IMPACT OF GESTATIONAL DIABETES ON FETAL PRECURSORS AND LIPOPROTEIN PROFILE: EFFECTS ON OFFSPRING

Francisco Algaba Chueca

Impact of gestational diabetes on fetal precursors and lipoprotein profile: effects on offspring

FRANCISCO ALGABA CHUECA

UNIVERSITAT
ROVIRA i VIRGILI

DOCTORAL THESIS

2020

Francisco Algaba Chueca

Impact of gestational diabetes on fetal precursors and lipoprotein profile: effects on offspring

DOCTORAL THESIS

Directed by:

Sonia Fernández Veledo, PhD

Ana Megía Colet, MD, PhD

Joan Vendrell Ortega, MD, PhD

UNIVERSITAT
ROVIRA i VIRGILI

2020

UNIVERSITAT ROVIRA i VIRGILI

- Sonia Fernández Veledo, PhD in Biomedicine by the University of Barcelona, co-head of the Diabetes and Metabolic Associated Diseases Research Group of the Pere Virgili Health Research Institute and associate professor of the Rovira i Virgili University.
- Ana Megía Colet, PhD in Medicine and Surgery by the Autonomous University of Madrid, medical specialist in endocrinology and nutrition, and associate professor of the Rovira I Virgili University.
- Joan Vendrell Ortega, PhD in Medicine and Surgery by the University of Barcelona, medical specialist in endocrinology and nutrition, co-head of the Diabetes and Metabolic Associated Diseases Research Group of the Pere Virgili Health Research Institute and full professor of the Rovira i Virgili University.

STATE THAT:

The study entitled "**Impact of gestational diabetes on fetal precursors and lipoprotein profile: effects on offspring**" has been developed by **Francisco Algaba Chueca** under our supervision for the award of the degree of Doctor with international mention.

And so that it is registered and has the appropriate effects, we signed this document in Tarragona, on April 29, 2020.

Dr. Sonia Fernández Veledo Dr. Ana Megía Colet Dr. Joan Vendrell Ortega

ACKNOWLEDGEMENTS/AGRADECIMIENTOS

Las casualidades no existen. No es casualidad que haya depositado mi tesis el 29 de abril, fecha de mi cumpleaños. Tampoco lo es que, durante estos casi cuatro años, haya trabajado con placentas, cuando el desprendimiento de una casi nos cuesta la vida a mi madre y a mí; o que trate sobre la diabetes, una enfermedad contra la cual mi abuela Vicenta batalló durante muchos años antes de dejarnos. Y, por supuesto, tampoco es casualidad haber tenido la oportunidad de realizar mi tesis en el Grupo de Investigación DIAMET.

Aún recuerdo como si fuera ayer la entrevista por Skype con la Dra. Sonia Fernández, en la cual escuché por primera vez las palabras placenta, diabetes gestacional y células madre en la misma frase. Y aunque al principio pensé: "*Madre mía… ¡Qué locura!*", a día de hoy sólo puedo agradecer una y mil veces a Sonia, Ana y Joan el haberme permitido formar parte de esta gran aventura.

Esta tesis doctoral representa la suma del trabajo y el entusiasmo de muchas personas a las que tengo mucho que agradecer:

A la Dra. Sonia Fernández, por haber sido toda una inspiración para mí y por todo lo que he aprendido a su lado; por su extraordinaria ayuda, confianza y dedicación durante todo este tiempo, así como por su admirable capacidad de liderazgo.

A la Dra. Ana Megía, por todas enseñanzas, por su paciencia y por haber confiado en mí en todo momento. Por transmitirme calma y perspectiva cuando más lo he necesitado y por enseñarme que por muy negras que se vean las cosas, todo saldrá adelante con trabajo y esfuerzo.

Al Dr. Joan Vendrell, por su inestimable y continua ayuda y generosidad; por su consejo, su liderazgo y por haber confiado en mí en múltiples aspectos más allá del doctorado.

A todos mis compañeros/as de DIAMET, donde me he sentido como en familia durante todo este periodo. Especialmente quiero agradecer al Dr. Enrique Calvo, a la Dra. Elsa Maymó, a la Dra. Carol Serena, a la Dra. Victoria Ceperuelo, al Dr. Brenno Astiarraga, a la Dra. Miriam Ejarque, a la Dra. Mar Rodríguez y a Cati Núñez su extraordinaria ayuda. Por supuesto, también a mi equipo "predoc": Marga, Noe, Joan y Ester, por su apoyo constante y por su incondicional

compañerismo; así como por todos los buenos momentos que hemos vivido juntos y que estoy convencido que seguiremos viviendo.

A todo el personal de los Servicios de Ginecología y Obstetricia y de Endocrinología del Hospital Universitari Joan XXIII de Tarragona, sin el cual esta tesis no hubiera sido posible. Por haberme aguantado día tras día y por haberme ayudado en todo lo que he necesitado (aunque no me negaréis que unas risas sí que nos hemos echado). Especialmente quiero destacar a la Dra. Mónica Ballesteros y al Dr. Albert Guarque, por su ejemplar colaboración y profesionalidad.

To all my mates from the Medical University of Graz, especially to Dr. Gernot Desoye, Dr. Alejandro Majali, Denise, Julia and Dr. Ilaria del Gaudio (I would like to be the first one in calling you doctor!). Also, to Paul and Daniel. For their great reception, hospitality and kindness; for everything I have learned at their side, both inside and outside the laboratory, and for making me have one of the best experiences of my life during my PhD stay.

A todos mis compañeros/as del mundo de la divulgación, especialmente a los miembros de l'Associació per a la Divulgació Científica al Camp de Tarragona (DivulgaTGN) y a mis chicos/as de Famelab, con los cuales he aprendido a amar y disfrutar la ciencia de una forma diferente.

A todas las personas que, de forma directa o indirecta, me han ayudado y enseñado en el mundo de la ciencia; sobre todo al equipo de CIBERehd Alicante y al Dr. José Manuel González, con el cual di mis primeros pasos en el mundo de la investigación.

A toda mi familia y mis amigos en general, especialmente a mi Tía Misi y a mi prima Cristina, cuyo cariño, apoyo y confianza han sido determinantes en mi educación y desarrollo, tanto personal, como profesional. A Paula, por sacar siempre lo mejor de mí y por haber estado a mi lado en todo momento. A la gente maravillosa que he conocido en Tarragona, sobre todo a Andrea, Víctor, Vero, Edna, José, Jesús y Nacho. A Asier, Sergio, Jaro y Juan Díaz, por ser una constante en mi vida en la cual me he refugiado innumerables veces en busca de ayuda y consejo. También a mis compañeros de carrera y máster.

Finalmente, quiero agradecer profundamente a todo el soporte institucional que me ha acompañado durante este tiempo, sobre todo al Institut d'Investigació Sanitària Pere Virgili (IISPV), la Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR), al Hospital Universitari de Tarragona Joan XXIII, la Universitat Rovira i Virgili y al Instituto de Salud Carlos III.

Esta tesis doctoral está dedicada íntegramente a mis padres, Kiko y Julia, y a mi hermano Jaime, cuyo esfuerzo, pasión y apoyo incondicional han construido la escalera que me ha permitido llegar hasta aquí. Gracias.

In loving memory of my grandparents Julián, Juan, Vicenta and Jerónima.

INDEX

ABBREVIATIONS

AGA: appropriate for gestational age

AM: amniotic membrane

AMSCs: amniotic mesenchymal stem cells

APCs: antigen presenting cells

BMI: body mass index

CD40: cluster of differentiation 40

CTSS: cathepsin S

DCs: dendritic cells

FATPs: fatty acids transport proteins

FABPs: fatty acids binding proteins

FFA: free fatty acids

GDM: gestational diabetes mellitus

GLUT4: glucose transporter 4

hCG: human chorionic gonadotropin

HDL: high-density lipoproteins

HLA: human leukocyte antigen complex

hPGH: human placental growth hormone

hPL: human placental lactogen

IADPSG: International Association of Diabetes and Pregnancy Study Groups

IDL: intermediate-density lipoproteins

IL: interleukin

IR: insulin resistance

IRS-1: insulin receptor substrate-1

JNK: c-Jun N-terminal kinase

LDL: low-density lipoproteins

LGA: large for gestational age

LPL: lipoprotein lipase

MCP-1: monocyte chemoattractant protein-1

MHC: major histocompatibility complex

MSCs: mesenchymal stem cells

NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells

NK: natural killer cells

NMR: nuclear magnetic resonance

PBMCs: peripheral blood mononuclear cells

PGE2: prostaglandin E2

PI3K: phosphoinositide 3-kinase

pMSCs: placental mesenchymal stem cells

PKC: protein kinase C

PPAR-γ: peroxisome proliferator–activated receptor gamma

PTGS2: prostaglandin-endoperoxide synthase 2

SGA: small for gestational age

VLDL: very low-density lipoproteins

Th: T-helper lymphocyte

TNF-α: tumor necrosis factor alpha

Tregs: T-regulatory lymphocytes

ABSTRACT

This work studies the impact of gestational diabetes mellitus (GDM) on the functionality of fetal precursor cells found in the amniotic membrane and on the morphological and functional characteristics of fetal lipoproteins, and also whether these potential disturbances could program the fetal metabolism and directly contribute to the higher predisposition to metabolic and cardiovascular diseases later in life.

BACKGROUND AND OBJECTIVES

Pregnancy is a dynamic state that encompasses changes in the metabolism of the mother to ensure an adequate growth and development of the fetus. GDM is one of the most common pregnancy complications and has been associated with maternal nutritional and metabolic alterations that disturbs the metabolic adaptations of pregnancy, including an exacerbated insulin resistance (IR) that favors an excessive nutrient availability and uptake by the fetus. These derangements have been associated with altered growth patterns and increased predisposition for developing later diseases in life by fetal programming.

Fetal precursor cells and lipid metabolism are key components of fetal programming that can be directly affected by GDM. Several studies pointed the great impact of GDM on the functionality of mesenchymal stem cells (MSCs) from extraembryonic tissues and the umbilical cord lipoprotein profile. The amniotic membrane (AM) is in contact with the amniotic fluid and the fetus and, as such, its stem cell component might be a good indicator of how the intrauterine environment impacts the fetus. On the other hand, ^{1}H-NMR-based lipoprotein tests have demonstrated higher detection capacity of subtle lipoprotein abnormalities and to have a greater ability to predict cardiovascular risk than classical cholesterol determinations.

For these reasons, we decided to study the influence of GDM on the functional characteristics of the amniotic mesenchymal stem cells (AMSCs) and on the fetal advanced lipoprotein profile across birth weight categories, in order to explore a potential relationship with fetal parameters associated with adverse outcomes that may have a prognostic value.

METHODS

We carried out two observational case-control studies. In the first one, AMSCs and resident macrophages were isolated from eighteen pregnant women (9 diagnosed with GDM and 9 with normal glucose tolerance) scheduled for cesarean section. After characterization,

functional characteristics of AMSCs were analyzed and correlated with anthropometrical and clinical variables from both mother and offspring. In the second one, 62 GDM and 74 normal glucose tolerant pregnant women and their offspring were included. Newborns were classified according to birth-weight as small, appropriate or large for gestational age (SGA, AGA and LGA, respectively). Advanced ^{1}H-nuclear magnetic resonance (NMR)-based lipoprotein test was used to profile cord blood serum lipoproteins. Height and weight data of the offspring up to two years was used to estimate age- and sex-specific body mass index.

RESULTS

We observed that GDM induces a pro-inflammatory profile in AMSCs accompanied by a higher chemotactic and invasive activities, and it also modifies their plasticity, affecting proliferation and differentiation potential. Consistently, AM-resident macrophages also displayed a more pro-inflammatory phenotype. Moreover, genes involved in AMSCs inflammatory response were associated with maternal insulin sensitivity and pre-pregnancy body mass index, as well as with fetal metabolic parameters, suggesting that the GDM environment could program stem cells and subsequently favor metabolic dysfunction later in life.

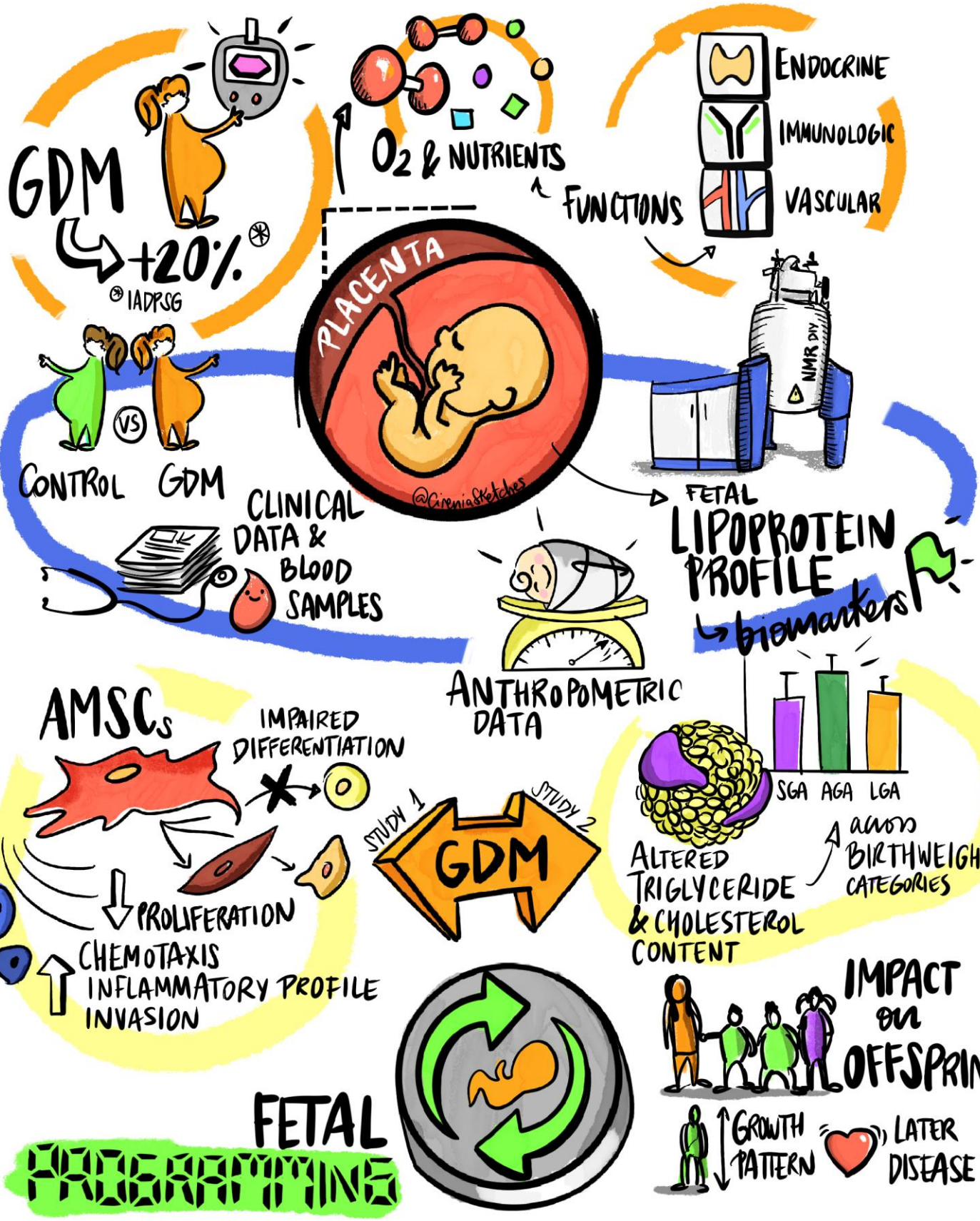
On the other hand, using an advanced ^{1}H-NMR-based lipoprotein test we found a disturbed triglyceride and cholesterol lipoprotein content in offspring of GDM mothers across birth categories. Concretely, AGA neonates born to GDM mothers showed a profile more similar to adults with dyslipidemia and atherosclerosis than to those born to control women. In addition, lipoprotein parameters were independently associated with offspring obesity at two years old, indicating a possible implication of an altered fetal lipoprotein profile in the greater predisposition to future adverse outcomes.

CONCLUSIONS

We demonstrate that GDM induces changes in the biological characteristics of AMSCs, many of which are related to fetal metabolic parameters, suggesting that the GDM environment could program stem cells and subsequently favor metabolic dysfunction later in life. On the other hand, we found a disturbed triglyceride and cholesterol lipoprotein content in offspring of GDM mothers across birth categories, with AGA neonates showing a profile more similar to adults with dyslipidemia and atherosclerosis than those born to control women. Moreover, we find that LDL particles are potential biomarkers of obesity later in life.

IMPACT OF GESTATIONAL DIABETES ON FETAL PRECURSORS AND LIPOPROTEIN PROFILE: EFFECTS ON OFFSPRING

Francisco Algaba-Chueca 2020



DIAMET

INTRODUCTION

INTRODUCTION

Fetal growth and development are particularly vulnerable periods in life, greatly affected by the maternal environment. Prenatal exposure to nutritional and metabolic stressors has been associated with fetal programming, a concept that alludes to an increased predisposition to later development of cardiovascular and metabolic disorders, including obesity and type 2 diabetes [1–4].

Maternal overnutrition over the lifetime leads to the development of obesity, which increments the possibility to develop gestational diabetes mellitus (GDM) during pregnancy[5,6]. Both conditions are characterized by an exacerbated insulin resistance (IR) and chronic low-grade inflammatory state[7,8]. In this line, several factors, such as adipokines, chemokines and cytokines, released mainly from adipocytes and immune cells[9,10] may activate a series of intracellular cascades that can boost the insulin resistant state[11,12], entering a vicious feedback loop whose side effects are magnified.

Maternal obesity and GDM are associated with childhood obesity, diabetes, metabolic syndrome and cardiovascular risk[13–21]. In this context, the role of inflammation as a mediator of the programming for metabolic disorders following adverse intrauterine exposure has gained increasing attention. Inflammatory mediators may modify placental and fetal metabolism and physiology, and program the fetus for later development of metabolic disorders associated with IR.

1. METABOLIC CHANGES DURING PREGNANCY

Human pregnancy is a dynamic state that encompasses substantial changes in the maternal metabolism in order to facilitate a constant net flow of glucose and other necessary nutrients to the growing fetus[22]. **Early gestation** is characterized by a series of anabolic processes that favors accumulation of fat storage in the mother. This stage includes the hyperplasia of pancreatic beta cells and the increase of insulin secretion, with normal or augmented insulin sensitivity, which facilitates the use of glucose for energy requirements and *de novo* lipogenesis, and that inhibits lipolysis and hepatic gluconeogenesis[22,23]. On the other hand, an increment in lipoprotein lipase (LPL) activity favors the hydrolysis of circulating triglycerides and facilitates accumulation of lipids in maternal fat depots (Figure 1).

In **late pregnancy** the requirements for metabolic substrates are augmented, therefore this phase is characterized by a series of catabolic processes triggered predominantly by a decreased insulin sensitivity, or what is the same, an increased IR. This situation results in higher postprandial maternal glucose and free fatty acids (FFA) concentration, allowing for greater substrate availability for fetal growth. In the fasted state, hepatic glycogenolysis and gluconeogenesis are augmented[24], along with an enhanced lipolytic activity in the maternal adipose tissue that accelerates the breakdown of stored triglycerides[25]. Hence, nutrients are stored in early pregnancy to meet the higher maternal and fetal demands of late gestation and lactation. Indeed, in healthy pregnancies there is a recovery of insulin sensitivity immediately post-delivery[26].

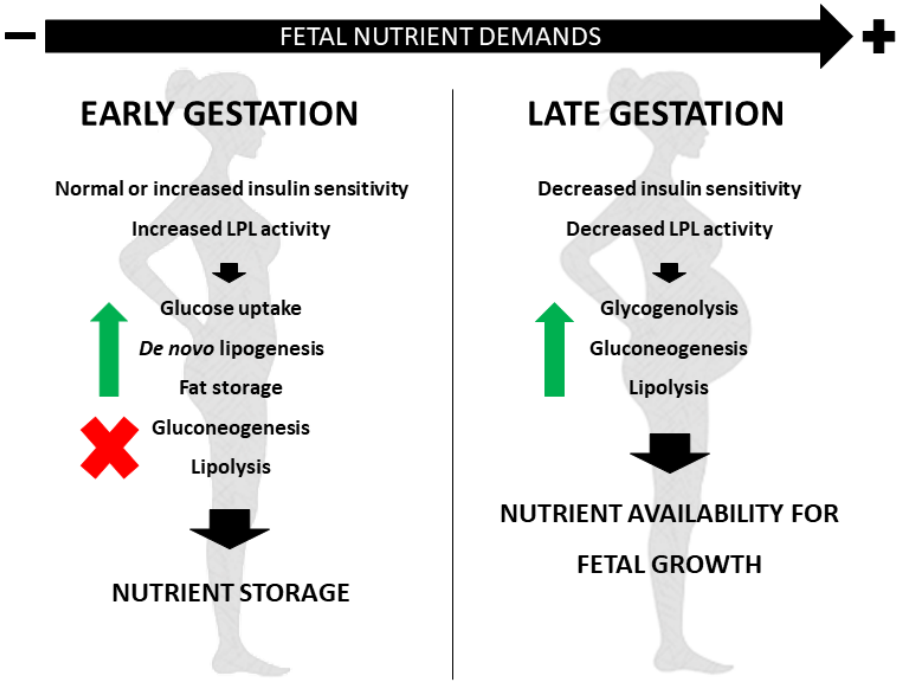


Figure 1. Main metabolic changes during pregnancy. LPL: lipoprotein lipase.

A fine-tuning regulation of all these metabolic changes is required for an adequate fetal growth and development. This situation can be threatened when any imbalance is present, as occurs with the onset of GDM, in which the metabolic stress of pregnancy reveals an underlying impaired insulin production, insulin sensitivity or insulin response[13].

1.1. Insulin resistance during pregnancy

IR is defined as a decreased capacity of target tissues, such as liver, adipose tissue and muscle, to respond to normal circulating concentrations of insulin, and it is predominantly caused by a defect in the insulin signaling pathway. In normal conditions, insulin binds to its membrane receptor, triggering its autophosphorylation and the subsequent activation of insulin receptor substrate-1 (IRS-1) by tyrosine-residue phosphorylation. IRS-1 binds to the phosphoinositide 3-kinase (PI3K) and subsequently activates several signaling mediators, resulting in a variety of tissue-specific metabolic effects. In adipose tissue and skeletal muscle, the activation of the insulin receptor predominantly promotes the translocation of glucose transporter 4 (GLUT4) to the membrane and therefore allowing glucose to enter the cells[27].

In late pregnancy, peripheral insulin sensitivity decreases up to almost 50-60% and the maternal pancreatic beta cells try to overcome this situation by increasing insulin secretion in order to maintain the glycaemia[28,29]. This decline in insulin sensitivity throughout pregnancy is a physiological adaptation to ensure an adequate supply of glucose for the rapidly growing fetus. It favors the use of fats instead of carbohydrates for energy in the mother, then sparing the latter for the fetus[30]. According to Freinkel's hypothesis[31], glucose and other nutrients pass across the placenta and stimulate fetal insulin secretion, which in turn will stimulate glucose uptake and utilization by peripheral tissues[32].

There are many mechanisms implicated in the development of IR during pregnancy and the placenta is thought to be one of the main drivers. Placental-derived hormones, such as progesterone, estrogen, human placental lactogen (hPL) or human placental growth hormone (hPGH), are augmented in the maternal circulation throughout pregnancy and have been related with changes in insulin sensitivity[27,33]. For instance, gradually increasing levels of progesterone have been reported to reduce the expression of IRS-1 and to inhibit the insulin-induced GLUT4 translocation and glucose uptake in adipocytes[34]. Accordingly, a downregulation of GLUT4 protein in adipose tissue has been observed when IR develops during pregnancy[35]. On the other side, estrogens contribute to the development of maternal hyperlipidemia and to diminish insulin sensitivity at high concentrations[22,36].

Other factors secreted by adipose tissue have been also implicated in the development of IR. In normal pregnancy, a tightly regulated equilibrium between pro- and anti-inflammatory cytokines is necessary at various stages from implantation to delivery[37] and any disturbance affecting this balance could impact on maternal and fetal health. In this context, tumor necrosis factor alpha (TNF-α) and interleukin (IL)-6 are a good example. They are pro-

inflammatory cytokines secreted by the adipose tissue in obesity that affect the insulin signaling cascade and therefore are involved in the development of IR[12]. During pregnancy, the levels of IL-6 and TNF-α increment at each trimester[37] and an inverse correlation between TNF-α and insulin sensitivity has been demonstrated throughout pregnancy[38]. TNF-α impairs insulin signaling by increasing the serine inhibitory phosphorylation of IRS-1[39,40] and diminishing the insulin receptor tyrosine kinase activity[41]. Moreover, TNF-α and IL-6 have been described to suppress the transcription of the peroxisome proliferator–activated receptor gamma (PPAR-γ) and its target gene adiponectin in adipocytes, which are central players in the regulation of insulin sensitivity, thus contributing to IR[42–44].

1.2. Maternal changes in glucose, lipid and protein metabolism

Changes in glucose, lipid and protein metabolism are essential for the adaptation of maternal energy homeostasis to the increasing requirements during pregnancy. As previously mentioned, the variation in the insulin sensitivity is the basis of the nutrient-related metabolic changes during gestation.

a) Glucose metabolism:

Basal glycaemia decreases as the pregnancy progresses[29] and several factors have been proposed as responsible. In early pregnancy, the expansion of plasma volume, hyperplasia of beta cells and the raise in insulin secretion, which might be accompanied by an augmented insulin sensitivity, seem to be the key elements, whereas in late pregnancy, the increment in the glucose consumption by the fetoplacental unit is the most important factor[45]. Nonetheless, the decrease in fasting glucose is accompanied by an increased hepatic glucose production throughout pregnancy[24]. When IR develops, there is a decreased capacity of the skeletal muscle and adipose tissue to uptake the glucose in the postprandial state, favoring transplacental transport to the fetus in favor of gradient[46,47].

b) Lipid metabolism:

The vast majority of FFA derived from maternal diet are esterified in triglycerides and transported along with cholesterol in lipoproteins through plasma. There are many types of plasmatic lipoproteins that differ in their lipid and protein composition, which give them different functional identity (Figure 2). Due to the augmented insulin sensitivity, *de novo* lipogenesis is favored in the first half of pregnancy. A higher uptake of circulating triglyceride-rich lipoproteins (chylomicrons and very low-density lipoproteins (VLDL)) is observed in parallel

to the augmented LPL activity and decreased lipolytic actions of the adipocytes[23]. These processes favor the maternal fat deposition[23]. By contrast, in the last trimester of pregnancy the increased IR facilitates the adipose tissue lipolysis (Figure 1), which raises the FFA and glycerol plasma concentrations, leading to maternal hyperlipidemia[23]. In the fasted state, FFAs can be used along with glycerol for ketogenesis and gluconeogenesis, respectively, whereas in the postprandial state, FFA can be re-esterified in triglycerides in the liver[23]. The presence of hypertriglyceridemia is a characteristic of the pregnancy-associated maternal hyperlipidemia and it is mainly due to an increment in VLDL, but also low- and high-density lipoproteins (LDL and HDL, respectively) concentrations[48].

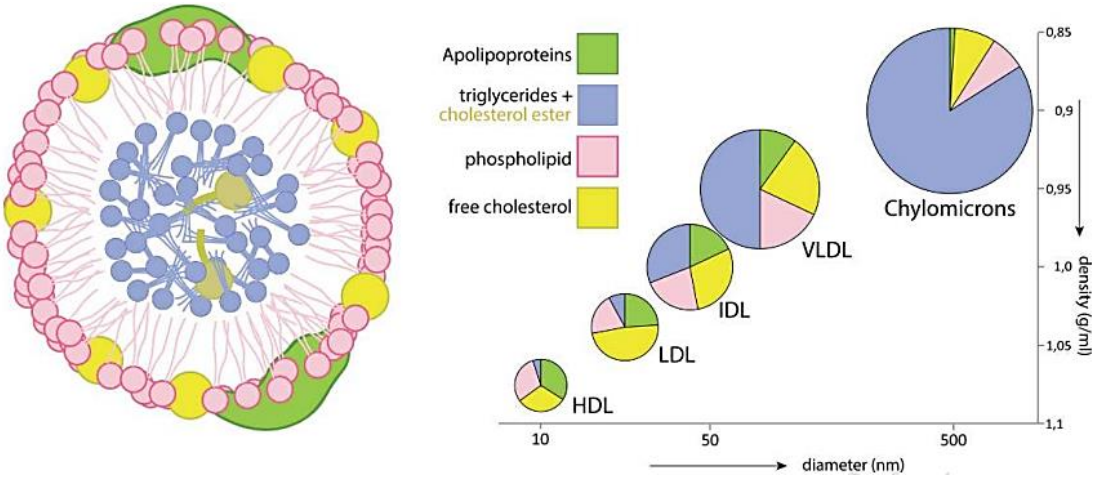


Figure 2. Composition and main physical-chemical properties of the major lipoprotein classes. From Van Leeuwen et al., 2018[49]. HDL: high-density lipoproteins; LDL: low-density lipoproteins; IDL: intermediate-density lipoproteins; VLDL: very low-density lipoproteins.

c) Protein metabolism:

Maternal protein synthesis is increased up to 25% from the first to the third trimester of pregnancy, which correlates with a decrease in circulating amino acids[50,51]. Several mechanisms have been proposed for the lower circulating amino acids concentrations, including an inhibition of skeletal muscle amino acids release by placental hormones, an accelerated muscle uptake as a result of postprandial hyperinsulinemia, and an augmented amino acids utilization by the fetus in late pregnancy[52,53].

1.3. Fetal metabolism

Throughout pregnancy, the fetus matures and metabolically adapts to the nutrients that come from the maternal circulation across the placenta. Nonetheless, the higher demand for nutrients occurs primarily during the last half of gestation when most of the fetal growth occurs, including the accumulation of adipose tissue[54].

a) Glucose metabolism

Glucose is the most demanded fetal energy substrate. During pregnancy, the fetus is constantly supported by maternal glucose, which passes across the placenta and stimulates the fetal insulin production[32]. Fetal insulin-expressing cells are the first endocrine cells that appear in the human pancreas. They can be detected as at 7-8 weeks of gestation and remain the most prevalent endocrine cell type during the first trimester[55]. Glucose-stimulated insulin secretion facilitates glucose uptake and utilization by peripheral tissues. In the liver, fetal insulin induces *de novo* lipogenesis from glycolytic precursors, resulting in non-essential FFA synthesis contributing to fetal fat accretion[56].

b) Lipid metabolism

Lipid molecules play a critical role in embryonic and fetal development. Cholesterol is an essential component of the cell membrane controlling fluidity and passive permeability, and a precursor for bile acids and steroid hormones, among others[22]. The great majority of cholesterol, especially in late pregnancy[57], is obtained by the fetus from *de novo* biosynthesis. However, a fraction can arise from maternal circulation and placental deposits[58]. FFA are important energy substrates, essential structural components of cell membranes and precursors for several signaling molecules[59], so the mechanisms regulating FFA oxidation are crucial for fetal health. Indeed, genetic alterations in the FFA oxidation pathway have been associated with several disorders, ranging from fetal prematurity and growth restriction to even coma and death[60]. Although the fetus can synthesize its own non-essential FFA, a great part of the required FFA is transported from maternal circulation through the placenta. Thus, an adequate transfer and availability of FFA is required for a correct fetal adiposity. Fat mass is the strongest determinant of newborn weight variability[54] so a progressive and well-regulated adipose tissue accretion is crucial for a correct growth and metabolic development, as we will discuss later.

c) Protein metabolism

Amino acids are important substrates for the fetal synthesis of proteins, nucleic acids and neurotransmitters. In parallel with the exponential growth of the fetus, the demand for amino acids and other nutrients rises in late pregnancy[52]. Maternal amino acids reach the fetal circulation after placental active transport[61,62]. Furthermore, the placenta has the capacity to synthesize some amino acids *de novo*, and to modify others[22]. Augmented fetal insulin secretion due to higher glucose transport from the mother in late pregnancy also influences amino acids metabolism by increasing protein synthesis[63].

2. FETAL GROWTH AND DEVELOPMENT

Fetal growth and development result from an integrated interplay between maternal, placental and fetal factors[64]. Fetal growth accelerates and most of the adipose tissue accretion occurs in late pregnancy[54]. Around week 25 of pregnancy, the accumulation of lipids in the fetal adipose tissue increases logarithmically with gestational age[65], which is sustained by the intense placental transfer of glucose, its use as a lipogenic substrate, as well as by the transfer of maternal FFA. Plasma concentrations of maternal triglycerides and FFA have been shown to correlate with fetal lipid concentrations and fetal growth[66]. Consistently, an exponential relation of gestational age with both infants' body weight and white adipose tissue lipid content has been observed[67]. Maternal nutrient availability and metabolic status, and the capacity of the placenta to transport them to the fetus are the main determinants of a normal fetal size. Abnormal growth patterns are associated with altered nutrient supply and fetal fat deposition, and have been related with metabolic changes in the fetus with later consequences in life[1–4,68].

Although there are some cases that would not fit it[69], most approaches for defining fetal growth use gestational age-based norms[68]. Infants are considered to be appropriate for gestational age (AGA) if the birth-weight is between the 10th and 90th percentiles for the infant's gestational age and sex reference growth charts, while those with a birth-weight over the 90th percentile are large for gestational age (LGA) and the remainder, with a birth-weight under the 10th percentile, are small for gestational age (SGA). Next, factors involved in fetal growth are discussed in greater detail.

2.1. Fetal inherited factors

About 25-31% of the variance of birthweight is explained by heritability[70,71]. Several factors such as race, sex, familial genetic inheritance, chromosomal abnormalities and dysmorphic syndromes have been shown to influence fetal growth[68]. Black and male fetuses showed an increase in birthweight compared with their white and female respective counterparts[72]. Parental genetic influence over fetal growth is likely to be polygenic[73,74] and a significant correlation between parental and offspring weight at birth has been observed, suggesting a familial trend[75,76]. Also, growth restriction is frequently present in infants born with malformations[77].

2.2. Maternal factors

Maternal health, constitutional factors and nutrition are key determinants of fetal growth. Fetuses from primiparous women displayed a higher weight at birth compared with those from multiparous[72]. Smoking is a well-known factor associated with small neonates at birth, but also with an increased weight and height catch-up at 1 year[78] and obesity in childhood[79]. Additionally, maternal diseases that affect uteroplacental blood flow, such as hypertension and preeclampsia[80], and genital infections were also related to reduced birth-weight, the latter because of an increased risk of preterm delivery[81]. On the other hand, maternal nutritional situation is one of the main determinants of fetal growth since it strongly influences each stage of embryonic and fetal development. Several studies indicate a direct relationship between maternal pre-pregnancy body mass index (BMI) and gestational weight gain, and the neonate weight and body fat mass[82–85]. In this aspect, both maternal over and undernutrition results in an adverse intrauterine environment that can modify placental and fetal gene expression, and result in fetal growth alterations and higher susceptibility for later diseases[68].

2.3. Placental factors

Placental nutrient transfer capacity is a critical factor for fetal growth. In some mammals, fetal weight near term is closely related to placental weight, as an indirect indicator of its size. Placental size, its morphology, the blood flow and the abundance of nutrient transporters are factors that modify the amount of nutrient supply through the placenta[86]. In addition, the placenta synthesizes and secretes hormones that are able to modulate fetal growth[87]. We will discuss this topic more in detail in the following section.

3. THE PLACENTA

The human placenta is a highly specialized and transient organ that constitutes a natural barrier between maternal and fetal circulation. It is comprised of several cell types specialized in nutrient transport and energy metabolism, but they also have important endocrine, vascular and immunological functions[88]. Therefore, the placenta has a fundamental role in nutrient uptake, gas exchange and waste elimination between the mother and the fetus and also in the induction of maternal immunotolerance by altering the local immune environment[46,88]. Besides, it is a source of hormones that support pregnancy and parturition, and that can influence fetal growth[87,88]. For these reasons, the functional integrity of the placenta is crucial to support fetal growth and development as well as to adapt to the maternal nutritional and metabolic status.

3.1. Structure and cellular composition of the placenta

The mean mature placenta is a discoidal organ of about 3 cm thick, 20 cm in diameter and 500-600g in weight, although these parameters can vary widely according to different conditions[89]. In the mature placenta, the fetal side consists in the chorionic plate, which is covered by the fetal membranes chorion and amnion (Figure 3). Amnion, or amniotic membrane (AM), is the innermost layer of the placenta and completely surrounds the embryo/fetus, thus delimiting the amniotic cavity, which is filled by amniotic fluid[90]. The chorionic plate carries the chorionic vessels that converge toward the umbilical cord vessels[88,89]. The maternal side of the placenta consist in the basal plate and is subdivided into 15-20 lobes or cotyledons, covered by a thin layer of decidua basalis. Placental cotyledons contain the main functional units of the placenta, the chorionic villi[88,89]. They are extensively branched structures where the large majority of materno-fetal exchange occurs. Each villus is surrounded by a continuous layer of syncytiotrophoblasts with several cytotrophoblasts beneath it. The surface of the syncytiotrophoblast is covered by a higher number of microvilli that greatly increase the total surface area of the placenta. The core of a villus consists of fetal blood vessels and stroma, which is composed of mesenchymal stem cells, macrophages (also known as Hofbauer cells), and fibroblasts[88,89]. Circulating maternal blood enters the intervillous space via spiral arteries, bathes the villi and drains back through endometrial veins. On the fetal side, oxygen and nutrient-deficient fetal blood passes, via two umbilical arteries and the branched chorionic arteries, to the extensive arterio-capillary-venous system within the chorionic villi. Then, the well-oxygenated fetal blood in the capillaries returns to the fetus, via the chorionic veins and the single umbilical vein[88,89].

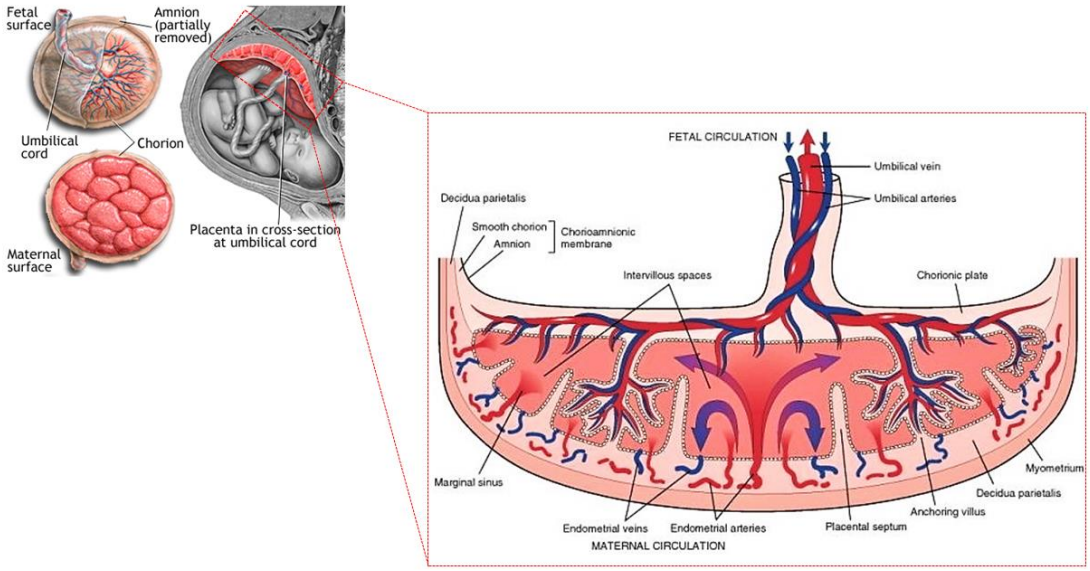


Figure 3. Anatomy of human placental. Adapted from biologydictionary.net/placenta/ and medlineplus.gov/ency/imagepages/17010.htm

3.2. Nutrient transport and endocrine functions

Adequate fetal growth and development is primarily determined by nutrient availability, which is intimately related to capacity of the placenta to transport them. The placenta is richly endowed with transport systems that ensure an adequate supply of glucose, lipids and amino acids to the fetus[46]. Placental nutrient transport encompasses a very dynamic set of processes, highly adaptable and responsive to nutrient fluctuations associated with maternal nutrient intake, before which it regulates blood flow and the levels of nutrient transporters and associated molecules[46]. Given this adaptability, the nutrient transfer capacity of the placenta has demonstrated to be highly sensitive to maternal disturbances, which can in turn permanently compromise its functional integrity and an adequate fetal nutrient availability, affecting normal growth and development. Indeed, alterations in placental transport function are present in maternal diseases associated with fetal growth disturbances[91,92], such as GDM and obesity[93,94].

After implantation, the major function of the placenta is to mediate and regulate nutrient uptake from the mother to the fetus, which is accomplished by forming a highly branched villous structure and establishing its own circulation connected to the umbilical cord[89]. To cross the placenta, nutrients must be taken up from the maternal plasma, cross the

syncytiotrophoblast, diffuse through the placental villous stroma and pass across the fetal capillary endothelium.

Glucose crosses the placenta more than any other substrate since it is the predominant energy source for the fetus and the placenta[95]. Net glucose transfer is predominantly mediated by placental GLUT transporters and depends on materno-fetal concentration gradient, which is favored by postprandial glucose levels[46,47]. There is evidence suggesting that fetal glucose demands dictate the transplacental transport rather than maternal circulating levels[96]. However, in certain pathological situations that course with an exaggerated IR, such as maternal obesity and GDM, an excessive gradient of glucose from maternal to fetal circulation leads to an abnormal increase in glucose availability to the fetus, and, subsequently, to an abnormal fetal growth[13]. Conversely, **amino acids** concentration is greater in the fetal than in the maternal circulation, so their transport generally relies on active transport systems that require energy expenditure[61,62]. Changes in the distribution, concentration and affinities of these systems may determine the supply of amino acids to the fetus and, subsequently, impact on fetal growth. In fact, a reduced Na^+/K^+ ATPase activity, which plays a crucial role in sodium-dependent amino acids transport system, has been found in placentas of intrauterine growth restricted babies[97].

A great part of the **FFA** required by the fetus comes from the maternal circulation. Maternal FFA are transported as triglycerides in triglyceride-rich lipoproteins through plasma. Since there is no direct placental transfer of maternal lipoproteins[57], FFA are hydrolyzed from chylomicrons and VLDL by placental LPL[98] and uptaken by fatty acids transport proteins (FATPs) into the cytosol of syncytiotrophoblast[99]. Then, they bind to fatty acid-binding proteins (FABPs) for cell trafficking, and finally, they are carried to the fetal liver preferentially bound to α-fetoprotein[100,101]. In the liver, they are re-esterified and released into the fetal circulation in the form of triglycerides[22]. On the other hand, the fetus obtains the vast majority of **cholesterol** from *de novo* biosynthesis, but a fraction can come from maternal deposits in the placenta[58]. Nonetheless, the detailed mechanisms of cholesterol transfer to the fetus are not fully understood.

On the other hand, the placenta has very important endocrine functions since it is a source of hormones that support the adaptation of the maternal body to pregnancy, establishment and maintenance of pregnancy, fetal growth and development and mechanisms involved in parturition[87]. Here are included the human chorionic gonadotropin (hCG), progesterone, estrogens, leptin, hPL or hPGH, many of which have been associated with the development of

IR in late pregnancy as we have discussed before[27,33]. Despite its importance, placental endocrine function will not be further addressed since it is beyond the scope of this thesis.

3.3. Immunological function and mesenchymal stem cells

The placenta is an immunological frontier between the mother and the semiallogenic fetus. On its surface, fetal cells exhibit human leukocyte antigen complex (HLA) (also known as major histocompatibility complex, MHC) proteins that differ from those of the mother so it is considered a ''not-self'' material that should be eliminated if no protective mechanism was present[102]. Numerous placental and maternal mechanisms work in concert to protect the fetus from immunological recognition and rejection[103]. A series of complex interactions between placental trophoblasts and the immune cells of the maternal decidual, including natural killer (NK) cells, dendritic cells, T-cells and macrophages, allow the embryo and then the fetus to properly develop in the uterus[102]. One of the main mechanisms for the immune tolerance at the interface between placenta and decidua, is ensured by the lack of expression of MHC-II complexes along with the expression of atypical MHC-I complexes, including HLA-E, -F and -G, which inhibit the maternal immune response on trophoblasts[103–106]. The fetal membranes themselves express high levels of HLA-G, which plays a critical role in materno-fetal tolerance[107,108]. On the other hand, Hofbauer cells are well known for maintaining an anti-inflammatory or M2 phenotype[109] and also play a critical role in maternal immunotolerance[110].

Recently, considerable attention has been paid to mesenchymal stem cells (MSCs) derived from both placental and umbilical tissues. These cells play an important role in immune tolerance and exhibit better immunomodulatory properties than those isolated from adult human tissues [111–118], although both type of MSCs show a similar immunophenotypic profile[111,112,119–121].

a) Mesenchymal stem cells

MSCs are multipotent adult stem cells that are present in different fetal and adult tissues, such as adipose tissue, umbilical cord, bone marrow and placenta[122]. These cells are able to differentiate into mesenchymal lineage cell types, including adipocytes, osteocytes and chondrocytes, and can modulate several immune cells, such as antigen presenting cells (APCs), T-cells, B-cells and NK cells by suppressing their proliferation and/or their production of cytokines and other factors[122,123] (Figure 4). Because of these properties, MSCs are a very promising tool for regenerative medicine [124]. However, their great plasticity makes these cells very sensitive to environmental stressors. Among other reports, previous work from our

laboratory have demonstrated the great impact of obesity and type 2 diabetes on the phenotype and functional capacity of MSCs derived from adipose tissue [125–127].

Fetal tissues are a well-documented source of MSCs[123]. Umbilical cord-derived MSCs have been extensively explored under the point of view of regenerative medicine, because of their feasibility to obtaining and their ability to modulate the immune response[128]. Emerging data point the placental MSCs (pMSCs) as a promising alternative to umbilical cord stem cells, since they seem to present better immunoregulatory properties[129]. As their analogues from other sources, pMSCs, including those from the chorionic villi, chorionic membrane, AM and decidua basalis, display low immunogenicity, important immunomodulatory capabilities and multilineage differentiation capacity *in vitro*, even across germinal boundaries outside of their specific lineage[130–133]. pMSCs perform their immunosuppressive actions on immune cells by both secretion of soluble mediators and direct cell-to-cell contact [123,134,135]. Several studies indicate that pMSC inhibit T-cell proliferation as well as cytokine secretion by T-helper (Th) 1 cells (i.e. IL-2, IL-12, TNF-α and interferon-γ), whereas they stimulate the expression and secretion of cytokines by the Th2 cells (i.e. IL-10). Furthermore, they induce the differentiation of T-regulatory lymphocytes (Tregs) and the induction of Th17 cells[115,136]. pMSCs can also inhibit T-cell proliferation indirectly by modulating the functions of macrophages and dendritic cells (DCs). On macrophages, pMSCs seem to stimulate a switch from a pro-inflammatory or M1 into M2 phenotype, including decreased levels of co-stimulatory molecules whereas increased expression of co-inhibitory molecules, along with a higher production of IL-10 and lower of IL-1β[137]. On the other hand, pMSCs inhibit the differentiation and maturation of monocytes into DCs, which reduces their ability to stimulate the proliferation T-cells[138,139] (Figure 4).

In summary, pMSCs from both the placental disk and the fetal membranes have aroused great interest because of their strong immune properties and ease of obtaining. In recent years, pMSCs derived from the AM have become the target of several studies because of their fetal origin and metabolic and immunomodulatory potential.

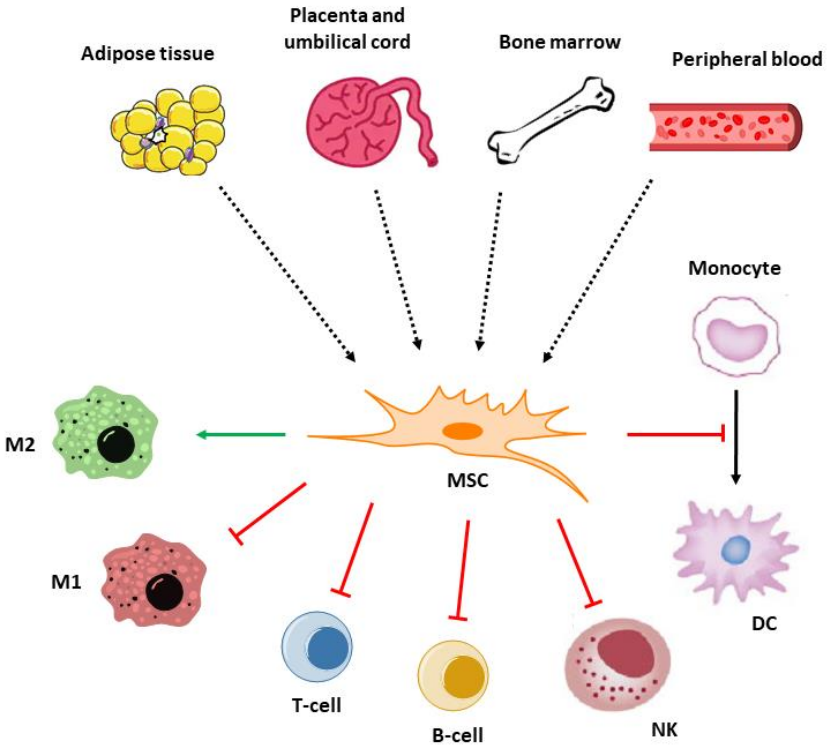


Figure 4. Sources of MSCs and their modulatory effects on immune system cells. MSC: mesenchymal stem cell; NK: natural killer cell; DC: dendritic cell.

b) Amniotic membrane

Fetal membranes are composed of chorion, an outer layer which contacts maternal cells, and amnion or AM, an inner thin layer on the inner side of the placenta[90] (Figure 3). AM has no vessels so it feeds by diffusion from the amniotic fluid and fetal surface vessels[140], using anaerobic glycolysis as a means of energy production[141]. AM has multiple metabolic functions such as the transport of water and soluble molecules and the production of bioactive factors, including vasoactive peptides, growth factors and cytokines[140]. It possesses anti-inflammatory[134,142], antibacterial and antiviral properties that contribute to materno-fetal tolerance, as well as anti-angiogenic and pro-apoptotic characteristics[140]. At birth, AM also secretes substances involved in the initiation and maintenance of uterine contractility[140]. Due to all its characteristics, it has been widely used in the area of regenerative medicine. Since its first reported use in 1910[143], AM has been used in for the treatment of several diseases including eyes-, skin- and genito-urinary-related pathologies[140,144].

Histologically, AM consists mainly of an epithelial layer, which is adjacent to the amniotic fluid, and a mesenchymal stromal layer, which is adjacent to the chorion[145,146]. The latter is comprised of mesenchymal stromal cells named **amniotic mesenchymal stem cells (AMSCs**). This specific cell population is responsible for many of the properties attributed to the AM and, especially promising for cell therapy because of their fetal origin, their immuno-privileged status[147] and their modulatory effect over immune cells[131]. For these reasons, AMSCs are considered as fetal precursor cells. Indeed, not just AM but also AMSCs are being extensively used in regenerative medicine, including cardiovascular, respiratory, gastrointestinal, endocrine and neurological preclinical applications[108]. AMSCs have showed low HLA-ABC and no HLA-DR expression[148], and abolishment of peripheral blood mononuclear cells (PBMCs) and T-cells proliferation[148–152]. They also exhibit a strong inhibition of DC generation, maturation and function[139,152], as well as they promote the secretion of anti-inflammatory and anti-fibrotic factors *in vitro*[131,153]. Besides, the conditioned medium from AMSCs have been reported to induce macrophages to display an M2-like phenotype[154].

Summarizing, the placenta is an organ highly adaptable and responsive to the maternal nutritional and metabolic status that could reflect the metabolic milieu of both mother and fetus. It contains a wide variety of cells devoted to nutrient transport, endocrine secretion and immune modulation and response. However, given its privileged position, it is also a target for potential disturbances reflected in either maternal or fetal circulation, which could lead to structural and functional modifications associated with potential consequences for the fetal health. Abnormalities in placental functions have been associated with poor pregnancy outcomes ranging from alterations in fetal growth pattern to implantation failure and fetal death[155]. Moreover, MSCs have been shown to be especially sensitive to changes in the metabolic environment[125–127], and particularly those derived from the placental tissues could be responsible for some of the lasting effects in the health of the offspring. In this context, the development of GDM has been associated with placental structural and functional alterations, which will be covered in the next section.

4. GESTATIONAL DIABETES MELLITUS (GDM)

GDM is one of the most common pregnancy complications and refers to any degree of glucose intolerance with onset or first recognition during pregnancy[156]. It is usually detected in late second trimester or early in the third trimester of gestation by routine glucose screening

and in most cases, it resolves after delivery. In recent years, the incidence of GDM has been increased in parallel with the increment in obesity rates[157], which is one of the main predisposing factors for GDM[6]. The prevalence of GDM varies substantially depending on the diagnostic criteria and the studied population. In Europe, a systematic review published in 2011 reported a prevalence of GDM between a 2-6% (Buckley et al 2011). Nonetheless, and according to the International Association of Diabetes and Pregnancy Study Groups (IADPSG) diagnostic criteria (7, 8 Egan 2017), GDM might occur in more than 20% of pregnancies, and these numbers are even higher when only obese women are considered (Egan 2017).

GDM raises the risk of perinatal complications including pre-eclampsia, fetal macrosomia, preterm delivery, caesarian section, neonatal hypoglycemia or hyperbilirubinemia, but also is a risk factor for long-term adverse outcomes, both in the mother and offspring[156]. Thus, GDM has been associated with later development of type 2 diabetes, metabolic syndrome and cardiovascular diseases in the mother[13,158,159]. In addition, the exposure to hyperglycemia and other nutrient stressors during intrauterine life increases the probability of developing later metabolic diseases in the offspring due to fetal programming[160], as it will be discussed in the following sections.

4.1. Physiopathology of GDM

Late pregnancy is normally associated with increased IR and a compensatory insulin secretion[29]. It is widely believed that most of the women that undergo GDM have a decreased insulin sensitivity and/or some defect in the beta cell function before pregnancy[13]. This defect is revealed as a consequence of the metabolic stress of pregnancy and is exacerbated in overweight or obese women[161–163] (Figure 5). Being overweight or obese before pregnancy is the most significant risk factor for GDM[5,6]. Concretely, obese women have 4-9 times greater risk for developing GDM than normal weight women and it has been estimated that approximately 45% of GDM cases might be preventable by maintaining a BMI<25kg/m^2 before pregnancy[164]. Therefore, it is not surprising that both pathological conditions share some of the adverse effects during pregnancy[165]. Advanced maternal age, ethnicity, previous history of gestational diabetes, family history of type 2 diabetes mellitus, tobacco consumption, genetic susceptibly and some sociodemographic and environmental factors have also been described as risk factors for GDM[156,166] (Figure 5).

In GDM women, beta-cell adaptation is unable to cope with the augmented demand of insulin secretion and hyperglycemia develops. Along with hyperglycemia, the metabolic derangements of GDM also include elevated FFAs and amino acids concentration in the

maternal circulation[156]. Serine inhibitory phosphorylation of IRS-1 and reduced insulin receptor autophosphorylation have been described in GDM pregnancies, which accentuates the state of IR[167,168]. Moreover, GLUT4 is downregulated in adipose tissue[35] and its insulin-induced translocation to plasma membranes is abnormal in GDM pregnant women[169]. GDM pregnancies, especially those accompanied by obesity, are associated with a state of **low-grade inflammation**[8,170], which is characterized by an increased circulating levels and adipose tissue secretion of proinflammatory mediators, such as TNF-α and IL-6[7,171,172]. As occurs with obesity-related IR and type 2 diabetes, many reports have highlighted the deep implication of inflammatory mediators and stress kinases in exacerbating IR during pregnancy leading to GDM[28,38,173]. Thus, GDM has been associated with higher levels of proinflammatory cytokines, such as TNF-α, and activation of kinases such as c-Jun N-terminal kinase (JNK)[174], nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB)[175] and protein kinase C (PKC)[176], which negatively modulate insulin signaling.

In recent years, the role of inflammation as a mediator of fetal growth alterations and the programming for metabolic disorders has gained interest. However, changes in maternal inflammatory mediators may not be reflected by similar changes in the fetus. The levels of leptin, IL-6 and TNF-α have been reported to be increased in GDM mothers while decreased in their neonates, despite birthweights were significantly higher compared to controls[8]. Either way, umbilical vein cytokine levels were described to be unaffected by maternal obesity[177]. In this aspect, the placenta has been proposed as an adaptor, sensing and responding to the maternal inflammatory environment in an attempt to diminish fetal exposure[178,179]. Even so, this adaptation could permanently disturb its functional capacity and compromise an adequate fetal nutrient availability and hormone regulation, thus affecting growth and later development.

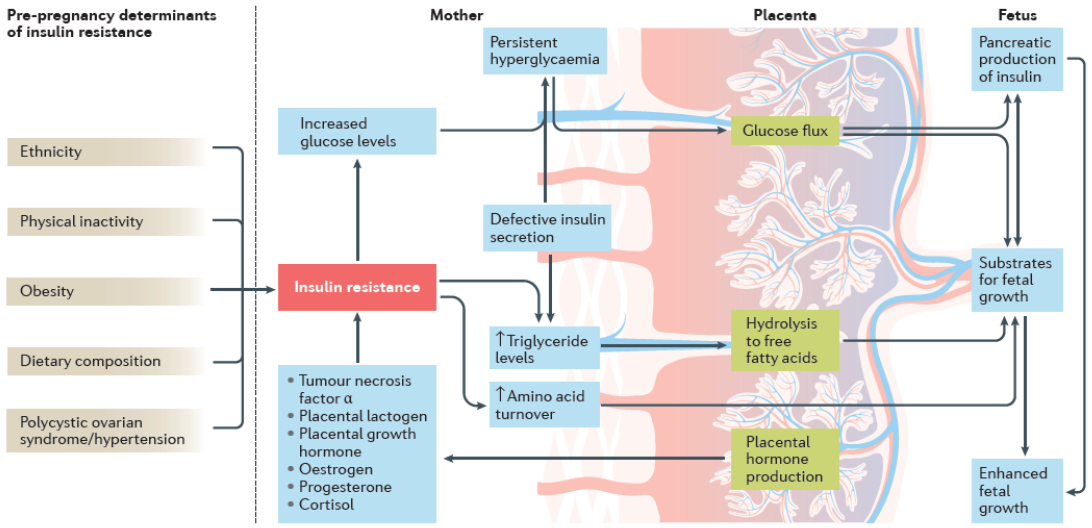


Figure 5. Pathophysiology of GDM. From Agha-Jaffar et al., 2017[180].

4.2. Placental alterations during GDM

As previously mentioned, the placenta is a primary target affected by both maternal and fetal metabolic derangements. This organ has evolved to acquire the capacity to buffer an adverse intrauterine environment by adapting its morphology and functions to minimize fetal exposure to inflammation and oxidative stress[178,179]. Nonetheless, this adaptive capacity is limited, and extreme perturbations of the maternal milieu, as in untreated GDM and/or obesity, may exceed the placental buffering capacity and thereby contribute to pathological effects in the fetus. The true extent of morphological and functional alterations of the placenta during GDM is still a matter of debate. Even so, an enlarged size, the presence of low-grade inflammation and an altered nutrient transport and vascular permeability are well recognized placental features of GDM[7,178]. Many other changes in the placenta of women with GDM include adaptive responses to protect both the placenta and fetus, such as the hypervascularization, or increased cholesterol clearance mechanisms[156].

Placental inflammation has been observed in pregnancies complicated by both GDM and obesity[7,171,177], where the placenta may act as both a target and a source of inflammatory cytokines. Indeed, placental cytokines were suggested to contribute to the physiological low-grade inflammatory state during the third trimester of gestation. Increased placental levels of leptin, IL-8, IL-1β and TNF-α have been observed during GDM[181–183]. Moreover, placental explants from GDM pregnancies showed higher secretion of TNF-α in response to high glucose

conditions[184], indicating that hyperglycemia might be the main driver in the enhanced placental cytokine production during GDM.

Maternal GDM has also been associated with increased **placental nutrient transport**[93,178]. Maternal hyperglycemia and hyperlipidemia raise the placental transport of glucose and FFA[178], which are accompanied by an augmented transport of amino acids[185] (Figure 5). The placenta does not actively enhance the transport of glucose during GDM as it is determined mostly by materno-fetal concentration gradient. By contrast, *in vitro* studies point to inflammatory cytokines as key mediators in the dysregulation of lipid[186,187] and amino acid transport across the placenta observed in GDM conditions[93,188]. These potential disturbances in nutrient transport might affect the fetal growth pattern and lead to the co-morbidities observed in these pregnancies.

GDM also alters the inflammatory profile of immune cells[11]. Given their great plasticity one of the most sensitive cells to maternal and fetal metabolic derangements are the **MSCs**, as previously mentioned, which are very abundant in fetal tissues. Similar to what happens in the placenta, the umbilical cord undergoes morphological alterations in GDM pregnancies that can impact the functionality of its MSCs, including dilated umbilical veins and eroded endothelial lining of the umbilical arteries[189]. Recent studies indicate the significant impact of GDM on human umbilical cord-derived MSCs in terms of proliferation[190,191], differentiation[192] mitochondrial dysfunction[192,193], and angiogenesis[190,193,194], regardless of the treatment followed or even in well-controlled patients[192]. In a similar way, it is reasonable to think that GDM could affect the niche of MSCs found in the placenta. In fact, the scarce data available has shown a decreased glucose uptake, marked IR and decreased clonogenicity and angiogenesis of pMSCs isolated from chorionic villi of GDM pregnant[195]. Studies about the potential impact of GDM in pMSCs from the AM are practically non-existent. Nonetheless, a significant decline in cell viability accompanied by an elevation of senescence and stress markers was observed in healthy but especially in GDM-derived AMSCs after the exposure to a long-term high glucose environment *in vitro*[196].

The impact of GDM on fetal precursor cells might play a key role in the etiology of metabolic diseases in offspring of GDM mothers. pMSCs, and especially AMSCs, are good indicators of the potential deleterious effects of maternal hyperglycemia and associated metabolic derangements of GDM on fetal tissues and organs, since they are genetically identical to those from the fetus. In addition, the placenta and fetal membranes are usually

discarded after delivery, so they are a very feasible source of stem cells, and the pMSCs lack many of the ethical considerations of using fetal tissues for research purposes.

4.3. Consequences of GDM on offspring

As previously stated, and according to the Freinkel's hypothesis[31], maternal hyperglycemia leads to increased transfer of glucose and other nutrients to the fetus; secondarily, fetal hyperinsulinism and persistent hyperglycemia lead to an accelerated fetal anabolism, augmented fetal growth and macrosomia. In GDM pregnancies, despite hyperglycemia is not as severe as in pregestational diabetes, infants are also at higher risk of overgrowing[197]. Besides, AGA neonates born to GDM mother seem to have a higher percentage of adipose tissue compared with those born to healthy controls, concordantly with an exaggerated adipose tissue accretion[197] (Figure 5). Both excessive accretion and asymmetrical distribution of fat increase the risk for dystocic delivery, including shoulder dystocia[198]. In the neonatal period, infants are at risk of hypoglycemia because of the intrauterine fetal beta cell hyperplasia[199], but also hypocalcemia, hyperbilirubinemia and respiratory distress are potential GDM-associated infant complications immediately after birth[200]. Follow-up studies indicated that offspring exposed to GDM are heavier, with higher fasting glucose levels, lower insulin sensitivity and defective insulin secretion, which make them more susceptible to be obese and to develop diabetes, metabolic syndrome and premature cardiovascular disease compared with the background population[13,14,16,17,19,20,201].

Although treatment of GDM has been shown to be effective in improving perinatal outcomes[156], its effect on long-term complications is far from clear. For this reason, a better understanding of the fetal metabolic derangements, its implications on the development of long-term complications and the search of biomarkers that could identify subjects at risk seem necessary. In this context, fetal lipid metabolism and the accurate determination of lipoprotein concentrations including their size and number might play an important role. On one hand, diabetes is not only a disorder of glucose metabolism, but also disturbs lipid and amino acid metabolism[31]. Similar to glucose, increased fetal FFA have been associated with fetal overgrowth[197] and maternal FFA and triglycerides concentrations close to delivery correlated with fetal plasma lipids, neonatal weight and fat mass in well-controlled GDM women[202]. All these data suggest that both maternal hyperlipidemia and fetal *de novo* synthesis of FFA contribute to fetal fat depots.

On the other hand, some studies suggest that cardiovascular diseases and atherosclerosis originate early in life[203], and alterations in fetal lipoprotein metabolism could be involved in

their development. In offspring born to GDM women, early markers of atherogenesis have been reported[203,204] and alterations in fetal lipoprotein metabolism are expected, although reports involving cord blood lipoprotein determination and function have been inconclusive[205–210]. Differences in the studied populations and the methods used for lipoprotein determination might be responsible for the incongruences. In this context, an extensive characterization of the main lipoproteins, with the assessment of the size and number of particle may give us a more accurate picture of the fetal lipoprotein disturbances and shed some light where standard lipid panels have failed, as it has been reported in dyslipidemia associated to diabetes[211]. Under this scenario, **^{1}H-nuclear magnetic resonance (NMR)-based lipoprotein tests** (Figure 6) have demonstrated that the number and size of LDL and HDL particles are a more powerful indicator of cardiovascular risk than classical cholesterol determinations in diabetes, given the large variability in the amount of cholesterol of the particles and the particle size[212]. Studies such as this have also demonstrated the incomplete conversion of VLDL into LDL, which results in a higher prevalence of VLDL and small and dense LDL particles[212]. These abnormal LDL particles increases the risk for atherosclerosis since they can be oxidized more easily, interacting badly with LDL receptors and associating more readily with proteoglycans on the surface of cells or in extracellular matrix[213–216].

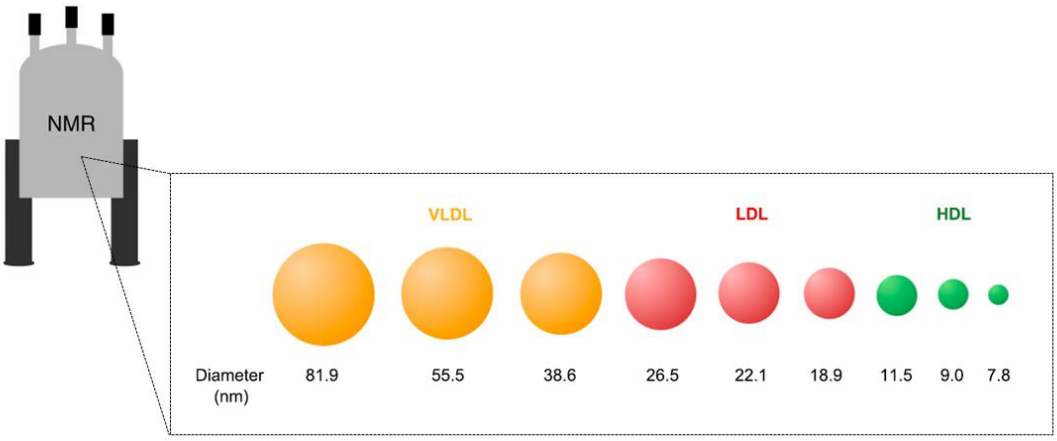


Figure 6. Lipoprotein subclasses identified by ^{1}H-NMR-based lipoprotein tests, named large, medium and small VLDL, LDL and HDL. Adapted from Mallol et al., 2015[211]. VLDL: very low-density lipoproteins; LDL: low-density lipoproteins; HDL: high-density lipoproteins.

In summary, a large body of experimental, clinical and epidemiological data support the link between intrauterine nutrition and the development of later metabolic and cardiovascular diseases. GDM disturbs maternal metabolism and exposes the fetus to an adverse intrauterine

milieu. This may increase the susceptibility for developing metabolic dysfunction and cardiovascular complications during childhood and adulthood, in a process known as fetal programming. Hyperglycemia, dyslipidemia, IR and the low-grade inflammatory state seem to be key elements in this situation, but less is known about the mechanisms involved in the appearance of long-term metabolic complications during childhood and adulthood. In this aspect, the study of the impact of GDM on fetal precursors found in the AM and fetal lipoprotein profile across birth-weight categories represent a novel approach that could help to uncover possible contributing factors to the development of later diseases.

HYPOTHESIS AND OBJECTIVES

HYPOTHESIS AND OBJECTIVES

Considering that:

1) Pregnancy is a dynamic state that encompasses changes in the metabolism of the mother to ensure an adequate growth and development of the fetus.
2) GDM has been associated with a disturbed maternal metabolism that favors an excessive nutrient availability and uptake by the fetus, altering fetal growth pattern and predisposing to later diseases in life by fetal programming.
3) Fetal precursor cells and lipid metabolism are key components of fetal programming that can be directly affected by GDM.
4) The AM is in contact with the amniotic fluid and the fetus and, as such, its stem cell component might be a good indicator of how the intrauterine environment impacts the fetus.
5) ^{1}H-NMR-based lipoprotein tests have demonstrated higher detection capacity of subtle lipoprotein abnormalities and to have a greater ability to predict cardiovascular risk than classical cholesterol determinations.

We hypothesized that:

GDM exerts deleterious effects on the functionality of fetal precursor cells found in the amniotic membrane and on the morphological and functional characteristics of fetal lipoproteins, which could program the fetal metabolism and directly contribute to the higher predisposition to metabolic and cardiovascular diseases later in life.

Objectives:

1. Main objective

To study the influence of GDM on the functional characteristics of the AMSCs and on the fetal advanced lipoprotein profile across birth-weight categories, in order to explore a potential relationship with fetal parameters associated with adverse outcomes that may have a prognostic value.

2. Specific objectives of study 1
    a. **Primary objective**: To determine whether GDM might leave an imprint in fetal precursors of the AM and whether it might be related to adverse outcomes in offspring.
    b. **Secondary objectives**:
        i. To study the biological features of AMSCs in a well-defined cohort of control and GDM-affected women, including their immunophenotypic, proliferative and differentiation capabilities.
        ii. To analyze the functional characteristics of these AMSCs, including their plasticity and their inflammatory, invasive and chemotactic profiles.
        iii. To explore the potential relationship between AMSCs-associated and clinical, anthropometric and metabolic parameters from mother and offspring.

3. Specific objectives of study 2
    a. **Primary objective**: To assess the impact of GDM on the cord blood lipoprotein profile across birthweight categories and on offspring outcomes by using advanced ^{1}H-NMR-based lipoprotein test.
    b. **Secondary objectives**:
        i. To analyze the impact of GDM on the size, lipid content, number and concentration of particles within their subclasses of the main lipoproteins in umbilical cord blood across both weight categories.
        ii. To explore the potential relationship between ^{1}H-NMR-based lipoprotein test data and parameters associated with obesity in the offspring at 2 years.

RESULTS

RESULTS

STUDY 1

Gestational diabetes impacts fetal precursor cell responses with potential implications for offspring.

Algaba-Chueca F, Maymó-Masip E, Ejarque M, Ballesteros M, Llauradó G, López C, Guarque A, Serena C, Martínez-Guasch L, Gutiérrez C, Bosch R, Vendrell J, Megía A, Fernández-Veledo S.

Stem Cells Transl Med. 2020 Mar;9(3):351-363. doi: 10.1002/sctm.19-0242. Epub 2019 Dec 27.

STUDY 2

Advanced lipoprotein testing in umbilical cord blood: impact of gestational diabetes across birth-weight categories.

Algaba-Chueca F, Maymó-Masip E, Ballesteros M, Guarque A, Freixes O, Amigó N, Fernández-Veledo S, Vendrell J, Megía A.

Submitted to The Journal of Clinical Endocrinology and Metabolism. Reference jc.2020-00724,

dated March 23, 2020.

Received: 1 August 2019 | Accepted: 5 November 2019

DOI: 10.1002/sctm.19-0242

FETAL AND NEONATAL STEM CELLS

STEM CELLS TRANSLATIONAL MEDICINE

Gestational diabetes impacts fetal precursor cell responses with potential consequences for offspring

Francisco Algaba-Chueca[1,2,3] | Elsa Maymó-Masip[1,2,3] | Miriam Ejarque[1,2,3] | Mónica Ballesteros[2,4] | Gemma Llauradó[3,5] | Carlos López[2,6] | Albert Guarque[2,4] | Carolina Serena[1,2,3] | Laia Martínez-Guasch[1,2,3] | Cristina Gutiérrez[1,2] | Ramón Bosch[2,6] | Joan Vendrell[1,2,3,7] | Ana Megía[1,2,3,7] | Sonia Fernández-Veledo[1,2,3]

[1]Servei d'Endocrinologia i Nutrició i Unitat de Recerca, Hospital Universitari de Tarragona Joan XXIII, Tarragona, Spain

[2]Institut d´Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain

[3]CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM)-Instituto de Salud Carlos III, Madrid, Spain

[4]Servei de Ginecologia i Obstetricia, Hospital Universitari de Tarragona Joan XXIII, Tarragona, Spain

[5]Department of Endocrinology and Nutrition, Hospital del Mar, Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain

[6]Department of Pathology, Plataforma de Estudios Histológicos, Citológicos y de Digitalización, Hospital de Tortosa Verge de la Cinta, Tortosa, Spain

[7]Departament de Medicina i Cirurgia, Universitat Rovira i Virgili, Reus, Spain

Correspondence
Sonia Fernández-Veledo, PhD, and Ana Megía MD, PhD, Research Unit, University Hospital of Tarragona Joan XXIII, c/ Dr. Mallafré Guasch, 4, 43007 Tarragona, Spain.
Email: sonia.fernandezveledo@gmail.com (S. F. V.) and ana.megia@gmail.com (A. M.)

Funding information
European Regional Development Fund (ERDF); Spanish Ministry of Economy and Competitiveness,

Abstract

Fetal programming has been proposed as a key mechanism underlying the association between intrauterine exposure to maternal diabetes and negative health outcomes in offspring. To determine whether gestational diabetes mellitus (GDM) might leave an imprint in fetal precursors of the amniotic membrane and whether it might be related to adverse outcomes in offspring, a prospective case-control study was conducted, in which amniotic mesenchymal stem cells (AMSCs) and resident macrophages were isolated from pregnant patients, with either GDM or normal glucose tolerance, scheduled for cesarean section. After characterization, functional characteristics of AMSCs were analyzed and correlated with anthropometrical and clinical variables from both mother and offspring. GDM-derived AMSCs displayed an impaired proliferation and osteogenic potential when compared with control cells, accompanied by superior invasive and chemotactic capacity. The expression of genes involved in the inflammatory response (*TNFα*, *MCP-1*, *CD40*, and *CTSS*) was upregulated in GDM-derived AMSCs, whereas anti-inflammatory *IL-33* was downregulated. Macrophages isolated from the amniotic membrane of GDM mothers consistently showed higher expression of *MCP-1* as well. In vitro studies in which AMSCs from healthy control women were exposed to hyperglycemia, hyperinsulinemia, and palmitic acid confirmed these results. Finally, genes involved in the inflammatory response were associated with maternal insulin sensitivity and prepregnancy body mass index, as well as with fetal metabolic parameters. These results suggest that the GDM environment could program stem cells and subsequently favor metabolic dysfunction later in life. Fetal adaptive programming in the setting of GDM might have a direct negative impact on insulin resistance of offspring.

Francisco Algaba-Chueca and Elsa Maymó-Masip contributed equally for this study.

Ana Megía and Sonia Fernández-Veledo are co-senior authors.

Grant/Award Numbers: PI17/01503, PI14/00228, RTI2018-093919-B-I00, SAF2015-65019-R, PI 18/00516, PI 15/01562

KEYWORDS

fetal precursors, gestational diabetes, offspring, placenta, programming, stem cells

1 | INTRODUCTION

Gestational diabetes mellitus (GDM) is associated both with short-term adverse obstetric and perinatal complications and with long-term metabolic health consequences for offspring.[1] In this context, fetal programming has been proposed as a key mechanism underlying the link between the intrauterine exposure to maternal diabetes and an increased risk for metabolic dysfunction in adulthood, leading to type 2 diabetes, obesity, and cardiovascular disease.[2] However, the ultimate mechanisms involved are not well known.

As a natural interface between mother and fetus, the functional integrity of the placenta is crucial to support fetal growth and development as well as to adapt to the maternal nutritional and metabolic status.[3] Thus, beyond its recognized endocrine, immunological, and vascular functions, the placenta is a highly structured organ with multiple cell types dedicated to nutrient transport and energy metabolism.[4] Although the extent to which maternal glycemic control contributes to placenta dysfunction remains a matter of debate,[5] an enlarged size, altered vascular permeability, and the presence of low-grade inflammation seem to be typical features of GDM.[4] In this aspect, the potential of these disturbances to imprint fetal precursor cells found in the placenta and/or umbilical cord is emerging as a novel pathway playing a key role in the etiology of metabolic diseases in offspring of mothers with GDM. Indeed, recent studies have revealed the significant impact of GDM on human umbilical cord-derived stromal cells in terms of proliferation,[6] mitochondrial dysfunction,[7] and angiogenesis.[8]

The amniotic membrane is the innermost layer of the placenta and possesses anti-inflammatory and antibacterial properties that contribute to materno-fetal tolerance. It also exerts many metabolic functions, such as the transport of water and soluble molecules and the production of bioactive factors.[9] Our study sought to investigate whether GDM might leave an imprint in fetal precursors found in the amniotic membrane and, if so, whether it might be related to adverse outcomes in offspring. More specifically, we focused on amniotic mesenchymal stem/stromal cells (AMSCs) which are mainly characterized by their low immunogenicity, immunomodulatory properties and mesodermal multilineage differentiation capacity in vitro.[10] We report evidence that maternal metabolic derangements during gestation disturb the biological properties of AMSCs. Of note, we found an association between the biological features of these fetal precursor cells and the maternal and fetal clinical and metabolic parameters, supporting the notion that fetal adaptive programming in the setting of GDM might have a direct impact on offspring.

Significance statement

Signatures of metabolic deregulation seem to remain in cells early in development. Given the location on the inner side of the placenta, amniotic membrane stem cells might be a good indicator of how the intrauterine environment impacts the fetus. This study showed, for the first time, how gestational diabetes disturbs both the phenotype and the functional characteristics of amniotic mesenchymal stem cells, and these alterations are related to maternal and fetal metabolic status, suggesting that fetal adaptive programming in the setting of gestational diabetes might have a direct impact on offspring.

2 | MATERIAL AND METHODS

2.1 | Study subjects

Eighteen pregnant women with a singleton pregnancy (9 with GDM and 9 with normal glucose tolerance acting as controls) scheduled for cesarean delivery were included in this study, which was performed at the Hospital Universitari de Tarragona Joan XXIII (HUJ23) according to the tenets of the Helsinki Declaration. The institutional review board (CEIm) approved the study protocol and all patients gave written informed consent before participating in the study. All mothers diagnosed with GDM were recruited at the Diabetes and Pregnancy Clinic of the HUJ23, while control subjects were recruited at the delivery suite. GDM was diagnosed according to the current criteria of the Spanish Diabetes in Pregnancy guidelines, which followed the National Data Group Criteria.[11,12] Four of the GDM women were treated only with diet whereas five were also treated with insulin. Timing of delivery was based primarily on obstetric indications. Gestational age was confirmed in all pregnant women by a routine ultrasonographic examination performed before 20 weeks of gestation. Exclusion criteria for all subjects were preexisting type 1 or type 2 diabetes, inflammatory or chronic diseases, current use of drugs known to affect carbohydrate metabolism, fetal anomalies identified at birth, smoking, or high blood pressure.

2.2 | Clinical and demographic data

Upon inclusion, demographic and historical data were collected paying special attention to third trimester HbA1c in GDM women, 1-hour 50-g glucose challenge test (GCT), pregravid weight,

gestational weight gain (GWG), and gestational age at delivery. Maternal anthropometric measurements of height (measured to the nearest 0.5 cm) and weight (measured to the nearest 0.1 kg) were obtained using a medical scale. Body mass index (BMI) was calculated using the formula BMI = weight (in kilograms)/height (in meters)^2. GWG was calculated as (final weight)−(pregravid weight). Neonatal length, weight, and waist circumference were measured at birth and the waist circumference/length ratio was calculated. Suprailiac skinfold thickness was measured at least three times using a Holtain skinfold caliper (Chasmors Ltd, London, UK) to obtain a consistent and stable reading.

2.3 | Sample collection and processing

Maternal blood samples were collected in the morning after an 8 hours fast and immediately before cesarean section, and umbilical vein cord blood was obtained at the time of delivery. Serum was immediately separated by centrifugation and stored at −80°C until analysis. Full-term placentas (37–39 weeks gestation) were collected after delivery and immediately processed under sterile conditions. The amniotic membrane was mechanically peeled free from underlying chorion and washed with phosphate-buffered saline (PBS) containing antibiotics.

2.4 | Laboratory measurements

Glucose, cholesterol, and triglycerides levels were determined using ADVIA 1800 and 2400 (Siemens AG, Munich, Germany) autoanalyzers by standard enzymatic methods. Fasting plasma insulin was determined by immunoassay on the Centaur XP platform (Siemens AG). Homeostasis model assessment-insulin resistance (HOMA-IR) was determined according to the equation HOMA-IR = fasting plasma glucose (mmol/L) × fasting plasma insulin (μU/mL)/22.5. HbA1c was estimated by high-performance liquid chromatography-based ion exchange chromatography (ADAMS-A1c HA-8160; Menarini Diagnostics, Florence, Italy).

2.5 | Histological study of placenta

Tissue sections from the maternal (facing the decidua) and fetal (facing the amniotic cavity) sides of the placentas were collected, washed, fixed in a 4% paraformaldehyde, embebbed in paraffin, cut into slices using a rotator microtome and mounted onto glass microscope slides. Tissue sections were then dewaxed and stained with Masson's trichrome to highlight connective tissue and collagen fibers of the extracellular matrix. Slides were observed microscopically at ×2.5 magnification using a Leica DM LB2 bright-field microscope (Leica Microsystems GmbH, Wetzlar, Germany). Ten different nonoverlapping fields randomly selected from each slide were visualized and captured at ×40 magnification using a Leica DFC320 Digital Camera (Leica Microsystems). Morphometric measurements were performed using Image-Pro Plus 5.0 software (MediaCybernetics Inc., Silver Springs, Maryland) programmed for multistep algorithms: one for the detection of the total area of the villous/image (in pixels) and a second for the detection of the total trichrome-positive green pixels/image (fibrotic area). The ratio between the fibrotic area and the total area of the villous was calculated for each image. Finally the average of the ratios of the 10 images was calculated for each case.

2.6 | Isolation and culture of human amniotic mesenchymal stem cells

AMSCs were isolated as previously described.[13,14] Briefly, the amniotic membrane was digested in a rotary incubator at 37°C with a solution of 0.25% trypsin-EDTA for 30 minutes and then with collagenase type IV solution (Gibco, Carlsbad, California) in complete medium comprising Dulbecco's modified Eagle's medium (DMEM)/F12, 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution, for 1.5 hours. After centrifugation and washing, cell pellet was resuspended in complete medium. Primary cultures of AMSCs at passage 0 were grown to 80%-90% confluence and harvested with 0.25% trypsin-EDTA. Non-adherent cells were removed by rinsing twice with PBS. All of the experiments were performed at passages 3-5 to ensure cell purity. For chemotaxis experiments and prostaglandin E2 experiments, 24-hour culture media containing 0.2% BSA and without FBS were collected and stored at −80°C.

2.7 | Isolation of amniotic membrane-resident macrophages

Macrophages were purified by adherence to tissue culture plates following an established procedure that results in >90% purity.[15] Briefly, the cell pellet from the digested membrane was resuspended in complete medium and allowed to attach for 10 minutes. The supernatant was then collected and seeded again in different flasks to obtain AMSCs. Culture plates were then washed twice with PBS. Cells that did not express the macrophage marker CD68 were discarded.

Since amniotic macrophages and AMSCs are isolated from the same primary sample, in order to assess their purity, the expression levels of typical markers of monocyte-lineage and antigen presenting cells, such as *CD80*, *CD86*, *CD163*, *CD206*, and *CD209* were compared in amniotic macrophages and AMSCs.

2.8 | Immunophenotyping

To assess mesenchymal lineage features and to determine whether they met the minimum criteria defined by the International Society of Cell Therapy,[16] AMSCs (1×10^5) were incubated with a panel of

primary antibodies (BD Pharmingen, San Diego, California) and their surface markers expression was analyzed by flow cytometry (FacsAriaIII, BD Biosciences, San Jose, California). Data analysis was performed using the FACSDiva software (BD Biosciences).

2.9 | Multilineage differentiation capacity

To determine the multilineage differentiation capacity of AMSCs, specific differentiation conditions were used to trigger cell differentiation into adipocytes, chondrocytes, and osteocytes as described.[17,18] Differentiated cells were stained with Oil Red O, Alcian Blue or Alizarin Red, respectively, and observed in a bright-field microscope (ZEISS Axio Vert A1, Oberkochen, Germany). Quantification of the differentiation capacity was assessed by extracting the dyes from cell cultures (isopropanol for Oil Red O and cetylpyridinium chloride for Alizarin Red) and by measuring their absorbance by spectrophotometry at 540 nm.

2.10 | Migration capacity

The migratory capacity of AMSCs was determined using a Transwell system (8-μm polycarbonate membrane; Corning, New York, New York), as described.[19] Briefly, lower chambers were filled with DMEM high glucose 0.2% BSA and cells (8×10^4) suspended in the same medium were added to the upper chambers. Transwells were then incubated for 24 hours at 37°C. Cells that migrated to the lower chamber were then collected and counted using the BioRad TC 10 Automated Cell Counter (BioRad, Hercules, California).

2.11 | Invasion capacity

The invasive capacity of AMSCs was determined as for the migration assay except that Transwell membranes were firstly coated with Matrigel (Corning) in DMEM high glucose 0.2% BSA for 2 hours at 37°C. Cells (8×10^4) were added to the upper chambers and incubated for 24 hours at 37°C and those ones that invaded into the lower chamber were collected and counted as above.

2.12 | Stimulation experiments

AMSCs from control pregnant women were cultured in 6-well plates (5×10^4) and allowed to attach for 24 hours. Cells were then stimulated for 24 hours with glucose, insulin and/or palmitic acid (PA) to a final concentration of 30 mM, 100 nM, and 0.4 mM, respectively. Absolute ethanol (EtOH) was used as vehicle to dissolve palmitic acid so a control of EtOH 0.4 mM was added in all the experiments. All conditions including PA were relativized to that control.

2.13 | Chemotaxis capacity

The migratory response of human monocytes (THP-1 cell line) and human T lymphocytes (Jurkat cell line) to the conditioned medium of AMSCs was determined as for the migration assay, except that a 5-μm polycarbonate membrane was used. Lower chambers were filled with 24-hour conditioned medium without FBS. In the stimulation experiments, 24-hour conditioned medium was collected 24 hours after removing the stimuli. THP-1 or Jurkat cells (1×10^5) suspended in DMEM/F12, 0.1% BSA were then added to the upper chambers and incubated overnight at 37°C. Cells that migrated to the lower chambers were collected and counted as above.

2.14 | MCP-1 blockage experiments

MCP-1 neutralization was performed as per the chemotaxis experiments with THP-1 cells, except that the 24-hour conditioned media without FBS were incubated with 20 μg/mL of an antibody against MCP-1 (CCL2 [MCP-1] Monoclonal Antibody 5D3-F7, eBioscience) for 30 minutes at room temperature before being added to the lower chamber of the Transwell System. A negative epitope control (Mouse IgG1 kappa Isotype Control, eBioscience) was included in each experiment. THP-1 cells (1×10^5) suspended in DMEM with 0.2% BSA were then added to the upper chambers and incubated overnight at 37°C. Cells that migrated to the lower chambers were collected and counted as mentioned above.

2.15 | Cell proliferation

Proliferation rate of AMSCs was determined by standard colorimetric 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetra-zolium bromide (MTT) incorporation experiments. Cells (1.6×10^3) were cultured in 96-well plates and allowed to attach for 24 hours. A MTT assay at day 1 was performed to count the initial number of cells. After 5 days, a second MTT assay was performed (day 7) and the difference in absorbance between day 7 and day 1 was considered the proliferation rate. In the AMSCs stimulation studies, the proliferation rate was measured at 24 hours after the addition of the stimuli. Absorbance was measured by spectrophotometry at 540 nm in all cases.

2.16 | Prostaglandin E_2 determination

Prostaglandin E_2 (PGE2) concentrations were measured, at 24 hours, in the conditioned medium of AMSCs by ELISA (R&D Systems) following the manufacturer's instructions.

2.17 | Gene expression analysis

Total RNA was isolated from cells using the RNeasy Mini kit (Qiagen, Valencia, California) and its quality was assessed by the OD260/OD280

ratio. For gene expression analysis, RNA was transcribed into cDNA with random primers using the Reverse Transcription System (Applied Biosystems, Foster City, California). Quantitative gene expression was evaluated by real-time polymerase chain reaction (RT-PCR) on a 7900HT Fast Real-Time PCR System using the following predesigned TaqMan primers (Applied Biosystems): *18S* (Hs03928985_g1), *ACACA* (Hs01046047_m1), *ALP* (Hs01029144_m1), *CCL3* (Hs00234142_m1), *CD40* (Hs 01002915_g1), *CD74* (Hs00269961_m1), *CD80* (Hs01045161_m1), *CD86* (Hs01567026), *CD163* (Hs00174705), *CD209* (Hs01588349), *COL1a1* (Hs00164004_m1), *COL2a1* (Hs00264051_m1), *COMP* (Hs00164359_m1), *CTSB* (Hs00947439_m1), *CTSS* (Hs00175407_m1), *FABP4* (Hs01086177_m1), *IL-1β* (Hs01555410_m1), *IL-6* (Hs00 174131_m1), *IL-10* (Hs00961622_m1), *IL-12b* (Hs01011518_m1), *IL-33* (Hs04931857_m1), *LPL* (Hs00173425_m1), *MCP-1* (Hs00234140_m1), *MRC-1* (Hs00267207), *PPARγ* (Hs01115513_m1), *PTGS2* (Hs00153133), *TGFβ1* (Hs00998133_m1), *TNFα* (Hs00174128_m1).

2.18 | Statistical analysis

Data were analyzed with SPSS software version 21.0 (IBM, Armonk, New York). The one-sample Kolmogorov-Smirnov test was performed to verify normal distribution of the quantitative variables. Quantitative variables are expressed as mean ± SD or SEM as indicated and categorical variables are reported as number (percentages). Student's *t* test was used to compare the mean values of continuous variables. To analyze differences in nominal variables between groups we used the chi-squared test. Pearson's correlation coefficient was used to analyze the univariate correlation between gene expression and clinical and metabolic parameters. A *P*-value <.05 was considered statistically significant in all analyses.

3 | RESULTS

3.1 | Clinical and placental histological data of the population studied

AMSCs were isolated from the amniotic membrane of mothers with a healthy pregnancy (control group, n = 9) and with GDM (n = 9). Clinical, anthropometric, and biochemical data from mothers and offspring are presented in Table 1. In the GDM group, no differences in maternal HOMA-IR and insulin concentrations were observed between diet and insulin treated women.

Placental abnormalities associated with GDM have been inconsistently reported in the literature, perhaps reflecting glycemic control, and prenatal care quality. First, we assessed macroscopic and histopathologic features of the collected placentas. The macroscopic study of the placentas failed to find difference between GDM and control groups. No differences were observed in placental weight and area (Table 1), and the umbilical cord was marginally inserted in most of the placentas studied (7/9 of the control group and in 5/9 of the GDM group).

TABLE 1 Clinical characteristics of the subjects included in the study

	Whole group (18)	Control (9)	GDM (9)	*P*
Maternal age, years	35.06 ± 5.34	34.11 ± 6.49	36.00 ± 4.06	.470
Parity ≥1, n (%)	10 (55.6)	4 (44.4)	6 (66.7)	.603
Prepregnancy BMI, kg/m^2	26.42 ± 4.43	24.85 ± 3.31	28.00 ± 5.03	.067
Gestational weight gain, kg	10.09 ± 5.10	11.83 ± 5.29	8.14 ± 4.37	.081
1-h 50-g glucose challenge, mg/dL	148.94 ± 37.35	120.44 ± 24.11	177.44 ± 23.57	<.001
Maternal glucose, mg/dL	79.23 ± 12.69	77.57 ± 7.68	81.17 ± 17.53	.632
HbA1c %	-	-	5.36 ± 0.25	
Maternal HOMA-IR	2.75 ± 1.93	1.70 ± 3.98	3.98 ± 2.20	.052
Maternal insulin	14.20 ± 8.31	10.13 ± 4.23	18.27 ± 9.59	.061
Maternal total cholesterol, mg/dL	212.31 ± 38.21	224.71 ± 42.59	197.83 ± 29.35	.220
Maternal triglycerides, mg/dL	224.38 ± 82.76	244.00 ± 87.93	201.50 ± 77.41	.379
Week at delivery, weeks	38.17 ± 0.99	37.78 ± 0.83	38.56 ± 1.01	.097
Newborn weight, g	3,396 ± 454	3,385 ± 448	3,408 ± 487	.517
Placental weight, g	661.67 ± 130.45	625 ± 66	698 ± 170	.460
Suprailiac skinfold, mm	3.35 ± 0.49	3.29 ± 0.39	3.42 ± 0.60	.917
Cord blood glucose, mg/dL	68.06 ± 11.96	64.25 ± 12.07	71.87 ± 11.32	.213
Infant male sex, n (%)	9 (50%)	5 (55.6)	4 (44.4)	.637
Cord blood insulin	6.70 ± 4.61	6.04 ± 3.53	7.35 ± 5.63	.567
Cord blood total cholesterol, mg/dL	60.44 ± 8.97	56.00 ± 3.70	64.88 ± 10.66	.054
Cord blood triglycerides, mg/dL	29.56 ± 18.30	23.75 ± 4.95	33.38 ± 24.82	.215

However, the histological study of placental tissue showed differences in the distribution of collagen deposition in villous stroma between groups. Staining quantification indicated lower collagen deposition on the maternal side of placentas obtained from GDM women (P = .0173) whereas it was higher on the fetal side compared the controls (P = .0067) (Figure 1). These results indicate that a diabetic environment modifies the collagen deposition within the placenta, highlighting the presence of fibrosis on the placenta's fetal side, which is characterized by an excessive connective tissue accumulation in response to tissue injury and inflammation.

3.2 | Isolation and characterization of amniotic mesenchymal stem cells from GDM and control women

Flow cytometry analysis of cell marker expression was consistent with the minimum criteria defined for AMSCs. Accordingly, cells were positive for the surface markers CD90, CD73, and CD105 and negative for CD45, CD34, CD31, CD14, and the human leukocyte antigen complex DR (HLA-DR) (Table 2). No significant differences were detected between groups. Likewise, the amniotic membrane-cell number ratio (number of cells obtained per gram of tissue) was similar between control and GDM women (data not shown). By contrast, cell proliferation was significantly lower in AMSCs isolated from GDM mothers as measured by MTT incorporation assays (Figure 2A). Both groups of AMSCs demonstrated similar adipogenic and chondrogenic differentiation capacities, typical of mesodermal cells. However, AMSCs from GDM mothers showed an impairment in the osteogenic lineage differentiation as revealed by a diminished Alizarin Red staining quantification and gene expression of typical osteogenic markers including alkaline phosphatase (*ALP*) and collagen type I alpha 1 chain (*COL1a1*) (Figure 2B,C).

3.3 | GDM displays an immune response in AMSCs

A gene expression signature of placental inflammation in pregnancies complicated by GDM has been previously reported. However, the cellular events underlying this process remain unknown or, at least, unclear. The GDM-AMSCs inflammatory expression profile presented an increase in the expression of genes encoding the pro-inflammatory cytokine tumor necrosis factor alpha (*TNFα*) and the chemokine monocyte chemoattractant protein 1 (*MCP-1*), as well as in genes involved in the inflammatory response such as *CD40* and cathepsin S (*CTSS*) compared to controls (Figure 3A). No differences were observed in the expression of interleukin 10 (*IL-10*), transforming growth factor beta 1 (*TGFβ1*), *CD74*, *CD80*, or cathepsin B (*CTSB*) (data not shown). By contrast, GDM-AMSC showed a significant reduction in the expression of *IL-33*, a cytokine with anti-inflammatory properties, and also of prostaglandin-endoperoxide synthase 2 (*PTGS2*), a key enzyme in prostaglandin biosynthesis (Figure 3A). Accordingly, prostaglandin E2 (PGE2) levels were lower in the conditioned medium from GDM-AMSCs compared to those from control cells (4237.28 ± 25.37 pg/mL vs 2476.62 ± 30.36 pg/mL,

TABLE 2 Immunophenotypic profile of AMSCs from pregnant control and gestational diabetes mellitus (GDM) women

Marker	Control (%)	GDM (%)
CD90	97.88 ± 1.35	96.02 ± 3.28
CD73	94.18 ± 5.36	93.28 ± 4.41
CD105	91.78 ± 5.40	88.24 ± 7.01
CD45	0.02 ± 0.045	0.025 ± 0.05
CD34	0 ± 0	0.24 ± 0.33
CD31	0.366 ± 0.45	0.26 ± 0.207
CD14	0.34 ± 0.47	0.34 ± 0.53

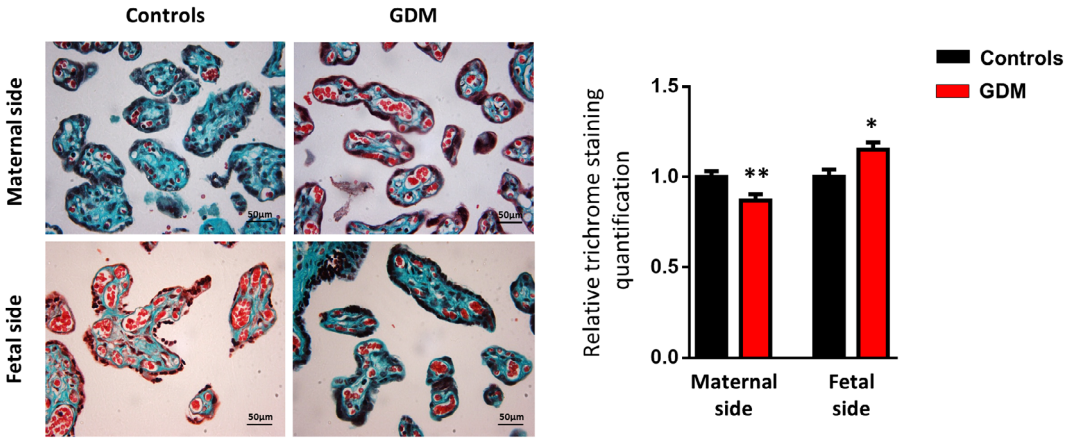


FIGURE 1 Histological features of the placental tissue from maternal and fetal sides obtained from gestational diabetes mellitus (GDM) and control women. Representative photomicrographs of terminal and intermediate villi in placental sections from pregnant control and GDM women, stained with Masson's trichrome to highlight connective tissue and collagen fibers (in green). Quantification of Masson's trichrome staining in villous stroma of placental sections is shown as percentage mean area ± SD (n = 6-8 per group). Results are shown as mean ± SEM from independent donor experiments performed in duplicate. *P < .05 vs controls, **P < .01 vs controls

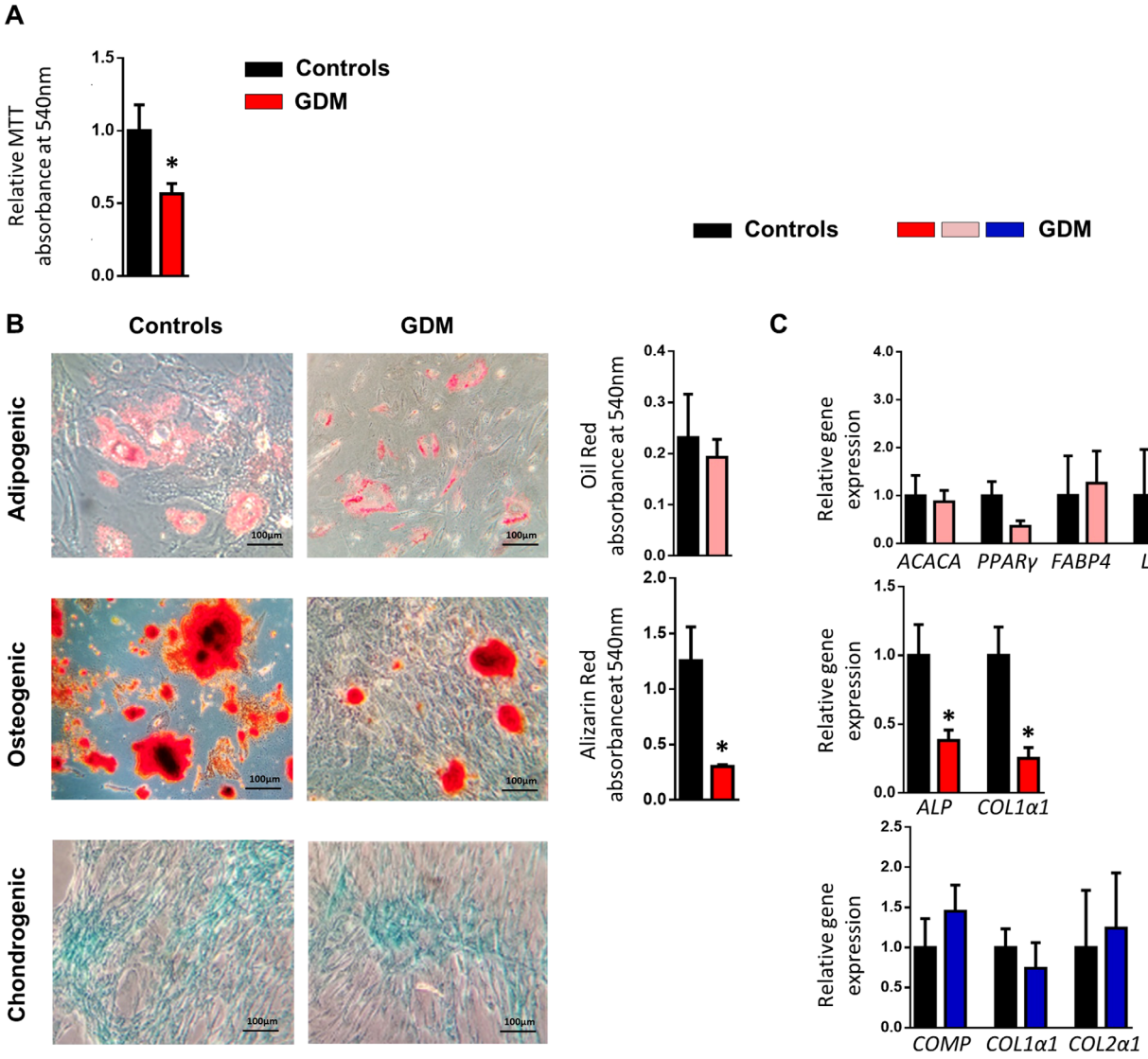


FIGURE 2 Gestational diabetes mellitus (GDM) affects the plasticity of fetal precursor cells from the amniotic membrane. A, MTT incorporation in proliferating amniotic mesenchymal stem cells (AMSCs) isolated from pregnant control and GDM women (n = 7-9 per group). B, Representative photomicrographs of AMSCs isolated from pregnant control and GDM women (n = 4 per group), differentiated into adipocytes, osteocytes, and chondrocytes and stained with Oil Red, Alizarin Red, and Alcian Blue, respectively (magnification ×200). Quantification of the differentiation capacity was assessed by extracting the staining dyes and measuring the absorbance by spectrophotometry at 540 nm. C, Gene expression of adipogenic (*ACACA*, *PPARγ*, *FABP4*, *LPL*), osteogenic (*ALP*, *COL1α1*), and chondrogenic (*COMP*, *COL1α1*, *COL2α1*) markers in AMSCs isolated from pregnant control and GDM women (n = 4 per group). In all cases, values of differentiated cells were normalized to their undifferentiated counterparts. Results are shown as mean ± SEM from independent donors experiments performed in duplicate. *P < .05 vs controls

P = .0109) (Figure 3B). At the same time, these findings are accompanied by an increase in GDM-AMSCs migratory and invasive capacities as compared to control cells (Figure 3C).

MCP-1 is a potent chemoattractant and PGE2 has been involved in the anti-inflammatory and immunosuppressive capacities of the AMSCs. According to these findings, the migration of monocytes and T-lymphocytes was significantly higher when exposed to the conditioned medium from GDM-AMSCs compared to those from control cells (Figure 3D) and this increased chemotactic capacity was significantly reversed when we treated the conditioned medium of GDM-AMSCs with an anti-MCP-1 specific antibody (Figure 3E). In addition, amniotic membrane-resident macrophages isolated from GDM mothers showed a higher expression of *MCP-1*, a gene associated with a pro-inflammatory (M1) phenotype (Figure 3F), whereas a similar but not significant trend was observed in the expression of pro-repair (M2) markers such as *MRC-1* (*CD206*), *CD163*, *CD209*, *peroxisome proliferator-activated receptor gamma* (*PPARγ*), and *IL-10* between groups (Supplementary Figure S1). Collectively, these results indicate that GDM alters the inflammatory expression profile of cells with immunological function within the amniotic membrane, and specifically modifies the immunomodulatory properties of AMSCs.

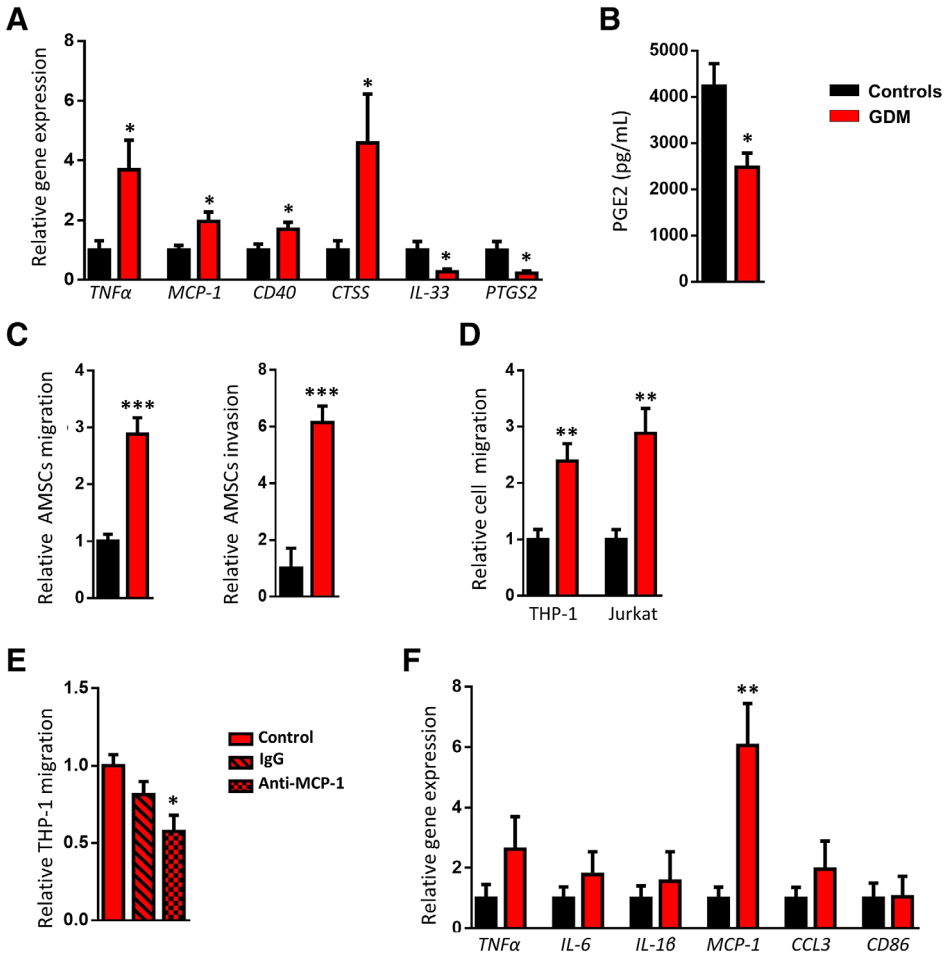


FIGURE 3 Disturbances in amniotic mesenchymal stem cells (AMSCs) and amniotic membrane-resident macrophages from placentas obtained from gestational diabetes mellitus (GDM) women. A, Gene expression analysis of the inflammatory markers *TNFα*, *MCP-1*, *CD40*, *CTSS*, *IL-33*, and *PTGS2* in AMSCs isolated from pregnant control and GDM women (n = 8 per group). B, Prostaglandin E_2 (PGE2) levels in the conditioned medium of AMSCs from pregnant control and GDM women (n = 6-7 per group). C, Migratory and invasive capacities of AMSCs isolated from pregnant control and GDM women assessed in Transwell assays (n = 7-9 per group). D, Migration of THP-1 and Jurkat cells to AMSC-conditioned medium assessed in Transwell assays (n = 5-7 per group). E, Migration of THP-1 cells to GDM-AMSC-conditioned medium after incubation with anti-MCP-1 antibody. F, Gene expression analysis of pro-inflammatory and chemotactic markers (*TNFα*, *IL-6*, *IL-1β*, *MCP-1*, *CCL3*, and *CD86*) in amniotic membrane-resident macrophages from pregnant control and GDM women (n = 6-8 per group). Results are shown as mean ± SEM from independent donor experiments performed in duplicate. **P* < .05 vs controls, ***P* < .01 vs control, ****P* < .0001 vs controls

3.4 | In vitro stimulation with GDM-like insults alters the inflammatory profile of control AMSCs

Next, we assessed whether the exposure to hyperglycemia, hyperinsulinemia, and dyslipidemia, which are typical features of the GDM metabolic environment, modified the inflammatory profile of AMSCs. We stimulated in vitro AMSCs from four control women with high levels of glucose, insulin, or palmitic acid separately and also with different combination of them for 24 hours. No significant changes in the AMSCs inflammatory gene expression profile were observed when each stimuli was applied separately nor combining glucose and insulin, but nevertheless there was a significant increase in the expression of genes involved in the inflammatory response such as *TNFα*, *MCP-1*, *CD40*, *IL-1β*, and *IL-6* when AMSCs were stimulated with the three stimuli together (Figure 4A). Moreover, AMSCs exposed to the three insults showed a significantly higher migratory capacity and an increased chemotactic activity over monocytes compared with the unstimulated ones (Figure 4B,C). Finally, in order to make sure that these results are specifically caused by the sum of the three stimuli tested and not by cell damage or death, we performed a MTT proliferation assay and found no significant differences in the proliferative capacity of the differentially stimulated AMSCs (Figure 4D). These data confirm that GDM adverse nutritional and metabolic environment alter the inflammatory profile of the amniotic membrane-derived fetal precursors.

3.5 | Biological properties of fetal precursor cells are related to maternal and infant clinical and metabolic parameters

Finally, we explored the potential relationship between maternal clinical and analytical parameters and AMSCs phenotype, and also whether their functional characteristics were associated with offspring's anthropometric and metabolic parameters. *MCP-1* expression levels were correlated with maternal biomarkers associated with adverse perinatal and long-term outcomes such as prepregnancy BMI and HOMA-IR, as well as with cord blood concentrations of triglycerides and insulin. A positive association was also observed between *CD40* and *CTSS* with GCT and prepregnancy BMI (Table 3).

Next, we analyzed the groups separately and regarding the control group, all associations observed disappear except for *CD40* and cord blood triglycerides (*r*: 0.775; *P* = .014). On the other hand, in the

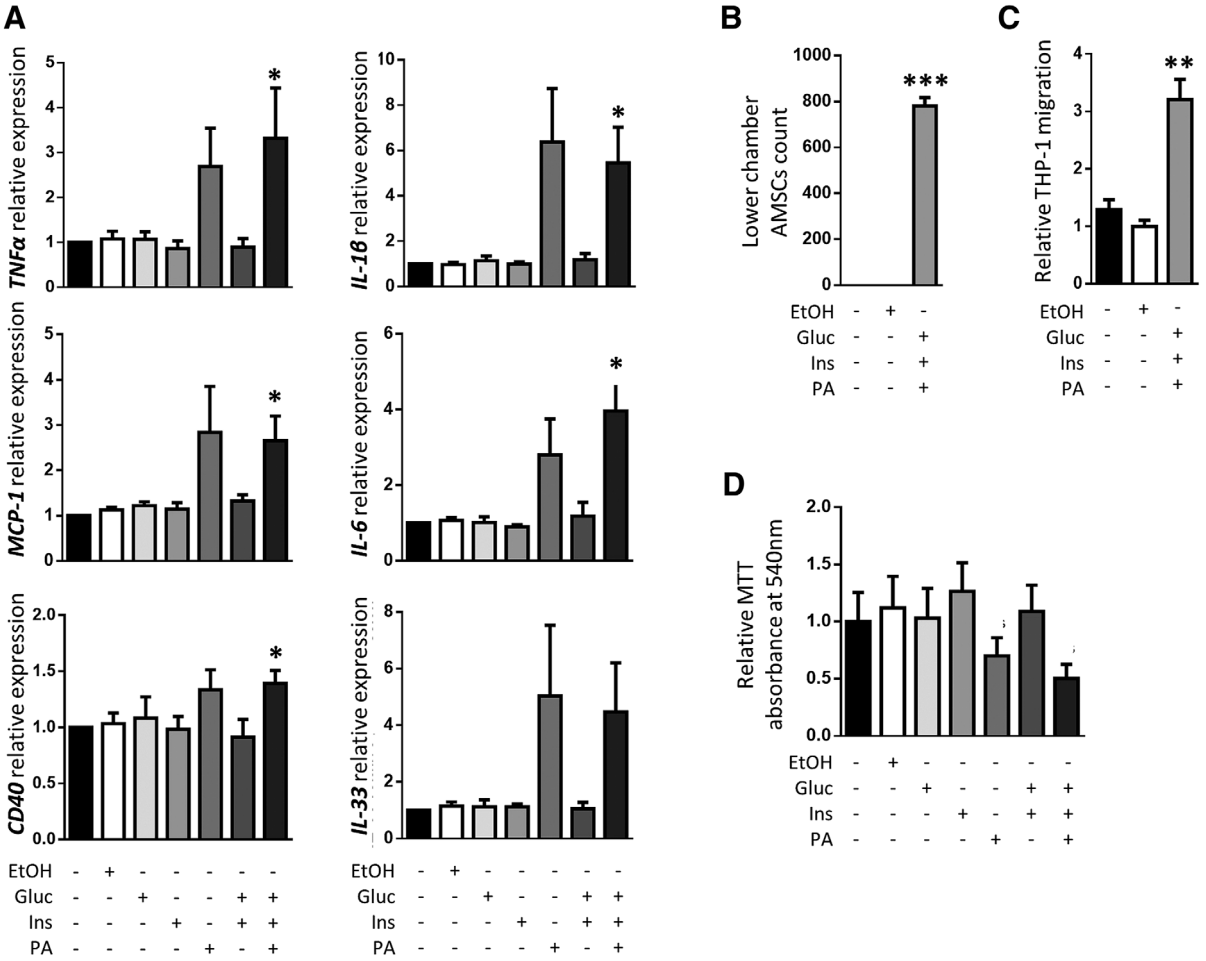


FIGURE 4 Phenotypic changes induced by a gestational diabetes mellitus (GDM)-like environment rich in glucose, insulin, and palmitic acid on amniotic mesenchymal stem cells (AMSCs) obtained from control pregnant women. A, Gene expression analysis of the inflammatory markers *TNFα*, *MCP-1*, *CD40*, *IL-1β*, *IL-6*, and *IL-33* in AMSCs isolated from control pregnant women stimulated with glucose (Gluc), insulin (Ins), and/or palmitic acid (PA; n = 4 per group). B, Migration capability of control AMSCs stimulated with glucose, insulin, and/or PA assessed by Transwell assays (n = 3 per group). C, Migration of THP-1 cells to the conditioned medium of control AMSCs stimulated with glucose, insulin, and/or PA assessed in Transwell assays (n = 4 per group). D, MTT incorporation in proliferating control AMSCs stimulated with glucose, insulin, and/or PA (n = 4 per group). **P* < .05 vs control, ***P* < .01 vs controls, ****P* < .0001 vs controls

TABLE 3 Relationship observed between maternal and offspring clinical and metabolic parameters with amniotic mesenchymal stem cells (AMSCs) phenotype

	TNFα	MCP-1	CD40	CTSS	IL-33
Prepregnancy BMI	0.057 (0.821)	0.525 (0.025)	0.528 (0.024)	0.504 (0.033)	−0.195 (0.438)
1-h 50 g challenge test	0.357 (0.159)	0.676 (0.003)	0.551 (0.022)	0.493 (0.044)	−0.232 (0.486)
Maternal glucose	−0.123 (0.688)	0.044 (0.888)	0.023 (0.940)	−0.102 (0.740)	−0.081 (0.749)
Maternal insulin	0.023 (0.941)	0.626 (0.022)	0.465 (0.108)	0.397 (0.179)	−0.003 (0.992)
Maternal HOMA-IR	−0.005 (0.988)	0.648 (0.017)	0.479 (0.098)	0.353 (0.237)	−0.007 (0.979)
Maternal Triglycerides	−0.076 (0.805)	−0.344 (0.250)	−0.300 (0.320)	−0.125 (0.685)	−0.005 (0.986)
Suprailiac skinfold	−0.168 (0.520)	0.074 (0.779)	−0.041 (0.876)	−0.064 (0.806)	0.050 (0.842)
Cord blood glucose	0.194 (0.471)	0.309 (0.244)	0.335 (0.205)	0.268 (0.316)	−0.506 (0.032)
Cord blood insulin	−0.465 (0.070)	0.517 (0.040)	0.148 (0.584)	0.158 (0.559)	0.022 (0.931)
Cord blood triglycerides	0.234 (0.382)	0.535 (0.033)	0.496 (0.051)	−0.016 (0.952)	0.032 (0.901)

GDM group, the correlations of *MCP-1* with cord blood insulin (*r*: 0.745; *P* = .021) and GCT (*r*: 0.753; *P* = .019), became stronger, whereas its association with maternal HOMA-IR (*r*: 0.638; *P* = .064) and prepregnancy BMI (*r*: 0.644; *P* = .061) followed a trend. The associations between *CD40* with maternal prepregnancy BMI (*r*: 0.683; *P* = .042) and GCT (*r*: 0.800; *P* = .010) remained significant. Overall, our data indicate that maternal metabolic phenotype during pregnancy could determine the biological characteristics of fetal precursors, which may be linked to metabolic characteristics in the offspring. These results become stronger in the context of GDM.

4 | DISCUSSION

It has been proposed that signatures of metabolic deregulation remain in cells early in development.[20] Given its location on the inner side of the placenta, the amniotic membrane is in contact with the amniotic fluid and the fetus and, as such, its stem cell component might be a good indicator of how the intrauterine environment impacts the fetus. Our results suggest that GDM modifies the plasticity of fetal precursor cells in the amniotic membrane. We show that GDM results in a deregulation of genes involved in inflammation in AMSCs, which have been associated with the development of insulin resistance, type 2 diabetes, obesity, and atherosclerosis, as well as with pro-inflammatory changes of amniotic membrane-resident macrophages. These data are supported by in vitro studies reproducing a GDM environment, in which the combination of hyperglycemia, hyperinsulinemia, and palmitic acid induced a similar phenotype in AMSCs obtained from control donors.

The novelty and the consistency of the clinical and experimental results are some of the strengths of our study. We have been able to reproduce our findings in vitro. AMSCs obtained from control women displayed an inflammatory gene expression profile similar to the pattern observed in GDM women when exposed to a GDM-like environment. Furthermore, their migration and chemotactic capacities were affected. It is important to note that all placentas were obtained from a well-characterized cohort of women scheduled for cesarean delivery in order to avoid any uncontrolled inflammatory stimuli linked to labor. Amniotic membrane samples were collected from the placental disk, halfway between the cord insertion point and the lateral edge, avoiding interregional differences. The sample size was similar to or even larger than other studies analyzing the plasticity of placental or umbilical cord mesenchymal stem cells.[6-8,21] However, it could be considered a limitation in the investigation of potential associations with newborn anthropometric variables, particularly because fetal growth is influenced not only by maternal metabolic and nutritional factors but also by the genetic potential and placental capacity to transfer nutrients. Nevertheless, *MCP-1* is associated with maternal anthropometry and with maternal and fetal metabolic parameters.

Although usually discarded following birth, placental membranes are an easily accessible and nontraumatic source of mesenchymal stem cells (MSCs),[22] and hence placental MSCs (pMSCs) are considered as a promising tool for cell-based therapy.[9] They can also shed light on how some diseases could modify intrauterine gene expression profiles. Data on the effect of GDM on pMSCs plasticity are scarce and somewhat discordant. The majority of studies have focused on chorionic[21] and umbilical cord-derived[6-8] stem cells, and whereas a decreased clonogenic potential seems to be well establish, their differentiation ability is poorly defined.[6-8,21] Kim et al observed impaired differentiation in umbilical cord MSCs obtained from GDM women as compared with controls, in contrast to other reports.[8,21] We show that AMSCs obtained from GDM women retain their multipotent characteristics, albeit with an impaired osteogenic differentiation capability. According with our findings, Chen et al reported that umbilical cord MSCs isolated from obese women exhibited a poor cell differentiation potential to osteoblasts,[23] and this was accompanied by a superior differentiation to adipocytes, which may favor adipogenesis. Therefore, our results support the hypothesis of maternal programming in GDM and identify AMSCs as new players in such a mechanism.

Previous reports showed that the conditioned medium from non-GDM AMSCs induced macrophages to become M2-like (anti-inflammatory or pro-repair) macrophages,[24] and that macrophages of the placental villous stroma (Hofbauer cells) maintained an M2-like phenotype in GDM,[25] which is consistent with the role of placental tissue in inducing fetal-maternal tolerance and for protection of fetus from low-grade inflammatory environment. Our study found that macrophages obtained from the amniotic membrane of GDM mothers presented a higher expression of *MCP-1* (and a notably trend in other pro-inflammatory genes) than those obtained from controls, suggesting they have a more pro-inflammatory profile predisposition, even in GDM women with a good metabolic control according to their third trimester HbA1c concentrations. Consistent with previous findings, the levels of PGE2, a potent immunomodulatory molecule secreted by mesenchymal stem cells deeply implied in their anti-inflammatory and immunosuppressive capacities,[26-29] are lower in the conditioned media from GDM-derived AMSCs. Likewise, these data are supported by a decreased expression of *PTGS2* in AMSCs from GDM women, a key enzyme involved in the PGE2 synthesis pathway.

A pro-inflammatory state is known to be associated with the development of insulin resistance, obesity, type 2 diabetes, and atherosclerosis.[30] *TNFα* expression is increased in placental tissue obtained from pregnancies complicated by GDM[31] and it is also released by the placenta under hyperglycemic conditions.[32] MCP-1 is a key element involved in the modulation and recruitment of macrophages, participates in the induction of fat tissue inflammation in type 2 diabetes and obesity[33] but also seems to have a pivotal role in adipogenesis.[34] We have observed an increased expression of *MCP-1* in AMSCs isolated from diabetic mothers along with an increased chemotactic capacity over monocytes and T-lymphocytes through their conditioned medium. These phenomena may participate in the onset of placental inflammation that accompanies GDM. In addition to *TNFα* and *MCP-1*, AMSCs isolated from GDM women showed an upregulation of genes involved in the inflammatory and immune response such as *CD40* and *CTSS*. Cathepsin is a cysteine protease implicated in the regulation of inflammatory activity and it has been postulated as a potential biomarker of

the development of insulin resistance and type 2 diabetes.[35] In agreement with epidemiological data, we observed an upregulation of *CTSS*, *TNFα*, and *MCP-1* in AMSCs isolated from GDM mothers, suggesting intrauterine programming/imprinting.

Less consistent is the behavior of IL-33, an interleukin involved in the maintenance of tissue homeostasis, resolution of inflammation, and repair of tissue damage.[36] IL-33 is considered to have pro- and anti-inflammatory actions and it is released after cell injury, acting as an alarm signal that alerts immune cells about tissue damage.[37,38] Our results showed a decreased *IL-33* expression in GDM-derived AMSCs whereas in vitro control AMSCs exposed to a GDM-like milieu showed an upward trend. Dalmas et al[39] observed that IL-33 was produced by islet mesenchymal cells in a diabetic milieu rich in glucose and palmitate but described a defective action in chronic obesity. Moreover, in some clinical studies IL-33 concentrations showed different behavior in health and disease,[38,39] suggesting a downregulation in some chronic inflammatory diseases such as type 2 diabetes and obesity, limiting inflammatory damage.

Our in vitro results confirm that AMSCs exposed to high concentrations of insulin, glucose and palmitic acid, simulating the hyperinsulinemia, hyperglycemia, and dyslipidemia observed in metabolic syndrome[40] but also in pregnancies complicated by diabetes,[41] reproduced the inflammatory expression profile, the migration capacity, and the chemotactic activity observed in GDM-derived AMSCs. Although we did not observe changes in the gene expression profile of control AMSCSs when exposed to insulin or glucose separately, a trend was seen when they were treated with palmitic acid. These results became evident when we treated them with a combination of the three stimuli, suggesting that saturated fatty acids were the main drivers of the metabolic activation but this effect was only significant in the context of hyperglycemia and hyperinsulinemia.

According to the fetal programming hypothesis,[42] a disturbed metabolic environment could permanently affect the health of offspring exposed and predispose them to obesity and/or type 2 diabetes. GDM has been associated with an increased risk of obesity, type 2 diabetes, and cardiovascular disease in offspring, but the underlying pathogenic mechanisms are unclear. Despite GDM's association with macrosomia and excessive fat accretion, we found similar birth weight and adiposity in GDM offspring compared with control offspring. *MCP-1* gene expression was positively correlated with both cord blood insulin and triglyceride concentrations, but not with birth weight or newborn adiposity. The lack of association between gene expression profiling and neonatal anthropometric parameters could be due, in part, to the sample size, the effect of nutritional therapy which normalizes fetal growth,[43] and/or other factors besides hyperglycemia.[44] On the other hand, we reported that maternal BMI and insulin resistance are related to gene expression of chemokines implicated in the modulation of migration and inflammation, but also involved in adipose tissue dysfunction and cardiovascular diseases. The upregulation of *MCP-1* and *CD40* by the adverse maternal environment seems to be specific to pregnancies complicated by GDM and suggests that maternal nutritional and metabolic status could induce changes in the placental gene expression profile even when they are adequately controlled. It suggests that normalizing birth weight and metabolic control is unable to revert the placental insult. Based on this evidence, we speculate that GDM environment could program stem cells and subsequently favor metabolic dysfunction later in life.

5 | CONCLUSION

For the first time, we demonstrate that maternal metabolic derangements in pregnancies complicated by GDM disturb both the phenotype and biological features of AMSC which are ultimately related to maternal and fetal nutritional and metabolic status, supporting the notion that fetal adaptive programming in the setting of GDM might have a direct impact on offspring. Our results also suggest that AMSCs might be a powerful tool for the indirect study of fetal cells in the context of hyperglycemia and insulin resistance, opening the possibility of new predictor or diagnostic approaches.

ACKNOWLEDGMENTS

We want to particularly acknowledge all the patients and volunteers involved in this study, and the BioBank IISPV (PT17/0015/0029) integrated in the Spanish National Biobanks Network for its collaboration. We also want to highlight the essential contribution of the midwives and the Gynecology and Obstetrics Service of the Joan XXIII University Hospital. This study was supported by grants from the Spanish Ministry of Economy and Competitiveness (PI 15/01562 and PI 18/00516 to A.M., SAF2015-65019-R and RTI2018-093919-B-I00 to S.F.-V., PI14/00228 and PI17/01503 to J.V.) co-financed by the European Regional Development Fund (ERDF). The Spanish Biomedical Research Center in Diabetes and Associated Metabolic Disorders (CIBERDEM) (CB07708/0012) is an initiative of the Instituto de Salud Carlos III. F.A.C. is recipient of a predoctoral fellowship from AGAUR, Spain (2017FI_B_00632). C.S. acknowledges support from the "Ramón y Cajal" program from MINECO (RYC2013-13186) and S.F.-V. the Miguel Servet tenure-track program (CP10/00438 and CPII16/00008) from the Fondo de Investigación Sanitaria, co-financed by the ERDF.

CONFLICT OF INTEREST

The authors indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

F.A.-C., E.M.-M., M.B., J.V., A.M., S.F.-V.: conception and design; F.A.-C., E.M.-M., M.B., A.G., L.M.-G., A.M.: provision of study material or patients; F.A.-C., E.M.-M., M.E., M.B., C.L., A.G., L.M.-G., C.G., R.B., A.M.: collection and/or assembly of data; F.A.-C., E.M.-M., M.E., G.L., C.L., C.S., R.B., J.V., A.M., S.F.-V.: data analysis and interpretation; F.A.-C., A.M., S.F.-V.: manuscript writing; F.A.-C., E.M.-M., M.B., J.V., A.M., S.F.-V.: final approval of manuscript; M.B., C.G., J.V., A.M., S.F.-V.: administrative support; C.S., L.M.-G.: article revision and final approval of manuscript; J.V., A.M., S.F.-V.: financial support.

DATA AVAILABILITY STATEMENT

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ORCID

Francisco Algaba-Chueca https://orcid.org/0000-0002-6537-1502
Carlos López https://orcid.org/0000-0003-1248-3065
Cristina Gutiérrez https://orcid.org/0000-0003-2626-1957
Ana Megía https://orcid.org/0000-0002-5101-9452
Sonia Fernández-Veledo https://orcid.org/0000-0003-2906-3788

REFERENCES

1. Mitanchez D, Yzydorczyk C, Siddeek B, Boubred F, Benahmed M, Simeoni U. The offspring of the diabetic mother—short- and long-term implications. *Best Pract Res Clin Obstet Gynaecol*. 2015;29:256-269.
2. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? *Physiol Rev*. 2014;94:1027-1076.
3. Jones HN, Powell TL, Jansson T. Regulation of placental nutrient transport—a review. *Placenta*. 2007;28:763-774.
4. Gauster M, Desoye G, Tötsch M, Hiden U. The placenta and gestational diabetes mellitus. *Curr Diab Rep*. 2012;12:16-23.
5. Huynh J, Dawson D, Roberts D, Bentley-Lewis R. A systematic review of placental pathology in maternal diabetes mellitus. *Placenta*. 2015;36:101-114.
6. Wajid N, Naseem R, Anwar SS, et al. The effect of gestational diabetes on proliferation capacity and viability of human umbilical cord-derived stromal cells. *Cell Tissue Bank*. 2015;16:389-397.
7. Kim J, Piao Y, Pak YK, et al. Umbilical cord mesenchymal stromal cells affected by gestational diabetes mellitus display premature aging and mitochondrial dysfunction. *Stem Cells Dev*. 2015;24:575-586.
8. Amrithraj AI, Kodali A, Nguyen L, et al. Gestational diabetes alters functions in offspring's umbilical cord cells with implications for cardiovascular health. *Endocrinology*. 2017;158:2102-2112.
9. Mamede AC, Carvalho MJ, Abrantes AM, Laranjo M, Maia CJ, Botelho MF. Amniotic membrane: from structure and functions to clinical applications. *Cell Tissue Res*. 2012;349:447-458.
10. Parolini O, Caruso M. Review: preclinical studies on placenta-derived cells and amniotic membrane: an update. *Placenta*. 2011;32:S186-S195.
11. Grupo Español de Diabetes y Embarazo (GEDE), Grupo Español de Diabetes y Embarazo. Asistencia a la gestante con diabetes. Guía de práctica clínica actualizada en 2014 | Avances en Diabetología. *Av en Diabetol*. 2015;31:45-59.
12. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes*. 1979;28:1039-1057.
13. Barbati A, Grazia Mameli M, Sidoni A, et al. Amniotic membrane: separation of amniotic mesoderm from amniotic epithelium and isolation of their respective mesenchymal stromal and epithelial cells. *Curr Protoc Stem Cell Biol*. 2012; Chapter 1:Unit 1E.8.
14. Pelekanos RA, Sardesai VS, Futrega K, Lott WB, Kuhn M, Doran MR. Isolation and expansion of mesenchymal stem/stromal cells derived from human placenta tissue. *J Vis Exp*. 2016;112:e54204.
15. Zhang X, Goncalves R, Mosser DM. The isolation and characterization of murine macrophages *Curr Protoc Immunol*. 2008; Chapter 14:Unit 14.1.
16. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8:315-317.
17. Ciuffreda MC, Malpasso G, Musarò P, et al. Protocols for in vitro differentiation of human mesenchymal stem cells into osteogenic, chondrogenic and adipogenic lineages. *Methods Mol Biol*. 2016;1416:149-158.
18. Bunnell BA, Estes BT, Guilak F, et al. differentiation of adipose stem cells. *Methods Mol Biol*. 2008;456:155-171.
19. Serena C, Keiran N, Ceperuelo-Mallafre V, et al. Obesity and type 2 diabetes alters the immune properties of human adipose derived stem cells. *Stem Cells*. 2016;34:2559-2573.
20. Catalano PM. Trying to understand gestational diabetes. *Diabet Med*. 2014;31:273-281.
21. Mathew SA, Bhonde R. Mesenchymal stromal cells isolated from gestationally diabetic human placenta exhibit insulin resistance, decreased clonogenicity and angiogenesis. *Placenta*. 2017;59:1-8.
22. Murphy S, Atala A. Amniotic fluid and placental membranes: unexpected sources of highly multipotent cells. *Semin Reprod Med*. 2013;31:062-068.
23. Chen J-R, Lazarenko OP, Blackburn ML, et al. Maternal obesity programs senescence signaling and glucose metabolism in osteoprogenitors from rat and human. *Endocrinology*. 2016;157:4172-4183.
24. Magatti M, Vertua E, De Munari S, et al. Human amnion favours tissue repair by inducing the M1-to-M2 switch and enhancing M2 macrophage features. *J Tissue Eng Regen Med*. 2017;11:2895-2911.
25. Schliefsteiner C, Peinhaupt M, Kopp S, et al. Human placental Hofbauer cells maintain an anti-inflammatory M2 phenotype despite the presence of gestational diabetes mellitus. *Front Immunol*. 2017;8:888.
26. Bouffi C, Bony C, Courties G, Jorgensen C, Noël D. IL-6-dependent PGE2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis. *PLoS One*. 2010;5:e14247.
27. Németh K, Leelahavanichkul A, Yuen PST, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E2–dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med*. 2009;15:42-49.
28. Mao Y-X, Xu J-F, Seeley EJ, et al. Adipose tissue-derived mesenchymal stem cells attenuate pulmonary infection caused by *P seudomonas aeruginosa* via inhibiting overproduction of prostaglandin E_2. *Stem Cells*. 2015;33:2331-2342.
29. Abumaree MH, Al Jumah MA, Kalionis B, et al. Human placental mesenchymal stem cells (pMSCs) play a role as immune suppressive cells by shifting macrophage differentiation from inflammatory M1 to anti-inflammatory M2 macrophages. *Stem Cell Rev Rep*. 2013;9:620-641.
30. de Luca C, Olefsky JM. Inflammation and insulin resistance. *FEBS Lett*. 2008;582:97-105.
31. Marseille-Tremblay C, Ethier-Chiasson M, Forest J-C, et al. Impact of maternal circulating cholesterol and gestational diabetes mellitus on lipid metabolism in human term placenta. *Mol Reprod Dev*. 2008;75:1054-1062.
32. Coughlan MT, Oliva K, Georgiou HM, Permezel JMH, Rice GE. Glucose-induced release of tumour necrosis factor-alpha from human placental and adipose tissues in gestational diabetes mellitus. *Diabet Med*. 2001;18:921-927.
33. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;112:1796-1808.
34. Ferland-McCollough D, Maselli D, Spinetti G, et al. MCP-1 feedback loop between adipocytes and mesenchymal stromal cells causes fat accumulation and contributes to hematopoietic stem cell rarefaction in the bone marrow of patients with diabetes. *Diabetes*. 2018;67:1380-1394.
35. Jobs E, Risérus U, Ingelsson E, et al. Serum cathepsin S is associated with decreased insulin sensitivity and the development of type 2 diabetes in a community-based cohort of elderly men. *Diabetes Care*. 2013;36:163-165.

36. Cayrol C, Girard J-P. Interleukin-33 (IL-33): a nuclear cytokine from the IL-1 family. *Immunol Rev*. 2018;281:154-168.
37. Miller AM. Role of IL-33 in inflammation and disease. *J Inflamm (Lond)*. 2011;8:22.
38. Altara R, Ghali R, Mallat Z, Cataliotti A, Booz GW, Zouein FA. Conflicting vascular and metabolic impact of the IL-33/sST2 axis. *Cardiovasc Res*. 2018;114:1578-1594.
39. Dalmas E, Lehmann FM, Dror E, et al. Interleukin-33-activated islet-resident innate lymphoid cells promote insulin secretion through myeloid cell retinoic acid production. *Immunity*. 2017;47:928-942.e7.
40. Kratz M, Coats BR, Hisert KB, et al. Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. *Cell Metab*. 2014;20:614-625.
41. Herrera E, Desoye G. Maternal and fetal lipid metabolism under normal and gestational diabetic conditions. *Horm Mol Biol Clin Investig*. 2016;26:109-127.
42. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care*. 2009;32:1076-1080.
43. Kurtzhals LL, Nørgaard SK, Secher AL, et al. The impact of restricted gestational weight gain by dietary intervention on fetal growth in women with gestational diabetes mellitus. *Diabetologia*. 2018;61:2528-2538.
44. Sacks DA, Liu AI, Wolde-Tsadik G, et al. What proportion of birth weight is attributable to maternal glucose among infants of diabetic women? *Am J Obstet Gynecol*. 2006;194:501-507.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Algaba-Chueca F, Maymó-Masip E, Ejarque M, et al. Gestational diabetes impacts fetal precursor cell responses with potential consequences for offspring. *STEM CELLS Transl Med*. 2019;1–13. https://doi.org/10.1002/sctm.19-0242

The Journal of Clinical Endocrinology & Metabolism

Advanced Lipoprotein Testing in Umbilical Cord Blood:Impact of Gestational Diabetes across Birth Weight Categories

--Manuscript Draft--

Manuscript Number:	
Article Type:	Clinical Research Article
Full Title:	Advanced Lipoprotein Testing in Umbilical Cord Blood:Impact of Gestational Diabetes across Birth Weight Categories
Short Title:	Advanced Lipoprotein Testing in Cord Blood
Corresponding Author:	Ana Megia, Ph.D.,M.D., Hospital Universitari Joan XXIII. IISPV. Universitat Rovira i Virgili. Ciberdem. Tarragona, SPAIN
Corresponding Author's Institution:	Hospital Universitari Joan XXIII. IISPV. Universitat Rovira i Virgili. Ciberdem.
Order of Authors:	Francisco Algaba-Chueca
	Elsa Maymó-Masip
	Mónica Ballesteros
	Albert Guarque
	Olga Freixes
	Núria Amigó
	Sonia Fernández-Veledo
	Joan Vendrell
	Ana Megia
Section/Category:	Diabetes, Pancreatic and Gastrointestinal Hormones
Manuscript Classifications:	Cardiovascular Endocrinology / Lipids; LDL; Triglycerides; Diabetes; Gestational diabetes
Keywords:	birth weight; gestational diabetes; lipoprotein profile; obesity; offspring; outcome

Abstract:	Context Abnormal lipid metabolism is observed in GDM and in neonates with abnormal fetal growth, however, how these alterations specifically affects the lipoprotein profile is not well understood. Objective To assess the impact of GDM on the cord blood lipoprotein profile across birth-weight categories and on offspring outcomes by using Advanced Lipoprotein Testing. Methods 74 control and 62 GDM pregnant women and their offspring. Newborns were classified according to birth-weight as small-(SGA), adequate-(AGA) or large-(LGA) for gestational age. Two-dimensional diffusion-ordered 1 H-NMR spectroscopy was used to profile serum lipoproteins. Height and weight data of the offspring up to two years was used to estimate age- and sex-specific body mass index. Results Baseline characteristics of the two groups were similar except for gestational weight gain. The size, lipid content, number and concentration of particles within their subclasses were similar between offspring born to GDM and control mothers. Using two-way analysis of variance, we observed an interaction between GDM and birth-weight categories for IDL-cholesterol content and IDL- and LDL-triglyceride content, and the number of medium VLDL and LDL particles, specifically in AGA neonates. Large and small LDL particles were independently associated with offspring obesity at two years. Conclusion GDM disturbs triglyceride and cholesterol lipoprotein content across birth categories, and AGA neonates born to GDM mothers have a profile more similar to adults with dyslipidemia and atherosclerosis than to those born to mothers with normal glucose tolerance. Cord blood lipoprotein profile emerges as a potential biomarker for future adverse outcomes in offspring.

Funding Information:	Instituto de Salud Carlos III (PI 15/01562 and PI 18/00516)	Ana Megia
	Instituto de Salud Carlos III (PI14/00228 and PI17/01503)	Joan Vendrell
	Ministerio de Economía y Competitividad (RTI2018-093919-B-I00)	Sonia Fernández-Veledo

Author Comments:	NA is a stockowner in Biosfer Teslab and has a patent for the lipoprotein profiling described in the present manuscript. The other authors report no conflicts of interest.
Suggested Reviewers:	
Opposed Reviewers:	
Additional Information:	
Question	**Response**

REPORTING GUIDELINES Does this manuscript report on the results of a clinical trial or an observational trial? If so, we encourage the authors to comply with the appropriate reporting guidelines, detailed in the author guidelines. For more information on the CONsolidated Standards of Reporting Trials (CONSORT) guidelines, please see http://www.consort-statement.org/consort-2010. For more information on the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines, please see https://www.strobe-statement.org/index.php?id=strobe-home.	No, this does not report on a clinical trial or observational study.
DATA REPOSITORIES AND DATA REGISTRATION: I have read and agree to take appropriate action to comply with the following Data Repositories and Data Registration guidelines and confirm that I have included the appropriate registration numbers / information in the text of the manuscript being submitted.	Not Applicable
CLINICAL TRIAL REGISTRATION: This study reports on a clinical trial and I provide the Clinical Trial Registration number on the title page of my manuscript as described in the Clinical Trials Registration guidelines.	Not applicable to this manuscript.
CELL LINE AUTHENTICATION: I have read and understood the Cell Line Authentication policy and describe my submission as follows:	My manuscript includes cell lines and DOES NOT meet the standards described in the Cell Line Authentication policy.
STEROID HORMONE MEASUREMENT: I have read and understood the Steroid Hormone Measurement policy and describe my submission as follows:	My manuscript includes steroid hormone assays and DOES NOT meet the standards described in the Steroid Hormone Measurement policy.

DATA AVAILABILITY: The Endocrine Society requires that authors provide a statement about the availability of data generated or analyzed in the submitted manuscript. This statement will be included in the final version of accepted manuscripts. For more information, see the Author Guidelines. Please select the statement below that describes the availability of the data generated or analyzed in your manuscript.	The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.
SPECIAL REQUESTS: In place of a cover letter, enter specific comments or requests to the editors here	We would like you to consider our work “Advanced Lipoprotein Testing in Umbilical Cord Blood: Impact of Gestational Diabetes across Birth Weight Categories” for publication as a research article in the Journal of Clinical Endocrinology and Metabolism. Gestational diabetes mellitus (GDM), which is characterized by glucose intolerance during pregnancy, affects 3–5% of all pregnancies and is a substantial health concern, with long-term and often serious consequences for both mother and offspring. In this study, we used advanced NMR-based lipoprotein profiling to identify subtle parameters of dyslipidemia in umbilical cord blood in women with normal pregnancy and GDM, across different birth-weight categories. We show that GDM disturbs triglyceride and cholesterol lipoprotein content across birth-weight categories. Of particular note, an analysis of the effect of both the birth-weight categories and GDM showed that specifically “adequate-for-gestational-age” neonates born to GDM mothers have a lipoprotein profile more similar to adults with dyslipidemia and atherosclerosis than to those born to mothers with normal glucose tolerance. Our data indicate that a normal birth weight does not equate to normal lipid metabolism. Moreover, we find that low-density lipoprotein particles are associated with obesity in offspring at two years of age. Consequently, cord blood lipoprotein profiles emerge as potential biomarkers for future adverse outcomes in offspring.

Advanced Lipoprotein Testing in Umbilical Cord Blood: Impact of Gestational Diabetes across Birth Weight Categories

Francisco Algaba-Chueca[1,2], Elsa Maymó-Masip[1,2], Mónica Ballesteros[3,5], Albert Guarque[3,5], Olga Freixes[1], Núria Amigó[2,4,5] Sonia Fernández-Veledo[1,2,5], Joan Vendrell[1,2,5*], Ana Megía[1,2,5*].

[1]Department of Endocrinology and Nutrition and Research Unit. Hospital Universitari de Tarragona Joan XXIII-Institut d'Investigació Sanitària Pere Virgili (IISPV), Tarragona, Spain.

[2]CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM)-Instituto de Salud Carlos III, Madrid, Spain.

[3]Department of Obstetrics and Gynecology. University Hospital of Tarragona Joan XXIII. Institut d'Investigació Sanitària Pere Virgili (IISPV). Tarragona. Spain.

[4] Bisofer Teslab SL, Reus, Spain

[5]Rovira i Virgili University, Tarragona, Spain

Short title: Advanced Lipoprotein Testing in Cord Blood

Key Words: birth weight, gestational diabetes, lipoprotein profile, obesity, offspring, outcome.

*Co-corresponding authors: Joan Vendrell (jvortega2002@gmail.com) and Ana Megía (ana.megia@gmail.com). Research Unit, University Hospital of Tarragona Joan XXIII,

c/ Dr. Mallafré Guasch, 4, 43007 Tarragona. Spain, Tel.: +34 977 29 58 00; Fax: +34 977 29 58 23.

Funding: This study was supported by grants from the Spanish Ministry of Economy and Competitiveness (PI 15/01562 and PI 18/00516 to AM, RTI2018-093919-B-I00 to SF-V, PI14/00228 and PI17/01503 to JV) co-financed by the European Regional Development Fund (ERDF) " A way to make Europe/Investing in your future". The Spanish Biomedical Research Center in Diabetes and Associated Metabolic Disorders (CIBERDEM) (CB07708/0012) is an initiative of the Instituto de Salud Carlos III. FAC is funded by a predoctoral fellowship from AGAUR, Spain (2017FI_B_00632). SFV acknowledges the Miguel Servet tenure-track program (CP10/00438 and CPII16/00008) from the Fondo de Investigación Sanitaria, co-financed by the ERDF.

NA is a stockowner in Biosfer Teslab and has a patent for the lipoprotein profiling described in the present manuscript. The other authors report no conflicts of interest.

ABSTRACT

Context: Abnormal lipid metabolism is observed in GDM and in neonates with abnormal fetal growth, however, how these alterations specifically affects the lipoprotein profile is not well understood.

Objective: To assess the impact of GDM on the cord blood lipoprotein profile across birth-weight categories and on offspring outcomes by using Advanced Lipoprotein Testing.

Methods: 74 control and 62 GDM pregnant women and their offspring. Newborns were classified according to birth-weight as small- (SGA), adequate- (AGA) or large- (LGA) for gestational age. Two-dimensional diffusion-ordered ^{1}H-NMR spectroscopy was used to profile serum lipoproteins. Height and weight data of the offspring up to two years was used to estimate age- and sex-specific body mass index.

Results: Baseline characteristics of the two groups were similar except for gestational weight gain. The size, lipid content, number and concentration of particles within their subclasses were similar between offspring born to GDM and control mothers. Using two-way analysis of variance, we observed an interaction between GDM and birth-weight categories for IDL-cholesterol content and IDL- and LDL-triglyceride content, and the number of medium VLDL and LDL particles, specifically in AGA neonates. Large and small LDL particles were independently associated with offspring obesity at two years.

Conclusion: GDM disturbs triglyceride and cholesterol lipoprotein content across birth categories, and AGA neonates born to GDM mothers have a profile more similar to adults with dyslipidemia and atherosclerosis than to those born to mothers with normal

glucose tolerance. Cord blood lipoprotein profile emerges as a potential biomarker for future adverse outcomes in offspring.

INTRODUCTION

Fetal growth and development is a particularly vulnerable period in life and is greatly affected by the maternal environment. Prenatal exposure to nutritional stressors has been associated with fetal programming, which can impact both metabolism and physiology and, consequently, can predispose offspring to later development of cardiovascular disease and related disorders, including obesity.[1] Maternal obesity and gestational diabetes mellitus (GDM) are associated with childhood obesity[2] and in this context, it has been proposed that cardiovascular disease can originate early in life,[3] and atherosclerosis may originate during the fetal period.[4]

Birth weight is strongly determined by neonatal fat mass, and fetal growth disorders can result from impaired maternal and fetal lipid metabolism. In fact, the levels and composition of cord blood lipids, apolipoprotein and lipoprotein are affected by both maternal and fetal factors.[5–7] Disturbed lipid profiles at birth have been described in small- and large-for-gestational-age (SGA and LGA, respectively) neonates.[7–10] When compared with adequate-for-gestational-age (AGA) peers, SGA neonates show higher levels of triglycerides, triglyceride-enriched very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), and lower levels of cholesterol.[7,10,11] By contrast, LGA neonates display higher LDL, HDL and total cholesterol levels than AGA neonates.[9]

Diabetic pregnancies are associated with a high incidence of fetal growth disorders and there is evidence that disturbances in maternal metabolism strongly contribute to these disorders.[12] Changes in cord blood lipoprotein concentrations have been reported in mothers with type 1 diabetes mellitus, including an increase in the cholesterol content of LDL and a decrease in HDL.[13,14] The situation appears more complex in GDM, with some studies showing no differences with normal glucose tolerant mothers and others

showing lower HDL- and higher VLDL- and LDL-cholesterol concentrations.[15] Additionally, qualitative changes in HDL remodeling resulting in an altered functionality have been reported in GDM neonates,[16] but this does not seem to affect newborn cholesterol metabolism in both obese and well controlled GDM mothers.[17]

Nuclear magnetic resonance (NMR)-based analysis of lipoproteins has established that the number of LDL and HDL particles is a more powerful index of cardiovascular risk than classical cholesterol determination, given the large variability in the amount of cholesterol per particle and particle size.[18] ^{1}H-NMR-based tests have also demonstrated the incomplete conversion of VLDL into LDL in diabetes, which results in a higher prevalence of VLDL and small- and dense-LDL particles.[18]

Given the heterogeneity of growth patterns and the inconclusive findings in the lipoprotein profile of cord blood in infants of GDM mothers across studies, a comprehensive characterization of the main lipoproteins, including the assessment of the size and number of particles, is necessary to identify possible alterations in fetal lipoprotein metabolism and their potential consequences for health. In the present study, we used the Liposcale test, a novel advanced lipoprotein assessment method based on 2D diffusion-ordered ^{1}H-NMR,[19] to examine for correlations between fetal growth disorders and differences in umbilical cord blood lipoprotein profile, and assess the impact of GDM on these differences and its potential association with offspring outcomes, including obesity, at two years of age.

MATERIAL AND METHODS

Study subjects

This is an observational case-control study of 136 neonates included in a prospective pre-birth cohort of control (N=74) and GDM (N=62) pregnant women undertaken at the Hospital Universitari de Tarragona Joan XXIII (Spain). This study was performed in accordance with the tenets of the Declaration of Helsinki and its protocol was reviewed and approved by the local Research Ethics Board. All participants provided informed consent before inclusion.

Neonates from women with singleton pregnancies and no major birth defects, with gestational age confirmed by ultrasound examination before 20 weeks of gestation and delivery at term, were included. Neonates with major congenital anomalies or born from women with chronic inflammatory disease were excluded.

All mothers were screened for GDM between 24–28 weeks of pregnancy following the Spanish Diabetes and Pregnancy Group recommendations.[20] Subjects with a 1-hour, 50-g glucose challenge test ≥140 mg/dL underwent a 3-hour 100-g oral glucose tolerance test. Subjects with 2 or more values above the threshold proposed by the National Diabetes Data Group[21] were considered to have GDM, whereas those with values below the threshold were classified as controls.

Clinical and demographic data

Demographic and obstetric information on participants was collected *via* an interviewer-administered questionnaire, which paid particular attention to GDM risk factors. Maternal anthropometry included height, pre-pregnancy weight and weight at the end of pregnancy. Pre-pregnancy body mass index (BMI) and gestational weight gain (GWG) were calculated according to the formulas:

$$Prepregnancy\ BMI = \frac{Prepregnancy\ Weight\ (kg)}{(Height\ (m))^2}$$

$GWG = Final\ weight - Pre\text{-}Pregnancy\ weight.$

Smoking status and laboratory data including blood lipid concentrations were collected during the third trimester of pregnancy.

Infant data included sex, gestational age, method of delivery and anthropometry. Neonatal length and weight were measured after delivery using a measuring board to the nearest 0.1 cm and a calibrated scale to the nearest 10 g. Ponderal index (PI) was calculated by the formula: $PI = \frac{Birth\ weight\ (g)}{(lenght(cm))^3}$. Suprailiac skinfold thickness was measured within the first 48 hours of life and was used to calculate fat mass percentage.[22] Neonates were classified according to gestational age- and sex-specific growth charts of the World Health Organization (WHO).[23] Infants were considered LGA (>90th percentile birth weight) or SGA (<10th percentile birth weight), and the remainder was considered AGA.

Infant growth and child BMI

Height and weight information from birth to two years of age was collected for 103 children. We defined obesity as a BMI ≥85th percentile according to age- and sex-specific BMI tables of the WHO growth standards.[23]

Umbilical cord blood collection

Umbilical cord blood was obtained immediately after delivery. Serum was immediately separated by centrifugation, divided into aliquots and stored at -80ºC until analysis.

Laboratory analysis

Serum fasting glucose, insulin, triglycerides, total and HDL-cholesterol were determined by standard enzymatic methods in maternal serum. LDL-cholesterol was

calculated using the Friedewald formula. Insulin resistance was estimated using homeostatic model assessment of insulin resistance (HOMA)-IR, as described.[24].

^{1}H-NMR spectroscopy-based cord blood lipoprotein profiling

Cord blood serum samples were analyzed using the Liposcale test (Biosfer Testlab, Reus, Spain), based on 2D diffusion-ordered ^{1}H-NMR.[19] The test provides information about size, lipid concentration (cholesterol and triglycerides) and number of particles for the main classes of lipoproteins, VLDL, LDL, intermediate-density lipoprotein (IDL) and HDL, as well as the concentration of particles within their subclasses (large, medium and small). ^{1}H-NMR spectra were recorded on a Bruker Avance III 600 spectrometer (Bruker BioSpin, Rheinstetten, Germany).

Statistical analysis

Statistical significance was set at $P<0.05$. Data were analyzed with SPSS software v20.0 (IBM, Armonk, NY). Data are presented as percentages for categorical variables, mean (SD) for normally-distributed continuous variables, and median (interquartile range) for non-normally distributed variables. Normality of the data was tested with the Kolmogorov-Smirnov test. Non-normally distributed quantitative variables were used after log10 transformation. For comparisons of proportions, differences between groups were analyzed using the chi-square test while for comparisons of normally- and non-normally-distributed quantitative variables an unpaired t-test or Mann-Whitney U test was applied. Two-way analysis of variance (ANOVA) was used to examine potential interactions between GDM and birth-weight categories, and the Bonferroni procedure for *post hoc* analyses was performed for multiple comparisons. Spearman's rank correlation coefficients were used for the analysis of the relationships between ^{1}H-NMR-assessed lipoprotein subclass profiles and maternal and offspring metabolic and

clinical variables, and the estimated significance levels (p) were adjusted by the false discovery rate (FDR) method. Logistic regression was applied to investigate the independency of the association between ^{1}H-NMR-assessed lipoprotein subclass profiles, the offspring catch-up and obesity (percentile ≥85% = 1) and the normal weight (percentile <85% = 0).

Funding was provided by the Spanish Ministry of Economy and Competitiveness (PI 15/01562 and PI 18/00516 to AM, RTI2018-093919-B-I00 to SF-V, PI14/00228 and PI17/01503 to JV) co-financed by the European Regional Development Fund (ERDF).

RESULTS

Clinical characteristics and advanced lipoprotein profile of the studied population

Clinical and metabolic characteristics of the two groups are shown in Table 1. Clinical and laboratory parameters and the ^{1}H-NMR-lipoprotein profile (Table 2) were similar between GDM and control groups with the exception of GWG, which was significantly lower in the GDM group.

One hundred and thirty-six neonates born to GDM (N=62) and control (N=74) mothers were categorized into three groups based on gestational age- and sex-weight specific charts (Table 3). As expected, there were significant differences in birth weight, percentage fat mass and PI across groups, increasing from the SGA to the LGA group. There were also differences between birth-weight groups for maternal GWG, maternal LDL-cholesterol and triglyceride concentrations, and for cord blood insulin and standard lipoprotein profile at birth.

Cholesterol content in VLDL, LDL and HDL lipoproteins was different between the three birth-weight groups, with the LGA and AGA groups showing the highest cholesterol content in VLDL and HDL lipoproteins, respectively, and the SGA group showing the lowest cholesterol content in LDL and HDL lipoproteins. Regarding triglyceride content, the SGA and AGA groups showed higher VLDL and HDL enrichment, respectively, than the other two groups. No differences were observed in LDL lipoproteins (Table 4)

Lipoprotein particle size and number was also different across the three groups. The number of VLDL-particles (VLDL-P) was highest in the SGA group and lowest in the LGA group, and was consistent with the differences observed among particle sizes (large, medium and small VLDL-P). The number of LDL-P was lower in the SGA group than in the AGA and LGA groups, and the same distribution was observed for large and small LDL-P. The number of HDL-P was highest in the AGA group and showed an inverse U distribution when compared with the LGA and SGA groups. This phenomenon was observed specifically for small size particles (Table 4).

GDM alters the cord blood lipoprotein profile across birth weight categories

While the cord blood lipoprotein profile was similar in offspring born to GDM and control mothers, we found some interactions when we assessed the effect of both the birth-weight categories and GDM. AGA neonates born to GDM mothers had higher IDL-cholesterol and -triglyceride content and LDL-triglyceride content than the SGA and LGA groups, whereas those of control mothers had lower concentrations. The same pattern was observed with medium VLDL-P and LDL-P, which followed an inversed U distribution (Figure 1).

We next examined the relationship between standard lipid analysis or ^{1}H-NMR-lipoprotein profiles and maternal and neonatal outcomes separately in both the GDM and control groups. Maternal age, pre-pregnancy BMI, gestational weight gain, maternal glucose concentrations and HOMA-IR were unrelated to offspring standard lipid and ^{1}H-NMR-lipoprotein profiles in both groups (Figure 2). Only maternal LDL-cholesterol was negatively associated with VLDL-P, VLDL-cholesterol content and IDL-triglyceride content. By contrast, gestational age was positively associated with cord blood standard triglyceride concentrations and ^{1}H-NMR-assessed triglyceride-rich lipoproteins. Some differences between groups emerged when we examined the relationship between cord blood insulin, birth weight and neonatal adiposity (assessed by fat mass percentage and PI) and cord blood lipid and lipoprotein profile. Standard total and LDL-cholesterol concentrations were negatively associated with birth weight in the GDM group, while no association was observed in the control group. Cord blood insulin was strongly and positively associated with LDL-P and LDL-cholesterol content in the control group, while a negative relationship was observed with VLDL-P and VLDL-triglyceride content in GDM offspring. In the control group, neonatal adiposity was negatively correlated with VLDL lipoproteins, VLDL-P, and IDL-triglyceride content, and positively associated with LDL-cholesterol content, LDL-P and HDL-P. The associations were weaker in the GDM group, with neonatal adiposity negatively associated with triglyceride-rich lipoproteins and with VLDL-P and its subfractions.

Cord blood ^{1}H-NMR-lipoprotein profile is associated with overweight and obesity at 24 months

To assess whether cord blood lipoprotein profile could be used as a biomarker for offspring outcomes, we explored its potential association with obesity at 2 years of life in a subset of participants. Of the 103 children studied, 78 were normal weight and 25

were obese. Obese children were born to women with higher BMI, had higher birth weight and were more exposed to GDM during the intrauterine life compared with normal weight children. They also had a higher number of cord blood small (352.00±28.86 vs 325.60±50.69 nmol/L; P=0.019) and large (98.10±11.24 vs 91.37±12.91 nmol/L; P=0.022) LDL-P. No other differences were observed between the two groups. To further assess the independence of these associations, we performed logistic regression analysis. We found that both small and large LDL-P were associated with infant obesity at two years when we adjusted for potential confounders including GDM, birth-weight categories, pre-pregnancy BMI categorized as normal weight (<25 kg/m^2), overweight (25 to <30 kg/m^2) or obese (≥30kg/m^2), weight gain during pregnancy and sex (Table 5).

DISCUSSION

Both GDM and abnormal growth patterns have been associated with long-term adverse outcomes in offspring, and changes in the lipoprotein composition have been proposed as potential markers of cardiovascular diseases later in life. To the best of our knowledge, no previous study has assessed the association between GDM and extreme fetal growth categories using advanced umbilical cord blood lipoprotein profiling. We demonstrate that GDM modifies the umbilical cord blood lipoprotein profile in AGA neonates, in particular IDL-lipoproteins, triglyceride content in LDL and medium-size VLDL-P and LDL-P. By contrast, GDM offspring in the LGA and SGA groups have a phenotype more similar to controls. In addition, we show that both cord blood large and small LDL-P, known to be associated with atherosclerosis development, are predictors of later obesity in the offspring.

Both under- and overnutrition *in utero* affects the lipoprotein profile of neonates.[4-7] SGA neonates are reported to exhibit high cord blood triglyceride concentrations,[7,10,25] high VLDL and IDL concentrations and low HDL concentrations when compared with equivalent AGA neonates.[11] Some of these findings have also been reported in fetal macrosomia[7,9] and in GDM pregnancies.[26] However, similar to our present results, standard lipid profiling fails to show differences between offspring born to control and GDM mothers.[27] We found that the interaction between GDM and fetal growth categories was evident only when ^{1}H-NMR-based lipoprotein profiling was performed. This methodology revealed that AGA neonates have a disturbed cholesterol and triglyceride lipoprotein metabolism, suggesting a decrease in the clearance of IDL lipoproteins in the liver by receptor-mediated endocytosis, leading to an increase in their half-life and allowing excessive transfer of triglycerides to LDL and the further action of hepatic lipase forming cholesterol-poor LDL particles.[28] This state is similar to dyslipidemia associated with diabetes and insulin resistant states, where an increased generation of IDL, small and dense LDL particles and triglyceride-enriched HDL particles is observed,[28] a pattern associated with increased atherogenic risk. These findings allow us to hypothesize that postnatal insulin resistance, which has been described in offspring of GDM women, may be programmed *in utero* and would be present even in AGA neonates, suggesting that GDM treatment may be unable to prevent long-term complications.

Previous studies exploring the potential relationship between prenatal lipid metabolism and adverse metabolic outcomes in offspring have generated inconsistent results.[29–34] In accordance with other reports,[35,36] we confirm that GDM, pre-pregnancy BMI and gestational weight gain during pregnancy are all associated with offspring obesity at early stages of life. We also found independent associations between small and large

LDL-P with outcomes in offspring even when other confounders were considered.[37] Given that disturbances in lipoprotein composition and concentration at birth have been shown to persist after one month,[38] and that an altered fetal lipoprotein profile is associated with aorta intima thickness in SGA and LGA neonates,[9,25] our findings support the notion that disturbances in the lipoprotein metabolism may have lasting effects.

Lipoproteins are complex particles containing proteins, cholesterol and phospholipids. Standard lipid panel typically provides information on cholesterol content from all circulating particles, but it lacks data on the protein component and lipoprotein particle size and number. Similar to what is seen in diabetic dyslipidemia, where standard lipid panels seem to be insufficient to predict cardiovascular diseases,[18] our findings suggest that an extensive characterization of cord blood lipoproteins could provide more accurate information on the regulation of lipoprotein metabolism in fetal life and its potential implications for metabolism in later life. Despite having a normal weight at birth, AGA neonates born to GDM mothers show a disturbed lipoprotein metabolism, which provides a pathophysiological explanation for the increased risk for developing metabolic disorders. We were unable to find an association between the high IDL lipoprotein concentrations and outcomes at two years, whereas a GDM-independent association was observed with LDL-P.

Unanswered question from our study include how GDM AGA offspring show a well-differentiated lipoprotein pattern compared with control neonates, and whether this is associated with atherogenic parameters at birth. Long-term studies might confirm whether cord blood ^{1}H-NMR-based lipoprotein profiling can be implemented as a biomarker of later metabolic diseases beyond two years of age.

As in any observational study, we cannot clarify whether the observed associations are causal effects or a result of unmeasured residual confounding. Nevertheless, the main prenatal factors were addressed and the groups were matched for maternal BMI and birth-weight categories. The potential loss of follow-up in children can also be considered a limitation. However, our study population did not substantially differ from excluded mother-child pairs in the main baseline outcomes, so we believe that it should not limit our results. The strengths of this study include a longitudinal birth cohort with almost complete maternal data that establish a temporal relationship between the outcome and exposure to GDM. The novelty of the lipoprotein assessment, which allows us to identify different fetal metabolic behaviors, is also a major strength. We are aware, however, that the SGA and LGA groups were overrepresented and further population-based studies are needed to determine the role of lipoprotein composition and subfractions in the pathogenesis of metabolic diseases in offspring.

CONCLUSION

GDM disturbs triglyceride and cholesterol lipoprotein concentrations across birth categories, with GDM AGA neonates showing a profile more similar to adults with dyslipidemia and atherosclerosis than those born to normal glucose tolerant mothers. Moreover, an altered fetal lipoprotein pattern is associated with obesity development at 2 years. Overall, these findings suggest that the fetal lipoprotein profile might be an early biomarker of development of later diseases.

Acknowledgements:

We want to particularly acknowledge all the patients and volunteers involved in this study for their collaboration. We also acknowledge the BioBank IISPV (PT17/0015/0029) integrated into the Spanish National Biobanks Network. Finally, we wish to highlight the essential contribution of the midwives and the Gynecology and Obstetrics Service of the Joan XXIII University Hospital.

Data availability:

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

.

REFERENCES:

1. Barker DJ. The fetal and infant origins of disease. *Eur J Clin Invest*. 1995;25(7):457-463.

2. Zhao P, Liu E, Qiao Y, et al. Maternal gestational diabetes and childhood obesity at age 9–11: results of a multinational study. *Diabetologia*. 2016;59(11):2339-2348. doi:10.1007/s00125-016-4062-9

3. Bao W, Srinivasan SR, Wattigney WA, Bao W, Berenson GS. Usefulness of Childhood Low-Density Lipoprotein Cholesterol Level in Predicting Adult Dyslipidemia and Other Cardiovascular Risks. *Arch Intern Med*. 1996;156(12):1315-1320. doi:10.1001/archinte.1996.00440110083011

4. Cohen MS. Fetal and childhood onset of adult cardiovascular diseases. *Pediatr Clin North Am*. 2004;51(6):1697-1719. doi:10.1016/j.pcl.2004.08.001

5. Loughrey CM, Rimm E, Heiss G, Rifai N. Race and gender differences in cord blood lipoproteins. *Atherosclerosis*. 2000;148(1):57-65. doi:10.1016/s0021-9150(99)00238-5

6. Lane DM, McConathy WJ. Factors Affecting the Lipid and Apolipoprotein Levels of Cord Sera. *Pediatr Res*. 1983;17(2):83-91. doi:10.1203/00006450-198302000-00001

7. Aletayeb SMH, Dehdashtian M, Aminzadeh M, et al. Correlation between umbilical cord blood lipid profile and neonatal birth weight. *Pediatr Pol*. 2013;88(6):521-525. doi:10.1016/J.PEPO.2013.08.004

8. Kumar A, Gupta A, Malhotra VK, Agarwal PS, Thirupuram S, Gaind B. Cord blood lipid levels in low birth weight newborns. *Indian Pediatr*. 1989;26(6):571-574.

9. Koklu E, Akcakus M, Kurtoglu S, et al. Aortic intima-media thickness and lipid profile in macrosomic newborns. *Eur J Pediatr*. 2007;166(4):333-338. doi:10.1007/s00431-006-0243-8

10. Miranda J, Simões R V., Paules C, et al. Metabolic profiling and targeted lipidomics reveals a disturbed lipid profile in mothers and fetuses with intrauterine growth restriction. *Sci Rep*. 2018;8(1):13614. doi:10.1038/s41598-018-31832-5

11. Kim S-M, Lee SM, Kim S-J, et al. Cord and maternal sera from small neonates share dysfunctional lipoproteins with proatherogenic properties: Evidence for Barker's hypothesis. *J Clin Lipidol*. 2017;11(6):1318-1328.e3. doi:10.1016/j.jacl.2017.08.020

12. Herrera E, Desoye G. Maternal and fetal lipid metabolism under normal and gestational diabetic conditions. *Horm Mol Biol Clin Investig*. 2016;26(2):109-127. doi:10.1515/hmbci-2015-0025

13. Fordyce MK, Duncan R, Chao R, et al. Cord blood serum in newborns of diabetic mothers. *J Chronic Dis*. 1983;36(3):263-268. doi:10.1016/0021-9681(83)90061-9

14. Kilby MD, Neary RH, Mackness MI, Durrington PN. Fetal and Maternal

Lipoprotein Metabolism in Human Pregnancy Complicated by Type I Diabetes Mellitus [1]. *J Clin Endocrinol Metab*. 1998;83(5):1736-1741. doi:10.1210/jcem.83.5.4783

15. Chan TC, Schwartz JJ, Garcia RE, Chin HP, Barndt R. Total serum cholesterol and plasma lipoprotein cholesterol concentrations in cord sera of newborns from hispanic mothers with gestational diabetes. *Artery*. 1988;15(4):203-216.

16. Sreckovic I, Birner-Gruenberger R, Besenboeck C, et al. Gestational diabetes mellitus modulates neonatal high-density lipoprotein composition and its functional heterogeneity. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2014;1841(11):1619-1627. doi:10.1016/j.bbalip.2014.07.021

17. Miettinen HE, Rönö K, Koivusalo SB, Eriksson JG, Gylling H. Effect of gestational diabetes mellitus on newborn cholesterol metabolism. *Atherosclerosis*. 2018;275:346-351. doi:10.1016/j.atherosclerosis.2018.06.879

18. Mallol R, Rodriguez MA, Brezmes J, Masana L, Correig X. Human serum/plasma lipoprotein analysis by NMR: Application to the study of diabetic dyslipidemia. *Prog Nucl Magn Reson Spectrosc*. 2013;70:1-24. doi:10.1016/j.pnmrs.2012.09.001

19. Mallol R, Amigó N, Rodríguez MA, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. *J Lipid Res*. 2015;56(3):737-746. doi:10.1194/jlr.D050120

20. Grupo Español de Diabetes y Embarazo (GEDE), Grupo Español de Diabetes y Embarazo. Asistencia a la gestante con diabetes. Guía de práctica clínica actualizada en 2014 | Avances en Diabetología. *Av en Diabetol*. 2015;31(2):45-59. doi:10.1016/j.avdiab.2014.12.001

21. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998;15(7):539-553. doi:10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S

22. Catalano PM, Thomas AJ, Avallone DA, Amini SB. Anthropometric estimation of neonatal body composition. *Am J Obs Gynecol*. 1995;173:1176-1181.

23. WHO Multicentre Growth Reference Study Group. *WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weightfor- Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development*. World Health Organization; 2006.

24. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412-419.

25. Koklu E, Kurtoglu S, Akcakus M, et al. Increased Aortic Intima-Media Thickness Is Related to Lipid Profile in Newborns with Intrauterine Growth Restriction. *Horm Res Paediatr*. 2006;65(6):269-275. doi:10.1159/000092536

26. Schaefer-Graf UM, Graf K, Kulbacka I, et al. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational

diabetes mellitus. *Diabetes Care*. 2008;31(9):1858-1863. doi:10.2337/dc08-0039

27. Schaefer-Graf UM, Meitzner K, Ortega-Senovilla H, 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):1053-1059. doi:10.1111/j.1464-5491.2011.03346.x

28. Hassing HC, Surendran RP, Mooij HL, Stroes ES, Nieuwdorp M, Dallinga-Thie GM. Pathophysiology of hypertriglyceridemia. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2012;1821(5):826-832. doi:10.1016/j.bbalip.2011.11.010

29. Shokry E, Marchioro L, Uhl O, et al. Transgenerational cycle of obesity and diabetes: investigating possible metabolic precursors in cord blood from the PREOBE study. *Acta Diabetol*. 2019;56(9):1073-1082. doi:10.1007/s00592-019-01349-y

30. Simpson J, Smith AD, Fraser A, et al. Cord Blood Adipokines and Lipids and Adolescent Nonalcoholic Fatty Liver Disease. *J Clin Endocrinol Metab*. 2016;101(12):4661-4668. doi:10.1210/jc.2016-2604

31. Hellmuth C, Uhl O, Standl M, et al. Cord Blood Metabolome Is Highly Associated with Birth Weight, but Less Predictive for Later Weight Development. *Obes Facts*. 2017;10(2):85-100. doi:10.1159/000453001

32. Stratakis N, Gielen M, Margetaki K, et al. Polyunsaturated fatty acid status at birth, childhood growth, and cardiometabolic risk: a pooled analysis of the MEFAB and RHEA cohorts. *Eur J Clin Nutr*. May 2018. doi:10.1038/s41430-018-0175-1

33. Standl M, Thiering E, Demmelmair H, Koletzko B, Heinrich J. Age-dependent effects of cord blood long-chain PUFA composition on BMI during the first 10 years of life. *Br J Nutr*. 2014;111(11):2024-2031. doi:10.1017/S0007114514000105

34. Donahue SMA, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. *Am J Clin Nutr*. 2011;93(4):780-788. doi:10.3945/ajcn.110.005801

35. Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles M-A, Pettitt DJ. Childhood Obesity and Metabolic Imprinting. *Diabetes Care*. 2007;30:2287-2292. doi:10.2337/dc06-2361.Additional

36. Voerman E, Santos S, Patro Golab B, et al. Maternal body mass index, gestational weight gain, and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. *PLoS Med*. 2019;16(2):e1002744. doi:10.1371/journal.pmed.1002744

37. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation*. 2009;119(7):931-939. doi:10.1161/CIRCULATIONAHA.108.816181

38. Merzouk H, Madani S, Prost J, Loukidi B, Meghelli-Bouchenak M, Belleville J.

Changes in serum lipid and lipoprotein concentrations and compositions at birth and after 1 month of life in macrosomic infants of insulin-dependent diabetic mothers. *Eur J Pediatr*. 1999;158(9):750-756. doi:10.1007/s004310051194

554 **Figure 1. Differences in ^{1}H-NMR-assessed lipoprotein pattern among growth**
555 **groups in GDM and control mothers.** Statistical analysis: two-way analysis of
556 variance. Data shown as mean±SD. AGA: adequate for gestational age, LGA: large for
557 gestational age, SGA: small for gestational age. GDM: gestational diabetes.

558

559 **Figure 2. Heat map of the associations between ^{1}H-NMR-assessed lipoprotein**
560 **subclass profiles and maternal and neonatal clinical variables in control (left**
561 **panel) and GDM (right panel) mothers.** % Fat Mass: percentage of fat mass, HDL:
562 high-density lipoprotein; LDL low-density lipoprotein; VLDL: very-low-density
563 lipoprotein; VLDL-C: cholesterol content in VLDL; VLDL-TG: triglyceride content in
564 VLDL; VLDL-P: VLDL particles; LDL-C: cholesterol content in LDL; LDL-TG:
565 triglyceride content in LDL; LDL-P: LDL particles; HDL-C: cholesterol content in
566 HDL; HDL-TG: triglyceride content in HDL; HDL-P: HDL particles; pre pregnancy
567 BMI: pregestational body mass index; HOMA-IR: homeostatic model assessment
568 Insulin Resistance. Spearman Correlation coefficients. *Indicates significant
569 associations after false discovery rate adjustment.

570
571
572
573
574
575
576
577
578
579
580

Table 1. Mother and neonate clinical characteristics according to glucose tolerance status

	Control	GDM	P-value
Maternal age (years)	32.5±5.4	33.5±4.3	0.257
Pregestational BMI (kg/m^2)	25.5±5.2	26.6±5.1	0.207
Gestational Weight Gain (kg)	12.3±6.2	8.6±4.9	<0.001
Smoking, n (%)	14 (18.9)	8 (12.9)	0.363
M cholesterol (mg/dL)	244.5±38.5	233.1±40.6	0.868
M HDL cholesterol (mg/dL)	77.50 (62.25-89.00)	70.30 (62.00-81.75)	0.050
M LDL cholesterol (mg/dL)	120.81±54	122.30±35.71	0.329
M triglycerides (mg/dL)	183.3±74.8	188.6±59.1	0.677
HOMA-IR	2.02 (1.19-3.26)	2.93 (1.66-4.22)	0.052
Gestational age (weeks)	39 (38-40)	39 (38-40)	0.749
Birth weight (g)	3258.5±602.5	3309.9±697.2	0.645
Fat mass (%)	11.74±4.31	11.94±3.84	0.789
Ponderal Index (g/cm^3)	2.74±0.30	2.72±0.26	0.633
Cb Insulin (mcUI/mL)	4.46 (2.08-8.04)	6.33 (2.94-12.09)	0.058
Cb Cholesterol (mg/dL)	59.00 (52.75-78.75)	63.50 (53.75-73.75)	0.772
Cb HDL-cholesterol (mg/dL)	25.00 (21.25-28.75)	24.50 (20.00-29.50)	0.634
Cb LDL-cholesterol (mg/dL)	26.70 (23.00-37.15)	30.36 (24.25-35.45)	0.707
Cb Triglycerides (mg/dL)	37.50 (26.50-52.75)	40.00 (26.25-60.00)	0.542

Data presented: mean±SD and median (IQR, 25–75), for parametric and nonparametric variables, respectively. Differences between variables: t-test and the Mann-Whitney U test as required. M=maternal blood, Cb=cord blood. BMI: body mass index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; LDL: Low density lipoproteins; HDL: High-density lipoproteins,

Table 2. Mother and neonate clinical characteristics according to birth-weight groups

	SGA	AGA	LGA	P-value
Maternal age (years)	31.6±4.6	33.91±5.0	32.8±5.0	0.102
Pregestational BMI (kg/m^2)	25.3±4.2	25.9±5.3	26.7±5.7	0.445
Gestational Weight Gain (kg)	8.6±5.0	10.0±5.3	12.9±6.3♯	0.002
Smoking, n (%)	11 (28.2)	5 (10.6)	6 (12)	0.053
M cholesterol (mg/dL)	243.7±35.2	235.0±46.4	241.9±35.4	0.589
M HDL cholesterol (mg/dL)	74.95 (65.63-88.25)	74.00 (63.25-91.00)	76.50 (59.00-83.25)	0.831
M LDL cholesterol (mg/dL)	130.56±36.04♯	115.02±35.50	96.64±32.46	0.015
M triglycerides (mg/dL)	166.3±57.1*	210.6±73.2✗	174.0±64.9	0.007
HOMA-IR	1.7 (1.2-3.2)	2.2 (1.4-4.5)	2.4 (1.6-3.4)	0.272
Gestational age (weeks)	39 (39-40)	39 (38-40)	39 (38-40)	0.599
Birth weight (g)	2595.8±279.3*	3268.4±269.8✗	3929.0±250.8♯	<0.001
Fat mass (%)	6.8±3.6*	11.6±2.4✗	12.0±2.0	<0.001
Ponderal Index (g/cm^3)	2.5±0.2*	2.8±0.3✗	2.9±0.2♯	<0.001
Cb insulin (mcUI(mL)	2.5 (1.1-4.3)	4.3 (2.2-4.7)	8.2 (6.2-14.1)♯	<0.001
Cb cholesterol (mg/dL)	80.00 (57.50-99.00)	63.00 (54.00-75.00)	56.00 (51.00-63.00)	0.005
Cb HDL-cholesterol (mg/dL)	27.00 (18.50-34.00)	25.00 (22.00-28.00)	23.00 (18.00-27.70)	0.133
Cb LDL-cholesterol (mg/dL)	37.40 (24.00-52.10)	29.80 (23.40-36.90)	24.60 (22.40-30.40)	0.027
Cb triglycerides (mg/dL)	37.40 (24.00-34.00)	40.00 (27.00-59.00)	34.00 (23.00-44.00)	0.008

Data presented: mean±SD and median (IQR, 25–75), for parametric and nonparametric variables, respectively. Two-way analysis of variance (ANOVA) and the Bonferroni procedure for *post hoc* analyses; *P<0.05 between SGA and AGA; ♯p<0.05 between SGA and LGA; ✗p<0.05 between AGA and LGA. M=maternal blood; Cb=cord blood. BMI: body mass index, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; LDL: Low density lipoproteins; HDL: High-density lipoproteins,

611 **Table 3.** ^{1}H-NMR-Lipoprotein content and particle profile according to gestational diabetes.

612

	Control	GDM	p
VLDL-cholesterol (mg/dL)	7.2±3.3	7.6±4.0	0.495
IDL-cholesterol (mg/dL)	4.6±1.6	5.0±2.0	0.291
LDL-cholesterol (mg/dL)	70.1±10.1	70.6±8.7	0.767
HDL-cholesterol (mg/dL)	40.9±8.8	40.6±8.1	0.827
VLDL-triglycerides (mg/dL)	29.4±8.6	30.7±9.9	0.415
IDL-triglycerides (mg/dL)	4.4±1.3	4.7±1.8	0.335
LDL-triglycerides (mg/dL)	4.5±1.8	4.4±1.9	0.925
HDL-triglycerides (mg/dL)	7.9±3.7	8.6±3.9	0.251
VLDL-P (nmol/L)	23.1±6.3	24.0±7.5	0.416
Large VLDL-P (nmol/L)	0.6 (0.5-0.8)	0.6 (0.5-0.8)	0.848
Medium VLDL-P (nmol/L)	1.1 (0.6-2.0)	1.1 (0.7-2.4)	0.471
Small VLDL-P (nmol/L)	21.0±5.1	21.8±6.3	0.408
LDL-P (nmol/L)	484.9±71.7	488.0±62.0	0.79
Large LDL-P (nmol/L)	92.5±13.0	93.1±11.0	0.782
Medium LDL-P (nmol/L)	61.9±39.3	62.1±36.9	0.973
Small LDL-P (nmol/L)	330.4±50.1	332.7±42.1	0.775
HDL-P (nmol/L)	17.8±4.2	17.4±4.1	0.591
Large HDL-P (nmol/L)	0.3±0.1	0.3±0.1	0.107
Medium HDL-P (nmol/L)	9.7±1.5	9.9±1.4	0.257
Small HDL-P (nmol/L)	7.8±3.8	7.1±3.9	0.306

613 Data presented: mean±SD and median (IQR, 25–75), for parametric and nonparametric
614 variables, respectively. Differences between variables: t-test and the Mann-Whitney U test as
615 required.VLDL: Very low-density lipoproteins; IDL: Intermediate-density lipoproteins; LDL:
616 Low density lipoproteins; HDL: High-density lipoproteins; VLDL-P: VLDL particles, IDL-P:
617 IDL particles; LDL-P: LDL particles; HDL-P: HDL particles.

618
619
620
621
622
623

Table 4. Cord blood ^{1}H-NMR-lipoprotein content and particle profile according to birth weight categories.

	SGA	AGA	LGA	P-value
VLDL-cholesterol (nmol/L)	8.49±3.61	8.06±3.45$^{✗}$	5.80±3.28$^{♯}$	0.001
IDL-cholesterol (nmol/L)	5.07±1.41	4.84±2.04	4.52±1.73	0.353
LDL-cholesterol (nmol/L)	66.27±11.34*	72.07±9.48	71.95±7.05$^{♯}$	0.005
HDL-cholesterol (nmol/L)	37.29±6.56*	44.16±8.69$^{✗}$	40.11±8.47	<0.001
VLDL-triglycerides (nmol/L)	34.10±10.07*	29.02±8.22	27.53±8.28$^{♯}$	0.002
IDL-triglycerides (nmol/L)	4.65±1.33	4.81±1.67	4.14±1.57	0.092
LDL-triglycerides (nmol/L)	4.48±1.66	4.55±2.05	4.30±1.74	0.797
HDL-triglycerides (nmol/L)	7.40±3.57*	10.11±3.19$^{✗}$	6.96±3.76	0.000
VLDL-P (nmol/L)	26.12±7.09	23.63±6.06	21.20±6.63$^{♯}$	0.003
Large VLDL-P (nmol/L)	0.81±0.30	0.67±0.26	0.59±0.26$^{♯}$	0.001
Medium VLDL-P (nmol/L)	1.97 (0.91-2.71)*	0.70 (0.53-1.50)	1.09 (0.64-1.50)$^{♯}$	0.001
Small VLDL-P (nmol/L)	23.20±5.71	21.68±4.82	19.35±5.84$^{♯}$	0.005
LDL-P (nmol/L)	455.67±79.74	504.03±62.62	493.80±50.75$^{♯}$	0.002
Large LDL-P (nmol/L)	86.16±12.56	94.12±10.59	97.00±10.85$^{♯}$	<0.001
Medium LDL-P (nmol/L)	67.45±32.05	59.00±44.62	60.55±35.68	0.555
Small LDL-P (nmol/L)	302.07±48.48	350.91±37.44	336.91±41.25$^{♯}$	<0.001
HDL-P (nmol/L)	15.83±3.72*	19.91±4.04$^{✗}$	16.74±3.48	<0.001
Large HDL-P (nmol/L)	0.35±0.08*	0.33±0.06	0.33±0.07	0.374
Medium HDL-P (nmol/L)	9.45±1.15	9.96±1.39	9.90±1.62	0.194
Small HDL-P (nmol/L)	6.03±3.92*	9.61±3.61$^{✗}$	6.51±3.02	<0.001

Data presented: mean±SD and median (IQR, 25-75) for parametric and nonparametric variables, respectively. Two-way analysis of variance (ANOVA) and the Bonferroni procedure for *post hoc* analyses. SGA: small for gestational age, AGA: adequate for gestational age; LGA: large for gestational age; VLDL: Very low-density lipoproteins; IDL: Intermediate-density lipoproteins; LDL: Low density lipoproteins; HDL: High-density lipoproteins; VLDL-P: VLDL particles, IDL-P: IDL particles; LDL-P: LDL particles; HDL-P: HDL particles. *P<0.05 between SGA and AGA; ♯p<0.05 between SGA and LGA; ✗p<0.05 between AGA and LGA.

637 **Table 5.** Independent association of large and small LDL particles with the
638 development of child obesity at two years of age.

	Model R^2	Exp (B)	95% CI for exp (B)	P-value
Large LDL-P* (nmol/L)	0.223	1.052	1.00-1.107	0.049
Small LDL-P* (nmol/L)	0.238	1.018	1.003-1.033	0.021

639 Statistical Analysis: Logistic regression analysis. *Adjusted for GDM, weight for gestational
640 age categories, sex, BMI and gestational weight gain. LDL-P: LDL particles. CI: confidence
641 interval.

642
643
644
645

Figure 1

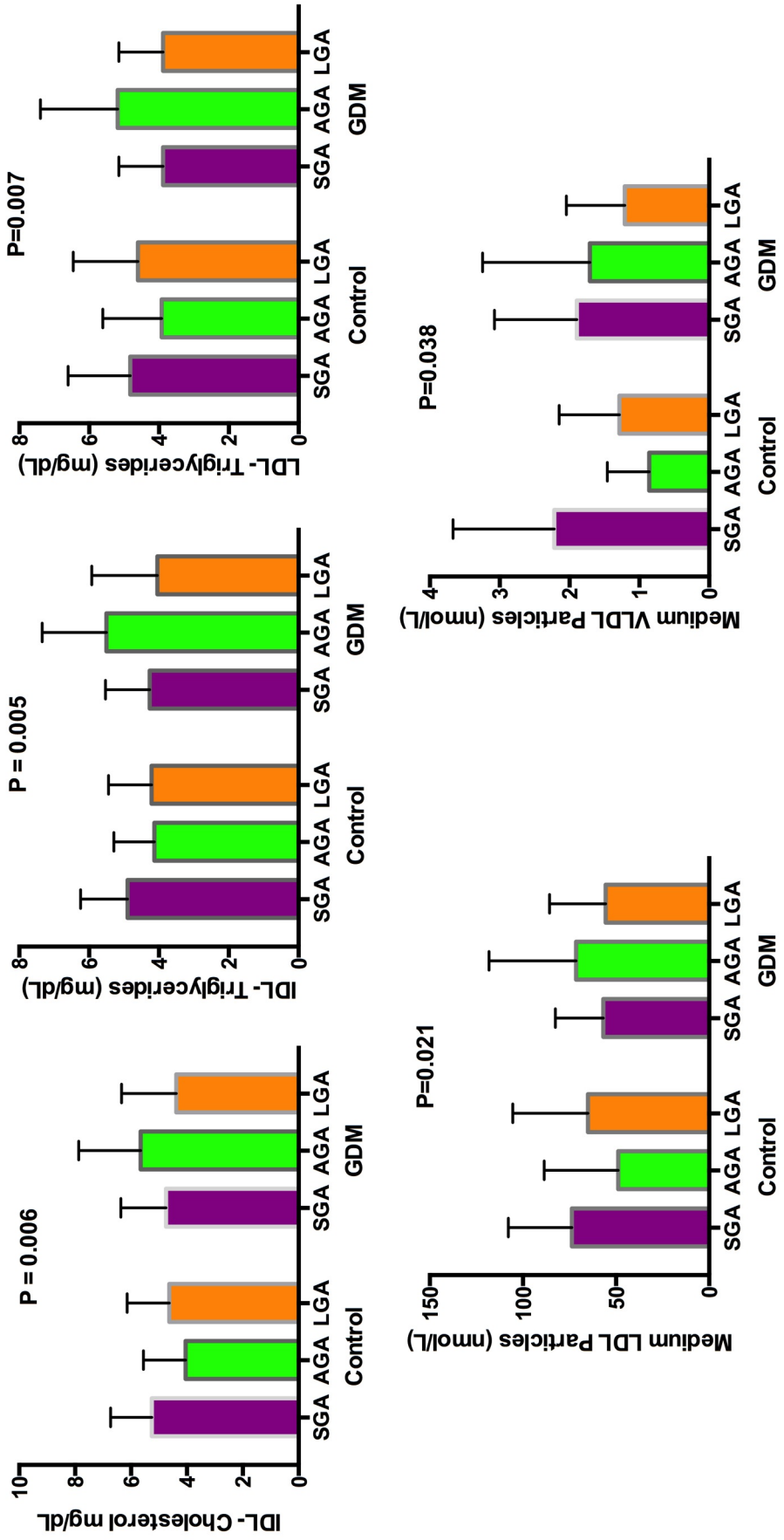
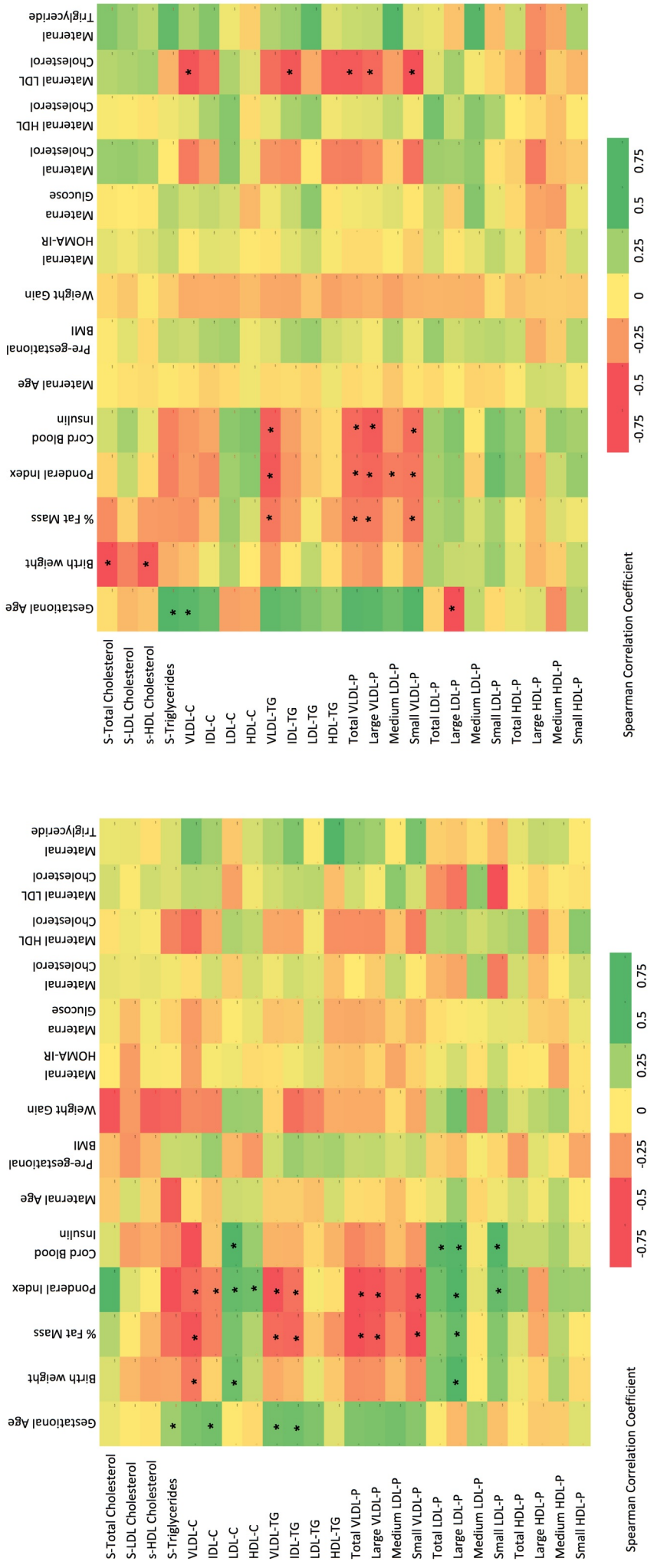


Figure 2



DISCUSSION

DISCUSSION

GDM has been associated with lasting effects in the offspring, such as obesity, type 2 diabetes, metabolic syndrome and early cardiovascular disorders[13,14,16,17,19,20,201]. There is scarce data about the molecular mechanisms responsible for its appearance but fetal programming has been identified as a key event.

Our studies report for the first time that GDM alters the plasticity and functionality of fetal precursors from the amniotic membrane, as well as the fetal lipoprotein profile and metabolism across birth-weight categories. Remarkably, both disturbances are related with potential adverse outcomes in the offspring. Indeed, several parameters of the umbilical cord lipoprotein profile are related to obesity in 2-year infants, which open new avenues in the field of predictive biomarkers to detect population at risk of the disorders mentioned above.

1. Fetal precursors from the placenta mirror fetal metabolic disturbances induced by GDM.

The influence of an adverse maternal environment on fetal outcomes is governed by the placenta, which reflects the metabolic milieu of both mother and fetus. During GDM, it undergoes morphologic and functional changes that might contribute to the increased risk of later metabolic dysfunction[7,178]. The AM is in contact with the amniotic fluid and the fetus, and AMSCs are genetically identical to those from the fetus[147], so they might reflect the deleterious effects of maternal metabolic derangements of GDM on fetal structures.

Our results demonstrate that GDM affects the plasticity and the functional characteristics of AMSCs. Of note, functional changes were not associated with immunophenotypic changes. We show a decreased proliferative capacity and diminished osteogenic differentiation potential in GDM-AMSCs. In this line, Wajid et al.[217] and Amrithraj et al.[194] observed a decreased proliferation rate of umbilical cord-derived MSCs, and a lower clonogenicity (but not proliferation) was also described in chorionic villi MSCs from GDM women[195]. This data is in contrast with previous reports on MSCs derived from other tissues. Several studies, including some from our laboratory, showed a higher proliferation of adipose tissue-derived MSCs isolated from obese and type 2 diabetic patients[125,126]. A possible explanation is that MSCs from gestational tissues display increased apoptosis compared with their control counteracts. Thus, Amrithraj et al. found decreased levels of antiapoptotic factors

in GDM-derived umbilical cord MSCs[194]. In our study, the production of prostaglandin E2 (PGE2) was decreased in AMSCs derived from GDM women, which has been described that could inhibit cell apoptosis[218,219] apart from its important anti-inflammatory and immunosuppressive properties[220], and this is in accordance with a down-regulation of *prostaglandin-endoperoxide synthase 2* (*PTGS2*). This might reflect a possible protective mechanism of the fetus by the AMSCs. However, further research is needed in this aspect. On the other hand, we observed that AMSCs isolated from women with GDM have a decreased differentiation ability into osteocyte, which was in concordance with Kong et al. and Chen et al. who reported that umbilical cord-derived MSCs exhibited a poor cell osteogenic differentiation potential in women with GDM[192] and obesity[221], respectively.

During pregnancy, there is a modulation of the immune system leading to differential responses that allow an adequate implantation and fetal development[102,103]. Healthy pregnancy requires a very well-balanced equilibrium between pro- and anti-inflammatory cytokines during all its stages[37,170]. A very important factor in pregnancy deeply involved in the anti-inflammatory and immunosuppressive properties of MSCs is PGE2. This soluble molecule has been described to suppress DCs maturation and T-cells activation[222] and to diminish inflammation and attenuate infections[223–225], among others. We show that GDM-AMSCs displayed lower PGE2 secretion and *PTGS2* expression, a key enzyme involved in the PGE2 synthesis pathway. According to these findings, higher migration and invasion, as well as increased chemotactic activity over T-cells and monocytes were observed in AMSCs from GDM women. With reference to the latter, we demonstrate that the higher chemotactic capacities of GDM precursors is consequence of an augmented monocyte chemoattractant protein-1 (MCP-1) secretion, a key element involved in the modulation and recruitment of macrophages that has been implicated in the induction of adipose tissue inflammation in type 2 diabetes and obesity[226]. Thus, the higher chemotaxis induced by the GDM-AMSCs was reversed when their conditioned medium was treated with an anti-MCP-1 specific antibody, which is in consistent with an augmented *MCP-1* expression in those cells.

GDM is associated with a pro-inflammatory environment, with the placenta being a source of pro-inflammatory factors. In this line, other inflammatory markers, such as, *TNF-α, cathepsin S (CTSS)* and *cluster of differentiation* 40 *CD40* were increased in AMSCs derived from GDM women. This has been also observed in adipose tissue-derived MSCs from obese and type 2 diabetic patients[125,126]. Our findings on AMSCs are in agreement with previous reports in placental tissue obtained from GDM pregnancies. Augmented placental *TNF-α* gene

expression[227] and release under hyperglycemic conditions[184] is well established in GDM women.

Notably, we were able to reproduce the dysfunction of AMSCs from GDM patients by mimicking a hyperglycemic, hyperinsulinemic and dyslipidemic environment, which are well-recognized features of the GDM-associated fetal metabolic environment. Thus, the combination of high levels of glucose, insulin and palmitic acid induced a similar phenotype in control AMSCs. Of note, we did not observe changes in the inflammatory expression profile when exposed to insulin and glucose, separately or together. However, a trend was observed when control AMSCs were treated with palmitic acid, and these results became evident when we treated them with a combination of the three stimuli. This indicate that FFA might be the main drivers of the functional alterations of AMSCs but a context of hyperglycemia and hyperinsulinemia is necessary. Our data demonstrate that the functional alteration found in GDM-AMSCs is induced by environmental factors and suggest that there must be a local increase of those factors impacting the cells in GDM women, despite a good metabolic control.

Consistently with the favored pro-inflammatory profile of AMSCs, we found that AM-resident macrophages displayed a significantly higher expression of *MCP-1* and notably trend in others pro-inflammatory markers. Our data demonstrate that GDM disturbs the expression profile of AM-resident macrophages and predispose them to a more pro-inflammatory profile even in GDM women with a good metabolic control. Nonetheless, based on our expression results, we were not able to find a deeper switch to a M1 phenotype, as it has been reported in adipose tissue-derived macrophages of obese patients[228]. This is consistent with Schliefsteiner et al., who showed that macrophages of the villous stroma maintained an M2-like pro-repair phenotype in GDM[109]. In this context, pMSCs[123] and AMSCs[154] seem to modulate the polarization of macrophages into a M2 phenotype. These data support the role of placental immune cells in inducing fetal-maternal tolerance and granting fetal protection from low-grade inflammation. However, we believe that the pro-inflammatory features of the AMSCs induced by GDM may modify their normal interaction with AM-resident macrophages, contributing to their increased expression of pro-inflammatory markers.

According to the fetal programming hypothesis, a disturbed metabolic environment could permanently affect the health of offspring exposed and predispose them to obesity, type 2 diabetes and cardiovascular disease[1–4]. In this aspect, given its location and genetic potential, AMSCs might be an optimal indicator of how the intrauterine environment impacts the fetus. We found that *MCP-1* gene expression was positively correlated with both cord blood insulin

and triglyceride concentrations, but not with birth-weight or newborn adiposity. The lack of association could be due to the small sample size of the study, the effect of the nutritional therapy[229] and the different variables responsible for birth-weight other than hyperglycemia[230]. On the other hand, we showed that gene expression of chemokines implicated in the modulation of migration and inflammation, as well as involved in adipose tissue dysfunction and cardiovascular diseases[231], were related to maternal BMI and insulin resistance. Moreover, the upregulation of *MCP-1* and *CD40* seems to be specific to GDM pregnancies, even when they are adequately controlled, indicating that birth-weight normalization and metabolic control might be unable to revert the insult. Overall, our results support the hypothesis that that GDM environment could program fetal stem cells and subsequently favor metabolic dysfunction later in life.

2. Advanced ^{1}H-NMR-based lipoprotein testing uncovers deep fetal lipoprotein alterations during GDM and it is a biomarker for later obesity.

In our second study, advanced ^{1}H-NMR-based lipoprotein test highlights a disturbed triglyceride and cholesterol content in lipoproteins of offspring born to GDM mothers across birth categories. Specifically, GDM alters the umbilical cord lipoprotein profile of AGA neonates, particularly affecting IDL-lipoproteins, triglyceride content in LDL and the number of particles of LDL, and showing a profile more similar to adults with dyslipidemia and atherosclerosis than to those born to control women.

Several maternal and fetal factors such as hypertension, diabetes, obesity and low or high birth-weight can influence plasma lipids[232–235]. Consistently, an increase in LDL- and decrease in HDL-cholesterol[236], and augmented FFAs, triglycerides, and total cholesterol have been found in newborns of well-controlled mothers with type 1 diabetes [237]. On the other hand, abnormal growth patterns are associated with altered nutrient supply and fetal fat deposition, with changes in circulating lipids and lipoprotein concentrations[238–242]. Moreover, altered fetal lipoprotein profile has been associated with aorta intima thickness in SGA and LGA neonates[243,244], supporting the notion that disturbances in the lipoprotein metabolism may have lasting effects. Nevertheless, we did not see an additional effect of GDM on the pattern that SGA and LGA showed in the lipoprotein profile assessed by ^{1}H-NMR, which was similar to the phenotype observed in control infants.

Standard lipid panels provide data on cholesterol content from all circulating particles but they lack information on the protein component and lipoprotein particle size and number,

thus failing to identify many lipoprotein abnormalities[211]. Similar to our results, standard lipid profiling did not show differences between offspring born to control and GDM mothers[245]. On the contrary, we observed an interaction between GDM and fetal growth categories when ^{1}H-NMR-based lipoprotein profiling was performed, which revealed that GDM AGA neonates have higher concentrations of IDL cholesterol and triglyceride content than AGA neonates born to control women. These findings suggest a decrease in the clearance of IDL lipoproteins in the liver, which would raise the IDL half-life and the transfer of triglycerides to LDL, resulting in LDL particles with decreased cholesterol content[246]. This is consistent with previous studies showing an incomplete conversion of VLDL to LDL, augmented generation of IDL and the prevalence of small and dense LDL in diabetic patients, which increases the risk for atherosclerosis[125,213–216,246,247].

Quantitative and qualitative alterations in plasma lipoproteins are one of the metabolic abnormalities characteristic of diabetic patients and a main risk factor for the development of cardiovascular disease[248–250]. Similarly, GDM has been associated with alterations in fetal lipoprotein metabolism and early markers of atherogenesis in the offspring[202]. Increased VLDL- and LDL-cholesterol concentrations have been described in cord blood from GDM mothers, along with a lower HDL-cholesterol concentration[251]. This is in concordance with changes in cord blood lipoprotein concentrations found in well-controlled type 1 diabetic mothers[236,237] but, nonetheless, other studies found no differences between GDM and normal glucose tolerant mothers[251]. Higher triglycerides content-particles are more atherogenic and has been associated with arterial inflammation, cardiovascular disease, diabetes and systemic inflammation[242,252–255]. On the other hand, HDL has potent antioxidant and anti-inflammatory activities and it has been inversely correlated with the incidence of cardiovascular disease[256]. In this context, triglyceride-enriched HDL particles have been found in diabetic patients, contributing to a higher risk for atherosclerosis[213–216,246,247].

Our results, as it has been observed in previous reports[257], show that gestational age was positively associated with triglyceride-rich lipoproteins. On the contrary, neonatal adiposity was negatively correlated with VLDL lipoproteins and the number of VLDL particles, and IDL-triglyceride content, while a positive association was observed with the number of particles of lipoproteins rich in cholesterol, such LDL and HDL. This might indicate a higher clearance of triglyceride-rich lipoprotein as the fat accretion increments. In this line, Ramy et al.[258] described a negative relationship between ponderal index with cord blood triglycerides accompanied by a positive association with HDL, although they indicate a negative association with LDL, but others failed to confirm these results[259]. Fetal hyperinsulinemia is related with an

enhanced VLDL synthesis[260], which may contribute to increased levels of plasma triglycerides and LDL-cholesterol[261]. However, we observed a negative relationship between cord blood insulin and VLDL particles and triglyceride content in GDM offspring, whereas a strong positive association with LDL particles and cholesterol content in the control group. This suggests that the metabolic changes induced by GDM may explain some of the changes in the fetal lipoprotein profile

Finally, our results indicate that both cord blood large and small LDL particles are independently associated with offspring obesity at two years. This highlights the potential predictive capacity of the ^{1}H-NMR-assessed lipoprotein profile on later obesity. Long-term studies are required to study whether the lipoprotein profile determined by advanced ^{1}H-NMR-based tests is able to identify obesity beyond two years, in order to confirm its possible use as a biomarker of later metabolic diseases that allows identifying the population at risk of future adverse outcomes.

In short, we used advanced ^{1}H-NMR-based lipoprotein profiling to identify subtle parameters of dyslipidemia in umbilical cord blood in women with normal pregnancy and GDM, across different birth-weight categories. We show that GDM disturbs triglyceride and cholesterol lipoprotein content across birth-weight categories. Of particular note, an analysis of the effect of both the birth-weight categories and GDM showed that specifically AGA neonates born to GDM mothers have a lipoprotein profile more similar to adults with dyslipidemia and atherosclerosis than to those born to mothers with normal glucose tolerance. Our data indicate that a normal birth-weight does not equal to normal lipid metabolism, which provides a pathophysiological explanation for the increased risk for developing metabolic disorders and suggests that GDM treatment may be unable to prevent long-term complications. Moreover, we find that LDL particles are associated with obesity in offspring at two years of age. Consequently, cord blood lipoprotein profiles emerge as potential biomarkers for future adverse outcomes in offspring. An extensive characterization of cord blood lipoproteins could provide more accurate information on the regulation of lipoprotein metabolism in fetal life and its potential implications for metabolism in later life.

Several limitations should be also considered. We are aware about the observational design of our studies, their limitations to infer causality and the possibility of the presence of confounders and selection biases. To avoid this, we have matched subjects in case-control studies and/or we have used statistical analysis to overcome these effects. In addition, the

data of the patients included was registered prospectively and following the same questionnaire, limiting some confounders. Noticeably, larger samples and less follow-up losses probably would have added robustness to our results, although it is worth mentioning that we had similar rate of follow-up losses in the control and GDM groups. Moreover, it is important to highlight the novelty of our findings, which set the basis for further studies in the field.

With our work we have revealed how GDM could affect the plasticity of AMSCs and modify their inflammatory profile and response. At the same time, from a more clinical point of view, we showed how the metabolic profile of newborns born to GDM mothers is different to controls despite a normal birthweight. Moreover, the advanced study of fetal lipoproteins might result a biomarker for future complications in the offspring. Overall, our data contribute to the knowledge about the pathophysiology behind GDM and its implication in a greater predisposition of offspring for developing later disease.

CONCLUSIONS

CONCLUSIONS

1. Gestational diabetes mellitus (GDM) impairs the plasticity fetal precursor cells from the amniotic membrane. Specifically, amniotic mesenchymal stem cells (AMSCs) from GDM women are les proliferative and have lower osteogenic differentiation capacity.
2. GDM induces a pro-inflammatory profile in AMSCs, including a higher expression of pro-inflammatory markers, increased chemotactic and invasive activities and lower production of prostaglandin E2 (PGE2).
3. Genes involved in the AMSCs inflammatory response were associated with maternal insulin sensitivity and pre-pregnancy body mass index, as well as with fetal metabolic parameters.
4. GDM disturbs triglyceride and cholesterol lipoprotein content in offspring of GDM mothers across birth categories. Concretely, appropriate for gestational age (AGA) neonates born to GDM mothers showed a profile more similar to adults with dyslipidemia and atherosclerosis than to those born to control women.
5. Low-density lipoprotein (LDL) particles are associated with obesity in offspring at two years of age, indicating the possible implication of an altered fetal lipoprotein profile in the greater predisposition to future adverse outcomes and the potential use of lipoprotein profile as biomarker for future adverse outcomes in offspring.

REFERENCES

REFERENCES

1. Lucas A. Programming by early nutrition in man. *Ciba Found Symp*. 1991.
2. Barker DJ. The fetal and infant origins of disease. *Eur J Clin Invest*. 1995;25(7):457-463.
3. Kwon EJ, Kim YJ. What is fetal programming?: a lifetime health is under the control of in utero health. *Obstet Gynecol Sci*. 2017;60(6):506-519. doi:10.5468/ogs.2017.60.6.506
4. Calkins K, Devaskar SU. Fetal origins of adult disease. *Curr Probl Pediatr Adolesc Health Care*. 2011. doi:10.1016/j.cppeds.2011.01.001
5. Zhang C, Ning Y. Effect of dietary and lifestyle factors on the risk of gestational diabetes: Review of epidemiologic evidence. In: *American Journal of Clinical Nutrition*. ; 2011. doi:10.3945/ajcn.110.001032
6. Chu SY, Callaghan WM, Kim SY, et al. Maternal obesity and risk of gestational diabetes mellitus. *Diabetes Care*. 2007. doi:10.2337/dc06-2559a
7. Pantham P, Aye ILMH, Powell TL. Inflammation in maternal obesity and gestational diabetes mellitus. *Placenta*. 2015. doi:10.1016/j.placenta.2015.04.006
8. Atègbo JM, Grissa O, Yessoufou A, et al. Modulation of adipokines and cytokines in gestational diabetes and macrosomia. *J Clin Endocrinol Metab*. 2006. doi:10.1210/jc.2006-0980
9. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. In: *Journal of Clinical Endocrinology and Metabolism*. ; 2004. doi:10.1210/jc.2004-0395
10. Cildir G, Akincilar SC, Tergaonkar V. Chronic adipose tissue inflammation: All immune cells on the stage. *Trends Mol Med*. 2013. doi:10.1016/j.molmed.2013.05.001
11. Gregor MF, Hotamisligil GS. Inflammatory Mechanisms in Obesity. *Annu Rev Immunol*. 2011. doi:10.1146/annurev-immunol-031210-101322
12. Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. *Diabetes Care*. 2007;30(SUPPL. 2). doi:10.2337/dc07-s202
13. Catalano PM. The impact of gestational diabetes and maternal obesity on the mother and her offspring. *J Dev Orig Health Dis*. 2010. doi:10.1017/S2040174410000115
14. Damm P, Houshmand-Oeregaard A, Kelstrup L, Lauenborg J, Mathiesen ER, Clausen TD. Gestational diabetes mellitus and long-term consequences for mother and offspring: a view from Denmark. *Diabetologia*. 2016;59(7):1396-1399. doi:10.1007/s00125-016-3985-5
15. Zhao P, Liu E, Qiao Y, et al. Maternal gestational diabetes and childhood obesity at age 9–11: results of a multinational study. *Diabetologia*. 2016;59(11):2339-2348. doi:10.1007/s00125-016-4062-9
16. Dabelea D, Pettitt DJ. Intrauterine diabetic environment confers risks for type 2 diabetes mellitus and obesity in the offspring, in addition to genetic susceptibility. *J Pediatr Endocrinol Metab*. 2001. doi:10.1515/jpem-2001-0803
17. Boney CM, Verma A, Tucker R, Vohr BR. Metabolic syndrome in childhood: Association

with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics*. 2005. doi:10.1542/peds.2004-1808

18. Drake AJ, Reynolds RM. Impact of maternal obesity on offspring obesity and cardiometabolic disease risk. *Reproduction*. 2010. doi:10.1530/REP-10-0077

19. Hillier TA, Pedula KL, Schmidt MM, Mullen JA, Charles MA, Pettitt DJ. Childhood obesity and metabolic imprinting: The ongoing effects of maternal hyperglycemia. *Diabetes Care*. 2007. doi:10.2337/dc06-2361

20. Clausen TD, Mathiesen ER, Hansen T, et al. Overweight and the metabolic syndrome in adult offspring of women with diet-treated gestational diabetes mellitus or type 1 diabetes. *J Clin Endocrinol Metab*. 2009. doi:10.1210/jc.2009-0305

21. Bridger T. Childhood obesity and cardiovascular disease. *Paediatr Child Health (Oxford)*. 2009. doi:10.1093/pch/14.3.177

22. Zeng Z, Liu F, Li S. Metabolic Adaptations in Pregnancy: A Review. *Ann Nutr Metab*. 2017;70(1):59-65. doi:10.1159/000459633

23. Herrera E, Ortega-Senovilla H. Lipid Metabolism During Pregnancy and its Implications for Fetal Growth. *Curr Pharm Biotechnol*. 2014. doi:10.2174/1389201015666140330192345

24. Catalano PM, Tyzbir ED, Wolfe RR, Roman NM, B. Amini S, Sims EAH. Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. *Am J Obstet Gynecol*. 1992. doi:10.1016/S0002-9378(12)80011-1

25. Catalano PM, Hoegh M, Minium J, et al. Adiponectin in human pregnancy: Implications for regulation of glucose and lipid metabolism. *Diabetologia*. 2006. doi:10.1007/s00125-006-0264-x

26. Ryan EA, O'Sullivan MJ, Skyler JS. Insulin action during pregnancy: Studies with the euglycemic clamp technique. *Diabetes*. 1985. doi:10.2337/diab.34.4.380

27. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev*. 2018. doi:10.1152/physrev.00063.2017

28. McLachlan KA, O'Neal D, Jenkins A, Alford FP. Do adiponectin, TNFα, leptin and CRP relate to insulin resistance in pregnancy? Studies in women with or without gestational diabetes, during and after pregnancy. *Diabetes Metab Res Rev*. 2006. doi:10.1002/dmrr.591

29. Catalano PM, Tyzbir ED, Roman NM, Amini SB, Sims EAH. Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. *Am J Obstet Gynecol*. 1991. doi:10.1016/0002-9378(91)90012-G

30. Sivan E, Homko CJ, Chen X, Reece EA, Boden G. Effect of insulin on fat metabolism during and after normal pregnancy. *Diabetes*. 1999. doi:10.2337/diabetes.48.4.834

31. Freinkel N. Banting Lecture 1980: of Pregnancy and Progeny. *Diabetes*. 1980. doi:10.2337/diab.29.12.1023

32. Farrar D. Hyperglycemia in pregnancy: Prevalence, impact, and management challenges. *Int J Womens Health*. 2016. doi:10.2147/IJWH.S102117

33. Ryan EA, Enns L. Role of Gestational Hormones in the Induction of Insulin Resistance. *J*

Clin Endocrinol Metab. 1988. doi:10.1210/jcem-67-2-341

34. Wada T, Hori S, Sugiyama M, et al. Progesterone inhibits glucose uptake by affecting diverse steps of insulin signaling in 3T3-L1 adipocytes. *Am J Physiol - Endocrinol Metab*. 2010. doi:10.1152/ajpendo.00649.2009

35. Okuno S, Akazawa S, Yasuhi I, et al. Decreased expression of the GLUT4 glucose transporter protein in adipose tissue during pregnancy. *Horm Metab Res*. 1995. doi:10.1055/s-2007-979946

36. Gonzalez C, Alonso A, Alvarez N, et al. Role of 17β-estradiol and/or progesterone on insulin sensitivity in the rat: Implications during pregnancy. *J Endocrinol*. 2000;166(2):283-291. doi:10.1677/joe.0.1660283

37. Christian LM, Porter K. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: Effects of maternal body mass index. *Cytokine*. 2014. doi:10.1016/j.cyto.2014.06.018

38. Kirwan JP, Hauguel-De Mouzon S, Lepercq J, et al. TNF-α is a predictor of insulin resistance in human pregnancy. *Diabetes*. 2002. doi:10.2337/diabetes.51.7.2207

39. Hotamisligil GS, Spiegelman BM. Tumor necrosis factor α: A key component of the obesity-diabetes link. *Diabetes*. 1994. doi:10.2337/diabetes.43.11.1271

40. Peraldi P, Hotamisligil GS, Buurman WA, White MF, Spiegelman BM. Tumor necrosis factor (TNF)-β inhibits insulin signaling through stimulation of the p55 TNF receptor and activation of sphingomyelinase. *J Biol Chem*. 1996. doi:10.1074/jbc.271.22.13018

41. Peraldi P, Spiegelman B. TNF-α and insulin resistance: Summary and future prospects. *Mol Cell Biochem*. 1998. doi:10.1023/A:1006865715292

42. Schoonjans K, Staels B, Auwerx J. The peroxisome proliferator activated receptors (PPARs) and their effects on lipid metabolism and adipocyte differentiation. *Biochim Biophys Acta - Lipids Lipid Metab*. 1996. doi:10.1016/0005-2760(96)00066-5

43. Zhang B, Berger J, Hu E, et al. Negative regulation of peroxisome proliferator-activated receptor-gamma gene expression contributes to the antiadipogenic effects of tumor necrosis factor-alpha. *Mol Endocrinol*. 1996. doi:10.1210/mend.10.11.8923470

44. Bruun JM, Lihn AS, Verdich C, et al. Regulation of adiponectin by adipose tissue-derived cytokines: In vivo and in vitro investigations in humans. *Am J Physiol - Endocrinol Metab*. 2003. doi:10.1152/ajpendo.00110.2003

45. Lain KY, Catalano PM. Metabolic changes in pregnancy. *Clin Obstet Gynecol*. 2007. doi:10.1097/GRF.0b013e31815a5494

46. Jones HN, Powell TL, Jansson T. Regulation of Placental Nutrient Transport – A Review. *Placenta*. 2007;28(8-9):763-774. doi:10.1016/j.placenta.2007.05.002

47. Illsley NP. Glucose transporters in the human placenta. *Placenta*. 2000. doi:10.1053/plac.1999.0448

48. Alvarez JJ, Montelongo A, Iglesias A, Lasunción MA, Herrera E. Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. *J Lipid Res*. 1996.

49. van Leeuwen EM, Emri E, Merle BMJ, et al. A new perspective on lipid research in age-related macular degeneration. *Prog Retin Eye Res*. 2018.

doi:10.1016/j.preteyeres.2018.04.006

50. Felig P, Kim YJ, Lynch V, Hendler R. Amino acid metabolism during starvation in human pregnancy. *J Clin Invest*. 1972. doi:10.1172/JCI106913

51. Schoengold DM, deFiore RH, Parlett RC. Free amino acids in plasma throughout pregnancy. *Am J Obstet Gynecol*. 1978. doi:10.1016/0002-9378(78)90108-4

52. Duggleby SL, Jackson AA. Protein, amino acid and nitrogen metabolism during pregnancy: How might the mother meet the needs of her fetus? *Curr Opin Clin Nutr Metab Care*. 2002. doi:10.1097/00075197-200209000-00008

53. Kalhan SC. Protein metabolism in pregnancy. In: *American Journal of Clinical Nutrition*. ; 2000. doi:10.1093/ajcn/71.5.1249s

54. Bernstein I. Assessment of fetal body composition [4]. *Ultrasound Obstet Gynecol*. 2004;23(3):310-311. doi:10.1002/uog.997

55. Jennings RE, Berry AA, Kirkwood-Wilson R, et al. Development of the human pancreas from foregut to endocrine commitment. *Diabetes*. 2013. doi:10.2337/db12-1479

56. Lewis RM, Wadsack C, Desoye G. Placental fatty acid transfer. *Curr Opin Clin Nutr Metab Care*. 2018;21(2):78-82. doi:10.1097/MCO.0000000000000443

57. Herrera E, Amusquivar E, López-Soldado I, Ortega H. Maternal lipid metabolism and placental lipid transfer. *Horm Res*. 2006;65(SUPPL. 3):59-64. doi:10.1159/000091507

58. Woollett LA, Heubi JE. *Fetal and Neonatal Cholesterol Metabolism*.; 2000.

59. Mennitti L V., Oliveira JL, Morais CA, 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. doi:10.1016/j.jnutbio.2014.10.001

60. Treem WR. *Fatty Acid Oxidation Disorders*. Third Edit. Elsevier Inc.; 2006. doi:10.1016/B978-0-7216-3924-6.50064-0

61. Regnault TRH, De Vrijer B, Battaglia FC. Transport and metabolism of amino acids in placenta. *Endocrine*. 2002. doi:10.1385/ENDO:19:1:23

62. Grillo MA, Lanza A, Colombatto S. Transport of amino acids through the placenta and their role. *Amino Acids*. 2008. doi:10.1007/s00726-007-0006-5

63. Mongelli M. Factors influencing fetal growth. *Ann Acad Med Singapore*. 2003;32(3):283-288. doi:10.1542/neo.2-6-e119

64. Sacks DA. Determinants of fetal growth. *Curr Diab Rep*. 2004;4(4):281-287. doi:10.1007/s11892-004-0080-y

65. Haggarty P. Fatty Acid Supply to the Human Fetus. *Annu Rev Nutr*. 2010. doi:10.1146/annurev.nutr.012809.104742

66. Nolan CJ, Riley SF, Sheedy MT, Walstab JE, Beischer N a. Maternal serum triglyceride, glucose tolerance, and neonatal birth weight ratio in pregnancy. *Diabetes Care*. 1995. doi:10.2337/diacare.18.12.1550

67. Kuipers RS, Luxwolda MF, Offringa PJ, et al. Gestational age dependent content, composition and intrauterine accretion rates of fatty acids in fetal white adipose tissue. *Prostaglandins Leukot Essent Fat Acids*. 2012. doi:10.1016/j.plefa.2011.10.007

68. Simmons R. *Abnormalities of Fetal Growth*. Ninth Edit. Elsevier Inc.; 2012. doi:10.1016/b978-1-4377-0134-0.10006-x

69. Zhang J, Merialdi M, Platt LD, Kramer MS. Defining normal and abnormal fetal growth: promises and challenges. *Am J Obstet Gynecol*. 2010. doi:10.1016/j.ajog.2009.10.889

70. Lunde A, Melve KK, Gjessing HK, Skjærven R, Irgens LM. Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. *Am J Epidemiol*. 2007. doi:10.1093/aje/kwk107

71. Magnus P, Gjessing HK, Skrondal A, Skjærven R. Paternal contribution to birth weight. *J Epidemiol Community Health*. 2001. doi:10.1136/jech.55.12.873

72. Zhang J, Bowes, WA. Birth-weight-for-gestational-age patterns by race, sex, and parity in the United States population. *Obstet Gynecol*. 1995. doi:10.1016/0029-7844(95)00142-E

73. Surani MAH, Barton SC, Norris ML. Nuclear transplantation in the mouse: Heritable differences between parental genomes after activation of the embryonic genome. *Cell*. 1986. doi:10.1016/0092-8674(86)90544-1

74. McGrath J, Solter D. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell*. 1984. doi:10.1016/0092-8674(84)90313-1

75. Langhoff-Roos J, Lindmark G, Gustavson K -H, Gebre-Medhin M, Meirik O. Relative effect of parental birth weight on infant birth weight at term. *Clin Genet*. 1987;32(4):240-248. doi:10.1111/j.1399-0004.1987.tb03307.x

76. Magnus P. Birth weight of relatives by maternal tendency to repeat small-for-gestational-age (SGA) births in successive pregnancies. *Acta Obstet Gynecol Scand Suppl*. 1997.

77. Paliwal A, Singh V, Mohan I, Choudhary RC, Sharma BN. Risk Factors Associated With Low Birth Weight in Newborns: a Tertiary Care Hospital Based Study. *Int J Curr Res Rev*. 2013;5(11):42. http://ezproxy.umng.edu.co:2048/login?url=http://search.proquest.com/docview/1399515555?accountid=30799%5Cnhttp://site.ebrary.com/lib/umng/Top?layout=search&nosr=1&frm=smp.x&p00=RISK+FACTORS+ASSOCIATED+WITH+LOW+BIRTH+WEIGHT+IN+NEWBORNS:+A+TERTIARY+CARE+HO.

78. Ong KKL, Preece MA, Emmett PM, Ahmed ML, Dunger DB. Size at birth and early childhood growth in relation to maternal smoking, parity and infant breast-feeding: Longitudinal birth cohort study and analysis. *Pediatr Res*. 2002. doi:10.1203/00006450-200212000-00009

79. Widerøe M, Vik T, Jacobsen G, Bakketeig LS. Does maternal smoking during pregnancy cause childhood overweight? *Paediatr Perinat Epidemiol*. 2003. doi:10.1046/j.1365-3016.2003.00481.x

80. Baird D. The epidemiology of low birth weight: Changes in incidence in aberdeen, 1948–72. *J Biosoc Sci*. 1974. doi:10.1017/S0021932000009688

81. Agrawal V, Hirsch E. Intrauterine infection and preterm labor. *Semin Fetal Neonatal Med*. 2012. doi:10.1016/j.siny.2011.09.001

82. Sewell MF, Huston-Presley L, Super DM, Catalano P. Increased neonatal fat mass, not

lean body mass, is associated with maternal obesity. *Am J Obstet Gynecol*. 2006. doi:10.1016/j.ajog.2006.06.014

83. Hull HR, Dinger MK, Knehans AW, Thompson DM, Fields DA. Impact of maternal body mass index on neonate birthweight and body composition. *Am J Obstet Gynecol*. 2008. doi:10.1016/j.ajog.2007.10.796

84. Estampador AC, Pomeroy J, Renström F, et al. Infant body composition and adipokine concentrations in relation to maternal gestational weight gain. *Diabetes Care*. 2014. doi:10.2337/dc13-2265

85. Carlsen EM, Renault KM, Nørgaard K, et al. Newborn regional body composition is influenced by maternal obesity, gestational weight gain and the birthweight standard score. *Acta Paediatr Int J Paediatr*. 2014. doi:10.1111/apa.12713

86. Fowden AL, Ward JW, Wooding FPB, Forhead AJ, Constancia M. Programming placental nutrient transport capacity. *J Physiol*. 2006;572(1):5-15. doi:10.1113/jphysiol.2005.104141

87. Evain-Brion D, Malassine A. Human placenta as an endocrine organ. *Growth Horm IGF Res*. 2003. doi:10.1016/S1096-6374(03)00053-4

88. Gude NM, Roberts CT, Kalionis B, King RG. Growth and function of the normal human placenta. *Thromb Res*. 2004;114(5-6 SPEC. ISS.):397-407. doi:10.1016/j.thromres.2004.06.038

89. Carlson BM. Formation of Chorionic Villi. *Placenta*. 2014:1-8. doi:10.1016/B978-0-12-801238-3.05435-0

90. Van Herendael BJ, Oberti C, Brosens I. Microanatomy of the human amniotic membrane. A light microscopic, transmission, and scanning electron microscopic study. *Am J Obstet Gynecol*. 1978. doi:10.1016/S0002-9378(16)33135-0

91. Jansson T, Powell TL. Human Placental Transport in Altered Fetal Growth: Does the Placenta Function as a Nutrient Sensor? - A Review. *Placenta*. 2006. doi:10.1016/j.placenta.2005.11.010

92. Sibley CP, Turner MA, Cetin I, et al. Placental phenotypes of intrauterine growth. *Pediatr Res*. 2005. doi:10.1203/01.PDR.0000181381.82856.23

93. Jansson T, Ekstrand Y, Björn C, Wennergren M, Powell TL. Alterations in the activity of placental amino acid transporters in pregnancies complicated by diabetes. *Diabetes*. 2002. doi:10.2337/diabetes.51.7.2214

94. Jansson N, Rosario FJ, Gaccioli F, et al. Activation of placental mTOR signaling and amino acid transporters in obese women giving birth to large babies. *J Clin Endocrinol Metab*. 2013. doi:10.1210/jc.2012-2667

95. Girard J, Ferre P, Pegorier JP, Duee PH. Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. *Physiol Rev*. 1992. doi:10.1152/physrev.1992.72.2.507

96. Holme AM, Roland MCP, Lorentzen B, Michelsen TM, Henriksen T. Placental glucose transfer: A human in vivo study. *PLoS One*. 2015. doi:10.1371/journal.pone.0117084

97. Johansson M, Karlsson L, Wennergren M, Jansson T, Powell TL. Activity and protein expression of Na+/K+ ATPase are reduced in microvillous syncytiotrophoblast plasma membranes isolated from pregnancies complicated by intrauterine growth restriction. *J*

Clin Endocrinol Metab. 2003;88(6):2831-2837. doi:10.1210/jc.2002-021926

98. McCoy MG, Sun GS, Marchadier D, Maugeais C, Glick JM, Rader DJ. Characterization of the lipolytic activity of endothelial lipase. *J Lipid Res*. 2002.

99. Kazantzis M, Stahl A. Fatty acid transport proteins, implications in physiology and disease. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2012. doi:10.1016/j.bbalip.2011.09.010

100. Neville MC. Adaptation of maternal lipid flux to pregnancy: Research needs. *Eur J Clin Nutr*. 1999. doi:10.1038/sj.ejcn.1600752

101. Gil-Sánchez A, Demmelmair H, Parrilla JJ, Koletzko B, Larqué E. Mechanisms involved in the selective transfer of long chain polyunsaturated fatty acids to the fetus. *Front Genet*. 2011. doi:10.3389/fgene.2011.00057

102. PrabhuDas M, Bonney E, Caron K, et al. Immune mechanisms at the maternal-fetal interface: Perspectives and challenges. *Nat Immunol*. 2015. doi:10.1038/ni.3131

103. Makrigiannakis A, Karamouti M, Drakakis P, Loutradis D, Antsaklis A. Fetomaternal Immunotolerance. *Am J Reprod Immunol*. 2008. doi:10.1111/j.1600-0897.2008.00655.x

104. Feinman MA, Kliman HJ, Main EK. HLA antigen expression and induction by γ-interferon in cultured human trophoblasts. *Am J Obstet Gynecol*. 1987. doi:10.1016/S0002-9378(87)80238-7

105. Hunt JS, Andrews GK, Wood GW. Normal trophoblasts resist induction of class I HLA. *J Immunol*. 1987.

106. King A, Boocock C, Sharkey AM, et al. Evidence for the expression of HLA-C class I mRNA and protein by human first trimester trophoblast. *J Reprod Immunol*. 1996. doi:10.1016/0165-0378(96)87783-7

107. Houlihan JM, Biro PA, Harper HM, Jenkinson HJ, Holmes CH. The human amnion is a site of MHC class Ib expression: evidence for the expression of HLA-E and HLA-G. *J Immunol*. 1995.

108. Lim R. Concise Review: Fetal Membranes in Regenerative Medicine: New Tricks from an Old Dog? *Stem Cells Transl Med*. 2017;6(9):1767-1776. doi:10.1002/sctm.16-0447

109. Schliefsteiner C, Peinhaupt M, Kopp S, et al. Human Placental Hofbauer Cells Maintain an Anti-inflammatory M2 Phenotype despite the Presence of Gestational Diabetes Mellitus. *Front Immunol*. 2017;8:888. doi:10.3389/fimmu.2017.00888

110. Svensson-Arvelund J, Mehta RB, Lindau R, et al. The Human Fetal Placenta Promotes Tolerance against the Semiallogeneic Fetus by Inducing Regulatory T Cells and Homeostatic M2 Macrophages. *J Immunol*. 2015. doi:10.4049/jimmunol.1401536

111. Choudhery MS, Badowski M, Muise A, Harris DT. Comparison of human mesenchymal stem cells derived from adipose and cord tissue. *Cytotherapy*. 2013. doi:10.1016/j.jcyt.2012.11.010

112. Barlow S, Brooke G, Chatterjee K, et al. Comparison of human placenta- and bone marrow-derived multipotent mesenchymal stem cells. *Stem Cells Dev*. 2008. doi:10.1089/scd.2007.0154

113. Danišovič L, Boháč M, Zamborský R, et al. Comparative analysis of mesenchymal stromal cells from different tissue sources in respect to articular cartilage tissue

engineering. *Gen Physiol Biophys*. 2016. doi:10.4149/gpb_2015044

114. Drela K, Lech W, Figiel-Dabrowska A, et al. Enhanced neuro-therapeutic potential of Wharton's Jelly-derived mesenchymal stem cells in comparison with bone marrow mesenchymal stem cells culture. *Cytotherapy*. 2016. doi:10.1016/j.jcyt.2016.01.006

115. Mareschi K, Castiglia S, Sanavio F, et al. Immunoregulatory effects on T lymphocytes by human mesenchymal stromal cells isolated from bone marrow, amniotic fluid, and placenta. *Exp Hematol*. 2016. doi:10.1016/j.exphem.2015.10.009

116. Li X, Bai J, Ji X, Li R, Xuan Y, Wang Y. Comprehensive characterization of four different populations of human mesenchymal stem cells as regards their immune properties, proliferation and differentiation. *Int J Mol Med*. 2014. doi:10.3892/ijmm.2014.1821

117. Lee JM, Jung J, Lee HJ, et al. Comparison of immunomodulatory effects of placenta mesenchymal stem cells with bone marrow and adipose mesenchymal stem cells. *Int Immunopharmacol*. 2012. doi:10.1016/j.intimp.2012.03.024

118. Macholdová K, Macháčková E, Prošková V, Hromadníková I, Klubal R. Latest findings on the placenta from the point of view of immunology, tolerance and mesenchymal stem cells. *Ces Gynekol*. 2019.

119. Avanzini MA, Bernardo ME, Cometa AM, et al. Generation of mesenchymal stromal cells in the presence of platelet lysate: A phenotypic and functional comparison of umbilical cord blood- and bone marrow-derived progenitors. *Haematologica*. 2009. doi:10.3324/haematol.2009.006171

120. Zhang X, Hirai M, Cantero S, et al. Isolation and characterization of mesenchymal stem cells from human umbilical cord blood: Reevaluation of critical factors for successful isolation and high ability to proliferate and differentiate to chondrocytes as compared to mesenchymal stem cells fro. *J Cell Biochem*. 2011. doi:10.1002/jcb.23042

121. Soncini M, Vertua E, Gibelli L, et al. Isolation and characterization of mesenchymal cells from human fetal membranes. *J Tissue Eng Regen Med*. 2007. doi:10.1002/term.40

122. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. *Cell Commun Signal*. 2011;9(1):12. doi:10.1186/1478-811X-9-12

123. Abumaree MH, Abomaray FM, Alshabibi MA, AlAskar AS, Kalionis B. Immunomodulatory properties of human placental mesenchymal stem/stromal cells. *Placenta*. 2017;59:87-95. doi:10.1016/j.placenta.2017.04.003

124. Han Y, Li X, Zhang Y, Han Y, Chang F, Ding J. Mesenchymal Stem Cells for Regenerative Medicine. *Cells*. 2019;8(8):886. doi:10.3390/cells8080886

125. Pachón-Peña G, Serena C, Ejarque M, et al. Obesity Determines the Immunophenotypic Profile and Functional Characteristics of Human Mesenchymal Stem Cells From Adipose Tissue. *Stem Cells Transl Med*. 2016;5(4):464-475. doi:10.5966/sctm.2015-0161

126. Serena C, Keiran N, Ceperuelo-Mallafre V, et al. Obesity and Type 2 Diabetes Alters the Immune Properties of Human Adipose Derived Stem Cells. *Stem Cells*. 2016;34(10):2559-2573. doi:10.1002/stem.2429

127. Kornicka K, Houston J, Marycz K. Dysfunction of Mesenchymal Stem Cells Isolated from Metabolic Syndrome and Type 2 Diabetic Patients as Result of Oxidative Stress and Autophagy may Limit Their Potential Therapeutic Use. *Stem Cell Rev Reports*.

2018;14(3):337-345. doi:10.1007/s12015-018-9809-x

128. Marino L, Castaldi MA, Rosamilio R, et al. Mesenchymal stem cells from the Wharton's jelly of the human umbilical cord: Biological properties and therapeutic potential. *Int J Stem Cells*. 2019;12(2):218-226. doi:10.15283/ijsc18034

129. Talwadekar MD, Kale VP, Limaye LS. Placenta-derived mesenchymal stem cells possess better immunoregulatory properties compared to their cord-derived counterparts-a paired sample study. *Sci Rep*. 2015;5(July):1-12. doi:10.1038/srep15784

130. Parolini O, Caruso M. Review: Preclinical studies on placenta-derived cells and amniotic membrane: An update. *Placenta*. 2011;32:S186-S195. doi:10.1016/j.placenta.2010.12.016

131. Parolini O, Alviano F, Bergwerf I, et al. Toward cell therapy using placenta-derived cells: Disease mechanisms, cell biology, preclinical studies, and regulatory aspects at the round table. *Stem Cells Dev*. 2010. doi:10.1089/scd.2009.0404

132. Parolini O, Knöfler M, Abumaree M. New frontiers in placenta stem cell research, translation, and clinical application. *Placenta*. 2017;59:73. doi:10.1016/j.placenta.2017.07.015

133. Antoniadou E, David AL. Placental stem cells. *Best Pract Res Clin Obstet Gynaecol*. 2016;31:13-29. doi:10.1016/j.bpobgyn.2015.08.014

134. Hao Y, Ma DHK, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. *Cornea*. 2000. doi:10.1097/00003226-200005000-00018

135. Stubbendorff M, Deuse T, Hua X, et al. Immunological properties of extraembryonic human mesenchymal stromal cells derived from gestational tissue. *Stem Cells Dev*. 2013. doi:10.1089/scd.2013.0043

136. Choi JH, Jung J, Na KH, Cho KJ, Yoon TK, Kim GJ. Effect of mesenchymal stem cells and extracts derived from the placenta on trophoblast invasion and immune responses. *Stem Cells Dev*. 2014. doi:10.1089/scd.2012.0674

137. Abumaree MH, Al Jumah MA, Kalionis B, et al. Human Placental Mesenchymal Stem Cells (pMSCs) Play a Role as Immune Suppressive Cells by Shifting Macrophage Differentiation from Inflammatory M1 to Anti-inflammatory M2 Macrophages. *Stem Cell Rev Reports*. 2013. doi:10.1007/s12015-013-9455-2

138. Abomaray FM, Al Jumah MA, Kalionis B, et al. Human Chorionic Villous Mesenchymal Stem Cells Modify the Functions of Human Dendritic Cells, and Induce an Anti-Inflammatory Phenotype in CD1+ Dendritic Cells. *Stem Cell Rev Reports*. 2015. doi:10.1007/s12015-014-9562-8

139. Magatti M, De Munari S, Vertua E, et al. Amniotic mesenchymal tissue cells inhibit dendritic cell differentiation of peripheral blood and amnion resident monocytes. *Cell Transplant*. 2009. doi:10.3727/096368909X471314

140. Mamede AC, Carvalho MJ, Abrantes AM, Laranjo M, Maia CJ, Botelho MF. Amniotic membrane: from structure and functions to clinical applications. *Cell Tissue Res*. 2012;349(2):447-458. doi:10.1007/s00441-012-1424-6

141. Benedetti WL, Sala MA, Alvarez H. Histochemical demonstration of enzymes in the umbilical cord and membranes of human term pregnancy. *Eur J Obstet Gynecol Reprod*

Biol. 1973. doi:10.1016/0028-2243(73)90061-0

142. Li H, Niederkorn JY, Neelam S, et al. Immunosuppressive factors secreted by human amniotic epithelial cells. *Investig Ophthalmol Vis Sci*. 2005. doi:10.1167/iovs.04-0495

143. Davis JS. Skin transplantation with a review of 550 cases at the Johns Hopkins Hospital. *Johns Hopkins Med J*. 1910.

144. Toda A, Okabe M, Yoshida T, Nikaido T. The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. *J Pharmacol Sci*. 2007;105(3):215-228. doi:10.1254/jphs.CR0070034

145. Bourne GL. The microscopic anatomy of the human amnion and chorion. *Am J Obstet Gynecol*. 1960. doi:10.1016/0002-9378(60)90512-3

146. Benirschke K, Burton GJ, Baergen RN. *Pathology of the Human Placenta, Sixth Edition*.; 2012. doi:10.1007/978-3-642-23941-0

147. Kubo M, Sonoda Y, Muramatsu R, Usui M. Immunogenicity of human amniotic membrane in experimental xenotransplantation. *Investig Ophthalmol Vis Sci*. 2001.

148. Wolbank S, Peterbauer A, Fahrner M, et al. Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: A comparison with human mesenchymal stem cells from adipose tissue. *Tissue Eng*. 2007. doi:10.1089/ten.2006.0313

149. Bailo M, Soncini M, Vertua E, et al. Engraftment potential of human amnion and chorion cells derived from term placenta. *Transplantation*. 2004. doi:10.1097/01.TP.0000144606.84234.49

150. Banas RA, Trumpower C, Bentlejewski C, Marshall V, Sing G, Zeevi A. Immunogenicity and immunomodulatory effects of amnion-derived multipotent progenitor cells. *Hum Immunol*. 2008. doi:10.1016/j.humimm.2008.04.007

151. Rossi D, Pianta S, Magatti M, Sedlmayr P, Parolini O. Characterization of the Conditioned Medium from Amniotic Membrane Cells: Prostaglandins as Key Effectors of Its Immunomodulatory Activity. *PLoS One*. 2012. doi:10.1371/journal.pone.0046956

152. Magatti M, Caruso M, De Munari S, et al. Human amniotic membrane-derived mesenchymal and epithelial cells exert different effects on monocyte-derived dendritic cell differentiation and function. *Cell Transplant*. 2015;24(9):1733-1752. doi:10.3727/096368914X684033

153. Cargnoni A, Ressel L, Rossi D, et al. Conditioned medium from amniotic mesenchymal tissue cells reduces progression of bleomycin-induced lung fibrosis. *Cytotherapy*. 2012. doi:10.3109/14653249.2011.613930

154. Magatti M, Vertua E, De Munari S, et al. Human amnion favours tissue repair by inducing the M1-to-M2 switch and enhancing M2 macrophage features. *J Tissue Eng Regen Med*. 2017;11(10):2895-2911. doi:10.1002/term.2193

155. Cross JC. Placental function in development and disease. *Reprod Fertil Dev*. 2006;18(1-2):71-76. doi:10.1071/RD05121

156. McIntyre HD, Catalano P, Zhang C, Desoye G, Mathiesen ER, Damm P. Gestational diabetes mellitus. *Nat Rev Dis Prim*. 2019;5(1). doi:10.1038/s41572-019-0098-8

157. Chen C, Xu X, Yan Y. Estimated global overweight and obesity burden in pregnant

women based on panel data model. *PLoS One*. 2018. doi:10.1371/journal.pone.0202183

158. Pallardo F, Herranz L, Garcia-Ingelmo T, et al. Early postpartum metabolic assessment in women with prior gestational diabetes. *Diabetes Care*. 1999. doi:10.2337/diacare.22.7.1053

159. Xu Y, Shen S, Sun L, Yang H, Jin B, Cao X. Metabolic syndrome risk after gestational diabetes: A systematic review and meta-analysis. *PLoS One*. 2014. doi:10.1371/journal.pone.0087863

160. Monteiro LJ, Norman JE, Rice GE, Illanes SE. Fetal programming and gestational diabetes mellitus. *Placenta*. 2016;48:S54-S60. doi:10.1016/j.placenta.2015.11.015

161. Catalano PM, Huston L, Amini SB, Kalhan SC. Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. In: *American Journal of Obstetrics and Gynecology*. ; 1999. doi:10.1016/S0002-9378(99)70662-9

162. Buchanan TA. Pancreatic B-Cell Defects in Gestational Diabetes: Implications for the Pathogenesis and Prevention of Type 2 Diabetes. *J Clin Endocrinol Metab*. 2001. doi:10.1210/jc.86.3.989

163. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: Risks and management during and after pregnancy. *Nat Rev Endocrinol*. 2012. doi:10.1038/nrendo.2012.96

164. Zhang C, Tobias DK, Chavarro JE, et al. Adherence to healthy lifestyle and risk of gestational diabetes mellitus: Prospective cohort study. *BMJ*. 2014. doi:10.1136/bmj.g5450

165. Dixit A, Girling JC. Obesity and pregnancy. *J Obstet Gynaecol (Lahore)*. 2008. doi:10.1080/01443610701814203

166. Solomon CG, Willett WC, Carey VJ, et al. A prospective study of pregravid determinants of gestational diabetes mellitus. *J Am Med Assoc*. 1997. doi:10.1001/jama.278.13.1078

167. Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. *Diabetes Care*. 2007. doi:10.2337/dc07-s202

168. Friedman JE, Ishizuka T, Shao J, Huston L, Highman T, Catalano P. Impaired glucose transport and insulin receptor tyrosine phosphorylation in skeletal muscle from obese women with gestational diabetes. *Diabetes*. 1999. doi:10.2337/diabetes.48.9.1807

169. Garvey WT, Maianu L, Zhu JH, Brechtel-Hook G, Wallace P, Baron AD. Evidence for defects in the trafficking and translocation of GLUT4 glucose transporters in skeletal muscle as a cause of human insulin resistance. *J Clin Invest*. 1998. doi:10.1172/JCI1557

170. Stewart FM, Freeman DJ, Ramsay JE, Greer IA, Caslake M, Ferrell WR. Longitudinal assessment of maternal endothelial function and markers of inflammation and placental function throughout pregnancy in lean and obese mothers. *J Clin Endocrinol Metab*. 2007. doi:10.1210/jc.2006-2083

171. Kleiblova P, Dostalova I, Bartlova M, et al. Expression of adipokines and estrogen receptors in adipose tissue and placenta of patients with gestational diabetes mellitus. *Mol Cell Endocrinol*. 2010;314(1):150-156. doi:10.1016/j.mce.2009.08.002

172. Morisset AS, Dubé MC, Côté JA, Robitaille J, Weisnagel SJ, Tchernof A. Circulating

interleukin-6 concentrations during and after gestational diabetes mellitus. *Acta Obstet Gynecol Scand*. 2011. doi:10.1111/j.1600-0412.2011.01094.x

173. Noureldeen AFH, Qusti SY, Al-Seeni MN, Bagais MH. Maternal leptin, adiponectin, resistin, visfatin and tumor necrosis factor-alpha in normal and gestational diabetes. *Indian J Clin Biochem*. 2014. doi:10.1007/s12291-013-0394-0

174. Aguirre V, Uchida T, Yenush L, Davis R, White MF. The c-Jun NH2-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser307. *J Biol Chem*. 2000. doi:10.1074/jbc.275.12.9047

175. Gao Z, Hwang D, Bataille F, et al. Serine phosphorylation of insulin receptor substrate 1 by inhibitor κB kinase complex. *J Biol Chem*. 2002. doi:10.1074/jbc.M209459200

176. Li Y, Soos TJ, Li X, et al. Protein kinase C θ inhibits insulin signaling by phosphorylating IRS1 at Ser1101. *J Biol Chem*. 2004. doi:10.1074/jbc.C400186200

177. Aye ILMH, Lager S, Ramirez VI, et al. Increasing Maternal Body Mass Index Is Associated with Systemic Inflammation in the Mother and the Activation of Distinct Placental Inflammatory Pathways1. *Biol Reprod*. 2014. doi:10.1095/biolreprod.113.116186

178. Gauster M, Desoye G, Tötsch M, Hiden U. The Placenta and Gestational Diabetes Mellitus. *Curr Diab Rep*. 2012;12(1):16-23. doi:10.1007/s11892-011-0244-5

179. Desoye G. The human placenta in diabetes and obesity: Friend or foe? The 2017 Norbert Freinkel award lecture. *Diabetes Care*. 2018;41(7):1362-1369. doi:10.2337/dci17-0045

180. Agha-Jaffar R, Oliver N, Johnston D, Robinson S. Gestational diabetes mellitus: Does an effective prevention strategy exist? *Nat Rev Endocrinol*. 2016;12(9):533-546. doi:10.1038/nrendo.2016.88

181. Kuzmicki M, Telejko B, Wawrusiewicz-Kurylonek N, et al. The expression of suppressor of cytokine signaling 1 and 3 in fat and placental tissue from women with gestational diabetes. *Gynecol Endocrinol*. 2012. doi:10.3109/09513590.2012.683055

182. Lepercq J, Cauzac M, Lahlou N, et al. Overexpression of placental leptin in diabetic pregnancy: A critical role for insulin. *Diabetes*. 1998. doi:10.2337/diabetes.47.5.847

183. Marseille-Tremblay C, Ethier-Chiasson M, Forest J-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):1054-1062. doi:10.1002/mrd.20842

184. Coughlan MT, Oliva K, Georgiou HM, Permezel JM, Rice GE. Glucose-induced release of tumour necrosis factor-alpha from human placental and adipose tissues in gestational diabetes mellitus. *Diabet Med*. 2001;18(11):921-927.

185. 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. doi:10.1016/j.placenta.2016.12.006

186. Lager S, Jansson N, Olsson AL, Wennergren M, Jansson T, Powell TL. Effect of IL-6 and TNF-α on fatty acid uptake in cultured human primary trophoblast cells. *Placenta*. 2011. doi:10.1016/j.placenta.2010.10.012

187. Varastehpour A, Radaelli T, Minium J, et al. Activation of phospholipase A2 is associated with generation of placental lipid signals and fetal obesity. *J Clin Endocrinol Metab*. 2006. doi:10.1210/jc.2005-0873

188. Jones HN, Jansson T, Powell TL. IL-6 stimulates system A amino acid transporter activity in trophoblast cells through STAT3 and increased expression of SNAT2. *Am J Physiol - Cell Physiol*. 2009. doi:10.1152/ajpcell.00195.2009

189. Devi Singh S. Gestational diabetes and its effect on the umbilical cord. *Early Hum Dev*. 1986. doi:10.1016/0378-3782(86)90114-3

190. Wajid N, Naseem R, Anwar SS, et al. The effect of gestational diabetes on proliferation capacity and viability of human umbilical cord-derived stromal cells. *Cell Tissue Bank*. 2015;16(3):389-397. doi:10.1007/s10561-014-9483-4

191. An B, Kim E, Song H, et al. Gestational diabetes affects the growth and functions of perivascular stem cells. *Mol Cells*. 2017. doi:10.14348/molcells.2017.0053

192. Kong CM, Subramanian A, Biswas A, et al. Changes in Stemness Properties, Differentiation Potential, Oxidative Stress, Senescence and Mitochondrial Function in Wharton's Jelly Stem Cells of Umbilical Cords of Mothers with Gestational Diabetes Mellitus. *Stem Cell Rev Reports*. 2019. doi:10.1007/s12015-019-9872-y

193. Kim J, Piao Y, Pak YK, et al. Umbilical cord mesenchymal stromal cells affected by gestational diabetes mellitus display premature aging and mitochondrial dysfunction. *Stem Cells Dev*. 2015;24(5):575-586. doi:10.1089/scd.2014.0349

194. Amrithraj AI, Kodali A, Nguyen L, et al. Gestational Diabetes Alters Functions in Offspring's Umbilical Cord Cells With Implications for Cardiovascular Health. *Endocrinology*. 2017;158(7):2102-2112. doi:10.1210/en.2016-1889

195. Mathew SA, Bhonde R. Mesenchymal stromal cells isolated from gestationally diabetic human placenta exhibit insulin resistance, decreased clonogenicity and angiogenesis. *Placenta*. 2017;59:1-8. doi:10.1016/j.placenta.2017.09.002

196. Chen L, Merkhan MM, Forsyth NR, Wu P. Chorionic and amniotic membrane-derived stem cells have distinct, and gestational diabetes mellitus independent, proliferative, differentiation, and immunomodulatory capacities. *Stem Cell Res*. 2019. doi:10.1016/j.scr.2019.101537

197. 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. doi:10.1016/S0002-9378(03)00828-7

198. Athukorala C, Crowther CA, Willson K, et al. Women with gestational diabetes mellitus in the ACHOIS trial: Risk factors for shoulder dystocia. *Aust New Zeal J Obstet Gynaecol*. 2007. doi:10.1111/j.1479-828X.2006.00676.x

199. Alam M, Raza SJ, Sherali AR, Akhtar SM. Neonatal complications in infants born to diabetic mothers. *J Coll Physicians Surg Pakistan*. 2006. doi:10.1016/j.earlhumdev.2008.09.040

200. Cordero L, Treuer SH, Landon MB, Gabbe SG. Management of infants of diabetic mothers. *Arch Pediatr Adolesc Med*. 1998. doi:10.1001/archpedi.152.3.249

201. Zhao P, Liu E, Qiao Y, et al. Maternal gestational diabetes and childhood obesity at age 9–11: results of a multinational study. *Diabetologia*. 2016. doi:10.1007/s00125-016-4062-9

202. Schaefer-Graf UM, Graf K, Kulbacka I, et al. Maternal lipids as strong determinants of fetal environment and growth in pregnancies with gestational diabetes mellitus.

Diabetes Care. 2008;31(9):1858-1863. doi:10.2337/dc08-0039

203. Cohen MS. Fetal and childhood onset of adult cardiovascular diseases. *Pediatr Clin North Am*. 2004;51(6):1697-1719. doi:10.1016/j.pcl.2004.08.001

204. Bao W, Srinivasan SR, Wattigney WA, Bao W, Berenson GS. Usefulness of Childhood Low-Density Lipoprotein Cholesterol Level in Predicting Adult Dyslipidemia and Other Cardiovascular Risks. *Arch Intern Med*. 1996;156(12):1315-1320. doi:10.1001/archinte.1996.00440110083011

205. Shokry E, Marchioro L, Uhl O, et al. Transgenerational cycle of obesity and diabetes: investigating possible metabolic precursors in cord blood from the PREOBE study. *Acta Diabetol*. 2019;56(9):1073-1082. doi:10.1007/s00592-019-01349-y

206. Simpson J, Smith AD, Fraser A, et al. Cord Blood Adipokines and Lipids and Adolescent Nonalcoholic Fatty Liver Disease. *J Clin Endocrinol Metab*. 2016;101(12):4661-4668. doi:10.1210/jc.2016-2604

207. Hellmuth C, Uhl O, Standl M, et al. Cord Blood Metabolome Is Highly Associated with Birth Weight, but Less Predictive for Later Weight Development. *Obes Facts*. 2017;10(2):85-100. doi:10.1159/000453001

208. Stratakis N, Gielen M, Margetaki K, et al. Polyunsaturated fatty acid status at birth, childhood growth, and cardiometabolic risk: a pooled analysis of the MEFAB and RHEA cohorts. *Eur J Clin Nutr*. May 2018. doi:10.1038/s41430-018-0175-1

209. Standl M, Thiering E, Demmelmair H, Koletzko B, Heinrich J. Age-dependent effects of cord blood long-chain PUFA composition on BMI during the first 10 years of life. *Br J Nutr*. 2014;111(11):2024-2031. doi:10.1017/S0007114514000105

210. Donahue SMA, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. *Am J Clin Nutr*. 2011;93(4):780-788. doi:10.3945/ajcn.110.005801

211. Mallol R, Amigó N, Rodríguez MA, et al. Liposcale: a novel advanced lipoprotein test based on 2D diffusion-ordered 1H NMR spectroscopy. *J Lipid Res*. 2015;56(3):737-746. doi:10.1194/jlr.D050120

212. Mallol R, Rodriguez MA, Brezmes J, Masana L, Correig X. Human serum/plasma lipoprotein analysis by NMR: Application to the study of diabetic dyslipidemia. *Prog Nucl Magn Reson Spectrosc*. 2013;70:1-24. doi:10.1016/j.pnmrs.2012.09.001

213. Krauss RM. Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care*. 2004. doi:10.2337/diacare.27.6.1496

214. Goldberg IJ. Clinical review 124: Diabetic dyslipidemia - Causes and consequences. *J Clin Endocrinol Metab*. 2001. doi:10.1210/jc.86.3.965

215. Ginsberg HN, Zhang YL, Hernandez-Ono A. Regulation of plasma triglycerides in insulin resistance and diabetes. *Arch Med Res*. 2005. doi:10.1016/j.arcmed.2005.01.005

216. Howard B V. Insulin resistance and lipid metabolism. In: *American Journal of Cardiology*. ; 1999. doi:10.1016/S0002-9149(99)00355-0

217. Wajid N, Naseem R, Anwar SS, et al. The effect of gestational diabetes on proliferation capacity and viability of human umbilical cord-derived stromal cells. *Cell Tissue Bank*. 2015. doi:10.1007/s10561-014-9483-4

218. Islam SU, Shehzad A, Lee YS. Prostaglandin E2 inhibits resveratrol-induced apoptosis through activation of survival signaling pathways in HCT-15 cell lines. *Animal Cells Syst (Seoul)*. 2015. doi:10.1080/19768354.2015.1101398

219. Ottonello L, Gonella R, Dapino P, Sacchetti C, Dallegri F. Prostaglandin E2 inhibits apoptosis in human neutrophilic polymorphonuclear leukocytes: Role of intracellular cyclic AMP levels. *Exp Hematol*. 1998.

220. Kalinski P. Regulation of Immune Responses by Prostaglandin E 2 . *J Immunol*. 2012. doi:10.4049/jimmunol.1101029

221. Chen J-R, Lazarenko OP, Blackburn ML, et al. Maternal Obesity Programs Senescence Signaling and Glucose Metabolism in Osteo-Progenitors From Rat and Human. *Endocrinology*. 2016;157(11):4172-4183. doi:10.1210/en.2016-1408

222. Yañez R, Oviedo A, Aldea M, Bueren JA, Lamana ML. Prostaglandin E2 plays a key role in the immunosuppressive properties of adipose and bone marrow tissue-derived mesenchymal stromal cells. *Exp Cell Res*. 2010. doi:10.1016/j.yexcr.2010.08.008

223. Bouffi C, Bony C, Courties G, Jorgensen C, Noël D. IL-6-dependent PGE2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis. *PLoS One*. 2010. doi:10.1371/journal.pone.0014247

224. Németh K, Leelahavanichkul A, Yuen PST, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E 2-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med*. 2009. doi:10.1038/nm.1905

225. Mao YX, Xu JF, Seeley EJ, et al. Adipose tissue-derived mesenchymal stem cells attenuate pulmonary infection caused by Pseudomonas aeruginosa via inhibiting overproduction of prostaglandin E2. *Stem Cells*. 2015. doi:10.1002/stem.1996

226. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest*. 2003;112(12):1796-1808. doi:10.1172/JCI19246

227. Marseille-Tremblay C, Ethier-Chiasson M, Forest JC, et al. Impact of maternal circulating cholesterol and gestational diabetes mellitus on lipid metabolism in human term placenta. *Mol Reprod Dev*. 2008. doi:10.1002/mrd.20842

228. Challier JC, Basu S, Bintein T, et al. Obesity in Pregnancy Stimulates Macrophage Accumulation and Inflammation in the Placenta. *Placenta*. 2008. doi:10.1016/j.placenta.2007.12.010

229. Kurtzhals LL, Nørgaard SK, Secher AL, et al. The impact of restricted gestational weight gain by dietary intervention on fetal growth in women with gestational diabetes mellitus. *Diabetologia*. 2018;61(12):2528-2538. doi:10.1007/s00125-018-4736-6

230. Sacks D a, Liu AI, Wolde-Tsadik G, Amini SB, Huston-Presley L, Catalano PM. What proportion of birth weight is attributable to maternal glucose among infants of diabetic women? *Am J Obstet Gynecol*. 2006;194(2):501-507. doi:10.1016/j.ajog.2005.07.042

231. Yadav A, Saini V, Arora S. MCP-1: Chemoattractant with a role beyond immunity: A review. *Clin Chim Acta*. 2010;411(21-22):1570-1579. doi:10.1016/j.cca.2010.07.006

232. Kherkeulidze P, Johansson J, Carlson LA. High density lipoprotein particle size distribution in cord blood. *Acta Paediatr Scand*. 1991;80(8-9):770-779.

233. Dolphin PJ, Breckenridge WC, Dolphin MA, Tan MH. The lipoproteins of human

umbilical cord blood apolipoprotein and lipid levels. *Atherosclerosis*. 1984;51(1):109-122. doi:10.1016/0021-9150(84)90147-3

234. Parker CR, Carr BR, Simpson ER, MacDonald PC. Decline in the concentration of low-density lipoprotein-cholesterol in human fetal plasma near term. *Metabolism*. 1983;32(9):919-923. doi:10.1016/0026-0495(83)90207-X

235. Parker CR, Fortunato SJ, Carr BR, Owen J, Hankins GDV, Hauth JC. Apolipoprotein A-1 in umbilical cord blood of newborn infants: Relation to gestational age and high-density lipoprotein cholesterol. *Pediatr Res*. 1988. doi:10.1203/00006450-198804000-00002

236. Fordyce MK, Duncan R, Chao R, et al. Cord blood serum in newborns of diabetic mothers. *J Chronic Dis*. 1983;36(3):263-268. doi:10.1016/0021-9681(83)90061-9

237. Kilby MD, Neary RH, Mackness MI, Durrington PN. Fetal and Maternal Lipoprotein Metabolism in Human Pregnancy Complicated by Type I Diabetes Mellitus [1]. *J Clin Endocrinol Metab*. 1998;83(5):1736-1741. doi:10.1210/jcem.83.5.4783

238. Loughrey CM, Rimm E, Heiss G, Rifai N. Race and gender differences in cord blood lipoproteins. *Atherosclerosis*. 2000;148(1):57-65. doi:10.1016/s0021-9150(99)00238-5

239. Lane DM, McConathy WJ. Factors Affecting the Lipid and Apolipoprotein Levels of Cord Sera. *Pediatr Res*. 1983;17(2):83-91. doi:10.1203/00006450-198302000-00001

240. Aletayeb SMH, Dehdashtian M, Aminzadeh M, et al. Correlation between umbilical cord blood lipid profile and neonatal birth weight. *Pediatr Pol*. 2013;88(6):521-525. doi:10.1016/J.PEPO.2013.08.004

241. Miranda J, Simões R V., Paules C, et al. Metabolic profiling and targeted lipidomics reveals a disturbed lipid profile in mothers and fetuses with intrauterine growth restriction. *Sci Rep*. 2018;8(1):13614. doi:10.1038/s41598-018-31832-5

242. Kim S-M, Lee SM, Kim S-J, et al. Cord and maternal sera from small neonates share dysfunctional lipoproteins with proatherogenic properties: Evidence for Barker's hypothesis. *J Clin Lipidol*. 2017;11(6):1318-1328.e3. doi:10.1016/j.jacl.2017.08.020

243. Koklu E, Akcakus M, Kurtoglu S, et al. Aortic intima-media thickness and lipid profile in macrosomic newborns. *Eur J Pediatr*. 2007;166(4):333-338. doi:10.1007/s00431-006-0243-8

244. Koklu E, Kurtoglu S, Akcakus M, et al. Increased Aortic Intima-Media Thickness Is Related to Lipid Profile in Newborns with Intrauterine Growth Restriction. *Horm Res Paediatr*. 2006;65(6):269-275. doi:10.1159/000092536

245. Schaefer-Graf UM, Meitzner K, Ortega-Senovilla H, 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):1053-1059. doi:10.1111/j.1464-5491.2011.03346.x

246. Hassing HC, Surendran RP, Mooij HL, Stroes ES, Nieuwdorp M, Dallinga-Thie GM. Pathophysiology of hypertriglyceridemia. *Biochim Biophys Acta - Mol Cell Biol Lipids*. 2012;1821(5):826-832. doi:10.1016/j.bbalip.2011.11.010

247. Chahil TJ, Ginsberg HN. Diabetic Dyslipidemia. *Endocrinol Metab Clin North Am*. 2006. doi:10.1016/j.ecl.2006.06.002

248. Grundy SM, Pasternak R, Greenland P, Smith S, Fuster V. Assessment of cardiovascular risk by use of multiple-risk-factor assessment equations: A statement for healthcare

professionals from the American Heart Association and the American College of Cardiology. *Circulation*. 1999. doi:10.1161/01.CIR.100.13.1481

249. Wilson PWF, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation*. 1998. doi:10.1161/01.CIR.97.18.1837

250. Morrish NJ, Wang SL, Stevens LK, Fuller JH, Keen H. Mortality and causes of death in the WHO multinational study of vascular disease in diabetes. *Diabetologia*. 2001. doi:10.1007/PL00002934

251. Chan TC, Schwartz JJ, Garcia RE, Chin HP, Barndt R. Total serum cholesterol and plasma lipoprotein cholesterol concentrations in cord sera of newborns from hispanic mothers with gestational diabetes. *Artery*. 1988;15(4):203-216.

252. Albers JJ, Slee A, Fleg JL, O'Brien KD, Marcovina SM. Relationship of baseline HDL subclasses, small dense LDL and LDL triglyceride to cardiovascular events in the AIM-HIGH clinical trial. *Atherosclerosis*. 2016. doi:10.1016/j.atherosclerosis.2016.06.019

253. März W, Scharnagl H, Winkler K, et al. Low-density lipoprotein triglycerides associated with low-grade systemic inflammation, adhesion molecules, and angiographic coronary artery disease: The Ludwigshafen Risk and Cardiovascular Health Study. *Circulation*. 2004. doi:10.1161/01.CIR.0000146898.06923.80

254. Libby P. Fat fuels the flame: Triglyceride-rich lipoproteins and arterial inflammation. *Circ Res*. 2007. doi:10.1161/01.RES.0000259393.89870.58

255. Gordts PLSM, Nock R, Son NH, et al. ApoC-III inhibits clearance of triglyceride-rich lipoproteins through LDL family receptors. *J Clin Invest*. 2016. doi:10.1172/JCI86610

256. Cho KH. Biomedicinal implications of high-density lipoprotein: Its composition, structure, functions, and clinical applications. *BMB Rep*. 2009. doi:10.5483/BMBRep.2009.42.7.393

257. Kharb S, Kaur R, Singh V, Sangwan K. Birth weight, cord blood lipoprotein and apolipoprotein levels in Indian Newborns. *Int J Prev Med*. 2010. doi:10.4103/ijpvm.ijpvm_72_17

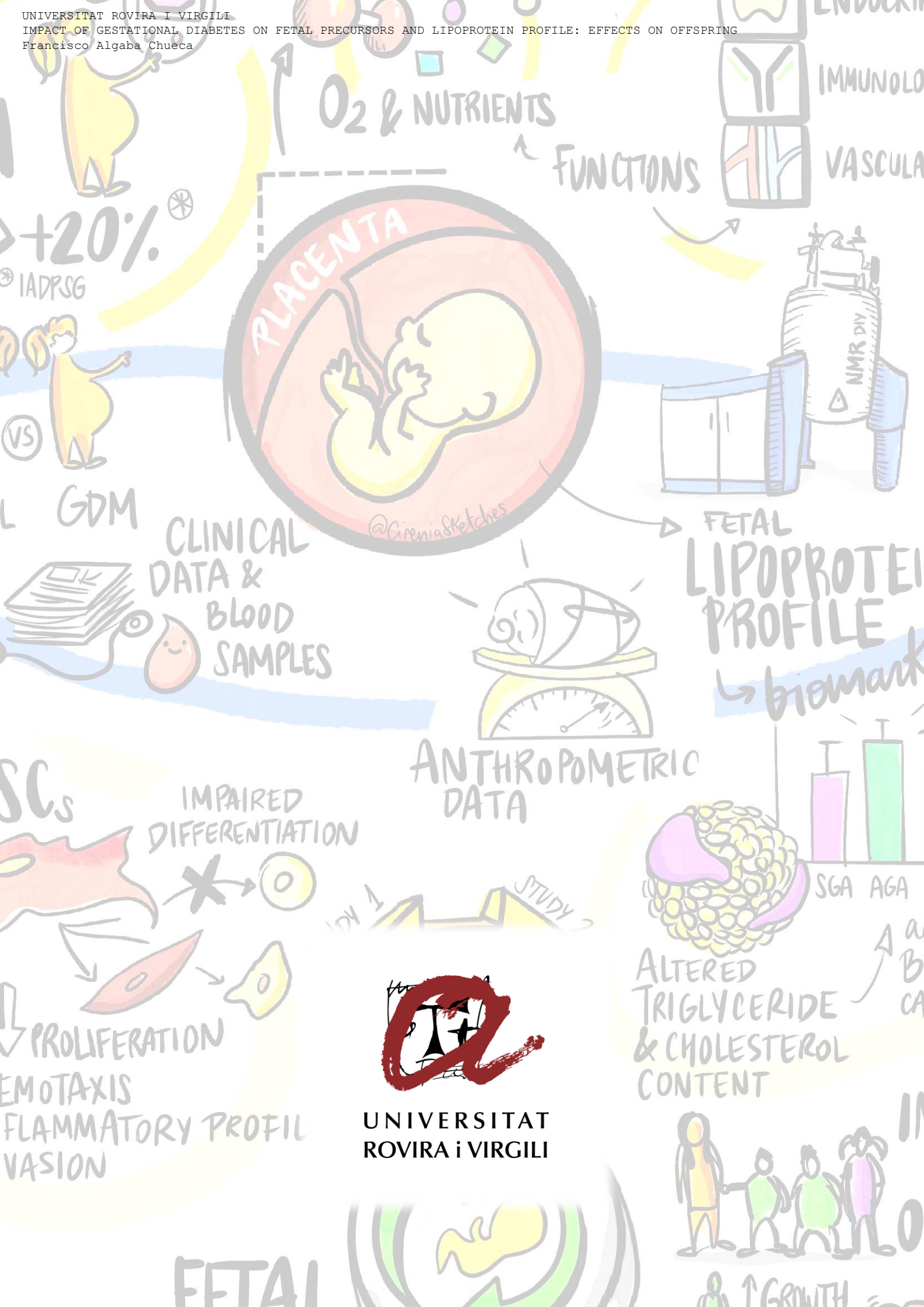
258. Ramy N, Zakaria M, El Kafoury M, Kamal M. Cord blood lipid profile in relation to anthropometric measures of newborns. *Kasr Al Ainy Med J*. 2017;23(1):54. doi:10.4103/kamj.kamj_5_17

259. Nayak CD, Agarwal V, Nayak DM. Correlation of cord blood lipid heterogeneity in neonates with their anthropometry at birth. *Indian J Clin Biochem*. 2013. doi:10.1007/s12291-012-0252-5

260. Horton JD, Shimano H, Hamilton RL, Brown MS, Goldstein JL. Disruption of LDL receptor gene in transgenic SREBP-1a mice unmasks hyperlipidemia resulting from production of lipid-rich VLDL. *J Clin Invest*. 1999. doi:10.1172/JCI6246

261. Tank S, Jain SK. Altered cord blood lipid profile, insulin resistance & growth restriction during the perinatal period & its potential role in the risk of developing cardiovascular disease later in life. *Indian J Med Res*. 2016. doi:10.4103/0971-5916.195021

UNIVERSITAT ROVIRA I VIRGILI
IMPACT OF GESTATIONAL DIABETES ON FETAL PRECURSORS AND LIPOPROTEIN PROFILE: EFFECTS ON OFFSPRING
Francisco Algaba Chueca



UNIVERSITAT
ROVIRA i VIRGILI