UNIVERSIDAD DE GRANADA

Facultad de Medicina

Departamento de Pediatría

EFFECT OF MATERNAL OBESITY AND GESTATIONAL DIABETES ON PLACENTAL FATTY ACID UPTAKE, METABOLISM AND TRANSFER TO THE FETUS

Efecto de la obesidad y diabetes gestacional maternas sobre la captación, metabolismo y transferencia transplacentaria de ácidos grasos

María Teresa Segura Moreno

Programa de Doctorado en Biomedicina

Granada, 2018

Editor: Universidad de Granada. Tesis Doctorales
Autor: María Teresa Segura Moreno
ISBN: 978-84-1306-149-8
URI: http://hdl.handle.net/10481/55425

Dissertation submitted by

María Teresa Segura Moreno

to obtain the Doctoral Degree in Biology from

The University of Granada with the "International Mention"

This Doctoral Thesis was directed by

Prof. Dra. Cristina Campoy Folgoso

Department of Pediatrics, School of Medicine

University of Granada

La doctoranda **María Teresa Segura Moreno** y la directora de la Tesis **Cristina Campoy Folgoso** garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por la doctoranda bajo la supervisión de la directora de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

We guarantee, by signing this doctoral thesis, that the work has been done by the doctoral candidate under the direction of the thesis supervisor and, as far as our knowledge reaches, in the performance of the work, the rights of other authors to be cited (when their results or publications have been used) have been respected.

Lugar y fecha/ Place and date:

Directora de la tesis / Thesis Supervisor:

Doctoranda / Doctoral candidate:

Firma / Signed

Firma / Signed

"Como todo el mundo, Darwin se equivocaba en ocasiones. Pero los fallos en los datos no le duelen tanto a un científico como los errores en las hipótesis, que son los hijos más queridos, el resultado no de la experiencia y de la práctica profesional (un producto de la edad, sin más), sino de la capacidad para el razonamiento abstracto, de la creatividad. El talento se expresa en las teorías, y son éstas las que hacen que alguien pase a la historia y sea recordado en el futuro como un gran descubridor… o ridiculizado para siempre como un gran tonto. Así de claro. En las grandes ideas es donde el aspirante a genio se la juega… si es que decide intentarlo. Muchos son tan prudentes que prefieren apuntarse, a posteriori, al consabido ya lo decía yo."

Juan Luis Arsuaga

El reloj de Mr. Darwin

FUNDING

The results presented in this Doctoral Thesis belongs to the Excellence Project P06-CTS-02341 (PREOBE) entitled: *"Role of mother's nutrition and genetics on the programming and development of foetal adipose tissue. Searching for early risk biomarkers to develop obesity"*, supported by Spanish Government, Innovation, Science and Company Ministry (Andalusian Government), and coordinated by Prof. Cristina Campoy. Additionally, this work has been financially supported in part by Abbott Laboratories (Granada, Spain) and by the Commission of the European Communities, within the 7th Framework Programme, Theme 2, Food, Agriculture and Fisheries, and Biotechnology, Early Nutrition Project (Grant Agreement "FP7-289346-EARLY NUTRITION").

María Teresa Segura Moreno performed a twenty-eight moths international predoctoral stay in Munich supported by a TALENTIA Scholarship from the Regional Ministry of Economy, Innovation, and Science of Andalusia, Spain (24 months), and of a scholarship from the Child Health Foundation (Stiftung Kindergesundheit), Munich, Germany (4 months). As consequence, part of this work was partially performed in two German research centers: The Division of Metabolic and Nutritional Medicine of the Dr. von Hauner Children´s Hospital (Ludwig-Maximilians-University of Munich) under the supervision of Prof. Berthold Koletzko and Dr. Hans Demmelmair and in The Comprehensive Pneumology Center of the Ludwig-Maximilians-University, and Helmholtz Center of Munich, under the supervision of Prof. Susanne Krauss-Etschmann.

AGRADECIMIENTOS

No es hasta el momento de finalizar la memoria de la tesis que una se da cuenta de la gran importancia que tiene este apartado, y es que al echar la vista atrás voy siendo consciente de que sin la colaboración, apoyo y cariño de tantas personas nunca podría haber realizado este trabajo. Y es que como dice el dicho popular "Solo llegarás más rápido, pero acompañado llegarás más lejos".

En primer lugar, quiero agradecer a la Profesora Cristina Campoy, supervisora de este trabajo y directora del "Centro de Excelencia en Investigación Pediátrica EURISTIKOS", por darme la primera oportunidad en el mundo de la investigación y por mantener la confianza en mi trabajo y criterio durante estos once años, por tu alegría y entusiasmo día tras día, porque hemos sido capaces de encontrar un equilibrio entre tu optimismo sin límites y mi realismo, fruto del cual es esta tesis.

A los Profesores Berthold Koletzko y Susanne Krauss-Etschmann, por acogerme en sus centros y hacerme sentir uno más del equipo. Por la supervisión de la publicación asociada a esta tesis. Al Dr. Hans Demmelmair, por las tardes de discusión científica que, aunque al principio las "odiaba" hoy las echo de menos, por empujarme a pensar y ser critica en la lectura. A todos los compañeros que conocí durante mi estancia en Munich, a aquellos que dedicaron parte de su tiempo a enseñarme en el laboratorio y a aquellos que me enseñaron que una sonrisa es suficiente para entenderse a pesar de las diferencias de idiomas. Esta estancia tuvo un gran impacto profesional, pero sobre todo personal, ayudándome a salir de mi zona de confort, a asumir mis limitaciones, y conocer mis virtudes.

A todos los miembros del Centro EURISTIKOS, muchos a lo largo de estos años. A los primeros que conocí a mi llegada y con los que compartí trabajo y buenos ratos hasta que me fui a Munich, por enseñarme la importancia del trabajo en equipo dentro y fuera del departamento, porque a pesar de ser una de las épocas más duras, a nivel de trabajo de campo, recuerdo esa etapa con mucho cariño, José Antonio, Elena, Miguel, Tania, Jole, Juan Carlos, Helena, Mº José, Iryna. A aquellos con los que me reencontré a mi vuelta y los que se han ido incorporando en los últimos años Luz, Paco, Cristina, Rosario, Pilar, Dani, Miriam, Signe, Mª Carmen, Staffan, Ana, Liliana, Tomás, Estefanía Parejo, Mireia, María, Erika, Clara, Mercedes, Concha, Elena, Estefanía Diéguez, Flo, Hatim, Natalia, Raquel, Santos y Facundo. Gracias a todos por los ratos

de revisiones, las risas nerviosas ante los plazos imposibles, y las celebraciones. ¡Que nos gusta una tarta! Especialmente a aquellos que se han tomado tiempo fuera del trabajo para conocerme, entenderme y quererme. Elena, Tania, Jole, Estefanía P, María "a secas", Ana, Mireia, Mercedes y Santos gracias por cruzar la línea que separa el compañerismo de la amistad.

Gracias a Luz, Tania, Juan Carlos, Cristina, Jole, Mº José, Iryna, Rosario y Pilar por la implicación directa en la recogida de muestras y datos incluidos en esta memoria de tesis. A Paco y Miriam por continuar con la complicada tarea del seguimiento de los niños del PREOBE.

A los obstetras y enfermeras que participaron en la colección de los datos ecográficos.

A todas las madres que participaron desinteresadamente en el proyecto PREOBE, contribuyendo con sus muestras y datos, pero sobre todo con su tiempo y confianza.

Por último, a mi familia y amigos que, aunque siguen sin tener claro a que me dedico, entienden y respetan mis ausencias y mi mal humor. **A mi padre**, mi primer y mayor ejemplo de trabajo y sacrificio, por tu capacidad de superación constante, por aconsejarme dándome la libertad de elegir mis batallas y apoyarme cuando me equivoco, por traerme hasta aquí. A mi **madre**, que desde su trocito de cielo sé que está orgullosa, por darme uno de los mejores consejos que he recibido y que poco cumplo "Aprovecha el tiempo". A mis hermanas, **Vero y Charo**, por ser las únicas personas capaces de sacarme de mis casillas y carcajadas, a veces las dos cosas a la vez, por vuestra energía, por no dejarme caer nunca. A **Paco**, por cuidar a mi hermana y mis sobrinas, por aguantar las invasiones de las hermanas Segura. A mis sobrinas, **Lucía y Alejandra**, por vuestro amor incondicional y alegría sin límites, por vuestra generosidad y porque por fin habéis aprendido que soy bióloga y trabajo en algo de análisis de la sangre. A **Santos**, por tu apoyo dentro y fuera del grupo EURISTIKOS, por tus consejos científicos, pero sobre todo por tu paciencia y cariño, por ver más allá de lo que muestro. A mis niñicas, Susana, Patri, Tere, Raquel, Ana, Vicky, Tamara, repartidas por tantas zonas geográficas, porque a pesar de las llamadas que se espacian en el tiempo, siempre estáis ahí. A mis cuñaooss, Laura y Noé, porque aun teniéndoos cerca os veo menos de lo que me gustaría.

¡Gracias a todos los que directa e indirectamente habéis contribuido a este trabajo!

¡Gracias a todos los que en algún momento os habéis interesado por la tesis! Y es que por fin puedo responder: ¿La tesis? ¡Acabada! **¡¡GRACIAS!!**

TABLE OF CONTENTS

RESUMEN

La prevalencia de obesidad se ha triplicado en los últimos treinta años, alcanzando la magnitud de epidemia. Los datos más recientes aportados por la Organización Mundial de la Salud son alarmantes. Aproximadamente el 40% y el 15 % de mujeres mayores de 18 años tienen sobrepeso y obesidad, respectivamente. Especialmente preocupantes son los datos en población infantil y adolescente. Desde 1975 hasta 2016 la prevalencia de sobrepeso y obesidad ha aumentado de un 4% a un 18%, siendo la prevalencia de obesidad en niños y adolescentes a nivel mundial de un 8% aproximadamente.

Los actuales patrones sociales han ido asociados a cambios en los hábitos alimentarios y de actividad física en la población. Entre los cambios en los patrones dietéticos cabe destacar el actual acceso a gran cantidad de alimentos procesados hipercalóricos, al bajo consumo de alimentos frescos, al aumento de las porciones y a horarios irregulares de comidas. Otros factores que han influido ampliamente en el aumento de esta epidemia han sido la industrialización, y los nuevos modelos de transporte y ocio. A pesar de todo esto, la obesidad debe considerarse como un complejo desorden multifactorial resultado de la interacción entre factores ambientales y genéticos.

Independientemente de la causa, la obesidad se ha convertido en un importante problema de salud pública a nivel mundial. El exceso de grasa corporal representa un factor de riesgo clave en la susceptibilidad a padecer enfermedades cardiovasculares, diabetes, enfermedades musculoesqueléticas y algunos tipos de cáncer.

Centrándonos en mujeres en edad reproductiva, el sobrepeso y la obesidad en el embarazo están asociados a un mayor riesgo de sufrir complicaciones obstétricas tales como aborto, diabetes gestacional, hipertensión, preeclampsia y cesárea. Pero la obesidad materna no solo afecta a la salud de la madre, los hijos de madres obesas presentan un mayor riesgo de macrosomía, defectos del tubo neural y enfermedades congénitas. Es más, el ambiente intrauterino asociado al fenotipo materno obeso aumenta el riesgo de complicaciones metabólicas futuras en la descendencia.

Barker *et al.* relacionaron por primera vez un ambiente desfavorable durante el embarazo con el origen de las enfermedades de la vida adulta, para lo que adoptaron el término de *"Early Programming"* o *"Programación Precoz"* de la salud y la enfermedad. Así, ciertas patologías metabólicas maternas, como la obesidad y la diabetes gestacional, están asociadas con un ambiente nutricional desfavorable.

Igualmente la malnutrición durante la gestación, tanto por déficit como por exceso de nutrientes, puede tener efectos a largo plazo sobre la salud de los hijos.

Se ha comprobado que los hijos nacidos de madres obesas o con diabetes gestacional presentan un mayor riesgo de desarrollar resistencia a la insulina, adiposidad, obesidad, diabetes tipo 2 y síndrome metabólico en la vida adulta.

Aunque los mecanismos implicados en la programación precoz de la salud y la enfermedad no están claros, la placenta podría jugar un papel importante en los efectos a largo plazo de las complicaciones maternas durante el embarazo. Las patologías maternas podrían comprometer la morfología y función placentarias alterando así su capacidad de transporte de nutrientes, y creando un ambiente intrauterino desfavorable para el feto en desarrollo.

A lo largo del embarazo el metabolismo materno va cambiando y adaptándose a las demandas nutricionales del feto. Estos cambios están mediados en gran medida por las hormonas maternas y placentarias. Uno de los mayores cambios que experimenta el cuerpo de la mujer embarazada es en el metabolismo lipídico. Durante el primer trimestre de gestación se produce un aumento de los niveles de estrógenos, progesterona e insulina, y aumenta la actividad de la enzima lipoproteína lipasa en el tejido adiposo materno, lo que promueve la acumulación lipídica en los tejidos maternos. A medida que el embarazo progresa el metabolismo va cambiando de la situación anabólica del comienzo a la catabólica del final. De esta forma se produce un aumento de la lipolisis y movilización de las grasas previamente depositadas en los tejidos maternos, y un aumento de la resistencia a la insulina; esto determina un aumento de las concentraciones plasmáticas de triglicéridos y colesterol en la embarazada. La hiperlipidemia observada en el tercer trimestre coincide con el periodo de mayor crecimiento fetal.

La resistencia a la insulina (adaptación fisiológica al final del embarazo) puede verse exacerbada en el caso de obesidad materna, ya que la obesidad *per se* conlleva una disminución a la sensibilidad a la insulina. En determinadas situaciones el páncreas materno es incapaz de sintetizar suficiente insulina para compensar dicha resistencia, desarrollándose diabetes gestacional, más frecuente en mujeres con obesidad pre-gestacional. Por este motivo, cabe esperar que la hiperlipidemia en embarazadas obesas sea mayor.

Los ácidos grasos juegan un papel clave en el mantenimiento de la salud y son fundamentales durante el desarrollo fetal. Las funciones de los ácidos grasos en el organismo se pueden clasificar en metabólicas, estructurales y reguladoras. Los ácidos grasos son incorporados como parte estructural en las membranas de las células plasmáticas, son precursores de moléculas reguladoras y proporcionan un sustrato para la producción de energía. Además, los ácidos grasos poliinsaturados de cadena larga, dada su composición química, son fundamentales para el desarrollo de las estructuras del sistema nervioso fetal.

El feto es capaz de sintetizar ácidos grasos saturados y monoinsaturados de cadena corta, mientras que para los ácidos grasos de cadena larga es totalmente dependiente del aporte materno a través de la placenta. La transferencia placentaria de ácidos grasos esenciales es un proceso complejo mediado por un gran número de proteínas transportadoras de membrana y de unión en el citosol, aunque los mecanismos exactos siguen siendo objeto de discusión.

La placenta tiene una capacidad de adaptación que protege al feto del exceso o déficit de nutrientes. En situaciones metabólicas particulares, como la obesidad y la diabetes gestacional, la duración del insulto intrauterino puede exceder la capacidad de adaptación de la placenta viéndose alterado el aporte de nutrientes hacia el feto, especialmente el de ácidos grasos, lo que podría comprometer el desarrollo correcto de las estructuras fetales. La deficiencia de ácidos grasos poliinsaturados de cadena larga, como el ácido docosahexaenoico y el ácido araquidónico, durante la gestación y los primeros meses de la vida, ha sido relacionada con defectos visuales y problemas cognitivos en los niños.

El objetivo principal de esta tesis doctoral es evaluar el efecto del sobrepeso materno pre-gestacional y la diabetes gestacional sobre el contenido de ácidos grasos en la placenta y la expresión de genes que codifican proteínas claves para la transferencia placentaria de ácidos grasos hacia el feto *(FATP: fatty acid transport protein, FABP: fatty acid binding protein, FAT/CD36: fatty acid translocase, EL: endothelial lipase and LPL: lipoprotein lipase).*

En el presente estudio se han analizado muestras de placentas recogidas durante el desarrollo del proyecto PREOBE. El proyecto PREOBE fue diseñado como un estudio observacional prospectivo en el que las mujeres incluidas fueron clasificadas en función

de su índice de masa corporal antes del embarazo y el control glucémico durante el mismo. Las participantes fueron clasificadas como normopeso ($18.5 \leq IMC < 25$ kg/m^2), sobrepeso ($25 \leq IMC < 30$ kg/m^2), obesas (IMC ≥ 30 kg/m^2), y mujeres con diabetes gestacional. A fin de evaluar el efecto *per se* de la diabetes gestacional en estudio solo se han incluido mujeres con diabetes gestacional con peso saludable.

Se realizó un seguimiento del embarazo con visitas programadas a las semanas 24 y 34 de gestación. Durante estas visitas se recogieron muestras de sangre venosa materna y datos ecográficos. En el momento del parto, se recogieron sangre venosa materna y de cordón umbilical, así como muestras de tejido placentario. Las muestras de sangre se usaron para la determinación de parámetros bioquímicos, y las muestras de tejido placentario se congelaron a -80ºC hasta el momento de su análisis. Las muestras de tejido placentario se emplearon para la cuantificación de ácidos grasos mediante cromatografía de gases y para el análisis de la expresión de los genes codificadores de las principales proteínas y enzimas involucradas en la transferencia placentaria de ácidos grasos mediante RT- PCR.

Los principales resultados obtenidos en esta tesis doctoral muestran que, tanto un Índice de Masa Corporal preconcepcional excesivo como el desarrollo de diabetes gestacional. alteran la expresión de los genes que codifican la síntesis de proteínas claves en la absorción, metabolismo y transferencia de ácidos grasos a través de la placenta. Además, la composición de ácidos grasos específicos es diferente en los grupos de estudio (sobrepeso, obesidad y GD) comparado con la composición obtenida en placentas de mujeres con un peso saludable antes del embarazo, mientras el contenido total de ácidos grasos no resultó alterado entre los grupos. El porcentaje del total de ácidos grasos saturados en placentas de mujeres obesas y afectadas con diabetes gestacional resultó más bajo que el observado en mujeres normopeso, mientras que el porcentaje de ácidos grasos poliinsaturados de cadena larga mostró la tendencia opuesta.

La superficie de la placenta expresa receptores para distintas moléculas y hormonas lo que sugiere que la placenta actúa como sensor nutricional regulando el transporte de nutrientes según las señales recibidas tanto del lado materno como del fetal. De esta forma, ante estímulos negativos la placenta puede activar mecanismos compensatorios que modifiquen la capacidad de transferencia de nutrientes. Los cambios observados en la expresión de los genes codificadores de las proteínas transportadoras *FATP1* y

FATP4, así como en la expresión de la enzima lipasa endotelial pueden representar un mecanismo protector limitando el exceso de nutrientes hacia el feto.

Curiosamente, los cambios en la expresión de los genes que regulan el transporte de ácidos grasos *(FATP)* son similares en los tres grupos de estudio; sin embargo, no sucede lo mismo con la expresión de los genes que regulan la síntesis de proteínas de unión de ácidos grasos en el citosol *(FABP)*. La expresión génica de *FABP4* fue menor en placentas de mujeres con sobrepeso y obesidad comparada con la observada en mujeres normopeso, mientras que no se observaron cambios en la expresión del *FABP4* en placentas de mujeres con diabetes gestacional. Esta diferencia en la expresión de *FABP4* entre placentas de mujeres obesas y mujeres con diabetes gestacional sugiere un mecanismo regulador diferente para la transferencia y metabolismo de los ácidos grasos en la placenta condicionado por estas condiciones metabólicas maternas.

Algunos autores han reportado un aumento de los niveles de triglicéridos y colesterol en plasma de mujeres obesas y con diabetes gestacional en el tercer trimestre de embarazo. Estas diferencias no fueron significativas en nuestra población de estudio, aunque se comprobó una tendencia a tener niveles más elevados de estos lípidos plasmáticos en ambos grupos. El porcentaje de hemoglobina glicosilada (HbA1c) resultó mayor en mujeres con sobrepeso, obesidad y diabetes gestacional que el obtenido en mujeres normopeso a lo largo del embarazo. Además, se ha podido comprobar una relación positiva entre el porcentaje de HbA1c en sangre materna y los marcadores de adiposidad fetal obtenidos por ecografía durante la gestación. Los fetos de mujeres obesas mostraron parámetros de adiposidad más elevados en las semanas 24 y 34 de gestación, mientras que los de mujeres con diabetes gestacional los mostraron solo en la semana 34. Estos datos sugieren que la exposición a un exceso de glucosa materna podría comenzar incluso antes del segundo trimestre de gestación en las embarazadas obesas.

El presente trabajo es el primero en estudiar de forma conjunta la expresión de genes implicados en la transferencia placentaria de ácidos grasos y el contenido de ácidos grasos en placentas de mujeres con sobrepeso. Es de interés resaltar el hecho de que, en estas embarazadas la expresión génica resultó más parecida a la observada en placentas de mujeres obesas, mientras que el contenido de ácidos grasos en la placenta fue similar al encontrado en mujeres con un índice de masa corporal saludable. Estos datos sugieren que el sobrepeso representa una situación intermedia en términos de disrupción

metabólica entre el normopeso y la obesidad. Además, las características fetales observadas también podrían justificar una situación intermedia en los procesos de alteración metabólica y funcional observados en mujeres con sobrepeso. Es decir, mientras que la adiposidad fetal en la semana 24 de gestación fue alta, los mecanismos compensatorios activados por la placenta parecen suficientes para que el feto alcance un desarrollo adiposo normal al final de la gestación.

A lo largo del embarazo la placenta debe integrar las señales procedentes tanto del lado materno como del fetal. Estas señales pueden resultar contradictorias en ocasiones. Por ejemplo, el exceso de nutrientes en el lado materno puede ser interpretado como un buen estado nutricional, lo que conllevaría un exceso de aporte de nutrientes al feto, mientras que los tejidos fetales pueden generar señales opuestas ante la sobreoferta de nutrientes. Aunque los cambios en la placenta encontrados en el presente estudio sugieren una respuesta de protección para mantener la salud fetal, la influencia de estos cambios en el desarrollo temprano fetal, así como las consecuencias a largo plazo en la etapa adulta no están claras.

CONCLUSIONES

1. La expresión de genes implicados en la transferencia de ácidos grasos a través de la placenta resulta afectada por un peso materno excesivo antes del embarazo y la diabetes gestacional, incluso en embarazos bien controlados. El sobrepeso, la obesidad y la diabetes gestacional maternas modifican de forma diferente la expresión de los genes codificadores de proteínas de unión a ácidos grasos en el citosol de las células placentarias.
2. La obesidad materna y la diabetes gestacional alteran la composición de ácidos grasos de la placenta sin comprometer el contenido total de ácidos grasos.
3. La placenta es un órgano heterogéneo cuya composición puede resultar comprometida, no solo por las patologías metabólicas maternas sino también por otros factores tales como el estado nutricional materno o el propio metabolismo de la placenta.
4. A las 34 semanas de gestación, se observa un mayor desarrollo de tejido adiposo fetal en los hijos de madres obesas y diabéticas, respecto a aquellos que se están desarrollando en madres sanas normopeso. Es posible que el tejido adiposo fetal

genere señales moleculares que podrían contribuir a la activación de rutas metabólicas reguladoras en la placenta.

5. El sobrepeso materno representa una situación metabólica intermedia entre el normopeso y la obesidad. El efecto del sobrepeso sobre la expresión génica placentaria es similar al observado en la obesidad. sin embargo, el contenido de ácidos grasos en la placenta de las gestantes con sobrepeso es parecido al encontrado en placentas de mujeres normopeso. Las características clínicas de los fetos y recién nacidos de mujeres con sobrepeso también sugieren un resultado intermedio entre el normopeso y la obesidad. Los mecanismos compensatorios activados durante la última parte del embarazo parecen ser suficientes para alcanzar un buen desarrollo fetal.
6. Nuestros resultados son consistentes con una adaptación funcional de la placenta para asegurar un aporte óptimo de nutrientes hacia el feto en crecimiento a través de la regulación de la expresión de genes codificadores del transporte y metabolismo de ácidos grasos.
7. A pesar de que, en el presente estudio, los cambios observados en la expresión génica en la placenta pueden representar un mecanismo protector para asegurar el óptimo desarrollo fetal, estas modificaciones pueden tener un profundo impacto en el fenotipo fetal con consecuencias desconocidas a largo plazo.
8. Los marcadores placentarios genéticos y bioquímicos analizados en el presente estudio se ven alterados por el peso pre-concepcional de la madre, y no por la ganancia de peso durante la gestación. Estos resultados sugieren que intervenciones que mejoren el peso pre-concepcional serán mucho más efectivas en la prevención del efecto intergeneracional de la obesidad, que las actuaciones centradas exclusivamente en la gestación.

SUMMARY

The prevalence of obesity has triplicate by the last thirty years, reaching the magnitude of epidemic. Recent data from the World Health Organization are alarming. Approximately the 40% and the 15% of women older than 18 years are overweight and obese, respectively. Specially worrying are data in respect infant and young population. From 1975 to 2016 the prevalence of overweight and obesity has increase to 18%, the worldwide prevalence of infant and teenager is about 8%.

Obesity must be considered as a complex multifactorial disorder resulting of the interaction of several environmental and genetic factors. Changes in dietary and physical activity patterns associated to social changes are disturbing especially in children and adolescent

Regardless of the cause, obesity represent an important global health concern. Excessive fat accumulation has been found to be a major risk factor for the four group of noncommunicable diseases: cardiovascular diseases, diabetes, musculoskeletal disorders and some kinds of cancer.

Obesity is specially worrying in women of reproductive age. Women with excessive body weight/fat accumulation at conception face many obstetric complications such as miscarriage, gestational hypertension, preeclampsia, gestational diabetes and cesarean delivery. In addition, offspring from obese women are in major risk of macrosomia, neural tube defects, and congenital heath disease. Furthermore, nutritional milieu associated to maternal obesity increases the risk of metabolic complications later in their descendance.

For first time, Barker *et al.* linked an unfavorable environment during pregnancy with the origin of diseases in adult life. Some maternal metabolic pathologies, such as obesity and gestational diabetes, are associated to an unfavorable nutritional status. Maternal malnutrition includes both excess and deficit of nutrients. Thus, offspring born from obese or diabetic mother shown increased insulin resistance, adiposity, and higher risk to develop metabolic syndrome later in life.

Although mechanism involved in early programming of health and disease remains unclear, the placenta may play an important role on the long-term consequences of maternal complications during pregnancy. Maternal pathologies may compromise the placental morphology and function, altering its nutrient transport capacity, and creating an unfavorable intrauterine environment for the developing fetus.

During pregnancy, maternal metabolism changes to adapt to the nutritional fetal requirements. Those changes are mediated by maternal and placental hormones. One of the biggest changes that pregnant women experience is in lipid metabolism. At the beginning of gestation, the level of estrogens, progesterone, insulin, and adipose tissue LPL activity increase which favor deposition of fat in maternal tissues. As pregnancy progress, maternal metabolism shift from anabolic to catabolic status. Hence, there are an increase of lipolysis, maternal fat mobilization, and insulin resistant condition. Consequently, the levels of triglycerides and cholesterol increase in maternal blood. The hyperlipidemia found in the third trimester of pregnancy overlap with the period of greatest fetal growth.

The insulin resistant condition is a physiological adaptation in normal pregnancies. This condition may result exacerbated in obese pregnant women, as obesity *per se* is a state of decreased insulin sensitivity. Furthermore, in some situation the maternal pancreas is unable to produce enough insulin to compensate such resistance, so gestational diabetes is developed. Thus, in those maternal conditions a higher increase of lipids in blood is expected.

Fatty acids play a key role to maintain the well-being and are essential for fetal development. The role of fatty acids in the human body can be classified as metabolic, functional and regulatory. For instance, fatty acids are incorporated in membranes of fetal structures, are precursors of regulatory molecules, and they provide energy substrate. Furthermore, due to their chemical structure long chain polyunsaturated fatty acids are essential for fetal nervous system development.

The fetus can synthesize saturated and short chain monounsaturated fatty acids, but for long chain fatty acids is dependent of maternal supply by the placenta. The placental transfer of essential fatty acids is a complex process mediated by transport and binding proteins, though exact mechanisms remains a matter of discussion.

The placental can protect the fetus from excess or deficit of nutrients. In some metabolic conditions, such as obesity and gestational diabetes, the duration of intrauterine insult may exceed the ability of the placental to adapt, which may alter the supply of nutrients to the fetus, especially fatty acids, with negative consequences for fetal structures development. For instance, the deficiency of long chain polyunsaturated fatty acids, such as DHA and AA, during gestation and first months of life has been associated with visual defects and cognitive problems in infants.

The general aim of this Doctoral Thesis was to investigate the potential effect of high pre-pregnancy body mass index and gestational diabetes on placental fatty acid content and expression of genes encoding for fatty acid metabolism and transport through the placenta (*FATP: fatty acid transport protein, FABP: fatty acid binding protein, FAT/CD36: fatty acid translocase, EL: endothelial lipase and LPL: lipoprotein lipase*).

In the present study placentas samples obtained within the PREOBE study were analyzed. The PREOBE study was designed as a prospective observational cohort study. Participant pregnant women were classified at study entry according to their body mass index and glucose tolerance during pregnancy. Thus, women were classified as normal weight ($18.5 \leq \text{BMI} < 25$ kg/m2), overweight ($25 \leq \text{BMI} < 30$ kg/m^2), obese (BMI ≥ 30 kg/m^2), and women with gestational diabetes. To evaluate the effect of gestational diabetes on placenta alone, without the combination of obesity, only normal weight diabetic women were included in this work.

Data from pregnancy and delivery were collected throughout the study. Maternal venous blood and fetal anthropometric measurements by ultrasound were collected at study visits performed at 24 and 34 weeks of gestation. At delivery, maternal and cord venous blood, and placental tissue were collected. Biochemical parameters were determined in serum samples. Placental samples were kept at -80º until their analysis. Fatty acids were quantified by gas chromatography form one piece of placental tissue. A second sample of placenta tissue was used for gene expression analysis by RT- PCR.

Our results showed that maternal excessive pre-conceptional body mass index and gestational diabetes alter the expression of key genes involved in the uptake, metabolism and transfer of fatty acids through the placenta. Moreover, the specific fatty acid composition was different in placentas from the studied group (overweight, obese and gestational diabetic) compared to normal weight ones, while total fatty composition resulted unchanged. The percentage of the total saturated fatty acids in placentas from obese and gestational diabetes women was lower than the percentages found in placentas from healthy weight women, while the percentage of total long chain polyunsaturated fatty acids showed the opposite trend.

The placental surface express receptors for different molecules and hormones, which suggest that the placenta acts as nutritional sensor. Thus, the placenta regulates nutrient supply according to signals generated in both maternal and fetal sides. In this way,

when negative stimulus are generated the placenta may activate compensatory mechanisms to modify its nutrient transfer capacity. The observed changes in gene expression of *FATP1*, *FAPT4* and *EL* may represent a protective mechanism to limit the excess of nutrient supplied to the fetus.

Interestingly, changes in *FATPs* expression were similar among studied groups while it was not the situation of *FABP* gene expression. The gene expression of *FABP4* was lower in placentas from overweight and obese women compared to those from healthy weight women, while it was unchanged in placentas from gestational diabetic women. The differences observed in placental *FABP4* mRNA expression between obese mothers and GDM suggest a differential regulatory pathway for placental FA handling and metabolism in both maternal conditions.

Some authors have reported an increase in levels of triglycerides and cholesterol in maternal plasma at third trimester of pregnancy. Such differences were no found in our population, but trend was higher in both groups. The percentage of glycosylate hemoglobin was higher in blood of overweight, obese and diabetic mother that in blood from normal weight women during pregnancy. Furthermore, a positive correlation was found between the percentage of glycosylated hemoglobin in maternal blood and ultrasound markers of fetal adiposity. Fetus from obese women showed higher markers of adiposity at 24 and 34 weeks of gestation, while fetus from diabetic women showed only at 34 weeks. Our data suggest that intrauterine insult to exacerbated glucose levels begins before second trimester of gestation in obese pregnancies.

Our work is the first to study placental expression of genes involved in lipid transport and metabolism in overweight pregnancies. Interestingly, gene expression in overweight group was like the placental expression from obese mothers, while fatty acid content was more similar to placentas from healthy women. Thus, overweight seems to be an intermediate group between normal weight and obese pregnant women in terms of metabolic disruption. The clinical characteristics of fetal and offspring represent an intermediate situation between normal weight and obesity as well. Fetal adiposity was higher at mid gestation, but it seems that compensatory mechanism activated during last part of gestation were enough to achieve a good fetal adipose tissue development at the end of pregnancy.

During pregnancy, the placenta must integrate nutritional and endocrine signals produced at both maternal and fetal side. Those signals may be sometimes

contradictories. For instance, while circulating maternal levels may indicate a good nutritional status, which lead to increased supply of nutrients, regulatory factors produced by fetal tissues may lead to the opposite placental response. Although placental modifications found in our study seem to be a response to protect and maintain the fetal health, the effect of these changes on fetal early programming and consequences in adult life are not yet clear.

INTRODUCTION

1 DEVELOPMENTAL ORIGINS OF ADULT HEALTH AND DISEASE

The Developmental Origins of Health and Disease (DOHaD) paradigm, originally proposed by Barker *et al*. (1), was arisen almost 30 years ago. Barker *et al.* published three articles in The Lancet that are considered pioneers in this area, reason why DOHaD paradigm is often known as "Barker's hypothesis" (2). Based on epidemiological studies, Barker *et al.* found a relationship between birthweight and adult mortality from heart diseases. In addition, they found a low rate of heart diseases in wealth regions compare to those with rising prosperity. These observations led to the hypothesis that *"an environment which produces poor fetal and infant growth is followed by an adult environment that determines high risk for ischemic heart disease"* (3), linking for the first time the maternal undernutrition during pregnancy to early origin of adult disorders.

Barker's hypothesis was the starting point for a very interesting and poorly studied field. In 2005, The International Society for Developmental Origins of Health and Disease was created to promote research into this topic.

Maternal, fetal and infant malnutrition (including both, excess or limitation of nutrients) are linked to a higher risk to develop non-communicable diseases, which have dramatically raised in the past decades. Offspring of obese or diabetic mothers show increased insulin resistance, adiposity, and consequent higher risk to develop metabolic syndrome later in life. In addition, maternal pathologies such as obesity and/or gestational diabetes (GDM) during pregnancy have severe consequences for the mother and are associated to higher risk of mortality, morbidity, miscarriage, and Caesarean section *inter alia* (4).

The long-term effect of these pregnancy complications may be mediated, at least in part, by an aberrant placental morphology and function, which alter its nutrient transport capacities (5-7) contributing to create an unfavorable in utero environment for the developing fetus.

2 THE PLACENTA: FUNCTIONS AND IMPORTANCE

The placenta is a temporary organ which faces both maternal and fetal circulation via the syncytiotrophoblast (Figure 1). Based on its syncytial nature the placenta plays a key role during pregnancy. The placenta regulates the exchange of a wide variety of molecules, which are important for fetal nutrition, molecular signaling, and immune tolerance (8, 9).

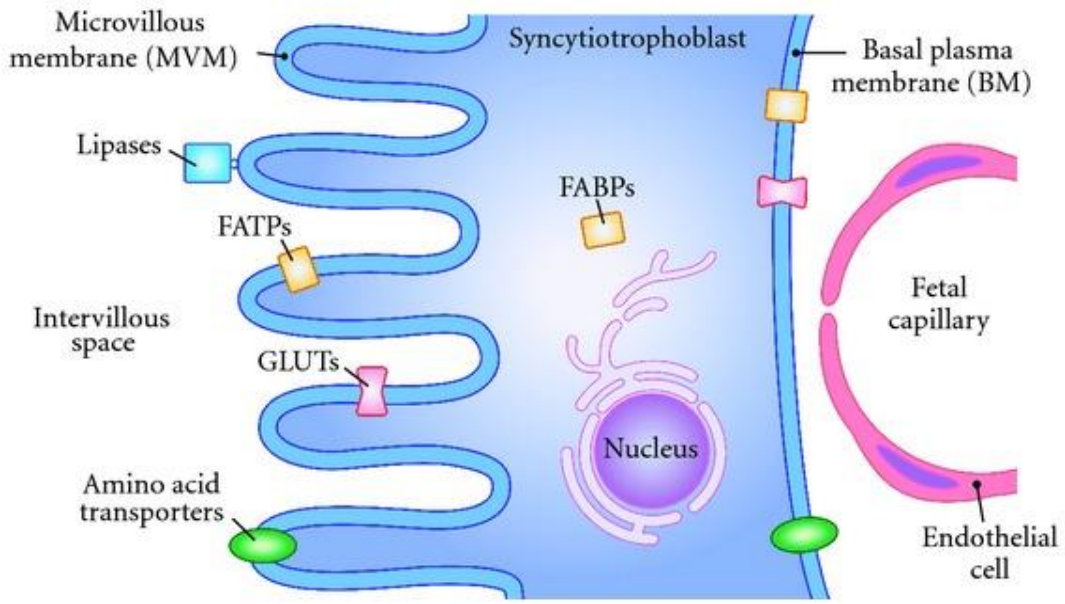

Figure 1. *Representation of the syncytiotrophoblast. Maternal blood pools in the intervillous space and bathes the microvillous membrane (MVM). The basal plasma membrane (BM) of the syncytiotrophoblast is oriented toward the fetal circulation. Transporters mediating the transfer of amino acids, glucose (GLUTs), and fatty acids (FATPs) are expressed in both plasma membranes of the syncytiotrophoblast. For transfer of lipids, extracellular lipases release fatty acids from maternal lipoproteins and intracellular binding proteins (FABPs) guide the fatty acids within the cytosol of the syncytiotrophoblast. Larger et al. (10).*

Regarding nutrition, the fetus relies on maternal supply of nutrients and excretion of wastes for proper development and growth. Fatty acids, glucose, lactate, cholesterol and amino acids are essential nutrients that are incorporated into fetal circulation by a complex process via the placenta (Figure 2).

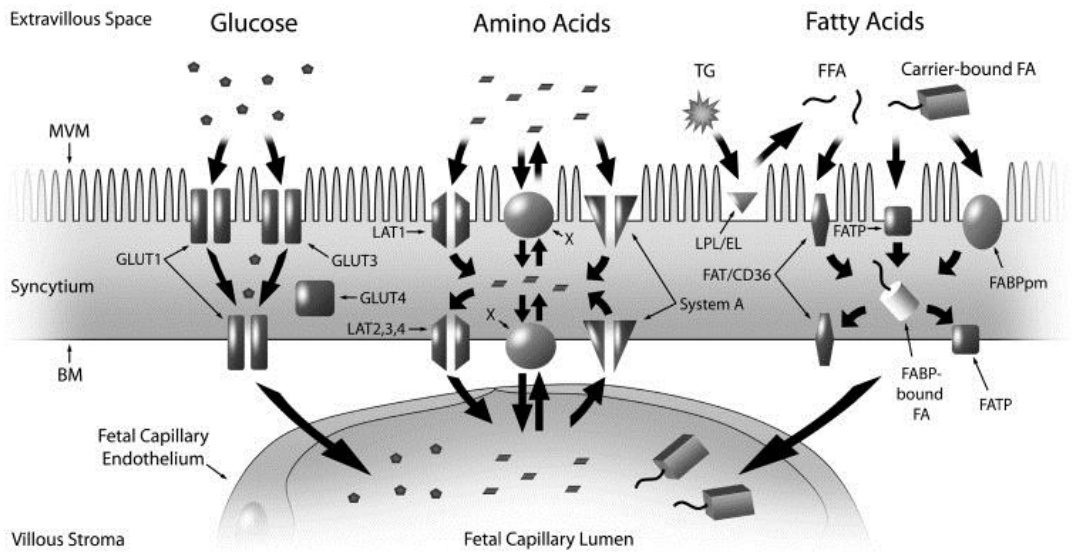

Figure 2. *Nutrient transport across the placenta. Nutrient transport across the placenta, featuring the SCTB and the fetal endothelium, and the location of key proteins involved in macronutrient (glucose, amino acids, fatty acids) transport at the MVM and BM. Abbreviations: SCTB: syncytiotrophoblast, MVM: microvillous membrane, BM: basal membrane, GLUT: glucose transporter, LAT: large neutral amino acid transport, TG: triglycerides, LPL: lipoprotein lipase, EL: endothelial lipase, FFA: free fatty acid, FAT/CD36: fatty acid translocase, FATP: fatty acid transport protein, FABP: fatty acid binding protein, FABPpm: plasma membrane fatty acid binding protein, X: exchangers. Brett et al. (11).*

Several studies, with both animal and human placentas, suggest that placenta acts as a nutrient sensor. Thus, based on signals from mother and fetus, the placenta may regulate itself and consequently influence the fetal outcome. For instance, low insulin and leptin levels in maternal blood conduce to a reduction of nutrient release to fetal circulation, while high maternal levels will be understood as a good nutritional status, which favors nutrient delivery (10).

The maternal and fetal blood does not establish direct contact; therefore, the molecule exchange involves numerous receptors and transporters expressed in the plasma membranes of the syncytiotrophoblast. Hence, molecules can cross the placental barrier via: 1) *passive diffusion* depending on exchange area and blood flow; 2) *facilitated diffusion* by carriers, which is affected by concentration gradients in both maternal and fetal sides; or 3) *active transport* which is an energy-dependent process that involves

expression of specific membrane transport proteins (11). Consequently, efficacy of transport mechanisms depends on placental exchange surface area, utero-fetus-placental blood flow and nutrient transporter capacity determined by total number, density, distribution and transporter complexes activity (12). In addition, placenta has a high metabolic activity as some molecules must be converted and modified in alternative substrates before to be released to the fetal circulation. Some of the metabolic processes performed by the placenta are glycolysis, gluconeogenesis, glycogenesis, oxidation, protein synthesis, amino acid interconversion, triglyceride synthesis, and chain lengthening or shortening of individual fatty acids (13, 14).

The placenta is not only a barrier and exchange unit between mother and fetus, but an important endocrine organ as well. Placental hormones have a significant effect on maternal metabolism by changing her physiology; as a consequence, there are an increase food intake and fat deposition at early gestation, and fat reserves mobilization for fetal growth at late gestation, as will be described in posterior section.

Due to its crucial regulatory functions in maternal-fetal communication proper placental development is essential for optimal fetal growth and well-being. Maternal obesity and/or GDM have been associated to adverse perinatal outcomes, but underlaying mechanisms are still poorly understood. In the case of obese women, the placental endocrine effect is modified by maternal adipose tissue derived hormones, which are released into maternal circulation. These changes determine an increase of leptin and interleukins levels and a reduction of adiponectin concentrations, compared to lean pregnant women. Hence, placental function and transfer capacity may be directly altered by maternal unfavorable metabolic conditions (15).

3 OVERWEIGHT AND OBESITY: CONCEPT AND PREVALENCE

Overweight and obesity are defined, by the World Health Organization (WHO), as an abnormal or excessive fat accumulation that represents a risk for health; however, an exact body fat quantification is not always easy and possible. The WHO recommends the Body Mass Index (BMI), calculated as the weight (kg) divided by the square of the height (m^2), as a simple and commonly used method to classify weight in adults.

According to WHO, adults with a BMI ≥25 kg/m^2 are considered overweight and obese when BMI ≥30 kg/m^2.

Despite overweight and obesity are preventable diseases, their prevalence has raised alarmingly by the last 30 years. Excessive fat accumulation has been found as a major risk factor for the four groups of noncommunicable diseases: *cardiovascular diseases, diabetes, musculoskeletal disorders and some kinds of cancer*. Currently, obesity has become a worldwide public health issue, as it is linked to more deaths than underweight. While in the past obesity was considered a wealth countries problem, its incidence is rising in low-middle income countries as well. Recent data shows that in Africa, the number of children with overweight/obesity has doubled in only 20 years. Data from WHO estimates that around 39% of women older than 18 years are overweight and 15% obese. Focusing in Spain, data are perturbing with around 55% and 24% of overweight and obese adult women, respectively (World Health Organization, 2014).

Obesity must be considered as a complex multifactorial disorder, resulting from the interaction of several environmental and genetic factors. Changes in dietary and physical activity patterns associated to social changes are disturbing especially in children and adolescent, while an elevated percentage of parents underestimate their child weight status (16, 17). There are important factors influencing this epidemic such as: 1) The actual access to an enormous diversity of low cost high fat/sugar processed food and beverages; 2) Irregular meats times; 3) Excessive big portion sizes; and, 4) Poor-quality diet due to the decrease in the consumption of fresh fish, fruit and vegetables, whole grain cereals and legumes (18).

Human nervous system (important role of hypothalamus), control a complex molecular and hormone signaling pathways which involve thousands of genes to guarantee body weight and homeostasis in a hostile environment. Those evolutionary pathways, beneficial in the past, seem to be an "enemy" in the modern world and their abundance of food (19). Thus, new theories consider obesity as a brain disorder where rewards and decision-making processes are altered, finding similarities between sugar and drug brain reward response (20). Indeed, neuroimaging studies have demonstrated brain alterations

in response to different stimuli regarding body weight (21, 22). Unfortunately, it remains still unclear if obesity is a cause or consequence of these alterations (Figure 3).

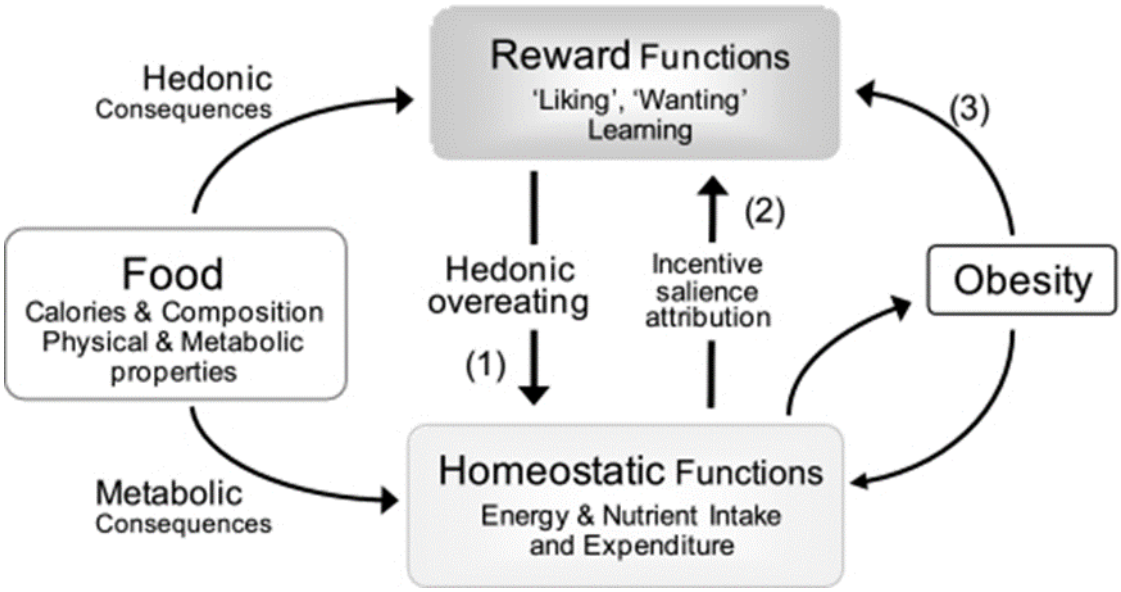

Figure 3. *Relationship between metabolic and hedonic controls of food intake and energy balance. The metabolic consequences of food are regulated by homeostatic functions and the hedonic consequences by reward functions. Hedonic and metabolic consequences are interdependent in that the hedonic value of food modulates caloric intake (*1*), and the metabolic status modulates hedonic processing (*2*). The obese state is associated with altered reward functions, but it is not clear whether these changes are the cause or consequence of obesity. Altered reward functions could cause obesity via increased intake of calories or fat (*1*), or alternatively, could result from consequences of the obese state (*3*), or could be a combination of both. Berthoud HR, (19).*

Regardless of the cause, obesity represents an important health concern, especially worrying in women of reproductive age. Women with excessive body weight/fat accumulation at conception face many obstetric complications such as miscarriage, gestational hypertension, gestational diabetes and Cesarean delivery (4). In addition, offspring from obese women are at major risk of macrosomia, neural tube defects, and congenital heart disease. Furthermore, nutritional milieu associated to maternal obesity increases the risk of later obesity, insulin resistance, adiposity, cardio-metabolic disease and type 2 diabetes and metabolic syndrome in the offspring. Epidemiological and

interventional studies in the same mothers with different *in utero* conditions among pregnancies strengthen that environmental conditions, both in addition to and independent of genetic susceptibilities, can program infant wellbeing during fetal programming as reviewed by Alfaradhi *et al.* (Figure 4) (23).

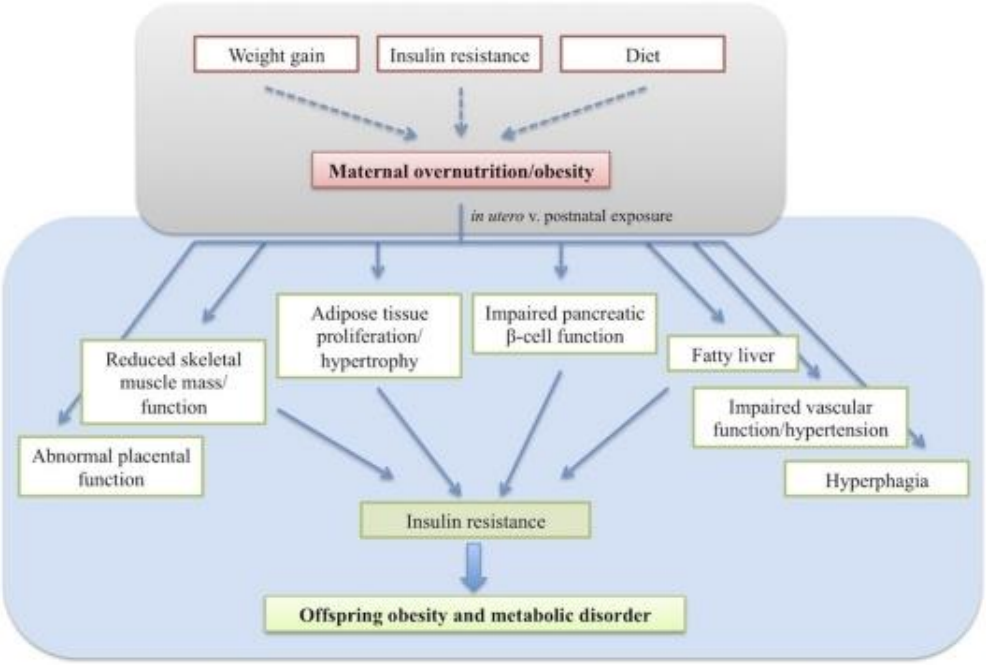

Figure 4. *The effects of maternal obesity on developmental programming on offspring phenotype resulting in insulin resistance and obesity. Alfaradhi et al. (23).*

4 GESTATIONAL DIABETES: CONCEPT AND PREVALENCE

Gestational diabetes is one of the most common health problems during pregnancy. According to WHO definition, GDM is a *"hyperglycemia with blood glucose values above normal but below those diagnostics of diabetes, with first recognition during pregnancy"*.

According to the American Diabetes Association (ADA), about 7% of all pregnancies are complicated by GDM. The prevalence is estimated between 1 to 14%, but it depends on the population and geographic area studied and the test used for its diagnostic as

well. Despite the implications of GDM during pregnancy its screening and diagnosis remain controversial. Thus, due to the several international criteria for diagnosis GDM, a woman may be diagnosed, or not, with GDM depending on the thresholds selected even by using the same test. This is a very important issue as some women without GDM can received excessive treatment while women with GDM will not receive any treatment at all (24, 25). In Spain, Ricart *et al.* compared the effect of to use the ADA criteria in our population instead of the current criteria of the National Diabetes Data Group (NDDG). About the 9% of the women screened met NDDG criteria, while by using ADA criteria the prevalence would increase up a 32%. Nevertheless, ADA diagnosis did not result in an increase of adverse GDM outcomes, so change to NDDG diagnosis criteria was not recommended by the authors (26).

The exact causes of GDM remains still unclear, but some clinical characteristics such as obesity, pregnancy over age 35, personal history of GDM and strong family history of diabetes are consistent with an increased risk of develop GDM. In normal pregnancies, around 20 weeks of gestation, maternal insulin sensitivity decreases while insulin requirements increase. Furthermore, placental hormones (*lactogen, human placental growth hormone, estrogen, progesterone, cortisol, and prolactin*) lead to an increase of maternal insulin resistance, as a physiological adaptation, to ensure fetal availability of nutrients to support fetal growth (27). Thus, GDM occurs when maternal body is not able to use and produce enough insulin required during pregnancy and, as a consequence, glucose levels increase in maternal blood. The excess of glucose goes through the placenta to fetal circulation, increasing fetal pancreatic insulin production. Those extra nutrients are stored in fetal adipose tissue, which may lead to fetal macrosomia. As GDM occurs mostly late in pregnancy, when fetal structures are developed, birth defects are less severe than those found in babies born from pre-gestational diabetic women. Nevertheless, newborns with extra production of insulin face several health problems and they are at higher risk to develop obesity and type 2 diabetes later in life.

5 METABOLIC ADAPTATIONS IN NORMAL, OBESE AND DIABETIC PREGNANCIES

Pregnancy is a special physiological situation where diverse setpoints are adjusted. Then, changes in maternal metabolism during pregnancy are essential to ensure nutrients supply for an optimal fetal growth and development. One of the most important changes that pregnant women experience is in lipid metabolism. These changes are manifested by an increase in maternal adipose tissue in early pregnancy and plasma hyperlipidemia in late gestation. These changes are strongly mediated by maternal and placental hormones and insulin sensitivity. The enzyme lipoprotein lipase (LPL) plays a key role in this process as well, and its activity is modified during pregnancy. LPL hydrolyzes plasmatic triglycerides in their derivates products, fatty acid and glycerol, which will be taken up by the subjacent tissue (liver, placenta or adipose tissue, mainly) (28).

In early pregnancy the increased levels of estrogen, progesterone, and insulin favor lipid synthesis and inhibit lipolysis, while increase of adipose tissue LPL activity enhance lipid deposition, promoting accumulation of maternal fat stores as previously mentioned (29). Fat may deposit as visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT). VAT plays an important metabolic role and its excess has been associated to higher risk of diabetes and dyslipidemia. Thus, effect of VAT deposition during pregnancy may be exacerbated in obese pregnant women.

The opposite situation occurs in late gestation, when metabolism shift from an anabolic to a catabolic state. Hormonal changes drive to an increase of adipose tissue lipolytic activity and a decrease of adipose tissue LPL activity, resulting in an increase of lipolysis and fat mobilization, and the consequent increase of fatty acid and glycerol in maternal plasma. These products are re-esterified by the maternal liver and they are released into the maternal circulation as triglycerides (TG) and cholesterol, contributing to create the hyperlipidemic status observed in the third trimester of pregnancy, when greatest fetal growth take place. Then, lipids are used as maternal energy source preserving glucose and amino acids for the growing fetus (29). In addition, it is known that insulin inhibits adipose tissue lipolytic activity, hepatic gluconeogenesis and ketogenesis, but increases adipose tissue LPL activity. The insulin-resistant condition

during late pregnancy contributes to net maternal fat breakdown, with may increase hyperlipidemia in GDM and obese pregnant women. Therefore, first indication of altered lipid metabolism associated with maternal pathologies can be observed at maternal plasma level. Both maternal TG and non-esterified fatty acids (NEFA) levels have been shown to correlate positively with both neonatal weight and fat mass, indicating that maternal hyperlipidemia in GDM actively enhances the availability of lipids to the fetus, contributing to fat depot accumulation (30). Unfortunately, other studies on maternal dyslipidemia reported contradictory results making difficult to understand the effect of maternal metabolic diseases on fetal development. Thus, Marseille-Treblay *et al.* reported no differences of maternal circulating TG in GDM compared to healthy controls (31), whereas Sanchez-Vera *et al.* found pronounced hypertriglyceridemia in diabetic mothers (32). Controversial results have also been reported for maternal plasma cholesterol, which has been found to be decreased (33), normal (34) or increased (32) in cases of GDM. Similarly, data about dyslipidemia in obese pregnant women are inconsistent and almost inexistent. Only a few studies aim to study the effect of this maternal metabolic condition on maternal plasma (35).

Carbohydrate metabolism is also affected by pregnancy. Dietary carbohydrates are broken into simple molecules, as glucose, during digestion. Briefly, glucose is absorbed by the intestine and it is release into circulation after its pass for the liver. Glucose metabolism is regulated by insulin, a dipeptide synthesized in the pancreas by the beta cells of Langerhans islets. But, the effect of insulin is the result of its interaction to other hormones produced by the whole body, such as glucagon, glucocorticoids and catecholamines. As described previously, early pregnancy is characterized by an elevated insulin sensitivity which declines as pregnancy advance. Around 20 weeks of gestation insulin resistance gradually increase as an adaptation to ensure continuous glucose supply to the growing fetus and favoring the use of lipids as maternal fuel. Exactly causes of insulin resistance are not fully understood, but free fatty acids, cytokines (IL-6, TNFα, plasminogen activator inhibitor, angiotensinogen), and hormones (leptin and adiponectin) secretes by adipose tissue strongly contributes to this situation, as observed in obese population. In normal pregnancy, a compensatory hyperinsulinemia, by enhanced insulin production, takes place in the third trimester of pregnancy. But beta cells capacity for insulin production is limited, and insulin

production declines as pregnancy advance. In this scenario, insulin production is not able to counteract the insulin resistant effect, leading to an elevated blood glucose levels, glucose intolerance and consequently gestational diabetes (36, 37).

Despite the fact the human placenta adapts to mild diabetes by limiting placental transfer of glucose, to protect the fetus to a certain degree from excessive glucose availability (38), knowledge about fatty acid metabolism and adaptations in response to obesity and/ or GDM in human placenta is far more limited.

6 PREGNANCY COMPLICATIONS ASSOCIATED TO OBESITY AND GDM

Maternal factors, such as excessive body mass index and gestational weight gain among others, has been associated with adverse pregnancy outcomes in both mother and offspring as summarized by Leddy *et al.* (Table 1) (39). In human, most of the existing data come from epidemiological studies. Unfortunately, they can only indicate associations even when those studies are based on very well controlled cohorts (40). Due to the growing prevalence of obesity and the associated economic and social cost increase, obesity has become a major public health concern. Obesity during pregnancy is associated with many obstetric complications and more than 50% of mortality cases during pregnancy, childbirth or the puerperium are in obese or overweight women. Many authors have tried to identify which adverse outcomes may be linked, at least in part, to obesity in pregnancy (39, 41-45).

Gestational diabetes mellitus

Chu *et al*. estimated that the risk of developing GDM is about two, four, and eight times higher among overweight, obese, and severely obese women, respectively, compared to lean pregnant women (46), and one in three pregnancies in obese women may be complicated by GDM (47). Pregnancy is characterized by a progressive decrease of insulin sensitivity and increase of insulin requirements to ensure fetal glucose availability. The underlying mechanism is a post receptor defect of decreased mobilization of Glucose transporter type 4 (GLUT 4) from the interior of the cell to the cell surface (48). Obesity is associated with an increase of insulin resistance and is the primary cause of type 2 diabetes, but exact causes have not yet been fully elucidated.

One mechanism supported by animal and human studies is the negative feedback that hyperinsulinemia have on insulin signaling pathway. Excessive fatty acid/glucose exposure induce hyperinsulinemia by enhancing of both pancreatic β cell number and function (49). Thus, insulin resistance state is exacerbated in pregnancies complicated by obesity with a 40% less insulin sensitivity compared to non-pregnant situation (50). In addition, GDM *per se* is associated with many obstetric complications, so early GDM diagnosis in obese pregnant women is highly recommended to improve clinical outcomes.

Maternal Hypertension and Preeclampsia

It is estimated that about 6-10% pregnancies are complicated by pregnancy-induced hypertension (PIH). PIH is defined as systolic blood pressure > 140 and diastolic blood pressure > 90 mmHg. PIH term include pre-existing hypertension, gestational hypertension and pre-eclampsia, pre-existing hypertension plus superimposed gestational hypertension with proteinuria and unclassifiable hypertension (51). PIH is a major cause of maternal, fetal and newborn morbidity and mortality, being the second cause of maternal death in USA and the commonest cause of fetal death (52, 53). Preeclampsia increases the risk of preterm delivery, fetal growth restriction, abruptio placenta, and fetal death (51). Maternal short-term complications associated with preeclampsia include central nervous system dysfunction, hepatocellular injury, thrombocytopenia, acute disseminated intravascular coagulation, oliguria, pulmonary edema, cerebrovascular events and placental abruption (53).

Inadequate maternal vascular response to placentation has been identified as one possible cause of PIH. Cardiovascular system suffers physiologic changes, as trophoblastic invasion on the walls of spiral arteries, from the beginning of pregnancy until 20 weeks of gestation as normal adaptation to pregnancy. Through those modifications uteroplacental vascularization results in a low-resistance, low-pressure, high-flow system. But, placental biopsies from hypertensive women did not show trophoblastic invasion of the spiral arteries as founded in biopsies from normotensive women (52, 54).

Others well established risk factors for development of preeclampsia are obesity, insulin resistance and hypertriglyceridemia (45). Obese women are at 10-15% more risk to

develop preeclampsia (55). Results from Generation R study showed associations between overweight and obesity and increased risk of gestational hypertension (OR 2.15 (95% CI 1.55, 2.97); OR 6.31 (95% CI 4.30, 9.26), respectively) and preeclampsia (OR 1.91 (95% CI 1.21, 3.00); OR 3.61 (95% CI 2.04,6.39, respectively) (44). Insulin resistance is strongly associated to development of preeclampsia. Kaaja *et al.* founded a low insulin sensitivity on those women who developed preeclampsia compared to normotensive women. Moreover, the decreased insulin sensitivity persisted for about 3 months postpartum (56). In addition, features associated with insulin resistance syndrome (i.e. glucose intolerance, hyperinsulinemia, lipid abnormalities, and high levels of leptin, testosterone, tissue plasminogen activator, plasminogen activator inhibitor-1, and tumor necrosis factor-alpha) are observed in women with PIH (57, 58).

Stillbirth

Based on the results of meta-analysis, obesity was identified to increase twice risk of stillbirth compared to normal weight pregnant women, but it remains unclear the independent effect of obesity or the associated comorbidities, such as GDM and hypertensive disorders (59). Stephansson *et al.* evaluated the risk of antepartum stillbirth of maternal overweight and obesity in primiparous women. When they excluded women affected by GDM or pre-eclampsia, the risk of antepartum death decreased in obese group, while such effect was not found in overweight pregnant women (60).

The mechanisms involved in the increased risk of stillbirth are unknown. Some possible causes are an accelerated fetal growth in combination with uteroplacental insufficiency and consequently hypoxia, endothelial cells damage through lipid peroxidases, or unhealthy lifestyle (smoking, physical activity, dietary factors) (60, 61).

Preterm birth

Preterm birth has been associated to maternal pregnancy complications such as obesity, diabetes and hypertension. Data regarding the risk of preterm birth associated to maternal obesity are inconclusive. About 5-8% of obese pregnant women have delivery before 37 weeks of gestation (62, 63) The risk of preterm delivery before 32 weeks increases as the maternal pre-pregnancy BMI increase (64, 65), but it seem to be more

related to other associated complications, as pre-eclampsia, instead obesity *per se*. On the other hand, Stotland *et al*. found an association between obesity and prolongation of pregnancy beyond 42 weeks (66).

Table 1. Obstetric Complications in Obese Pregnant Women

Complication	OR (95% CI) or % vs Normal Weight
Early pregnancy	
Spontaneous abortion (miscarriage)	
After spontaneous conception	1.2 (1.1–1.5)
After IVF conception	1.8 (1.1–3.0)*
Recurrent miscarriage	3.5 (1.1–21.0)
Congenital anomalies	
Neural tube defects	1.8 (1.1–3.0)*
Spina bifida	2.6 (1.5–4.5)*
Congenital heart disease	1.2 (1.1–1.3)*
Omphalocele	3.3 (1.0–10.3)*
Late pregnancy	
Hypertensive disorder of pregnancy	
Gestational nonproteinuric hypertension	2.5 (2.1–3.0)**
Preeclampsia	3.2 (1.8–5.8)*
Gestational diabetes mellitus	2.6 (2.1–3.4)**
Preterm birth	1.5 (1.1–2.1)*
Intrauterine fetal demise (stillbirth)	2.8 (1.9–4.7)**
Peripartum	
Cesarean delivery	47.7% vs 20.7%*
Decreased VBAC success	84.7% vs 66%
Operative morbidity	33.8% vs 20.7%*
Fetal/neonatal complications	
Fetal macrosomia (EFW ≥ 4500 g)	2.2 (1.6–3.1)**
Shoulder dystocia	3.6 (2.1–6.3)**
Birth weight < 4000 g	1.7 (1.4–2.0)**
Birth weight < 4500 g	2.0 (1.4–3.0)**
Childhood obesity	2.3 (2.0-2.6)*

*95% CI: 95% confidence interval, EFW: estimated fetal weight, IVF: in vitro fertilization, OR: odds ratio, VBAC: vaginal birth after cesarean. Significantly different to normal weight women: *P<0.05, **P<0.001. Table modified from Leddy at al., 2008 (39).*

Mode of delivery

In the retrospective cohort study conducted by Lynch *et al.* it was found that there was a decrease of vaginal delivery rate with increasing BMI. Obesity was associated with a two-three folds increase risk to have delivery by Cesarean section (67). The exact causes remains unclear but some plausible explanations are that Cesarean section is indicated in the management of pregnancy complication associated to obesity, such as gestational diabetes and pre-eclampsia; or failure of vaginal delivery due the increase amount of soft tissue in maternal pelvis (45). Additionally, fetal macrosomia associated to maternal obesity and GDM may contribute to the increase rate of Cesarean section among these populations (68).

Infant birth weight and fetal programming of obesity

Maternal obesity has been associated with high birth weight and has been found a major risk for develop obesity later in life, but the underlying mechanism are poorly understood. Obesity is the result of complex interactions between genes, dietary intake, physical activity, and the environment (69). The genetic component cannot explain the increasing prevalence of obesity itself. Thus, the hypothesis of fetal programming of obesity is gathering strength.

There is clear evidence from animal studies that in utero environment may determine adult life. Several rodents models where maternal obesity was induced by obesogenic diet (palatable high fat and sugar content) had as results offspring hyperphagic, insulin resistant, hypertensive, glucose intolerant, and exhibited adiposity, cardiovascular and metabolic dysfunction in adulthood (70, 71). Interestingly, Bayol *et al.* (72) found that offspring born to mother fed with "junk food" showed exacerbated preference for this kind of food after weaning and had high body weight compared to offspring born to mother fed with a balance diet. Bayol *et al.* study also identified the lactation period as a critical window, as animal exposed to "junk food" during gestation but not at lactation did not show hyperphagia.

In human those associations between periconceptual, fetal and infant stages and development of obesity and metabolic syndrome later in life are difficult to establish due to the influence of many uncontrollable variables. For instance, obesity is usually

associated with a calorie dense and unbalance diet, but knowledge about the effect of a high calorie diet have on offspring from lean mother is non-existed so far. In addition, it is likely that children and adolescents follow the same dietetic patterns that their parents. Therefore, it remains unclear if adverse perinatal and postnatal outcomes are the result of an obesogenic diet or maternal metabolic status associated with obesity *per se* (23). Moreover, obesity and diabetes during pregnancy may alter the placental function, leading to an increase of fatty acids transfer to the fetus with negative consequences for the structural and regulatory development (73).

7 FATTY ACIDS: CONCEPT AND METABOLISM

Fatty acids (FA) are molecules involve in a wide range of biological process including, *inter alia*, cellular signaling, regulation of immune responses and expression control of a varied array of genes. Thus, they are essential molecules for health maintenance and well-being, and alterations in their composition may lead to different diseases. Usually the importance of fatty acids in health was mainly focus in cardiovascular disease. Today, there are raising evidence on their influence in non-communicable diseases such as type 2 diabetes, metabolic syndrome, inflammatory diseases, and cancer. Despite their importance, fatty acids are commonly described by their chemical structure rather than their biological function. Then, we can classify them according the number of carbon atoms and the absence or presence of double bonds in their structure as saturated FAs (SFAs), monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs), but this gives poor information about their specific role in human health. So, even when traditionally we talk about generic fatty acid class, we must have in mind that every fatty acid plays a specific molecular and cellular action. For instance, linoleic acid (LA, 18:2n6) deficiency is associated with loss of skin integrity, while arachidonic acid (AA, 20:4n6) is the principal precursor for eicosanoids synthesis.

It is estimated that, in healthy people, around the 95% of dietary FAs are available in the bloodstream. Briefly, after a complex process of digestion and absorption dietary fats are hydrolyzed producing free fatty acids and other derivates. After hydrolysis, short and medium chain FAs are transported to the liver where they are quickly oxidized. The other hydrolysis derivates (long chain FA, monoacylglycerol, cholesterol,

lysophospholipids) are re-esterified in triglycerides, phospholipids and cholesterol esters.

FAs can also be synthesized *de novo* from non-lipid precursors such as glucose in rich-carbohydrates diets or from other FA by desaturase and elongase enzymes activity. Mammalian tissues express Δ9-desaturase activity, but they lack of Δ12 and Δ15 desaturases enzymes, so, double bound between position 10 and methyl end cannot be introduced. Thus, *de novo* synthesis is stopped with the formation of n-9 MUFAs. PUFAs synthesis require the so-called essential fatty acids, as they cannot be synthesized by mammalian tissues and must be provided by diet. So, PUFAs can be synthesized after ingestion of plant-oil derived linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3) (Figure 5) (74-77). Nevertheless, conversion efficiency of essential fatty acids into their derivates is small. For instance, docosahexaenoic acid (DHA ,22:6n-3) obtained from ALA represent less than 5% of whole-body conversion. Also, both metabolic pathways (ω-3 and ω-6) share the Δ5 and Δ6 desaturases, so LA, ALA and derivates compete for these enzymes (76). Thus, in abundance of ALA, AA production results less efficient that eicosapentaenoic acid (EPA, 20:5n-3) and DHA. For this reason, in case of some pathologies (heart diseases, atherosis and autoimmune diseases) ingestion of high amount of ω-3 FAs series are recommended as it results in a reduced production of AA derived prostanoids (e.g. prostaglandin E_2 and thromboxane A_2) (76).

Since lipids are molecules water insoluble, they must be transported in the bloodstream as lipoproteins. Lipoproteins are complex molecules with a central core containing cholesterol esters and triglycerides surrounded by free cholesterol, phospholipids, and apolipoproteins, which create the required polar surface to be mobile in aqueous mediums (78). Into the circulation, lipoproteins are found as 7 different kinds based on lipid composition, size and apolipoproteins, playing different roles. Thus, once lipids are release into the circulation, as NEFA and lipoproteins, they are transferred to different body compartments and tissue where they will be used for different purposes.

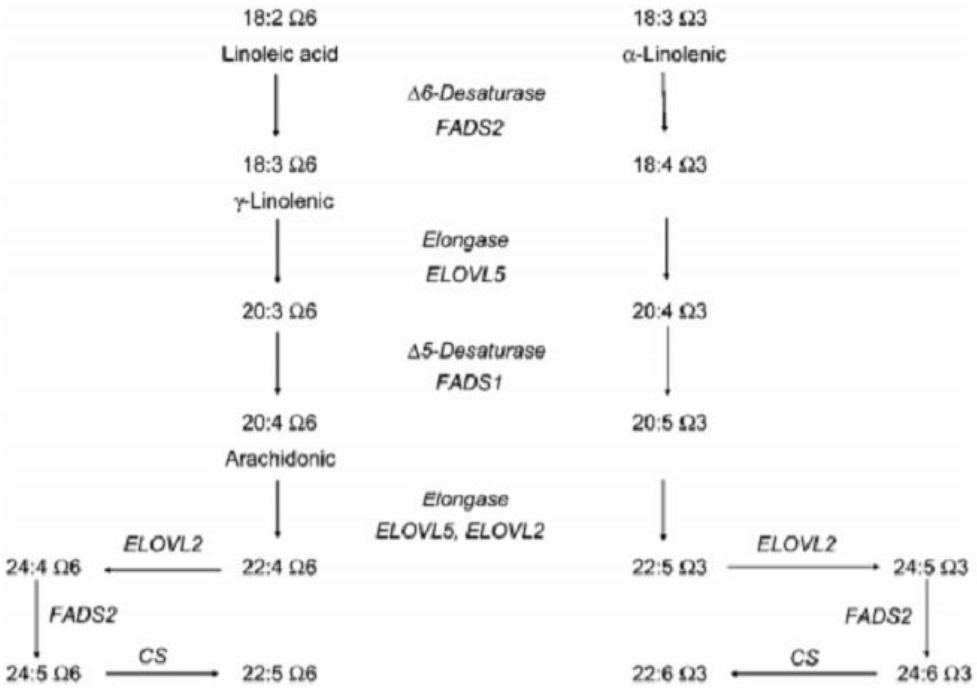

Figure 5. *Metabolic conversion of the essential fatty acid linoleic acid (omega-6 series) and alpha-linolenic acid (omega-3 series) to long-chain polyunsaturated fatty acids (LC-PUFA), FADS: fatty acid desaturase, ELOVL: fatty acid elongase, CS: Chain shorten. Koletzko et al., 2008 (77).*

7.1 Fatty acids role on human health

In the early XX´s, Burr & Burr demonstrated, with their experiments in rats, that fats are more than a simple source of calories, but critical to health. At that time, nutritional experts considered fats as non-necessary for a complete diet. By studying the deficiency of vitamin E, they fed rats with a free-fat diet. After a few months, rats on the fat-free diet group experienced skin problems, reddened in the hind paws, loss of fur around face and throat, weight loss and finally death. Also, animals autopsy reveals significant kidneys and urinary tracts damage. Interestingly, when they added three drops of lard to the feed animals started to recovery. With their experiments, they discovered the essential fatty acids by serendipity (79).

Despite of this knowledge, fats are become very unpopular in the past decades maybe due to the popular association between intake of saturate fats and cardiovascular diseases. This theory, developed by Ancel Keys, was recognized by the American Heart

Association and the U.S. Federal Government who recommended to low the intake of saturated fat. By that time John Yudkin stated for the first time, with less successful than is counterpart, that sugar is more related to cardiovascular diseases and mortality than saturated fatty acids. Today, we know that replacement of saturated fats by refined carbohydrates in the diet leads to changes in low- density lipoprotein (LDL), high-density lipoprotein (HDL) and TG which may imply many health risks (80). However, it is important to recognized that FAs are not only determinants on the development of chronic diseases, but they play numerous biological functions essentials for human health and well-being maintenance (Figure 6).

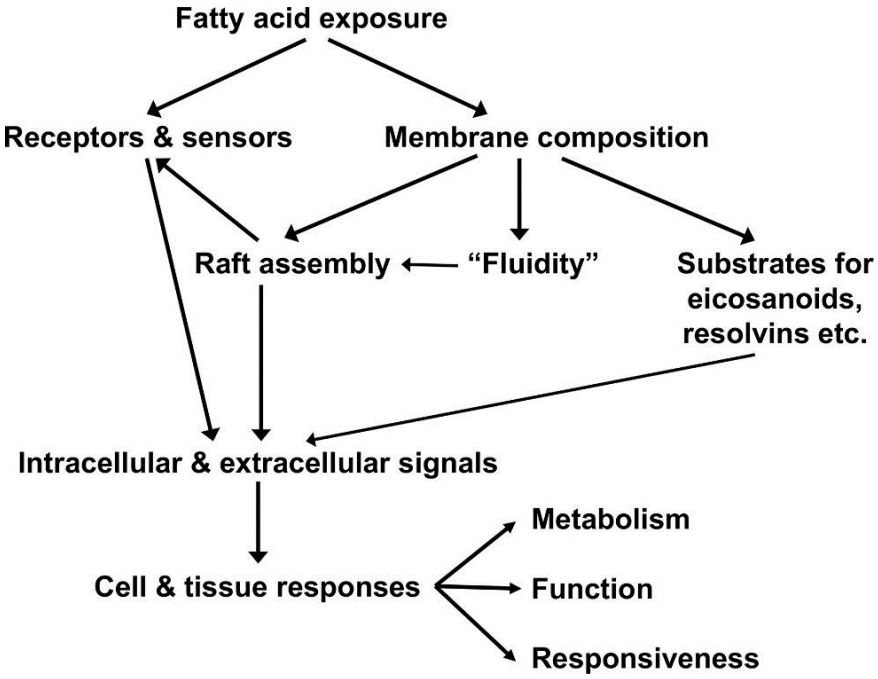

Figure 6. *Scheme relating the impact of fatty acid exposure on cell and tissue responses and the mechanisms involved proposed by Calder, 2015 (74).*

Mainly, fatty acids role in the human health may be grouped as metabolic, functional and regulatory. As metabolic role, FAs may by oxidized to obtains energy by most tissues, but not the brain. So, in situations of glucose limitation FA oxidation become a good alternative energy source to preserve glucose. Thus, excessive dietary FAs are stored in adipose tissue and they are mobilized when required.

In addition to energy store, in adipose and muscle tissue, FAs play important roles in health maintenance. FAs are important structural components of cell membranes phospholipids. The phospholipid FA composition vary from cells and membranes, and it is determinant of their function. Thereby, the specific FAs composition influence the membrane structure, its fluidity and its interaction with membrane proteins. For instance, plasma membrane microdomains (lipid rafts) that serve as cell signaling platforms are rich in saturated FAs, membrane DHA optimize the rhodopsin function in signal transduction, and the linoleic acid content in skin ceramides preserves its integrity and prevent water loss (74, 75).

As regulatory, FAs serves as signaling molecules themselves or as precursors of a wide range of signaling and regulatory molecules. AA, for example, is the substrate for the synthesis of eicosanoids, such as prostaglandins, thromboxanes, and leukotrienes. It is unclear if excessive intake of AA is harmful or beneficial for health. A procoagulatory, proinflammatory, proallergic, and protumor environment is expected when cell membranes contain high amounts of AA but cohort studies did not find negative association between circulating levels of AA and low-grade inflammation or cardiovascular diseases (74). Moreover, as reviewed by Lee *et al.* (75) treatment with PUFAs seems to be beneficial on the prevention of non-alcoholic fatty liver disease, autoimmune responses, cardiovascular disease, cancer and diabetes. Studies in animal models have found a reduction of insulin resistance and glucose tolerance improvement by supplementing animals with n-3 PUFAs (81).

FAs regulate the expression and activity of many genes and transcription factors, influencing the cell protein production. Several authors have revised the role of FA on gene regulation and their underlaying mechanism (75, 81, 82). For instance, FAs and derivates activate the expression of peroxisome proliferator–activated receptors (PPARs). PPAR activated by FAs promote FA oxidation in the mitochondria and peroxisome, stimulate TGs synthesis, and regulate other lipid-related pathways, inflammatory pathways, and glucose metabolism. Those mechanisms protect against lipotoxicity by inhibiting the hydrolysis of circulating TGs and consequently FAs uptake and intracellular accumulation. Also, dietary PUFAs inhibit *de novo* lipogenesis by suppressing the expression of the sterol regulatory element binding protein (SREBP). The role of FAs on inflammatory response has been very studied as well. Saturated FAs

shown a proinflammatory effect by direct activation of Toll-like receptor 4 (TLR4), whereas n-3 PUFAs seems to have anti-inflammatory properties. But the anti-inflammatory effect of EPA and DHA may be predominantly due the inhibition of AA metabolism.

In resume, each type of FA (SFA, MUFA or PUFA) have a specific structural and regulatory role in human health. They may contribute to health maintenance and well-being or enhancing risk of diseases by influencing a variety of biological activities, which affect cell and tissues composition, metabolism, function and response to stimulus.

7.2 Importance of fatty acids during pregnancy and fetal development

Several factors such as maternal diet, metabolic status, placental transport activity and timing of nutrients release influence not only in the quantity but also in the quality of nutrients delivery to the fetus. Grieger *et al.* reviewed the impact of maternal intake of several nutrients on infant outcome (83). They highlighted that both under and overnutrition influence fetal growth and metabolism with not only postnatal effect but also contributing to an increased risk of disease later in life.

Glucose is considered the major oxidative substrate used by the fetus. As reviewed by Herrera *et al.* several studies positively relate glucose levels in maternal plasma and fetal growth in both healthy and pregnant women who develop GDM. This correlation was not found by other authors in diabetic women, suggesting that other factors, as maternal lipid profile during pregnancy, may contribute to fetal growth (84). Gademan *et al.* observed that both pre-pregnancy excessive BMI and higher levels of FFAs in maternal blood during pregnancy independently influence the children BMI at 5-6 years. Furthermore, they found an association between maternal FFA and offspring adiposity, BMI and risk of overweight (85).

Woman physiological adaptations to pregnancy, as described in previous sections, lead to changes in maternal lipid metabolism throughout pregnancy. At third trimester of gestation FAs are mobilized from maternal tissues and liberated into maternal circulation making them available for placental uptake. Once NEFAs are released into

the fetal bloodstream they are used by fetal liver to form TAG. In addition, onset of fetal *de novo* saturated and monounsaturated FAs synthesis capacity is established around 28th weeks. So, by the end of pregnancy fetal fat accretion is the result of interaction between maternal and fetal metabolism (Figure 7) (84). On the contrary, PUFAs levels are almost exclusively dependent on maternal supply due the fetal physiological immaturity to PUFAs synthesis, and the lack of placental desaturase and elongase enzymes. Maternal LCPUFA *de novo* synthesis rate is also limited so not only essential fatty acids (LA and ALA) but also DHA, EPA and AA must be introduced with maternal diet.

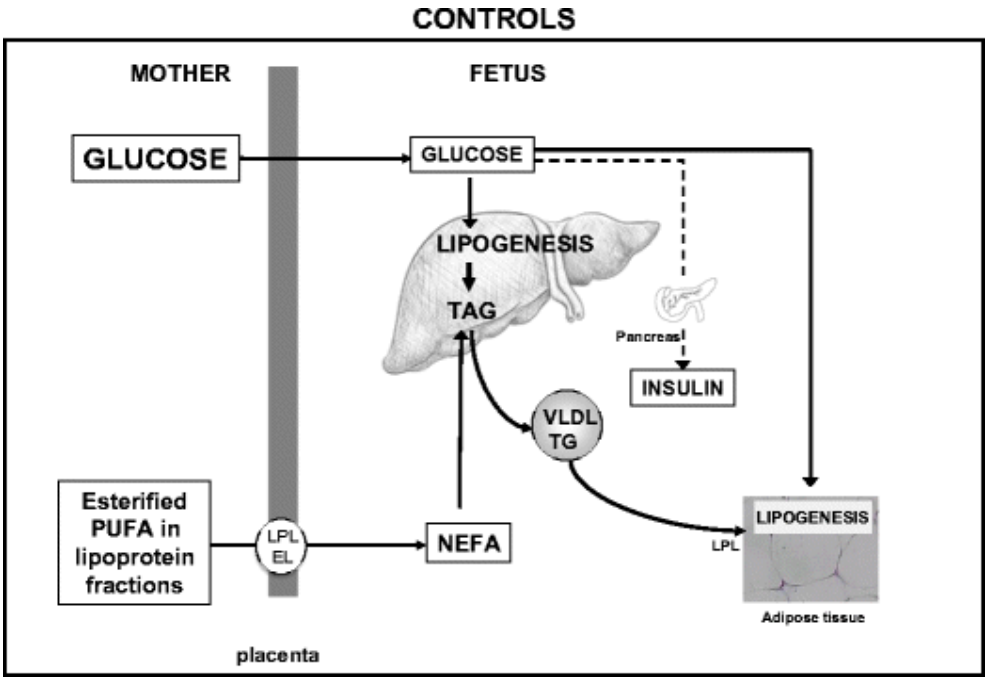

Figure 7. *Schematic representation of the mechanism of fetal fat mass development in healthy controls proposed by Herrera et al. (84). TAG: triacylglycerols, NEFA: non-esterified fatty acids, LPL: lipoprotein lipase, EL: endothelial lipase, PUFA: polyunsaturated fatty acids, VLDL TG: very low-density lipoprotein triacylglycerols. Herrera et al., 2018 (84).*

Nonetheless, maternal dietary intake includes a wide mix of FAs, with different effect in maternal and fetal metabolism. Most of the studies performed during pregnancy and lactation are focused on the structural role of LC-PUFAS. LC-PUFAs are essential for cell membrane phospholipids synthesis. Also, phospholipid derivates play a role as

signaling molecules and are required for normal cell function. Specific LC-PUFAs, such as DHA and AA, have been found essential for proper brain and visual system development. The effect of n-3 and n-6 fatty acids on nervous system development has been extensively studied by many authors (86, 87). While it is clear that DHA, AA and EPA are essential for proper fetal development, a consensus about daily intake requirements has not been reached (88, 89). Several studies have explored the effect of PUFAs in adipose tissue development as well, but data are inconclusive. Disbalance between maternal intake of n-6 and n-3 PUFAs, rather than consumption of n-6 or n-3 PUFAs *per se,* during pregnancy seems to negative influence the fetal adipose tissue development (90, 91).

Mennitti *et al*. carefully reviewed the effect of different types of FAs on programming the health and disease of the offspring (92). They highlighted that excess of some FAs may compromise the availability of others. For instance, placental LC-PUFAs binding sites can be occupied by *trans* fatty acids (93), which difficult the placental transfer of LC-PUFAs to the fetus. In addition, a negative association has been found, in both human and animal models, between maternal intake of trans fatty acids and levels of LC-PUFAs in different fetal compartments (94). Furthermore, rats exposed to trans fatty acids diet during fetal development showed an altered appetite signaling mechanism later in life (95).

The high SFA diet exposure during gestation has been widely explored in animal models, while less information regarding SFA limitation is available. Offspring of dams fed with a SFAs rich diet shown hyperglycemia, hyperinsulinemia, hyperleptinemia, and increased insulin resistance, body mass index, visceral fat and adipocyte hypertrophy as summarized by Mennitti *et al.* (92). Those negative outcomes can be the result of functional damage in liver and pancreatic structures (96-98). Moreover, excessive adipose tissue may alter several pathways involved in body weight regulation, energy metabolism and glucose homeostasis, among others, contributing to develop metabolic diseases in adult life (99). Furthermore, maternal obesity and high fat diet alter the circulating levels of leptin, insulin and glucose, which have a deep impact in the development of the hypothalamic melanocortin system, which regulate the appetite control and energy consumption (100). Leptin and insulin act as neural signals in the

hypothalamus, thus altered hormonal levels compromise the development of neuro-regulatory signals that control eating behavior (101).

Disrupted placental FAs transfer at the third trimester of pregnancy, when the development of hypothalamic melanocortin system take place, may alter hypothalamic peptides in the fetal brain that will be responsible of hyperphagia and excessive weight gain later in life (102).

8 FATTY ACID TRANSFER ACROSS THE PLACENTA

The fetus requires fatty acids for energy substrate, structures development and growth. Fetus may synthesize SFA and short chain monounsaturated acids, but for LCPUFAs depends on maternal supply. During third trimester fetal requirements of FAs increase, with the highest accretion rates about the last 5-10 weeks of gestation (103). In this period, maternal diet is not able to support alone the raised fetal demands of FAs, so the fat deposited in maternal tissues during first part of pregnancy must be mobilized and incorporated into the maternal circulation. Otto *et al.* showed that the DHA concentration in maternal plasma increase after 14 weeks of gestation, even when maternal intake of DHA was not increased (104). Al *et al.* reported lower levels of DHA in plasma from multigravidae women compared to primigravidae ones, which suggest a reduction of maternal DHA stores after each pregnancy (105). Thus, during pregnancy both maternal metabolism and placental function coordinately adapt to ensure continuous supply of n-3 and n-6 LCPUFA to the fetus (106). Once FAs reach fetal circulation they are bound to albumin and α fetoprotein to be distributed to the liver, brain and other peripheral fetal tissues such as adipose tissue. LCPUFAS, specially DHA and AA, are the principal components of brain, retina, and central nervous system, while palmitic acid constituted the 45-50% of fetal adipose tissue (107).

During pregnancy maternal and fetal circulations never stablish contact, thus the FAs exchange is mediated by the placenta. At the third trimester, both maternal hyperlipidemia and fetal fat deposition exponentially increase, while levels of NEFA in fetal circulation decrease. In addition, concentration of albumin in fetal plasma is estimated about 10-20 per cent higher than in maternal circulation (108). As consequence the unbound NEFA circulating levels are around three times higher in

maternal than in fetal plasma, creating a concentration gradient which favors the FAs deliver to the fetus. Perazzolo *et al.* highlighted the importance of placental metabolic pool in the uptake and transfer of FAs. Their transfer model suggested that FAs diffuse easily to the maternal side which results in a decrease of FAs available in the syncytiotrophoblast. Based on their model results, they proposed that FAs trafficking to the fetus is not only determined by microvillous membrane uptake, but also by placental metabolism and basal membrane transfer (109). In fact, the placental ability to extract LCPUFAs from maternal circulation can be observed at term, when the relative concentration of DHA and AA are increased in cord blood and neonate tissues compare to maternal plasma. The exact mechanism involved in this phenomenon, called biomagnification, are not fully elucidated yet. Some authors have reported a preferential placental transfer of DHA over other FAs. Haggarty *et al.* reported a selectively uptake of AA, DHA, αLN and LA (from more to less), while the transfer to fetus was quite different (DHA>αLN>LA>AA) (110). This transfer order was found changed when levels of AA were increased in the simulated maternal circulation (111). Studies in vivo performed by Larqué's group with orally administrated labelled FAs to pregnant women who underwent to elective Cesarean section showed a preferential incorporation of DHA in placenta lipids (112) and cord plasma (113). They demonstrated that incorporation of dietary FAs in maternal lipids and placental transfer of FAs is a slow process that requires about 12 hours (114). However, the specific mechanism involved in placental LCPUFAs uptake and release is unclear. All FAs may cross lipid bilayers but since only about a 10% of cellular long chain fatty acids (LCFA) acquisition occurs via simple diffusion, this mechanism seems insufficient to satisfy the great fetal demand of LCFA. Hence, because the importance of LCPUFAs for proper neurological structures development and regulatory molecules synthesis, the placental transfer to the fetus requires a more efficient mechanism mediated by transport proteins.

8.1 Mechanism of placental uptake of LCPUFAs

The placenta takes the FAs from maternal circulation as free fatty acids. Although their concentration increases as pregnancy advance, NEFA represent only a small proportion of maternal circulating FAs. About the 97-99% of FAs are esterified in triglycerides, phospholipids, and cholesterol ester, and due to their low solubility, they must be

transferred in maternal circulation incorporated into lipoproteins. Thus, as those complexes cannot cross the placenta several mechanisms have been proposed for FA release prior placental uptake (Figure 8) (115-118). The MVM express specific receptors for very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (84). Once maternal lipoproteins have been bound to the placental binding sites they may be taken up by receptor-mediated endocytosis and hydrolyzed by intracellular lipases (119) or hydrolyzed by the adjacent placental lipases and take up by fatty acids carrier proteins present in the MVM (120).

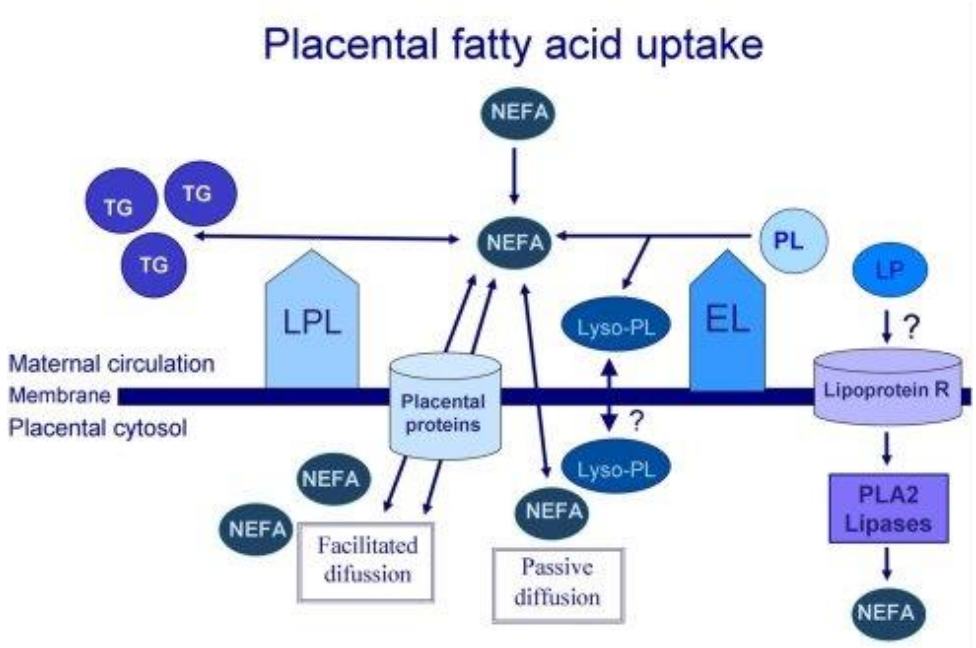

Figure 8. *Placental fatty acid uptake process. TG: triglycerides, NEFA: non-esterified fatty acids, PL: phospholipids, Lyso-PL: lysophospholipids, LP: lipoproteins, R: receptor, LPL: lipoprotein lipase, EL: endothelial lipase, PLA2: phospholipase A2. Gil-Sanchez et al. (115).*

Several extracellular lipases, such as phospholipase A_2 type II, hormone sensitive lipase, endothelial lipase and lipoprotein lipase, have been recognized in the surface of the placenta. Lipoprotein lipase (LPL) and endothelial lipase (EL) are identified as the more active lipases in the placenta. LPL is expressed in the MVM, while EL is found in both MVM and the membrane of capillary endothelial cells. LPL hydrolyzes maternal plasma triglycerides into FFA. LPL was originally considered the only active lipase in the placenta. Since LPL is a triglyceride hydrolase maternal TG were thought to be the only source of FFA (117). The identification of the EL in the placenta, which shown

predominantly phospholipase activity, demonstrated that both TG and PL can provided FFA for the fetus. LPL preferentially hydrolyze TG fatty acids in the sn-2 position (generally more unsaturated) (117), whereas EL hydrolyze PL fatty acids in the sn-1 position (predominantly saturated) (121).

Once FAs are released into the maternal circulation as NEFA they can cross the placental membranes (MVM and BM) by both simple or facilitated diffusion. Facilitated FAs diffusion is mediated by a family of transmembrane fatty acid transporters (FATP), fatty acid binding proteins (FABP) and fatty acid translocase (FAT/CD36).

FAT/CD36 and FATPs have been identified in both MVM and basal membrane which suggest that placenta allow a bi-directional flux of FAs between maternal and fetal circulation.

FAT/CD36 is a membrane protein involved in a large variety of adhesive processes. The absence of FAT/CD36 expression in liver, an organ with a high capacity of FA uptake suggest that FAT/CD36 is not directly involved in FAs uptake. Based on its function, as a cell adhesion molecule, it seems to be involved in binding and concentrating of NEFA at the cell surface, and to transfer to FATPs. (122).

FATPs are a large family of well conserved protein in mammals, reflecting that they are key genes for essential functions for life. Six members of the *FATP (FATP1-FATP6)* family have been identified in humans with different tissue expression patterns. Al least one or more members of the *FATP* family are found in organs with major FAs utilization (122). Expression of five of them have been identified in the human placenta (123). Localization of FATP4 expression in trophoblasts of mice and early embryonic lethality of homozygous deletion of FATP4 in mice suggests a key role of this gene in fat absorption in early embryogenesis (124). In addition, FATP1 and FATP4 are the predominant FATP in brain, supporting the hypothesis that they are responsible for long-chain polyunsaturated fatty acids (LC-PUFAs) uptake, as LCPUFAs are highly concentrated in brain (125). Larqué *et al.* found a positive correlation between placental gene expression of *FATP1* and *FATP4* and maternal plasmatic and placental percentage of DHA in phospholipid fraction, while DHA in cord plasma phospholipids was only correlated to FATP4 mRNA expression (126). Nevertheless, little is known about

mechanism involved in LCFAs uptake by FATPs. FATPs do not show similarities to other transporters families, so evidence that FATPs are authentic transporters is limited to the observation that overexpression of the genes increases FA transport, while reduced expression results in a decrease of FA transport. Other mechanism propose are that FATPs are enzymes with fatty acid CoA synthetase activity, or dual-function proteins (122, 127, 128).

8.2 Placental intracellular movement of fatty acids

Once NEFAs are introduced in the cytoplasm they must be bound to FABP for trafficking due their insoluble properties. FABPs are a family of 9 small, highly conserved, cytoplasmic proteins that bind LCFAs and other hydrophobic ligands. As occurs with FATPs, FABP family members shown different tissue expression patterns. FABPs are found in tissues with high FA metabolism. Human placental syncytiotrophoblast express FABP1, FABP3, FABP4, FABP5 and FABP7 (118).

FABPs remove fatty acids bound to FATP in the inner cellular membrane participating in the trafficking of them to their respective cell compartment, including endoplasmic reticulum, mitochondria, lipid droplets, peroxisomes and nucleus (116, 120). Although no affinities have been demonstrated for specific FAs, it seems that all FABPs are able to bind LCFAs, with increasing affinity as number of double bond increase and decreasing as fatty acid chain length reduce (129).

FABPs are not only relevant for uptake and transport but also for fatty acid metabolism. In the cytosol, bound FAs may follow various pathways (Figure 9) (130). For instance, FAs are essential for placental own metabolism, growth and development. NEFAs may be oxidized in the mitochondria to produce energy, used as precursor for synthesis of eicosanoids or re-esterified as TG, PL and cholesterol for storage in placental lipid droplets. Furthermore, transcription factors, such as the peroxisome proliferator-activated receptor (PPAR), can be activated in the nucleus by FAs and derivates delivery by FABPs (131). The remaining NEFAs can be modified by elongation and desaturation reactions and release into fetal blood stream.

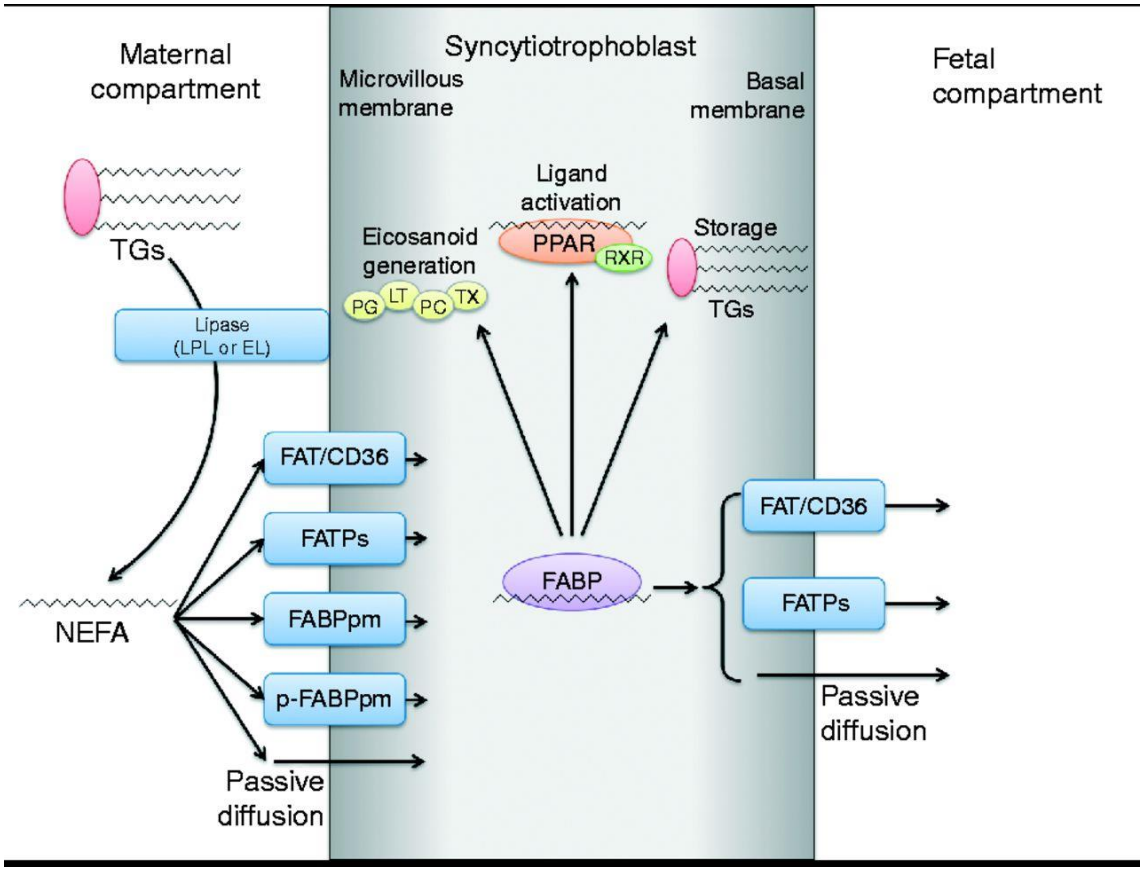

Figure 9. *Schematic representation of placental fatty acid transport mechanisms and potential metabolic fates within the placental tissue. TGs: tricyglycerides, LPL: lipoprotein lipase, EL: epithelial lipase, NEFA: non-esterified fatty acid, FAT/CD36: fatty acid translocase, FATPs: fatty acid transport proteins, FABP: fatty acid-binding protein, FABPpm: plasma membrane FABP, p-FABPpm: placental plasma membrane FABP, PG: prostaglandin, LT: leukotriene, PC: prostacyclin, TX: thromboxane, PPAR: peroxisome proliferator-activated receptor; RXR: retinoic acid X receptor. Jones et al. (130)*

8.3 Fatty acid release in fetal circulation

Part of NEFA introduced into the cytosol can be directly incorporated into fetal circulation through the basal membrane, either by simple diffusion or facilitate process mediated by transporters expressed in the membrane. Evidences from perfused placenta studies, where unlabeled FA release was observed, suggest that placental esterified pools contribute to FAs transfer as well. Madsen *et al*. reported that placenta is capable to synthesize and secrete apoB-100 containing lipoproteins (132). An additional transfer

of FAs in their esterified form from placental lipid pools has been proposed, but this mechanism have not been demonstrated yet (128).

8.4 Placental FA transfer in complicated pregnancies

Placental cells must respond to hormonal and nutritional signals generated from both mother and fetus. Hence, placental function may be compromised as result of the cross-talk between the mother and fetus/placenta unit. Maternal pathologies create a negative intrauterine environment that may dysregulate the expression of enzymes, transporters and binding proteins imply in placental FA supply, as they are mostly regulated at transcriptional level. Despite of placental response to maternal stimulus has been subject of many investigations and reviews, reported data are inconclusive and many times contradictories. For example, Gauster *et al.* reported upregulation of *EL* in placental homogenates from obese women affected by GDM, whereas such differences were not observed in placentas from obese or GDM pregnant women (121). *LPL* expression was found unchanged in pregnancies affected with GDM by Magnusson *et al.* (133), while expression was increased in placentas from insulin-dependent diabetic women. Intrauterine growth restriction has been associated with increased expression of placental *LPL* and decreased expression of *EL* (134, 135). Dubé *et al.* (136) found increased placental FAT/CD36 mRNA, while FATP4 mRNA was decreased in placentas from obese women.

Furthermore, a reduced DHA content has been reported in cord blood vein of offspring born of women affected with GDM (137, 138). Although those results suggest an impaired DHA transfer in pregnancies complicates with GDM, as far our knowledge there are not available studies regarding FATPs expression in placentas from GDM women. A similar scenario is found regarding maternal weight. Only Dubé *et al.* (136) aimed to explore the effect of maternal pre-pregnancy obesity on placental expression of genes imply on FAs transfer, where information about the impact of overweight and gestational weight gain is non-existent.

HYPOTHESIS AND OBJECTIVES

The placenta plays a key role in mediating the transfer of nutrients between the mother and the fetus and regulating the maternal and fetal metabolism. But placenta is more than a physical barrier and transfer unit. Placenta is metabolically active and requires nutrients for its own growth and development. Thus, optimal maternal delivery of nutrients throughout pregnancy is essential for both placental and fetal development and growth. Specifically, fatty acids play an essential functional and regulatory role during pregnancy.

It is established that plasma and placental composition are strongly influenced by maternal nutritional status. Maternal pathologies such as obesity or gestational diabetes have been associated with an altered nutritional status, with excess of some nutrients and deficit of others. Specifically, disrupted plasmatic fatty acid composition has been associated to maternal obesity and impaired glucose control.

The exposure to adverse intrauterine environment, associated to obesity and GDM, may alter placental function in many ways. Within the maternal and placental physiological capacity of adaptation, the developing fetus is protected from nutrient deficiency or excess in the maternal diet. However, this capacity is limited, so in utero insults during critical periods or "windows" may affect placental development and function and, thereby influencing not only fetal outcomes but compromising long-term health. Hence, infants born from mothers with suboptimal metabolic status are at higher risk to develop obesity, type 2 diabetes, cardio-metabolic disease later in life. Also, improper weight gain during pregnancy has been associated with obstetric complications and unfavorable infant outcomes. Knowledge regarding the effect of overweight during pregnancy is more limited.

Thus, we hypothesized that placental FAs transfer and metabolism may be compromised under maternal metabolic diseases, with unfavorable consequences for the offspring.

The principal aim of this study is:

- To investigate the potential effect of high pre-pregnancy body mass index or GDM on fatty acids content and expression of genes encoding proteins involved in fatty acid metabolism and transport in the placenta.

Secondary objectives of this work are:

- To analysis the differences in maternal and fetal clinical and biochemical parameters among study groups.
- To evaluate the influence of maternal pathologies on fetal and newborn outcomes and their possible relationship with placental FAs transfer.
- To elucidate the effect of weight gain during pregnancy on placental FAs composition and regulation.

MATERIALS AND METHODS

9 STUDY DESIGN AND SUBJECTS

This work was conducted as a case-control study nested within the Excellence Project entitled *"Role of mother's nutrition and genetics on the programming and development of fetal adipose tissue. Searching for early risk biomarkers to develop obesity"* (PREOBE study) supported by Spanish Government, Innovation, Science and Company Ministry (Andalusian Government) (Project Id. P06-CTS-02341) (Clinicaltrial.gov identifier: NCT01634464) (139). The project was approved by the Bioethical Committees for Clinical Research of the Clinical University Hospital San Cecilio and the Mother-Infant University Hospital of Granada, Granada, Spain. An ethical approval was also obtained by the Research Bioethical Committee of the University of Granada.

The PREOBE study was designed as a prospective observational cohort study. Participants were recruited at the Clinical University Hospital San Cecilio and the Mother-Infant University Hospital of Granada between 2008 and 2012. Pregnant women attending antenatal clinical visits were invited to participate in the study. Detailed oral and written information about study aims and procedures was provided to the mothers. The inclusion criteria were: single pregnancy at 12-20 weeks of gestation, pre-pregancy BMI (pBMI) $\geq$18.5 kg/m^2, age between 18 and 45 years, no simultaneous participation in any other research study, no drug treatment, no vegan diet, and no diagnosed diseases other than obesity, overweight or gestational diabetes.

From all contacted subjects, 474 agreed to participate and provided a written inform consent before any study procedure were performed. Out 474 subjects, 143 were consider as screen failure (124 declined to attend the first study visit and 19 were found not eligible after screening visit and prior first study visit) (Figure 10) (140). From 331 women finally enrolled, the current study was only performed in those participants which placental tissue was collected at delivery (total n = 151).

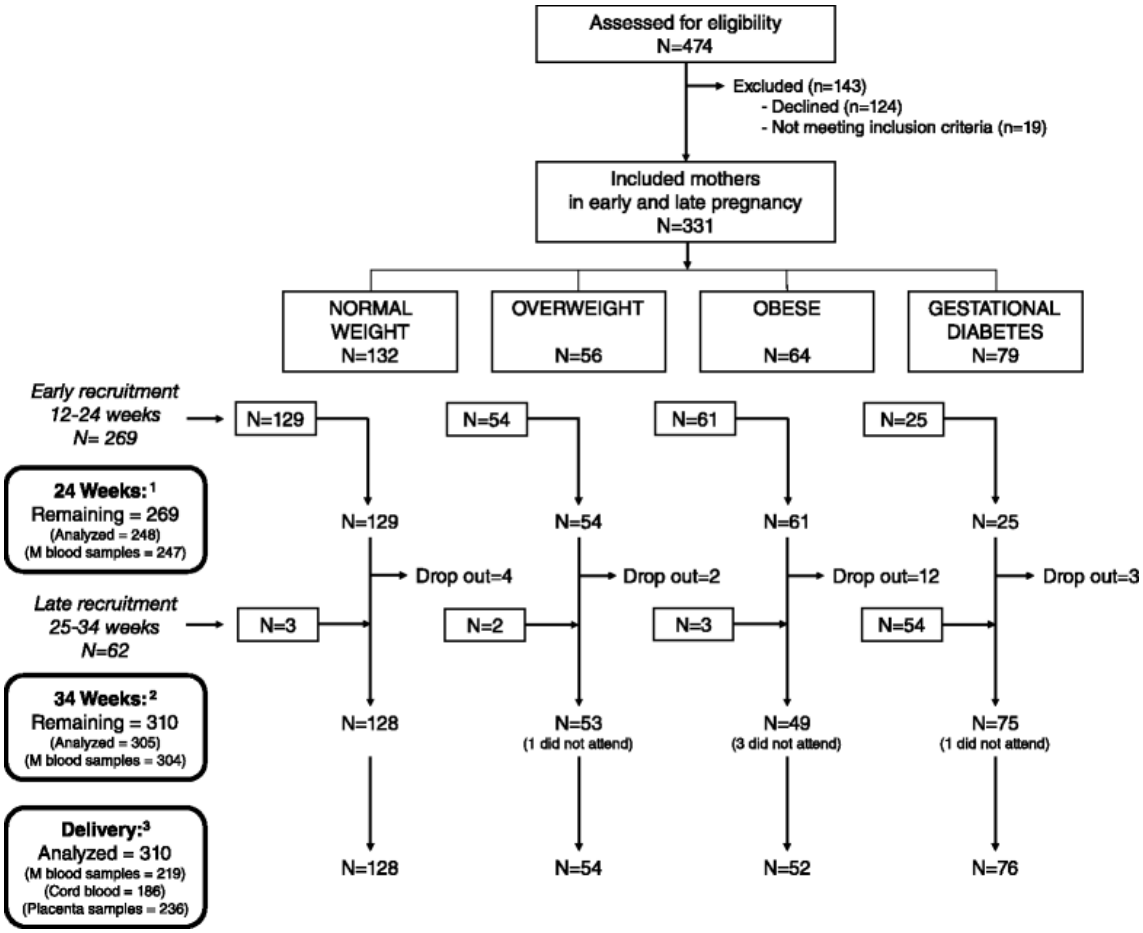

Figure 10. *Flow chart representing the PREOBE subject follow up. [1]Of the 269 mothers included before 24 weeks, 248 remained at delivery. [2]At 34 weeks, 21 subjects had dropped out and 5 cases abstained the visit, two of them due to preterm birth. [3]At delivery, 245 cases called the study staff to collect blood samples from mothers and umbilical cord, and placental samples. For remaining cases, only delivery record data was collected. Berglund et al. (140).*

According to the WHO criteria for pBMI (141), subject were classified in 3 groups as normal weight, overweight and obese. BMI calculation was done based in self-reported pre-gravide weight and height measured at study entry. Those normal weight women who developed GDM were included in a fourth group (Table 2) (142). The diagnosis of GDM was made by the local clinicians at the hospital following the Andalusian Healthcare Process during pregnancy. As standard of care all pregnant women were screening for GDM at 24-28 weeks of pregnancy with a 50 g 1-hour glucose load test (O'Sullivan test). Subject with positive screening test (venous plasma glucose ≥ 140 mg/dL one hour after glucose challenge) were asked to perform a 100 g 3-hours oral glucose tolerance test (OGTT). Women were diagnosed with GDM when two or more glucose values meet or exceed the criteria of The National

Diabetes Data Group *(fasting, 1-hour, 2-hour, and 3-hour plasma glucose levels of 105 mg/dL, 190 mg/dL, 165 mg/dL, and 145 mg/dL, respectively).* Since GDM was diagnosed around 28 weeks, those subjects enrolled into the study before were shifted from the correspond BMI group to the GDM one. Due to the limited number of mothers diagnosed with GDM after the study entry, 54 subjects were recruited directly after GDM diagnosis at the prenatal endocrinology consulting (late recruitment 25-34 weeks, see figure 10). For this work, we selected only gestational diabetic pregnant women with normal weight to evaluate the effect of gestational diabetes on placenta alone, without the combination of obesity.

Table 2. Group classification for the PREOBE study

Normal weight (control) (Kg/m^2)	$18.5 \leq BMI < 25$
Overweight (Kg/m^2)	$25 \leq BMI < 30$
Obese (Kg/m^2)	$BMI \geq 30$
Gestational Diabetes Mellitus	Positive Oral Glucose Tolerance Test

10 DATA AND SAMPLES COLLECTION

Data from pregnancy and delivery were collected throughout the study. At screening/recruitment visit, data regarding maternal pre-pregnancy weight, age, educational level, parity and smoking habit were collected. Gestational age was estimated by obstetricians according to the last menstrual period and confirmed by first-trimester ultrasonography. Maternal venous serum was collected and fetal anthropometric measurements by ultrasound were performed at study visits (24 and 34 weeks of gestation).

Study participants were asked to contact the research team when they were admitted at the hospital for delivery. Whenever possible, study staff collected placental tissue and cord blood samples. Also, placental weight and length were measured. Maternal weight at delivery was collected from medical records and weight gain during pregnancy was calculated as the difference between weight at delivery and pre-gestational weight. Gestational weight gain was classified as low, recommended or excessive based on 2009 Institute of Medicine (IOM) guidelines (Table 3) (143). After delivery, data regarding newborn weight, length, sex, gestational age and delivery mode were collected from medical records.

Table 3. Total gain weight recommendation during pregnancy according pre-pregnancy BMI

Pre-pregnancy BMI	Total weight gain	
	Range in kg	Range in Ibs
Underweight (< 18.5 kg/m^2)	12.5–18	28–40
Normal weight 18.5 ≤ BMI < 25 18.5 kg/m^2)	11.5–16	25–35
Overweight (25 ≤ BMI <30 kg/m^2)	7–11.5	15–25
Obese (BMI ≥30 kg/m^2)	5-9	11-20

Institute of Medicine recommendations, 2009 (143).

10.1 Blood Samples collection

Maternal venous blood was collected at study visits and delivery using a Vacutainer system. Venous cord blood was sampled immediately after clamping the cord by a member of the research team. Blood for serum analysis were collected in serum tube containing clot activator and gel for serum separation (Vacutainer, Becton Dickinson, USA) and were centrifuged at 3500 r.p.m. for 15 min. Samples were sent to the hospital local laboratory for biochemical analysis.

10.2 Placental samples collection

Placenta was collected, weighed and measured after vaginal delivery or caesarean section within less than 30 minutes. Approximately 1g of placenta tissue was cut, immediately frozen and stored at -80°C until lipid analysis.

A second placental piece of ~0.5 x 0.5 x 0.5 cm was cut with a sharp knife, washed several times in 0.9% NaCl solution to eliminate blood residues and immediately placed into a nuclease free vial (Greiner Bio One, USA) containing 2ml RNAlater solution (RNA Stabilization Reagent, QIAGEN, Hilden, Germany) for RNA stabilization. Placental samples were stored at 80°C until genes expression analysis.

11 SAMPLE ANALYSIS

11.1 Biochemical analysis

Glucose, total cholesterol, HDL cholesterol, triglycerides levels were measured at the hospital local laboratory by enzyme-colorimetric automated methods for clinical chemistry (Modular Analytics EVO, Roche, Neuilly sur Seine Cedex, France). LDL cholesterol was estimated using Friedewald's formula (Friedewald, 1972).

11.2 Placental fatty acids content analysis

The placental FA content analysis were performed in the Division of Metabolic and Nutritional Medicine of the Dr. von Hauner Children´s Hospital (Ludwig-Maximilians-University of Munich) in Munich, Germany.

Lipid extraction

Total lipids were extracted from placental tissue according to a modified Folch procedure developed by Klingler *et al*. using chloroform:methanol (2:1 vol/vol) (144). Butylated hydroxytoluene (BHT) was used as antioxidant for minimize oxidation of PUFAs during sample preparation. 0.2-0.3 g of placenta tissue was weighed and washed several times in 0.9% NaCl solution to eliminate blood residues as much as possible and 250 µl of lipid standard was added. Placenta tissue was homogenized for 1 minute in 3 mL of [Chloroform:Methanol (2:1 vol/vol) + 5g/L BHT] using a metal-blade homogenizer (DIAX 100: Heidolph, Schwabach, Germany). Homogenizer and tube were rinsed three times with 3 mL of chloroform/methanol (2:1 vol/vol), and the washing solution was combined with the extraction solution. The mixture was heated at 35ºC for 20 minutes and filtered through a Whatman nº5 filter paper. After this, 4 mL potassium hydroxide solution (0.1 M) were added, samples were shaken carefully and centrifugated at 1500 x g for 30 minutes at 10ºC. After incubation for 20 minutes at room temperature two phases were observed. The aqueous phase, the upper one, was discarded, and the nonpolar phase containing the lipids was filtered over sodium sulfate and taken to dryness under reduced pressure.

Lipid isolation

Lipid fractions were isolated by Thin Layer Chromatography using heptane, diisopropyl ether, and acetic acid (60:40:3, vol/vol/vol) as mobile phase. For this, the dried lipid extract was dissolved in 400 µL of chloroform/methanol (1:1 vol/vol) and deposited in a 20x20 cm

silica gel plate (Merck, Darmstadt, Germany). PL, NEFA, TG and cholesterol ester fractions were visualized under 254 nm ultraviolet light after spray a solution of ethanol:2',7'-dichlorofluorescein (0.2%). The bands containing the PL and TG fractions were scraped and transferred to 4 mL glass vial provided with Teflon-lined screw caps.

Fatty acid methylation

Fatty acid methyl-esters (FAME) of PL and TG fractions were obtained by transmethylation reaction by heating at 85ºC for 30 minutes with 1.5 mL of 3 M methanolic hydrochloric acid (Supelco, Bellfonte, PA, USA). The solution was neutralized with sodium sulfate/ sodium hydrogen carbonate/ sodium carbonate (2/2/1). Methyl esters were extracted twice by adding 1 mL of hexane and centrifugation at 400 x g for 3 minutes, and the combined extracted were dryness under a gently nitrogen stream.

Fatty acid methyl esters quantification

FAME were analyzed by capillary gas-liquid chromatography with Helium as carrier gas on a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard, Waldbronn, Germany) equipped with a BPX70 column (SGE, Weiterstadt, Germany, 25 m in inner diameter 0.22 mm). Initial temperature of 130ºC was kept for 30 seconds, then heated to 150ºC at a rate of 3ºC/min, and to 180ºC at a rate of 1.5ºC/min. The rate was increased to 3ºC/min until reached final temperature of 210ºC, which was kept during 23 minutes before fall to the initial 130ºC at a rate of 15ºC/min.

Fatty acids were identified by the retention time comparison with fatty acid standards (Nu-Chek-Pre, Elysian, MN).

11.3 Placental Gene expression analysis

The placental gene expression analysis was performed in the Comprehensive Pneumology Center of the Ludwig-Maximilians-University, and Helmholtz Center of Munich, Germany.

RNA isolation

Total RNA was extracted from placental tissue stored in RNAlater solution. Frozen placenta pieces of 50-100 mg were placed in tubes prefilled with ceramic beads (MagNa Lyser Green Beads, Roche Applied Science, Germany) and 700 µl of QIAzol Lysis Reagent (Qiagen, Hilden, Germany). Placenta tissue was homogenized using a MagNA Lyser Instrument (Roche Applied Science) at 65000 rpm for 20 seconds. This step was repeated 3 times and

samples were cooled down for 60 seconds between runs using a rotor cooling block (Roche Applied Science, Germany). Total RNA was isolated from the homogenate using miRNeasy kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The RNA concentration was calculated by determining the absorbance at 260 nm with a Nanodrop-1000 spectrophotometer (NanoDrop Fluorometer, Thermo Scientific, Wilmington, DE, USA). The A260/280 ratio was used to determine protein contamination. Only samples with a ratio above 1.8 were included into this study. RNA integrity was checked by loading 1µg of RNA in 1.5% agarose gel in TBE (Tris-borate-EDTA Buffer) and visualized with SYBR Green II at 1:100 dilution. A second aliquot of 1µg of RNA was heated at 37ºC for 1 hour and loaded in 1.5% agarose gel to discard RNAasas contamination. Only samples that showed clear 28S and 18S bands were selected for further analysis.

Complementary DNA (cDNA) synthesis

cDNA was synthesized with QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) from 1.5 µg mRNA, according to the manufacturer´s instructions.

Primers design

A set of primers for every target and reference gene (Table 4) was designed by SciEd Central (1998-1999) using gene sequences from NCBI Reference Sequence Database (http://www.ncbi.nlm.nih.gov/gene). Several reference genes (*SDHA*, *B2M*, *ACTB*, *BRD1*, *KCTD2*, *HPRT*, *YWHAZ* and *TBP*) were tested for stable and constant expression in placental tissue (145). *B2M* and *YWHAZ* were selected for normalization due to their low variance among subjects and groups.

The target sequence was tested using NCBI basic nucleotide BLAST search engine (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Standard curves of serial dilutions from pooled cDNA of randomly selected samples were used to calculate the PCR efficiency for every primer pair.

Table 4. Characteristics of primers used for RT-PCR

Gene	NCBI Ref. Sequence	Forward sequence	Reverse sequence	Efficiency
FATP1	NM_198580.1	GCTAAGGCCCTGATCTTTGG	CCAAGTCTCCAGAGCAGAAC	1.89
FATP4	NM_005094.3	TGGCGCTTCATCCGGGTCTT	CGAACGGTAGAGGCAAACAA	1.92
FATP6	NM_014031.3	TGGAGAACTTTGTCGCTACC	CCATACATCACTCCGTATGC	2
FABP3	NM_004102.3	GGTGGAGTTCGATGAGACAA	TCAATTAGCTCCCGCACAAG	1.96
FABP4	NM_001442.2	GGTACCTGGAAACTTGTCTC	TTAGGTTTGGCCATGCCAGC	1.88
FABP7	NM_001446.3	CAGGCCTACCTTACTGGTGA	ACCTCGGTGGTGGACACGTT	1.97
LPL	NM_000237.2	AGTCCCGGCTTCGCCATTCA	TCACAAATACCGCAGGTGCC	2
EL	NM_006033.2	GGCCACATTGACATCTACCC	ACGGCTCGCTCATGCTCACA	1.93
FAT/ CD36	NM_000072.3	GGTACAGATGCAGCCTCATT	AGGCCTTGGATGGAAGAACA	1.98
YWHAZ	NM_001135699.1	ACTGGGTCTGGCCCTTAAC	GCGTGCTGTCTTTGTATGAC	1.94
B2M	NM_004048.2	AGTATGCCTGCCGTGTGAAC	TGCGGCATCTTCAAACCTCC	1.94

FATP: Fatty acid transport protein, FABP: Fatty acid binding protein, LPL: Lipoprotein lipase, EL: Endothelial lipase, FAT/CD36: Fatty acid translocase, YWHAZ: Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Zeta, B2M: Beta-2-Microglobulin.

Quantitative real-time PCR (RT-PCR)

PCR reactions were set up with 2.5 µl cDNA, 0,6 µl primer (1:10 dilution), 1.9 µl nuclease free water (Roche Applied Science, Germany) and 5 µl 2X SYBR Green Master Mix (Roche Applied Science, Germany). Each sample was analyzed by triplicated. Reactions were performed in 96-wells plates using Light Cycler 480 system (Roche Applied Science, Germany). Cycling conditions for the RT-PCR included an initial denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 15 s, synthesis at 72°C for 10 s and a final elongation step at 60°C for 1 min. The final products were verified by melting curve analysis by using the provided software (Roche, Mannheim, Germany).

Expression values of target genes were normalized to the geometric mean of the expression of B2M and YHWAZ housekeeping genes using the software GenEx Pro (http://genex.gene-quantification.info/).

12 DATA ANALYSIS

Statistical analyses were performed using SPSS 24.0 software (SPSS Inc., Chicago, IL. USA). Firs, we determined the data distribution by Kolmogorov-Smirnov test. Parametric test was used for normally distributed variables. Thus, fatty acid composition was evaluated by analysis of variance (ANOVA) followed by multiple comparisons with Bonferroni correction, and their values were expressed as Mean and Standard Deviation (SD). Maternal serum biochemistry parameters and mRNA expression showed non-normal distribution; therefore, group comparison to normal weight group was performed by Mann-Whitney U test. Data with non-normal distribution were expressed as median and interquartile range. Correlations between different variables were assessed by Spearman's correlation coefficients. P values < 0.05 were considered statistically significant.

RESULTS

13 Clinical characteristics

Participants were classified at study entry according to their BMI in three groups: normal weight, overweight and obese at study entry. Mothers with normal weight who developed GDM were included in a fourth group. The clinical characteristics of study participants are listed in **Table 5**. As expected, pre-pregnancy BMI was high in overweight and obese group but not in GDM compared to normal weight. The maternal age average was similar among groups. The gestational weight gain was significantly lower in the obese and GDM group compared to normal weight ($P < 0.005$). Obese mothers achieved the average lower gain weight values (6 kg). According to the IOM gestational gain weight guidelines (143), more than 40% of participants included in normal, obese and GDM groups had a gestational weight gain below recommendation, while the 43.3% of overweight subjects achieve the recommended weight gain. Not significant differences were found in respect delivery in terms of gestational age, mode of delivery, baby sex, number of term pregnancies and smoking during pregnancy compared to normal weight group (Table 5 and 6). Blood pressure was slightly higher in overweight and obese pregnant women at 24 weeks of gestation, but no women developed PIH. Both placenta and newborn weight were significantly higher in the obese group compared to normal weight mothers ($P < 0.05$), but placenta efficiency estimated by the ratio of newborn and placenta weight did not differ between groups. At birth, abdominal circumference was higher in infants born to obese mothers compared to those born to mothers with normal weight. No differences were found in other anthropometric measures including head circumference, length and ponderal index among (Table 6).

Biochemical parameters were also analyzed in maternal blood during pregnancy and at delivery. Due to late GDM diagnosis, only 3 cases were included in the GDM group at 24 weeks (Table 7). Glycated hemoglobin (HbA1c) was significantly higher in overweight, obese and GDM compared to normal weight women at 24 and 34 weeks of gestation. Serum glucose level was also significantly higher in the three studied groups at 24 weeks but not at 34 weeks and delivery (Table 7). Total cholesterol and triglycerides levels were higher in obese women through pregnancy, but these differences were not statistically significant. HDL cholesterol was lower in overweight, obese and GDM women at 24 weeks of gestation, while only was found lower in obese

mothers at 34 weeks. At delivery, percentage of HbA1c was significantly higher, but within the normal range, in overweight, obese and GDM compared to normal weight women. There were no significant differences among groups in other biochemical parameters analyzed, neither in maternal nor in venous cord blood (Table 7).

Table 5. Maternal clinical characteristics during pregnancy and at delivery

	Normal weight (n = 56)	Overweight (n = 35)	Obese (n = 36)	GDM (n = 24)
Maternal Age (years)	31 (5)	31(6)	31 (6)	32 (6)
pBMI (Kg/m^2)	21.79(2.78)	26.92 (2.20)*	32.86 (3.73)*	22.18 (3.39)
SBP (mmHg) at 24 weeks	114.69 (17)	123.50 (24) **	130 (18)**	113 (20)
DBP (mmHg) at 24 weeks[a]	65 (11)	71.50 (10)**	75 (12)**	69.50 (10)
SBP (mmHg) at 34 weeks[a]	121 (18)	120 (16)	122 (14)	111 (15)*
DBP (mmHg) at 34 weeks[a]	70 (11)	73 (13)	73 (15)	69 (12)
Gestational weight gain (Kg)	12(6)	10.95 (5)	6 (10)*	7.50 (9.50)*
Gestational Age (weeks)	39 (2)	40 (2)	40 (2)	39.50 (1)
IOM GWG recommendation				
Lower (% participants)	43.8	20.0	43.3	66.7
Recommended (% participants)	37.5	43.3	23.3	27.8
High (% participants)	18.8	36.7	33.3	5.6
Epidural Anesthesia (%)	57.70	54.20	53.80	45.50
Vaginal delivery (%)	86.50	65.50	70.40	75.00
Nulliparous (%)	52.80	55.60	47.10	57.10
Non-smoker (%)	86.30	93.90	90.90	95.00

*Data are expressed as median and interquartile range (in parenthesis); Mann-Whitney U tests were performed to investigate differences between groups; Highlighted text box: *Statistically significant different compared to normal weight women (P < 0.05), **Statistically significantly different compared to normal weight women (P< 0.001). pBMI: Pre-gestational Body Mass Index; GDM: Gestational Diabetes Mellitus; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure, IOM: Institute of Medicine, GWG: gestational weight gain.*

Table 6. Placental and neonate characteristics at birth

	Normal weight (n = 56)	Overweight (n = 35)	Obese (n = 36)	GDM (n = 24)
Head circumference (cm)	34.50 (1.75)	34.50 (1.13)	35 (2.25)	35 (1.5)
Arm circumference (cm)	11 (1.25)	11 (1.50)	11.40 (1.50)	11.25 (1.38)
Chest circumference (cm)	33 (2)	33.25 (2.38)	34.75 (3)	34 (2.88)
Abdominal circumference (cm)	33 (2)	31.65 (2.25)	34 (3.53)*	33 (2.90)
Length (cm)	50 (2)	50 (1)	51 (3)	50 (1)
Birth weight (g)	3260 (445)	3320 (710)	3540 (637)*	3470 (580)
Ponderal Index (g/cm^3x100)	2.54 (0.32)	2.66 (0.44)	2.61 (0.26)	2.61 (0.50)
Placental weight (g)	500 (140)	500 (180)	550 (222)*	510 (185)
Newborn/placental weight ratio	6.92 (2.06)	6.31 (1.51)	6.33 (1.66)	6.34 (2.03)
Gender (Male) (%)	51.80	45.70	63.90	50.00

*Data are expressed as median and interquartile range (in parenthesis); Mann-Whitney U tests were performed to investigate differences between groups; Highlighted text box: *Statistically significant different compared to normal weight women (P < 0.05)*

Table 7. Maternal and cord serum biochemistry

	Normal weight	Overweight	Obese	GDM
***24 weeks of gestation*[1]**	(n = 49)	(n = 27)	(n = 27)	(n = 3)
Glucose (mg/dl)	75 (11.50)	86 (22)*	83 (24)*	104*
HbA1c (%)	4.2 (0.35)	4.5 (0.70)*	4.8 (0.80)*	6.5*
Total Cholesterol (mg/dl)	244 (52)	253 (49)	259 (59)	213
Triglycerides (mg/dl)	145 (123)	171(80)	181 (108)	192
HDL Cholesterol (mg/dl)	81(23)	64 (23)*	74 (27)*	65*
LDL Cholesterol (mg/dl)	126 (49)	137 (36)	140 (56)	96
***34 weeks of gestation*[1]**	(n = 49)	(n = 24)	(n = 26)	(n= 16)
Glucose (mg/dl)	81 (23.50)	85 (14.25)	87 (23.25)	88 (12.50)
HbA1c (%)	4.4 (0.40)	5.05 (0.98)*	4.85 (0.83)*	4.8 (0.68)*
Total Cholesterol (mg/dl)	257 (58.50)	259 (66.75)	265.5 (71)	248 (68.50)
Triglycerides (mg/dl)	237 (86.50)	240 (139)	276.5 (118.50)	228 (128.75)
HDL Cholesterol (mg/dl)	68 (23)	71 (21.75)	64.5 (9.75)*	73.5 (15.75)
LDL Cholesterol (mg/dl)	143 (67)	142 (50.75)	146.5 (59.25)	137.5 (70.38)
***Delivery*[1]**	(n = 44)	(n = 27)	(n = 26)	(n = 17)
Glucose (mg/dl)	81 (25)	87.50 (35)	92 (57)	104 (50)
HbA1c (%)	4.55 (0.40)	5.05 (0.63)*	5.20 (1.03)*	5.10 (0.70)*
Total Cholesterol (mg/dl)	248 (55)	246 (35)	261 (65)	231 (78)
Triglycerides (mg/dl)	215 (99)	228 (79)	242 (122)	215 (57)
HDL Cholesterol (mg/dl)	70 (29)	62 (23)	70 (18)	65 (43)
LDL Cholesterol (mg/dl)	127 (52)	139 (30)	140 (67)	113 (59)
Umbilical Cord Serum	(n =31)	(n =20)	(n =20)	(n = 12)
Glucose (mg/dl)	71 (28)	64 (30)	66 (38)	71 (24)
Total Cholesterol (mg/dl)	68 (25)	64 (11)	64 (16)	72 (20)
Triglycerides (mg/dl)	42 (10)	52 (29)	47 (30)	43 (20)
HDL Cholesterol (mg/dl)	29 (21)	21 (12)	26 (8)	28 (12)
LDL Cholesterol (mg/dl)	31 (17)	29 (10)	28 (10)	28 (10)

[1]*Maternal samples. Data are expressed as median and interquartile range (in parenthesis); Mann-Whitney U tests were performed to investigate differences between groups; Highlighted text box: *Statistically significant different compared to normal weight women (P<0.05). HbA1c: Glycated hemoglobin; HDL: High density lipoprotein; LDL: Low density lipoprotein.*

Fetal biometry was measured by ultrasound at 24 and 34 weeks of gestation. Sonographic markers of fetal adiposity (anterior abdominal wall thickness, upper and lower limb fat mass) and estimated fetal adiposity at 24 and 34 weeks of gestation were higher in fetus from obese women compared to those from normal weight participants. At 34 weeks of gestation estimated fetal adiposity was significantly higher in overweight and GDM group as well (Table 8).

Table 8. Fetal anthropometric measurements during gestation

	24 weeks of gestation				***34 weeks of gestation***			
	NW (n = 53)	OW (n = 28)	OB (n = 33)	GDM (n = 8)	NW (n = 53)	OW (n = 31)	OB (n = 31)	GDM (n = 21)
Biparietal diameter (cm)	5.92 (0.48)	6.11 (0.52)*	6.10 (0.52)*	6.06 (0.59)	8.50 (0.30)	8.59 (0.30)	8.63 (0.59)*	8.70 (0.61)
Head Circumference (cm)	22.22 (1.05)	22.35 (1.95)	22.28 (1.22)	22.60 (2.31)	30.45 (1.50)	30.40 (1.33)	30.87 (1.51)	31 (2.67)
Abdominal circumference (cm)	19.97 (1.39)	20.33 (1.69)	20.40 (1.73)	20.55 (2.59)	30.02 (1.76)	30.22 (1.97)	30.51 (2.49)	30.22 (2.20)
Femur length (cm)	4.35 (0.32)	4.45 (0.48)	4.44 (0.27)	4.40 (0.61)	6.61 (0.20)	6.62 (0.28)	6.63 (0.52)	6.60 (0.38)
Estimated fetal weight (g)	699 (131)	740 (184)*	732 (146)	780 (241)	2362 (423)	2406 (423)	2419 (533)	2574 (585)
Check to check diameter (cm)	3.24 (0.48)	3.11 (0.87)	3.11 (0.89)	3.74 (0.74)	4.30 (0.63)	4.11 (1.21)*	4.10 (1.67)*	4.52 (1.10)
Anterior abdominal wall thickness (cm)	0.34 (0.09)	0.33 (0.12)	0.41 (0.18)*	0.37 (0.19)	0.44 (0.16)	0.55 (0.24)*	0.60 (0.24)*	0.71 (0.59)*
Upper limb fat mass (cm^2)	0.30 (0.06)	0.29 (0.09)	0.33 (0.19)*	0.35 (0.18)*	0.38 (0.10)	0.44 (0.15)	0.46 (0.27)*	0.52 (0.40)*
Lower limb fat mass (cm^2)	0.33 (0.10)	0.33 (0.15)	0.38 (0.16)*	0.37 (0.30)	0.41 (0.12)	0.46 (0.16)	0.53 (0.29)*	0.59 (0.32)*
Estimated fetal adiposity (arbitrary units)	-0.22 (0.55)	-0.22 (0.88)	0.17 (1.63)*	0.46 (3.22)	-0.32 (0.52)	-0.08 (1.18)*	0.22 (0.98)*	0.78 (2.03)*

*Data are expressed as median and interquartile range (in parenthesis); Mann-Whitney U tests were performed to investigate differences between groups Highlighted text box: *Statistically significant different compared to normal weight women (P<0.05). NW: normal weight, OW: overweight, OB: obese, GDM: diabetes gestation*

14 Placental Fatty acids content

The mean percentage FA of phospholipid and triglyceride fractions of the four groups are presented in tables 9 and 10, respectively. Not significant differences among study groups were observed in both total FA phospholipids and total FA triglycerides. Full term placentas from both obese and GDM women showed significantly lower levels of both total SFA phospholipids and triglycerides than those from normal weights. The decreases are due mainly to a reduction in both stearic acid (18:0) and lignoceric acid (24:0) ($P < 0.05$).

Percentages of eicosapentaenoic acid (EPA, 20:5n3), docosatetraenoic acid (22:4n6), and docosapentaenoic acid (22:5n3) in phospholipid fraction were higher in placenta from overweight, obese and GDM than the control group. As consequence, total LCPUFAs tend to be higher in the three studied groups, but such differences were found only statistically significant in placentas from obese women (Table 9).

While differences in some specific fatty acids were observed in triglyceride fractions, total MUFAs, SCPUFAs and LCPUFAs were found unchanged among groups (Table 10).

Table 9. Percentage of FA of full-term placental phospholipids fraction

Fatty acids	Normal Weight (n =36)	Overweight (n = 18)	Obese (n = 17)	GDM (n = 16)
14:0	0.38 (0.08)	0.37 (0.10)	0.31 (0.08)	0.36 (0.05)
16:0	26.42 (1.21)	25.72 (0.9)	26.69 (0.92)	26.37 (0.88)
17:0	0.31 (0.05)	0.31 (0.03)	0.29 (0.04)	0.33 (0.04)
18:0	11.99 (0.76)	12.69 (0.72)*	11.54 (0.64)	11.35 (0.90)*
20:0	0.31 (0.06)	0.33 (0.10)	0.29 (0.07)	0.27 (0.04)
22:0	1.38 (0.23)	1.48 (0.34)	1.25 (0.25)	1.06 (0.10)**
24:0	1.65 (0.28)	1.67 (0.45)	0.97 (0.57)**	1.25 (0.29)**
Total SFA	42.47 (1.24)	42.59 (1)	41.36 (1.18)*	41.06 (1.06)**
16:1n7	0.43 (0.11)	0.40 (0.12)	0.37 (0.10)	0.38 (0.05)
18:1n9	8.89 (1.19)	8.56 (1.12)	8.45 (0.66)	9.28 (0.82)
18:1n7	1.61 (0.17)	1.74 (0.18)	1.57 (0.15)	1.60 (0.12)
20:1n9	0.16 (0.05)	0.19 (0.05)	0.19 (0.02)	0.21 (0.03)*
22:1n9	0.18 (0.10)	0.12 (0.05)	0.19 (0.08)	0.28 (0.22)
24:1n9	1.60 (0.39)	1.40 (0.48)	1.20 (0.35)*	1.20 (0.35)*
Total MUFA	12.90 (1.54)	12.42 (1.60)	11.99 (0.86)	12.97 (1.05)
18:2n6	9.46 (1.08)	9.19 (1.35)	9.27 (1.16)	9.20 (1.02)
18:3n6	0.13 (0.04)	0.11 (0.04)	0.10 (0.03)	0.09 (0.02)*
18:3n3	0.02 (0.01)	0.02 (0.01)	0.01 (0.01)	0.02 (0.00)
18:4n3	0.05 (0.07)	0.07 (0.06)	0.02 (0.03)	0.02 (0.01)
Total SCPUFA	9.67 (1.08)	9.40 (1.38)	9.42 (1.38)	9.35 (1.03)
20:2n6	0.42 (0.10)	0.39 (0.10)	0.44 (0.07)	0.43 (0.05)
20:3n9	0.12 (0.04)	0.13 (0.06)	0.10 (0.04)	0.14 (0.02)
20:3n6	4.88 (0.93)	4.51 (0.94)	4.65 (0.76)	4.33 (0.68)
20:4n6	21.36 (1.46)	21.74 (1.61)	22.32 (1.70)	22.33 (1.69)
20:3n3	0.09 (0.05)	0.12 (0.03)	0.10 (0.036)	0.10 (0.02)
20:5n3	0.04 (0.06)	0.12 (0.05)**	0.10 (0.06)*	0.11 (0.07)*
22:2n6	0.25 (0.05)	0.25 (0.05)	0.24 (0.05)	0.22 (0.03)
22:4n6	1.44 (0.24)	1.73 (0.19)**	1.74 (0.17)**	1.65 (0.17)*
22:5n6	0.93 (0.24)	0.98 (0.16)	1.70 (0.55)**	0.99 (0.24)
22:5n3	0.31 (0.35)	0.66 (0.27)**	0.54 (0.23)	0.57 (0.19)*
22:6n3	4.52 (0.68)	4.40 (0.64)	4.49 (0.73)	4.89 (0.87)
Total LCPUFA	34.40 (1.77)	35.09 (2.17)	36.47 (1.82)*	35.81 (1.91)
Total FA (mg/g weight)	7.33 (1.31)	7.11 (1.53)	7.48 (1.23)	6.89 (0.69)

*Data are expressed as mean and standard deviation (in parenthesis). One-way analysis of variance (ANOVA) was performed to evaluate group differences. Highlighted text box: *Statistically significant different compared to normal weight women (P<0.05) **Statistically significant different compared to normal weight women (P< 0.001). FA: Fatty acid, SFA: saturated FA, MUFA: monounsaturated FA, SCPUFA: short chain polyunsaturated FA, LCPUFA: long chain polyunsaturated FA, GDM: Gestational Diabetes.*

Table 10. Percentage of FA of full-term placental triglycerides fraction

Fatty acids	Normal Weight (n =36)	Overweight (n = 18)	Obese (n = 17)	GDM (n = 16)
14:0	2.86 (1.53)	1.64 (1.20)*	2.46 (1.31)	1.63 (0.58)*
16:0	24.76 (2.77)	25.51 (3.15)	23.64 (3.19)	23.49 (1.66)
17:0	0.59 (0.30)	0.45 (0.10)	0.45 (0.21)	0.46 (0.11)
18:0	9.39 (1.71)	8.38 (1.47)	7.85 (0.80)*	8.18 (1.01)*
20:0	0.26 (0.12)	0.23 (0.05)	0.23 (0.09)	0.28 (0.09)
22:0	0.28 (0.14)	0.22 (0.15)	0.24 (0.08)	0.21 (0.10)
24:0	0.16 (0.12)	0.23 (0.17)	0.07 (0.09)*	0.04 (0.05)*
Total SFA	38.33 (4.56)	36.69 (3.47)	34.96 (3.94)*	34.32 (1.73)*
16:1n7	2.05 (0.77)	2.42 (1.13)	2.06 (0.68)	1.86 (0.37)
18:1n9	18.30 (3.15)	18.76 (4.38)	17.95 (3.97)	18.91 (2.90)
18:1n7	1.74 (0.35)	1.95 (0.38)	1.79 (0.35)	1.75 (0.18)
20:1n9	0.56 (0.24)	0.35 (0.09)	0.49 (0.17)	0.53 (0.13)
22:1n9	0.21 (0.26)	0.17 (0.13)	1.17 (2.36)	2.10 (3.15)
24:1n9	0.32 (0.24)	0.28 (0.25)	0.22 (0.15)	0.27 (0.23)
Total MUFA	23.19 (3.30)	23.95 (4.20)	23.70 (4.30)	25.45 (3.33)
18:2n6	12.76 (2.27)	13.69 (2.32)	13.25 (2.02)	12.33 (3.81)
18:3n6	0.29 (0.12)	0.27 (0.08)	0.34 (0.07)	0.32 (0.07)
18:3n3	0.17 (0.05)	0.18 (0.05)	0.28 (0.39)	0.18 (0.06)
18:4n3	0.18 (0.15)	0.54 (1.19)*	0.10 (0.13)	0.10 (0.16)
Total SCPUFA	13.42 (2.31)	14.71 (2.59)	13.98 (2.16)	12.95 (3.85)
20:2n6	0.79 (0.38)	0.47 (0.17)*	0.56 (0.20)*	0.56 (0.15)*
20:3n9	0.11 (0.09)	0.12 (0.07)	0.13 (0.04)	0.13 (0.06)
20:3n6	5.15 (1.82)	4.57 (1.63)	5.10 (1.57)	4.84 (1.43)
20:4n6	10.27 (2.17)	10.70 (2.98)	11.17 (2.29)	11.10 (2.28)
20:3n3	0.06 (0.08)	0.10 (0.06)	0.06 (0.04)	0.06 (0.03)
20:5n3	0.17 (0.15)	0.30 (0.23)	0.25 (0.13)	0.34 (0.19)*
22:2n6	0.05 (0.05)	0.07 (0.03)	0.07 (0.06)	0.10 (0.04)*
22:4n6	1.30 (0.44)	1.47 (0.35)	1.55 (0.28)	1.47 (0.37)
22:5n6	0.84 (0.25)	0.87 (0.23)	1.24 (0.41)*	1.03 (0.29)
22:5n3	0.25 (0.34)	0.43 (0.28)	0.52 (0.30)*	0.55 (0.37)*
22:6n3	3.20 (1.06)	3.07 (1.07)	3.41 (1.12)	4.03 (1.11)
Total LCPUFA	22.24 (4.90)	22.23 (5.65)	24.10 (4.64)	24.25 (4.93)
Total FA (mg/g weight)	0.42 (0.19)	0.52 (0.46)	0.44 (0.16)	0.39 (0.13)

*Data are expressed as mean and standard deviation (in parenthesis). One-way analysis of variance (ANOVA) was performed to evaluate group differences. Highlighted text box: *Statistically significant different compared to normal weight women (P<0.05) **Statistically significant different to normal weight women (P<0.001). FA: Fatty acids, SFA: saturated FA, MUFA: monounsaturated FA, SCPUFA: short chain polyunsaturated FA, LCPUFA: long chain polyunsaturated FA, GDM: Gestational Diabetes.*

The effect of weight gain during pregnancy on placental fatty acids was evaluated as well. Participants were classified according the IOM GWG guidelines in three groups and percentage of placental FAs in both phospholipid and triglyceride fraction were compared among groups.

The percentages of FAs were found unchanged among groups. Only total FA in triglyceride fraction was lower in placentas from women with GWG below recommendations (Table 11).

Table 11. Placental FA according to IOM GWG classification

	Low GWG (n = 30)	Recommended GWG (n = 25)	High GWG (n = 12)
Phospholipids fraction			
SFA (%)	41.79 (1.34)	42.34 (1.03)	41.93 (1.46)
MUFA (%)	13.21 (1.65)	12.66 (1.43)	12.22 (1.35)
SCPUFA (%)	9.14 (1.12)	9.97 (0.69)	9.58 (1.37)
LCPUFA (%)	35.36 (1.99)	34.56 (1.60)	35.79 (2.74)
Total FA (mg/ g weight)	7.43 (1.32)	7.08 (1.12)	7.49 (1.37)
Triglyceride fraction			
SFA (%)	36.74 (4.39)	36.71 (4.46)	36.68 (3.37)
MUFA (%)	24.90 (4.02)	23.83 (3.59)	23.88 (3.88)
SCPUFA (%)	13.12 (2.67)	13.87 (3.02)	13.66 (2.91)
LCPUFA (%)	22.78 (5.26)	23.08 (5.17)	23.39 (5.22)
Total FA (mg/ g weight)	0.37 (0.14)*	0.56 (0.41)	0.43 (0.16)

*Data are expressed as mean and standard deviation (in parenthesis). One-way analysis of variance (ANOVA) was performed to evaluate group differences. Highlighted text box: *Statistically significantly different compared to normal weight women (P<0.05). IOM: Institute of Medicine, GWG: Gestational weight gain, FA: Fatty acids, SFA: saturated FA, MUFA: monounsaturated FA, SCPUFA: short chain polyunsaturated FA, LCPUFA: long chain polyunsaturated FA.*

15 Placental Gene Expression

The relative mRNA expression of placental fatty acid transporters (*FATP1, FATP4, FATP6, FAT/CD36*), fatty acid binding proteins (*FABP3, FABP4, FABP7*) and lipases (*EL, LPL*) were evaluated in our population.

Due the way of delivery may influence RT-PCR results, we first checked if delivery by caesarean section affected mRNA expression levels. Since no differences were found between way of delivery, we proceeded by analyzing the relative placental expression level among the four study groups (normal weight (n =37), overweight (n = 31), obese (n = 31) and women with GDM (n = 19)).

The placental relative expression of *FATP1* and *FATP4* were decreased in the three studied groups compared to normal weight. *FAT/CD36* mRNA increased among groups, while *FATP6* mRNA expression was higher in both obese and GDM (Figure 11).

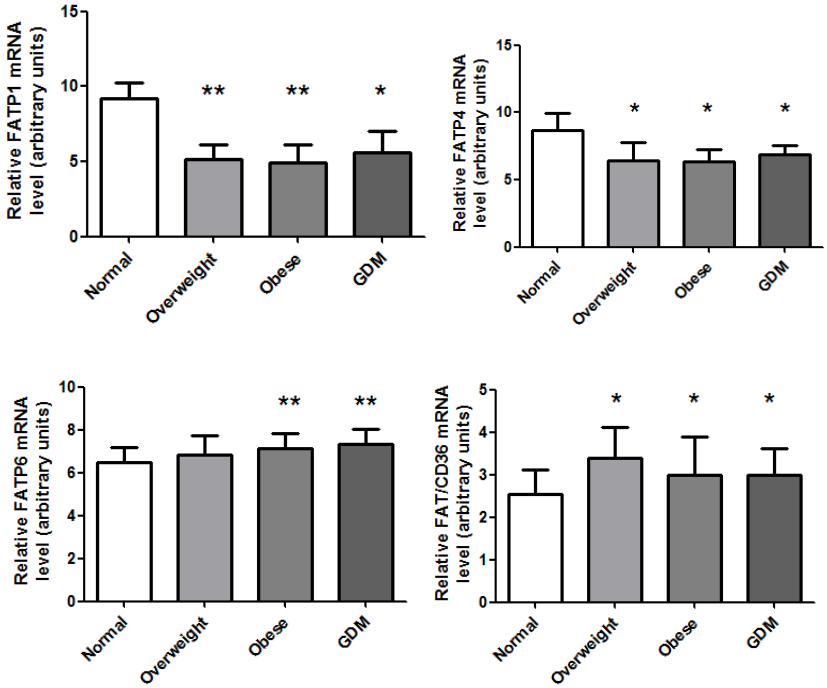

Figure 11. *Relative placental mRNA expression of fatty acid transporters. Normalized mRNA expression, to B2M and YHWAZ, is presented as median and interquartile range (IQR). *Statistically significant different compared to normal weight women (P< 0.05). **Significantly different to normal weight women (P< 0.001). FATP1: Fatty acid transporter protein 1, FATP4: Fatty acid transporter protein 4, FATP6: Fatty acid transporter protein 6, FAT/CD36: Fatty acid translocase.*

FABP4 and *FABP7* mRNA were decreased in both obese and overweight women, but *FABP3* mRNA only slightly decreased in obese group. Interestingly, *FABPs* were not significantly affected by GDM (Figure 12).

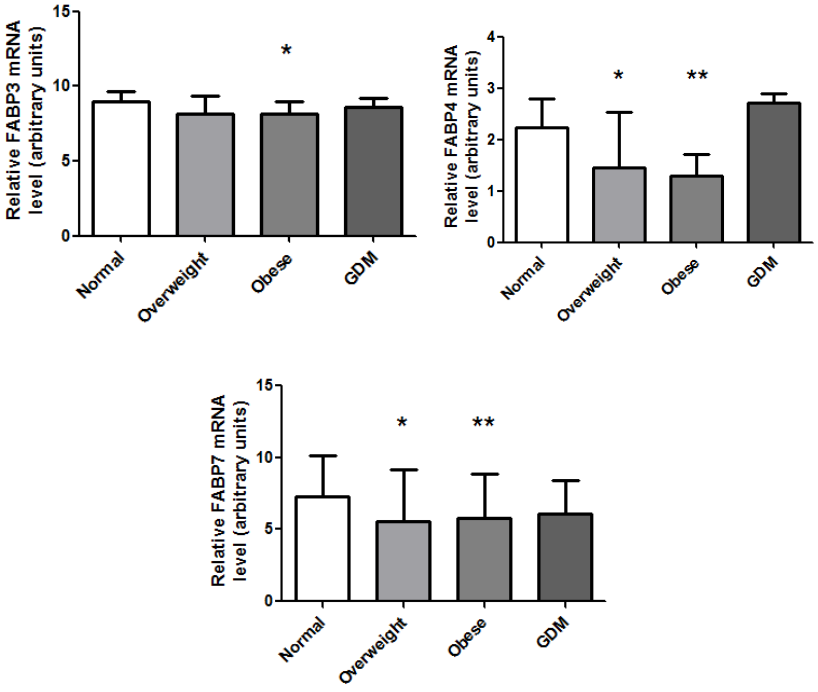

Figure 12. *Relative placental mRNA expression of fatty acid binding proteins. Normalized mRNA expression, to B2M and YHWAZ, is presented as median and interquartile range (IQR). *Statistically significantly different compared to normal weight women (P< 0.05). **Statistically significantly different compared to normal weight women (P< 0.001). FABP3: Fatty acid binding protein 3, FABP 4: Fatty acid binding protein 4, FABP7: Fatty acid binding protein 7.*

Regarding the enzymes, *EL* mRNA expression was decreased in the three studied groups compared to normal weight, but the relative expression of *LPL* mRNA remains unchanged among groups (Figure 13).

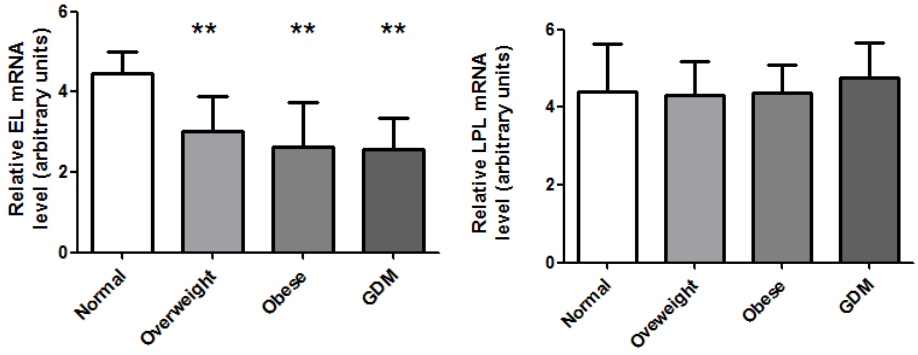

Figure 13. *Relative placental mRNA expression of enzymes. Normalized mRNA expression, to B2M and YHWAZ, is presented as median and interquartile range (IQR). *Statistically significant different compared to normal weight women (P< 0.05). **Statistically significant different compared to normal weight women (P< 0.001). EL: Endothelial lipase, LPL: Lipoprotein lipase.*

The effect of GWG on placental mRNA expression was explored as well. When women were classified according their GWG instead of their pre-pregnancy way mRNA expression was almost unchanged. Only the expression of FABP4 decreased in placentas from women with weight gain beyond IOM recommendation compared to those from women who achieve the recommended IOM weight gain (Table 12).

Table 12. Placental gene expression according to IOM GWG classification

Placental gene expression (arbitrary units)	Low GWG (n = 35)	Recommended GWG (n = 24)	High GWG (n = 26)
FATP1	5.61 (4.07)	5.71 (5.167)	5.24 (6.36)
FATP4	6.75 (2.69)	7.06 (2.31)	6.83 (3.07)
FATP6	6.91 (1.16)	6.49 (1.63)	6.64 (1.97)
FABP3	8.52 (1.62)	8.25 (2.05)	8.82 (2.13)
FABP4	1.92 (1.33)	2.30 (1.86)	1.20 (1.09)*
FABP7	6.07 (2.05)	6.58 (3.09)	5.66 (2.19)
EL	3.65 (1.83)	3.62 (2.31)	3.20 (1.94)
LPL	4.77 (2.33)	3.81 (2.25)	3.98 (2.12)
FAT/CD36	3.03 (1.21)	2.86 (1.63)	2.96 (1.81)

*Normalized mRNA expression, to B2M and YHWAZ, is presented as median and interquartile range (IQR).; Mann-Whitney U tests were performed to investigate differences between groups. Highlighted text box: *Statistically significant different compared to IOM recommended gestational weight gain (P<0.05). IOM: Institute of Medicine; GWG: Gestational weight gain, FATP1: Fatty acid transporter protein 1, FATP4: Fatty acid transporter protein 4, FATP6: Fatty acid transporter protein 6, FABP3: Fatty acid binding protein 3, FABP 4: Fatty acid binding protein 4, FABP7: Fatty acid binding protein 7, EL: Endothelial lipase, LPL: Lipoprotein lipase, FAT/CD36: Fatty acid translocase.*

16 Correlation analysis

We performed several correlation analyses to explore the relationship between the different variables included in this Doctoral Thesis. Thus, we evaluated the association between both maternal and infant clinical characteristics and placental outcomes.

16.1 Maternal, fetal and neonatal Clinical characteristics

Maternal pBMI was positively correlated to anterior abdominal wall thickness (r=0.226, P <0.01), placental (r=0.212, P <0.01) and newborn weight (r=0.206, P < 0.05). Moreover, we found a negative correlation between maternal pBMI and gestational weight gain (r=-0.232, P <0.01). There was a positive correlation between placental and newborn weight (r=0.531, P < 0.01).

The associations between maternal biochemical parameters and fetal anthropometric measurements were explored. Maternal percentage of glycosylated hemoglobin at 24 and 34 weeks of gestation were positively correlated to fetal adiposity markers at 24 and 34 weeks of gestation, respectively. Furthermore, those associations were stronger at 34 weeks of gestation. Other parameters, such as glucose, cholesterol and triglycerides, did not show association with fetal sonographic measurements (Table 13 and 14).

Table 13. Spearman correlation analysis between maternal biochemistry and fetal biometry at 24 weeks of gestation

	Maternal serum biochemistry at 24 weeks of gestation					
Fetal anthropometry at 24 weeks of gestation	Glucose (n = 118)	Glycosylated Hemoglobin (n = 104)	Total Cholesterol (n = 120)	Triglycerides (n = 120)	HDL-Cholesterol (n = 120)	LDL-Cholesterol (n = 120)
Biparietal diameter	0.224*	0.291**	0.033	0.120	-0.026	0.033
Head circumference	0.134	-0.017	-0.006	0.097	-0.040	-0.009
Abdominal circumference	0.072	0.099	0.110	0.148	-0.008	0.087
Femur length	0.168	0.133	-0.025	0.062	-0.079	-0.006
Estimated fetal weight	0.179	0.206*	0.112	0.167	-0.014	0.088
Anterior abdominal wall thickness	0.150	0.399**	-0.007	-0.029	0.092	-0.055
Lower limb fat mass	0.163	0.355**	-0.031	-0.003	0.090	-0.091
Upper limb fat mass	0.097	0.422**	0.022	-0.028	0.061	0.003
Check to check diameter	-0.101	0.037	-0.078	0.082	-0.005	-0.106
Estimated fetal adiposity	0.147	0.421**	-0.006	-0.022	0.087	-0.051

*Highlighted text box: *Correlation statistically significant at 0.05 level. ** Correlation statistically significant at 0.01 level.*

Table 14. Spearman correlation analysis between maternal biochemistry and fetal biometry at 34 weeks of gestation

	Maternal serum biochemistry at 34 weeks of gestation					
Fetal anthropometry* at *34 weeks of gestation	Glucose (n = 133)	Glycosylated Hemoglobin (n = 134)	Total Cholesterol (n = 133)	Triglycerides (n = 133)	HDL-Cholesterol (n = 133)	LDL-Cholesterol (n = 133)
Biparietal diameter	0.079	0.145	0.037	0.179*	-0.028	0.027
Head circumference	-0.079	-0.112	0.033	0.072	-0.060	0.085
Abdominal circumference	0.008	-0.020	0.079	0.198*	-0.090	0.065
Femur length	-0.064	-0.026	0.063	0.187*	-0.024	0.046
Estimated fetal weight	0.020	0.016	0.062	0.217*	-0.042	0.043
Anterior abdominal wall thickness	0.042	0.501**	-0.013	-0.072	-0.138	0.034
Lower limb fat mass	0.047	0.425**	-0.014	-0.002	-0.122	0.010
Upper limb fat mass	0.002	0.398**	-0.031	0.042	-0.149	-0.027
Check to check diameter	0.061	-0.211*	0.111	0.112	-0.020	0.109
Estimated fetal adiposity	0.027	0.488**	-0.012	-0.027	-0.155	0.023

*Highlighted text box: *Correlation statistically significant at 0.05 level. ** Correlation statistically significant at 0.01 level.*

16.2 Placental gene expression

To obtain insight in potential functional relationship between the mRNA expression of placental transporters, binding proteins and enzymes was studied. A strong positive correlation was found between *FATP1*, *FATP4*, *FABP3*, *FABP4*, *FABP7*, *EL* and *LPL*. *FATP6* only was significantly correlated to *LPL* and *FAT/CD36*. Expression of *FAT/CD36* correlate negatively with the expression of *FATP1, FATP4, FABP3, FABP7* and *EL*. *LPL* expression correlate positively with the expression of all studied genes with exception of *FAT/CD36*, which expression did not show significant correlation (Table 15).

16.3 Placental gene expression and clinical characteristics

The relationship between maternal, fetal and infant characteristics and placental gene expression were evaluated. Maternal pre-pregnancy BMI was negatively correlated to the placental expression of *FATP1* ($r = -0.27$, $P<0.05$), *FATP4* ($r=-0.32$, $P<0.05$), *FABP3* ($r=-0.19$, $P<0.001$), *FABP4* ($r=-0.40$, $P<0.001$), *FABP7* ($r=-0.26$, $P<0.001$), and EL ($r=-0.29$, $P<0.001$) but positively to *FAT/CD36* ($r=0.18$, $P<0.05$). Maternal serum biochemistry parameters at visit 34 did not show correlation to placental expression of target genes, with exception of glycosylated hemoglobin which was negatively correlated to *FATP1, FATP4, FABP4, FABP7* and *EL*, and positively to *FATP6* and *FAT/CD36* (Table 16).

Correlations coefficients between fetal anthropometry at study visits and placental expression are presented in tables 17 and 18. Briefly, expression of *FATP6* positively correlated to all markers of fetal adiposity (included estimated fetal adiposity) at 24 weeks of gestation, while *EL* expression was negatively correlated to most of them and estimated fetal weight. The expression of *FABPs* did not correlate to any fetal measure (Table 17). At 34 weeks of gestation, the anterior abdominal wall thickness was negatively correlated to *FATP1*, *FATP4*, *FABP7* and *EL*, while it was positive to *FATP6* and *FAT/CD36*. Similar associations were found for estimated fetal adiposity and lower limb fat mass (Table 18). While not significantly correlations were found between placental gene expression and neonate anthropometry at birth (data not shown). A negative correlation was found between *EL* mRNA expression and placental weight ($r = -0.251$, $P = 0.008$).

Table 15. Spearman correlation analysis among fatty acid transporters, binding proteins and enzymes

Full term placental gene expression	*FATP1* (n= 101)	*FATP4* (n= 101)	*FATP6* (n= 101)	*FABP3* (n= 101)	*FABP4* (n= 101)	*FABP7* (n= 101)	*EL* (n= 101)	*LPL* (n= 101)	*FAT/CD36* (n= 101)
FATP1		0.885**	-0.132	0.735**	0.505**	0.804**	0.601**	0.330**	-0.602**
FATP4	0.885**		0.019	0.688**	0.463**	0.723**	0.584**	0.420**	-0.507**
FATP6	-0.132	0.019		-0.034	0.075	-0.018	-0.137	0.344**	0.306**
FABP3	0.735**	0.688**	-0.034		0.416**	0.630**	0.366**	0.221*	-0.356**
FABP4	0.505**	0.463**	0.075	0.416**		0.602**	0.558**	0.356**	-0.119
FABP7	0.804**	0.723**	-0.018	0.630**	0.602**		0.600**	0.302**	-0.420**
EL	0.601**	0.584**	-0.137	0.366**	0.558**	0.600**		0.346**	-0.283**
LPL	0.330**	0.420**	0.344**	0.221*	0.356**	0.302**	0.346**		-0.151
FAT/CD36	-0.602**	-0.507**	0.306**	-0.356**	-0.119	-0.420**	-0.283**	-0.151	

*Highlighted text box: *Correlation statistically significant at 0.05 level. ** Correlation statistically significant at 0.01 level. FATP1: Fatty acid transporter protein 1, FATP4: Fatty acid transporter protein 4, FATP6: Fatty acid transporter protein 6, FABP3: Fatty acid binding protein 3, FABP 4: Fatty acid binding protein 4, FABP7: Fatty acid binding protein 7, EL: Endothelial lipase, LPL: Lipoprotein lipase, FAT/CD36: Fatty acid translocase*.

Table 16. Spearman correlation analysis between placental gene expression and maternal serum biochemistry at 34 weeks of gestation

	Full term placental gene expression								
Maternal serum biochemistry at 34 weeks of gestation	*FATP1* (n= 111)	*FATP4* (n=110)	*FATP6* (n=111)	*FABP3* (n=110)	*FABP4* (n=110)	*FABP7* (n= 110)	*EL* (n= 111)	*LPL* (n= 111)	*FAT/CD36* (n= 111)
Glucose	-0.147	-0.181	0.116	-0.096	-0.174	-0.142	-0.056	0.106	0.058
Glycosylated Hemoglobin	-0.308**	-0.281**	0.401**	-0.119	-0.210*	-0.230*	-0.373**	0.151	0.201*
Total cholesterol	0.011	0.008	-0.109	-0.024	0.113	0.054	0.042	-0.031	-0.049
Triglycerides	0.087	0.083	0.014	0.122	-0.087	0.007	0.022	-0.017	-0.029
HDL-Cholesterol	0.082	0.075	0.027	0.106	0.170	0.052	0.057	0.125	-0.078
LDL-Cholesterol	-0.049	-0.045	-0.158	-0.091	0.074	0.035	0.016	-0.092	-0.024

*Highlighted text box: *Correlation statistically significant at 0.05 level. ** Correlation statistically significant at 0.01 level. FATP1: Fatty acid transporter protein 1, FATP4: Fatty acid transporter protein 4, FATP6: Fatty acid transporter protein 6, FABP3: Fatty acid binding protein 3, FABP 4: Fatty acid binding protein 4, FABP7: Fatty acid binding protein 7, EL: Endothelial lipase, LPL: Lipoprotein lipase, FAT/CD36: Fatty acid translocase.*

Table 17. Spearman correlation analysis between placental gene expression and fetal anthropometry at 24 weeks of gestation

	Full term placental gene expression								
Fetal anthropometry at 24 weeks of gestation	*FATP1* (n= 91)	*FATP4* (n= 91)	*FATP6* (n= 91)	*FABP3* (n= 90)	*FABP4* (n= 90)	*FABP7* (n= 90)	*EL* (n= 91)	*LPL* (n= 91)	*FAT/CD36* (n= 91)
Biparietal diameter	-0.246*	-0.228*	0.244*	-0.105	-0.083	-0.042	-0.300**	0.062	0.269**
Head circumference	-0.101	-0.075	0.071	-0.038	0.022	0.003	-0.134	-0.083	0.162
Abdominal circumference	-0.175	-0.162	0.139	-0.117	-0.093	-0.096	-0.143	-0.055	0.185
Femur length	-0.259*	-0.258*	0.208*	-0.125	-0.177	-0.077	-0.296**	-0.145	0.269**
Estimated fetal weight	-0.224*	-0.211*	0.178	-0.127	-0.083	-0.087	-0.228*	-0.077	0.261*
Anterior abdominal wall thickness	-0.165	-0.166	0.258*	-0.085	-0.007	-0.095	-0.136	0.257*	0.194
Lower limb fat mass	-0.144	-0.180	0.206*	-0.103	-0.108	-0.122	-0.258*	0.202	0.096
Upper limb fat mass	-0.289**	-0.273**	0.251*	-0.153	-0.164	-0.188	-0.347**	0.092	0.203
Check to check diameter	0.027	0.078	0.290**	0.157	0.013	-0.059	-0.118	-0.016	0.117
Estimated fetal adiposity	-0.205	-0.219*	0.262*	-0.106	-0.107	-0.148	-0.270**	0.213*	0.166

*Highlighted text box: *Correlation statistically significant at 0.05 level. ** Correlation statistically significant at 0.01 level. FATP1: Fatty acid transporter protein 1, FATP4: Fatty acid transporter protein 4, FATP6: Fatty acid transporter protein 6, FABP3: Fatty acid binding protein 3, FABP 4: Fatty acid binding protein 4, FABP7: Fatty acid binding protein 7, EL: Endothelial lipase, LPL: Lipoprotein lipase, FAT/CD36: Fatty acid translocase.*

Table 18. Spearman correlation analysis between placental gene expression and fetal anthropometry at 34 weeks of gestation

	Full term placental gene expression								
Fetal anthropometry at 34 weeks of gestation	*FATP1* (n= 101)	*FATP4* (n= 101)	*FATP6* (n= 101)	*FABP3* (n= 101)	*FABP4* (n= 101)	*FABP7* (n= 101)	*EL* (n= 101)	*LPL* (n= 101)	*FAT/CD36* (n= 101)
Biparietal diameter	-0.231*	-0.241*	0.086	-0.168	-0.390*	-0.164	-0.323*	-0.047	0.146
Head circumference	-0.041	-0.032	-0.038	-0.066	-0.237*	0.015	-0.127	-0.024	0.091
Abdominal circumference	-0.011	0.012	0.110	-0.140	-0.049	0.080	-0.017	0.048	0.129
Femur length	0.083	0.085	-0.036	0.013	-0.107	0.121	0.054	0.030	-0.004
Estimated fetal weight	-0.043	-0.019	0.107	-0.106	-0.131	0.039	-0.082	0.044	0.126
Anterior abdominal wall thickness	-0.289**	-0.316**	0.292**	-0.178	-0.189	-0.200*	-0.303**	0.016	0.224*
Lower limb fat mass	-0.213*	-0.227*	0.245*	-0.092	-0.159	-0.128	-0.307*	0.061	0.142
Upper limb fat mass	-0.074	-0.128	0.126	-0.019	-0.004	0.020	-0.145	0.072	0.031
Check to check diameter	0.136	0.238*	-0.033	0.087	0.212*	0.158	0.281*	0.128	-0.109
Estimated fetal adiposity	-0.214*	-0.252*	0.242*	-0.085	-0.142	-0.113	-0.277**	0.041	0.152

*Highlighted text box: *Correlation statistically significant at 0.05 level. ** Correlation statistically significant at 0.01 level. FATP1: Fatty acid transporter protein 1, FATP4: Fatty acid transporter protein 4, FATP6: Fatty acid transporter protein 6, FABP3: Fatty acid binding protein 3, FABP 4: Fatty acid binding protein 4, FABP7: Fatty acid binding protein 7, EL: Endothelial lipase, LPL: Lipoprotein lipase, FAT/CD36: Fatty acid translocase.*

16.4 Placental fatty acid content and gene expression

The relationship between placental gene expression of *FATPs*, *FABPs*, *FAT/CD36* and lipases was evaluated. A positively correlation was found between total LCPUFAs from TG fractions and all analyzed genes, except for the expression of *FAT/CD36*. The percentage of total MUFAs from TG fraction was negatively correlated to placental expression of *FATP1*, *FATP4*. *FABP7*, *EL* and *LPL*. Placental expression of *FABP3* was negatively related to percentage of total SFA in PL fraction. No other correlations were found between placental gene expression and FAs content (Table 19).

16.5 Placental fatty acid content and fetal characteristics.

A negative correlation was found between SFA in triglycerides fraction of placental tissue and fetal anterior abdominal wall thickness, upper limb fat mass, check to check diameter and estimated fetal adiposity at 34 weeks of gestation. No other correlations were found between placental FA content and fetal characteristics (Table 20).

Table 19. Spearman correlation analysis between placental gene expression and fatty acid composition at term

	Percentage of fatty acid in full term placenta							
Placental gene expression	SFA PL (n = 52)	MUFAs PL (n = 53)	SCPUFAs PL (n = 52)	LCPUFAs PL (n = 53)	SFAs TG (n = 52)	MUFAs TG (n = 52)	SCPUFAs TG (n = 53)	LCPUFA TG (n = 53)
FATP1	-0.110	-0.113	0.163	0.030	-0.176	-0.357**	0.024	0.427**
FATP4	-0.137	-0.029	0.199	-0.025	-0.234	-0.322*	0.002	0.441**
FATP6	-0.211	0.054	0.059	0.039	-0.098	-0.183	-0.022	0.278*
FABP3	-0.319*	-0.079	0.037	0.272	-0.218	-0.167	-0.126	0.459**
FABP4	-0.061	0.191	0.072	-0.080	-0.124	-0.114	-0.042	0.314*
FABP7	-0.088	-0.148	0.179	0.071	-0.262	-0.296*	0.152	0.420**
EL	0.161	-0.198	0.061	-0.012	-0.246	-0.298*	0.196	0.342*
LPL	-0.025	-0.092	-0.052	0.176	0.000	-0.366**	-0.131	0.451**
FAT/CD36	0.131	0.216	-0.145	-0.136	0.156	0.159	-0.068	-0.104

*Highlighted text box: *Correlation statistically significant at 0.05 level. ** Correlation statistically significant at 0.01 level. FATP1: Fatty acid transporter protein 1, FATP4: Fatty acid transporter protein 4, FATP6: Fatty acid transporter protein 6, FABP3: Fatty acid binding protein 3, FABP 4: Fatty acid binding protein 4, FABP7: Fatty acid binding protein 7, EL: Endothelial lipase, LPL: Lipoprotein lipase, FAT/CD36: Fatty acid translocase, FA: Fatty acids, SFA: saturated FA, MUFA: monounsaturated FA, SCPUFA: short chain polyunsaturated FA, LCPUFA: long chain polyunsaturated FA.*

Table 20. Spearman correlation analysis between placental fatty acid composition and fetal anthropometry at 34 weeks of gestation

	Percentage of fatty acid in full term placenta							
Fetal anthropometry* at *34 weeks of gestation	SFA PL (n = 85)	MUFAs PL (n = 85)	SCPUFAs PL (n = 85)	LCPUFAs PL (n = 85)	SFAs TG (n = 85)	MUFAs TG (n = 85)	SCPUFAs TG (n = 85)	LCPUFA TG (n = 85)
Biparietal diameter	-0.114	0.081	0.046	-0.019	-0.084	0.003	-0.012	0.038
Head circumference	-0.003	-0.129	0.005	0.064	0.016	-0.125	0.050	0.027
Abdominal circumference	0.042	0.044	-0.035	-0.038	-0.121	-0.055	0.004	0.095
Femur length	-0.142	-0.080	-0.023	0.118	-0.169	-0.054	0.085	0.114
Estimated fetal weight	-0.122	0.049	-0.028	0.020	-0.143	-0.009	0.008	0.084
Anterior abdominal wall thickness	-0.056	0.005	0.128	-0.055	-0.298**	-0.127	0.149	0.194
Lower limb fat mass	0.001	0.195	-0.011	-0.077	-0.172	0.044	0.011	0.069
Upper limb fat mass	-0.059	0.125	0.095	-0.062	-0.266*	0.067	0.147	0.128
Check to check diameter	-0324**	0.070	0.028	0.119	-0.236*	0.089	0.060	0.144
Estimated fetal adiposity	-0.025	0.112		-0.082	-0.297**	-0.013	0.102	0.154

*Highlighted text box: *Correlation statistically significant at 0.05 level. ** Correlation statistically significant at 0.01 level. FA: Fatty acids, SFA: saturated FA, MUFA: monounsaturated FA, SCPUFA: short chain polyunsaturated FA, LCPUFA: long chain polyunsaturated FA*

DISCUSSION

The placenta is a temporary organ that plays a key role during pregnancy. The placenta acts not only as a physical barrier, facilitating the nutrient and waste exchange between mother and fetus, but also as an endocrine organ involved in the regulation of a wide range of processes. Throughout pregnancy, placenta endocrine activity has an enormous impact on maternal physiology and metabolism. Under normal conditions, placenta responds to signals generated from both mother and fetus, and the interplay between maternal, placental and fetal hormones ensure an adequate supply of oxygen and nutrients to the fetus without compromising maternal reserves (146). In case of maternal metabolic diseases, such as obesity and GDM, placenta is able to adapt its metabolism and structure to ensure optimal fetal development. For instance, early onset of GDM is associated with higher placental weights and morphological changes at microanatomical level, while late onset of GDM is mainly related to modified placental gene expression (147). On the other hand, data related to macroscopic structural modifications in placentas from obese mothers still remain unknown, although animal studies suggest that maternal obese phenotype alters placental function by increasing nutrient availability (120). However, if duration or extent of the insults produced by maternal illness exceeds placental adaptation capacity, fetal development may be compromise (148, 149). Despite of the rising evidence of association between maternal metabolic diseases and the risk to develop long-term diseases, such obesity, diabetes and cardiovascular diseases, the placental role on those adverse outcomes has not been well established (150). In this regard, studies about placental regulation are conflicting and mostly limited to animal models and food deprivation during pregnancy. In addition, most of the studies on the expression of FA transporters in human placenta are performed in pregnancies complicated by both obesity and GDM, but independent effects of those metabolic complications remain unclear. Having these considerations in mind, as a primary objective of this Doctoral Thesis, we aimed to separately investigate the effect of maternal excessive pre-pregnancy weight and GDM on placental fatty acid composition and expression of key genes involved in FA trafficking and handling.

Our results show that maternal high pre-pregnancy BMI and GDM alter the placental mRNA expression of main genes involved in FA uptake, transfer and metabolism. These changes potentially affect the placental specific FA composition without affecting total FA acid content. Our findings suggest that pre-pregnancy obesity and GDM lead to a selective mobilization and utilization of specific FA. In fact, the percentage of

placental saturated FA was significantly lower in obese and GDM groups compared to normal weight. However, placental LCPUFAs content, mainly percentage of eicosapentaenoic acid (EPA, 20:5n3) in the phospholipid fraction, was higher in the overweight, obese and GDM groups than normal weight pregnant women.

Traditionally, maternal lipids were almost exclusively considered an energy source for the mother, while maternal glucose is mostly destined for fetal growth. Current evidence, namely the limited fetal hepatic *de novo* lipogenesis, support that both glucose and lipids supply play a key role in fetal growth and fat accretion. In normal pregnancies, maternal lipid concentration increases by 2nd and 3rd trimester. Some authors have shown that circulating levels of TG are approximately 30-40% higher in pregnancies affected by obesity or GDM than in normal weight women (151, 152), while others did not reported such differences (153), making those results inconsistent and difficult to interpreted. In our study, obese women tend to have higher total cholesterol and triglycerides levels, although not reaching statistically significance. Nevertheless, due the own placental metabolism and endocrine activity, maternal circulating levels of lipids may not represent exact concentrations in the placental intervillous space. For instance, the concentration of glucose and free FA may increase due to anti-insulin effect of the human placental lactogen. Also, the own trophoblast metabolism consume oxygen and nutrients, which decrease substrates available for the transfer to the fetus affecting in turn the gradient concentration between placental sides (149). It is well established that placental nutrient transfer depends on concentration gradients between maternal and fetal circulation, placental morphology and specific transport mechanism. Hence, the placental function and efficiency of transport mechanisms are determined by the number, density and activity of carrier proteins and lipolytic enzymes (7). In addition, the maternal microvillous plasma membrane (MVM) expresses receptors for insulin and leptin, among others, indicating that placental nutrient transport is strongly regulated by maternal factors, particularly metabolic hormones (10). As proposed by Lager *et al.* alterations in maternal side signals such as nutrient supply, adipokines, cytokines, and hormone levels may lead to modification in placental transport function (10). Our group previously reported that obesity and GDM impact the energy sensing by dysregulation of placental expression of genes involved on those pathways (154). Thus, under unfavourable conditions, placenta may modify the gene expression as compensatory mechanism to regulate the uptake, storage and

delivery of nutrients. For instance, *FATPs* expression is regulated by a positive feedback loop that permits to syncytiotrophoblast cells incorporate FA as much as they are present in maternal circulation. Mouse studies have found a positive regulation of *FATP* by ligands such as FA and their derivate, which activate *PPAR* expression, resulting in higher incorporation of FA at high maternal plasma concentrations (155). On the contrary, GDM and insulin resistance have a negative effect on placental expression of *PPARs* (156-158). Interestingly, we not only observed a lower expression of *FATP1* and *FATP4*, but also a negative correlation between the expression of those transporters and glycosylated hemoglobin at 34 weeks of gestation. Thus, our results might suggest a protective placental mechanism to limit both excessive nutrient transfer to the fetus and lipotoxicity by down-regulation of *PPARs*.

Our results are consistent with data reported by Ye *et al*. (159) and Tian *et al.* (160), which showed decreased mRNA expression of FATP1 and FATP4 in placentas from a diet-induced obesity rat model and obese pig, respectively. However, unlike our findings, FAT/CD36 mRNA expression was also decreased in these animal studies. In this regard, Tian *et al.* reported an increased placental LPL activity and elevated levels of maternal circulating NEFA, which may explain the decreased expression of FAT/CD36. In our study, the *LPL* mRNA expression remained unchanged among groups, supporting that diabetes or obesity alone do not lead to significant overexpression of placental lipases, as previously reported by Magnusson *et al*. (133), Lindergaard *et al.* (161) and Dubé *et al.* (136). We also observed a lower expression of EL in in non-normal weight pregnancy women and GDM women, which might indicate a reduced lipolytic activity and subsequent decreased availability of "free" FA for placental uptake, as EL has been identified as the major extracellular placental lipase in term placentas (5). In this scenario, expected lower NEFA concentrations on the maternal side of the placenta could mediate an enhanced expression of *FAT/CD36*, which would support the hypothesis that CD36 is relevant for FA uptake and optimal LCFA supply to the developing fetus under limiting conditions (157). Furthermore, a reduced maternal local NEFA concentration might also contribute to lowered expression of *FATP1* and *FATP4* observed in the three groups affected by maternal metabolic pathology. Our hypotheses are supported by the relationship found between placental FA transporters and lipase enzymes, where expression of *EL* was positively associated

to the expression of *FATP1* and *FATP4* and negatively related to *FATP6* and *FAT/CD36* expression.

Interestingly, we also observed a negative correlation between *EL* expression and placental weight and adiposity biomarkers, including estimated fetal weight and adiposity at 24 weeks, anterior abdominal wall thickness and estimated fetal adiposity at 34 weeks of gestation. With these considerations in mind, we suggest that, after reaching a determine weight and fetal growth, human placenta could limit the availability and trafficking of FA to the fetus. Similar results have been previously reported in the activity of the MVM system A amino acid transporter, which was inversely correlated to placental weight and fetal size, particularly to neonatal abdominal circumference (162).

It is important to note that *FATP* family members show a high degree of homology among them, suggesting that *FATP* members can functionally replace each other (122). This scenario may explain the higher expression found of *FATP6* and *FAT/CD36*. In addition, placental expression of *FATPs, FABPs, EL* and *LPL* was positively correlated with percentages of total LCPUFAs in triglyceride fraction, while percentages of MUFAs in triglyceride fraction was negatively correlated with expression of most of studied genes. Thus, changes in placental pattern expression suggest a placental protective effect to ensure an optimal DHA and AA supply for the developing fetal brain, while limiting the excess of other non-essential FAs.

In contrast to FA transporters, expression of fatty acid binding proteins was not similarly affected by excessive BMI during pregnancy and GDM. In fact, expression of *FABP3* and *FABP7* in placentas from GDM subjects resulted slightly but not significantly decreased when compared to those from normal weight. Moreover, obese pregnant women showed a decreased expression of major intracellular fatty acid carriers, which could not only be related to differences in placental FA transfer, but also influence the expression of *PPAR*-related genes, and the efficiency of agonistic FA activity (8). These findings are in agreement to Dubé *et al.* (136) and Magnusson *et al*. (133) in obese and GDM pregnancies, respectively. Interestingly, differences observed in placental *FABP4* mRNA expression between obese mothers and GDM suggest a differential regulatory pathway for placental FA handling and metabolism in both maternal conditions. In this regard, Li *et al.* reported an increased *FABP4* expression in placentas from GDM compare to those from healthy pregnant women, as well as in pre-

adipocytes cultured with serum from GDM pregnant women (163). Authors suggested that elevated circulating levels of placental hormones, such human placenta lactogen and progesterone, could induce overexpression of *FABP4* (163). Ning *et al.* have proposed plasma *FABP4* as predictor of GDM, but it remains unclear if increased levels of plasma *FABP4* are a cause or a consequence for develop GDM (164). Nonetheless, placenta tissue from GDM has been associated with higher proportions of macrophages compared to healthy placentas (6), while no differences in immune cells populations have been described in the placental villi from obese mothers (165). As *FABP4* is expressed by macrophages as well (166), its high expression in GDM compare to obese women could be a consequence of elevated macrophages population instead of trophoblast cells.

When we put together our two set of findings, changes on placental FA profile could be explained by changes on gene expression. Thus, lower expression of *FATP1, FATP4* and *FABPs* may lead to a decreased placental transference of some LCPUFAs to the fetus, increasing their retention in placental tissue. Unfortunately, the requirements and mechanism implicate on FA uptake by placental FATPs are poorly understood and there are no clear evidence on FA specificity/affinity transporters to support this idea (157). Placental own metabolism may provide an alternative explanation for changes in specific FAs. As suggested by Perazzolo *et al.* (109), the incorporation and release of fatty acid to the fetus is determined by the metabolic pool, which may buffer the transfer of fatty acids to the fetal circulation. A decreased phospholipase EL A1 activity is consistent with a reduction in placental SFAs content, as position 1 of PL is normally esterified by saturated fatty acids (167). Thus, limiting the SFA availability, the placenta reduces their transfer to the fetus, which will be beneficial in the obese and GDM groups, where fetal anterior abdominal wall thickness and estimated adiposity were found higher at 34 weeks of gestation. This hypothesis is supported by the negative correlation found between anterior abdominal thickness and estimated fetal adiposity at 34 weeks with SFA content in triglyceride fraction in placental tissue at term. Nevertheless, it is unclear if the decrease in placental SFA is due to a higher incorporation of SFA in fetal tissues, a lower incorporation in placental tissue, or both. In addition, although LCPUFAs play an essential role in fetal nervous structures development, their excess may have adverse effects. In our study, percentages of DHA and AA were not found affected by the maternal metabolic status. It is estimated that

absolute amount of DHA deposited in fetal brain is closed to the amount of DHA incorporated in the placenta (106); thus, a correct central nervous system development is expected in our studied groups. Nevertheless, our data suggest a good DHA and AA placental uptake, but placental release and fetal utilization is unclear. Our results suggest that, in case of maternal pathologies, placenta focuses on PUFAs storage, modifying its transport and metabolic mechanisms to ensure the availability of PUFAs for the fetus.

Regarding clinical characteristics, serum glucose, total cholesterol and triglycerides did not greatly differ between studied groups and normal weight. Our results are broadly in line with those previously reported by Schaefer-Graf *et al.*(153) in well-controlled gestational diabetic pregnancies. These results could be due in part to a different intake of fats, as suggested by the lower gain weight observed in all the study groups compared to normal weight pregnant women. Other possible explanations for these findings may be related to higher FA sequestering and accumulation in other maternal tissues different that placenta (i.e. skeletal muscle, liver, adipose tissue), as a consequence of pronounce insulin resistance associated to obesity and GDM (168). In addition, a higher incorporation of maternal circulating FA into fetal adipose tissue is expected in obese and GDM pregnant women due higher fetal adiposity markers found in those groups. Interestedly, the percentage of glycosylate hemoglobin (which reflects average plasma glucose over the previous 12 weeks and is a reliable parameter even in non-fasting condition) at both 24 and 34 weeks of gestation was higher in our three groups compared to healthy control women. This result suggests that, fetus from overweight, obese and GDM mothers are exposed to high amounts of glucose during pregnancy, which may result in fetal hyperglycemia and consequently higher levels of insulin due to its secretion from fetal pancreatic beta-cell. As the fetal insulin sensitivity has been estimated to be more pronounced that in adults, an increased glucose and FA uptake by adipose tissue and fat deposition is expected under the hyperinsulinemic state (84). Furthermore, the positive correlations between maternal glycosylated hemoglobin and all sonographic markers of fetal adiposity and estimated fetal adiposity at 24 and 34 weeks of gestation indicate that continued exposure to glucose induces fat accumulation and growth disorders in the fetus, without compromising newborn weight (169-171). Curiously, fetus from obese mothers have more adiposity at 24 and 34 weeks compared to those from lean participants, while fetus from lean GMD women showed high

adiposity at 34 weeks. These results suggest that intrauterine insult to exacerbated glucose levels begins earlier in obese pregnancies.

We observed significant differences in both placental and birth weight in obese group compared to normal weight group, but no changes were found in newborn/placental weight ratio. The absence of differences in the newborn/placental weight ratio, together with the strong positive correlation between placental and birth weight, suggest that differences in placental weight could compensate higher demands of nutrient and oxygen by bigger babies instead reflecting a macroscopic modification of placental morphology (170, 171). Coan *et al.* reported that mouse placenta adopts different strategies to maintain fetal growthin response to fetal nutrient demands. They also found that changes in placental morphology takes place earlier in gestation while placental functional changes occur near to term (172). Constancia *et al.* showed that decreased fetal nutrient demands reduced placental expression of System A amino acid transport and expression of glutamine transporter Slc38a2 in late gestation, thus evidencing that placenta responds to fetal signals by regulation of expression of placental transport systems (173). Having these considerations in mind, similar newborn/placental weight ratio and differences in placental gene expression found in the current study indicate a successful placental morphological and functional adaptation, limiting nutrient transfer to avoid fetal overgrowth in the obese and GDM group (12, 174-176). Although babies from GDM and obese groups tend to be heavier than those from control group, they do not reach the macrosomia criteria (>4500 g) and shown an adequate weight to their gestational age. However, data from epidemiological studies suggest that early programming of obesity, type-2 diabetes and metabolic syndrome occurs within normal birth size (69). In addition, in our study, ponderal index at birth was similar in the four groups. Although BMI, or ponderal index at birth, are widely used as clinical measure of obesity, they are not always a robust estimation of adiposity. The ponderal index only considers around the 15% of the variance in adiposity in the neonate, but not discriminate the body compartment where fat is deposited (48, 177). For instance, visceral fat deposition has been associated with increased insulin resistance and, therefore, it is considered a risk factor for development of metabolic syndrome (178, 179). Unfortunately, skinfold thickness measurements were not collected in the PREOBE study at birth, but abdominal circumference was found higher in offspring

from obese mothers, which may be a predictor of excessive visceral fat and consequently for insulin resistance and related metabolic disorders (180, 181).

To the best of our knowledge, our work is the first to study placental expression of genes involved in lipid transport and metabolism in overweight pregnancies. Interestingly, overweight pregnant women showed gene expression closely resembles from obese mothers, but FA content was more similar to placentas from normal weight pregnant women. Thus, overweight seems to be an intermediate group between normal weight and obese pregnant women in terms of metabolic disruption. The clinical characteristics of fetal and offspring born to overweight mothers also showed an intermediate situation between normal weight and obesity. Fetal adiposity was higher at mid gestation, but it seems that compensatory mechanism activated during last part of gestation was enough to achieve, at least apparently, an optimal fetal development. This hypothesis is supported by the lack of differences between offspring born from overweight mothers compared to those born from lean participants.

On the other hand, the lack of changes in several metabolic parameters found in our study suggests that, when subjects are clinically diagnosed with a metabolic disease, they are not severely affected. Interestingly, in well-controlled pregnancies, we found significant differences in placental composition, gene expression and fetal characteristics in the studied groups compared to healthy pregnancy. While some physiological and metabolic changes are associated with pregnancy, others could be considered as anticipatory response to an unfavorable internal milieu. For instance, maternal obesity has been associated with an altered placental metabolome profile, lipotoxic environment (182-184), alterations in placental mitochondria function and subsequent changes in energy metabolism and consumption of oxidative substrates (185). Moreover, the effect of the maternal metabolic status is exacerbated in those obese pregnancies complicated with diabetes (186). Thus, chronic metabolic insults produced by obesity and diabetes may compromise regulatory pathways, resulting in ineffective adaptive response which lead to metabolic disorders for both mother and fetus (4). Nonetheless, the placenta is exposed to very diverse and sometimes contradictory signals from maternal and fetal side; for example, circulating maternal levels may indicate a good nutritional status, increasing supply of nutrients, but regulatory factors produced by fetal tissues may lead to the opposite placental response. As consequence, the placenta must integrate nutritional and endocrine signals to ensure

pregnancy progress and fetal development (186). Hence, successful or failed placental adaptation clearly depends on the nature, severity and duration of the environmental adversity, thereby avoiding maternal metabolic phenotype is always associated to fetal hazard (187). Although placental modifications found in our study seem to be a response to protect and maintain the fetal health, the influence of these changes on fetal early programming and consequences in adult life is not clear.

In human, data regarding placental function and adaptations to obesity and GDM are restricted to full term placenta due the impossibility to collect placental tissue at different gestational age. Thus, obesity- and GDM-related changes in the placenta throughout pregnancy and the consequences for fetal development are limited to animal models. In this regard, Zhu *et al.* (188) reported upregulation in placental FA transporter expression in the obese ewe at midgestation, but this expression was downregulated at late gestation. This study also showed that altered maternal environment at the beginning-midgestation leads to severest consequences for the fetus than modifications at the end of gestation, raising the risk of obesity and metabolic diseases in later life. In addition, the possibility to perform experimental interventions in human are limited and the consequences of modifications of maternal milieu during pregnancy (via diet intervention *inter alia*) on fetal programming are still largely unknown. Furthermore, most of epidemiological data available to the date are focus on nutrient limitation and growth restriction. But epidemiological studies in human can only indicate associations, even when data collection is very carefully controlled (40). Therefore, differences on the study design, inclusion/exclusion criteria, clinical management of the population and control of confounding variables may lead to different, and sometimes, contradictory results. Studies in animal models allow to perform interventions with well-controlled factors to assess causality. But the mechanisms involved on fetal programming are so complex, that even in animal models is difficult to establish how maternal obesity impacts on fetal programming. For instance, high-fat diet-induced obesity is often used in animal models, but this type of diet is maintained during gestation, making it difficult to separately evaluate the effect of maternal obesity *per se* and diet. Moreover, many mechanisms implicated in the offspring phenotype may be affected by paternal factors at different stages of development, even prior conception (40). In this regard, we observed that placental expression of *FATPs*, *FABPs* and lipases is more affected by pre-pregnancy weight than for gestational weight gain. Similarly,

expression of those target genes was not affected by nutritional intervention during pregnancy (126), suggesting that placental function is more affected by maternal metabolic status than by diet. Furthermore, changes in gene expression and specific fatty acids in placentas from obese and gestational diabetic women may have a marked effect on fetal membrane composition, and consequently on cell structure and function. Even more, these changes may create an adverse metabolic and neuroendocrine environment, which negatively contribute to program appetite control and regulation of energy balance in the fetus and newborn, leading to high risk of metabolic diseases (189). Due to intergenerational transmission of obesity could contribute to an increased risk of metabolic diseases, further studies are not only still needed, but also major effort must be done by the professionals and health care institutions to ensure a healthy weight before conception.

Our study has some strengths and limitations. First, the PREOBE study was designed as an observational study, so functional data cannot be provided. In fact, membrane transporters are often regulated on the post-transcriptional level, thus changes in gene expression presented in this Doctoral Thesis may or may not correlate with changes in protein insertion of the transporter into the plasma membrane. As consequence, to demonstrate functional relevance of our findings, further studies on protein expression are still needed. Also, placenta samples were collected from similar location thus, our results may not represent the placental expression as whole. Maternal blood at delivery were not collected in the same conditions due labor complications itself. Moreover, we have not evaluated both maternal and fetal circulating levels of hormones (including leptin and insulin) and inflammation markers, which are all relevant to clarify the role of maternal metabolic diseases, such as obesity and gestational diabetes, on placental fatty acid transport (159). As strengths, we are the first to jointly study, in both overweight and lean gestational diabetic women, the placental fatty acid content and expression of key genes involved in the placental metabolism, uptake and transfer of fatty acid to the fetus, which provide new data to the placental scientific community.

CONCLUSIONS

1. Placental gene expression of genes implicated on fatty acid uptake, transfer and metabolism is affected by maternal pre-pregnancy unhealthy weight and gestational diabetes, even in well controlled pregnancies. The overweight, obesity, and gestational diabetes differently modify the expression of placental fatty acid binding protein.
2. Maternal obesity and gestational diabetes alter the placental fatty acid composition without compromise the placental fatty acid total content.
3. The placenta is a heterogeneous organ which composition may be compromise not only by maternal metabolic pathologies but also by other factors such as maternal nutritional status or placental own metabolism.
4. At 34 weeks of gestation, fetus from obese and gestational diabetic pregnant women showed a higher development of adipose tissue that fetus from healthy weight mothers. Possibly, molecular signals secreted by fetal adipose tissue contribute to active placental regulatory pathways.
5. Maternal overweight represents an intermediate metabolic situation between normal weight and obese pregnancies. Effect of overweight was similar to obesity in terms of placental gene expression, while placental fatty acid content was like normal weight ones. The clinical characteristics of fetal and offspring suggest an intermediate situation between normal weight and obesity as well. It seems that compensatory mechanism activated during last part of gestation were enough to achieve a good fetal development.
6. Our results are consistent with placental functional adaptation to ensure an optimal nutrient supply to the growing fetus by regulating the expression of fatty acid transporters and enzymes.
7. Despite changes found in our study on gene expression may represent a protective effect to ensure optimal fetal development, those modifications may have a deep impact on fetal phenotype with unknown long-term consequences.
8. The genetic and biochemical placental parameter analyzed in our study resulted more affected by pre-pregnancy weight than for gestational weight gain. Those results suggest that interventions to reduce obesity prior conception will be more effective to reduce the intergenerational effect of obesity than those performed exclusively during pregnancy.

ABBREVIATIONS

ADA	American Diabetes Association
ALA (αLA)	α-linolenic acid
AA	Arachidonic Acid
BM	Basal membrane
BMI	Body Mass Index
DHA	Docosahexaenoic Acid
DOHaD	Developmental Origins of Health and Adult Disease
EL	Endothelial lipase
ELOVL	Fatty acid elongase
EPA	Eicosapentaenoic Acid
FA	Fatty Acids
FABP	Fatty acid binding protein
FABPpm	Plasma membrane fatty acid binding protein
FADS	Fatty acid desaturase
FAT/CD36	Fatty acid translocase
FATP	Fatty acid transport protein
FFA	Free fatty acid
GDM	Gestational Diabetes Melitus
GLUT 4	Glucose Transporter type 4
HDL	High-Density Lipoprotein
IL-6	Interleukin 6
LA	Linoleic Acid
LAT	Large neutral amino acid transport
LCFA	Long chain fatty acid
LCPUFA	Long chain polyunsaturated fatty acid
LDL	Low- Density Lipoprotein
LPL	Lipoprotein Lipase
LPL	Lipoprotein lipase
MUFA	Monounsaturated Fatty Acids
MVM	Microvillous membrane
NDDG	National Diabetes Data Group
NEFA	Non-esterified fatty acids

OR	Odd ratio
pBMI	Pre-pregnancy BMI
PIH	Pregnancy-induced hypertension
PPAR	Peroxisome proliferator–activated receptors
PUFA	Polyunsaturated Fatty Acids
SCAT	Subcutaneous adipose tissue
SCFA	Short chain fatty acid
SCTB	Syncytiotrophoblast
SFA	Saturated Fatty Acids
SREBP	Sterol Regulatory Element Binding Protein
TAG	Triacylglycerols
TG	Triglycerides
TLR4	Toll-like receptor 4
TNFα	Tumor Necrosis Factor α
VAT	Visceral adipose tissue
VLDL TG	Very low-density lipoprotein triacylglycerols
WHO	World Health Organization

REFERENCES

1. Barker DJP, Godfrey KM, Gluckman PD, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. The Lancet. 1993;341(8850):938-41.

2. Barker DJ. The origins of the developmental origins theory. J Intern Med. 2007;261(5):412-7.

3. Wadhwa PD, Buss C, Entringer S, Swanson JM. Developmental origins of health and disease: brief history of the approach and current focus on epigenetic mechanisms. Semin Reprod Med. 2009;27(5):358-68.

4. Power ML, Schulkin J. Maternal obesity, metabolic disease, and allostatic load. Physiology & Behavior. 2011(0).

5. Gauster M, Desoye G, Tötsch M, Hiden U. The Placenta and Gestational Diabetes Mellitus. Curr Diab Rep. 2012;12(1):16-23.

6. Vambergue A, Fajardy I. Consequences of gestational and pregestational diabetes on placental function and birth weight. World J Diabetes. 2011;15(2):196-203.

7. Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M, Burton GJ. Placental efficiency and adaptation: endocrine regulation. The Journal of Physiology. 2009;587(14):3459-72.

8. Duttaroy AK. Transport of fatty acids across the human placenta: a review. Prog Lipid Res. 2009;48(1):52-61.

9. Fowden A. Endocrine regulation of fetal growth. Reproduction, Fertility and Development. 1995;7(3):351-63.

10. Lager S, Powell TL. Regulation of nutrient transport across the placenta. J Pregnancy. 2012;2012:179827.

11. Brett KE, Ferraro ZM, Yockell-Lelievre J, Gruslin A, Adamo KB. Maternal-fetal nutrient transport in pregnancy pathologies: the role of the placenta. Int J Mol Sci. 2014;15(9):16153-85.

12. Fowden AL, Sferruzzi-Perri AN, Coan PM, Constancia M, Burton GJ. Placental efficiency and adaptation: endocrine regulation. J Physiol. 2009;587(Pt 14):3459-72.

13. Hay WW, Jr. The placenta. Not just a conduit for maternal fuels. Diabetes. 1991;40 Suppl 2:44-50.

14. Gallo LA, Barrett HL, Dekker Nitert M. Review: Placental transport and metabolism of energy substrates in maternal obesity and diabetes. Placenta. 2017;54:59-67.

15. Higgins L, Greenwood SL, Wareing M, Sibley CP, Mills TA. Obesity and the placenta: A consideration of nutrient exchange mechanisms in relation to aberrant fetal growth. Placenta. 2011;32(1):1-7.

16. de Ruiter I, Olmedo-Requena R, Jimenez-Moleon JJ. Parental and Child Factors Associated with Under-Estimation of Children with Excess Weight in Spain. Matern Child Health J. 2017.

17. Remmers T, van Grieken A, Renders CM, Hirasing RA, Broeren SM, Raat H. Correlates of parental misperception of their child's weight status: the 'be active, eat right' study. PLoS One. 2014;9(2):e88931.

18. Brantley PJ, Myers VH, Roy HJ. Environmental and lifestyle influences on obesity. J La State Med Soc. 2005;157 Spec No 1:S19-27.

19. Berthoud HR, Lenard NR, Shin AC. Food reward, hyperphagia, and obesity. Am J Physiol Regul Integr Comp Physiol. 2011;300(6):R1266-77.

20. Burger KS, Stice E. Variability in reward responsivity and obesity: evidence from brain imaging studies. Curr Drug Abuse Rev. 2011;4(3):182-9.

21. Verdejo-Roman J, Vilar-Lopez R, Navas JF, Soriano-Mas C, Verdejo-Garcia A. Brain reward system's alterations in response to food and monetary stimuli in overweight and obese individuals. Hum Brain Mapp. 2017;38(2):666-77.

22. Stice E, Yokum S, Burger KS, Epstein LH, Small DM. Youth at risk for obesity show greater activation of striatal and somatosensory regions to food. J Neurosci. 2011;31(12):4360-6.

23. Alfaradhi MZ, Ozanne SE. Developmental programming in response to maternal overnutrition. Front Genet. 2011;2:27.

24. Agarwal MM. Gestational diabetes mellitus: An update on the current international diagnostic criteria. World J Diabetes. 2015;6(6):782-91.

25. Ashwal E, Hod M. Gestational diabetes mellitus: Where are we now? Clin Chim Acta. 2015;451(Pt A):14-20.

26. Ricart W, Lopez J, Mozas J, Pericot A, Sancho MA, Gonzalez N, et al. Potential impact of American Diabetes Association (2000) criteria for diagnosis of gestational diabetes mellitus in Spain. Diabetologia. 2005;48(6):1135-41.

27. Ryan EA. Hormones and insulin resistance during pregnancy. Lancet. 2003;362(9398):1777-8.

28. Herrera E, Ortega-Senovilla H. Disturbances in lipid metabolism in diabetic pregnancy - Are these the cause of the problem? Best Pract Res Clin Endocrinol Metab. 2010;24(4):515-25.

29. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr. 2000;71(5 Suppl):1256S-61S.

30. Schaefer-Graf UM, Graf K, Kulbacka I, Kjos SL, Dudenhausen J, Vetter K, et al. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus. Diabetes Care. 2008;31(9):1858-63.

31. Marseille-Tremblay C, Ethier-Chiasson M, Forest JC, Giguere Y, Masse A, Mounier C, et al. Impact of maternal circulating cholesterol and gestational diabetes mellitus on lipid metabolism in human term placenta. Mol Reprod Dev. 2008;75(6):1054-62.

32. Sanchez-Vera I, Bonet B, Viana M, Quintanar A, Martin M, Blanco P, et al. Changes in plasma lipids and increased low-density lipoprotein susceptibility to oxidation in pregnancies complicated by gestational diabetes: consequences of obesity. Metabolism. 2007;56(11):1527-33.

33. Hollingsworth DR, Grundy SM. Pregnancy-associated Hypertriglyceridemia in Normal and Diabetic Women: Differences in Insulin-dependent, Non-insulin-dependent, and Gestational Diabetes. Diabetes. 1982;31(12):1092-7.

34. Montelongo A, Lasunción MA, Pallardo LF, Herrera E. Longitudinal Study of Plasma Lipoproteins and Hormones During Pregnancy in Normal and Diabetic Women. Diabetes. 1992;41(12):1651-9.

35. Bozkurt L, Gobl CS, Hormayer AT, Luger A, Pacini G, Kautzky-Willer A. The impact of preconceptional obesity on trajectories of maternal lipids during gestation. Sci Rep. 2016;6:29971.

36. Sonagra AD, Biradar SM, K D, Murthy DSJ. Normal pregnancy- a state of insulin resistance. J Clin Diagn Res. 2014;8(11):Cc01-3.

37. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26(2):19-39.

38. Alonso A, Del Rey CG, Navarro A, Tolivia J, Gonzalez CG. Effects of gestational diabetes mellitus on proteins implicated in insulin signaling in human placenta. Gynecol Endocrinol. 2006;22(9):526-35.

39. Leddy MA, Power ML, Schulkin J. The Impact of Maternal Obesity on Maternal and Fetal Health. Rev Obstet Gynecol. 2008;1(4):170-8.

40. Zambrano E, Nathanielsz PW. Mechanisms by which maternal obesity programs offspring for obesity: evidence from animal studies. Nutr Rev. 2013;71 Suppl 1:S42-54.

41. Bautista-Castano I, Henriquez-Sanchez P, Aleman-Perez N, Garcia-Salvador JJ, Gonzalez-Quesada A, Garcia-Hernandez JA, et al. Maternal obesity in early pregnancy and risk of adverse outcomes. PLoS One. 2013;8(11):e80410.

42. Marchi J, Berg M, Dencker A, Olander EK, Begley C. Risks associated with obesity in pregnancy, for the mother and baby: a systematic review of reviews. Obes Rev. 2015;16(8):621-38.

43. Thornburg LL. Antepartum obstetrical complications associated with obesity. Semin Perinatol. 2011;35(6):317-23.

44. Gaillard R, Durmus B, Hofman A, Mackenbach JP, Steegers EA, Jaddoe VW. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity (Silver Spring). 2013;21(5):1046-55.

45. Ramachenderan J, Bradford J, McLean M. Maternal obesity and pregnancy complications: a review. Aust N Z J Obstet Gynaecol. 2008;48(3):228-35.

46. Chu SY, Callaghan WM, Kim SY, Schmid CH, Lau J, England LJ, et al. Maternal obesity and risk of gestational diabetes mellitus. Diabetes Care. 2007;30(8):2070-6.

47. Farren M, Daly N, O'Higgins AC, McKeating A, Maguire PJ, Turner MJ. The interplay between maternal obesity and gestational diabetes mellitus. J Perinat Med. 2015;43(3):311-7.

48. Catalano PM. Obesity, Insulin Resistance and Pregnancy Outcome. Reproduction. 2010;140(3):365-71.

49. Ye J. Mechanisms of insulin resistance in obesity. Front Med. 2013;7(1):14-24.

50. Sivan E, Chen X, Homko CJ, Reece EA, Boden G. Longitudinal study of carbohydrate metabolism in healthy obese pregnant women. Diabetes Care. 1997;20(9):1470-5.

51. Helewa ME, Burrows RF, Smith J, Williams K, Brain P, Rabkin SW. Report of the Canadian Hypertension Society Consensus Conference: 1. Definitions, evaluation and classification of hypertensive disorders in pregnancy. Cmaj. 1997;157(6):715-25.

52. Sibai BM, Ewell M, Levine RJ, Klebanoff MA, Esterlitz J, Catalano PM, et al. Risk factors associated with preeclampsia in healthy nulliparous women. The Calcium for Preeclampsia Prevention (CPEP) Study Group. Am J Obstet Gynecol. 1997;177(5):1003-10.

53. Kintiraki E, Papakatsika S, Kotronis G, Goulis DG, Kotsis V. Pregnancy-Induced hypertension. Hormones (Athens). 2015;14(2):211-23.

54. Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L, et al. Placental bed spiral arteries in the hypertensive disorders of pregnancy. Br J Obstet Gynaecol. 1991;98(7):648-55.

55. Barton JR, Sibai BM. Prediction and prevention of recurrent preeclampsia. Obstet Gynecol. 2008;112(2 Pt 1):359-72.

56. Kaaja R, Laivuori H, Laakso M, Tikkanen MJ, Ylikorkala O. Evidence of a state of increased insulin resistance in preeclampsia. Metabolism. 1999;48(7):892-6.

57. Solomon CG, Seely EW. Brief review: hypertension in pregnancy : a manifestation of the insulin resistance syndrome? Hypertension. 2001;37(2):232-9.

58. Seely EW, Solomon CG. Insulin resistance and its potential role in pregnancy-induced hypertension. J Clin Endocrinol Metab. 2003;88(6):2393-8.

59. Chu SY, Kim SY, Lau J, Schmid CH, Dietz PM, Callaghan WM, et al. Maternal obesity and risk of stillbirth: a metaanalysis. Am J Obstet Gynecol. 2007;197(3):223-8.

60. Stephansson O, Dickman PW, Johansson A, Cnattingius S. Maternal weight, pregnancy weight gain, and the risk of antepartum stillbirth. Am J Obstet Gynecol. 2001;184(3):463-9.

61. Tenenbaum-Gavish K, Hod M. Impact of maternal obesity on fetal health. Fetal Diagn Ther. 2013;34(1):1-7.

62. Wang T, Zhang J, Lu X, Xi W, Li Z. Maternal early pregnancy body mass index and risk of preterm birth. Arch Gynecol Obstet. 2011;284(4):813-9.

63. Johnson TS, Rottier KJ, Luellwitz A, Kirby RS. Maternal prepregnancy body mass index and delivery of a preterm infant in missouri 1998-2000. Public Health Nurs. 2009;26(1):3-13.

64. Torloni MR, Betran AP, Daher S, Widmer M, Dolan SM, Menon R, et al. Maternal BMI and preterm birth: a systematic review of the literature with meta-analysis. J Matern Fetal Neonatal Med. 2009;22(11):957-70.

65. Madan J, Chen M, Goodman E, Davis J, Allan W, Dammann O. Maternal obesity, gestational hypertension, and preterm delivery. J Matern Fetal Neonatal Med. 2010;23(1):82-8.

66. Stotland NE, Washington AE, Caughey AB. Prepregnancy body mass index and the length of gestation at term. Am J Obstet Gynecol. 2007;197(4):378.e1-5.

67. Lynch CM, Sexton DJ, Hession M, Morrison JJ. Obesity and Mode of Delivery in Primigravid and Multigravid Women.

68. Johnson JW, Longmate JA, Frentzen B. Excessive maternal weight and pregnancy outcome. Am J Obstet Gynecol. 1992;167(2):353-70; discussion 70-2.

69. Li M, Sloboda DM, Vickers MH. Maternal Obesity and Developmental Programming of Metabolic Disorders in Offspring: Evidence from Animal Models. Exp Diabetes Res. 2011;2011.

70. Samuelsson AM, Matthews PA, Argenton M, Christie MR, McConnell JM, Jansen EH, et al. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. Hypertension. 2008;51(2):383-92.

71. Nivoit P, Morens C, Van Assche FA, Jansen E, Poston L, Remacle C, et al. Established diet-induced obesity in female rats leads to offspring hyperphagia, adiposity and insulin resistance. Diabetologia. 2009;52(6):1133-42.

72. Bayol SA, Farrington SJ, Stickland NC. A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring. Br J Nutr. 2007;98(4):843-51.

73. Berti C, Cetin I, Agostoni C, Desoye G, Devlieger R, Emmett PM, et al. Pregnancy and Infants' Outcome: Nutritional and Metabolic Implications. Crit Rev Food Sci Nutr. 2016;56(1):82-91.

74. Calder PC. Functional Roles of Fatty Acids and Their Effects on Human Health. JPEN J Parenter Enteral Nutr. 2015;39(1 Suppl):18s-32s.

75. Lee JM, Lee H, Kang SB, Park WJ. Fatty Acid Desaturases, Polyunsaturated Fatty Acid Regulation, and Biotechnological Advances. Nutrients. 82016.

76. Cetin I, Alvino G, Cardellicchio M. Long chain fatty acids and dietary fats in fetal nutrition. J Physiol. 2009;587(Pt 14):3441-51.

77. Koletzko B, Lien E, Agostoni C, Böhles H, Campoy C, Cetin I, et al. **The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations**. Journal of Perinatal Medicine. 2008;36(1):5-14.

78. Feingold K, Grunfeld C. Introduction to Lipids and Lipoproteins. Endotext [Internet]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK305896/.

79. Spector AA, Kim HY. Discovery of essential fatty acids. J Lipid Res. 562015. p. 11-21.

80. DiNicolantonio JJ, Lucan SC, O'Keefe JH. The Evidence for Saturated Fat and for Sugar Related to Coronary Heart Disease. Prog Cardiovasc Dis. 2016;58(5):464-72.

81. Masi L, Rodrigues A, Curi R. Fatty acids regulation of inflammatory and metabolic genes. Current Opinion in Clinical Nutrition and Metabolic Care. 2013;16(4):418-24.

82. Georgiadi A, Kersten S. Mechanisms of Gene Regulation by Fatty Acids. Adv Nutr. 32012. p. 127-34.

83. Grieger JA, Clifton VL. A Review of the Impact of Dietary Intakes in Human Pregnancy on Infant Birthweight. Nutrients. 72015. p. 153-78.

84. Herrera E, Ortega-Senovilla H. Implications of Lipids in Neonatal Body Weight and Fat Mass in Gestational Diabetic Mothers and Non-Diabetic Controls. Curr Diab Rep. 2018;18(2):7.

85. Gademan MG, Vermeulen M, Oostvogels AJ, Roseboom TJ, Visscher TL, van Eijsden M, et al. Maternal prepregancy BMI and lipid profile during early pregnancy are independently associated with offspring's body composition at age 5-6 years: the ABCD study. PLoS One. 2014;9(4):e94594.

86. Campoy C, Escolano-Margarit MV, Anjos T, Szajewska H, Uauy R. Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. Br J Nutr. 2012;107 Suppl 2:S85-106.

87. Makrides M, Smithers LG, Gibson RA. Role of long-chain polyunsaturated fatty acids in neurodevelopment and growth. Nestle Nutr Workshop Ser Pediatr Program. 2010;65:123-33; discussion 33-6.

88. Koletzko B, Uauy R, Palou A, Kok F, Hornstra G, Eilander A, et al. Dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in children – a workshop report. Nutrition. 2010;103(6):923-8.

89. Kris-Etherton PM, Grieger JA, Etherton TD. Dietary reference intakes for DHA and EPA. Prostaglandins Leukot Essent Fatty Acids. 2009;81(2-3):99-104.

90. Korotkova M, Gabrielsson B, Lonn M, Hanson LA, Strandvik B. Leptin levels in rat offspring are modified by the ratio of linoleic to alpha-linolenic acid in the maternal diet. J Lipid Res. 2002;43(10):1743-9.

91. Ailhaud G, Guesnet P, Cunnane SC. An emerging risk factor for obesity: does disequilibrium of polyunsaturated fatty acid metabolism contribute to excessive adipose tissue development? Br J Nutr. 2008;100(3):461-70.

92. Mennitti LV, Oliveira JL, Morais CA, Estadella D, Oyama LM, Oller do Nascimento CM, et al. Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. J Nutr Biochem. 2015;26(2):99-111.

93. Campbell FM, Gordon MJ, Dutta-Roy AK. Preferential uptake of long chain polyunsaturated fatty acids by isolated human placental membranes. Mol Cell Biochem. 1996;155(1):77-83.

94. Enke U, Jaudszus A, Schleussner E, Seyfarth L, Jahreis G, Kuhnt K. Fatty acid distribution of cord and maternal blood in human pregnancy: special focus on individual trans fatty acids and conjugated linoleic acids. Lipids Health Dis. 2011;10:247.

95. Albuquerque KT, Sardinha FL, Telles MM, Watanabe RL, Nascimento CM, Tavares do Carmo MG, et al. Intake of trans fatty acid-rich hydrogenated fat during pregnancy and lactation inhibits the hypophagic effect of central insulin in the adult offspring. Nutrition. 2006;22(7-8):820-9.

96. Savage DB, Semple RK. Recent insights into fatty liver, metabolic dyslipidaemia and their links to insulin resistance. Curr Opin Lipidol. 2010;21(4):329-36.

97. Dongiovanni P, Stender S, Pietrelli A, Mancina RM, Cespiati A, Petta S, et al. Causal relationship of hepatic fat with liver damage and insulin resistance in nonalcoholic fatty liver. J Intern Med. 2018;283(4):356-70.

98. Gregorio BM, Souza-Mello V, Mandarim-de-Lacerda CA, Aguila MB. Maternal high-fat diet is associated with altered pancreatic remodelling in mice offspring. Eur J Nutr. 2013;52(2):759-69.

99. Oller do Nascimento CM, Ribeiro EB, Oyama LM. Metabolism and secretory function of white adipose tissue: effect of dietary fat. An Acad Bras Cienc. 2009;81(3):453-66.

100. Kirk SL, Samuelsson AM, Argenton M, Dhonye H, Kalamatianos T, Poston L, et al. Maternal obesity induced by diet in rats permanently influences central processes regulating food intake in offspring. PLoS One. 2009;4(6):e5870.

101. Bouret SG. Role of early hormonal and nutritional experiences in shaping feeding behavior and hypothalamic development. J Nutr. 2010;140(3):653-7.

102. Kabaran S, Besler HT. Do fatty acids affect fetal programming? J Health Popul Nutr. 33. London2015.

103. Kuipers RS, Luxwolda MF, Offringa PJ, Boersma ER, Dijck-Brouwer DA, Muskiet FA. Fetal intrauterine whole body linoleic, arachidonic and docosahexaenoic acid contents and accretion rates. Prostaglandins Leukot Essent Fatty Acids. 2012;86(1-2):13-20.

104. Otto SJ, Houwelingen AC, Antal M, Manninen A, Godfrey K, Lopez-Jaramillo P, et al. Maternal and neonatal essential fatty acid status in phospholipids: an international comparative study. Eur J Clin Nutr. 1997;51(4):232-42.

105. Al MD, van Houwelingen AC, Hornstra G. Relation between birth order and the maternal and neonatal docosahexaenoic acid status. Eur J Clin Nutr. 1997;51(8):548-53.

106. Haggarty P. Fatty acid supply to the human fetus. Annu Rev Nutr. 2010;30:237-55.

107. Innis SM. Palmitic Acid in Early Human Development. Crit Rev Food Sci Nutr. 2016;56(12):1952-9.

108. Benassayag C, Mignot TM, Haourigui M, Civel C, Hassid J, Carbonne B, et al. High polyunsaturated fatty acid, thromboxane A2, and alpha-fetoprotein concentrations at the human feto-maternal interface. J Lipid Res. 1997;38(2):276-86.

109. Perazzolo S, Hirschmugl B, Wadsack C, Desoye G, Lewis RM, Sengers BG. The influence of placental metabolism on fatty acid transfer to the fetus. J Lipid Res. 2017;58(2):443-54.

110. Haggarty P, Page K, Abramovich DR, Ashton J, Brown D. Long-chain polyunsaturated fatty acid transport across the perfused human placenta. Placenta. 1997;18(8):635-42.

111. Haggarty P, Ashton J, Joynson M, Abramovich DR, Page K. Effect of maternal polyunsaturated fatty acid concentration on transport by the human placenta. Biol Neonate. 1999;75(6):350-9.

112. Larque E, Demmelmair H, Berger B, Hasbargen U, Koletzko B. In vivo investigation of the placental transfer of (13)C-labeled fatty acids in humans. J Lipid Res. 2003;44(1):49-55.

113. Gil-Sanchez A, Larque E, Demmelmair H, Acien MI, Faber FL, Parrilla JJ, et al. Maternal-fetal in vivo transfer of [13C]docosahexaenoic and other fatty acids across the human placenta 12 h after maternal oral intake. Am J Clin Nutr. 2010;92(1):115-22.

114. Larque E, Demmelmair H, Gil-Sanchez A, Prieto-Sanchez MT, Blanco JE, Pagan A, et al. Placental transfer of fatty acids and fetal implications. Am J Clin Nutr. 2011;94(6 Suppl):1908s-13s.

115. Gil-Sanchez A, Demmelmair H, Parrilla JJ, Koletzko B, Larque E. Mechanisms involved in the selective transfer of long chain polyunsaturated Fatty acids to the fetus. Front Genet. 2011;2:57.

116. Larque E, Ruiz-Palacios M, Koletzko B. Placental regulation of fetal nutrient supply. Curr Opin Clin Nutr Metab Care. 2013;16(3):292-7.

117. Haggarty P. Placental regulation of fatty acid delivery and its effect on fetal growth--a review. Placenta. 2002;23 Suppl A:S28-38.

118. Cunningham P, McDermott L. Long chain PUFA transport in human term placenta. J Nutr. 2009;139(4):636-9.

119. Desoye G, Gauster M, Wadsack C. Placental transport in pregnancy pathologies. Am J Clin Nutr. 2011;94(6 Suppl):1896s-902s.

120. Cetin I, Parisi F, Berti C, Mando C, Desoye G. Placental fatty acid transport in maternal obesity. Journal of Developmental Origins of Health and Disease. 2012;3(6):409-14.

121. Gauster M, Hiden U, van Poppel M, Frank S, Wadsack C, Hauguel-de Mouzon S, et al. Dysregulation of placental endothelial lipase in obese women with gestational diabetes mellitus. Diabetes. 2011;60(10):2457-64.

122. Stahl A, RE G, LA T, Lodish H. Fatty acid transport proteins: a current view of a growing family. Trends Endocrinol Metab. 2001;12(6):266-73.

123. Campbell FM, Bush PG, Veerkamp JH, Dutta-Roy AK. Detection and cellular localization of plasma membrane-associated and cytoplasmic fatty acid-binding proteins in human placenta. Placenta. 1998;19(5-6):409-15.

124. Gimeno RE, Hirsch DJ, Punreddy S, Sun Y, Ortegon AM, Wu H, et al. Targeted Deletion of Fatty Acid Transport Protein-4 Results in Early Embryonic Lethality. Journal of Biological Chemistry. 2003;278(49):49512-6.

125. Larqué E, Demmelmair H, Gil-Sánchez A, Prieto-Sánchez MT, Blanco JE, Pagán A, et al. Placental transfer of fatty acids and fetal implications. The American Journal of Clinical Nutrition. 2011;94(6 Suppl):1908S-13S.

126. Larque E, Krauss-Etschmann S, Campoy C, Hartl D, Linde J, Klingler M, et al. Docosahexaenoic acid supply in pregnancy affects placental expression of fatty acid transport proteins. Am J Clin Nutr. 2006;84(4):853-61.

127. Kazantzis M, Stahl A. Fatty acid transport proteins, implications in physiology and disease. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2012;1821(5):852-7.

128. Lewis RM, Childs CE, Calder PC. New perspectives on placental fatty acid transfer. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2018;138:24-9.

129. Chmurzynska A. The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. J Appl Genet. 2006;47(1):39-48.

130. Jones ML, Mark PJ, Waddell BJ. Maternal dietary omega-3 fatty acids and placental function. Reproduction. 2014;147(5):R143-52.

131. Smathers RL, Petersen DR. The human fatty acid-binding protein family: evolutionary divergences and functions. Hum Genomics. 2011;5(3):170-91.

132. Madsen EM, Lindegaard ML, Andersen CB, Damm P, Nielsen LB. Human placenta secretes apolipoprotein B-100-containing lipoproteins. J Biol Chem. 2004;279(53):55271-6.

133. Magnusson A, Waterman I, Wennergren M, Jansson T, Powell TL. Triglyceride hydrolase activities and expression of fatty acid binding proteins in the human placenta in pregnancies complicated by intrauterine growth restriction and diabetes. J Clin Endocrinol Metab. 2004;89(9):4607-14.

134. Gauster M, Hiden U, Blaschitz A, Frank S, Lang U, Alvino G, et al. Dysregulation of placental endothelial lipase and lipoprotein lipase in intrauterine growth-restricted pregnancies. J Clin Endocrinol Metab. 2007;92(6):2256-63.

135. Tabano S, Alvino G, Antonazzo P, Grati FR, Miozzo M, Cetin I. Placental LPL gene expression is increased in severe intrauterine growth-restricted pregnancies. Pediatr Res. 2006;59(2):250-3.

136. Dubé E, Gravel A, Martin C, Desparois G, Moussa I, Ethier-Chiasson M, et al. Modulation of Fatty Acid Transport and Metabolism by Maternal Obesity in the Human Full-Term Placenta1. Biology of Reproduction. 2012;87(1):1-11.

137. Pagan A, Prieto-Sanchez MT, Blanco-Carnero JE, Gil-Sanchez A, Parrilla JJ, Demmelmair H, et al. Materno-fetal transfer of docosahexaenoic acid is impaired by gestational diabetes mellitus. Am J Physiol Endocrinol Metab. 2013;305(7):E826-33.

138. Wijendran V, Bendel RB, Couch SC, Philipson EH, Cheruku S, Lammi-Keefe CJ. Fetal erythrocyte phospholipid polyunsaturated fatty acids are altered in pregnancy complicated with gestational diabetes mellitus. Lipids. 2000;35(8):927-31.

139. Campoy C, Martin-Bautista E, Garcia-Valdes L, Florido J, Agil A, Lorente JA, et al. Study of maternal nutrition and genetic on the foetal adiposity programming (The PREOBE study). Nutr Hosp. 2008;23(6):584-90.

140. Berglund SK, Garcia-Valdes L, Torres-Espinola FJ, Segura MT, Martinez-Zaldivar C, Aguilar MJ, et al. Maternal, fetal and perinatal alterations associated with obesity, overweight and gestational diabetes: an observational cohort study (PREOBE). BMC Public Health. 2016;16:207.

141. (NIDDK) NHaLINaNIfDaDaKD. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults--The Evidence Report. National Institutes of Health. Obes Res. 1998;6(2):51S-209S.

142. ACOG technical bulletin. Diabetes and pregnancy. Number 200--December 1994 (replaces No. 92, May 1986). Committee on Technical Bulletins of the American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet. 1995;48(3):331-9.

143. Institute of Medicine and National Research Council Committee to Reexamine IOMPWG. The National Academies Collection: Reports funded by National Institutes of Health. In: Rasmussen KM, Yaktine AL, editors. Weight Gain During Pregnancy: Reexamining the Guidelines. Washington (DC): National Academies Press (US)

National Academy of Sciences.; 2009.

144. Klingler M, Demmelmair H, Larque E, Koletzko B. Analysis of FA contents in individual lipid fractions from human placental tissue. Lipids. 2003;38(5):561-6.

145. Meller M, Vadachkoria S, Luthy DA, Williams MA. Evaluation of housekeeping genes in placental comparative expression studies. Placenta. 2005;26(8-9):601-7.

146. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. Philos Trans R Soc Lond B Biol Sci. 3702015.

147. Radaelli T, Lepercq J, Varastehpour A, Basu S, Catalano PM, Hauguel-De Mouzon S. Differential regulation of genes for fetoplacental lipid pathways in pregnancy with gestational and type 1 diabetes mellitus. Am J Obstet Gynecol. 2009;201(2):209 e1- e10.

148. Desoye G, Hauguel-de Mouzon S. The Human Placenta in Gestational Diabetes Mellitus: The insulin and cytokine network. Diabetes Care. 2007;30(Supplement 2):S120-S6.

149. Burton GJ, Fowden AL. Review: The placenta and developmental programming: balancing fetal nutrient demands with maternal resource allocation. Placenta. 2012;33 Suppl:S23-7.

150. Castillo-Castrejon M, Powell TL. Placental Nutrient Transport in Gestational Diabetic Pregnancies. Front Endocrinol (Lausanne). 2017;8:306.

151. Geraghty AA, Alberdi G, O'Sullivan EJ, O'Brien EC, Crosbie B, Twomey PJ, et al. Maternal and fetal blood lipid concentrations during pregnancy differ by maternal body mass index: findings from the ROLO study. BMC Pregnancy Childbirth. 2017;17(1):360.

152. Barbour LA, Farabi SS, Friedman JE, Hirsch NM, Reece MS, Van Pelt RE, et al. Postprandial Triglycerides Predict Newborn Fat More Strongly than Glucose in Women with Obesity in Early Pregnancy. Obesity (Silver Spring). 2018;26(8):1347-56.

153. Schaefer-Graf UM, Meitzner K, Ortega-Senovilla H, Graf K, Vetter K, Abou-Dakn M, et al. Differences in the implications of maternal lipids on fetal metabolism and growth between gestational diabetes mellitus and control pregnancies. Diabet Med. 2011;28(9):1053-9.

154. Martino J, Sebert S, Segura MT, Garcia-Valdes L, Florido J, Padilla MC, et al. Maternal Body Weight and Gestational Diabetes Differentially Influence Placental and Pregnancy Outcomes. J Clin Endocrinol Metab. 2016;101(1):59-68.

155. Motojima K, Passilly P, Peters JM, Gonzalez FJ, Latruffe N. Expression of Putative Fatty Acid Transporter Genes Are Regulated by Peroxisome Proliferator-activated Receptor α and γ Activators in a Tissue- and Inducer-specific Manner. Journal of Biological Chemistry. 1998;273(27):16710-4.

156. Arck P, Toth B, Pestka A, Jeschke U. Nuclear Receptors of the Peroxisome Proliferator-Activated Receptor (PPAR) Family in Gestational Diabetes: From Animal Models to Clinical Trials. Biology of Reproduction. 2010;83(2):168-76.

157. Stahl A, Gimeno R, Tartaglia L, Lodish H. Fatty acid transport proteins: a current view of a growing family. Trends Endocrinol Metab. 2001;12(6):266-73.

158. Holdsworth-Carson SJ, Lim R, Mitton A, Whitehead C, Rice GE, Permezel M, et al. Peroxisome proliferator-activated receptors are altered in pathologies of the human placenta: Gestational diabetes mellitus, intrauterine growth restriction and preeclampsia. Placenta. 2010;31(3):222-9.

159. Ye K, Li L, Zhang D, Li Y, Wang HQ, Lai HL, et al. Effect of Maternal Obesity on Fetal Growth and Expression of Placental Fatty Acid Transporters. J Clin Res Pediatr Endocrinol. 2017;9(4):300-7.

160. Tian L, Dong SS, Hu J, Yao JJ, Yan PS. The effect of maternal obesity on fatty acid transporter expression and lipid metabolism in the full-term placenta of lean breed swine. J Anim Physiol Anim Nutr (Berl). 2018;102(1):e242-e53.

161. Lindegaard ML, Damm P, Mathiesen ER, Nielsen LB. Placental triglyceride accumulation in maternal type 1 diabetes is associated with increased lipase gene expression. J Lipid Res. 2006;47(11):2581-8.

162. Godfrey KM, Matthews N, Glazier J, Jackson A, Wilman C, Sibley CP. Neutral amino acid uptake by the microvillous plasma membrane of the human placenta is inversely related to fetal size at birth in normal pregnancy. J Clin Endocrinol Metab. 1998;83(9):3320-6.

163. Li L, Lee SJ, Kook SY, Ahn TG, Lee JY, Hwang JY. Serum from pregnant women with gestational diabetes mellitus increases the expression of FABP4 mRNA in primary subcutaneous human pre-adipocytes. Obstet Gynecol Sci. 2017;60(3):274-82.

164. Ning H, Tao H, Weng Z, Zhao X. Plasma fatty acid-binding protein 4 (FABP4) as a novel biomarker to predict gestational diabetes mellitus. Acta Diabetol. 2016;53(6):891-8.

165. Roberts KA, Riley SC, Reynolds RM, Barr S, Evans M, Statham A, et al. Placental structure and inflammation in pregnancies associated with obesity. Placenta. 2011;32(3):247-54.

166. Floresta G, Pistara V, Amata E, Dichiara M, Marrazzo A, Prezzavento O, et al. Adipocyte fatty acid binding protein 4 (FABP4) inhibitors. A comprehensive systematic review. Eur J Med Chem. 2017;138:854-73.

167. Wang TY, Liu M, Portincasa P, Wang DQ. New insights into the molecular mechanism of intestinal fatty acid absorption. Eur J Clin Invest. 2013;43(11):1203-23.

168. Anastasiou CA, Kavouras SA, Lentzas Y, Gova A, Sidossis LS, Melidonis A. Diabetes mellitus is associated with increased intramyocellular triglyceride, but not diglyceride, content in obese humans. Metabolism. 2009;58(11):1636-42.

169. Jansson T, Cetin I, Powell TL, Desoye G, Radaelli T, Ericsson A, et al. Placental transport and metabolism in fetal overgrowth -- a workshop report. Placenta. 27 Suppl A. Netherlands2006. p. S109-13.

170. Catalano PM, Thomas A, Huston-Presley L, Amini SB. Increased fetal adiposity: a very sensitive marker of abnormal in utero development. Am J Obstet Gynecol. 2003;189(6):1698-704.

171. Schaefer-Graf UM, Kjos SL, Buhling KJ, Henrich W, Brauer M, Heinze T, et al. Amniotic fluid insulin levels and fetal abdominal circumference at time of amniocentesis in pregnancies with diabetes. Diabet Med. 2003;20(5):349-54.

172. Thame M, Osmond C, Bennett F, Wilks R, Forrester T. Fetal growth is directly related to maternal anthropometry and placental volume. Eur J Clin Nutr. 2004;58(6):894-900.

173. Winick M, Coscia A, Noble A. Cellular growth in human placenta. I. Normal placental growth. Pediatrics. 1967;39(2):248-51.

174. Coan PM, Angiolini E, Sandovici I, Burton GJ, Constancia M, Fowden AL. Adaptations in placental nutrient transfer capacity to meet fetal growth demands depend on placental size in mice. J Physiol. 2008;586(18):4567-76.

175. Constancia M, Angiolini E, Sandovici I, Smith P, Smith R, Kelsey G, et al. Adaptation of nutrient supply to fetal demand in the mouse involves interaction between the Igf2 gene and placental transporter systems. Proc Natl Acad Sci U S A. 2005;102(52):19219-24.

176. Sibley CP, Brownbill P, Dilworth M, Glazier JD. Review: Adaptation in placental nutrient supply to meet fetal growth demand: implications for programming. Placenta. 2010;31 Suppl:S70-4.

177. Hayward CE, Lean S, Sibley CP, Jones RL, Wareing M, Greenwood SL, et al. Placental Adaptation: What Can We Learn from Birthweight:Placental Weight Ratio? Front Physiol. 2016;7:28.

178. Sandovici I, Hoelle K, Angiolini E, Constancia M. Placental adaptations to the maternal-fetal environment: implications for fetal growth and developmental programming. Reprod Biomed Online. 2012;25(1):68-89.

179. Barker DJ. Obesity and early life. Obes Rev. 2007;8 Suppl 1:45-9.

180. Kwon JH, Jang HY, Oh MJ, Rho JS, Jung JH, Yum KS, et al. Association of Visceral Fat and Risk Factors for Metabolic Syndrome in Children and Adolescents. Yonsei Med J. 2011;52(1):39-44.

181. Maffeis C, Morandi A. Body composition and insulin resistance in children. Eur J Clin Nutr. 2018;72(9):1239-45.

182. Hirschler V, Aranda C, Calcagno Mde L, Maccalini G, Jadzinsky M. Can waist circumference identify children with the metabolic syndrome? Arch Pediatr Adolesc Med. 2005;159(8):740-4.

183. Janssen I, Heymsfield SB, Allison DB, Kotler DP, Ross R. Body mass index and waist circumference independently contribute to the prediction of nonabdominal, abdominal subcutaneous, and visceral fat. Am J Clin Nutr. 2002;75(4):683-8.

184. Saben J, Lindsey F, Zhong Y, Thakali K, Badger TM, Andres A, et al. Maternal obesity is associated with a lipotoxic placental environment. Placenta. 2014;35(3):171-7.

185. Malti N, Merzouk H, Merzouk SA, Loukidi B, Karaouzene N, Malti A, et al. Oxidative stress and maternal obesity: feto-placental unit interaction. Placenta. 2014;35(6):411-6.

186. Altmae S, Segura MT, Esteban FJ, Bartel S, Brandi P, Irmler M, et al. Maternal Pre-Pregnancy Obesity Is Associated with Altered Placental Transcriptome. PLoS One. 2017;12(1):e0169223.

187. Hastie R, Lappas M. The effect of pre-existing maternal obesity and diabetes on placental mitochondrial content and electron transport chain activity. Placenta. 2014;35(9):673-83.

188. Mando C, Anelli GM, Novielli C, Panina-Bordignon P, Massari M, Mazzocco MI, et al. Impact of Obesity and Hyperglycemia on Placental Mitochondria. Oxid Med Cell Longev. 2018;2018:2378189.

189. Vaughan OR, Fowden AL. Placental metabolism: substrate requirements and the response to stress. Reprod Domest Anim. 2016;51 Suppl 2:25-35.

190. Zhu MJ, Ma Y, Long NM, Du M, Ford SP. Maternal obesity markedly increases placental fatty acid transporter expression and fetal blood triglycerides at midgestation in the ewe. Am J Physiol Regul Integr Comp Physiol. 2010;299(5):R1224-31.

191. Kabaran S, Besler HT. Do fatty acids affect fetal programming? J Health Popul Nutr. 2015;33:14.

ANEXES

THESIS ASSOCIATED PUBLICATION

Part of the results includes in this Doctoral thesis have been previously published as:

Maternal BMI and gestational diabetes alter placental lipid transporters and fatty acid composition. **Segura MT**, Demmelmair H, Krauss-Etschmann S, Nathan P, Dehmel S, Padilla MC, Rueda R, Koletzko B, Campoy C.Placenta. 2017 Sep;57:144-151. doi: 10.1016/j.placenta.2017.07.001. Epub 2017 Jul 3.PMID: 28864004

Placenta Journal quality key indicators from 2016 Journal Citation Reports (JCR Index): Impact factor: 2.759; 5 years impact factor: 2.982; Total cites: 8159; Q1 in Obstetrics & Gynecology, position 19 of 80.

OTHER PUBLICATIONS RELATED TO THE TOPIC OF THIS DOCTORAL THESIS

The Impact of Maternal Pre-Pregnancy Body Weight and Gestational Diabetes on Markers of Folate Metabolism in the Placenta. Martino J, **Segura MT**, García-Valdés L, Padilla MC, Rueda R, McArdle HJ, Budge H, Symonds ME, Campoy C. Nutrients. 2018 Nov 13;10(11). pii: E1750. doi: 10.3390/nu10111750. PMID:30428605

Maternal Pre-Pregnancy Obesity Is Associated with Altered Placental Transcriptome. Altmäe S, **Segura MT**, Esteban FJ, Bartel S, Brandi P, Irmler M, Beckers J, Demmelmair H, López-Sabater C, Koletzko B, Krauss-Etschmann S, Campoy C.
PLoS One. 2017 Jan 26;12(1):e0169223. doi: 10.1371/journal.pone.0169223. eCollection 2017. PMID:28125591

Maternal, fetal and perinatal alterations associated with obesity, overweight and gestational diabetes: an observational cohort study (PREOBE). Berglund SK, García-Valdés L, Torres-Espinola FJ, **Segura MT**, Martínez-Zaldívar C, Aguilar MJ, Agil A, Lorente JA, Florido J, Padilla C, Altmäe S, Marcos A, López-Sabater MC, Campoy C; PREOBE team. BMC Public Health. 2016 Mar 1;16:207. doi: 10.1186/s12889-016-2809-3. PMID:26931143

Maternal Body Weight and Gestational Diabetes Differentially Influence Placental and Pregnancy Outcomes. Martino J, Sebert S, **Segura MT**, García-Valdés L, Florido J, Padilla MC, Marcos A, Rueda R, McArdle HJ, Budge H, Symonds ME, Campoy C.

J Clin Endocrinol Metab. 2016 Jan;101(1):59-68. doi: 10.1210/jc.2015-2590. Epub 2015 Oct 29. PMID:26513002

Effects of obesity and gestational diabetes mellitus on placental phospholipids. Uhl O, Demmelmair H, **Segura MT**, Florido J, Rueda R, Campoy C, Koletzko B. Diabetes Res Clin Pract. 2015 Aug;109(2):364-71. doi: 10.1016/j.diabres.2015.05.032. Epub 2015 May 16. PMID:26021978

Determination of acylglycerols from biological samples with chromatography-based methods.Hellmuth C, Uhl O, **Segura-Moreno M**, Demmelmair H, Koletzko B. J Sep Sci. 2011 Dec;34(24):3470-83. doi: 10.1002/jssc.201100556. Epub 2011 Oct 17. Review. PMID:22002927

COMMUNICATIONS TO INTERNATIONAL MEETINGS

Title of the work: Maternal pre-pregnancy obesity alters term placental microRNA expression

Name of conference: 51st ESPGHAN Annual Meeting

Type of participation: Poster

Year: 2018

Geographic area: Geneva, Switzerland

Authors Maria Teresa Segura, Signe Altmäe, Francisco Esteban, Sabine Bartel, Hans Demmelmair, Berthold Koletzko, Susanne Krauss-Etschmann, Cristina Campoy

---

Title of the work: Placental expression of fatty acid transporter related to maternal pre-pregnancy weight.

Name of conference: The Power of Programming, 13-15th October 2016

Type of participation: Oral presentation (New Investigator Award)

Year: 2016

Geographic area: Munich, Germany

Authors: Segura MT, Escudero M, Parejo E, Demmelmair H, Krauss-Etschman S, Rueda R, Koletzko B, Campoy C

Title of the work: Maternal LCPUFAS status and fetal neurodevelopment in obese women

Name of conference: 47th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition

Type of participation: Oral presentation

Year: 2014

Geographic area: Jerusalem, Israel

Authors: Maria Teresa Segura Moreno; Francisco Jose Torres-Espínola; Maria Carmen Lopez-Sabater; Luz Garcia-Valdes; Ricardo Rueda; Tania Anjos; Pilar Brandi-Blanco; Cristina Campoy and the PREOBE Group

---

Title of the work: Obesity and gestational diabetes mellitus cause similar alterations in placental phospholipid species

Name of conference: 47th Annual Meeting of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition

Type of participation: Poster

Year: 2014

Geographic area Jerusalem, Israel

Authors: Olaf Uhl, Hans Demmelmair, **María Teresa Segura**, Cristina Campoy, Berthold Koletzko