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Sex differences in the developmental origins of cardiometabolic disease following exposure to maternal obesity and gestational diabetes.

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Abstract

Over the last 30 years, the worldwide prevalence of obesity has nearly doubled. In addition, more and more women in their child bearing years are overweight or obese, which increases the risk for gestational diabetes mellitus (GDM). It is increasingly accepted by the scientific community that the early life exposure to environmental stress influences the long-term health of an individual, which has been termed the Developmental Origins of Health and Disease (DOHaD) theory. Evidence from human cohorts, epidemiological and animal studies have reported that maternal obesity and GDM alter the epigenome of the next generation, conditioning the offspring for cardiometabolic disease development. These effects are most likely regulated by epigenetic mechanisms; however, biological sex is an important factor in defining risk for the development of several metabolic health disorders. The aim of this review is to describe current evidence from human cohort and animal model studies that implicates sex differences in the developmental origins of cardiometabolic disease following exposure to maternal obesity and GDM. Additionally, this review will address the potential mechanisms involved in these sex differences. In many studies, sex is ignored as an important variable in disease development; however, the results presented in this review highlight important differences between sexes in the developmental programming of biological responses to exposures during the fetal stage. This knowledge will ultimately help for the development of effective therapeutic strategies for the treatment of cardiometabolic diseases that exhibit sexual dimorphism.

Keywords: Gestational diabetes, maternal obesity, cardiometabolic disease, Developmental Origins of Health and Disease, endocrine hormones, estrogen, testosterone, insulin resistance, metabolism.
1. Introduction

The prevalence of obesity is increasing at an alarming rate, across all populations and age groups. In addition, obesity in pregnant women increases the risk of pregnancy complications, such as gestational diabetes mellitus (GDM). In Canada, it has been estimated that, in 2009, 16% of women starting a pregnancy were obese (Fuchs et al. 2017). In addition to the effects on maternal health, maternal obesity as well as GDM increases the risk of long-term health complications in the offspring, including obesity, type 2 diabetes (T2D), and heart disease (Gaillard et al. 2013; Godfrey et al. 2017). Data from human observational and animal studies have been recently reviewed by our group, and we summarized that maternal obesity and GDM alter the epigenome of the next generation, conditioning the offspring for cardiometabolic disease development (Agarwal et al. 2018). However, biological sex modifies the risks for cardiometabolic disease in the offspring that are a consequence of early life exposure to maternal obesity or diabetes during pregnancy (Figure 1). In this review, we focus on how maternal obesity and GDM influence the cardiometabolic health of the next generation in a sex-specific manner. For this, we will discuss the current evidence from human cohort and animal model studies, as well as the potential mechanisms involved in these sex differences.

2. Current state in the epidemic of chronic metabolic disease in children and adults

According to the World Health Organization, in 2016, over 650 million of the adults worldwide were obese, with 41 million children under the age of 5 being overweight or obese. The prevalence of obesity has doubled in nearly 30 years, with 10% of adult men and 14% of adult women worldwide being obese. In addition, 74 million of boys and 50 million of girls aged between 5 and 19 years old suffer from obesity (NCD-RisC 2017). In Canada, it has been estimated that approximately 1 out of 4 adults, and 1 out of 7 children and adolescents are obese (Bancej et al. 2015). Obesity is a major risk factor for the development of several metabolic
disorders, such as cardiovascular diseases, hypertension, insulin resistance and T2D. In parallel with these trends, the global adult diabetes prevalence drastically increased from 4.3% to 9.0% in men and from 5.0% to 7.9% in women in about 30 years (Rosengren 2018). In total, it is estimated that over 400 million people worldwide, and 3.5 million people in Canada suffer from T2D (Lipscombe and Hux 2007). In addition, in 2001, the prevalence of T2D in North America was estimated to be 42 cases per 100,000 children and adolescents aged between 10–19 years (Chen et al. 2011). Childhood obesity is associated with a higher risk for metabolic disease, such as metabolic syndrome, atherosclerosis, dyslipidemia, hypertension and T2D (Halpern et al. 2010). Lifestyle changes, such as dietary habits or lack of physical activity, enhances the development of obesity and T2D.

3. The influence of biological sex on chronic metabolic disease risk

Sex-based differences in physiology and metabolism are important factors that likely define the differences between rates of cardiometabolic disease and risk for disease development among men and women. For example, males and females have differences in systemic glucose regulation (Basu et al. 2006; Flanagan et al. 2006). In addition, pre-menopausal women tend to have peripheral fat accumulation in contrast to the visceral fat in men, contributing to a greater insulin sensitivity at higher levels of body mass index in women as compared to men (Wannamethee et al. 2012). Consequently, women require a greater level of metabolic disorder before overt T2D diagnosis. While pre-menopausal women typically present with cardiovascular disease approximately a decade later than men (Norhammar and Schenck-Gustafsson 2013), women with diabetes experience a greater relative risk for major cardiovascular disease events compared to men (Kannel et al. 1974; Laverty et al. 2017). These observations highlight the need to consider the influence of sex differences in cardiometabolic disease research.
4. The Developmental Origins of Health and Disease theory (DOHaD)

As a consequence of an increased prevalence of obesity and diabetes in young women in their child bearing years, the incidence of maternal obesity and diabetes during pregnancy are also on the rise (Kaseva et al. 2018). Because evidence shows that there is a link between these changes and negative consequences for fetal development, it is also evident that fetal exposure to maternal obesity and GDM increases the chances for the development of severe metabolic diseases at a later stage of lifetime of the offspring (i.e. the Developmental Origins of Health and Disease theory (DOHaD)) (Wadhwa et al. 2009).

The DOHaD theory states that exposure to environmental stressors early in life, such as during the fetal stage, has profound consequences on the vulnerability to develop diseases later in life, mostly during adulthood (Gluckman et al. 2010; Mochizuki et al. 2017). The maternal metabolic milieu appears to be a key determinant of insulin resistance in the offspring (Lacroix et al. 2013). Foundational population health, human cohort and animal model studies that were used to develop the DOHaD theory have already been reviewed in detail by our group (Agarwal et al. 2018). With respect to the exposure of offspring to maternal obesity and diabetes during pregnancy, the collection of studies we reviewed shows effects on the development of several metabolic disorders, including obesity, nonalcoholic fatty liver disease, high blood pressure, renal and heart disease, and T2D in the offspring, consistent with the DOHaD theory (Agarwal et al. 2018). The mechanisms behind these effects are complex and most likely involve epigenetic modifications, such as histone modifications, non-coding RNA mediated regulation, and DNA methylation; the latter being the most studied and best characterized. These epigenetic mechanisms partly mediate developmental plasticity in order for the organism to adapt to environmental signals, and disruption of these epigenetics mechanisms is related to the development of metabolic disorders (Gluckman et al. 2011). However, less is known about how
maternal obesity and diabetes during pregnancy interact with the biological sex of the offspring and influence mechanisms that define offspring health outcomes.

5. Sex differences in the developmental origins of cardiometabolic disease following exposure to maternal obesity and gestational diabetes

It is well known that sex hormones regulate many genes involved in biological processes and disease development (Arnold and Lusis 2012; Arnold et al. 2009). As previously reviewed, females and males display differences in adipose tissue storage, energy balance, glucose homeostasis and diseases predisposition, i.e. men being more prone to develop diabetes, while women being more predisposed to obesity and metabolic syndrome (Mauvais-Jarvis 2015). Results from the Helsinki Birth Cohort Study showed that the association between maternal BMI and T2D development in the offspring was stronger in women than in men. In addition, higher maternal BMI during pregnancy was associated with greater incidence of stroke only in female offspring, while association with coronary heart disease was greater in male offspring (Eriksson et al. 2014). Furthermore, waist circumference was found to be a predictor of T2D in Chinese women aged between 18-79 years, regardless of the BMI status, while both waist circumference and BMI were positively associated with T2D risk in Chinese men. The authors suggested that differences in fat deposition between men and women could be a potential explanation for this observation (Tian et al. 2018). Rodent studies have reported that there were sex differences in the fat accumulation and blood pressure phenotypes of the offspring exposed to maternal obesity and diabetes during pregnancy, with males displaying increased adipose tissue lipogenesis and visceral fat accumulation, whereas females had elevated blood pressure (Agarwal et al. 2018). Differences in genes expression and methylated regions (Keleher et al. 2018; Mischke et al. 2013) between the liver tissues of female and male offspring in response to maternal obesity have also been reported. Feeding Western-style high fat diets to mouse
dams affected lipid metabolism in the offspring in a sex-specific manner. Indeed, genes related to cholesterol homeostasis were found to be upregulated in male offspring, whereas genes involved in cholesterol biosynthesis were mainly up-regulated in female. In addition, hepatic gene expression profiles were changed in offspring exposed to maternal high fat diet, with only 10% of these changed genes being common to both sexes (Mischke et al. 2013). Differences in DNA methylation between sexes could be due to the fact that more differentially methylated regions were found to be located on the X-chromosome in the female mouse offspring exposed to maternal high fat diet, as compared to the male offspring, which may be a result of the inactivation of the X-chromosome (Keleher et al. 2018). In addition, maternal diet not only influences sex-specific responses the sex of the offspring, as well as lead to. It has indeed been reported that diets high in fat and low in carbohydrate generally favor birth of males over females, while the opposite is observed for low-fat and high carbohydrate diets (Rosenfeld and Roberts 2004). Female fetuses appear to respond more strongly to maternal diets than males, as indicated by the marked upregulation of genes in the female mouse placentae from high fat diet-fed dams. The increased expression of genes involved ion balance and chemoreception could allow the females’ placenta to more acutely sense minor dietary components as compared to males on the same diet (Mao et al. 2010). As males and females distribute fat differentially, maternal dietary fat content is a critical variable to consider when studying sex differences. The section below will summarize the sex differences in body weight, insulin resistance, pancreatic β-cell development and fatty liver disease in offspring exposed to maternal obesity and GDM.

5.1 Sex differences in Body weight/Obesity in the offspring

*Human*
Cohort studies reported that metabolic health outcomes associated with GDM exposure are different in male and female offspring. Maternal glycemia appears to be the main predictor of adiposity in male infants, whereas the main predictor in female infants is maternal BMI (Lingwood et al. 2011). Male offspring exposed to GDM were reported to be heavier and taller than female offspring (Persson and Fadl 2014).

A recent study performed in an African population showed that fetal abdominal circumference was larger in fetus exposed to GDM as compared to non-exposed fetus. This association only held up when the fetus was male, while there was no difference among female fetus (Macaulay et al. 2018). These findings are consistent with a previous study in which male offspring exposed to GDM, but not female, were found to have a higher BMI and increase risk to develop obesity as compared to non-exposed offspring. This was true only in male offspring and persisted from late childhood (< 12 years) and adolescence (12 to 18 years) to early adulthood (≥ 18 years) (Li et al. 2017), suggesting that GDM has long-term adverse outcomes in the offspring in a sex-specific manner.

**Animal models**

Many studies have evaluated the effects of maternal high fat diets (Table 2), and fetal exposure to maternal obesity and diabetes during pregnancy on the offspring in animal models (Agarwal et al. 2018); however, these experimental studies have primarily examined effects on male offspring. Studies that compared effects on male and female offspring are summarized in Table 1 and reviewed below. Results from a study performed in rat dams fed 20 % energy from animal lard to induce maternal obesity showed no effect on body weight in both male and female offspring fed with a control chow diet at 36 and 110 days old (Zambrano et al. 2016). Nonetheless, the percentage of body fat, and circulating triglyceride and leptin levels were
lower in female offspring exposed to maternal obesity as compared with male offspring. A previous study using a diet-induced model of maternal obesity (17% energy from fat) showed that, from 4 weeks of age, exposed male offspring fed a chow diet underwent greater postnatal weight gain than exposed female offspring (Ornellas et al. 2013). Maternal obesity exposure also induced a larger increase in food intake in male offspring as compared to females. Consistent with these findings, our group observed that in rats up to 105 days of age, the male offspring of GDM dams fed either low fat or high fat and sucrose diets throughout the postnatal period gained significantly more weight compared to the male offspring of lean dams (Pereira et al. 2015). Interestingly, we did not observe an effect of GDM on the body weights of female offspring in the first 105 days of life. Higher levels of circulating cholesterol, triglycerides, leptin and insulin levels, and lower levels of circulating adiponectin in male offspring were factors that likely contributed to the sex differences in the maternal obesity-induced weight gain. Both sexes exposed to maternal obesity presented higher expression of pro-inflammatory adipokines, i.e. tumor necrosis factor-alpha (TNFα), interleukin-6 (IL-6), and lower expression of adiponectin as compared to control non-obese mice; however, effects were larger in male offspring (Ornellas et al. 2013). In another study, at 21 days old, male offspring displayed a 30% increase in body weight, whereas HF female offspring only showed a 10% increase. In addition, males were predisposed to visceral fat accumulation, whereas this was not observed in female offspring (Lecoutre et al. 2016). Differences in gene expression were also found. Nine-month-old male offspring, but not female, exposed to maternal obesity that were fed a low-fat control diet (10% of total calories as fat) displayed in perirenal white adipose tissue higher mRNA levels of leptin, as well as of fatty acid synthase (FAS) and sterol regulatory element-binding protein-1 (SREBP1), both promoting de novo lipogenesis. Additionally, genes involved in adipogenesis, such as peroxisome proliferator-activated receptor gamma (PPARγ) and 11β-hydroxysteroid dehydrogenase type 1 (11β-Hsd1) were decreased in male offspring.
but not in female. In female offspring, only CCAAT/enhancer-binding protein alpha (C/EBP-α) was increased, which was not observed in males (Lecoutre et al. 2016). It was recently shown that maternal consumption of a cafeteria diet (Table 2) resulted in an increase in fat mass in both male and female rat offspring fed with a control diet (3.3 kcal/g) and aged of 3 months old, while the body weight was not increased. It was suggested by the authors that it might be a consequence of lower protein intake and high-fat consumption (Pomar et al. 2017).

5.2 Sex differences and Insulin Resistance in the offspring

**Human**

In an Indian cohort, female offspring exposed to GDM have been found to have higher insulin concentrations as compared to male at age 5, suggesting increased insulin resistance in female offspring (Krishnaveni et al. 2005). Similarly, 9 year-old Indian children from mothers that had diabetes during pregnancy have higher glucose concentrations, are more insulin resistant compared to children from non-diabetic pregnancies, and the prevalence of insulin resistance was greater in the girls than the boys (Krishnaveni et al. 2010). This is consistent with another study that reported that newborn girls from healthy mothers have higher cord blood insulin and proinsulin concentrations than boys, which points to greater insulin resistance in girls both in utero and post partum (Shields et al. 2007). Treatment of mild-GDM was found to lower fasting glucose levels in female offspring aged between 5 and 10 years old, while interestingly no effect was found in males (Landon et al. 2015).

**Animal models**

Several studies have compared insulin resistance in male and female offspring (Table 1). In rat dams fed 20% animal lard, maternal obesity induced a greater level of insulin resistance in male
110 day-old chow-fed (5 % energy from fat) offspring compared to females (Zambrano et al. 2016). This is in line with a previous report suggesting that female mice are protected against insulin resistance due to the beneficial effect of estrogens (Riant et al. 2009). However, in rats, maternal consumption of a cafeteria diet during lactation induced higher circulating glucose levels at 3 and 6 months of age in the female offspring fed with a standard chow diet (3.3 kcal/g) as compared to controls, while the elevated blood glucose was not observed in the male offspring of cafeteria diet fed dams (Pomar et al. 2017). In addition, 3 month-old female offspring from obese dams that were fed a postnatal standard chow diet have reduced expression of IRS-1, as well as impaired Akt phosphorylation and PI3K activity in muscle tissue, that was not observed in male (Shelley et al. 2009). Interestingly, the combination of maternal high-fat diet (containing 45% fat) with probiotics improved offspring metabolism and was associated with a decrease in leptin levels in both male and female aged of 42 days and fed with a postnatal standard chow diet (10% fat). A reduction in glucose and insulin levels were also observed but only in the female offspring (Guo et al. 2018). The discordant effects on offspring insulin resistance observed between animal and human studies might be attributable to species-specific differences in how biological sex regulates gene and protein expression as well as intracellular signaling to control glucose and insulin regulation (Chandrasekera and Pippin 2014).

5.3 Sex differences in pancreatic β-cell development and function in the offspring

*Human*

It was recently reported that both the male and female offspring (≥18 years of age) of mothers with type 1 diabetes (T1D) displayed reduced insulin secretion in response to oral glucose, as compared with offspring of T1D fathers which were the control group. However, only females exposed in utero to maternal type 1 diabetes exhibited a decreased insulin secretion in response to intravenous glucose infusion. It was suggested by the authors that, in contrast to females, the
direct β-cell stimulation of insulin secretion by glucose is preserved in male offspring (Gautier et al. 2018), which might be attributed to the action of testosterone via the androgen receptor (Navarro et al. 2016). Another explanation could be that the effects of gut incretin hormones, i.e. glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), on insulin secretion might be altered in offspring exposed to GDM. Indeed, the incretin effect has been reported to be reduced in women with GDM and in individuals with T2D, due to an impairment in β-cell sensitivity (Bonde et al. 2013; Foghsgaard et al. 2017; Kosinski et al. 2013). In addition, offspring exposed to GDM also display an altered incretin effect (Chandler-Laney et al. 2014; Kelstrup et al. 2015). In contrast to the study of Gautier et al., it has been previously suggested that women carrying a male fetus display higher risk for the development of GDM, as compared to women carrying a female fetus. These women also had reduced pancreatic β-cell function, as measured by the insulinogenic index divided by HOMA-IR during pregnancy (Retnakaran et al. 2015). Nevertheless, few studies have investigated the impact of maternal obesity and GDM on human offspring β-cell development and function in a sex-specific manner, highlighting the need for more longitudinal studies in that area of research.

**Animal models**

Only a few studies have evaluated whether maternal obesity affects the endocrine pancreas of the offspring in a sex specific manner (Table 1). Using dams with the Agouti yellow modification, Han et al. tested the effect of maternal obesity on β-cell function in the offspring (Han et al. 2005). This study showed that female offspring of 50 weeks of age and fed either a control postnatal diet (22% energy from fat) or a high-fat diet (60% energy from fat) display impaired glucose tolerance, while this was not seen in male offspring. They also showed that the ability of glucose to stimulate insulin secretion was reduced in isolated islets from female offspring. It was therefore suggested by the authors that the glucose intolerance in the offspring
was due to fetal programming of an inherent defect in insulin secretion by pancreatic β-cells. In addition, the activity of several metabolic enzymes was found to be reduced in islets from female offspring, such as transketolase, GAPDH and PFK. These results taken together may explain the decline in islet function observed (Han et al. 2005). However, more recently, it was shown that male offspring from obese dams and fed a high-fat postnatal diet (62% energy from fat) display a decrease in the average islet area and its insulin content at 20 weeks of age, as compared to male fed a control postnatal diet (11% energy from fat), suggesting an impaired pancreatic β-cell function (Yokomizo et al. 2014). However, defective insulin content and area was not observed in female offspring. This may be related to the increase in oxidative stress observed in male islets, that was not observed in the female offspring.

5.4 Sex differences and fatty liver diseases in the offspring

Human

The Raine study provided quality evidence between parental characteristics and diagnosis of nonalcoholic fatty liver disease (NAFLD) in their adolescent children. The Raine study is a prospective study that was performed in Australia from 1989 to 1992, and that included 2 868 live-born children from 2 900 pregnancies. It was demonstrated that maternal obesity was associated with NAFLD in 17 years old adolescent females, whereas lower family socioeconomic status at birth was associated with NAFLD in male offspring (Ayonrinde et al. 2018).

Animal models

The influence of maternal obesity on the development of NAFLD in male and female offspring has been examined in several experimental animal model studies (Table 1). Male offspring from
obese female Wistar rats fed with a control diet (5% energy from fat) and aged 110 days old were shown to exhibit more pronounced physiological, biochemical, and histological changes related to NAFLD (Lomas-Soria et al. 2018). In addition, downregulation of several genes involved in liver function were reported in the male offspring exposed to maternal obesity as compared to female offspring. A previous study found similar findings in rats, where the 35 week-old male offspring exposed to maternal high-fat diet displayed reduction of the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) mRNA abundance in the liver, a gene involved in gluconeogenesis in the liver, cellular cholesterol homoeostasis and the development of obesity. In contrast, PGC-1α was increased in female offspring. In addition, abnormal liver and abdominal fat accumulation was observed in male offspring exposed to maternal obesity, which was not significant in the females (Burgueno et al. 2013). These sex specific differences were suggested to be related to the protective effect of estrogens.


Differences between men and women are caused by several factors, including differences in sex chromosomes, sex-specific gene expression of autosomes, sex hormones, and subsequently their effects on organ systems. Sex hormones have an important impact on body composition, men having greater visceral fat mass and women more subcutaneous fat mass, and on adipokines, leptin and adiponectin levels being higher in women as compared to men. The number of X chromosomes also contribute to sex differences in adiposity (Kautzky-Willer et al. 2016). Below, we review how several of these mechanisms could modulate fetal programming effects of maternal obesity and diabetes during pregnancy exposures in the offspring.
6.1 The role of steroid hormones

A possible explanation for the sex differences reviewed in the preceding sections could be differences in steroid hormones. Sex hormones play an important role in the maintenance of energy metabolism, body composition, and sexual function. Estrogens in women have been demonstrated to protect β-cells from apoptosis, as well as stimulate insulin secretion, improve insulin sensitivity, and regulate adipose tissue deposition and function (Kautzky-Willer et al. 2016; Palmer and Clegg 2015). In men, testosterone inhibits lipoprotein lipase and the uptake of triglycerides by the adipose tissue, increases lipolysis via β-adrenergic receptors, and promotes the increase of fat-free mass and muscle insulin sensitivity via increasing mitochondrial capacity (Kautzky-Willer et al. 2016). Imbalances in sex hormones are associated with cardiometabolic disturbances and similarities are observed between women with androgen excess and men with androgen deficiency (Kautzky-Willer et al. 2016). As recently reviewed by Mauvais-Jarvis, testosterone deficiency contributes to the development of hyperglycemia and diabetes in men, while estrogen deficiency was reported to increase diabetes risk in women due to alterations in insulin secretion, insulin sensitivity, and glucose effectiveness (Mauvais-Jarvis 2018). In addition to the reproductive organs, brain and adipose tissue are also important sites of action of steroid hormones. As previously reviewed, differences in the action of steroid hormones on the hypothalamic-pituitary-growth hormone axis play a major role in determining growth and body composition differences between the sexes. Growth hormone secretion increases similarly in boys and girls during adolescence, whereas gender differences are observed in adults, suggesting divergent actions of testosterone and estrogen. Indeed, testosterone stimulates growth hormones action by increasing growth hormones stimulation of IGF-1 (insulin-like growth factor-1), while estrogen attenuates growth hormones action by reducing IGF-1. In addition, treatment with growth hormones induces a greater increase in lean mass and decrease in fat mass in male than in female. (Meinhardt and
Steroid hormones are also important regulators of adipose tissue distribution and function, which can most likely be explained by the differential expression of sex steroid receptors in adipose tissues. Indeed, subcutaneous tissue has higher concentrations of estrogen receptors, while visceral adipose tissue has a higher concentration of androgen receptors (Chang et al. 2018). Finally, steroid hormones are also responsible for sex differences in protein metabolism and muscle mass. Testosterone increases muscle mass via increasing muscle protein synthesis and net muscle protein balance. So far, no study has investigated the role of estrogen on muscle mass in humans; however, in vitro and rats studies suggest that ovarian hormones inhibit muscle protein synthesis (Tipton 2001).

6.2 The role of metabolic hormones

Sensitivity to metabolic hormones, such as leptin and adiponectin, strongly vary between male and female. Indeed, it is now well recognized that women have higher concentrations of both leptin and adiponectin, as compared to men (Lubkowska et al. 2015). A recent study has shown that the sex differences in the concentrations of leptin and adiponectin could be explained by differences in fat distribution between both sexes. Indeed, differences in total body fat percentage between men and women were correlated with sex differences in leptin concentrations. However, differences in adiponectin concentrations were only partly explained by differences in visceral fat, and thus require further investigation (Christen et al. 2018). While melanocortins are known to mediate many of the hypothalamic effects of leptin on energy balance, no sex differences have been found in the sensitivity to melanocortin in a rat model (Clegg et al. 2003). In addition, men display lower plasma levels of ghrelin than women (Makovey et al. 2007; Patel et al. 2013). Ghrelin was found to be inversely associated with BMI and total fat mass in women, whereas in men associations with abdominal fat mass were observed (Makovey et al. 2007).
6.3 The role of the placenta

As the placenta is a key organ involved in the communication between the mother and the fetus, it most likely has a role in the consequences of maternal obesity and GDM on the offspring. Thus, placenta dysfunction likely plays an important role in the DOHaD concept (Rosenfeld 2015).

In response to maternal high-fat diet (60% energy from fat), placenta from female mice showed increased global placental gene expression profiles, among them genes coding for olfactory receptors and genes regulating the uptake of nutrients and ions, suggesting an increased sensitivity of the female placenta in order to adapt and be protective to changes in the maternal environment (Mao et al. 2010). In a separate study, male placentas from dams fed a diet enriched in fat (45%) and/or salt (4%), used to mimic a Western diet, displayed an increase in the expression of proinflammatory cytokines, such as IL-1β, TNFα, and CD68, which through their signaling could induce programming effects on the health of the offspring (Reynolds et al. 2015). In addition, a decrease in placental PGC-1α, which play a key role in the regulation of mitochondrial biogenesis and energy metabolism, was found in both male and female offspring exposed to GDM. However, only male offspring exposed to GDM displayed a reduction in placental mitochondria transcription factor A (TFAM) and mitochondrial DNA content, two downstream targets of PGC-1α, while this was not observed in female offspring (Jiang et al. 2017). It has been therefore suggested that female placenta can adapt more easily to adverse events in utero (Aiken and Ozanne 2013).

6.4 Differences in microbiota
An additional explanation for the sex differences in the development of metabolic disorders in offspring could be changes in the gut microbiota. Alterations in gut microbiota induce obesity (Dahiya et al. 2017), and a recent study from Guo et al. (Guo et al. 2018) has shown in mice fed with a high fat diet an alteration in the composition of the offspring gut microbiota in a sex specific way. In this study, Bacteroidetes species decreased while the Firmicutes increased in female offspring, whereas the opposite was observed in male offspring (Guo et al. 2018). Additional studies exploring sex differences in the effects of the vaginal microbiota and breast milk microbiota composition are also warranted.

6.5 Epigenetic differences

One of the main epigenetic modifications is DNA methylation, consisting of cytosine methylation of CpG sequences and mainly occurring at the fifth position of the cytosine ring (Bansal and Simmons 2018). As recently reviewed by our group, exposure to maternal obesity and/or diabetes has been reported to be associated with altered epigenetic differences and to increase the risk of T2D in the offspring (Agarwal et al. 2018). Sex differences in DNA methylation in various tissues have been reported in several studies (as reviewed by (Davegardh et al. 2018). Higher mRNA levels of the DNA methyltransferase enzyme (DNMT3b) were reported in female liver tissue as compared to males (Xiao et al. 2008). In addition, the sex differences in DNMTs might be attributed to sex hormones, as mRNA levels of DNMTs, such as DNMT3a and DNMT3b, were found to be changed in the human endometrium during the menstrual cycle (Yamagata et al. 2009), and a maternal high-fat diet (60% of energy from fat) led to DNA hypomethylation in placenta from female mice offspring only (Gallou-Kabani et al. 2010). In addition, experiments on human pancreatic islets showed that DNA methylations in candidate genes T2D and for related metabolic traits differed between the sexes. In addition, sex differences in DNA methylation were associated with higher glucose-stimulated insulin
secretion in female islets as compared to male islets (Hall et al. 2014). In addition, two specific microRNAs located on the X chromosome, hsa-mir-660 and hsa-miR-532, were differently expressed and methylated in female islets, i.e. higher expression concomitant with a decreased methylation. Target genes of these microRNAs, such as exportin-T (XPOT) and spire-type actin nucleation factor 1 (SPIRE1), were differently expressed in the islets in a sex-specific manner (Hall et al. 2014). As previously reviewed (Sharma and Eghbali 2014), microRNAs are differentially regulated in males versus females, which appears to be a result of both hormonal and genetic differences. Indeed, it has been suggested that microRNAs are regulated by estrogens and that the X chromosome encodes more microRNAs than the Y chromosome. However, only a few studies have investigated the influence of sex on miRNAs gene regulation so far, which warrants additional investigation in a wide array of metabolically relevant tissues. Moreover, regulation of microRNA expression by DNA methylation is an understudied area of research that could link discrete mechanisms responsible for the DOHaD theory at several levels. Thus, exposure to maternal obesity and GDM appear to program long-term cardiometabolic health in the offspring that involves sex specific epigenetic mechanisms, though the complexity of these interactions highlights the need for further research.

7. Conclusions and future perspectives

Numerous lines of research emphasize that diabetes and obesity during pregnancy affect fetal growth and increase the development of several metabolic health disorders in the offspring. The mechanisms are complex and biological sex appears to be an important factor. Indeed, the results presented in this review highlight that it is relevant to study both sex in the development of metabolic diseases, such as obesity and diabetes (Figure 1). More complete data evaluating biological sex will ultimately assist in the discovery and development of sex-appropriate targets for therapeutic intervention. However, to date, many studies have ignored sex as an important
variable in disease development. Indeed, the majority of experimental studies failed to examine sex differences and were only performed in males to avoid the effect of hormonal changes on the parameters of interest. In addition, when both sexes are studied, results are often adjusted for this variable considered as a confounder. However, when both males and females were studied separately, sex-associated differences were often found, as summarized in Table 1. In addition, males and females display differences in their susceptibility to develop metabolic diseases, with men being more prone to develop diabetes, while women are more predisposed to obesity and metabolic syndrome (Mauvais-Jarvis 2015). It is therefore crucial for upcoming studies to study individual sexes, in order to set the basis for the development of effective therapeutic strategies for the treatment of cardiometabolic diseases that exhibit sexual dimorphism.

Acknowledgments

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Conflicts

The authors have no conflicts of interest to report.
References


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Table 1: Sex differences in offspring exposed to gestational diabetes or maternal obesity

<table>
<thead>
<tr>
<th></th>
<th>GDM-exposed offspring</th>
<th>Maternal obesity-exposed offspring</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male versus Female</td>
<td>Male Versus Female</td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>↑ body weight&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↑ body weight&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(Macaulay et al. 2018; Persson and Fadl 2014); (Lecoutre et al. 2016; Ornellas et al. 2013; Zambrano et al. 2016)</td>
</tr>
<tr>
<td></td>
<td>↑ fetal abdominal circumference&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↑ visceral fat accumulation&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>↑ food intake&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>↑ fat %; TG; CH; insulin&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<td></td>
<td></td>
<td>↑ pro-inflammatory adipokines&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Insulin resistance</td>
<td>↓ cord blood insulin and proinsulin concentrations&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↑ insulin resistant&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(Krishnaveni et al. 2010; Shields et al. 2007); (Zambrano et al. 2016)</td>
</tr>
<tr>
<td></td>
<td>↓ glucose concentrations&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<td></td>
<td>↓ insulin resistant&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Pancreatic β-cell</td>
<td>Type 1 diabetes: ↑ β-cells function&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↓ average islet area&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(Gautier et al. 2018); (Yokomizo et al. 2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ oxidative stress in islets&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Fatty liver disease</td>
<td>↑ NAFLD associated changes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(Lomas-Soria et al. 2018)</td>
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<tr>
<td><strong>Metabolic hormones</strong></td>
<td></td>
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<tr>
<td>↑ Leptin&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td>(Ornellas et al. 2013; Zambrano et al. 2016)</td>
<td></td>
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<tr>
<td>↓ adiponectin&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Gene expression</strong></td>
<td>↑ FAS and SREBP1 mRNA in adipose tissue&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(Burgueno et al. 2013; Lecoutre et al. 2016)</td>
<td></td>
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<tr>
<td>↓ PPARγ and 11β-Hsd1 mRNA in adipose tissue&lt;sup&gt;b&lt;/sup&gt;</td>
<td>↓ PGC-1α mRNA in the liver&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td><strong>Placenta</strong></td>
<td>↓ mitochondrial biogenesis&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↓ sensitivity to environmental changes&lt;sup&gt;b&lt;/sup&gt;</td>
<td>↑ proinflammatory cytokines&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>: human studies; <sup>b</sup>: animal studies.

TG: triglycerides; CH: Cholesterol; FAS: fatty acid synthase; SREBP1: sterol regulatory element-binding protein-1; PPARγ: peroxisome proliferator-activated receptor gamma; 11β-Hsd1: 11β-hydroxysteroid dehydrogenase type 1; NAFLD: non-alcoholic fatty liver disease; PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha
Table 2: Nutritional composition of the maternal diet of animal models and sex specific outcomes in the offspring

<table>
<thead>
<tr>
<th>Maternal phenotype</th>
<th>Nutritional composition of the maternal diet</th>
<th>Sex specific outcomes in the offspring</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese</td>
<td>High fat diet 13.8% of proteins and 40% (w/w) bovine and porcine fat added to the standard chow (total energy: 5.34 -5.46 kcal/g)</td>
<td>↓ PGCG-1α mRNA in males</td>
<td>(Burgueno et al. 2013)</td>
</tr>
<tr>
<td>Obese</td>
<td>High fat diet 19% protein, 49% lipids (200 g of animal lard and 70 g of soybean oil/kg diet and 32% carbohydrates (total energy: 20.7 kJ/kg)</td>
<td>↑ postnatal weight gain and food intake in males</td>
<td>(Ornellas et al. 2013)</td>
</tr>
<tr>
<td>Obese</td>
<td>High fat diet 18.2% protein, 62.2% fat and 19.6% carbohydrate (total energy: 5.06 kcal/g)</td>
<td>↓ in the average islet area and the insulin content in males</td>
<td>(Yokomizo et al. 2014)</td>
</tr>
<tr>
<td>Obese</td>
<td>High fat diet</td>
<td>↑ visceral fat accumulation in males</td>
<td>(Lecoutre et al. 2016)</td>
</tr>
</tbody>
</table>
| Obese | High fat diet | - 20% protein, 40% of total calories as fat consisting of soybean oil (5.6%) and lard (54.4%) and 20% as carbohydrate (total energy: 5.24 kcal/g) | - ↑ mRNA levels of leptin, FAS and SREBP1 in males 
- ↓ mRNA levels of PPARγ and 11β-Hsd1 in males |
|-------|---------------|-------------------------------------------------|-------------------------------------------------|
| Obese | High fat diet | 23.5% protein, 20.0% animal lard, 5.0% corn oil, 21% polysaccharide, 21% simple sugars, 5.0% fiber (total energy: 4.9 kcal/g) | - no effect on body weight for both male and female 
- ↑ % fat, TG and leptin levels in females 
- ↑ insulin resistance in males (Zambrano et al. 2016) |
| Obese | High fat diet | 20% protein, 45% fat and 35% carbohydrate (total energy: 4.7 kcal/g) | - ↓ bacteroidetes species in females 
- ↑ firmicutes in females (Guo et al. 2018) |
| Obese | High fat diet | 23.5% protein, 20% animal lard, 5% corn oil, 21% polysaccharide, 21% simple sugars, 5% fiber (total energy: 4.9 kcal/g) | - ↓ genes involved in liver function in males (Lomas-Soria et al. 2018) |
| Metabolic syndrome | Cafeteria diet | 12.8% protein, 36.7% fat and 50.5% carbohydrates | - ↑ fat mass in both male and female (Pomar et al. 2017) |
| GDM | High fat and sucrose diet 20% protein, 45% fat and 35% carbohydrate (total energy: 4.7 kcal/g) | - ↑ circulating glucose levels in females | - ↑ body weight in males (Pereira et al. 2015) |

GDM: gestational diabetes mellitus; TG: triglycerides; FAS: fatty acid synthase; SREBP1: sterol regulatory element-binding protein-1; PPARγ: peroxisome proliferator-activated receptor gamma; 11β-Hsd1: 11β-hydroxysteroid dehydrogenase type 1; PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-alpha
Figure 1: Mechanisms involved in the developmental origins of cardiometabolic disease in the offspring following exposure to maternal obesity and gestational diabetes.

Exposure to environmental stressors early in life, such as diabetes and obesity during pregnancy, affect fetal development. In this context, epigenetic processes result in adaptations across the cellular, tissue, and organ system levels. These epigenetic changes together with postnatal diet, lifestyle factors as well as sex hormones have profound consequences on the vulnerability to the offspring to develop metabolic disorders later in life, mostly during adulthood.
Maternal diabetes and obesity

EPIGENETIC PROCESSES

Stem Cell Lineages
Increased adipocytes

Cell Proliferation
Beta-cell numbers

Organ Growth
Cardiomyocyte Hypertrophy

Metabolic Control
Mitochondrial Dysfunction

Testosterone Dependent

Estrogen Dependent

Obesity
Hyperlipidemia
Insulin Resistance
Type 2 Diabetes
Cardiovascular Dysfunction
Inflammation

Lifecourse

Fetal Adaptations

Adult Offspring Phenotype

POSTNATAL DIET/LIFESTYLE

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