The Impact of Maternal Obesity in Pregnancy on Placental Glucocorticoid and Macronutrient Transport and Metabolism

Membrane Transporters and Receptors in Pregnancy Metabolic Complications

Title: The Impact of Maternal Obesity in Pregnancy on Placental Glucocorticoid and Macronutrient Transport and Metabolism

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Abstract

Maternal obesity is the most common metabolic disturbance in pregnancy affecting more than 1 in 5 women in some countries. Babies born to obese women are heavier with more adiposity at birth, and are vulnerable to obesity and metabolic disease across the lifespan suggesting offspring health is 'programmed' by fetal exposure to an obese intra-uterine environment. The placenta plays a major role in dictating the impact of maternal health on prenatal development. Maternal obesity impacts the function of integral placental receptors and transporters for glucocorticoids and nutrients, key drivers of fetal growth, though mechanisms remain poorly understood. This review aims to summarise current knowledge in this area, and considers the impact of obesity on the epigenetic machinery of the placenta at this vital juncture in offspring development. Further research is required to advance understanding of these areas in the hope that the trans-generational cycle of obesity can be alleviated.

Introduction

Obesity is one of the greatest public health challenges of the 21st century. Since 1980, the prevalence of obesity (body mass index [BMI] ≥ 30kg/m^2) has doubled in more than seventy countries and has steadily increased in most other countries, across the range of socioeconomic development [1]. In 2016, over 650 million adults were estimated to be obese, representing approximately 13% of the world's adult population. At this time, over 340 million children and adolescents aged 5 to 19 years were estimated to be overweight or obese, a prevalence of 18% in this age group [2]. In this context, it is unsurprising that rates of maternal obesity are also rising. Estimates of the prevalence of pre-pregnancy obesity across Europe in 2010 ranged from 7.1% in Poland to 20.7% in Scotland [3]. In 2011-12, the prevalence of obesity in women age 20-39 years in the United States was approximately 31.8% [4].

Obesity during pregnancy is an important risk factor for adverse health outcomes in the mother and offspring. Obese women have an increased risk of pregnancy complications compared to normal weight women including gestational diabetes mellitus, gestational hypertension, pre-eclampsia, caesarean section, postpartum haemorrhage and stillbirth [5,6]. Adverse phenotypes in the offspring are evident in the neonatal setting, with babies born to obese mothers demonstrating greater adiposity and increased umbilical cord blood insulin and inflammatory cytokine levels compared to babies born to lean mothers [7]. Maternal BMI correlates positively with birthweight and the offspring are typically born heavier than those born to lean women [8,9]. Maternal obesity is associated with an elevated offspring risk of metabolic and cardiovascular disease in child- and adulthood including obesity, type 2 diabetes mellitus, coronary heart disease, stroke and cardiovascular disease [10–12]. The offspring of obese women also have an elevated risk of childhood asthma, type 1 diabetes (in the absence of parental diabetes), and may display poorer cognitive performance than those born to lean mothers [13–16]. Intrauterine growth restriction (IUGR) is less common in the context of maternal obesity, occurring in approximately 5 to 7% of pregnancies, and may relate to placental vascular dysfunction, for example in the context of pre-eclampsia [17,18]. Offspring who fail to reach their genetically

determined growth potential in-utero display increased growth velocity during childhood but remain shorter and lighter than their normal counterparts aged 9 years and have an increased risk of short and long-term adverse health outcomes [19,20].

The relationship between maternal health and fetal development is closely mediated by the placenta, which controls many aspects of the intra-uterine environment including oxygen delivery, nutrient transport and hormone production and transfer to the developing fetus. The placenta therefore plays a key role in developmental 'programming', the phenomenon whereby early life development determines susceptibility to disease in adulthood [21]. Mechanistic studies demonstrate that maternal obesity is associated with changes in placental function and structure which likely impact on fetal growth and development. For example, increased gene expression of the chemotactic cytokines interleukin (IL) 1β, IL 8 and monocyte chemoattractant protein-1 (MCP-1) and increased macrophage infiltration are evident in the placenta of obese women, suggesting an exaggeration of the inflammatory state which occurs in normal pregnancy [22,23]. Studies of placental histology indicate obesity is associated with hyperplasia of the muscular tissue of the tunica media in placental vessel walls and immaturity of the placental villous tree, as evidenced by fewer villi of larger diameter and with abnormal angiogenesis [22,24].

Alterations in placental nutrient and hormone transporter capacity have been demonstrated in human and animal models of obesity and are hypothesised as a mechanism leading to an accelerated fetal growth trajectory and macrosomia [25,26]**.** The Pedersen hypothesis, postulated in the 1920's, stated that maternal hyperglycaemia leads to fetal hyperglycaemia, fetal islet cell hypertrophy and fetal insulin hypersecretion. However, it has since been recognised that when present in excess, other macronutrients such as fatty acids and amino acids may behave as insulin secretagogues and contribute to accelerated fetal growth [27]. It is also now recognised that glucocorticoids are vitally important for fetal growth and that circulating cortisol levels are influenced by maternal obesity [28,29]. Placental handling of glucocorticoids therefore presenting a potential mechanism through which maternal obesity influences fetal development in-utero. Furthermore, the potential role of epigenetic processes during pregnancy to adversely programme the health of offspring has received increasing attention and is important to

consider. This review will focus on the impact of maternal obesity on placental glucocorticoid and macronutrient transfer and epigenetic processes, considering the mechanisms which may influence maternal and fetal health in this setting.

Glucocorticoids

Glucocorticoids in fetal development

Glucocorticoids are essential for fetal growth, maturation and survival, and are transported from the maternal to the fetal circulation via placental syncytiotrophoblasts. In-utero glucocorticoid exposure, which is dependent on the activity of the maternal hypothalamic-pituitary-adrenal (HPA) axis and placental glucocorticoid transfer, has a crucial impact on both fetal development and, potentially, life-long health [28]. In humans, levels of the major circulating glucocorticoid cortisol are under regulation of the HPA axis, which undergoes dramatic activation in pregnancy. During the second and third trimesters, the placenta secretes high levels of corticotrophin-releasing hormone into the maternal circulation, promoting release of adrenocorticotrophic hormone (ACTH) and cortisol from the anterior pituitary and adrenal glands respectively [30]. Rising cortisol levels in the maternal circulation stimulate further corticotrophin-releasing hormone release from the placenta, resulting in a positive feedback loop [31]. Additionally, the high oestrogen state of pregnancy promotes increased hepatic production of cortisol binding globulin (CBG). CBG is the principal plasma binding protein for cortisol, and in order to maintain the proportion of bound versus unbound cortisol, there is an associated increase in serum levels of free, or active, cortisol [30]. As a consequence of these mechanisms, there is a three-fold increase in total serum levels of cortisol, between the first and third trimesters [32]. The fetal adrenal glands produce cortisol transiently from approximately 7 to 10 weeks gestation, with progressively increasing production occurring during the third trimester [33].

Studies of the HPA axis in obese pregnant women show that total cortisol levels are lower throughout pregnancy than in lean women [29]. HPA axis activity appears blunted in these women with a lack of rise of corticotrophin-releasing hormone and cortisol binding globulin and a reduction in the pulse frequency of cortisol secretion [29,34]. This failure of the

maternal HPA axis to activate as observed in lean pregnant women, results in lower than normal fetal glucocorticoid exposure, and has been postulated as a factor contributing to macrosomia and later gestation at delivery in severely obese women [29,35]. Conversely, over-exposure to glucocorticoids in-utero is associated with intrauterine growth restriction, shorter gestation at delivery, and increased risk of cardiometabolic disease and psychiatric disease in adulthood [28,36,37].

Placental glucocorticoid receptors and transporters

Fetal exposure to glucocorticoids is mediated by a range of key placental receptors and transporters (Figure 1). Glucocorticoids elicit most of their effects through the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). These intracellular, structurally similar receptors are members of a superfamily of ligand-dependent transcription factors [38]. The GR is ubiquitously expressed throughout the body and encoded by the gene NR3C1 (nuclear receptor subfamily 3, group C, member 1) on chromosome 5 [39]. In-utero, GR expression is evident in most fetal tissues from mid-gestation onwards, and is highly expressed in the placenta and fetal membranes [40]. The human placenta possesses at least eight isoforms of GR: GRα-A, GRβ, GRα-C, GRP, GRA, GRα-D1, GRα-D2 and GRα-D3 [41]. Differential expression of GR isoforms have been suggested to influence placental glucocorticoid sensitivity and fetal growth. For example, GRβ and GRα-D1 expression were significantly higher in term placentae associated with a small for gestational age fetus [41]. The MR is coded for by the NR3C2 gene (nuclear receptor subfamily 3, group C, member 2) on chromosome 4 and is expressed in the syncytiotrophoblast, cytotrophoblast and interstitial cells of the villous core [42]. Whilst it has a similar binding affinity for mineralocorticoids and glucocorticoids, its major role is to mediate the effects of the vasoactive hormone aldosterone to control sodium and water balance [43]. Aldosterone levels rise during pregnancy and observations that adrenalectomized ewes demonstrate reduced placental mass despite cortisol supplementation, but not when supplemented with aldosterone, suggest aldosterone plays an important role in placental growth [44,45]. The relative density of MR and GR receptors in the placenta across gestation is not known. Aldosterone has been suggested to act as a growth factor for several tissues including the placenta, renal mesangial cells and cardiac

fibroblasts however the mechanism of this effect is uncertain [44,46,47]. Inactivation of cortisol by 11β-HSD-2 allows aldosterone to effectively stimulate the MR and limits activation of GR. 11β-HSD-2 activity may be altered in association with pregnancy metabolic complications, resulting in a change in MR/GR receptor activation. For example, in the context of maternal obesity, it is hypothesised that 11β-HSD-2 activity is increased, resulting in comparatively less GR and more MR receptor activation.

Another family of membrane proteins, the ATP-binding cassette (ABC) transporters, are implicated in the placental regulation of fetal glucocorticoid exposure. These transporters efflux a wide variety of compounds against their concentration gradient across intra- and extracellular membranes by utilising energy created through ATP hydrolysis [48]. In the placenta, several ABC transporters have been identified in the syncytiotrophoblast and fetal vessel endothelium (Table 1) [49]. As ABC transporters efflux substrates out of the cell, their placement on the opposing microvillous membrane (MVM) and basolateral membrane (BM) of the syncytium perhaps provides insight into their physiological roles [49]. Key transporters implicated in placental glucocorticoid transfer include permeability glycoprotein (encoded by ABCB1), breast cancer resistance protein (encoded by ABCG2) and the multidrug resistance associated protein-1 (encoded by ABCC1) [48]. ABCC1 exports corticosterone (not cortisol) and is predominantly localised on the fetal facing BM [49,50]. Corticosterone circulates at 10-20-fold lower concentrations than cortisol in the human plasma, and its physiological role remains uncertain [51]. On the other hand, ABCB1 exports cortisol (not corticosterone) and is located on the maternal facing MVM. Thus, these membrane transporters likely play a role in fine-tuning fetal glucocorticoid exposure. Further investigation is required to clarify the role of ABC transporters, including whether this is altered in context of maternal obesity, in regulating placental glucocorticoid sensitivity and transfer to the developing fetus.

Throughout the body, glucocorticoid activity is mediated by the action of microsomal enzymes which function as pre-receptor signalling pathway modulators. The enzymes believed to most crucially regulate maternal-fetal cortisol transfer are the two known human isoforms of 11β-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD-2 is located in the syncytiotrophoblast (Figure 2) and converts biologically active maternal cortisol into

inactive cortisone. This enzyme acts as a 'barrier' to prevent excessive fetal exposure to cortisol derived from the activated maternal system [52,53]. It was previously reported that placental 11β-HSD-2 inactivates the majority of maternal cortisol, however a recent study examining placental glucocorticoid transfer reported that even when 11β-HSD-2 activity was inhibited with carbenoxolone, (a potent HSD inhibitor), less than 10% of maternal cortisol crossed the placenta. This finding suggests other mechanisms are active in protecting the fetus from high maternal cortisol levels [53]. It has been suggested that due to the significantly higher cortisol concentrations in the maternal circulation compared to the fetal circulation, relatively small changes in 11β-HSD-2 activity may result in clinically significant alterations in fetal glucocorticoid exposure [28]. For example, one small study demonstrated that a 500nmol/l increase in maternal cortisol concentrations (a 1.5 standard deviation change) resulted in a 30nmol/l increase in fetal cortisol concentrations (a 1.1 standard deviation change) [54]. In contrast to 11β-HSD-2, 11β-HSD-1 re-activates maternal cortisone to cortisol and is located in extravillous trophoblasts, endothelial cells lining blood vessels in placental villous tissue and umbilical cord, and fetal membranes, with no presence detected in syncytiotrophoblasts [55]. The role of 11β-HSD-1 in determining maternal or fetal cortisol exposure is less well described.

Table 1: Human placental ABC transporters (adapted from [49])

Transporter	Syncytiotrophoblast location	Substrates/function
ABCA1	Microvillous	Cholesterol, phospholipids
ABCB1	Microvillous	Cortisol, drug resistance (antibiotics, antiemetic, cardiac drugs, HIV protease inhibitors)
ABCB4	Basolateral	Bile acids
ABCC1	Basolateral, microvillous	Corticosterone, folate
ABCC2	Microvillous	Folate, bilirubin, role in chemoprotection and detoxification
ABCC3	Microvillous	Bilirubin
ABCC4	Microvillous	Conjugated bile acids

ABCC5	Basolateral	Cyclic nucleotides
ABCC7	Microvillous	Chloride transport
ABCG1	Basolateral	Cholesterol, phospholipids
ABCG2	Microvillous	Drug resistance

ABC: ATP-binding cassette, HIV: human immunodeficiency virus

Obesity and placental glucocorticoid sensitivity and transfer

The impact of obesity on glucocorticoid sensitivity and transfer is yet to be established. However, unpublished data from our group *(Reynolds et al., unpublished)* suggests that placental 11β-HSD-2 messenger ribonucleic acid (mRNA) levels correlate positively with maternal BMI and are highest in term samples from obese women with macrosomic (>4000g) infants compared to lean women. This exploratory finding raises the possibility that an up-regulated placental 11β-HSD-2 barrier is an important mechanism contributing to reduced fetoplacental glucocorticoid exposure in obese pregnant women, potentially contributing to the prolonged gestation and higher birthweight observed in this population [56]. Further research is required to replicate this finding in a larger cohort, and to characterise the impact of obesity on the activity and function of placental glucocorticoid receptors and ABC transporters during pregnancy.

In addition to obesity, other maternal factors have been shown to regulate placental glucocorticoid sensitivity and transfer. For example, low maternal education status was associated with increased placental GR and 11β-HSD-1 mRNA expression in a Finnish cohort of 67 healthy pregnant women [57]. Abnormal mood is also an important factor, with maternal depressive symptoms across pregnancy in a group of 56 lean women shown to correlate with increased placental mRNA expression of GR and MR [58]. In a follow-up study of this cohort, increased regulatory behavioural challenges in the offspring at mean age 15.6 days were associated with increased placental expression of GR, 11β-HSD-1 and 11β-HSD-2 [59]. This result highlights the potential for placental adaptations to obesity to have short and long-term implications for the neurocognitive development of offspring. Compared to their lean counterparts, obese pregnant women are more likely to have a low education

status, to reside in an area of socioeconomic deprivation, and are around 40% more likely to develop antenatal depression [60,61]. Further research into these exposures is therefore crucial to advance the health of obese pregnant women and their offspring across the generations.

Placental nutrient transporters

Glucose

Glucose is the primary energy source for the fetus and placenta. The fetus produces minimal endogenous glucose and is therefore reliant on the transplacental supply from the maternal circulation. Glucose is transported, down its concentration gradient, by facilitated carrier-mediated diffusion across glucose transport proteins (GLUTs) (Figure 3). GLUT1 is the primary placental isoform and is present on the MVM and BM of the syncytiotrophoblast [62]. It is present in abundance from early pregnancy and expression continues to increase until the late second trimester, remaining stable thereafter [63]. GLUT-1 is distributed more densely on the MVM than the BM, suggesting that BM transport may provide the rate-limiting step for placental glucose transport [64]. The syncytiotrophoblast cells express a range of other GLUT isoforms including GLUT-3, GLUT-4, GLUT-8, GLUT-9 and GLUT-10 [65]. GLUT-3 and GLUT-4 are expressed most highly in the first trimester and are therefore thought to play a more important role in glucose transport in early pregnancy [66,67].

The impact of obesity and infant birthweight on placental glucose transport was examined in a cohort of predominantly Hispanic, non-diabetic, overweight/obese women (n=32) and lean controls (n=20) [68]. Maternal BMI demonstrated a positive correlation with umbilical vein glucose, a surrogate marker of fetal serum glucose levels. Analysis of isolated BMs demonstrated that increasing birthweight was associated with increased protein expression of GLUT-1 (r^2=0.14, p=0.03). However, birthweight did not correlate with glucose transport activity at the BM or MVM. There was no correlation between birthweight and expression of MVM GLUT-1, BM GLUT-9 or MVM GLUT-9. Increasing birthweight demonstrated a positive correlation with maternal BMI (r^2=0.16, p=0.03), umbilical vein glucose (r^2=0.24, p=0.008), umbilical vein insulin (r^2=0.19, p=0.04) and placental weight (r^2=0.34, p=0.001).

There was no association between maternal fasting glucose and umbilical vein glucose. These findings raise the possibility that heavier placentas from overweight and obese women possess a greater surface area to facilitate glucose transport, or that placenta glucose metabolism is decreased in this cohort, in either case resulting in greater glucose availability for the fetus. The correlation of birthweight and umbilical vein glucose and insulin levels supports the presence of fetal insulin resistance in babies born to overweight and obese mothers, findings replicated in another obese cohort [7].

Colomiere et al investigated the effect of obesity on glucose transporters and insulin signalling molecules including insulin receptor substrate 1 and 2, PI3K 85α and PI3K 110 α [69]. PI3K (phosphatidylinositol-3-kinase) proteins are a family of heterodimeric enzymes with key roles in insulin signalling pathways, including the ability to regulate glucose transporter activity [69]. They consist of a p85 regulatory subunit and a p110 catalytic subunit [70]. Comparing lean and obese women with normal glucose tolerance (n=6 per group), a significant reduction in GLUT-4 mRNA in was observed in obese women. This was not associated with any difference in GLUT-4 protein expression. There was no difference in protein or mRNA expression for GLUT-1, insulin receptor β (IR-β) or insulin receptor substrate 1 between groups. An increase in insulin receptor substrate 2 protein expression and decrease in PI3K p85a mRNA and protein expression was noted in obese women compared to lean women. Whilst these findings suggest obesity is associated with alterations in post-receptor insulin signalling, reproduction of this finding further study in a larger sample would be valuable.

A C57/BL6 mouse model of maternal obesity demonstrated significant increases in transplacental glucose transport [26]. Female mice, fed a high-fat or control diet for 8 weeks before mating and throughout pregnancy, were studied at embryonic day 18.5. Mean fetal weight at E18.5 was increased by 43% in the high fat diet group, with no difference observed in mean placental weight between groups. MVM GLUT-1 expression was increased 5-fold in high fat diet mice compared to controls. Similarly, placental glucose transport quantified by in vivo studies using a [^{3}H]methylglucose tracer, was increased 5-fold in the high fat diet group compared to controls. There was no difference in GLUT-3 expression, measured in total placental homogenate, between the two diet groups. BM GLUT

expression was not examined in this study. The finding of increased placental glucose transport and unchanged placental weight in obese mice compared to controls is in contrast to the results reported by Acosta et al [68], highlighting potential limitations of the murine model in modelling a chronic metabolic disease.

Inflammation and glucose transport

During pregnancy, obese women demonstrate elevated plasma levels of pro-inflammatory mediators and enhanced immune cell infiltration in the placenta [23,71]. It is hypothesised that a pro-inflammatory in utero environment alters placental glucose transport in the context of obesity. The pro-inflammatory cytokine hepatocyte growth factor is expressed strongly in the placental syncytiotrophoblast, extravillous trophoblast and amniotic epithelium. Elevated serum hepatocyte growth factor levels have been reported in the non-pregnant obese population, partly attributed to elevated synthesis within the adipose tissue. [72,73]. Through its action binding to the transmembrane c-met receptor, hepatocyte growth factor has numerous growth-promoting functions including regulation of glucose and lipid metabolism in pancreatic beta cells, adipocytes and skeletal muscle cells [74]. Levels of total and activated- hepatocyte growth factor, quantified by enzyme-linked immunosorbent assay, were significantly increased in the amniotic fluid of obese women (n=12; approximately 30ng/mL) compared to lean women (n=29, approximately 24 ng/mL) [75]. Western blot analysis revealed a 3-fold increase in hepatocyte growth factor protein levels in the placental tissue of obese compared to lean women. The role of hepatocyte growth factor in glucose transport was examined using placental explants preincubated in the presence or absence of hepatocyte growth factor. Uptake of 2-deoxyglucose was significantly increased in the presence of hepatocyte growth factor with a maximal increment of 30% noted at the 30ng/mL concentration. This was accompanied by an approximate 20% increase in glycolysis within the explants. Further Western blot analysis of placental explant samples from control participants (n=4) revealed incubation in 30ng/mL hepatocyte growth factor for 18 hours was associated with a 25% increase in GLUT-1 expression. The impact of hepatocyte growth factor on placental glucose uptake was abolished completely by inactivation of the PI3K pathway with wortmannin (a PI3K pathway

inhibitor) suggesting this pathway is involved in downstream signalling following c-met activation by hepatocyte growth factor.

Another pro-inflammatory cytokine, resistin, is elevated in the serum of non-pregnant obese people and is associated with insulin resistance [76]. Serum levels also increase in the third trimester of pregnancy, a trend thought to relate, at least in part, to increased trophoblast resistin production [77]. In vitro studies using normal cytotrophoblast cells and BeWo choriocarcinoma cells (a cell line commonly used to model the first trimester trophoblast) found exposure to recombinant resistin was associated with increased glucose uptake, GLUT-1 messenger ribonucleic acid expression and protein expression in both cell types [78]. Extracellular signal-regulated kinase 1 and 2 protein phosphorylation was highlighted as a potential mechanism through which resistin modifies placental GLUT-1 expression. The importance of inflammatory cytokines including hepatocyte growth factor and resistin in modulation of glucose transport in vivo during obese pregnancy requires further study.

Fatty acids

Fatty acids are vital macronutrients in fetal development required as an energy source, a structural component of cell membranes, tissues and organs, and as a precursor to many essential bioactive compounds. Fatty acids taken up by the placenta originate from two main sources in the maternal circulation: esterified fatty acids in triglycerides and nonesterified fatty acids. Fatty acids in triglycerides are broken down by hydrolysis into nonesterified fatty acids to facilitate transport across the placental syncytium (Figure 3). This process is catalysed by lipases such as lipoprotein lipase and endothelial lipase in the MVM [65]. Lipoprotein lipase preferentially hydrolyses triglycerides in chylomicrons and very low-density lipoproteins, whilst endothelial lipase typically hydrolyses high density lipoproteins and phospholipids [65]. Free fatty acids are transported across the syncytiotrophoblast MVM by transport proteins including fatty acid transport proteins (FATP), fatty acid translocase (FAT/CD36) and plasma membrane fatty acid binding protein (FABPpm) [79]. Once inside the cell, free fatty acids bind to fatty acid binding proteins where they may be beta-oxidised to produce energy, esterified for use as structural

components, or transferred through FATPs or FAT/CD36 in the BM and released into the fetal circulation [79]. FATPs are integral membrane proteins with a key role in long-chained fatty acid uptake. There are six members of the FATP family, five of which are present in the human placenta (FATP1-4 and FATP6; encoded by SLC27A1-4 and 6).

Obesity may influence fatty acid transport in several ways leading to alterations in fetal development and growth trajectories. Placental samples obtained at term from lean (n=34) and obese (n=55) women showed placentas from obese pregnancies contained a higher triglyceride content [80]. Obesity was associated with increased expression of six genes related to lipid transport and storage including FATP1, FATP3, adipose triglyceride lipase (a lipase localized in the syncytiotrophoblast) and CGI-58 (comparative gene identification-58; a regulator of hydrolysis) ($p<0.05$). In a ewe model of obesity at mid gestation (day 75), placental mRNA expression of FATP-1 and FAT/CD36 were significantly increased compared to normal controls ($p<0.05$), alongside protein expression of FATP-1 and FATP-4 ($p<0.05$) [81]. An increase in FATP-4 mRNA expression was also noted which trended towards significance ($p=0.08$). Similar findings were noted near term (day 135) with mRNA expression of FATP-1, FATP-4 and FAT/CD36 all increased in the obese group ($p<0.05$), although only FATP-4 protein expression was higher in this group ($p<0.05$). No difference in lipoprotein lipase mRNA expression was noted between groups at either gestation. Interestingly, fetuses of obese ewes were significantly heavier than fetuses of control ewes on day 75 (185.7± 6.89 vs 234.4±6.61g), but this difference did not persist at day 150 (5.05 ± 0.28 vs 5.02 ± 0.25kg). The same pattern was also described in the placental weight between groups. Whilst the reason for these observations are unknown, this presents an interesting model for further study to enhance the understanding of placental development and function in the context of obesity. Placental endothelial lipase activity did not appear to differ comparing term placental samples from obese (n=170) and lean (n=71) women [82]. This was in contrast to placental samples from obese women with gestational diabetes mellitus (n=26), where expression was increased 1.9-fold ($p<0.05$) [82].

The potential of the pro-inflammatory obesogenic environment to influence fatty acid metabolism was examined in a cohort of lean (n=7) and overweight/obese (n=5) women [83]. IL-6, but not tumour necrosis factor-α (TNF-α), was found to stimulate fatty acid

accumulation in primary cultured trophoblast cells from lean, overweight and obese women. No changes were noted in expression of FATP, lipoprotein lipase, or other examined markers, therefore the mechanism of this association is unclear.

Amino acids

Amino acids are vital substrates for the fetal production of nucleic acids and proteins, facilitate fetal growth and stimulate insulin release from the fetal pancreas [84]. Placental amino acid transport occurs against its concentration gradient across the syncytiotrophoblast, with intervillous blood amino acid concentrations generally more than two-fold greater than maternal concentrations [85]. Transport by active transport through accumulative transporters and exchangers on the MVM and BM (Figure 3). Accumulative transporters generally increase intracellular concentrations of amino acids by mediating uptake often by co-transporting extracellular sodium [86]. Exchangers alter amino acid concentrations by swapping amino acids between intracellular and extracellular compartments. Placental amino acid transport is tightly regulated with more than 20 known amino acid transporters. These include seven neutral amino acid transporters, including system A and system L. These systems are distinct in their substrate specificity and sodium dependency [87]. The role of amino acid transport activity in modulating fetal growth in obesity has been demonstrated in studies investigating various regulatory pathways.

The hormone leptin, produced by adipocytes and placenta, is a key regulator of appetite and is present in elevated concentrations in obesity [88]. In term primary villous fragments obtained from uncomplicated, normal-weight pregnancies, leptin was shown to stimulate placental system A transport activity through phosphorylation of STAT3 (a transcription factor) and activation of the JAK-STAT signalling pathway [89]. In a study of obese (n=7) and lean (n=7) pregnant women, obesity was associated with decreased placental system A sodium-dependent neutral amino acid transporter (SNAT) activity ($p=0.005$), maternal hyperleptinemia ($p=0.01$) and decreased syncytiotrophoblast expression of leptin receptor ($p=0.01$) and SNAT-4 ($p<0.001$) [90]. In addition, placental amino acid uptake was significantly stimulated by leptin in the lean women compared to the obese women. These findings were despite similar gestational weight gain and birthweights between groups. In

contrast, no significant difference in system L activity was identified comparing primary human trophoblast cells from overweight/obese (n=24) and lean (n=20) women [91].

The mammalian target of rapamycin (mTOR) pathway is thought to be a principal regulator of placental amino acid transport [92]. Activity of mTOR signalling was assessed alongside other key signalling pathways, including insulin/insulin-like growth factor-1, in term placental samples from 16 healthy pregnant women across a range of BMI [25]. BMI was found to negatively correlate with activity of AMPK, which inhibits mTOR, positively correlate with insulin/insulin-like growth factor-1 signalling, which stimulates mTOR. mTOR activity was also found to positively correlate with increasing birthweight ($p<0.001$). In this cohort, SNAT-1 and SNAT-4 expression were not significantly correlated to birthweight or maternal BMI. However, SNAT-2 expression was positively correlated with maternal early pregnancy BMI ($p<0.05$) and birthweight ($p<0.01$). On the contrary, placental mTOR gene expression was reduced in Spanish cohort of obese women (n=21) compared to lean women [93]. Gene expression of an upstream mTOR regulator (Akt1) and a downstream regulator (p70S6KB1) were unchanged. Interestingly these findings differed from obese women with gestational diabetes mellitus (n=11), who demonstrated increased mRNA expression of p70S6KB1.

Oleic acid, a nonesterified fatty acid, comprises 30% of circulating nonesterified fatty acids during pregnancy [94]. In cultured human trophoblast cells from term placentas obtained from lean, overweight and obese pregnancies, exposure to oleic acid doubled system A activity ($p<0.05$), increased STAT3 phosphorylation ($p<0.05$), but had no effect on system L or mTOR activity. Obese pregnant women have elevated plasma fatty acid levels therefore these findings reveal a novel potential mechanism through which maternal obesity is associated with increased amino acid transport and subsequent fetal growth.

Studies in a rat model of intra-uterine growth restriction using a low protein diet reported that down-regulation of amino acid transport predated the onset of intra-uterine growth restriction, rather than occurring as an adaptive response to growth restriction. Whilst the temporal nature of altered amino acid transport has yet to be determined in the context of

maternal obesity, this finding raises the possibility that up-regulation of amino acid transport may have a role in precipitating fetal overgrowth [95].

Epigenetics and maternal obesity

During prenatal development, cells and tissues acquire different patterns of gene expression in the absence of changes to the DNA sequence. These stable but reversible alterations, known as epigenetic modifications or marks, include DNA methylation, histone posttranslational modifications and non-coding RNAs [16]. Crucial timepoints for epigenetic programming during development include gametogenesis, as primordial germ cells differentiate into mature sperm and oocytes, following fertilisation and during pre-implantation development [96]. DNA methylation, which primarily occurs at cytosines in cytosine-guanine (CgP) dinucleotides, is the most widely studied epigenetic process [97]. As epigenetic modifications may be transmitted through generations of cell divisions, epigenetics has received great attention as a potential causal mechanism through which environmental exposures during early development mediate their effect on the long-term health of the offspring. Human studies examining the relationship between maternal obesity and DNA methylation changes in placenta, umbilical cord and cord blood of offspring are summarised in a 2017 review by Godfrey et al [16]. The following studies are of particular relevance to epigenetic changes in the placenta of obese women.

Michels et al. studied the association between maternal BMI and methylation of Long Interspersed Nuclear Elements (LINE) 1 (LINE-1) [98]. Approximately 50% of the human genome is composed of repetitive sequences including LINE and Short Interspersed Nuclear Elements (SINE). LINE-1 methylation decreases with advancing age, and hypomethylation has been associated with cancers and implicated as a poor prognostic indicator in the oncology setting [98]. In placental samples from 316 mother-child dyads, no association was found between maternal pre-pregnancy BMI, gestational weight gain or birthweight/placental weight ratio with LINE-1 methylation. The only significant result in placental sample analysis was an association between low birthweight (<2500g, 29 dyads) and a 1.41% increase in LINE-1 methylation, compared to infants of normal birthweight (95% CI 0.18-2.63, p=0.025). This association was strengthened in a sub-group analysis of

term infants, with a difference of 2.6% methylation compared to normal birthweight infants (p=0.0014). Interestingly, associations with reduced LINE-1 methylation were noted in the umbilical cord blood samples of low birthweight babies (-0.82%, 95% CI -1.42 to –0.23, p=0.007) and also macrosomic babies (62 dyads; -0.43%, 95% CI -0.84 to -0.03, p=0.036).

A comparison of the pattern of genome-wide DNA methylation and hydroxymethylation, known as the 'methylome', was performed by Mitsuya and colleagues using placental samples from obese and normal weight pregnancies (n=10 placentas combined in each group) [99]. Samples were obtained after delivery by elective caesarean section at term in the absence of labour, from women with no medical co-morbidities. Differences in methylated and hydroxymethylated regions were identified in a widespread pattern across the genome with a 21% increase in the number of methylated regions and 31% decrease in the number of hydroxymethylated regions in the obese compared to lean group. Changes were identified in diverse sites across the genome including around transcription start sites, CpG rich areas (CpG islands) and regions adjacent to CpG rich areas (CpG shores and shelves). Regions with differential methylation and hydroxymethylation patterns (i.e. increased methylation and decreased hydroxymethylation) were identified around transcription start sites of multiple genes in two pregnancy-associated gene clusters: the growth hormone-chorionic somatomammotropin hormone cluster on chromosome 17q24 and pregnancy-specific glycoprotein gene cluster on chromosomes 19q13. The growth hormone-chorionic somatomammotropin hormone encodes hormones vital for fetal growth, metabolism and stimulation of lactation whilst pregnancy-specific glycoprotein is thought to have a vital immune-modulating function [99,100]. Increased methylation was only associated with decreased mRNA expression of some genes in these clusters, suggesting further research into the clinical significance of these findings is required.

A second genome-wide study of the methylome in maternal obesity, performed in a multi-ethnic cohort, found global methylation levels were higher in placental samples from obese women (n=18, 56.1%) compared to lean women (n=32, 53.64%, p=0.01) [101]. This finding remained significant in a model adjusted for patient demographics (p=0.01). There was no difference in methylation noted between obese and lean groups on analysis of umbilical cord blood samples. Of note, these analyses did not control for concomitant medical

diagnoses of pre-eclampsia or gestational diabetes in adjusted analyses. There was also no association demonstrated between global methylation (in placenta and umbilical cord blood) with birth outcomes including birth weight and gestational age at birth.

A murine model was used to evaluate the impact of maternal obesity and pre-conception weight loss on the expression of epigenetic genes in two distinct placental zones: the placental labyrinth, known as the fetal placenta in humans, and the junctional zone, referred to as the basal plate or implantation site in humans [102,103]. For four months prior to conception, female mice were fed a control diet, a high fat diet, or a weight loss diet (high fat for two months followed by control diet for two months). This model of obesity was associated with fetal growth restriction in the offspring (an expected pattern in the murine model), which was partially restored in the weight loss group. Expression of 60 'epigenetic machinery' genes implicated in metabolic diseases including obesity and type 2 diabetes, and 32 genes involved in developmental conditioning, were examined using low density arrays. By hierarchical cluster analysis, the obese group demonstrated a change in mean expression of the 86 analysed genes in the labyrinth zone, compared to the control and weight loss groups. This finding was not replicated in the junctional zone. Maternal obesity was associated with altered expression of nine epigenetic genes in the labyrinth zone compared to the control group including downregulation of histone acetylation enzymes, 11βHSD1 and insulin receptor substrate 1. In the weight loss group, several genes were down-regulated in a similar pattern to the obese group, however others were measured at levels similar to the control group. In the junctional zone, one histone acetylation enzyme (Kat3b) was upregulated in the weight loss group compared to control, with no investigated genes affected in the obese group. These findings may be of relevance to histone acetylation pathways in the human fetal placenta and highlight the potential for distinct epigenetic patterns to affect varying placental sites.

Finally, methylation of the GR gene promoter region 1F in placental samples was shown to associate with birthweight category in a study involving 480 participants [104]. After Bonferroni correction for multiple comparisons, only one CpG site (out of thirteen) and the mean across all CpG sites showed a significantly difference, with increased methylation in the large for gestational age group (>90th percentile of weight for gestational age, n=35)

compared to the appropriate and small for gestational age groups (n=343 and n=102 respectively). Adjusted analyses revealed birthweight was significantly correlated with mean GR methylation extent (r=0.16, p=0.0004). Unfortunately, data on maternal BMI or weight was not available in this cohort. Altered methylation of the GR may lead to dysregulation of offspring glucocorticoid homeostasis, and potentially contribute to the development of metabolic disease including type 2 diabetes and obesity. For these reasons, further investigation into GR methylation and other epigenetic marks in the context of maternal obesity would be valuable.

Despite the potential for epigenetic studies to provide major breakthroughs into our understanding of developmental programming, it is important to acknowledge their limitations. Firstly, the clinical significance of the epigenetic markings discussed in this section is unknown. The magnitude of the role, if any, these changes have in contributing to the adverse phenotypes experienced in later life by the offspring of obese women remains to be elucidated [16]. Indeed, it is unknown if these changes occur in response to the obesogenic intra-uterine environment to aid fetal adaptation, or if they are simply a downstream consequence of changes in fetal growth which occur in the context of obesity. It has also been speculated that term placental samples reflect an organ at the end of its life cycle, and therefore a declining metabolic rate may be reflected in results relating to epigenome activity [98]. Finally, the placenta is known to consist of different cell populations, each which has the potential to exhibit a unique epigenetic pattern [16]. This adds a further layer of complexity in interpreting the results of mechanistic studies. At present there are no longitudinal studies following-up the impact of epigenetic changes observed at birth in the offspring of obese women on health in later life. Whilst this would provide invaluable insight into the role of epigenetics in developmental programming, it would also be practically challenging to perform.

Conclusion

The placenta is developmentally plastic and adapts to the maternal environment in order to optimise fetal growth and development. In the setting of maternal obesity, the placenta must function despite a milieu of inflammatory and metabolic disturbances. The findings

discussed in this review highlight the complexity of cellular pathways which may be altered, at least in part, by the obesogenic environment. Maternal obesity blunts the HPA axis activation associated with pregnancy, however the impact of obesity on placental glucocorticoid metabolism has yet to be determined. In particular, the impact of gestation and obesity on glucocorticoid sensitivity and ABC transporters presents a major knowledge gap. Maternal obesity is associated with excessive macronutrient exposure precipitating excess fetal growth. Despite detailed knowledge of the relevant placental transport mechanisms, further research is required to elucidate the mechanisms regulating these transporters, and the impact of obesity in this process. Furthermore, whilst differences in the epigenetic profile, specifically DNA methylation, of placentas from obese and lean women have been identified, the clinical implications of these findings are unknown. More detailed understanding in these fields may provide an opportunity to develop targeted novel interventions to offset the impact of obesity on the developing fetus, and potentially benefit the ever-growing numbers of offspring who are adversely impacted by their prenatal environment.

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Figure 1. Schematic diagram representing the transport and metabolism of glucocorticoids in the fetal, syncytiotrophoblast and maternal compartments during pregnancy.

Legend: Cortisol and corticosterone are metabolised to their inactive metabolites (cortisone and 11-dehydrocorticosterone) by the enzyme 11β-HSD-2 in the syncytiotrophoblast. Some of these inactive glucocorticoids are re-activated by 11β-HSD-1 in the fetal and maternal compartments. Cortisol is effluxed from the syncytiotrophoblast to the maternal circulation, whilst corticosterone is effluxed to the fetal compartment. ABCG2 is expressed on the microvillous membrane. The role of placental ABC transporters in glucocorticoid transport has yet to be fully established. Mineralocorticoid receptors are expressed in the syncytiotrophoblast, maternal and fetal compartments (not depicted).

Figure 2: 11β-HSD-2 staining in the syncytiotrophoblast of term placental tissue from a normal weight woman.

Legend: Immunohistochemistry staining for 11β-HSD-2 protein (brown) with nuclear counterstaining (blue) in term trophoblast tissue collected from a normal weight woman, A: 50x magnification, B: 400x magnification of placental villi.

Figure 3: Schematic diagram representing the transport of glucose, amino acids and fatty acids from the maternal to the fetal circulation across the syncytiotrophoblast.

Legend: Maternal blood in the intervillous space of the placenta bathes the microvillous membrane. Glucose transport proteins, amino acid transporters and fatty acid transporters are present on both sides of the syncytiotrophoblast, allowing movement of macronutrients across the syncytiotrophoblast from the maternal to the fetal circulation. Lipoproteins containing fatty acids, for example triglycerides, are cleaved by lipases attached to the microvillous membrane (MVM), resulting in the release of free fatty acids (FFAs). FFAs are transported across the MVM by fatty acid transporters, then bound to fatty acid binding proteins (FABP) within the cytosol of the syncytiotrophoblast which guide further transport.

MVM: microvillous membrane, BM: basal membrane, GLUT: glucose transport protein, AA: amino acid, TG: triglyceride, LPL: lipoprotein lipase, EL: endothelial lipase, FFA: free fatty acids, FABPpm: plasma membrane fatty acid binding protein, FATP: fatty acid transport protein, FAT/CD36: fatty acid translocase.

List of Abbreviations

11β-HSD: 11β-hydroxysteroid dehydrogenase
ABC: ATP-binding cassette
ACTH: Adrenocorticotrophic hormone
Akt1: Protein kinase B
AMPK: 5' adenosine monophosphate-activated protein kinase
ATP: Adenosine triphosphate
BM: Basal membrane
BMI: Body mass index
CGI: Comparative gene identification
DNA: Deoxyribonucleic acid
FABPpm: Plasma membrane fatty acid binding protein
FAT/CD36: Fatty acid translocase
FATP: Fatty acid transport protein
FFA: Free fatty acid
GLUT: Glucose transporter
GR: Glucocorticoid receptor
HPA: Hypothalamic-pituitary-adrenal
IL: Interleukin
IR: Insulin receptor
Kat: Lysine acetyltransferase
LINE: Long Interspersed Nuclear Elements
MR: Mineralocorticoid receptor
MCP-1: Monocyte chemoattractant protein-1
MVM: Microvillous membrane
mRNA: Messenger ribonucleic acid

mTOR: Mammalian target of rapamycin

NR3C1: Nuclear receptor subfamily 3, group C, member 1

NR3C2: Nuclear receptor subfamily 3, group C, member 2

PI3-K: Phosphatidylinositol-3-kinase

SNAT: Sodium-dependent neutral amino acid transporter

STAT: Signal transducer and activator of transcription

TNF-α: Tumour necrosis factor-α

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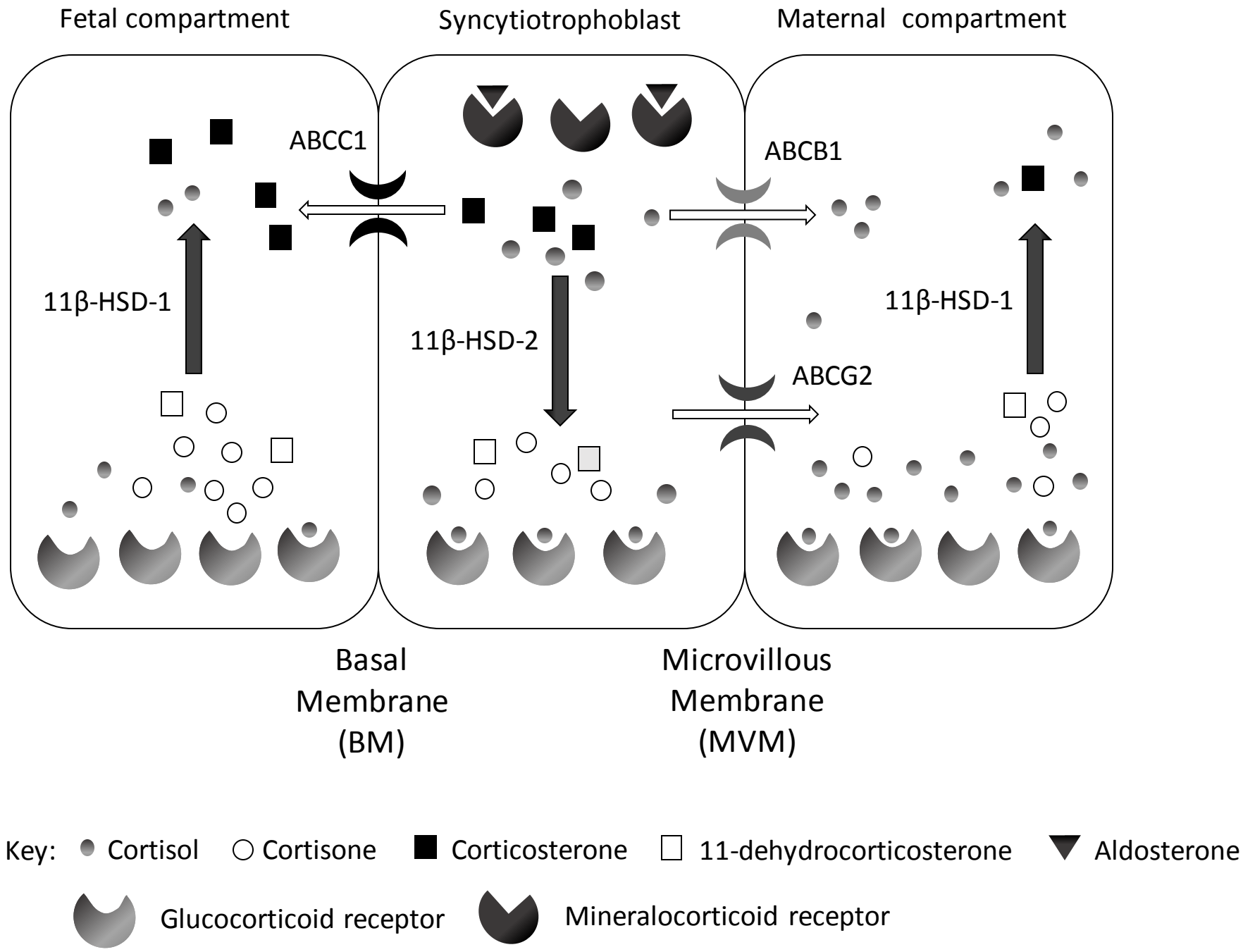
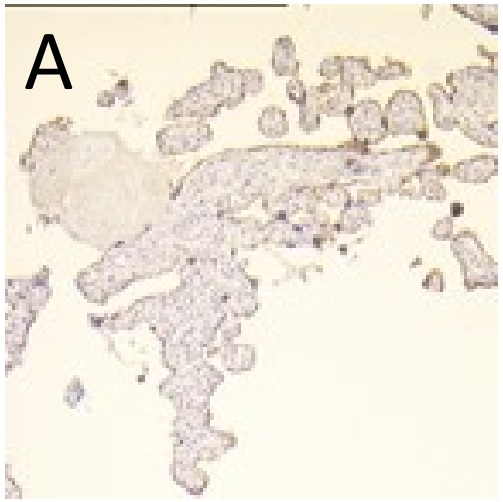

Fetal compartment
Syncytiotrophoblast
Maternal compartment
ABCC1
ABCB1
11β-HSD-1
11β-HSD-2
11β-HSD-1
ABCG2
Basal Membrane (BM)
Microvillous Membrane (MVM)
Key:
Cortisol
Cortisone
Corticosterone
11-dehydrocorticosterone
Aldosterone
Glucocorticoid receptor
Mineralocorticoid receptor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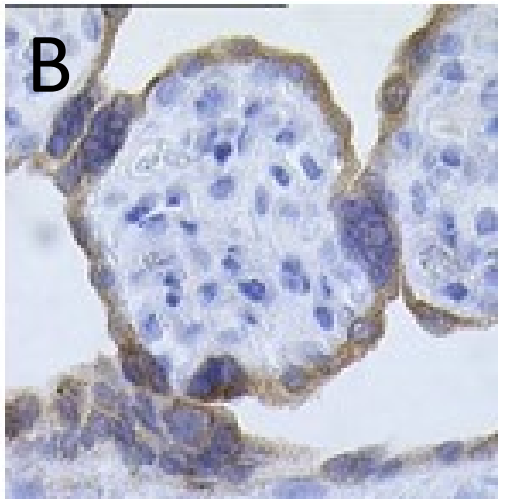
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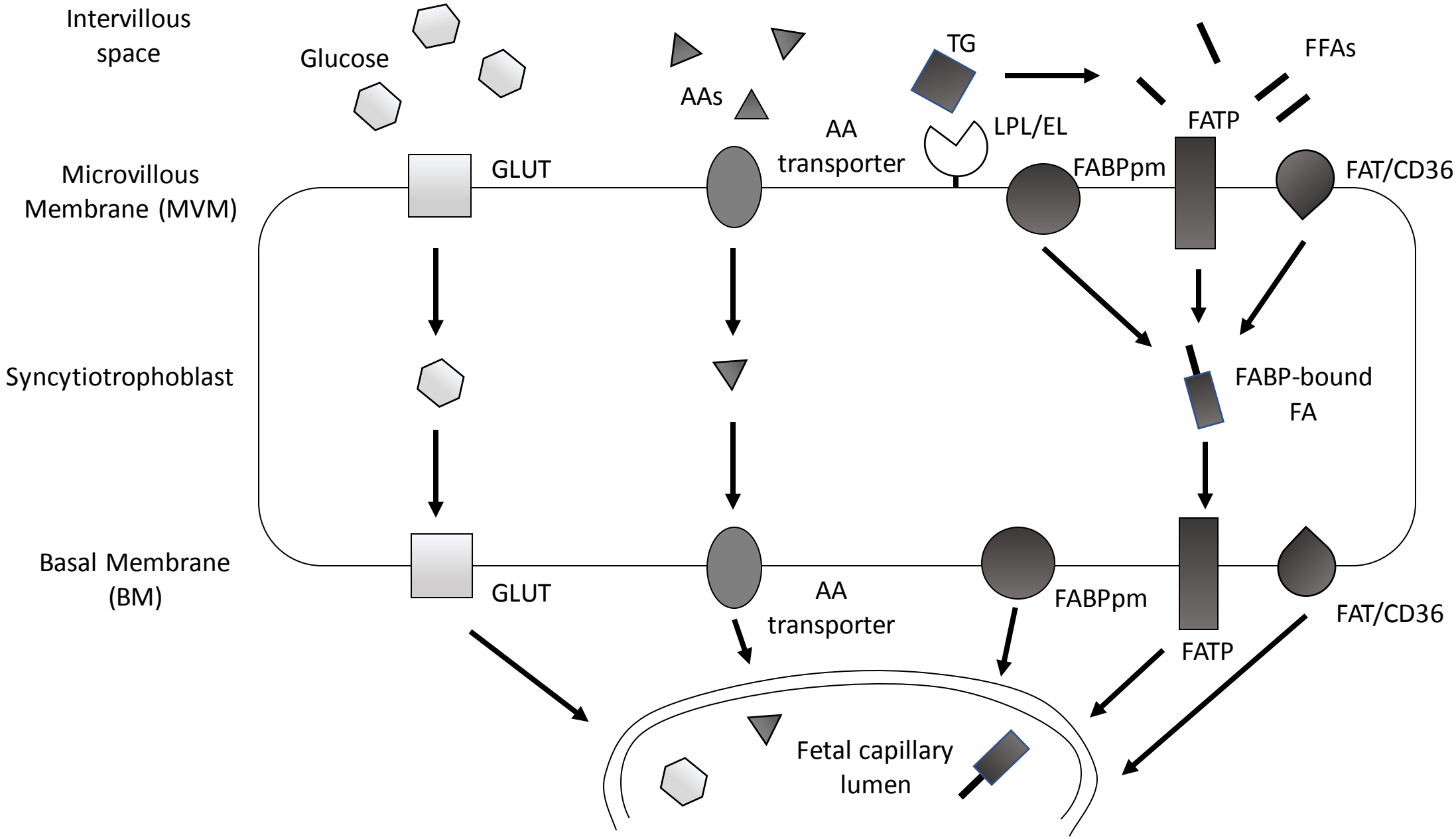
Intervillous space
Glucose
AAs
TG
FFAs
LPL/EL
FATP
AA transporter
Microvillous Membrane (MVM)
GLUT
FABPpm
FAT/CD36
Syncytiotrophoblast
FABP-bound FA
Basal Membrane (BM)
GLUT
AA transporter
FABPpm
FATP
FAT/CD36
Fetal capillary lumen