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Sex Differences in the Developmental Programming of Adult Disease

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1. Introduction

A significant body of knowledge has established that stressors in early life have long-term health consequences on the adult organism. This has given rise to the Developmental Origins of Health and Adult Disease (DOHAD) hypothesis. Among the several broad themes that have emerged from the clinical and experimental investigations into the DOHAD hypothesis; perhaps none is as intriguing as the role of biological sex and sex hormones in the progression and development of adult diseases. Despite the significant progress in recent years, many uncertainties remain with respect to the roles of biological sex and gonadal steroids in the progression of human diseases in general, and in the mechanisms underlying sex differences in developmental programming in particular.

While sexual dimorphism is widely recognized in the progression of many diseases (e.g. cardiovascular), it appears that the primary pathways leading to these differences exert distinct influences during fetal and adult life. The mechanisms by which biological sex contributes to these processes is a rapidly expanding area of investigation drawing upon studies interrogating systems at the molecular, cellular and whole organism physiological levels. From these investigations several intriguing hypotheses have been proposed. These include developmental programming due to: 1) endocrine disruption resulting from exogenous sex steroids and/or analogs or nutritional stress during development; 2) chromosomal regulation of sex dimorphism in the transcriptome of mammalian tissues; and 3) sex specific responses to stressors during fetal life. The goal of this chapter is to place into perspective the current body of knowledge in the rapidly growing area of sex differences in developmental programming with a primary focus on cardiovascular diseases.

2. Periods of susceptibility to developmental programming

Developmental programming can be defined as the response by the developing organism to specific stimuli during critical periods of organogenesis that results in persistent effects on the adult phenotype. It is now recognized as an important determinant of adult health. Acceptance and understanding of this concept derives from human epidemiological studies suggesting that many metabolic diseases such as cardiovascular disease (Barker, 1993), chronic kidney disease (Li *et al.*, 2007), type II diabetes mellitus (Hovi *et al.*, 2007;Hofman *et al.*, 1997), and hypertension (Roseboom *et al.*, 2001) are associated with low birth weight. Since developing organisms pass more physiological benchmarks prior to birth than during

any other time in life, it is not surprising that deviations in the timing or nature of these developmental steps have functional consequences in later life. Hence, it is vitally important to understand early life gene-environment interactions that can increase predisposition to adult disease. Figure 1 provides an overview of the timing of the susceptibilities for various organs systems and processes in developmental programming.

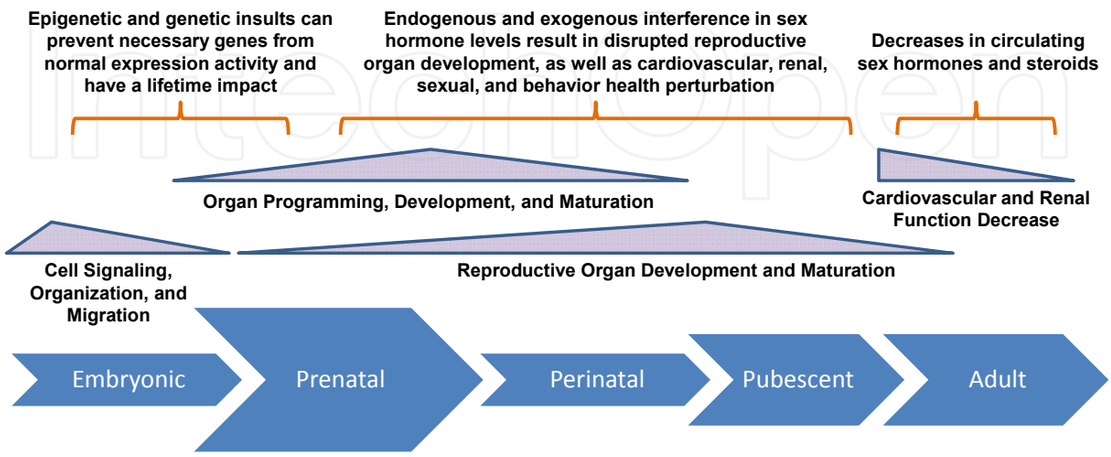

Fig. 1. *Organogenesis and Maturation Disruption.* Timeline depicts separate stages of mammalian organ development and maturation and the relative sensitivity to various programming stimuli. Listed above are necessary processes during this period for normal growth and the activity (light blue). At the top are the perturbations that can interfere with proper development.

2.1 Embryonic development

Susceptibility to programming events begins very early in life and surprisingly sex differences are already present. The transcriptome of male and female pre-implantation embryos differ such that several genes located on the X chromosome are more expressed in bovine and human female versus male embryos (Gutierrez-Adan *et al.*, 2000;Taylor *et al.*, 2001;Wrenzycki *et al.*, 2002;Peippo *et al.*, 2002) while autosomal genes expressed in trophoblast cells, such as those for interferon-γ (Larson *et al.*, 2001), human choriogonadotropic hormone (Haning, Jr. *et al.*, 1989), and numerous other imprinted genes (Paldi *et al.*, 1995;Kovtun *et al.*, 2000;Durcova-Hills *et al.*, 2004) are also not expressed or methylated the same across the sexes. Morphological differences exist as well; male and female embryos differ in rates of development as early as the first few days post-fertilization. Bovine (Avery *et al.*, 1992;Yadav *et al.*, 1993), murine (Valdivia *et al.*, 1993) and ovine embryos (Bernardi & Delouis, 1996) produced *in vitro* often fall into fast-cleaving and slow-cleaving groups that are predominantly male and female, respectively. Interestingly, Sood *et al.* reported a sex dichotomy in the genes expressed in male and female placentas (Sood *et al.*, 2006) using microarray analysis and identified genes in villous samples such as JAK1, IL2RB, Clusterin, LTBP, CXCL1, and IL1RL1 that were expressed at higher levels in female placentas.

2.2 Fetal development

The fetal period is a critical time for organogenesis. As gestation progresses and the embryo becomes a fetus, the sex dimorphism of early development re-appears around mid-gestation

as male fetuses become larger than age-matched females (Hindmarsh *et al.*, 2002;Crawford *et al.*, 1987;Parker *et al.*, 1984). From clinical and experimental studies we know that this size difference persists to term (Hindmarsh *et al.*, 2002;Parker *et al.*, 1984;Gilbert *et al.*, 2007a;Gilbert *et al.*, 2006a). Underlying these morphological differences are specific sex related differences in endocrinology and metabolism. While androgens are recognized for their role in male maturation, they are also essential to development of the female fetus. Production of androgens in both the ovaries and the adrenal cortex in females is essential to folliculogenesis and mammary development. Levels below the required amount for normal development have been shown to diminish the development of the tissues aforementioned. Exposure of female fetuses to androgens may not necessarily disrupt normal development of the ovaries, but may result in altered expression of steroidogenic proteins (Hogg *et al.*, 2011). Increased androgens are also associated with female fetuses developing male-like sexual behavior, increased aggression, delayed vaginal opening (Meisel & Ward, 1981).

Sex differences at the molecular level also persist from embryonic into fetal life. Baserga *et al.* have reported that gestation in the rat cyclooxygenase-2 (COX-2) levels were higher in the female than the male kidney at day of gestation (DG) 8, although not significantly increased at DG 21. In contrast, 11β-Hydroxysteroid Dehydrogenase 2 (11β-HSD2) levels were higher in the male control kidney at DG 21. Both of these gene products play important roles in renal function and alterations in either could have developmental and/or functional effects in the kidney (Baserga *et al.*, 2007b). Similarly, sex differences have also been reported in the ontogeny of gene expression in the renal RAS (Gilbert *et al.*, 2007a).

Similar to the kidney, the mammary gland undergoes discrete phases of development. However, in contrast to the kidney, the mammary has important developmental phases that extend into adulthood. In early pregnancy, the processes of fetal mammary development are thought to occur independently of influences from systemic hormones (Hennighausen & Robinson, 2001). After mid-gestation, placental hormones enter fetal circulation and initiate canalization of the early ductal system. It is during this period that exposure to endocrine mimetics may exert an influence on subsequent risk of breast cancer in the offspring (Xue & Michels, 2007). While the origin and the purpose of these sex differences in fetal development remain unclear, it may simply reflect different trajectories of fetal development between the sexes; however, this may also underlie sex differences in developmental programming.

2.3 Post-natal

It has long been observed that growth restricted fetuses which develop metabolic syndrome often experience "catch-up growth" and surpass normal birth weight controls (Hales & Ozanne, 2003). While it is clear that catch-up growth plays a role in the manifestations of developmental programming it has been difficult to identify the specific peri-natal *vs.* post-natal influences involved. Disordered vascular function is thought to contribute to programming of cardiovascular health but the cause and effect relationships remain uncertain (Martin *et al.*, 2000;Leeson *et al.*, 2001;Goodfellow *et al.*, 1998). There are recognized sex differences in arterial pressure and the progression of renal disease, both of which are thought to involve interactions of the renin angiotensin system and sex steroids (Sandberg & Ji, 2003;Silva-Antonialli *et al.*, 2004). Most current evidence points to sex steroids as the most important factor influencing sex differences in post-natal cardiovascular function.

In contrast to organs such as the kidney that complete development *in utero*, several reproductive organs such as the mammary undergo significant developmental changes during post-natal life and sometimes well into adulthood. A significant portion of mammary development begins at puberty and continues throughout an individual's reproductive years (Hinck & Silberstein, 2005) and renders this particular organ to more critical periods susceptible to programming influences. Aberrant signaling during these phases may initiate abnormal growth of the ductal epithelium, possibly resulting in alterations in risk for subsequently developing mammary cancer.

Interestingly, Wlodek, *et al.* found that uteroplacental insufficiency, via uterine restriction in rats, resulted in reduced alveolar proliferation (Wlodek *et al.*, 2009). We have reported that growth restricted female rats from hypertensive mothers have a much higher incidence of mammary tumors when exposed to N-nitroso-N-methylurea than normal birthweight control rats (Gingery *et al.*, 2011). Thus, differentiation events of the mammary epithelium occurring mid- to late-gestation may provide a substrate sensitive to sub-optimal intrauterine conditions or environmental exposures that could set the stage for subsequent development of cancer.

The transcriptome continues to display sex differences in adulthood, such as in differences in expression of mRNA for osmoregulatory, drug and steroid metabolizing proteins in the murine kidney and liver (Rinn *et al.*, 2004). It is therefore not unreasonable to hypothesize sex differences exist within a molecular framework and that there are many potential avenues, from embryonic life on into adulthood, through which sex differences may interact with developmental programming stimuli to result in sex specific alterations.

3. Endocrine disruption

It has become nearly axiomatic that endocrine signaling by gonadal steroids like estradiol and testosterone are important contributors to the development and maintenance of long-term health and/or disease. Endocrine disruption generally occurs as a consequence of one or more of four main characteristics of the compound under study: agonist, antagonist, modification, and/or altering synthesis (Derfoul *et al.*, 2003). Some compounds can have a pleiotropic effect in which at least two signaling pathways known to be independent from each other are impacted. Moreover, endogenous sexual hormones and mimetics include: estrone, estriol, estradiol, human chorionic gonadotropin, testosterone, progesterone, prostaglandins, and several other estrogens and androgens. While endocrine disruptors are traditionally considered to be environmental pollutants, there are numerous physiological stressors that may generate disturbances of endocrine signaling pathways. Further, in Table 1 we highlight the main classes of steroidogenic endocrine disruptors and the manners in which they become accessible to organisms. To this end, we have considered a variety of physiological models under the general theme of endocrine disruption.

3.1 Endogenous hormones/mimetics

Females on average are at lesser of a risk for cardiovascular disease during the premenopausal state, but significantly are at increased risk for cardiovascular and renal disease after menopause, nearing comparable rates to male disease development (Gilbert & Nijland, 2008;Sakemi *et al.*, 1995). In adult growth-restricted females, an ovariectomy can lead to an increase in renal-induced hypertension, compared to subjects with the ovaries still intact(Ojeda *et al.*, 2007a). These observations allude to the idea that estrogens have a cardiac

Sex Hormone Disruption	Reported Mimetics	Reported Conduits of Exposure
Estrogens	Polychlorinated (PCB) and polybrominated (PBB) biphenyls, dichlorodiphenyltrichloroethane (DDT), Dichlordiphenyldichloro-ethylene (DDE), methoxychlor, diethylstilbestrol (DES), alkylphenols, cadmium, bisphenyl-A (BPA).	Dielectric fluids and electronics; hard plastics; pesticides; insecticides; synthetic estrogens; sewage degradation, batteries and television screens; plastics and dental sealants
Androgens	Kepone , procymidone, dichlorodiphenyldichloroethylene (DDE), vinclosolin, 2,3,7,8-tetrachlorodibenzodioxin (TCDD)	Insecticides; pesticides; fungicides; herbicides
Prostaglandins	Phthalates, COX inhibiting pharmaceuticals	Soft plastics, paints, inks; prescription pharmaceuticals

Table 1. *Overview of exogenous steroid mimetics.* Exogenous endocrine mimetics have been reported to have agonistic and/or antagonistic behavior in mammals. Exposures to these compounds occur through various types of materials in a variety of settings.

and renal protective component that is attenuated during post-menopausal state (Rubinow & Girdler, 2011;Ojeda *et al.*, 2007a). But, estrogen differences between the sexes cannot alone explain the disease development differences because androgens have been observed to have cardioprotective properties as well (Manolakou *et al.*, 2009).

Androgens are important in fetal development regardless of sex. During the first trimester of development, the male fetus maturation is dictated by the presence of androgens, which if disrupted can lead to several conditions such as testicular cancer, lower sperm count and motility, and cryptorchidism (Manikkam *et al.*, 2004;Bormann *et al.*, 2011;Recabarren *et al.*, 2008). With below-normal levels of testosterone, the male fetus fails to properly develop the testes, known as testicular dysgenesis. In contrast, excess testosterone is linked to altered development of the seminiferous tubules and lower sperm count and motility. Taken together these studies show proper control of androgen levels in males is essential to normal reproductive development (Manikkam *et al.*, 2004;Bormann *et al.*, 2011).

In growth restricted males, increased testosterone levels have been shown to lead to an increase in angiotensin II sensitivity and this in turn may lead to increased susceptibility to hypertension (Ojeda *et al.*, 2010). Recently, Ojeda et al. showed high incidence of hypertension coinciding with growth restriction is dependent on circulating testosterone levels. The castration of adult growth restricted males resulted in mitigation of the hypertension, which contrasted the observations of no blood pressure change after castration of normal growth, hypertensive male rats (Ojeda *et al.*, 2007a).

The concept of endocrine disruption leading to developmental programming can be extended to the fetal renin-angiotensin system (RAS) as well. Interestingly, this system is responsive not only to pharmacological manipulation but also to nutritional stress as well. Indeed, work from several laboratories has provided insights regarding the role of the RAS in cardiac development (Beinlich *et al.*, 1991;Beinlich & Morgan, 1993;Beinlich *et al.*, 1995;Samyn *et al.*, 1998;Segar *et al.*, 1997;Segar *et al.*, 2001;Sundgren *et al.*, 2003). In particular, Sundgren *et al.* demonstrated that Ang II promotes hyperplastic growth during early gestation, whereas Beinlich *et al.* have reported neonatal hypertrophic growth in the pig

(Beinlich *et al.*, 1995;Sundgren *et al.*, 2003). The intra-cardiac RAS also appears to be sensitive to nutritional stress as demonstrated recently by Gilbert et al. in a study that shows decreased immunoreactive AT1 and AT2 in the mid-gestation left ventricle of fetal sheep gestated in ewes that were subjected to 50% global nutrient restriction (Gilbert *et al.*, 2005b).

3.2 Exogenous hormones/mimetics/antagonists

Exposure to a variety of environmental factors may generate exogenous interference with endocrine systems through mimetic or antagonistic activity. This is a rapidly growing area of research with implications for both environmental and public health. Several known exogenous estrogen antagonists include polychlorinated (PCB) and polybrominated (PBB) biphenyls, dichlorodiphenyltrichloroethane (DDT), methoxychlor, diethylstilbestrol, 17β-estradiol, alkylphenols sewage degradation, cadmium, and the infamous bisphenyl-A (BPA) (Sonnenschein & Soto, 1998;Derfoul *et al.*, 2003). Xenoestrogens may exist in the system at levels that do not elicit strong or detectable estrogenic effects individually, but it has been shown that these have additive effects and several xenoestrogens in the system can act together to induce estrogenic activity. (Sonnenschein & Soto, 1998) This additive effect brings rise to the idea that some materials we are exposed to may pass inspection individually for tolerable levels of these endocrine disrupting compounds, but in concert with low levels of the xenoestrogens may have phenotypic effects *in utero*. Since estrogen is mainly required in maturation and growth, it is common to not see congenital endocrine complications until adolescence or even as late as adulthood (Derfoul *et al.*, 2003). Overexposure of estrogens to the fetus has been suggested to stunt growth and alter bone development (Derfoul *et al.*, 2003;Sonnenschein & Soto, 1998). In contrast to the large number of estrogen disruptors, only several androgen antagonists have been identified and studied. These are insecticide ingredients such as kepone and procymidone, dichlorodiphenyldichloroethyle (DDE), vinclosolin, and 2,3,7,8-tetrachlorodibenzodioxin (TCDD) (Sonnenschein & Soto, 1998). While very little to no androgen agonists have been discovered (Sonnenschein & Soto, 1998) it has been reported that exposure to androgen antagonists like DDE is linked to development of recurrent respiratory tract infection (Carey *et al.*, 2007).

Prostaglandins are important to many sexual processes in both men and women, but little has been done on that research to describe endocrine disruption targeting prostaglandins. Interference in prostaglandin pathways has been associated with the development of several types of cancer and cardiovascular disorders. The alteration of synthesis of prostaglandins from arachadonic acid through the COX enzyme has been shown disrupt endocrine processes. Several phthalates that are similar to pharmaceutical COX inhibitors, were found to disrupt the levels of prostaglandin synthesis (Kristensen *et al.*, 2011). Chronic inhibition of COX activity is known to have deleterious effects on renal and cardiovascular function, resulting in mild to moderate hypertension and even renal failure. Developmental sex differences in this system have been reported and show that renal COX-2 expression is higher in female fetuses at gestational day 21 than age matched male fetuses (Baserga *et al.*, 2007a). Prostaglandin synthesis pathways are relatively understudied but may provide important insights into the development of sex differences in adult disease.

4. Sex differences in developmental programming

Numerous studies have documented sex-differences in the incidence and severity of cardiovascular diseases such as coronary artery disease, heart failure, cardiac hypertrophy,

and sudden cardiac death (Gilbert *et al.*, 2006b;Ojeda *et al.*, 2008;Grigore *et al.*, 2008). These differences in the expression of cardiovascular disease may be related in part to intrinsic sex-differences in myocardial function. Many recent studies have provided evidence that indicates a sex dichotomy also exists in the physiological responses to developmental challenges as they relate to the programming of subsequent cardio-renal function. These studies have largely been interpreted in one of two ways: 1) that male and female fetuses adapt differently to developmental stressors; or 2) that male and female sex steroids have a profound influence on the development and progression of developmentally programmed disease states. Moreover, since sex differences are apparent quite early in embryonic development and are independent of sex hormones; developing a third line of reasoning to suggest innate differences between the sexes play a role in the response of the developing organism to stressors may yield useful insights. Viewed in concert several primary remaining questions emerge: Do innate sex differences originating in fetal life predispose organisms to adult diseases in general and developmentally programmed outcomes in particular? Do post-natal sex differences drive specific fetal adaptations to *in utero* stressors that generate differential outcomes? Or perhaps it is a combination of these scenarios?

4.1 Human studies

A small number of clinical studies have investigated sex differences in renal function as it relates to developmentally programmed hypertension. The larger body of work in this area has detailed differences in cardiovascular parameters and stress responses. Nevertheless, several interesting findings have been reported that confirm the idea that women are "reno-protected" during early adulthood. A recent report from the Nord Trøndelag Health Study (1995-1997) in Norway found intrauterine growth restriction (IUGR), high blood pressure and low normal renal function were associated in 20-30 year olds (Hallan *et al.*, 2008). Although the degree of impaired renal function was small in these young adults, it was significant and more consistent in men than women (Hallan *et al.*, 2008). Similarly, Kistner *et al.* reported women born pre-term had increased blood pressure but no signs of adverse renal function as young adults (Kistner *et al.*, 2000).

Other studies have evaluated cardiovascular responses between male and female subjects that were growth restricted *in utero*. In one such study, Ward and colleagues reported women born small were far more susceptible to stress-induced increases in systolic blood pressure (Ward *et al.*, 2004). A recent study by Jones *et al.* has shown that there are marked sex differences in the way size at birth is associated with alterations in cardiovascular physiology established in childhood (Jones *et al.*, 2008). Further evidence that markers of impaired fetal growth are related to autonomic cardiovascular control involving modulation of both sympathetic and parasympathetic function but in a sex-specific manner has also been provided in an adult Australian cohort by the same group (Jones *et al.*, 2007). The authors reported women, but not men, who were small at birth demonstrated increased low-frequency blood pressure variability at rest and during stress, reduced levels of high-frequency heart period variability and a reduction in baroreflex sensitivity.

4.2 Animal studies

Studies utilizing animal models have employed a range of stressors in a variety of species to induce fetal growth restriction and test hypotheses regarding the developmental origins of disease (summarized in Table 2). Perhaps the most common model to date has focused on maternal nutrient restriction (MNR), either as a decrease in total caloric intake or an

isocaloric decrease in protein content; studies to understand the consequences of maternal obesity from the DOHAD perspective are gaining (Grigore *et al.*, 2008;Mcmillen & Robinson, 2005;Gallou-Kabani *et al.*, 2007;Khan *et al.*, 2005;Khan *et al.*, 2003;Taylor *et al.*, 2004).

Programming Insult	Species	Adult outcomes reported in the literature
Maternal overnutrition	Rat, mouse, sheep	Hypertension (females), reduced vascular compliance, Endothelial dysfunction, aortic hypoplasia, decreased renal $Na^{+,}$ K^{+} ATPase activity, decreased locomotor activity (female>male)
Maternal undernutrition	Rat, sheep, baboon	Hypertension (male>female), growth restriction, altered expression of renin-angiotensin system
Placental insufficiency	Rat	Hypertension (male>female), growth restriction, glucose intolerance
Maternal renal insufficiency	Rat, rabbit	Hypertension (female>male)
Ang II receptor inhibition	Rat	Hypertension, decreased nephron number, glomerulosclerosis (male>female), interstitial fibrosis (male>female)
Glucocorticoid excess	Rat, sheep	Hypertension (sex and age dependent), glomerulosclerosis
Androgen excess	Rat, mouse, sheep, human	Hypertension (male>female), decreased endothelial function, ovarian dysgenesis, increased ANG-2 sensitivity (GR males), increased male-like sexual behavior (female), growth retardation, delayed vaginal opening, increased aggression
Estrogen deficiency	Rat, mouse, sheep, human	Hypertension (postmenopausal women), infertility, abnormal mammary growth

Table 2. *Summary of developmental programming studies and outcomes.* A variety of developmental insults lead to long-term health consequences for the offspring. (References found in Sections 2 and 3.)

4.2.1 Models of nutrient restriction

Others have shown that considerable sex differences are observed in the response to MNR between male and female baboon fetuses near term (Cox *et al.*, 2008). Evidence from MNR studies suggest female progeny are less affected than their male siblings (Ozaki *et al.*, 2001;Woods *et al.*, 2005;McMullen & Langley-Evans, 2005b;McMullen & Langley-Evans, 2005a) although these observations may depend on the extent of the nutrient restriction (Hoppe *et al.*, 2007). These studies generally show decreased nephron endowment and altered expression of components of the intra-renal renin-angiotensin system (Ozaki *et al.*, 2001;Woods *et al.*, 2005;McMullen & Langley-Evans, 2005b;McMullen & Langley-Evans, 2005a;Hoppe *et al.*, 2007). Hemmings *et al.* have reported impairment of the myogenic

response in the mesenteric vascular bed of pregnant adult females exposed to MNR during development (Hemmings *et al.*, 2005). MNR during the pre-implantation period in the rat resulted in elevated BP in male offspring only (Kwong *et al.*, 2000). Restriction of specific nutrients other than protein has also been evaluated. A maternal low-sodium diet in rats has recently been associated with increased maternal plasma renin activity and correlated with IUGR, increased blood pressure, and reduced creatinine clearance in female offspring but not in males (Battista *et al.*, 2002).

Similar to the results observed in many small animal models, not all large animal models show clear effects of MNR on the offspring. In addition, only a subset of these studies has been evaluated for sex differences. Previous work has shown male sheep and baboon fetuses are more susceptible to the effects of poor maternal nutrition (Gilbert *et al.*, 2005a;Gilbert *et al.*, 2006a;Gilbert *et al.*, 2007a). Studies in sheep have shown global caloric restriction impairs nephrogenesis and alters intrarenal immunoreactive AT_1, AT_2 and renin expression in gestational age and gender specific ways (Gilbert *et al.*, 2007a). Further, only male offspring of these NR ewes are hypertensive (Gilbert *et al.*, 2005a). While the mechanisms by which NR alters gene expression remains unclear in our model, data from Lillycrop *et al.* and Burdge *et al.,* both employing protein restriction in the rat suggests deficiency of methyl donors may alter gene methylation patterns and in turn effect changes in gene expression (Lillycrop *et al.*, 2005;Burdge *et al.*, 2007;Lillycrop *et al.*, 2007;Lillycrop *et al.*, 2008). It remains unclear whether the increased risk to the males is a result of gene-environment interactions originating during or after gestation. Further studies are needed to thoroughly investigate these possibilities.

4.2.2 Models of utero-placental and renal insufficiency

Models of utero-placental insufficiency are quite intriguing as they are relevant to multiple maternal health issues as well as to the developmental programming of hypertension. Alexander *et al.* have shown that reduced uterine perfusion pressure during the last trimester of pregnancy in the rat programs hypertension in the offspring and in a sex specific manner (Grigore *et al.*, 2007;Alexander, 2003). Further, in this model both the RAS and sex steroids have been implicated in the observed sex differences in hypertension (Ojeda *et al.*, 2007b;Ojeda *et al.*, 2007a;Grigore *et al.*, 2007). In contrast, the two kidney-one wrapped kidney (2K,1W) model of hypertension resulted in hypertension in 30 week old female offspring only (Denton *et al.*, 2003). Interestingly, plasma renin activity was significantly lower in the female offspring of hypertensive mothers at 10 weeks of age ($P<0.05$), suggesting that development of the renin-angiotensin system was altered. The differences in the factors elaborated by the ischemic placenta and poorly perfused kidney illustrate the complexity of the interactions between the maternal endocrine milieu and fetal development. Whereas reduced renal perfusion primarily activates the RAS, the ischemic placenta produces a variety of humoral and locally acting factors such as sFlt-1 (soluble fms-like tyrosine kinase-1) and tumor necrosis factor (TNF)-α that have far reaching effects.

Recent studies in the rat and baboon have shown chronic reductions of utero-placental blood flow elevates levels of sFlt-1 in the placenta, amniotic fluid and maternal plasma (Gilbert *et al.*, 2007b;Makris *et al.*, 2007). In the rat, this has been associated with decreased fetal growth and subsequent hypertension that is sex dependent (Alexander, 2003;Ojeda *et al.*, 2007a). Recent studies in rodents have shown elevated sFlt-1 levels alone results in fetal growth restriction (Lu *et al.*, 2007b;Bridges *et al.*, 2008). Furthermore, Lu *et al.* have followed

the mouse offspring of these pregnancies and reported sex specific effects regarding the development of hypertension as only male mice have higher blood pressure in this model (Lu *et al.*, 2007a). Viewed together, these studies strongly suggest that in addition to the immediate well being of the mother, a long term outlook with regards to the well being of the fetus must also be considered during complicated and/or high risk pregnancies.

4.2.3 Maternal obesity

Maternal obesity is associated with a variety of conditions including maternal hypertension, hypertriglyceridemia, hyperglycemia and insulin resistance (Wilson & Grundy, 2003), that are independently correlated with a suboptimal *in utero* environment and consequently linked to DOHAD. Several human studies have described a positive correlation between maternal weight and/or adiposity and blood pressure of teenage children (Lawlor *et al.*, 2004;Cho *et al.*, 2000;Laor *et al.*, 1997), leading Boney *et al* to conclude from their examination of large for gestational age babies and the incidence of childhood metabolic syndrome, that "given the increased obesity prevalence in children exposed to either maternal diabetes or maternal obesity, there are implications for perpetuating the cycle of obesity, insulin resistance, and their consequences in subsequent generations." Few, if any, of the studies in humans include offspring sex as a co-variable (Boney *et al.*, 2005).

Important information with regard to maternal nutrient excess and sex-associated difference comes largely from animal models. Studies show hypertension in male rat offspring after exposure to a maternal diet high in saturated fat (or low in linoleic acid) that is not present in females (Langley-Evans, 1996). In contrast, Elahi *et al.* reported mice fed high fat diets long before the onset of gestation are hypercholesterolemic, hypertensive and produce hypertensive, hypercholesterolemic female offspring (Elahi *et al.*, 2008). Moreover, treatment of the dams with pravastatin lowered blood pressure and cholesterol levels in those offspring (Elahi *et al.*, 2008). Because the numerous pleiotropic effects of statins the mechanisms for these effects remain unclear, nevertheless these observations provide insights for further studies.

In a model more resembling high fat food consumption in humans, Armitage *et al.* demonstrated that a diet rich in fat fed to pregnant rats results in male offspring gaining more body weight and presenting with decreased renal renin activity when compared to females (Armitage *et al.*, 2005). Offspring from this model are reportedly hypertensive, exhibit increased aortic stiffness, decreased aortic smooth muscle cell number, endothelial dysfunction and decreased renal Na+, K+-ATPase activity. The bulk of these changes were independent of sex except for increased blood pressure where female offspring were hypertensive while the males were not (Khan *et al.*, 2003;Samuelsson *et al.*, 2008). Further, Khan et al. reported female offspring have reduced locomotor activity at 180 days of age compared to male offspring of pregnant rats fed a high fat diet during pregnancy (Khan *et al.*, 2003). In addition, this research group used cross-fostering techniques after birth to show that the hypertension in females is attained whether exposure to maternal high fat diet occurs before and during pregnancy or during the suckling period (Khan *et al.*, 2005). While the mechanisms responsible for programming due to high fat diets remain unclear, the report that statin treatment has beneficial effects on the offspring highlights at least one potential mechanism, alterations in lipid metabolism (Elahi *et al.*, 2008). In addition, it has been suggested that high levels of butyric acid that may result from a high fat diet could lead to changes in chromatin structure and result in epigenetic alterations (Junien, 2006). Taken together these observations highlight an important role for nutrition and intermediate metabolites in developmental programming.

4.2.4 Endocrine disruption

The importance of environmental exposures to endocrine disruptors during pregnancy has long been noted. Factors derived from *Pinus ponderosa* needles (e.g. isocupressic acid) and leaves from *Veratrum californicum* have long been observed to have profound impacts on the pregnancies of livestock (Short *et al.*, 1995;Panter *et al.*, 1992;Wu *et al.*, 2002). Moreover, the observations that ingestion of *Veratrum californicum* by sheep at specific times of gestation resulted in fetal malformations and prolonged gestation laid the foundation for experimental evidence that supports a crucial role for glucocorticoids and the fetal hypothalamic pituitary axis in the onset of parturition (Liggins, 1994;Challis *et al.*, 2000). Similarly, carbenoxolone, an active ingredient of licorice may also inhibit production of cortisol and disrupt normal HPA signaling between the mother and fetus. These findings point to a role for maternal and fetal stressors that alter glucocorticoid levels during pregnancy as important mediators of developmental programming. One such physiological stressor is exercise during pregnancy which has been reported to have a variety of effects on the offspring in hypertensive rats (Gilbert *et al.*, 2008). Whereas moderate exercise lowered blood pressure in female offspring and increased body density in both male and female progeny, a high volume of exercise resulted in post-natal growth failure followed by catch-up growth but only females suffered exacerbated hypertension (Gilbert *et al.*, 2002). Using a dexamethazone injection model, O'Reagan *et al.* showed similar effects on BP in males and females but the magnitude of hypertension and a greater stress-induced hypertension was observed in males. In another study, prenatal dexamethasone (DEX) treatment significantly enhanced the arterial pressure response to acute stress only in female Wistar rats, while DEX augmented the elevation in heart rate during stress only in male rats (Bechtold *et al.*, 2008).

Ortiz *et al.* have shown antenatal DEX elevates blood pressure in female offspring at three weeks of age while only male offspring had increased blood pressure at six months of age (Ortiz *et al.*, 2003). Interestingly, despite the observation only male DEX-treated rats were hypertensive at six months of age, both male and female offspring showed signs of glomerulosclerosis when compared to control rats (Ortiz *et al.*, 2003). Similar work has shown that a postnatal diet rich in ω-3 (n-3) fatty acids attenuates the effects of DEX on blood pressure in the offspring (Wyrwoll *et al.*, 2006) in a sex independent manner. With the wide ranging effects reported in the glucocorticoid models, continued studies are required to tease out the mechanisms of sex-specific responsivity in this programming model.

Another intriguing area of investigation garnering attention involves the role of the maternal RAS during pregnancy and/or lactation in pregnancy outcome and offspring health. These approaches may be in the form of administration of RAS inhibitors (Salazar *et al.*, 2008) or altered sodium diet as described above (Battista *et al.*, 2002). RAS inhibition at the level of the AT_1 receptor is reported to have several sex specific effects that manifest post-partum (Loria *et al.*, 2007b;Saez *et al.*, 2007;Salazar *et al.*, 2008). Saez *et al.* found that AT_1 inhibition reduces nephron number similarly in male and female rats, but the subsequent glomerulosclerosis and interstitial fibrosis are greater in males than in females. Further, the male rats are also reported to have a significant papillary atrophy (Saez *et al.*, 2007). Functional differences include impaired urinary-concentrating ability during a prolonged dehydration in the male offspring (Loria *et al.*, 2007b) and impaired excretory capacity following acute volume expansion (Loria *et al.*, 2007a).

Although the present data clearly indicate inhibition of the RAS during pregnancy has well defined and deleterious effects on renal development and function in the offspring, current studies are less clear on the effects of more subtle perturbations of the RAS (e.g. via dietary

alterations, etc.) on the long term health of the offspring. Further work in these areas will help define the importance of these pathways in the developmental programming of health and disease.

5. Potential mechanisms underlying sex differences in developmental programming

A variety of mechanisms have been postulated with regard to DOHAD (summarized in Figure 2). While the contribution of sex to the developmental origins of disease is widely recognized, it seems sex may exert distinctly different influences during fetal and adult life. For example, while male fetuses may be more susceptible to *in utero* nutrient privation (Gilbert *et al.*, 2007a), female fetuses may have increased susceptibility to gestational over-nutrition (Khan *et al.*, 2003). The reasons for this remain nebulous; however, one clue may be held in the long observed differences in growth rates exhibited by male and female fetuses *in utero* (Parker *et al.*, 1984). Hence, a faster growing male fetus may experience greater or lesser degrees of these nutritional insults compared to a female counterpart. Differences in the rate at which the male develops compared to the female likely contribute to gender differences in stress responses during pregnancy (Ozaki *et al.*, 2001). It remains unclear whether male fetuses have increased metabolism compared to female fetuses. Hence, the

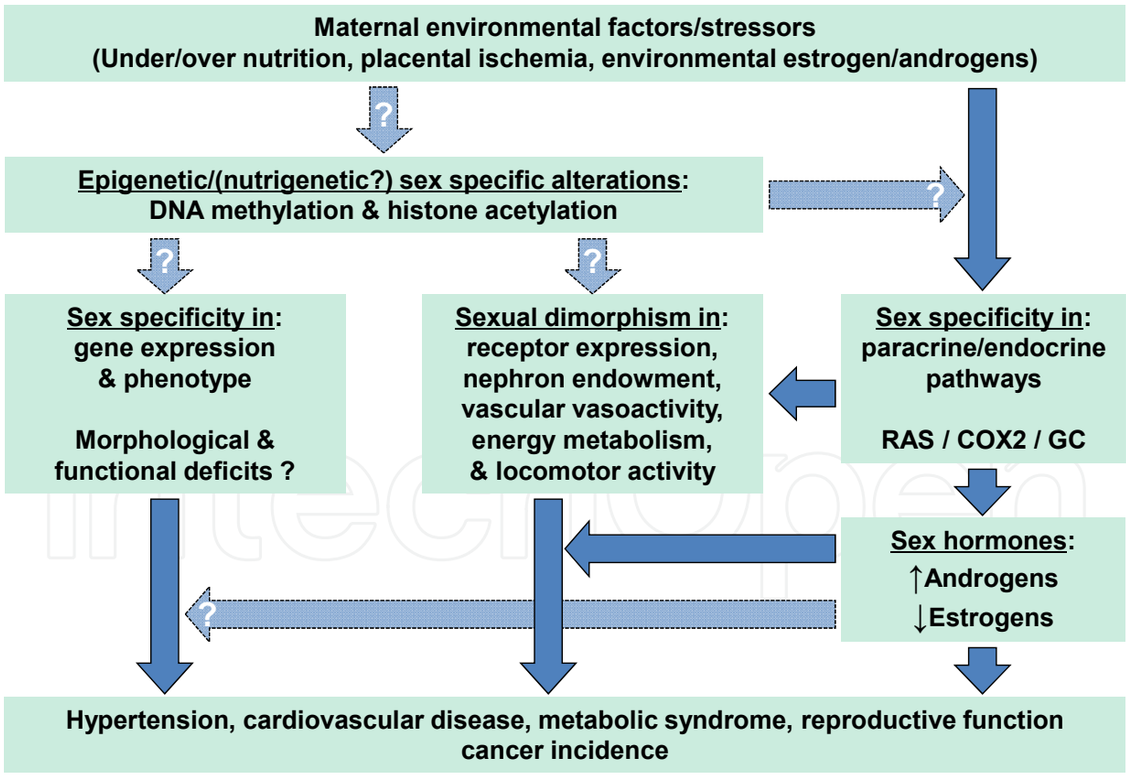

Fig. 2. *Proposed mechanisms of sexual dimorphism in developmental programming*. Research has revealed the dependence on the stimuli that result in normal development, but several connections are yet to be defined. Dark blue arrows with solid outline indicate observed pathways, like blue arrows with dotted outline represent putative connections.

chromosomal complement of the fetus may affect maternal metabolism and as the mother carrying a male fetus endures NR, the male fetus will face greater hardship than a female fetus in an equivalent pregnancy. In contrast, the female fetus in a pregnancy with an over-nourished mother could face similar hardship via different pathways.

5.1 Innate sex differences

While the existence of sexually dimorphic phenotypes is rather obvious, the mechanisms that underlie this process have remained a matter of interest. Using a theoretical model to examine the evolutionary association between X-linkage and sexually dimorphic phenotypes, Rice concluded that "sex chromosomes facilitate the evolution of sexual dimorphism and that X-linked genes have a predominant role in coding for sexually dimorphic traits" (Rice, 1984). In the ensuing twenty-five years support for this thesis has grown to include functional grouping of X chromosome gene content. Genes expressed in brain (Zechner *et al.*, 2001), for example, are particularly abundant on the X chromosome. In contrast, and perhaps of importance to potential paternal contributions to the interactions between fetus and the maternal environment, placentally expressed genes are relatively rare on the X chromosome (Ko *et al.*, 1998).

It has been recognized in humans that blood pressure is higher in men than in women (Burt *et al.*, 1995) and this difference originates during adolescence and persists into adulthood (Yong *et al.*, 1993). Further, males show an enhanced propensity to progress towards renal injury and decreased renal function than do females in several species (Neugarten *et al.*, 2002;Reckelhoff *et al.*, 1998;Sandberg & Ji, 2003). Although the roots of this difference have been linked to the RAS (Miller *et al.*, 1999), a role for an alteration in the ratio of sex steroids has also been proposed. Androgens have been linked with the progression of renal injury (Reckelhoff *et al.*, 1998;Sandberg & Ji, 2003) while estrogens have been proposed as being protective of renal function (Sandberg & Ji, 2003). Moreover, it seems that sex may exert distinctly different influences during fetal and adult life. Whereas male fetuses may be more susceptible to *in utero* nutrient privation (Gilbert *et al.*, 2007a), female fetuses appear to have increased susceptibility to gestational over-nutrition (Khan *et al.*, 2003). The reasons for this are not clear; however, one clue may be held in the long observed differences in growth rates exhibited by male and female fetuses *in utero* (Parker *et al.*, 1984). Despite findings that seem to clearly identify sex hormones as a likely culprit, recent efforts have raised many further questions and much remains unclear regarding the role of innate sex *vs.* sex steroids in developmental programming.

5.2 Epigenetic mechanisms

Epigenetic phenomena appear to be central to the induction of persistent and heritable changes in gene expression that occur without alteration of DNA sequence (Akintola *et al.*, 2008;Bird, 1986;Holliday & Ho, 2002;Wyrwoll *et al.*, 2007). While most cells in an organism contain the same DNA, gene expression varies widely across various tissues. Epigenetic mechanisms underlie this tissue- and cell-type-specific gene expression (Waterland & Michels, 2007) and include CpG methylation, histone modification (acetylation) and the activity of autoregulatory DNA-binding proteins (Kelly & Trasler, 2004). Moreover, since DNA methylation and histone acetylation are implicated in the silencing of gene expression, X-inactivation and X-linked dosage differences (Chow *et al.*, 2005), one might argue that sex-bias in differential gene expression linked to DOHAD also has its roots in methylation. Indeed, these processes appear to have many sex specific features.

Because moderate folate depletion can induce genome-wide DNA methylation (Jacob *et al.*, 1998), genomic methylation may be useful as an integrative biomarker of methyl donor nutritional status (Mason, 2003). While considerable work has been initiated in this area with regards to developmental programming, little work has focused specifically on sex differences. Interestingly, sheep exposed to a methyl deficient diet during pregnancy produce hypertensive male offspring compared to females of similar rearing, as well as to male and female controls (Sinclair *et al.*, 2007). The authors then evaluated 1400 CpG sites (primarily gene promoter associated) in fetal liver at 90 days of gestation (term=150d) and reported that more than half of the affected loci were specific to males. These observations suggest male-specific demethylation that could provide a mechanistic basis for the phenotypic sex differences observed in that study (Sinclair *et al.*, 2007). In addition, the emerging fields of nutrigenetics and metabolomics (Mutch *et al.*, 2005;Goodacre, 2007) seem poised to shed further light on these operational characteristics of these mechanisms.

Alternatively, it has also been hypothesized that when genes are expressed in multiple tissues or serve several functions they should show less sex bias than genes that are more specialized (Ellegren & Parsch, 2007). The genes such as those involved in the RAS are certainly expressed in multiple tissues, yet these genes are also closely associated with sex differences in the developmental origins of cardio-renal diseases. Clearly there is a tremendous gap in our understanding of these complex topics and further studies are needed to clarify these matters particularly in the light of the differences reported regarding fetal gender and the developmental response to maternal over- and under-nutrition.

5.3 Sex steroids

In contrast to the sex-related dichotomy observed in response to nutritional stressors, when faced with a robust stressor such as AT_1 antagonism (Loria *et al.*, 2007a;Loria *et al.*, 2007b;Saez *et al.*, 2007;Salazar *et al.*, 2008), severe protein restriction (Woods *et al.*, 2001), or chronic reductions uterine perfusion pressure (Ojeda *et al.*, 2007a;Alexander, 2003) both male and female fetuses are affected similarly *in utero.* Nonetheless a dichotomy emerges later in life with females being less impacted by their suboptimal *in utero* experience (Loria *et al.*, 2007a;Loria *et al.*, 2007b;Saez *et al.*, 2007;Salazar *et al.*, 2008). The apparent benefit of being female in scenarios such as this are supported by recent work that suggested estrogens confer a protective effect on intrauterine growth restricted females that prevents the development of programmed hypertension (Ojeda *et al.*, 2007a). Moreover, the observation that ovariectomy leads to a significant increase in blood pressure in growth-restricted females with no significant effect in controls makes a strong case for the post-developmental involvement of estrogens. Indeed, estrogen replacement reversed the effect of ovariectomy on blood pressure in growth-restricted offspring as did renin angiotensin system blockade (Ojeda *et al.*, 2007a).

Studies on the role of sex hormones in expression of components of renal renin angiotensin in healthy Sprague Dawley rats, have suggested that an estrogen-mediated attenuation of renal AT_1 binding is a potential mechanism by which estrogen exerts protection from vascular and renal disease in females (Rogers *et al.*, 2007). When this inhibition is lifted following ovariectomy in their model, or in diabetes or menopause, the resulting increased angiotensin II signaling increases both the degree of susceptibility to vascular and renal disease and the rate of existing disease progression (Rogers *et al.*, 2007).

Testosterone has also been implicated in the progression of hypertension in male growth restricted offspring (Ojeda *et al.*, 2007b). The potential underlying mechanisms have been

studied by Sullivan (Sullivan *et al.*, 2007) who has described a relationship between androgens and the development of albuminuria, and the renal protection afforded by estrogen, in spontaneously hypertensive rats. There is some evidence to suggest that both over activity of the renin angiotensin system and oxidative stress likely contributing to sex differences in the progression to renal injury. Treatment with either an AT_1 blocker and/or an ACE inhibitor blunts the occurrence of renal injury in males (Lazaro *et al.*, 2005). Male spontaneously hypertensive rats (SHR), which exhibit some signs of a programming model such as smaller size at birth when compared to Wistar-Kyoto control rats, exhibit androgen-dependent increases in blood pressure and albuminuria that are independent of renal cortical angiotensin II levels and oxidative stress (Sullivan *et al.*, 2007). In contrast, a female specific form of hypertension during pregnancy, preeclampsia, is reportedly not to be influenced by the levels of circulating testosterone levels during pregnancy (Tuutti *et al.*, 2011).

Interestingly, the cardio-renal protective effects of estrogens has not been a universal finding (Salazar *et al.*, 2008). Considering the differences between the models employed by different laboratories, one possibility could be the magnitude of the insult to the kidney during development has an influence on the extent of protection that may be afforded by female sex hormones in later life. It is widely recognized that differences in sex hormones contribute to considerable sexual dimorphism in the transcriptome of a variety of mammalian tissues and organs (Rinn & Snyder, 2005); however, it has only recently been recognized that androgen/estrogen independent mechanisms may operate at the transcriptional level to regulate sex differences (Tullis *et al.*, 2003). This possibility represents an alternate pathway that may be at work contributing to the observations that the relationship between sex hormones and blood pressure is far more complex than simply the balance of estrogen *vs.* testosterone (Ojeda *et al.*, 2007a). Taken together, it appears that the influence of sex on the developmental origins of disease may reach far beyond the widely recognized role of sex hormones. Alternatively, recent work implicates growth hormone (GH) in sex dependent differences in renal expression of glomerular AT_1 during hypertrophy following uninephrectomy; male rat kidneys show increased glomerular AT_1 expression, whereas females do not (Mok *et al.*, 2003). Because there is sexual dimorphism in GH release these observations may hold implications for both normal and pathological growth and development of the kidney.

6. Concluding remarks

From a clinical perspective it is hoped that increased understanding and awareness of developmental programming will lead to better diagnostic, preventative and therapeutic measures. The persistence of programmed effects is likely due to covalent modifications of the genome resulting from changes in promoter methylation and histone acetylation. The emerging fields of metabolomics and nutrigenetics suggest many of these alterations are likely a result of changes in the metabolic flux during critical periods of development. While epigenetic phenomena are central to the induction of persistent and heritable changes in gene expression that occur without alteration of DNA sequence, their contribution to the intensively studied sex differences in developmental programming remains uncertain. While reversal of these molecular changes may be possible and to improve long-term health outcomes if interventions are timed appropriately, loss of function in existing structures may be difficult to overcome if developmental plasticity is no longer present. For example it is difficult to see how any deficit in nephron endowment can be remedied. Nevertheless,

continued investigation using hypothesis driven mechanistic studies that incorporate sexual dimorphism into the models rather than attempt to control for sex differences by omitting male and/or female subjects are needed to identify target pathways for possible intervention.

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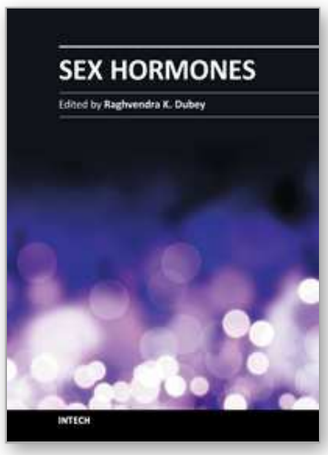

Sex Hormones

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Sex Hormones not only regulate reproductive function, but they also play a prominent role in the biology and physiology of several organs/tissues and in the pathophysiology of several diseases. During the last two decades, the information on the mechanisms of action of sex hormones, such as estrogens and androgens, has rapidly evolved from the conventional nuclear receptor dependent mechanisms to include additional non-nuclear, non-genomic and receptor-independent mechanisms. This highlights the need to update the current knowledge on sex hormones and their mode of action. Increasing evidence that exogenous/epigenetic factors can influence sex hormone production and action highlights the need to update our knowledge on the mechanisms involved. This book provides a systematic and updated overview of the male/female sex-hormones and their impact in the biology and physiology of various organs. Additionally, the book discusses their positive and negative association with the pathophysiology of various diseases (e.g. osteoporosis, cardiovascular-disease, hypogonadism, reproduction, cancer) and their therapeutic potential.

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