The Early Origins of Obesity and Insulin Resistance: Timing, Programming and Mechanisms

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Abstract

Maternal obesity is associated with an increased risk of developing gestational diabetes mellitus (GDM) and it also results in an increased risk of giving birth to a large baby with increased fat mass. Furthermore, it is also contributes to an increased risk of obesity and insulin resistance in the offspring in childhood, adolescence and adult life. It has been proposed that exposure to maternal obesity may therefore result in an 'intergenerational cycle' of obesity and insulin resistance. There is significant interest in whether exposure to maternal obesity around the time of conception alone contributes directly to poor metabolic outcomes in the offspring and whether dieting in the obese mother before pregnancy or around the time of conception has metabolic benefits for the offspring. This review focusses on experimental and clinical studies that have investigated the specific impact of exposure to maternal obesity during the periconceptional period alone or extending beyond conception on adipogenesis, lipogenesis and on insulin signalling pathways in the fat, liver and muscle of the offspring. Findings from these studies highlight the need for a better evidence base for the development of dietary interventions in obese women before pregnancy and around the time of conception to maximize the metabolic benefits and minimize the metabolic costs for the next generation.

1. Introduction

As the prevalence of obesity has increased worldwide, more women are entering pregnancy overweight or obese (1, 2). This is associated with increased risk of impaired glucose tolerance and GDM (3). Consequently, these women also have higher risk of having a large for gestational age (LGA) baby and these babies have an increased risk of obesity and impaired glucose tolerance in later life. This has led to a concern that the increased prevalence of pre-pregnancy obesity will lead to propagation of an 'intergenerational' cycle of obesity and insulin resistance (1, 4). There is, therefore, significant interest in understanding the type of maternal dietary and lifestyle interventions, which may lead to optimal outcomes for mother and child (4, 5). This review summarises the epidemiological and clinical studies highlighting the association between maternal obesity and the later onset of obesity and insulin resistance in her offspring in childhood and adult life. Some of the potential benefits and risks of maternal dietary restriction and weight loss will also be highlighted.

As most women who are obese at conception remain obese through their pregnancy, it is difficult, in human studies, to determine the separate or interdependent contributions of maternal pre-pregnancy body mass index (BMI), gestational weight gain and glycaemic control on the metabolic outcomes for the offspring. This review will, therefore, also focus on the importance of experimental studies in animals, which have investigated whether exposure to maternal obesity during critical windows of development has a sequential impact on adipogenic and insulin signalling pathways in tissues of metabolic importance of the offspring.

Finally, this review will highlight some of the epigenetic mechanisms that may be recruited within the developing embryo exposed to maternal obesity or imposed dietary restriction, which impact on programming of metabolic pathways in later life.

2. Maternal obesity and short and longer term pregnancy outcomes

The prevalence of obesity in women of reproductive age is around 15 - 28% in women in the US, UK and Australia (1, 2, 4). Maternal obesity results in increased rates of twinning and miscarriage in early pregnancy and increased rates of gestational hypertension, preeclampsia, venous thromboembolism and the requirement for obstetric intervention (7). Maternal obesity also has longer term impact on offspring metabolic health, extending into adult life.

2.1 Maternal obesity and developmental programming of obesity and insulin resistance: human studies

Maternal obesity and/or insulin resistance results in exposure of the fetus to a hyperglycemic and hyperinsulinemic environment leading to increased fetal growth and fetal body fat mass (8). Indeed, observational studies have shown that there is a positive and incremental association between maternal and offspring adiposity (9, 10). It has been argued, however, that observational studies are ill-equipped to distinguish the contributions of genetic, intrauterine or postnatal mechanisms to the programming of obesity in the offspring (9, 10). Studies investigating the relationship between offspring adiposity with both maternal and paternal BMI have found, in some instances, stronger associations of offspring adiposity with maternal BMI (9, 11) whilst others have found that the magnitudes of associations of both maternal and paternal BMI with offspring adiposity are similar suggesting that such associations are indeed driven by shared familial genetic or lifestyle characteristics (9).

Interestingly, the offspring of obese and/or diabetic mothers are not only heavier at birth but remain so throughout childhood and adult life. According to a recent systematic review, an increase in pre-pregnancy overweight/obesity in women increases the risk of having a LGA baby and these babies subsequently have an increased risk of overweight/obesity in later life (12). Furthermore, pre-pregnancy BMI is also associated with increased odds of rapid postnatal weight gain (13). Offspring of Pima Indian women with pre-existent T2DM and GDM were larger for gestational age at birth and, at every age, were heavier than the offspring of pre-diabetic or non-diabetic women (14, 15). Boney and colleagues also reported that obesity in children at 11 years of age was a strong predictor of insulin resistance (16). Furthermore, children of obese mothers that were born LGA were at twice the risk of developing the metabolic syndrome accompanying childhood obesity at this age (16). A longitudinal study of the 1958 British birth cohort concluded that maternal weight or her BMI largely explained the association between high birth weight and high adult BMI (17).

Recently, a longitudinal study of 421 mother-daughter pairs (18) has highlighted the concern that increased prevalence of pre-pregnancy obesity will, in turn, lead to propagation of an 'intergenerational' cycle of obesity and insulin resistance (1, 4). Kubo *et. al.* found that girls who were exposed to maternal GDM and hyperglycemia *in utero* had a higher risk of increased adiposity and that this risk increased if the mother was overweight/obese (18). Moreover, the risk of obesity was highest among offspring of mothers with GDM and pregravid obesity (18). Interestingly, a recent study that sought to characterize the cord blood metabolome of infants at risk for childhood obesity, before the onset of obesity found that the most differentially regulated metabolites in newborns with and without subsequent accelerated early childhood weight gain were related to food and plant components (13). This highlights the fact that maternal dietary patterns *per se* (and not just her BMI) influence the risk of offspring obesity.

2.2 Maternal obesity and developmental programming of obesity and insulin resistance: animal studies

Studies in rodents have investigated the effects of maternal consumption of either a high-fat only (19, 20) or high-fat, high-sugar junk food diet, which replicates an obesogenic Western diet in humans from before and throughout pregnancy (21, 22) and during lactation (21, 22). Despite the differences in diet composition, length of maternal overnutrition and whether the offspring were weaned onto a standard chow or high-fat diet, these maternal diets all resulted in increased offspring adiposity (19-23). Importantly, exposure to a maternal high-fat diet resulted specifically in increased visceral fat mass in the offspring (24, 25), which is associated with the development of insulin resistance (26). Interestingly, inflammatory changes in subcutaneous fat may also have a role to play in the development of insulin resistance in offspring exposed to maternal high fat feeding (27). Furthermore, fat also secretes a number of adipokines, the dysregulation of which is associated with pathogenesis of obesity-associated comorbidities (28). Some of the more functionally prominent adipokines include adiponectin, leptin, resistin and retinol binding protein-4 (RBP4). Adiponectin, which has anti-inflammatory and insulin-sensitising properties is reduced in obese patients with insulin resistance/T2DM (30). Leptin, which is a satiety factor (31) acts as a circulating signal of fat mass (32). Indeed, there is a direct relationship between cord blood concentrations of leptin at delivery and birth weight or neonatal adiposity both in normal pregnancies (33) and in pregnancies complicated by maternal diabetes (34). Resistin, which is highly expressed in visceral fat antagonises insulin action (35). Consequently, resistin-deficient animals are protected from obesity, whereas elevated resistin is associated with insulin resistance (36). Finally, RBP4 expression, which has been shown to be lowered by administration of the insulin-sensitiser, Rosiglitazone, is inversely regulated by expression of insulin-dependent GLUT-4 (37). This raises the interesting possibility of adipokines as

novel biomarkers of metabolic disease (38) especially if these changes are present in early life.

Maternal overnutrition in rodents is also associated with poor glucose tolerance and T2DM in the offspring (19, 21, 22, 24). This has been attributed to a combination of pancreatic β-cell dysfunction (20, 24) and insulin resistance in postnatal life (21, 22, 24). The former has been attributed to the negative impact of maternal obesity on molecular events involved in maintaining β-cell number, volume (20, 39) and insulin secretory capacity in the fetal pancreas (24, 39) and the latter has been considered a result of altered expression of key components of the insulin-signalling pathway in tissues of metabolic importance (40). It is interesting to note that a recent systematic review of rodent models of maternal high-fat feeding found that maternal obesity was not a pre-requisite for dysregulated glycemic control in the offspring; insulin resistance and impaired glucose tolerance were also present in offspring of non-obese, high-fat fed dams (41).

Inflammation and oxidative stress have also been implicated in pathological processes leading to obesity and T2DM (42-44). Indeed, obese and glucose intolerant offspring of obese dams have an altered redox state from as early as the pre-implantation stage of development leading to mild oxidative stress, which persists throughout gestation and early life (43).

Similar observations linking maternal obesity and development of adiposity and insulin resistance in the offspring have also been made in a primate model of maternal overnutrition. One of the earliest studies conducted in the baboon showed that overnutrition in the pre-weaning period permanently increased offspring adiposity as a consequence of fat cell hypertrophy (45). Sheep have also been used as a large animal model to study the impact of maternal overnutrition/obesity on the developmental programming of obesity and insulin

resistance in the fetus and in the offspring. The sheep fetus is similar to the human fetus in its dependence on glucose as a major source of energy (46-48). Moreover, both sheep and humans are precocial species, exhibit the same newborn-to-maternal weight ratios and have the same temporal pattern of fetal organ development throughout pregnancy (47, 49). A series of studies on the effects of maternal overnutrition/obesity in the sheep from 60 days before conception and throughout pregnancy found increased adiposity in the offspring in late gestation (50), which persists after birth (51). These changes were attributed to increased gene expression and protein abundance of fatty acid (FA) and glucose transporters as well as increased expression of enzymes mediating FA biosynthesis in fat depots of the offspring (50). Maternal obesity also resulted in decreased pancreatic weight and β-cell number in the fetus in late gestation and reduced plasma insulin concentration at term (52). Moreover, these offspring displayed upregulation of inflammatory signalling pathways in skeletal muscle, which promotes adipogenesis and downregulates myogenesis (42, 44). This along with the increased adiposity would result in an increased risk of insulin resistance and T2DM in the postnatal animal. Indeed, the offspring of obese ewes subjected to *ad libitum* feeding at maturity showed increased appetite, growth rates and adiposity and decreased glucose tolerance and insulin sensitivity (53).

2.3 Exposure to maternal obesity during gestation and the epigenetic origins of obesity

A number of recent studies in rodents have investigated the impact of maternal high-fat diet throughout gestation on epigenetic changes in adipose tissue and liver of the offspring (54-56). Changes in adiponectin and leptin expression in adipose tissue of offspring exposed to maternal high-fat diet were found to be due to alterations in both acetylation and methylation of histone H3K9 within the adiponectin promoter and changes in methylation of histone H4K20 within the leptin promoter (55). The offspring of high-fat fed dams also showed

increased hepatic expression of the cytosolic isoform of the gluconeogenic gene, phospho*enol*pyruvate carboxykinase, which was attributed to histone modifications associated with transcriptional activation (56). Importantly and most concerning is that the effects of maternal high-fat feeding appear to be transgenerational. F_2 offspring derived from both grand-maternal and maternal obesity appeared to be extremely susceptible to developing obesity due to transgenerational accumulation of epigenetic modifications including histone methylation, which then contribute to increased lipogenesis (54).

2.4 The 'intergenerational cycle' of obesity and insulin resistance

Although there is variation in the duration of feeding regimes and types of dietary challenges, studies in both small and large animals show that exposure to maternal overnutrition/obesity consistently results in a similar phenotype in the offspring including high body fat mass and in most cases impaired insulin signalling in the liver and muscle (4). Together, these findings implicate a possible 'intergenerational cycle' of obesity and insulin resistance. In a recent study by Graus-Nunes *et. al.*, a maternal high fat diet consumed before and throughout pregnancy and lactation resulted in impaired whole-body metabolism as well as altered development of the pancreas in the F_1 and F_2 offspring (57). The maternal contribution to the intergenerational transmission of obesity and insulin resistance appears to be mediated, at least in part, by transgenerational accumulation of epigenetic modifications (54). This has led to significant interest in the type of dietary and lifestyle interventions that could be imposed before and during pregnancy that could lead to optimal metabolic outcomes for the mother and the offspring (4, 58).

3. The periconceptional and late pregnancy periods as critical windows for developmental programming of obesity

Most epidemiological, clinical and experimental studies have focused on identifying the impact of exposure to maternal obesity across the period before and throughout pregnancy on the health of the offspring. Only a limited number of studies have identified specific or critical periods in development during which exposure to maternal obesity programs specific metabolic changes in the offspring. In order to develop targeted interventions, it is important to determine the separate or interdependent contributions of maternal pre-pregnancy BMI, gestational weight gain and glycemic control on the longer term metabolic outcomes for the offspring.

Currently, it appears that for obese women, pre-pregnancy BMI, rather than gestational weight gain, is associated with an increased risk of pre-eclampsia, GDM and the delivery of a macrosomic infant (59). It has also been reported that siblings born to women who had undergone bariatric surgery for the treatment of severe obesity had a lower BMI and obesity risk than their siblings who were born prior to maternal surgery and weight loss (60). It has been shown that even in women who are ovulating regularly, an increased BMI correlates with reduced conception rates (61, 62). This suggests that maternal obesity affects reproductive health in the period around the time of conception. Indeed, in recent years, a number of studies have highlighted the association between maternal pre-pregnancy BMI and her offspring's BMI as well as other metabolic parameters including waist-to-height ratio, insulin and triglycerides (63, 64). Interestingly, Maftei and colleagues found that the relationship between maternal pre-pregnancy BMI and child insulin resistance was non-linear; there was a clear positive, progressive association between maternal body size prior to pregnancy and insulin resistance in her child only until the 'obese' category but not thereafter. The exact functional form of this relationship remains unknown (65).

Studies in mice have shown that when cultured *in vitro*, oocytes from obese mice exhibited slower development from the four to eight cell stage and through to the blastocyst stage (66).

These blastocysts also have reduced level of mitochondrial DNA and these changes were still present in the fetus at 14.5 days even though the blastocysts were transferred to normal weight surrogates (67). Furthermore, the negative effects of maternal obesity can be improved by treatment with insulin-sensitisers administered around the time of conception indicating that maternal obesity and peripheral insulin sensitivity during the periconceptional period is an important determinant of the developmental outcomes of the offspring (66). The periconceptional period, which includes some or all of the following early developmental stages: oocyte maturation and follicular development i.e. pre-pregnancy events, conception and embryo/blastocyst growth up until implantation (4) is, therefore, a critical period during which exposure to maternal obesity may contribute to poor metabolic outcomes in the offspring.

4. Exposure to maternal obesity in the periconceptional period and the programming of obesity and insulin resistance in the offspring

In order to determine whether exposure to maternal obesity in the periconceptional period alone has any specific impact on adiposity and metabolic function in the offspring, we have developed an embryo transfer model in sheep (68-72). In this model, non-pregnant donor ewes were either overnourished or normally nourished for at least four months before conception. One week after conception, single embryos were transferred from obese or normal weight 'donor' ewes to non-obese 'recipient' ewes, which were maintained on a control diet for the remainder of pregnancy (68-72). Thus, exposure to a high nutrient environment encompassed oocyte maturation, follicular development, conception and growth of the early pre-implantation embryo. This model is unique in that exposure of the offspring to maternal obesity is confined strictly to the periconceptional period. Donor ewes that were overnourished during the periconceptional period were heavier than controls at four weeks before conception (71) and had an obese phenotype at conception (71). Furthermore, similar

to obese humans (73), these obese donor ewes also had increased circulating insulin but not glucose concentrations in the period around conception (71).

4.1 Impact of exposure to obesity in the periconceptional period on fat mass in the offspring

There is a sex-specific effect of exposure to maternal obesity during the periconceptional period on the body fat mass of lambs at four months of age (71). Female but not male lambs, conceived in obese ewes had an increased total fat mass and this appeared to be a consequence of an increase in visceral adiposity (71). There was also a significant relationship between total fat mass of female lambs at four months of age and the weight of donor ewes at conception (71). Interestingly, the increased adiposity was not attributed to changes in expression of adipogenic, lipogenic and adipokine genes peroxisome proliferator-activated receptor (PPAR) γ, glyceraldehyde 3-phosphate dehydrogenase, lipoprotein lipase, leptin and adiponectin in the perirenal, omental or subcutaneous fat depots in the offspring (71). Further studies on the role of the insulin signalling and other key metabolic pathways which regulate lipogenesis within these fat depots are required.

4.2 Impact of exposure to obesity in the periconceptional period on insulin signalling in the liver and muscle in the offspring

Follow up studies have also been carried out to determine whether exposure to maternal obesity during the periconceptional period may result in insulin resistance in the offspring either as a consequence of the impact of increased adiposity on insulin sensitive tissues such as the liver and muscle (74, 75) or as a consequence of the programming of specific changes in the abundance of insulin signalling molecules in these tissues of metabolic importance (76-78).

While exposure to maternal obesity around the time of conception resulted in an increase in body fat mass in female lambs, we found that there were programmed changes in gene expression and protein abundance of key insulin signalling molecules in the liver and to a more limited extent in skeletal muscle of both male and female lambs conceived in obese ewes (68, 69). There was a decreased hepatic abundance of the insulin receptor as well as phosphorylated Akt (Ser 473) and FoxO1 (Thr 24) in the young offspring of obese ewes. Interestingly, however, there was a paradoxical effect on the expression of key factors which regulate hepatic gluconeogenesis; expression of 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) and the cytosolic (PEPCK-C) and mitochondrial (PEPCK-M) isoforms of phospho*enol*pyruvate carboxykinase were decreased in lambs exposed to maternal obesity (69) (Figure 1a). These contrasting changes suggest that there are distinct mechanisms involved, which are programed by maternal obesity during the periconceptional period and which are impacting hepatic insulin signalling and gluconeogenic factors separately. Findings in skeletal muscle of the offspring, however, showed that exposure to maternal obesity during the periconceptional period did not appear to impact directly on the early components of the insulin signalling pathway. Instead, this exposure resulted in specific changes in the abundance of molecules downstream of Akt (68) (Figure 1b). Given that the changes in the insulin signalling pathway in the liver and muscle were present in both male and female lambs, in contrast to the increased adiposity which was present only in female lambs, exposure to maternal obesity during the periconceptional period appears to act directly to program changes within the insulin signalling pathway in the liver and skeletal muscle (Figure 3).

4.3 Impact of exposure to obesity in the periconceptional period on hepatic fatty acid metabolism in the offspring

The liver is also an important site for the removal of circulating free FAs, the synthesis and esterification of FAs and for FA oxidation (79). In addition to defects in the insulin signalling network, increased hepatic fat deposition is also a common feature of obesity and insulin resistance (80, 81). Indeed, maternal obesity both before and throughout pregnancy has been shown to result in alterations in hepatic FA oxidation and lipogenesis in the offspring (82, 83). In addition to changes in the abundance of hepatic insulin signalling molecules, we also found that exposure to maternal obesity during the periconceptional period resulted in down regulation of hepatic PPARγ co-activator 1-α (PGG1-α) and PPARα and also resulted in a compensatory increase in the abundance of AMP-activated protein kinase (AMPK) α1 and α2, which may initially limit the impact of these changes on intra-hepatic FA oxidation in the young offspring (70). Furthermore, hepatic expression of sterol regulatory element-binding protein 1, a key lipogenic gene, was also increased in these lambs. It is possible that with aging and/or exposure to a high-caloric diet, these offspring may be susceptible to hepatic lipid accumulation and steatosis (70).

5. Exposure to maternal obesity during the periconceptional period and the epigenetic origins of obesity

During the early stages of development, the differentiation and development of different cell types is regulated by epigenetic mechanisms, which play a role in modulating chromatin architecture (84). Furthermore, epigenetic regulation plays a key role in conferring phenotype plasticity, which allows organisms to adapt their gene expression and function in response to the environment (85). Each cell type, therefore, has its own epigenetic signature which reflects genotype, developmental history and environmental influences. This is ultimately

reflected in the phenotype of the cell and organism (86). Early embryogenesis in mammals is a critical period for the establishment of the epigenome (87); during the period between conception and implantation there is de-methylation of the genome followed by a wave of re-methylation shortly after implantation (86). This period, therefore, represents a critical window in development during which the embryo is vulnerable to environmental and/or nutritional cues that disrupt the establishment of epigenetic marks such as DNA methylation, histone modification and microRNAs (miRs); (88) a class of small, non-coding RNAs that play an important role in post-transcriptional gene regulation either by cleavage or translational repression of their specific target mRNAs. Importantly, although the genome of an individual is largely stable, the epigenome has the potential to be reversibly modified by exposure to a range of nutritional and environmental factors (89). Parental nutrition has been shown to permanently influence metabolism of the offspring through epigenetic mechanisms and these changes also appear to be stable and transgenerational (90).

Using the embryo transfer model described above (68-72), we found that exposure of the oocyte/early embryo to maternal obesity resulted in up-regulation of hepatic expression of miR-29b, miR-103 and miR-107 (69) (Figure 1a). Expression of these miRs have been shown to be related to decreased insulin signalling in adipocytes (91) and liver (92) and is increased in murine models of obesity and T2DM (91-93).

Defects in insulin signalling are among the earliest indicators that an individual is predisposed to the development of insulin resistance and T2DM (94). Our results suggest that miRs may be potential epigenetic regulators that are sensitive to programming by the metabolic and nutritional environment associated with maternal obesity specifically during the periconceptional period and that miRs could, therefore, play a key role in the transduction of the metabolic consequences of maternal obesity from the mother to the offspring.

Importantly, miRs have recently emerged as potential early biomarkers in metabolic and cardiovascular disease (95).

6. Exposure to maternal obesity in late gestation and the programming of obesity and insulin resistance in the offspring

Late gestation has also been identified as a period during which exposure to maternal obesity can result in programming of obesity in the offspring. We have used a sheep model to investigate the impact of exposure to maternal overnutrition in late pregnancy on the offspring. Pregnant ewes were overfed for the last month of pregnancy (term= 150 ± 3 days gestation) to determine the impact on fetal and postnatal adiposity (96-98). Exposure to maternal overnutrition during this period resulted in an increase in fetal glucose and insulin concentrations and an up-regulation of key adipogenic, lipogenic and adipokine genes, including PPARγ, leptin and adiponectin in perirenal adipose tissue of the sheep fetus in late gestation (97) as well as increased fasting plasma glucose concentrations and a higher relative subcutaneous fat mass in the first month of life in the postnatal lamb (96). Furthermore, leptin expression in both the perirenal and subcutaneous fat depots was higher in the postnatal lamb of the overnourished ewe (99). Thus, it appears that overnutrition in late gestation results in an initial up-regulation of adipogenic and lipogenic genes in the perirenal fat in fetal life followed by an up-regulation in leptin expression in the perirenal and subcutaneous fat depots and the emergence of leptin resistance in the hypothalamic network which regulates appetite in postnatal life.

Similar to our findings in lambs exposed to maternal obesity during the periconceptional period, exposure to maternal overnutrition in the last month of gestation also resulted in decreased hepatic expression of PEPCK-M (but not of PEPCK-C or glucose-6-phosphatase) and this was present before birth and persisted in postnatal life (100). Exposure to maternal

overnutrition in late gestation had no impact on DNA methylation at each of the three CpG sites (−49, −58, and −88 relative to the transcription start site) in the promoter of PEPCK-C in the liver of the fetal and postnatal offspring (100). The level of methylation at each CpG site increased significantly, however, after the transition from fetal to postnatal life (100). There was also no impact of maternal overnutrition during this period on hepatic expression of PGC-1, PPARα and 11βHSD1 in the postnatal lamb. This is in contrast to the changes that occurred in the offspring of ewes that were overnourished from before and for one week after pregnancy. Thus, maternal overnutrition in late pregnancy may have a greater impact on the programming of the fat-brain axis than on hepatic glucose metabolism.

7. Maternal obesity and the programming of obesity in the offspring – the 'two hit' hypothesis

We postulate that exposure to maternal obesity in the periconceptional period and in late pregnancy represent 'two hits' which result in programming of obesity in the offspring. The first 'hit' occurs in the periconceptional period and results in increased fat mass in female offspring with the greatest impact on the visceral fat depots (69) and also results in programming of changes in the insulin signalling and FA metabolism pathways in the liver and skeletal muscle of both male and female lambs (68-70). The second 'hit' then occurs with exposure to maternal obesity in later gestation and leads to an increase in subcutaneous fat mass, an increase in leptin expression in visceral and subcutaneous fat depots and the emergence of central leptin resistance in postnatal life.

8. Weighing up the benefits and costs of maternal dietary and lifestyle interventions in obese mothers for their offspring

Clinical and experimental studies have provided clear evidence that exposure to a maternal obesogenic environment from before pregnancy and around the time of conception has long

lasting metabolic consequences for the offspring. Thus, intervention to limit the impact of maternal obesity in the periconceptional period may limit the negative impact of the first and possibly also the second 'hit' of maternal obesity on adiposity and metabolic outcomes in the offspring. In the context of the concerns of the emergence of an intergenerational cycle of obesity there has, therefore, been a focus on the type of weight loss intervention(s) that can be safely introduced in the obese woman seeking to become pregnant (4). Previous studies have suggested that childhood obesity may be prevented by normalizing body composition and nutrition and improving the general health of young women of childbearing age before becoming pregnant (101). Other studies have focused on limiting gestational weight gain through both physical activity and the reduction of dietary intake (102-104). Some of the beneficial effects of exercise during pregnancy for the mother include reduced risk for GDM, pre-eclampsia and cardiovascular function and benefits for the infant include improved risks for prematurity and improved fetal growth. It is not known, however whether there are longer-term benefits of exercise during pregnancy for the obese mother and her offspring (105). Furthermore, as traditional "diet and exercise" approaches do not often achieve robust and sustained weight loss (106), surgical approaches have also emerged as an effective albeit complex strategy to promote durable weight loss, improve insulin resistance and improve or reverse T2DM especially in morbidly obese women (106, 107). A number of studies have found a decreased prevalence of obesity in the offspring of mothers who underwent maternal surgical weight loss prior to pregnancy (108, 109). Furthermore, a recent study by Guénard and colleagues found that the improved cardiometabolic risk profiles of offspring born after maternal weight loss surgery were associated with epigenetic changes including differential methylation patterns of glucoregulatory genes (110).

In contrast to human studies, there have been relatively few experimental animal studies, which have investigated whether maternal dietary restriction and/or exercise are able to

reverse the outcomes associated with maternal obesity. We have shown in the sheep that dietary restriction and weight loss experienced by the mother in the periconceptional period alone is able to ablate the effects of a high maternal pre-pregnancy weight on offspring adiposity (71). Furthermore, in a study where the nutritional intake of female rats, which were previously on a high-fat diet was "restricted" to normal chow from one month prior to conception and throughout pregnancy and lactation, dietary "restriction" was able to normalise fat mass, serum triglycerides, leptin and insulin levels in three-week old male offspring (111). Interestingly, these changes occurred even though these dams that were exposed to dietary "restriction" remained heavier than their control counterparts at conception (111) indicating that in this instance, it is the metabolic response to dietary restriction rather than a lower maternal body weight, which confers metabolic benefits in the offspring. Studies investigating the impact of exercise from before and/or throughout pregnancy in rodents have found that exercise is able to ablate the increase in plasma leptin and triglycerides caused by exposure to maternal obesity (112). Expression of key genes involved in glucose and lipid metabolism as well as markers of inflammation were also reduced to control levels in skeletal muscle and adipose tissue of offspring from dams that underwent voluntary exercise during pregnancy (113).

While maternal dieting before pregnancy has metabolic benefits, there are also potential metabolic and endocrine costs for the offspring. Studies of people born at the time of the Dutch famine in 1944-1945 have shown that exposure to undernutrition during both early and mid-pregnancy in a population that was previously well nourished was associated with a reduction in glucose tolerance and increased insulin concentration at age 50 and 58 (114). Furthermore, experimental evidence in sheep has shown that nutritional restriction imposed in ewes with a normal body across both the periconceptional and early gestation periods (from 60 days before until 30 days after conception) has an adverse impact on the glucose-

insulin axis of the offspring in postnatal life (115). This impaired glucose tolerance also persists in the adult offspring (116). It is not known, however, whether a similar period of dietary restriction and weight loss imposed on obese ewes will also have consequences for the metabolic health of the offspring.

We have also shown that in most instances dietary restriction and weight loss in the periconceptional period were unable to ablate the effects of maternal obesity on the abundance of insulin signalling proteins in both the liver (69) and skeletal muscle (68) or on signalling molecules involved in hepatic lipid metabolism in the postnatal lamb (70). Instead, exposure to dietary restriction in either normal weight or obese ewes resulted in a down-regulation of a different subset of insulin signalling proteins within both liver and skeletal muscle compared to offspring exposed to maternal obesity. Furthermore, the reduced abundance of some insulin signalling molecules were conserved both in liver and skeletal muscle of offspring exposed to maternal dietary restriction during the periconceptional period (68, 69) (Figure 2a & b). This suggests that the mechanism involved is one that is able to impact tissues that arise from different cell lineages as the liver and skeletal muscle arise from the endoderm and mesoderm respectively (117). This leads us to ask the question of whether the oocyte/early embryo is most sensitive to perturbations during the period encompassing one month before and one week after conception. Moreover, since the specification of different cell types is regulated by epigenetic mechanisms such as histone or DNA modification, which can modulate chromatin architecture (84), it is, possible that changes in the abundance of insulin signalling molecules in liver and skeletal muscle of the offspring are due to the recruitment of epigenetic mechanisms within the developing embryo. We have presented clear evidence that metabolic and endocrine changes occur in the offspring as a result of exposure of the oocyte and/or the early embryo to maternal dietary restriction and weight loss, independent of the body weight of the ewe at the time of

conception. These findings are of particular importance in the context of current clinical practice whereby dietary restriction is recommended to overweight and obese women who are seeking to become pregnant and highlight the need for a better evidence base for the development of dietary interventions in obese women before pregnancy which maximize the metabolic benefits and minimize the metabolic costs for the offspring. Furthermore, given the sensitivity of the oocyte/early embryo to changes in the maternal milieu, there is also a need for caution around the use of nutritional supplements and also medicines especially during the periconceptional period.

9. Conclusion

There is compelling evidence that exposure to maternal obesity or to impaired glucose tolerance during pregnancy is associated with an increased risk of obesity and features of insulin resistance in the offspring thus potentially fuelling an 'intergenerational cycle' of obesity and insulin resistance. Importantly, we have identified that the oocyte and/or early pre-implantation embryo appear to be particularly vulnerable to the maternal metabolic milieu associated with overnutrition/obesity. This vulnerability manifests as long lasting endocrine and metabolic effects for the offspring even after the transition to postnatal life. These key findings highlight the need for emphasis to be placed on ensuring that both young women and in particular young girls maintain a healthy body weight and be in good metabolic health.

While the impact of maternal obesity on the metabolic health of her offspring has been described, there are relatively few studies which have provided a detailed characterisation of the maternal metabolic features associated with obesity that are directly associated with the developmental programming of changes within the metabolic tissues of the offspring.

Investigations into the molecular mechanisms underlying the programming of obesity and insulin resistance in liver, muscle and adipose tissue have highlighted the sensitivity of the offspring epigenome to maternal obesity. Epigenetic changes within these tissues, which are recruited within the developing embryo and/or fetus, may be the conduit through which a life of metabolic vulnerability is programmed in tissues of metabolic importance. Furthermore, the impact of maternal obesity in different insulin sensitive tissues in the male and female offspring may be programmed independently rather than as a result of the indirect effects of an increase in body fat mass (Figure 3).

Finally, it is clear that dietary restriction alone in overweight/obese women prior to and around the time of conception may not be the optimal intervention to break the 'intergenerational cycle' of obesity and insulin resistance (Figure 4). In light of these findings, it is important to determine whether maternal exercise in overweight/obese mothers during the preconception period has long term consequences for insulin signalling pathways within the liver, muscle and/or fat of her offspring (118, 119). Studies highlighted in this review show that there is an important and immediate need for evidence-based interventions, which inform and motivate pregnant women to adopt a healthy lifestyle both before and during pregnancy. Moreover, it is important that there is a greater understanding of the long term consequences of any maternal dietary or lifestyle interventions introduced in the periconceptional period on the metabolic health of the offspring.

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Figure Legends

Figure 1: The impact of exposure to maternal obesity during the periconceptional period on the abundance of molecules involved in insulin signalling and gluconeogenesis in liver (a) and the abundance of insulin signalling molecules in skeletal muscle (b) of male and female lambs at four months of age

Figure 2: The impact of exposure to maternal dietary restriction during the periconceptional period in obese ewes on the abundance of molecules involved in insulin signalling and gluconeogenesis in liver (a) and the abundance of insulin signalling molecules in skeletal muscle (b) of male and female lambs at four months of age

Figure 3: The impact of maternal obesity during the periconceptional on different insulin sensitive tissues in the male and female offspring may be programmed independently rather than as a result of the indirect effects of an increase in body fat mass. Epigenetic changes within these tissues may be the conduit through which a life of metabolic vulnerability is programmed in tissues of metabolic importance

Figure 4: Both maternal obesity and dietary restriction during the periconceptional period program separate and distinct changes in hepatic and skeletal muscle insulin signalling in the offspring. Thus, although dietary restriction is able to ablate some of the effects of maternal obesity, it is clear and both maternal obesity and dietary restriction may independently confer an increased risk for metabolic disease in the offspring in later life.

Figure 1

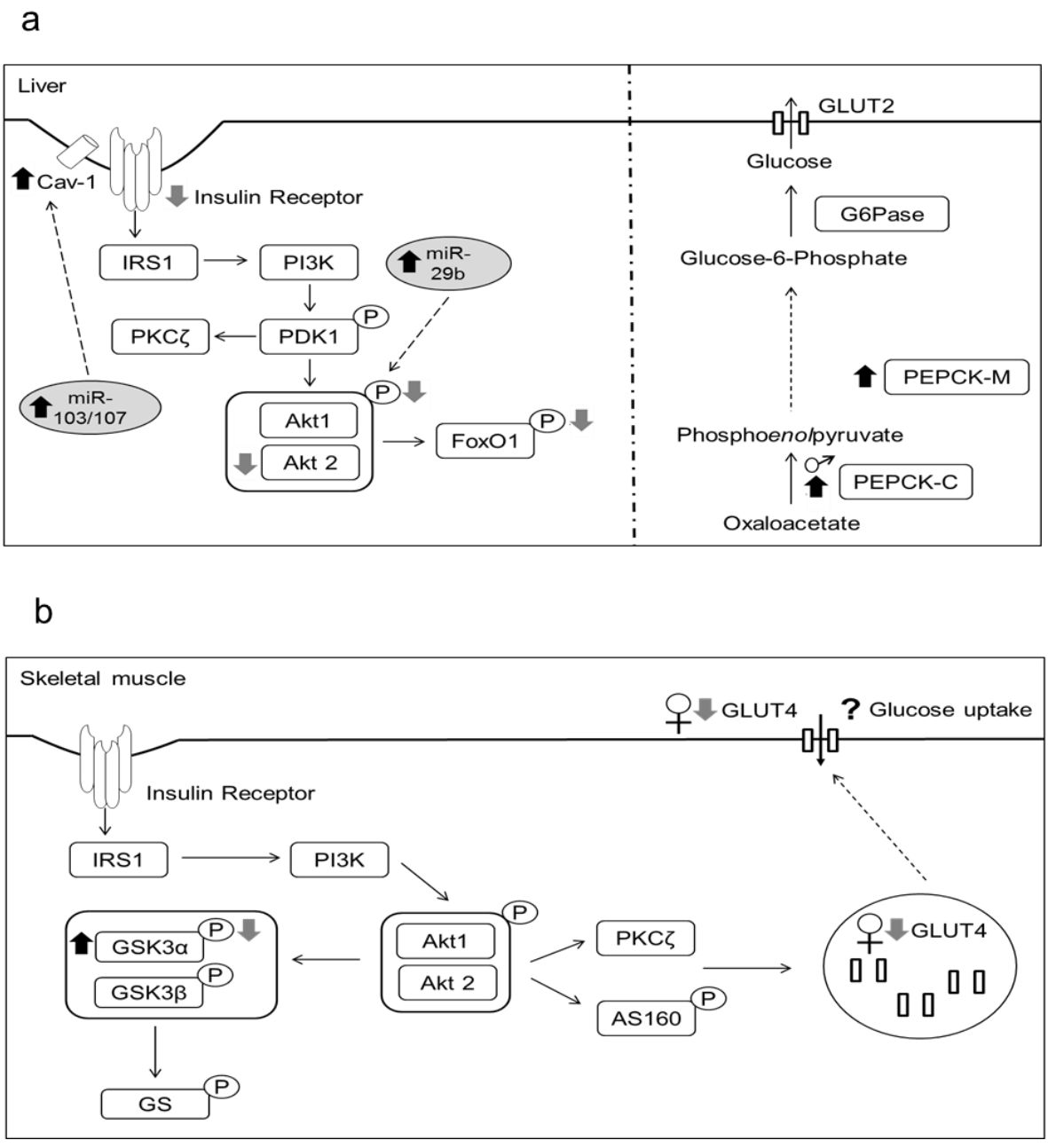

a
Liver
Cav-1
Insulin Receptor
IRS1
PI3K
miR-29b
PKCζ
PDK1
P
miR-103/107
Akt1
Akt 2
FoxO1
GLUT2
Glucose
G6Pase
Glucose-6-Phosphate
PEPCK-M
Phosphoenolpyruvate
PEPCK-C
Oxaloacetate
b
Skeletal muscle
GLUT4
? Glucose uptake
Insulin Receptor
IRS1
PI3K
GSK3α
GSK3β
Akt1
Akt 2
PKCζ
AS160
GLUT4
GS

Figure 2

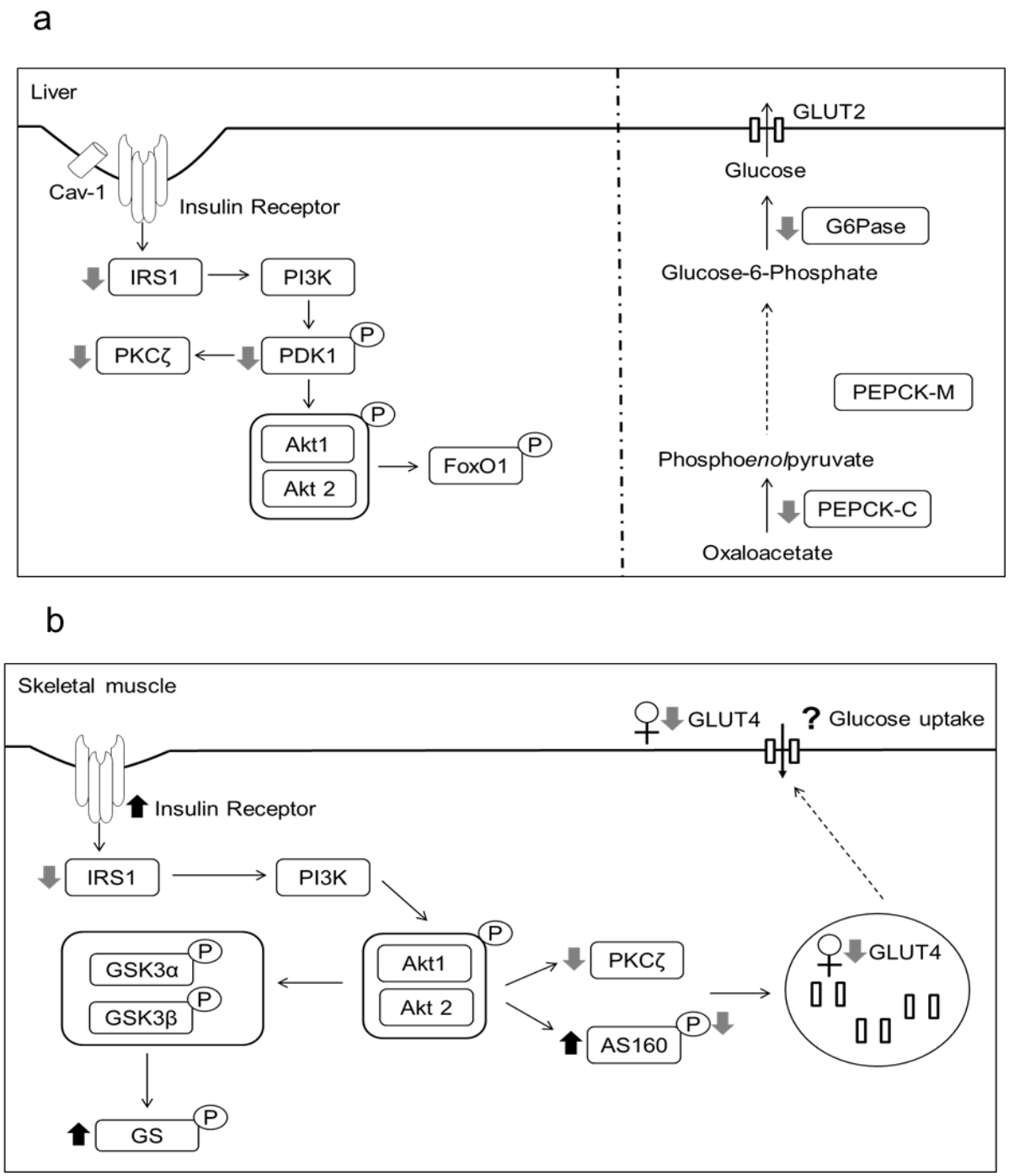

a
Liver
GLUT2
Glucose
Cav-1
Insulin Receptor
G6Pase
IRS1
PI3K
Glucose-6-Phosphate
PKCζ
PDK1
P
PEPCK-M
Akt1
Akt 2
FoxO1
Phosphoenolpyruvate
PEPCK-C
Oxaloacetate
b
Skeletal muscle
GLUT4
? Glucose uptake
Insulin Receptor
IRS1
PI3K
GSK3α
GSK3β
Akt1
Akt 2
PKCζ
GLUT4
AS160
GS

Figure 3

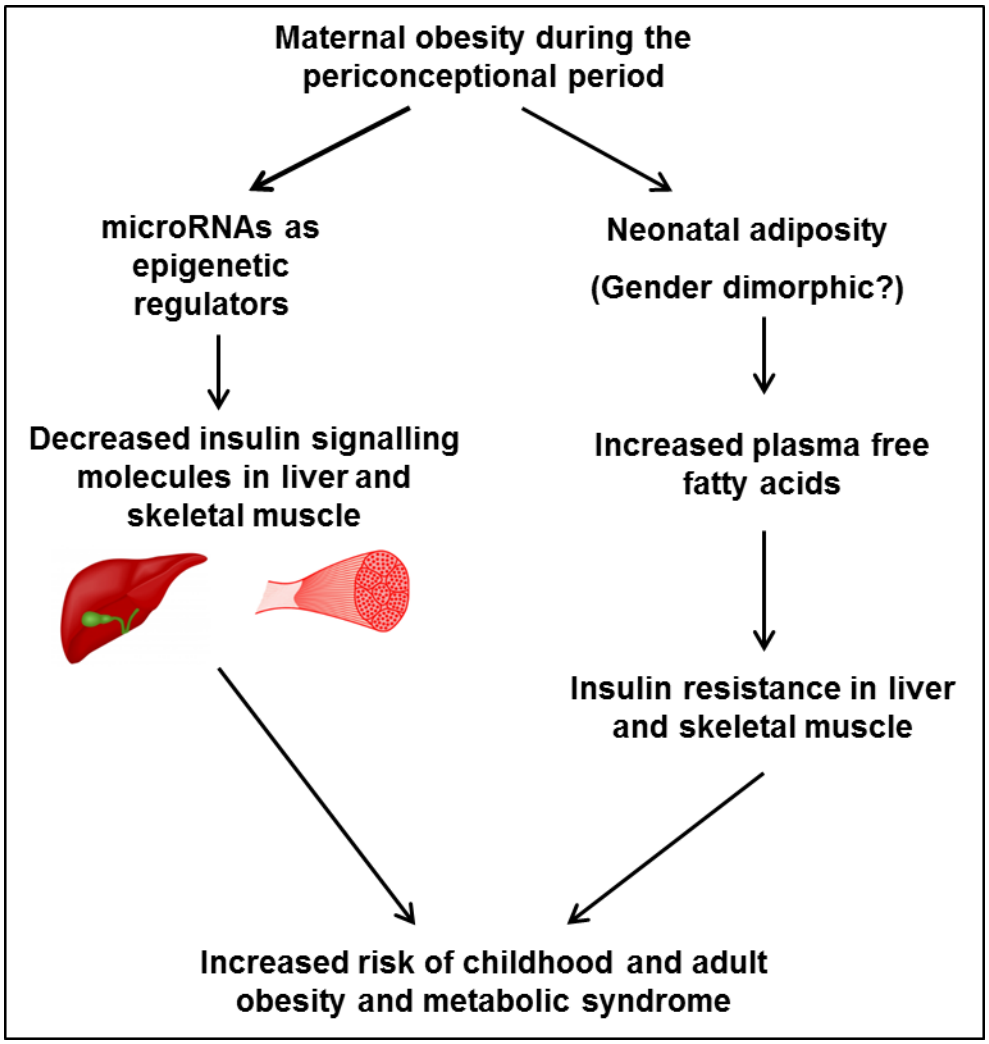
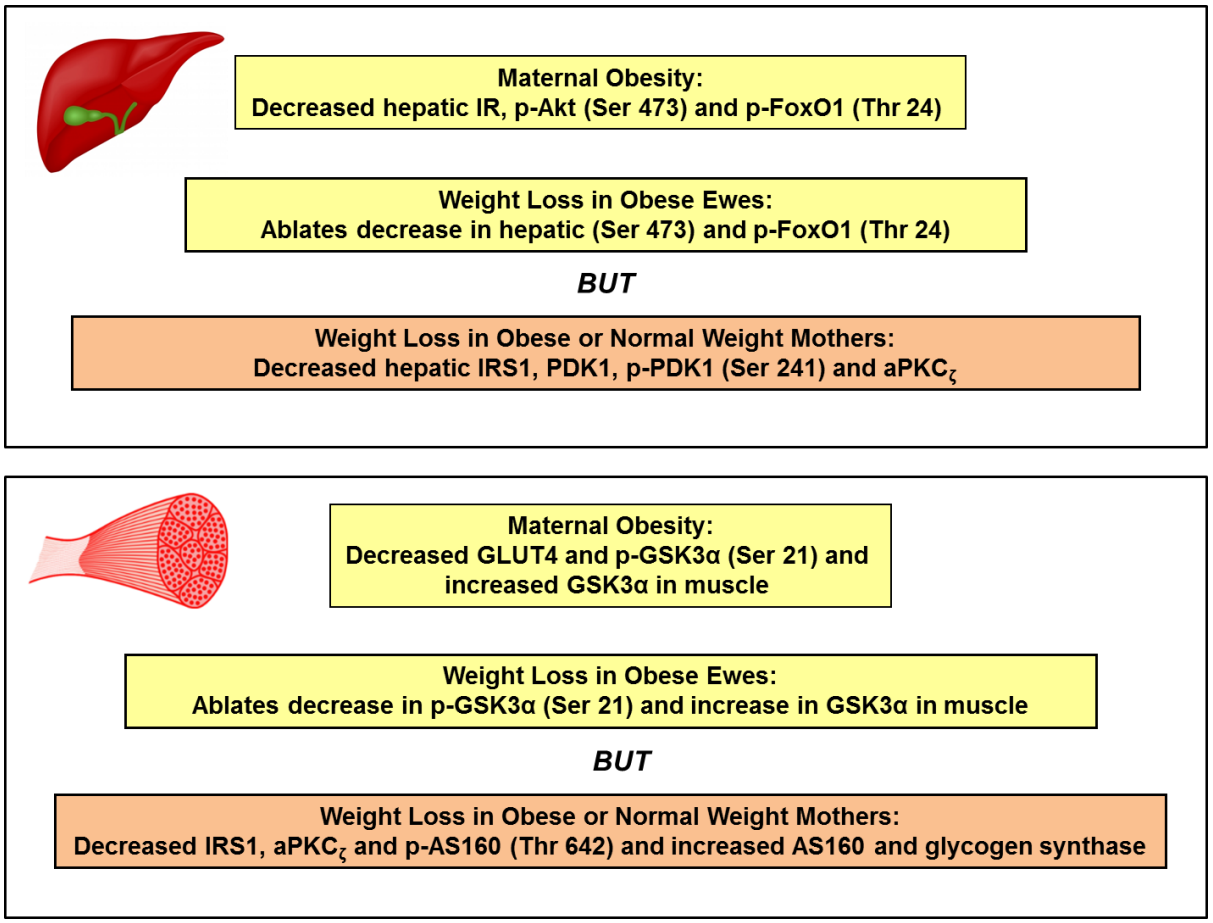

Maternal obesity during the periconceptional period
microRNAs as epigenetic regulators
Neonatal adiposity
(Gender dimorphic?)
Decreased insulin signalling molecules in liver and skeletal muscle
Increased plasma free fatty acids
Insulin resistance in liver and skeletal muscle
Increased risk of childhood and adult obesity and metabolic syndrome

Figure 4


Maternal Obesity:
Decreased hepatic IR, p-Akt (Ser 473) and p-FoxO1 (Thr 24)
Weight Loss in Obese Ewes:
Ablates decrease in hepatic (Ser 473) and p-FoxO1 (Thr 24)
BUT
Weight Loss in Obese or Normal Weight Mothers:
Decreased hepatic IRS1, PDK1, p-PDK1 (Ser 241) and aPKCζ
Maternal Obesity:
Decreased GLUT4 and p-GSK3α (Ser 21) and increased GSK3α in muscle
Weight Loss in Obese Ewes:
Ablates decrease in p-GSK3α (Ser 21) and increase in GSK3α in muscle
BUT
Weight Loss in Obese or Normal Weight Mothers:
Decreased IRS1, aPKCζ and p-AS160 (Thr 642) and increased AS160 and glycogen synthase