Developmental programming of insulin resistance: are androgens the culprits?

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Abstract

Insulin resistance is a common feature of many metabolic disorders. The dramatic rise in the incidence of insulin resistance over the past decade has enhanced focus on its developmental origins. Since various developmental insults ranging from maternal disease, stress, over/undernutrition, and exposure to environmental chemicals can all program the development of insulin resistance, common mechanisms may be involved. This review discusses the possibility that increases in maternal androgens associated with these various insults are key mediators in programming insulin resistance. Additionally, the intermediaries through which androgens misprogram tissue insulin sensitivity, such as changes in inflammatory, oxidative, and lipotoxic states, epigenetic, gut microbiome and insulin, as well as data gaps to be filled are also discussed.

Introduction

Insulin resistance is a characteristic feature of many metabolic disorders, including the metabolic syndrome, type 2 diabetes mellitus (T2DM), obesity, non-alcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), gestational diabetes mellitus, and others. Traditionally, genetics and lifestyle are considered the main contributors to the development of these disorders of insulin resistance (Nilsson & Ling 2017, Zheng et al. 2018). However, the dramatic rise in incidence of this pathology during the last decades cannot be adequately explained by genetic predisposition and lifestyle changes alone. Accumulating evidence suggests factors acting during early fetal development are additional contributors (Franks et al. 2013, Vaiserman & Lushchak 2019). As fetal growth and differentiation are largely dependent on the maternal and intrauterine hormonal milieu during early development, hormones and factors that change the hormonal milieu during the fetal ontogeny may have an important role in the development of insulin resistance.

Hormonal influences during fetal differentiation are so critical that even mild and transient changes in maternal and intrauterine hormone levels within accepted physiological limits can affect the trajectory of normal fetal growth (Miranda & Sousa 2018). Of these hormones, steroid hormones play a significant and widespread role in the long-term organization of fetal organ systems (Fowden & Forhead 2004, Seckl & Holmes 2007). Steroidal influences on tissue organization stem from the plasticity of the fetus, a feature entrenched in the physiological process of fetal development and differentiation, which is most clearly evident in the process of sexual differentiation. Prior to sexual differentiation primitive gonads and reproductive structures are bipotential, and based on the genetic sex and under the influence of sex hormones, they develop into either male or female gonads and reproductive organs (Jost et al. 1973, Jost 1983). The presence of the Y chromosome in the male, for example, leads to production of...
testis-determining factor (TDF/sex-determining region Y (SRY)), a transcription factor that directs the development of bipotential gonads into testes. The developing testes produce hormones, including Mullerian-inhibiting substance/anti-Mullerian hormone (MIS/AMH) and testosterone. This hormonal milieu induces regression of the Mullerian ducts, which are otherwise destined to form female reproductive structures, and promotes the development of Wolffian ducts into male reproductive structures. The steroid hormone testosterone further promotes maturation of these structures and organization of the reproductive neuroendocrine axis and reproductive behavior. Gonadal sexual differentiation begins early in gestation in humans (Rabinovici & Jaffe 1990), and during this critical window, inappropriate exposure of the female fetus (XX chromosomal complement) to excess testosterone leads to virilization of reproductive organs and masculinization of reproductive behaviors (Berenbaum 2002). These observations are embedded in classical physiology and demonstrate the plasticity of the developing fetus and the importance of the maternal and fetal steroid hormone milieu in shaping fetal developmental trajectories (McEwen 1992).

This plasticity allows the fetus to respond to the prevailing maternal environment, which induces cellular and molecular changes to allow phenotypic adaptations. This process is termed ‘developmental plasticity’, and in theory aids in improving the offspring’s chances for survival in the external environment (Laubach et al. 2018, Lema 2020). However, the organizational changes that allow short-term survival often prove to be maladaptive in the long term by augmenting risk for non-communicable diseases such as T2DM (Hales & Barker 1992), metabolic syndrome (Rinaudo & Wang 2012), and obesity (Breier et al. 2001). This concept has been formalized as the Developmental Origins of Health and Disease (DOHaD) hypothesis and is of relevance to many maternal and environmental factors. Data from the Helsinki Birth Cohort have shown that abnormal placenta are linked with development of adult onset cardiometabolic diseases in the offspring (Thornburg et al. 2010). Therefore, conditions that compromise placental function either through direct maternal-fetal transfer or through altered placental steroidogenesis (e.g. preeclampsia, gestational diabetes, and endocrine-disrupting chemicals (EDCs)) (Nugent & Bale 2015) contribute to inappropriate fetal exposure to steroids with potential consequences on fetal development and the long-term health of the offspring.

Androgen programming of insulin resistance

Insulin secreted from pancreatic beta-cells maintains postprandial nutrient homeostasis through stimulation of anabolic processes: increasing glucose influx into muscle and adipose tissue, protein and glycogen synthesis in muscle and liver, lipid synthesis and storage in liver and adipose tissue, and through inhibition of fatty acid oxidation, lipolysis, glycogenolysis, and gluconeogenesis (Guo 2014). A failure to respond to insulin and achieve these physiological effects defines insulin resistance and results in compensatory increases in beta-cell insulin

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secretion. Lack of responsiveness to insulin occurs at systemic, tissue, and cellular levels as a result of increases in inflammation, oxidative stress, hyperadiponectinemia, and dyslipidemia; all of which negatively affect insulin action and tissue function (Hocking et al. 2013, Burgos-Moron et al. 2019, Iwabu et al. 2019). At the cellular level, reduced insulin response may result from insulin receptor desensitization or reduced expression, suppression of members of the insulin receptor signaling pathway and associated regulatory network, and mitochondrial and endoplasmic reticulum dysfunction (Flamment et al. 2012, Guo 2014, Gonzalez-Franquesa & Patti 2017).

Developmental exposure to various insults can lead to programming of insulin resistance as evident from clinical, epidemiological, and experimental studies (Table 1). While steroid hormones can directly influence insulin action as evident from sex-specific differences in glucose homeostasis and adipose distribution (Mauvais-Jarvis 2015, Varlamov et al. 2015), the evidence that developmental insults can lead to changes in maternal steroids especially androgens (Table 2) and induce insulin resistance (Table 1) point to androgens as potential culprits in the developmental programming of insulin resistance. Examples of conditions that result in excessive maternal androgen exposure include virilizing congenital adrenal hyperplasia (CAH) and the PCOS. In CAH, mutations in the 21-hydroxylase enzyme, which is required for biosynthesis of mineralocorticoids and glucocorticoids, results in androgen excess with consequential fetal exposure (Parsa & New 2017); importantly, these offspring exhibit postnatal signs of reduced insulin sensitivity (Tamhane et al. 2018). Evidence indicates that during pregnancy women with PCOS have higher androgen levels with associated disruptions in placental steroidogenesis (Sir-Petermann et al. 2002, Maliqueo et al. 2013), which likely combine to result in excess fetal androgen exposure. Daughters of these women show signs of metabolic derangements, including hyperinsulinemia during adolescence (Sir-Petermann et al. 2009). Maternal stress not only increases maternal glucocorticoids levels (Dunkel Schetter 2011), but it also induces a hyperandrogenic maternal hormonal milieu (Barrett & Swan 2015). A 30-year follow-up study reported hyperinsulinemia among offspring born to mothers administrated glucocorticoids during pregnancy (Dalziel et al. 2005). Maternal under- and over-nutrition have both been linked with the programming of obesity, T2DM, and insulin resistance (Duque-Guimaraes & Ozanne 2013, Vaizerman 2017). In addition to direct effects of the altered nutritional state, these conditions also alter the maternal androgenic milieu (Mossa et al. 2019, Pasquali & Oriolo 2019). For example, maternal obesity is associated with increased androgen levels (Whyte et al. 2007, Arnon et al. 2016, Maliqueo et al. 2017, Pasquali & Oriolo 2019), suggesting a potential role for androgens in the programming of offspring phenotypes in such nutritional models.

Environmental EDCs, especially those that have the capacity to influence androgen biosynthesis or bioavailability as well as those that modulate androgen receptor signaling, are also potential contributors to androgenic programming of metabolic disorders such as insulin resistance (Diamanti-Kandarakis et al. 2009, Gore et al. 2015). Rapid industrialization has led to widespread fetal and offspring exposure to EDCs through maternal-fetal transfer both across the placenta and via lactation, resulting in exposure during critical susceptibility windows (Masuda et al. 1978, Ando et al. 1985, Bowman & Choudhury 2016). Many naturally occurring compounds (e.g. phytoestrogens, including genistein and coumestrol) as well as anthropogenic chemicals (e.g. industrial solvents and lubricants (e.g. polychlorinated biphenyls (PCBs), polybrominated biphenyls, and dioxins), plasticizers (e.g. bisphenol A (BPA)), pesticides (e.g. dichlorodiphenyltrichloroethane (DDT)), fungicides (e.g. vinclozolin), and pharmaceutical agents (e.g. diethylstilbestrol) (Kabir et al. 2015) are EDCs that can serve as steroid mimics and activate steroid receptor signaling. Meta-analyses conducted by the National Toxicology Program and analyses of the National Health and Nutrition Examination Survey 2003–2004 data have shown strong associations between the incidence of T2DM and persistent organic pollutants (DDT and PCBs) and BPA (Lang et al. 2008, Taylor et al. 2013). Especially concerning is the fact that many of these EDCs are detectable in pregnant women and cord blood (Woodruff et al. 2011, Lee et al. 2017, Goodrich et al. 2019), thus suggesting the potential for these chemicals to disrupt fetal and offspring developmental trajectories. Maternal exposure to BPA and phthalates have been shown to alter maternal androgen milieu (Takeuchi et al. 2004, Sathyanarayana et al. 2017) raising the possibility that the programming effects of these EDCs may be mediated via alterations in the maternal androgenic milieu. Apart from their agonistic/antagonistic actions (De Falco et al. 2015), EDCs can indirectly affect the prevailing steroid milieu by modulating rate-limiting enzymes involved in steroid hormone biosynthesis and/or metabolism (Sanderson 2006, Tabb & Blumberg 2006). Evidence that EDCs affect steroid biosynthesis comes from data demonstrating reduced placental aromatase
### Table 1: Developmental insults with consequential insulin resistance in the offspring.

<table>
<thead>
<tr>
<th>Maternal insult</th>
<th>Disease state</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dutch Hunger Winter</td>
<td>Virilizing CAH (Tamhane et al. 2018)</td>
<td>Macaques: See Table 3 for gestational hyperandrogenic experimental models</td>
</tr>
<tr>
<td>Dutch and Chinese Famines and Obesity during pregnancy (Painter et al. 2005)</td>
<td>See Table 3 for gestational hyperandrogenic experimental models</td>
<td>Murabashy et al. 2013, Chang et al. 2019</td>
</tr>
<tr>
<td>High fat diet fed rhesus macaques (McCurdy et al. 2009)</td>
<td>Western diet/high fat-high sugar diet (Nvoit et al. 2009); early postnatal overnutrition and catch-up growth (Berends et al. 2013)</td>
<td>Western diet/high fat high sugar diet (Samuelsson et al. 2008)</td>
</tr>
<tr>
<td>NHANES survey (Lang et al. 2008)</td>
<td>Gestational BPA treatment (Ma et al. 2013, Li et al. 2014)</td>
<td>Gestational and lactational D(2-ethylhexyl) phthalate treatment (Lin et al. 2011a)</td>
</tr>
<tr>
<td>EDC - Bisphenol A (BPA)</td>
<td>Gestational BPA treatment (Ma et al. 2013, Li et al. 2014)</td>
<td>Gestational and lactational D(2-ethylhexyl) phthalate treatment (Hunt et al. 2017)</td>
</tr>
</tbody>
</table>

CAH, congenital adrenal hyperplasia; EDC, endocrine-disrupting chemical; NHANES, National Health and Nutrition Examination Survey; PCOS, polycystic ovary syndrome; TNDM29, transient neonatal diabetes mellitus locus 29.
Table 2  Induction of hyperandrogenemic maternal hormone milieu by developmental insults.

<table>
<thead>
<tr>
<th>Maternal insult</th>
<th>Maternal androgen milieu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease state</td>
<td></td>
</tr>
<tr>
<td>• PCOS</td>
<td>Sir-Petermann et al. 2002</td>
</tr>
<tr>
<td>• Virilizing CAH</td>
<td>Parsa and New 2017</td>
</tr>
<tr>
<td>• Gestational diabetes</td>
<td>Morisset et al. 2013</td>
</tr>
<tr>
<td>• Preeclampsia</td>
<td>Acromite et al. 1999</td>
</tr>
<tr>
<td>Undertreatment</td>
<td>Mossa et al. 2019</td>
</tr>
<tr>
<td>Stress</td>
<td>Barrett and Swan 2015</td>
</tr>
<tr>
<td>EDC - Bisphenol A</td>
<td>Takeuchi et al. 2004, Rutkowska and Ranchon 2014</td>
</tr>
<tr>
<td>EDC - Phthalates</td>
<td>Sathyanarayana et al. 2017</td>
</tr>
</tbody>
</table>

CAH, congenital adrenal hyperplasia; EDC, endocrine-disrupting chemical; PCOS, polycystic ovary syndrome.

(CYP19) expression in trophoblast cultures treated with BPA (Chu et al. 2018), reduced δ-hydroxysteroid dehydrogenase and CYP19 in the JEG 3 placental cell line treated with phthalate metabolites (Xu et al. 2016), and increased 7α-hydroxylase/17,20-lyase (CYP17) expression in ovarian cells exposed to BPA (Zhou et al. 2008). In addition, EDC-mediated disruption in hepatic steroid hormone metabolism could influence steroid bioavailability. EDCs have been shown to bind nuclear receptors such as the aryl hydrocarbon receptor (AhR) and pregnane X receptor (PXR) and regulate the expression of hepatic enzymes that metabolize steroid hormones (Mikamo et al. 2003, Sanderson 2006, Tabb & Blumberg 2006). For example, AhR binding of PCB or dioxin upregulates expression of CYP1A1, CYP1A2, and CYP1B1 (Kohn et al. 1993, Wakui et al. 2006, Tarnow et al. 2019), while alkylphenols and DDT acting through PXR inhibit hepatic clearance of estradiol in rats (El-Hefnawy et al. 2017). Overall these data indicate the capacity for EDCs to act indirectly as well as directly to influence the maternal and fetal steroid milieu and contribute to the programming of insulin resistance, a premise requiring further study.

Experimental animal models provide robust direct evidence in support of steroidal programming of insulin resistance (Table 3). For instance, gestational androgen treatment culminates in insulin resistance during adulthood in female offspring of rodents, sheep, and non-human primates (Roland et al. 2010, Abbott et al. 2016, Cardoso & Padmanabhan 2019). In sheep, days 30–90 of gestation encompass the period of organization of metabolic systems (Padmanabhan & Veiga-Lopez 2014). The main metabolic defects observed in adult sheep treated prenatally with testosterone from days 30 to 90 of gestation include peripheral insulin resistance with compensatory hyperinsulinemia and associated dyslipidemia, oxidative stress, and hepatic steatosis (Padmanabhan et al. 2010b, Veiga-Lopez et al. 2013, Puttabyatappa & Padmanabhan 2017). Similarly, animal models of gestational glucocorticoid administration have documented adverse fetal programming of the hypothalamic-pituitary-adrenal (HPA) axis and long-term effects during adulthood on cardiometabolic function (Moisidi & Matthews 2014, Constantinof et al. 2016). Gestational exposure to EDCs in various animal models also provide direct evidence for the role of steroidal disruption in programming insulin resistance. Such evidence is available for a wide range of EDCs, including BPA and phthalate metabolites (Strakovsky et al. 2015, Provvisiero et al. 2016, Veiga-Lopez et al. 2016). The developmental impact of protein restriction (a model of undernutrition) and high fat/western diet feeding (a model of overnutrition) have been studied in both rodents and nonhuman primates (Friedman 2015, Neri & Edlow 2015, Ong & Ozanne 2015, Nicholas et al. 2016). Although all these conditions are associated with offspring insulin resistance with coincident increases in maternal androgens (Table 2), androgen ablation studies are required to ascertain whether androgens are indeed the programmers of insulin resistance in these scenarios.

Androgen programming of peripheral insulin resistance

Peripheral insulin resistance is characterized by clinical findings of hyperglycemia, hyperinsulinemia, and impaired glucose tolerance. Evidence supportive of androgen programming of peripheral insulin resistance in the offspring comes from studies in mice, sheep, and rhesus macaques treated with testosterone during gestation (Roland et al. 2010, Abbott et al. 2016, Cardoso & Padmanabhan 2019). Comparative studies of sheep treated prenatally with testosterone and dihydrotestosterone (DHT, a non-aromatizable and more
# Table 3  Developmental programming of insulin resistance by gestational androgen.

| Species                  | Treatment                  | Insulin resistance | Remarks                                                                 |
|--------------------------|                           |                   |                                                                        |
|                          | (term 160)                |                   | Infants: increased basal insulin and beta cell number (Nicol *et al.* 2014) |
|                          |                            |                   | Adulthood: impaired disposition index, a measure of beta cell function and the ability to dispose glucose load (Eisner *et al.* 2000) |
|                          |                            |                   | Impaired glucose tolerance in adulthood (Eisner *et al.* 2000) |
| Sheep (Suffolk)          | Testosterone GD 100–115   | Yes               | Juvenile period: decreased insulin sensitivity (Recabarren *et al.* 2005, Cardoso *et al.* 2016) |
|                          | (term 164)                |                   | Prepubertal period: decreased (Padmanabhan *et al.* 2010a) or normal (Recabarren *et al.* 2005, Cardoso *et al.* 2016) insulin sensitivity based on proximity to puberty |
|                          |                            |                   | Postpubertal/early adulthood: normal (Recabarren *et al.* 2005, Cardoso *et al.* 2016) or increased (Veiga-Lopez *et al.* 2013) insulin sensitivity |
|                          |                            |                   | Adulthood: Reduced insulin sensitivity (Padmanabhan *et al.* 2010b) |
|                          |                            |                   | Reduced insulin sensitivity at prepubertal age (Padmanabhan *et al.* 2010a) |
| Sheep (Suffolk)          | Testosterone GD 30–90     | Yes               | Reduced insulin sensitivity in adulthood (Padmanabhan *et al.* 2010b) |
|                          | (term 147)                |                   |                                                                        |
| Sheep (Suffolk)          | DHT GD 30–90 (term 147)   | Yes               |                                                                        |
| Sheep (Scottish Greyface x Texel) | Testosterone GD 62–102 (term 147) | Yes | Adult age: normal glucose homeostasis but elevated basal insulin levels (Hogg *et al.* 2011) |
| Rat (Wistar)             | Testosterone GD 20 (term 21) | Yes | Single injection of testosterone during gestation impaired insulin sensitivity with normal HOMA index and glucose tolerance (Noroozzadeh *et al.* 2015) |
| Rat (Wistar)             | Testosterone GD 15–19     | Yes               | Female offspring were insulin resistant compared male offspring, and this phenotype was prevented by flutamide or tamoxifen co-administration (Hu *et al.* 2015) |
| Rat (Sprague Dawley)     | DHT GD 16–19 (term 21)    | Yes               | Insulin resistance observed at pubertal age (Yan *et al.* 2013) |
| Rat (Sprague Dawley)     | Testosterone (5.0 mg daily) GD 16–19 (term 21) | No | Administration of 5 mg testosterone daily did not affect insulin sensitivity, but animals developed lipid disturbances and hepatic steatosis (Sun *et al.* 2012) |
| Rat (Sprague Dawley)     | DHT Day 35–125 postnatal  | Yes               | Hyperglycemia and hyperinsulinemia with increased body weight and perirenal fat at the end of the treatment period (Yanes *et al.* 2011) |
| Rat (Wistar)             | DHT Day 21–111 postnatal  | Yes               | Decreased insulin sensitivity at the end of 90-day DHT treatment (Mannerås *et al.* 2007) |
| Rat (Wistar)             | Letrozole Day 21–111      | No                | No effect on insulin sensitivity at the end of 90-day DHT treatment (Mannerås *et al.* 2007) |
| Rat (Wistar)             | Estradiol Valerate Single injection Day 56 postnatal | No | No change in insulin sensitivity or androgen levels (Stener-Victorin *et al.* 2005) |
| Rat (Sprague Dawley)     | DHEA Day 21–41 postnatal  | Yes               | Higher fasting glucose and insulin* (Wang *et al.* 2004) |
| Rat (Sprague Dawley)     | Testosterone Day 28–48    | Yes               | Hyperinsulinemia with reduced rate of glucose uptake* (Holmång *et al.* 1990) |
| postnatal                | Letrozole Day 28–88       | Yes               | Impaired glucose tolerance at unspecified age* (Kauffman *et al.* 2015) |
| Mouse (C57BL/N6)         | DHT GD 16–18 (term 20)   | No                | No effect on fasting glucose levels or insulin levels at 3 and 16 weeks of age (Caldwell *et al.* 2014) |
| Mouse (C57BL/6j)         | DHT Day 21–111 postnatal  | No                | No effect on fasting glucose levels or insulin levels at 16 weeks of age (Caldwell *et al.* 2014) |
| Mouse (C57BL/6j)         | DHEA Day 21–111 postnatal | No | No effect on fasting glucose levels or insulin levels at 16 weeks of age (Caldwell *et al.* 2014) |
| Mouse (C57BL/6j)         | Letrozole Day 21–111      | No                | No effect on fasting glucose levels or insulin levels at 16 weeks of age (Caldwell *et al.* 2014) |
| Mouse (C57BL/6j)         | DHT Day 19–109 postnatal  | Yes               | Glucose intolerant at the end of a 90-day DHT treatment (van Houten *et al.* 2012) |
| Mouse (CBB6/F1)          | DHT GD 16–18 (term 20)   | Yes               | Prepuberty through adulthood; impaired glucose intolerance (Roland *et al.* 2010) |

*Observations made either during treatment or end of treatment period – whether these effects are programmed or due to activational effects is not known.

DHT, dihydrotestosterone; GD, gestational days.
active metabolite of testosterone) provide direct evidence that insulin resistance is programmed specifically through the androgenic actions of testosterone (Padmanabhan et al. 2010b). The involvement of androgens in the programming of insulin resistance is also supported by evidence of hyperinsulinemia during adolescence in daughters born to women with PCOS (Sir-Petermann et al. 2009) who have higher androgen levels during pregnancy (Sir-Petermann et al. 2002). The insulin resistance evident in offspring from pregnancies with CAH (Reisch et al. 2011), maternal disease states such as PCOS and gestational diabetes (Sir-Petermann et al. 2009, Bianco & Josefson 2019), nutritional deficit/excess (Godfrey et al. 2017, Ong & Guest 2018), stress (Moisidis & Matthews 2014, Facchi et al. 2019), and environmental EDC exposures (Gore et al. 2015, Veiga-Lopez et al. 2016, Sargis & Simmons 2019) along with the evidence that these conditions induce increased maternal androgen levels (Table 2) (Takeuchi et al. 2004, Whyte et al. 2007, Rutkowska & Rachon 2014, Barrett & Swan 2015, Arnon et al. 2016, Maliqueo et al. 2017, Parsa & New 2017, Sathyarayaraya et al. 2017), point to androgens as a likely culprit in the programming of peripheral insulin resistance, a premise that needs to be formally tested using interventional strategies such as androgen antagonism.

**Androgen programming of tissue-specific insulin resistance**

Disruptions in both insulin-responsive tissues (liver, muscle, and adipose tissue) as well as in insulin-producing tissues (pancreas) collaborate in the development of systemic and tissue-level insulin resistance. Tissue effects of insulin involve ligand binding to the insulin receptor (IR) that triggers downstream signaling events, including phosphorylation of protein kinase B (AKT), mitogen-activated protein kinase/extracellular signal–regulated kinase (MAPK/ERK), and the mammalian target of rapamycin (mTOR) (Saltiel & Kahn 2001, Guo 2014). Collectively, these signaling events promote glucose uptake as well as lipid and protein anabolic metabolism. Therefore, impairment at any level of the insulin signaling pathway may result in insulin resistance with consequential hyperglycemia (Rask-Madsen & Kahn 2012). Evidence to date points to the role that developmental steroid exposures, especially androgens, play in programming tissue-specific insulin sensitivity. The following sections address the androgen programming of insulin sensitivity at metabolic targets, namely, liver, muscle, adipose tissue, pancreas, brain, and cardiac tissues.

**Liver**

Liver is a critical regulator of metabolic physiology, with insulin playing a central role in glucose, protein, and lipid metabolism. In the liver, insulin reduces blood glucose levels by inhibiting gluconeogenesis and glycogenolysis while promoting glycogen synthesis. Gluconeogenesis is inhibited by insulin-stimulated AKT-mediated phosphorylation of the transcription factor forhead box protein O1 (FOXO1), which downregulates expression of gluconeogenic enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) (Guo 2014). Glycogen synthesis is stimulated through AKT-dependent phosphorylation and inhibition of glycogen synthase kinase-3 beta (GSK3B), which promotes the dephosphorylation and activation of glycogen synthase. AKT-dependent inhibition of tuberous sclerosis protein-2 (TSC2) consequently activates mTORC1 and ribosomal protein S6 kinase (S6k), which promote protein synthesis (Inoki et al. 2002) and stimulate transcription of sterol regulatory element-binding protein 1 (SREBP1), resulting in increased expression of lipogenic genes (Rui et al. 2014).

Studies addressing the programming of offspring hepatic insulin sensitivity by maternal disease state are not available in humans; however, data from animal models of developmental insults show defects at multiple levels of the insulin signaling pathway. For instance, both male and female offspring born to obese sheep manifest decreased protein abundance of IR, phosphorylated AKT, and phosphorylated FOXO1 (Nicholas et al. 2013). Similarly, male offspring of obese mice manifest decreased levels of insulin receptor substrate (IRS) 1, phosphorylated IRS1, and phosphorylated AKT as well as increased levels of GSK3B (Martin-Gronert et al. 2010). Hyperinsulinemia and hyperglycemia observed in male offspring born to pregnant rats treated with dexamethasone, a model of fetal exposure to excess glucocorticoids, is also accompanied by increased expression and activity of the gluconeogenic enzyme PEPCK, suggesting defects in hepatic insulin signaling (Drake et al. 2005). Gestational treatment with BPA in mice also reduced expression of Irs2, Akt2, G6pc, and Srebp1 in the liver of both male and female offspring (Van der Meer 2015). All of the above discussed maternal insults (obesity, stress, and EDC exposure) provide evidence in support of the hypothesis that gestational insults that program the development of hepatic insulin resistance and systemic hyperinsulinemia are also associated with increases in maternal androgens (Table 2) (Takeuchi et al. 2004, Whyte et al. 2007, Rutkowska & Rachon 2014, Barrett & Swan 2015,
Arnon et al. 2016, Maliqueo et al. 2017). The commonality of androgen excess in these pathophysiological states suggests that androgens are a likely contributor to the programming of these metabolic defects. Importantly, the exact developmental changes observed after these programming events may vary depending on both the timing of the insult and the life period during which analyses are performed. For example, while gestational testosterone treatment induces higher mTOR protein level and greater phosphorylation of S6K and GSK3B in the livers of female fetal day 90 sheep suggesting an insulin sensitive state (Lu et al. 2016), IR2, IRS2, AKT, and mTOR mRNA are downregulated in adult life (Nada et al. 2010) indicative of an insulin resistant hepatic phenotype. The reduced hepatic insulin sensitivity during adult life is supported by evidence of reduced phosphorylation of AKT under insulin-stimulated conditions in prenatally testosterone-treated female sheep (Lu et al. 2016). Co-treatment with an androgen antagonist prevented the fetal, but not the adult, changes in insulin signaling, indicating that the consequences of androgen programming during adult life may be subject to activational inputs and thus masked or unmasked by the prevailing adult hormone milieu. For example, increases in modulators of insulin sensitivity such as lipid accumulation in the liver leading to hepatic steatosis, inflammation, and oxidative stress during adult life of all animal models discussed above (McCurdy et al. 2009, Ashino et al. 2012, Lynch et al. 2017, Puttabyatappa et al. 2017, 2019b, Shimpi et al. 2017) may aid in maintaining the insulin resistant state programmed by gestational insults. Although hepatic lipid accumulation is also observed in offspring of gestationally DHT-treated mice, the mechanisms by which this develops and the disruptions in the insulin signaling cascade remains to be determined (Caldwell et al. 2014).

Muscle

Muscle is largely responsible for postprandial glucose disposal, with insulin promoting glucose uptake through AKT stimulation of GLUT4 protein translocation to the cell membrane (Dimitriadis et al. 2011). While disease states such as PCOS are associated with aberrant skeletal muscle gene expression and signaling pathways (Nilsson et al. 2018, Hansen et al. 2019, Shen et al. 2019, Stepto et al. 2019), generational studies addressing developmental insults in androgen programming of muscle-specific insulin signaling disruptions is lacking in humans. However, in animal models of maternal obesity, which show increased maternal androgen levels (Whyte et al. 2007, Arnon et al. 2016, Maliqueo et al. 2017, Pasquali & Oriolo 2019) and offspring insulin resistance, muscle-specific disruptions in insulin signaling and GLUT4 levels are evident. One study of male offspring born to obese pregnant rats found reduced levels of skeletal muscle Glut4 expression (Simar et al. 2012), while another study in rat offspring (of unspecified sex) had reduced expression of Ir and Glut4 in muscle (Bayol et al. 2005). In sheep models of maternal obesity, insulin resistance in the offspring is associated with reduced expression of insulin signaling intermediates when both male and female offspring were assessed together (Yan et al. 2010). Similarly, female mice born to obese dams exhibited reduced IRS1 and PI3K protein levels as well as decreased AKT phosphorylation at serine residue 473 (i.e. site of activation by insulin) in the skeletal muscle (Shelley et al. 2009). The associated increased levels of maternal androgens during pregnancy that occur in both mice and humans with obesity (Whyte et al. 2007, Arnon et al. 2016, Maliqueo et al. 2017, Pasquali & Oriolo 2019) suggest that androgens may be involved in the programming of offspring muscle insulin resistance in the context of maternal obesity. Studies of gestationally testosterone-treated sheep support this premise with the observation of decreased phosphorylated-GSK3B and GLUT4 protein levels in female fetuses at fetal day 90 and impaired insulin stimulation of AKT phosphorylation in adult female offspring (Lu et al. 2016). Although GLUT4 levels have not been examined during adulthood, the reduced AKT phosphorylation is likely to result in reduced GLUT4 membrane translocation in adult sheep as well, likely resulting in increased glucose levels.

Adipose

As in muscle, adipose tissue glucose uptake is primarily driven by insulin; however, because adipose tissue accounts for only 5–10% of whole body glucose uptake, it has only a minor role in postprandial glucose regulation (Dimitriadis et al. 2011). Despite this fact, adipose tissue influences the insulin sensitivity of other tissues through release of free fatty acids and adipokines (Rosen & Spiegelman 2006, Tchernof & Despres 2013); as such, increased adiposity and adipose dysfunction are major risk factors for the development of insulin resistance (Kahn & Flier 2000). Because of this role of adipose tissue, the majority of studies examining adipose programming by developmental insults such as maternal obesity is focused on the assessment of adipose tissue phenotype.

The hyperandrogenic state of PCOS likely contributes to the insulin resistance in the absence or presence of obesity (Dumesic et al. 2007). Importantly, the daughters...
born to mothers with PCOS also manifest insulin resistance (Sir-Petermann et al. 2009), suggesting that the maternal hyperandrogenic state of PCOS may contribute to the developmental programming of insulin resistance across generations. At the level of adipose tissue, several studies in women with PCOS, mostly conducted on subcutaneous adipose tissue (SAT), have found adipose tissue to be insulin resistant (Cree-Green et al. 2015, 2017, Dumesic et al. 2019); other studies have found adipose tissue to be more insulin sensitive (Corbould & Dunai 2007, Ciaraldi et al. 2009). In studies demonstrating adipose insulin resistance, disruptions are evident at multiple levels of insulin signaling, including: (1) a rightward shift in the insulin dose–response curve for insulin-stimulated glucose uptake in isolated adipocytes (Ciaraldi et al. 1992); (2) reduced phosphorylation of IRS1 and IRS2 (Qiu et al. 2005, Wang et al. 2005); (3) hyperactivation of GSK3B that is resistant to insulin-stimulated downregulation (Chang et al. 2008); and (4) decreased expression of GLUT4 (Rosenbaum et al. 1993). These findings are supportive of androgens gestationally programming adipose insulin resistance. Considering that the majority of women with PCOS are also obese, the increased adiposity per se may further amplify the programmed insulin sensitivity defects induced by androgen excess. This premise is supported by several observations, including: (1) treatment of female rhesus macaques with testosterone from menarche (a developmental programming window) coupled with a Western diet leads to greater insulin resistance than that observed in either the testosterone-treated or Western diet-fed conditions alone (True et al. 2017); and (2) gestational testosterone treatment coupled with postnatal obesity in female sheep leads to earlier impairments in insulin sensitivity compared to offspring only exposed to testosterone (Padmanabhan et al. 2010b). Interestingly, gestational testosterone treatment promoted preferential accumulation of metabolically deleterious visceral adipose tissue (VAT) in female rhesus macaques (Eisner et al. 2003), indicating that the steroidal programming of metabolism includes disruption of adipocyte insulin signaling and adipose mass and distribution. Male offspring from dams gestationally stressed by diet-induced obesity in mice (Fernandez-Twinn et al. 2014) and caloric-restricted rats (Berends et al. 2013) manifest insulin resistance and impaired adipose tissue insulin signaling characterized by reduced levels of Ir2, Iris1, phosphatidylinositol-3-kinases (P13k), and Akt. Associated increases in maternal androgen levels in these models (Table 2) (Maliqueo et al. 2017, Mossa et al. 2019) suggest that programming of insulin resistance in adipose tissue may be androgen dependent.

In stark contrast, a lack of change in the phosphorylation states of Akt, mTOR, ERK, GSK3B, and S6K during fetal life as well as increased mRNA expression of IR2, mTOR, Akt, PI3K, and peroxisome proliferator-activated receptor gamma (PPARγ) in the VAT with increased insulin-stimulated phosphorylated-AKT in the VAT and SAT in adult female offspring of gestationally testosterone-treated sheep (Nada et al. 2010, Lu et al. 2016) reflect an insulin-sensitive state in adipose tissue. Whether these differences in adipose insulin sensitivity programmed by gestational insults is due to differences in the adipose depot examined, species- or sex-specific differences, developmental time point studied, or differences in adipocyte differentiation (adipocyte hypertrophy in PCOS and offspring of obese mice (Manneras-Holm et al. 2011, Caldwell et al. 2014, Ibanez et al. 2018) vs adipocyte hypotrophy in gestational testosterone-treated sheep (Cardoso et al. 2016)) requires further examination.

**Pancreas**

As insulin resistance is often accompanied by compensatory increases in insulin secretion, impairment in pancreatic beta-cells can contribute to compromised insulin sensitivity, potentially through inappropriate insulin secretion (Shanik et al. 2008). In pregnant women hyperandrogenemia during pregnancy (Gozukara et al. 2015) is associated with the development of gestational diabetes leading to the development of insulin resistance in the offspring (Bianco & Josefson 2019), disruptions in beta-cell function and development of compensatory hyperinsulinemia (Singh et al. 2006, Kelstrup et al. 2013). Similarly, in rat models of maternal high fat feeding, male offspring have disrupted beta-cell gene expression characteristic of impaired function (Agarwal et al. 2019) and impaired insulin signaling (e.g. reduced IRS1 and Akt with increased FOXO1 expression) (Bringhenti et al. 2016). As high-fat diet-fed dams exhibit hyperandrogenemia during gestation (Whyte et al. 2007, Arnon et al. 2016, Maliqueo et al. 2017), androgens may program beta-cell disruptions in the offspring. The identification of pancreatic beta-cell defects with associated elevations in basal insulin secretion in female fetuses born to gestationally testosterone-treated sheep support such a notion (Rae et al. 2013). Another study of testosterone administration at gestational days 62 and 82 showed that female fetuses had increased beta-cell numbers at both fetal and adolescent ages with elevated basal insulin secretion (Ramaswamy et al. 2016), suggesting androgen programming of beta-cell dysfunction that leads to hyperinsulinemia. The observation that this phenotype
was evident only in animals that received testosterone and not diethylstilbestrol or dexamethasone during gestation imply specific involvement of androgens in the steroidal programming of beta-cell dysfunction (Ramaswamy et al. 2016).

Heart
Cardiac tissue uses free fatty acids as its primary source of energy; however, it also utilizes glucose during fetal life and periods of stress (Abel 2004). As in skeletal muscle, insulin influences glucose uptake and storage in the myocardium. Maternal weight gain and obesity are associated with the development of cardiovascular defects (Gaillard 2015). In sheep models of maternal obesity, offspring manifest hyperinsulinemia with increased activation of AKT, ERK, and mTOR in cardiac tissues with cardiac hypertrophy (Fernandez-Twinn et al. 2012). As gestational obesity is associated with increases in maternal androgens (Table 2) (Whyte et al. 2007, Arnon et al. 2016, Maliqueo et al. 2017), these cardiac changes may be programmed by androgens. In support of this, gestationally testosterone-treated sheep also exhibit elevated expression of mTORC1 and increased phosphorylation of PI3K, Akt, and mTOR with associated cardiac hypertrophy (Vyas et al. 2016).

Brain
The function of insulin in the brain is not confined to regulation of glucose availability from the periphery. Insulin influences neuronal function, and disruptions in brain insulin action is thought to be a basis for neurodegenerative and mood disorders as well as the metabolic syndrome (Kleinridders et al. 2014). In addition, there is crosstalk between insulin and appetite regulators, including orexigenes such as neuropeptide Y (NPY) and anorexigenes such as proopiomelanocortin (POMC) (Breton 2013, Vogt & Bruning 2013), which influence systemic tissue-level insulin sensitivity. Gestational insults that program peripheral insulin resistance (e.g. diet-induced maternal obesity) also induce insulin resistance in the male rat hippocampus with reduced levels of Ir2, Pi3k, and Akt levels (Gomes et al. 2018). Similar changes have also been observed in female offspring of gestationally testosterone-treated sheep with reduced IR2 levels in the hypothalamic arcuate nucleus, an effect that was reversed with gestational androgen antagonist treatment (Cernea et al. 2016). These data coupled with the fact that maternal androgens are elevated in animal models of diet-induced obesity (Whyte et al. 2007, Arnon et al. 2016, Maliqueo et al. 2017) indicate that insulin resistance in these neurons may be programmed by androgens. Surprisingly, male offspring of gestationally estradiol-treated mice had decreased hypothalamic IR mRNA and protein expression associated with the development of insulin resistance (Wang et al. 2018), indicating the potential for estrogenic mediation of insulin resistance as well. However, the finding that androgen receptor antagonists reversed the decrease in IR expression in the hypothalamic arcuate nucleus in prenatal testosterone-treated sheep effectively rules out estrogen as the mediator in this model and point to androgens as the culprits.

Mediators of androgen programming of insulin resistance
Steroid hormones elicit their actions through direct actions and via intermediaries to reorganize pathways involved in insulin production and function. Direct actions may involve signaling through their nuclear or membrane receptors with consequential alterations in gene transcription and signal transduction that regulate metabolic functions (Faulds et al. 2012). Indirect actions may be facilitated via alterations in the inflammatory, oxidative, or lipotoxic state as well as through the involvement of epigenetic modifications. More recently, the role of the gut microbiome in the developmental programming and pathogenesis of insulin resistance has also been suggested.

Inflammation
Inflammation is a complex process that involves the production of cytokines and other inflammatory mediators as well as the activation and invasion of immune cells; these effects are associated with the metabolic dysfunction associated with obesity and T2DM (Esser et al. 2014). While physiological inflammatory modulation of processes regulated by adrenocortical steroids are involved in the processes of lung maturation and parturition (Keelan 2018) as well as in the sex steroid programming of behavioral sexual dimorphism (McCarthy et al. 2017), maternal disease states (e.g. gestational diabetes (Pantham et al. 2015), preeclampsia (Gomez-Lopez et al. 2019, Tenorio et al. 2019), maternal stress, obesity, and exposure to EDCs) that lead to the development of offspring insulin resistance (Godfrey et al. 2017, Facchi et al. 2019, Sargs & Simmons 2019) are also associated with pathophysiological maternal
inflammation (Schmatz et al. 2010, Ingvorsen et al. 2015, Ozias et al. 2015, Alfaradhi et al. 2016, Dewi et al. 2017, Dudele et al. 2017, Bansal et al. 2018, Gu et al. 2018, Zota et al. 2018, Desplats et al. 2019, Kelley et al. 2019a, Song et al. 2020) and an altered androgen milieu (Table 2) (Acromite et al. 1999, Takeuchi et al. 2004, Whyte et al. 2007, Morisset et al. 2013, Rutkowska & Rachon 2014, Barrett & Swan 2015, Arnon et al. 2016, Maliqueo et al. 2017, Sathyarayana et al. 2017) in both humans and animals. These findings implicate androgens and inflammatory processes in the programming of insulin resistance. Strong associations exist between inflammatory biomarkers and cortisol in mothers with major depressive disorder (Osborne et al. 2018) as well as placental inflammation following maternal exposure to BPA in the sheep (Song et al. 2020). As these states are associated with an increased androgenic maternal milieu (Takeuchi et al. 2004, Rutkowska & Rachon 2014, Barrett & Swan 2015), it is possible that inflammatory state in these situations also involve androgens. The finding that gestational treatment with testosterone leads to placental inflammation in sheep (Kelley et al. 2019b), and the female offspring of these animals develop insulin resistance (Padmanabhan et al. 2010b) lend support to the possibility that gestational androgen-mediated programming of offspring insulin resistance may involve inflammatory pathways. Further studies are needed to establish the absolute contribution of inflammatory processes to programmed metabolic dysfunction and to determine if inflammation is the key mediator of the androgenic programming of insulin resistance.

**Oxidative stress**

Physiological levels of reactive oxygen species (ROS) aid in placentation and fetal development by virtue of their role in cell signaling and gene transcription. However, breakdown of antioxidant defense mechanisms and accumulation of ROS lead to oxidative stress (Lushchak 2014), with consequential damage to cellular components, including proteins, lipids, DNA, and cellular organelles (Valko et al. 2007). Developmental stress arising from maternal disease states (Zhu et al. 2015, Tenorio et al. 2019), maternal obesity (Valko et al. 2007), and stress (Lorigooini et al. 2019, Venkatesh et al. 2019), as well as from environmental chemical exposures (Ferguson et al. 2014, Neier et al. 2015, Veiga-Lopez et al. 2015, Watkins et al. 2015, Puttabyatappa et al. 2019a) are associated with maternal oxidative stress (Luo et al. 2006, Valko et al. 2007), a known contributor to the developmental origins of insulin resistance (Thompson & Al-Hasan 2012, Rodriguez-Rodriguez et al. 2018). Because these conditions are also associated with changes in the maternal androgen milieu (Table 2) (Acromite et al. 1999, Sir-Petermann et al. 2002, Takeuchi et al. 2004, Whyte et al. 2007, Morisset et al. 2013, Rutkowska & Rachon 2014, Barrett & Swan 2015, Arnon et al. 2016, Maliqueo et al. 2017, Sathyarayana et al. 2017), the fetal programming of insulin resistance likely involve crosstalk between androgen signaling and oxidative stress pathways. Evidence for involvement of oxidative stress in the androgen programming of insulin resistance also comes from strong associations between steroid hormone-disrupting EDCs (e.g. phenols, parabens, and phthalate metabolites) and maternal and fetal oxidative stress in epidemiologic studies (Ferguson et al. 2014, Veiga-Lopez et al. 2015, Watkins et al. 2015, Puttabyatappa et al. 2019a) along with the induction of offspring insulin resistance after gestational exposure to these same EDCs in animal models (Angle et al. 2013, Rajesh & Balasubramanian 2014, Veiga-Lopez et al. 2016). Apart from their steroid potential, EDCs such as BPA and phthalates are also associated with increases in maternal androgen levels (Takeuchi et al. 2004, Rutkowska & Rachon 2014, Sathyarayana et al. 2017) implicating androgens as potential mediators in inducing a pro-oxidant state. Other studies have shown that high-fat diet induces obesity (Hariri & Thibault 2010), placental oxidative stress (Lin et al. 2011b), and insulin resistance in offspring (Matsuzawa-Nagata et al. 2008) and antioxidant supplementation during pregnancy ameliorates hyperinsulinemia in offspring (Sen & Simmons 2010). These findings suggest a direct involvement of maternal oxidative stress in the programming of offspring insulin resistance. Since maternal obesity is associated with increases in maternal androgen levels (Whyte et al. 2007, Arnon et al. 2016, Maliqueo et al. 2017), androgens may be involved in increasing the oxidative stress. This premise is supported by the observation that gestational exposure to testosterone in sheep leads to increases in placental oxidative stress (Kelley et al. 2019b) and induction of insulin resistance in the female offspring (Padmanabhan et al. 2010b). However, key questions remain regarding the relative contribution of androgen excess and oxidative stress and their interactions in the programming of offspring insulin resistance.

**Lipotoxicity**

Excess accumulation of triglycerides in non-adipose tissues results in both acute and chronic cellular dysfunction,
a process termed lipotoxicity (Weinberg 2006). These effects may be due either to direct lipotoxic effects or indirect induction of inflammation and oxidative stress as a result of lipid peroxide production. Observations in women with gestational diabetes and obesity as well as in animal models mimicking these conditions show increased maternal, placental, and fetal lipid accumulation (Diamant et al. 1982, McCurdy et al. 2009, Li et al. 2011, Dong et al. 2013, Visiedo et al. 2013, Pruis et al. 2014, Saben et al. 2014, Jeve et al. 2015). Since these conditions also result in offspring insulin resistance (Matsuzawa-Nagata et al. 2008, Bianco & Josefson 2019), lipotoxicity may be a contributing factor in the developmental programming of insulin action. Since these maternal conditions are also associated with increased maternal androgens (Table 2) (Whyte et al. 2007, Morisset et al. 2013, Arnon et al. 2016, Maliqueo et al. 2017), androgens may also play a role in the development of lipotoxicity. The observation in sheep models that gestational testosterone treatment increases placental lipid accumulation (Kelley et al. 2019b) supports such a possibility. Similar increases in placental lipotoxicity are also observed in sheep models of gestational BPA treatment (Song et al. 2020). As BPA exposure during pregnancy can increase maternal androgens (Takeuchi et al. 2004, Rutkowska & Rachon 2014) and are known to induce insulin resistance (Veiga-Lopez et al. 2016), maternal androgens might contribute to development of later-life insulin resistance through lipotoxicity.

Epigenetics

Another mechanism by which steroids program metabolic pathways in offspring involves epigenetic alterations. Epigenetic changes include changes in DNA methylation, histone modifications, and expression of noncoding RNAs, all of which can lead to alterations in gene expression. Considering steroids are known to modulate developmentally programmed epigenetic changes (Forger 2018), androgen-induced epigenetic modifications of genes involved in insulin action in insulin target tissues or insulin production from pancreatic beta-cells are likely contributors to the developmental origins of insulin resistance (Sharma et al. 2017, Ebrahimi et al. 2019, Kerr et al. 2019).

Epigenetic modulation via DNA methylation involves addition of a methyl group to cytosines by DNA methyltransferases (DNMTs), resulting in changes in gene transcription (Ciccaroni et al. 2018). DNA methylation is involved in the developmental programming of insulin resistance (Davegardh et al. 2018, Zhou et al. 2018), including in conditions associated with maternal disease states (Elliott et al. 2019, Kamrani et al. 2019), obesity (Fernandez-Twinn et al. 2019), stress (Cao-Lei et al. 2016), and following exposure to EDCs (Stel & Legler 2015, Rahmani et al. 2018). Additional support comes from observations of (1) DNA methylation of hepatic genes involved in insulin and glucocorticoid receptor signaling in mice with fetal exposure to glucocorticoids under conditions of maternal food restriction (Ogawa et al. 2014); (2) the strong association that exists between gestational BPA levels and genome-wide DNA methylation and transcriptome changes in human fetal liver (Faulk et al. 2015); and (3) hypermethylation of DNA in the pancreas and liver of offspring from gestationally BPA-treated mice and rats, respectively (Rajesh & Balasubramanian 2014, Bansal et al. 2017). Because all of these conditions are also associated with increases in maternal androgen levels (Table 2) (Sir-Petermann et al. 2002, Takeuchi et al. 2004, Whyte et al. 2007, Morisset et al. 2013, Rutkowska & Rachon 2014, Barrett & Swan 2015, Arnon et al. 2016, Maliqueo et al. 2017), the possibility exists that DNA methylation changes underlying the programming of insulin resistance in these scenarios may be a consequence of increased maternal androgens. Confirmation of the involvement of androgen-mediated DNA methylation in the programming of insulin resistance requires ablation studies with androgen antagonists and prevention of DNA methylation changes via maternal folic acid supplementation (Ly et al. 2016).

Epigenetic regulation through histone modifications includes acetylation, methylation, ubiquitination, phosphorylation, and sumoylation, all of which affect transcriptional activity through modification of the structure and conformation of chromatin and/or by the differential recruitment of transcriptional co-repressors or co-activators (Bannister & Kouzarides 2011). Histone alterations are brought about by enzymes that include ‘writers’ such as histone acetyltransferases (HATs) and histone methyltransferases (HMTs) that modify histones as well as ‘erasers’ such as histone deacetylases (HDACs) and lysine demethylases (KDMs) that remove these modifications (Hyun et al. 2017). The impact of developmental insults on the programming of insulin resistance through histone modifications is understudied (Vaiserman & Lushchak 2019). Evidence of a possible role of steroid-mediated histone modifications in fetal programming comes from the reprogramming of stress responses by fetal glucocorticoid exposures that induce transcriptional changes of genes involved in hypothalamic-pituitary-adrenal axis function through
histone modifications (Dirven et al. 2017). Altered expression of the pancreatic beta-cell differentiation gene PDX1 in gestationally protein-restricted and BPA-treated rats (Park et al. 2008, Chang et al. 2016) as well as altered hepatic gene expression in gestationally high-fat diet-fed macaques and BPA-treated mice (Aagaard-Tillery et al. 2008, Strakovsky et al. 2015) are both associated with histone modifications. Of interest, these gestational manipulations lead to increases in maternal androgen levels (Takeuchi et al. 2004, Whyte et al. 2007, Rutkowska & Rachon 2014, Barrett & Swan 2015, Arnon et al. 2016, Maliqueo et al. 2017) and offspring insulin resistance (Rinaudo & Wang 2012). Gestational testosterone treatment in sheep also increased expression of PDX1 in female fetuses (Rae et al. 2013), lending support to the possibility that androgen programming of insulin homeostasis may occur via histone modifications, a premise that needs formal testing.

The epigenetic regulation of insulin resistance may also be mediated via changes in noncoding RNAs (Desai et al. 2015, De Rosa et al. 2018), which include microRNAs (miRNAs), short (~18-25 nucleotide), long (exceeding 200 nucleotides), and circular RNAs, of which miRNAs are the best studied. The evidence that steroidal programming involves miRNAs comes from sexual differentiation of neonatal mouse brain (Morgan & Bale 2017). The role of miRNAs in androgen programming of insulin resistance is supported by observations that miRNA expression is increased after maternal insults that promote an androgenic maternal hormone milieu (Table 2) and program insulin resistance, such as maternal disease states (Poirier et al. 2017, Hemmatzadeh et al. 2020), diet-induced obesity (Benatti et al. 2014, Mendez-Mancilla et al. 2018, Puppala et al. 2018), growth restriction (Zheng et al. 2017, Hromadnikova et al. 2019), stress (Cao-Lei et al. 2016, Wu et al. 2016), and BPA exposure (Lin et al. 2017, Martinez-Ibarra et al. 2019) in both humans and animal models. The observation that gestational testosterone treatment increases expression of miRNAs that target insulin signaling, albeit in fetal ovaries (Luense et al. 2011), along with findings that their offspring develop insulin resistance during adulthood (Padmanabhan et al. 2010b), raise the possibility that reprogramming of insulin resistance by androgens may also involve changes in expression of miRNA associated with insulin signaling genes in metabolic tissues.

Microbiome

The identification of bacteria and/or their DNA in amniotic fluid, fetal membranes, umbilical cord blood, placenta, and meconium indicate that gut colonization begins during in utero life (Li et al. 2014a). Gut microbiota are required for host metabolism and fetal development, while reduced microbial diversity and dysbiosis is associated with gastrointestinal and systemic disorders, including obesity and insulin resistance (Wang et al. 2016). This is confirmed by studies in germ-free animals that consume more calories, become overweight, and exhibit insulin resistance and dyslipidemia (Laugerette et al. 2011). This association of dysbiosis with the development of insulin resistance (Wang et al. 2016, Codagnone et al. 2019) coupled with the observation that infants born to mothers with obesity or stress have altered gut microbiota (Collado et al. 2010, Zijlmans et al. 2015, Codagnone et al. 2019) and increased maternal androgens (Table 2) with consequential programing of offspring insulin resistance collectively suggest the potential for steroidal programming of insulin resistance through alterations in the microbiome. Although the influence of steroids on the gut microbiota have been explored (Huang et al. 2015, Tetel et al. 2018), the role of the microbiome in androgen programming is not established. There is evidence that gestational exposure to stress in mice (Gur et al. 2017), treatment of pregnant rats with the native steroid testosterone (Gur et al. 2017), or exposure of pregnant mice to EDCs such as BPA or synthetic estrogens (Javurek et al. 2016) all lead to altered gut microbiota. Since these gestational insults are associated with increased maternal androgens (Table 2) and can program insulin resistance, it is possible that androgen-induced gut microbial changes may be involved in androgen reprogramming of insulin responsiveness and development of insulin resistance. The finding that transplantation of gut microbiota from woman with PCOS into mice resulted in insulin resistance (Qi et al. 2019) indicates that further studies using fecal microbiota transplantation can be used to assess the relative contribution of gut microbiota in androgen programming of insulin resistance.

Insulin

An intriguing hypothesis set forth by published data and evident in clinical practice is the capacity of insulin to drive the development of insulin resistance (Gavin et al. 1974, Rizza et al. 1985, Shanik et al. 2008, Erion & Corkey 2017). In the context of developmental programming, it is interesting to note that gestational hyperinsulinemia observed in gestational diabetes mellitus and obesity are associated with the development of insulin resistance-associated conditions in the offspring later in life.
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Importantly, a major hormonal change associated with increased maternal androgens is hyperinsulinemia as androgen-mediated increases in insulin levels during pregnancy is evident in hyperandrogenic pregnant women with PCOS (Radon et al. 1999, Sir-Petermann et al. 2007) and pregnant sheep treated with testosterone (Abi Salloum et al. 2015). As such, programming of offspring insulin resistance by gestational testosterone excess may be mediated via increased levels of insulin itself. Indeed, since multiple conditions are also associated with increase in maternal androgens (Table 2) (Whyte et al. 2007, Morisset et al. 2013, Arnon et al. 2016, Maliqueo et al. 2017), the possibility androgen-mediated increases in maternal insulin levels contribute to programming of insulin resistance cannot be excluded. Moreover, it is interesting to speculate how such a scenario could establish a potential positive feedback loop in which persistent or even transient androgen excess increases insulin levels that then increase insulin resistance prompting further insulin release and potentiation of the effect. Intriguingly, insulin’s potential role as a mediator of androgen-induced insulin resistance is supported by evidence that the metabolic disruptions observed in prenatally testosterone-treated sheep can be ameliorated by co-treatment with an insulin sensitizer (Puttabyatappa et al. 2017). Importantly, while suggesting a potential route by which androgens induce insulin resistance, such a mechanism would also provide support for potential interventions, including insulin sensitizers as well as exercise.

Conclusions and future directions

The ‘thrifty phenotype hypothesis’ proposed by Hales and Barker first explained how malnutrition during pregnancy leads to poor fetal growth and the programming of maladaptive metabolic responses in the developing fetus (Barker 2005). One of the key metabolic reprogramming events is the establishment of insulin responsiveness,
which leads to the development of insulin resistance. Since then this hypothesis has evolved into the DOHaD hypothesis, which captures the impact of wide ranging insults occurring during critical in utero and postnatal developmental windows on the reprogramming of metabolic functions. Critically, the mechanisms by which these developmental insults program later-life insulin resistance are still unresolved. As discussed above, a commonality among the various insults promoting insulin resistance (e.g. maternal disease states, stress, over/undernutrition, lifestyle, and exposure to EDCs) is alterations in the maternal steroid hormone milieu with specific increases in androgen levels (Fig. 1 and Table 2) (Takeuchi et al. 2004, Whyte et al. 2007, Rutkowska & Rachon 2014, Barrett & Swan 2015, Arnon et al. 2016, Maliqueo et al. 2017). Since steroids, and especially androgens, play a major role in establishing the program of sexual differentiation at both the gonadal and brain levels, exposure to excess androgens during critical periods of organization of metabolic functions may also influence the programming of metabolic tissues, potentially resulting in maladaptive changes that manifest as adult onset metabolic dysfunction, including insulin resistance. Human observations of insulin resistance among offspring born to mothers with high testosterone levels during pregnancy (e.g. women with PCOS) (Sir-Petermann et al. 2009) as well as in animal models of gestational treatment with androgens (Table 3) (Roland et al. 2010, Abbott et al. 2016, Cardoso & Padmanabhan 2019) support this conjecture. Despite this evidence, however, further studies are needed to confirm the relative contribution of androgens in programming insulin resistance due to other maternal insults through the use of specific androgen receptor antagonists or other molecular approaches.

Another key observation arising from this discussion is that many of the studies conducted thus far have been confined to one sex of the offspring. Studies using rodent models have also grouped both sexes together to interpret findings. As it is now established that developmental insults have sex-specific effects (Dearden et al. 2018), future studies in both humans and animal models are required to be performed in both sexes. As sexual dimorphism is also apparent in placental function (Kalisch-Smith et al. 2017), studying the sex-based differences in this key mediator of maternal-fetal transport are also required. Another challenge to consider is the sensitivity of specific developmental periods to respective in utero or postnatal insults since the same exposure at different times during prenatal life can lead to a wide spectrum of effects (Selevan et al. 2000). Additionally, as studies in animal models allow longitudinal and direct assessment of relevant target tissues to empower investigation of potential inter- and trans-generational transfer of traits, the comparability of the most sensitive window(s) for exposures in animal models are essential to provide comparison data across species, which in turn will identify those critical windows of vulnerability to be targeted for policy development and risk management. Considering pregnancies associated with hyperandrogenic state such as PCOS, preeclampsia, obesity, and stress are common in clinical practice, the establishment of androgens as mediators driving adverse metabolic programming in offspring will aid in the development of targeted clinical interventions to overcome the long-term effects of developmental insults that drive insulin resistance by restoring and supporting a normal maternal steroid milieu. Developing such interventions may hold immense potential for addressing the early-life origins of metabolic dysfunction that are epidemic across the globe.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Therapeutic strategies for obesity and related disease, including polycystic ovary syndrome, may involve manipulation of androgen levels. Developmental exposure to androgens during pregnancy can lead to long-term health effects in their female offspring. This review highlights the role of the estrogen-aryl hydrocarbon receptor signaling pathways and their interaction with increased sympathetic nervous system activity. Reproductive Biology and Endocrinology 3 44. (https://doi.org/10.1186/1477-7827-3-44)


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