hours)
- **Teaching 2020/2021**
  Cellular and Human Physiology I. Bachelor's Degree in Pharmacy (5 hours)
  Human Physiology. Bachelor's Degree in Human Nutrition and Dietetics (10 hours)
  Functional Tests: Applications for Nutrition. Bachelor's Degree in Human Nutrition and Dietetics (15 hours)
- **Teaching 2019/2020**

Human Physiology. Bachelor's Degree in Sport and Exercise Sciences (15 hours)
Clinical Physiology and Biochemistry. Bachelor's Degree in Pharmacy (22.5 hours)
Physiopathology. Bachelor's Degree in Pharmacy (5.5 hours)
Physiopathology. Bachelor's Degree in Human Nutrition and Dietetics (17 hours)

- **Teaching** **2018/2019**
  Cellular and Human Physiology. Bachelor's Degree in Sport and Exercise Sciences (45 hours)
  Physiopathology. Bachelor's Degree in Human Nutrition and Dietetics (15 hours)

Supervising activities

- **Professional tutor** **2021/2022**
  Professional tutor of external practices. Certificate of Higher education in Nutrition and Dietetics, University of Granada, Spain (300 hours)
- **Mentor** **2021/2022**
  Mentor of Master's Program. Master in Human Nutrition, University of Granada, Spain.
- **Mentor** **2021/2022**
  Mentor of Degree's Program. Degree in Human Nutrition and Dietetics, University of Granada, Spain.
- **Mentor** **2020/2021**
  Mentor of Master's Program. Master in Human Nutrition, University of Granada, Spain.
- **Professional tutor** **2020/2021**
  Professional tutor of external practices. Certificate of Higher education in Nutrition and Dietetics, University of Granada, Spain (300 hours)
- **Professional tutor** **2018/2019**
  Professional tutor of external practices. Certificate of Higher education in Nutrition and Dietetics, University of Granada, Spain (300 hours)
- **Professional tutor** **2017/2018**
  Professional tutor of external practices. Certificate of Higher education in Nutrition and Dietetics, University of Granada, Spain (380 hours)

13.10.12. Courses attended (Transferable skills)

- **Course** **18/01/2021-22/01/2021**
  PhD course: Techniques for Risk Assessment of Exposure to Endocrine Disrupters
- **Course** **03/06/2020**
  PhD course: Science popularisation as a communication communication tool and professional alternative for doctoral students
- **Course** **22/05/2020**
  PhD course: How do I manage my Google Scholar profile?
- **Course** **13/04/2020**
  PhD course: The triforce of Open Science
- **Course** **25/03/2020**
  PhD course: Practical workshop for the preparation of the Juan de la Cierva, Marie Curie and Ramón & Cajal postdoctoral calls for proposals

- **Course** **25/03/2020**
  PhD course: E-learning and emerging pedagogies emerging pedagogies for a confined university.
- **Course** **24/03/2020**
  PhD course: How to select the right a journal to publish my scientific article?
- **Course** **12/03/2020**
  PhD course: Preparation and preparation and elaboration of research projects
- **Course** **14/05/2019-23/05/2019**
  PhD course: Scientific writing: How to write a paper
- **Course** **15/01/2019-24/01/2019**
  PhD course: Statistical techniques applied in the field of nutrition and health
- **Course** **22/11/2018-23/11/2018**
  III Conference of initiation to university teaching for pre-doctoral FPU and FPI contracts organised by the Quality, Innovation and Foresight Unit of the University of Granada.
- **Course** **05/03/2018-11/05/2018**
  Basis statistics with SPSS (XII Edition)

13.10.13. Outreach activities

- **European Researchers' Night 2019** **27/09/2019-28/09/2019**
- **European Researchers' Night 2022** **30/09/2022**