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Genetic Basis for Sex Differences in Obesity and Lipid Metabolism

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Abstract

Men and women exhibit significant differences in obesity, cardiovascular disease, and diabetes. To provide better diagnosis and treatment for both sexes, it is important to identify factors that underlie the observed sex differences. Traditionally, sex differences have been attributed to the differential effects of male and female gonadal secretions (commonly referred to as sex hormones), which substantially influence many aspects of metabolism and related diseases. Less appreciated as a contributor to sex differences are the fundamental genetic differences between males and females, which are ultimately determined by the presence of an XX or XY sex chromosome complement. Here, we review the mechanisms by which gonadal hormones and sex chromosome complement each contribute to lipid metabolism and associated diseases, and the current approaches that are used to study them. We focus particularly on genetic approaches including genome-wide association studies in humans and mice, -omics and systems genetics approaches, and unique experimental mouse models that allow distinction between gonadal and sex chromosome effects.

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SEX AND METABOLISM

Why Is It Important to Understand Sex Differences in Metabolism?

The most prevalent diseases in developed countries are cardiovascular disease, diabetes, and some forms of cancer (35, 95). The incidence of these diseases increases in the presence of a group of risk factors known as metabolic syndrome (MetSyn). MetSyn is defined by the presence of three of the following five risk factors: abdominal obesity, elevated triglyceride levels, low high-density lipoprotein (HDL) cholesterol levels, high blood pressure (hypertension), and elevated fasting blood glucose levels (73). Sex differences exist in nearly all the components of MetSyn (**Figure 1**). For example, females have evolved specific mechanisms to favor adipose tissue storage, whereas mobilization of fat stores tends to be more efficient in males (40, 60, 77, 90). Compared with men, women tend to have greater insulin sensitivity, and the sexes also differ in lipoprotein profiles (30, 85). It has become clear that understanding sex differences in metabolism is critical to optimize prevention, diagnosis, and therapeutic intervention for both sexes.

Here we review sex differences in obesity, other components of MetSyn, and related factors such as the gut microbiome, with an emphasis on the genetic mechanisms underlying these differences. In the sections below, we first discuss the components of sex and approaches used to study sex effects. We then review sex differences in obesity and other MetSyn components in humans, and we highlight studies in experimental models that shed light on the mechanisms involved.

Components of Sex: Gonadal Hormones and Sex Chromosome Complement

An understanding of why one sex is more susceptible to specific disease processes requires analysis of the component parts of sex. Differences between men and women can be influenced by sex and

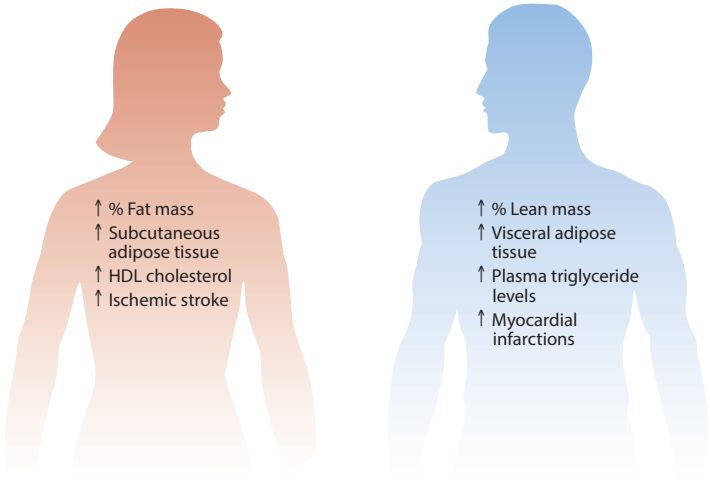

Figure 1

Sex differences in metabolic syndrome (MetSyn) components. Risk factors for MetSyn include visceral obesity, elevated triglyceride levels, low high-density lipoprotein (HDL) levels, hypertension, and elevated fasting glucose levels. Sex differences occur in most of these traits. Women tend to have increased fat mass proportional to their body weight, increased subcutaneous adipose tissue, and elevated HDL cholesterol levels. Men generally have greater proportional lean mass, increased visceral adipose tissue, and elevated plasma triglyceride levels. Cardiovascular disease incidence also differs by sex: Women have higher incidence of ischemic stroke, and men have higher incidence of myocardial infarction.

gender. Sex refers to the biological attributes of males and females that result from the presence of female (XX) or male (XY) sex chromosomes, which ultimately determine the levels and types of gonadal hormones (**Figure 2**). Gender refers to characteristics that a society or culture defines as masculine or feminine. In humans in particular, gender may influence behaviors that affect metabolism and related diseases. However, gender effects are challenging to assess experimentally, and data are lacking on their impact on metabolism. We therefore limit our review to the biological components of sex differences.

The most widely studied sex difference between males and females has been the effect of gonadal hormones. In human studies, the contribution of gonadal hormones to a trait of interest is often addressed by comparing women before and after menopause, and differences between the two are attributed to the reduced levels of ovarian hormones after menopause. Studies of this sort have demonstrated that postmenopausal women have altered body fat distribution and increased incidence of cardiovascular disease, hypertension, diabetes, and other disorders (41, 68, 74, 122). Androgen levels also influence metabolic disease. Testosterone levels in men typically diminish with age, and low testosterone levels in men are associated with increased body fat and cardiovascular disease (3, 12, 99). However, these studies are confounded by the fact that the pre- and postmenopausal (or high and low testosterone) groups usually comprise different individuals owing to the difficulty of performing longitudinal studies for several years in humans. Therefore, comparisons between groups could be influenced by genetic and environmental heterogeneity. Studies in humans are also complicated by the fact that reductions in gonadal hormone levels in older men and women cannot necessarily be distinguished from independent changes associated with aging.

In addition to gonadal hormones, sex chromosome complement could contribute to sex differences in metabolic traits (8, 69). It has been difficult to address whether hormone-independent effects of gonadal sex occur in humans because, typically, XX chromosomes and ovaries occur

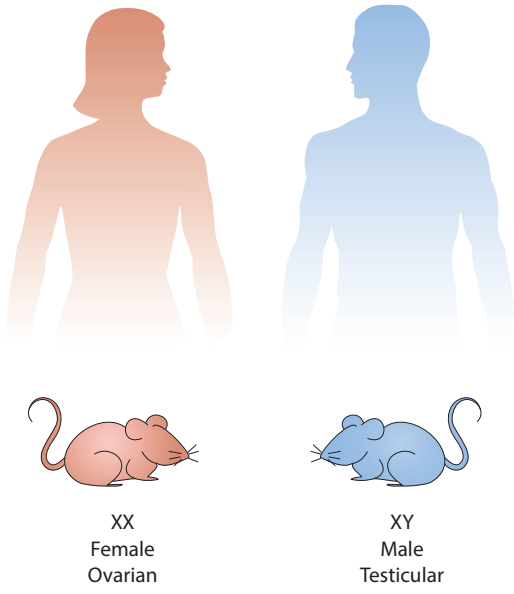

Figure 2

Genetic and hormonal components of sex. Male and female sex differences may result from genetic or hormonal components. Normal females and males differ in their sex chromosome complement of XX or XY, respectively. They also differ in the presence of ovarian or testicular gonadal hormones. In standard humans and mouse models, the genetic and gonadal contributions to sex differences cannot be easily distinguished.

together, and XY chromosomes and testes occur together (**Figure 2**). Some studies of metabolic factors have been performed on individuals with sex chromosome anomalies (Turner syndrome or Klinefelter syndrome) and have reported increased adiposity and other features of MetSyn (13, 14, 19). However, it is difficult to tease apart the role of sex chromosome complement from gonadal hormones in these individuals because alterations in chromosome complement occur in concert with abnormal hormone levels. Individuals with Turner syndrome (45, XO) have low endogenous levels of ovarian hormones such that it is impossible to distinguish the effects of the single X chromosome from those resulting from abnormal ovarian hormone levels (44, 48). Furthermore, Turner syndrome subjects often have congenital heart defects, which lead to heart disease independent of MetSyn (31). Individuals with Klinefelter syndrome (47, XXY) have reduced endogenous androgen levels and are often treated with exogenous androgens, which makes it difficult to distinguish effects of the XXY chromosome complement from those of the altered androgen levels (53). In addition to the factors outlined above, interpretation of metabolic disease traits in Turner and Klinefelter subjects has been limited by small available cohort sizes that preclude meaningful conclusions about metabolic parameters.

Approaches to Study Sex Differences

To overcome some limitations in studying the components of sex effects in humans, experimental animal models are valuable; unique genetic tools in the mouse make it a particularly useful animal model. Unlike humans, inbred mice allow for analysis of high and low gonadal hormone levels on identical genetic backgrounds. The action of gonadal hormones can be classified into two temporal classes: the permanent effects of gonadal hormones that lead to the development of sex differences during fetal and neonatal development, and the acute actions of gonadal hormones that cause

specific responses on numerous processes throughout life. The acute effects of gonadal hormones are reversible, and a standard way to identify acute hormone effects is to compare adult mice with intact gonads to those whose gonads were removed in adulthood. Sex differences caused by acute hormonal effects will be abolished in gonadectomized mice, whereas sex differences resulting from the permanent effects of gonads during development will remain.

The origin of male and female gonads and the hormones they produce traces back to the presence of either XX or XY sex chromosomes (**Figure 2**). Furthermore, at a more fundamental level than hormone action, the simple presence of either XX or XY chromosomes leads to sex differences at the cellular level because of the differential action of genes on the X and Y chromosomes (7). For example, during early embryonic growth prior to the presence of gonads or gonadal hormones, male embryos are larger than female embryos in mice, humans, and several other mammals (16). In the mouse, these differences have been attributed to a combination of effects from the presence of the Y chromosome in male embryos and the sexual imbalance between the number of X chromosomes in males versus females (17, 18). Following differentiation of the gonads, it is difficult to cleanly separate effects due to hormones from those resulting from the sex chromosome complement. This led to the generation of mouse models that allow for independent segregation of gonadal type from sex chromosome complement.

The most widely used model to distinguish gonadal and chromosome sex effects is known as the four core genotypes (FCG) mouse model (**Figure 3**). This model generates mice with XX chromosomes on both male and female gonadal backgrounds and XY mice on male and female gonadal backgrounds (4–6). This is accomplished by using a Y chromosome from which the testis-determining *Sry* gene has been deleted. Inheritance of the *Sry*-deleted Y chromosome fails to specify male gonads, and female gonads develop instead, allowing production of XY female mice. In addition, an *Sry* transgene is inserted into chromosome 3, such that it segregates independently of the sex chromosomes (56). Inheritance of the *Sry* transgene, even in the presence of XX sex chromosomes, results in development of male gonads. The FCG model is valuable for identifying whether sex differences in a trait of interest are associated with gonadal type or sex chromosome complement (**Figure 4**). For example, if a trait is influenced by gonadal sex, male mice with XX or XY chromosomes will be similar but will differ from female mice with XX or XY chromosomes. However, if a trait is influenced by sex chromosome complement, XX female and male mice will be similar to one another but will differ from XY female and male mice (**Figure 4**). This model also allows for detection of interactions between gonadal hormones and chromosomal sex, and the effects of acute hormones can be assessed by comparing gonadally intact with gonadectomized mice, as described above. This model has revealed that sex differences in obesity, lipid levels, fatty

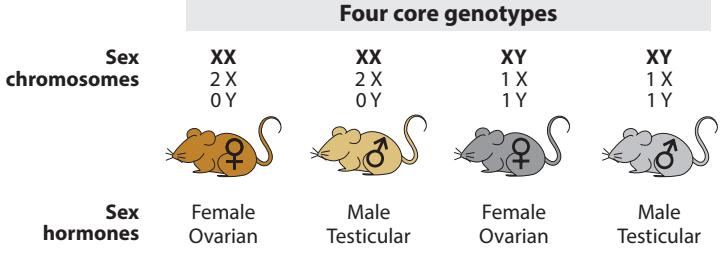

Figure 3

Distinction between gonadal and chromosomal contributions to sex differences using the four core genotypes (FCG) mouse model. The FCG mouse model breaks sex into gonadal type and sex chromosome complement as independent determinants. The FCG can be used to study the contribution of gonadal and chromosomal sex to traits of interest.

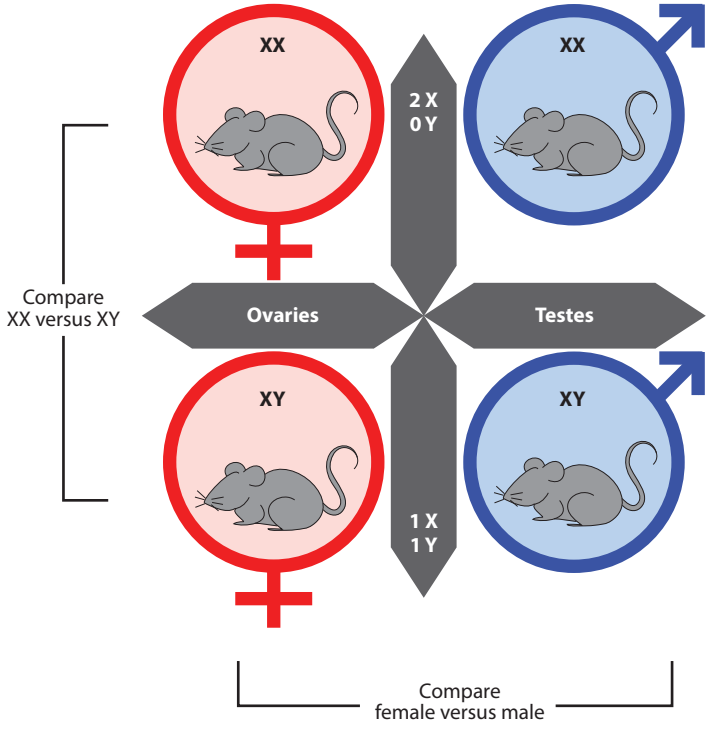

Figure 4

Use of the four core genotypes (FCG) mouse model to study sex differences. Studies with the FCG are performed in a 2 × 2 comparison, using gonadal type (ovaries versus testes) and chromosomal type (XX versus XY) as the categories. Comparison of XX versus XY mice (on both gonadal backgrounds) allows detection of effects due to sex chromosome complement. Comparison of female versus male mice (with both sex chromosome genotypes) allows detection of effects due to gonadal type.

liver, glucose homeostasis, and other metabolic traits are influenced by XX versus XY chromosome complement (**Figure 5**) (described below).

GENETIC CONTRIBUTIONS TO SEX DIFFERENCES IN ADIPOSITY AND OBESITY

Sex-Specific Obesity Loci

The heritability of obesity is estimated to be approximately 50–70%, indicating that genetics play a large role in fat accumulation (reviewed in Reference 52). Recently, large-scale genome-wide association studies (GWAS) have identified loci linked to fat accumulation and distribution. A large meta-analysis of 32 GWAS, with a follow-up of 16 loci in 29 additional studies, identified 14 genome-wide significant loci that were associated with waist-to-hip ratio, a proxy for adipose distribution (50). In sex-specific meta-analyses, 12 of these 14 single-nucleotide polymorphisms were significant in women, whereas only three were significant in men. The 14 loci collectively explained 1.34% of the waist-to-hip ratio variance in women and 0.46% of the variance in men. Subsequent studies confirmed these loci in genome-wide associations with subcutaneous and visceral adipose tissue measured by computed tomography scan (37, 104). When sex-stratified studies

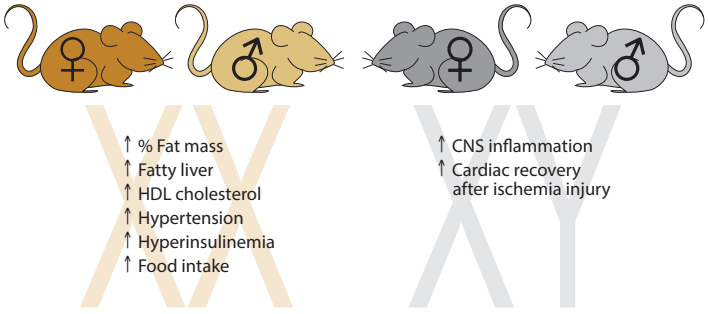

Figure 5

Sex chromosome complement confers sex differences in metabolic syndrome (MetSyn) traits. Studies using the four core genotypes mouse model have revealed that sex differences in some traits associated with MetSyn are influenced by the sex chromosome complement. For example, XX mice, regardless of whether they have ovaries or testes, have increased fat mass relative to body weight, are susceptible to fatty liver, and exhibit increased high-density lipoprotein (HDL) cholesterol. XY mice have greater inflammation in the central nervous system (CNS) (33) and improved cardiac recovery after ischemia injury (67).

were performed, the reported loci had statistically significant associations with fat distribution in women, but not in men. Concordant with these findings, another large meta-analysis of nearly 225,000 individuals reported 49 loci associated with waist-hip ratio adjusted for body mass index, and 20 of these 49 loci had significant sexual dimorphism (103). For sexually dimorphic loci, 19 of 20 had a larger effect in women than in men. Taken together, these GWAS findings highlight the importance of performing sex-stratified GWAS to better understand genetic determinants of adiposity. These findings also emphasize that a large proportion of the genetic variants that contribute to obesity in both sexes remain unidentified.

Sex Differences in Regional Adipose Tissue Deposition

It is well appreciated that there are inherent differences between men and women in fat distribution and properties of fat cells within anatomical depots (**Figure 1**). When normalized to waist circumference or to total body fat, men have more visceral adipose tissue (fat within the abdominopelvic cavity) than premenopausal women (64, 66). Compared with women, men also tend to lose relatively more visceral adipose tissue owing to calorie restriction (63). By contrast, women typically have more subcutaneous adipose tissue (fat underlying the dermis) (66). Numerous epidemiological studies have linked increased visceral fat to MetSyn, and others have suggested that subcutaneous adipose tissue may even play a protective role against glucose dysregulation and other components of MetSyn (38, 71, 96, 108). Thus, in the basal state, the greater subcutaneous to visceral adipose tissue composition in women may be a beneficial trait relative to MetSyn development.

Beyond sex differences in fat mass and distribution, metabolic differences between men and women have been identified in adipocyte biology that ultimately may contribute to the likelihood for abdominal obesity that contributes to MetSyn. For example, a comparison of adipocytes isolated from the subcutaneous and visceral compartments of abdominal fat in healthy women showed that the intraperitoneal adipocytes were 20–30% smaller than those from the subcutaneous depot, whereas men exhibit a similar adipocyte size from these two areas of abdominal fat (106). This is relevant because larger adipocytes have higher rates of lipolysis, are correlated with insulin resistance, and have increased expression of proinflammatory adipokines. Changes in the

basal characteristics of adipocytes in women with abdominal obesity may be highly detrimental and may contribute to the development of MetSyn.

Gonadal Hormone Signaling Effects on Adiposity and Potential Effectors

By manipulating receptors for gonadal hormone signaling, experimental models have been extensively used to investigate mechanisms underlying sex differences in adiposity. In particular, ablation of estrogen and androgen receptors in the brain and peripheral tissues in the mouse results in striking metabolic outcomes. For example, knockout of estrogen receptor α (ERα) resulted in increased adiposity in both male and female mice (51, 87). Knockout of ERα in hypothalamic neurons also leads to dysregulation of energy balance, resulting in increased abdominal fat (84, 117). Whereas deficiency of ERα affects both sexes, deficiency of androgen receptor results in late-onset obesity only in males (36). Interestingly, adipose-specific knockout of androgen receptor causes hyperleptinemia, but not increased adiposity. This suggests that androgen receptor is required in adipocytes for normal levels of leptin, but not for expansion of fat tissue.

Although gonadal hormones have an irrefutable role in shaping and distributing fat, the specific mechanisms have not been fully elucidated. One possible mechanism is a role for gonadal hormones, along with nutrients and other signals, as cues to signal adipocyte progenitor cells to proliferate and/or differentiate. Indeed, ovarian hormones appear to influence fat-pad expansion in a sex-specific manner (57). In mice fed a high-fat diet, adipocyte precursor cells proliferate and differentiate into mature adipocytes to a much greater degree than in mice fed a standard chow diet, as would be expected. Interestingly, the removal by ovariectomy of acute hormonal effects in females blunts the proliferative capacity of preadipocytes in the subcutaneous fat pad, but not the visceral fat pad. These results suggest that gonadal hormones affect precursor cells differentially in distinct fat depots. In addition, elegant adipose tissue transplant studies revealed that adipocyte precursor cell behavior is dictated by the recipient depot, implicating environment rather than cell-intrinsic properties as a determinant of fat tissue hyperplasia (57).

Another potential effector of sex hormone effects on adipose tissue depot expansion may be the enzyme aldehyde dehydrogenase 1 (Aldh1a1) (119). Deletion of *Aldh1a1* abrogated diet-induced expansion of subcutaneous fat in male and female mice compared with wild-type animals. However, compared with the corresponding wild-type mice, visceral fat mass was reduced in female but not male *Aldh1a1*$^{-/-}$ mice. The female-specific reduction in visceral fat of *Aldh1a1*$^{-/-}$ mice was attributed to differential expression of adipose triglyceride lipase, which was likely induced by a buildup of retinaldehyde in *Aldh1a1*$^{-/-}$ females. The investigators (119) postulate that the retinoic acid produced by Aldh1a1 action may activate retinoic acid receptor response elements (such as in the promoter of the *PPARγ* gene) to a greater degree in female versus male visceral adipose tissue. Though this study identified an interesting potential role of retinoic acid in mediating sex differences in fat depots, more research is needed to understand how sex-biasing factors, such as sex hormones and sex chromosomes, interact with retinaldehyde and retinoic acid signaling pathways.

Sex-biasing factors, such as sex hormones and sex chromosomes, likely mediate sexually dimorphic traits via changes in gene expression. Global gene expression analysis has identified thousands of sexually dimorphic genes in adipose tissue in mice (45, 118). These genes were enriched in functional categories such as immune response, lipid metabolism, and insulin signaling. The mice used in these studies were fed a high-fat diet (45–47% calories from fat) to increase adiposity, such that some of the observed sexually dimorphic gene expression may reflect sex-dependent responses to nutritional excess and the ensuing metabolic dysregulation. Interestingly, only approximately 100 genes were differentially expressed between ovariectomized females and gonadally intact females, suggesting that circulating gonadal hormones do not regulate most sexually dimorphic genes (45).

One factor that may contribute to sexually dimorphic gene expression is regulator of sex-limitation 1 (RSL1), a zinc-finger protein that affects the expression of many transcripts in liver and adipose tissue. Deletion of *Rsl1* in mice results in sex-specific weight gain (61). *Rsl1*$^{-/-}$ females gain significantly more weight compared with wild-type females, whereas *Rsl1*$^{-/-}$ and wild-type males have equal weight gain on a high-fat diet. In addition, *Rsl1* deficiency enhanced sexual dimorphism in adipose tissue gene expression compared with wild-type mice, suggesting that RSL1 normally suppresses sex differences in adipose tissue.

Sex Chromosome Complement Influences Adiposity

The obesity loci discovered in GWAS and the studies from experimental models discussed above account for a small proportion of the estimated genetic heritability of obesity. The missing heritability may arise from a number of factors, including an inability to detect the combined effects of numerous loci, which, individually, may have insignificant associations with adiposity traits. In addition, few GWAS reports stratify associations by sex, thus potentially obscuring our understanding of complex diseases. To determine genetic causes of sex differences in adiposity, one must consider the fundamental definition of sex in a cell: the sex chromosome complement.

In many cases, the X chromosome is not included in GWAS because arrays do not have enough X chromosome variants or because imputation is needed for combined-sex studies (116). This practice impedes discovery of novel associations, especially with sexually dimorphic traits such as adiposity. There are more than 1,600 coding and hundreds of noncoding genes on the X chromosome, which represent greater than 5% of all human genes. The number of X chromosomes, the ability to randomly inactivate an X chromosome, and X chromosome imprinting are sources of sex differences between females and males that reside in each nucleated cell of every tissue.

Mouse models have been used to demonstrate an effect of XX versus XY chromosome complement on fat accumulation and obesity comorbidities. Chen et al. used the FCG mouse model (**Figure 3**) to distinguish effects of sex hormones from effects of sex chromosomes on obesity (21). All genotypes were on an inbred C57BL/6 background, ruling out genetic differences besides sex chromosome complement. To remove acute effects of circulating hormones, mice were gonadectomized in early adulthood. Mice with two X chromosomes, regardless of whether they originally had ovaries or testes, gained nearly twice as much fat as mice with X and Y chromosomes (**Figure 5**). Remarkably, the enhanced weight gain in XX mice was observed on a standard chow diet. The weight difference between XX and XY mice was amplified by a high-fat diet, becoming statistically significant after merely 3 days on the diet. Increased obesity in XX mice was associated with increased food intake during the light (inactive) phase of the circadian cycle (21, 22). Along with increased adiposity, XX mice developed fatty liver and had elevated levels of fasting insulin (**Figure 5**), both of which are comorbidities of obesity in humans.

The provocative finding that XX mice have greater adiposity than XY mice raised the question of whether the effect was due to the presence of two X chromosomes or to the absence of a Y chromosome. To address this, investigators generated mice with XX, XY, XO, and XXY sex chromosome complements. When body weight of these mice was followed for several months following gonadectomy, the XX and XXY mice had greater body weight than did XY and XO mice (21). Thus, the presence of two X chromosomes drove increased body weight, irrespective of inclusion of a Y chromosome. Interestingly, another mouse model of altered sex chromosome number also showed increased body weight in mice with two X chromosomes compared with those with one X, regardless of the presence of a Y chromosome (23).

The studies described above implicate X chromosome dosage as a risk factor for obesity. It will be important to further define the mechanisms involved. One hypothesis is that increased

expression of genes escaping X chromosome inactivation in XX mice may cause phenotypic differences between XX and XY mice (21, 69, 70). This can be investigated by modulating the dosage of individual genes that escape X chromosome inactivation in cell or mouse models to determine the effects on downstream gene expression and physiology.

SEX DIFFERENCES IN OBESITY COMORBIDITIES

In this section, we review sex differences in comorbidities of obesity that are also components of MetSyn. The prevalence of MetSyn varies across world populations and is clearly influenced by ethnicity, sex, age, and socioeconomic status (47, 97, 98). The prevalence of MetSyn in the US population is similar in age-adjusted men and women (34.9% of men, 33.3% of women) (82), but this may differ in other countries (98). The combination of components that most often lead to a diagnosis of MetSyn differs between the sexes (97, 98). For example, the predominant component of MetSyn in women is abdominal obesity (often measured as waistline circumference), whereas the predominant component is men is hypertension (62). In both men and women, these two components occur most frequently in combination with low HDL cholesterol levels and elevated triglyceride levels to form the triad of factors used to define MetSyn. Additional components of MetSyn and other obesity comorbidities exhibit substantial sex differences in both humans and mouse models, as reviewed below and summarized in **Figure 1**.

Plasma Lipid Levels

Dyslipidemia is a key component of MetSyn and often occurs together with obesity. A widely held generalization regarding lipid levels and cardiovascular disease is that elevated levels of low-density lipoproteins (LDL) are detrimental, whereas elevated HDL are beneficial (20). However, the reality is more complex than this. Recent work indicates that the context in which HDL levels occur (i.e., levels of LDL and triglycerides) influences the utility of HDL as a predictor of cardiovascular disease risk (9). Furthermore, specific subspecies of each of the lipoprotein classes appear to be more relevant to cardiovascular disease prediction. In the broadest terms, premenopausal women have higher levels of HDL cholesterol than do men, and men have higher LDL cholesterol levels (11, 39, 43, 58, 75). Within the lipoprotein classes are subclasses that differ in size, lipid and protein composition, and, potentially, function. An examination of the lipoprotein subclasses reveals that men tend to have a profile that includes several characteristics associated with cardiovascular disease risk (small LDL, small HDL, large very-low-density lipoproteins), whereas women are likely to have higher concentrations of large HDL particles, which are associated with athero-protection (58).

Gonadal hormones (either directly or indirectly) influence the lipoprotein profile. Lipoprotein profiles are altered in women after menopause, and this can be partially reversed by exogenous hormone replacement therapy (11, 24, 29, 113). Androgen levels in men also appear to influence lipid levels and cardiovascular disease risk. Conflicting data exist, but at least some data indicate that androgen action is likely not the major determinant of LDL cholesterol levels (54, 81, 113, 115). Much work remains to be done to determine the relevance of specific lipid profile characteristics on cardiovascular disease risk in men and women. Understanding these mechanisms is important, however, as they will inform therapeutic approaches and management of cardiovascular disease.

To understand basic mechanisms that influence lipid levels differentially in males and females, animal models may provide some insight. For example, a study performed in the FCG mouse model identified some aspects of lipid levels that are determined predominantly by the presence of male or female gonads and other aspects that are determined by the presence of 2 X chromosomes (as

found in XX animals) versus 1 X chromosome (as found in XY animals) (70). Whereas humans transport the majority of cholesterol in the form of LDL particles, mice transport the majority of cholesterol in the form of HDL particles. However, when fed a cholesterol-enriched diet, mice increase LDL cholesterol levels substantially to levels resembling those of humans. Studies of lipid levels in mice of the FCG (XX female, XX male, XY female, and XY male mice) revealed that under basal conditions in which the mice were fed a chow diet, plasma HDL cholesterol levels were higher in animals with XX versus XY chromosome complement as well as in mice with male versus female gonads (**Figure 5**). LDL cholesterol levels, which were only approximately one-fifth of HDL levels on the chow diet, were similar across the four genotypes. By contrast, triglyceride and free fatty acid levels were higher in males versus females, regardless of sex chromosome complement. To determine the contribution of acute-acting gonadal hormones, adult mice were gonadectomized. This led to a loss of male/female differences and accentuated the effects of XX chromosome complement on promoting higher HDL cholesterol and free fatty acid levels. When fed a cholesterol-enriched diet, LDL cholesterol levels increased and were similar among the four genotypes. However, XX mice had higher levels of HDL cholesterol, whereas XY mice had higher levels of triglycerides and free fatty acids. Importantly, investigators used a separate mouse model with XX, XXY, and XY genotypes to demonstrate that HDL cholesterol levels are elevated in XX and XXY mice compared with XY mice, indicating that the presence of two X chromosomes, rather than absence of a Y chromosome, confers the high HDL phenotype (70). These results reveal that specific components of the lipid profile may be influenced independently by gonadal hormones and/or sex chromosomes and that the underlying sex chromosome complement, rather than sex hormones, may be a key determinant of HDL cholesterol levels.

Glucose Dysregulation

Common comorbidities of obesity are impaired glucose homeostasis and insulin resistance. Standard measures of glucose homeostasis are determinations of fasting glucose levels and glucose tolerance, the latter measured as the kinetics of glucose clearance from the blood following an acute glucose load. Abnormal values for either of these traits may indicate impaired glucose metabolism, but the mechanisms underlying each are distinct. Indeed, only a minority of individuals with dysregulated glucose metabolism (approximately one-fourth) exhibits impairment in both fasting glucose and glucose tolerance (49). Elevated fasting glucose levels could result from inadequate levels of insulin secretion and/or impaired hepatic insulin sensitivity, leading to dysregulated glucose production by the liver. By contrast, impaired glucose tolerance is more likely a result of peripheral insulin resistance at the sites of postprandial glucose disposal, especially skeletal muscle. Notably, men are more likely to develop elevated fasting glucose levels, whereas women are more likely to develop impaired glucose tolerance, as shown in a meta-analysis of more than 20 studies from European and Asian populations (111). Assessments of insulin resistance show greater occurrence in men than women, even after adjusting for age and body mass index (42, 59, 80, 86). Key mechanisms that have been implicated include effects of gonadal hormone–dependent and –independent sex differences in regional adipose tissue distribution, production of cytokines and adipokines, hepatic gluconeogenesis and glycogenolysis, and glucose uptake by skeletal muscle (1, 86, 102). Sex differences in the mechanisms of dysglycemia raise a concern that diagnosis of MetSyn based on fasting glucose levels may not reliably identify women that have MetSyn and should undergo some intervention.

Sex differences in glucose metabolism have also been described in rodent models. In a screen of ~100 inbred mouse strains, Park et al. compared males and females for development of insulin resistance as assessed using a homeostatic model assessment of insulin resistance after feeding a high-fat/high-carbohydrate diet (93). In nearly all strains, males were more prone to insulin

resistance than were females. This model assessment was also correlated with total body fat and mesenteric fat-pad weights in both males and females, but only females showed a strong correlation with additional visceral fat pads (gonadal and retroperitoneal fat depots). A set of three inbred mouse strains were studied with intact gonads or gonadectomy to determine the impact of gonadal hormones on the development of diet-induced insulin resistance. Ovariectomy improved insulin sensitivity in females of all three strains examined, but gonadectomy in male mice improved insulin sensitivity only in one of the three strains, demonstrating a hormone-gene interaction (93). Genetic association analyses identified distinct loci in males and females for plasma insulin and glucose levels (93), underscoring that mechanistic differences exist in the regulation of glucose metabolism between males and females.

Another approach to identify mechanisms underlying sex differences in the regulation of glucose homeostasis is to compare sex chromosome and gonadal sex contributions to glucose metabolism parameters. In most cases in mice and humans, increased adiposity correlates with insulin resistance. In the FCG mouse model, glucose and insulin levels were analyzed in mice that were gonadectomized to remove the effects of acute gonadal hormones. As described above, XX mice (with either male or female original gonads) gained more fat than did XY mice (of either gonadal type) when fed a chow diet (21). Despite this, chow-fed XX mice had glucose and insulin levels similar to those of XY mice. The ability to maintain glucose homeostasis despite higher body fat could indicate that compensatory changes occur in XX mice, as suggested by enhanced fatty acid oxidation gene expression profiles in muscle and liver (21). When animals are made obese by feeding them a high fat-high carbohydrate diet, the XX mice appear to be more prone to insulin resistance (**Figure 5**): Compared with XY mice, they have twofold higher plasma insulin levels to maintain similar plasma glucose levels (21). This effect may be secondary to increased obesity in XX mice.

Nonalcoholic Fatty Liver

A common comorbidity of obesity and insulin resistance is nonalcoholic fatty liver disease (NAFLD). This condition is characterized by the accumulation of neutral lipids in hepatocytes and represents the initial stage of a continuum of conditions that can progress to include severe inflammation (steatohepatitis) and, further, fibrosis and cirrhosis (2, 28). Data show increased cardiovascular disease mortality and overall mortality in individuals with NAFLD/steatohepatitis (34, 88), but it is difficult to disentangle this risk from that due to conditions that typically occur in tandem, such as MetSyn (120). Sex differences have been noted in the prevalence of NAFLD, but these vary depending on the criteria used to define NAFLD, ethnicity, and other clinical characteristics of the subjects (91). In population-based studies, which may have less ascertainment bias than case-control studies, men have higher incidence of NAFLD than do women (reviewed in 91). In some, but not all, cases, the greater occurrence of NAFLD in men remained after adjustment for body weight and insulin resistance (15, 25). Interestingly, in a study where individuals are segregated into lean and overweight/obese groups, lean women had a higher incidence of NAFLD than lean men (121). There may be distinct mechanistic etiologies for fatty liver that occur in the context of obesity compared with what occurs in lean individuals.

As with most metabolic phenotypes discussed here, gonadal hormones likely contribute to differences in fatty liver development, either directly through estrogen and androgen receptor signaling or indirectly through effects on adiposity, insulin resistance, etc. Studies in male rats that were gonadectomized and treated with estradiol or dihydrotestosterone showed that both of these hormones decreased lipid accumulation in the liver (124). Estradiol appeared to reduce fatty acid synthesis, and testosterone increased cholesterol synthesis, each in specific hepatic zones.

Studies using the FCG mouse model indicate that gonad-independent mechanisms also influence fatty liver development. Mice that were gonadectomized as adults and then fed a high-fat/high-carbohydrate diet for 12 weeks gained substantial body weight and fat, regardless of gonadal and chromosomal sex (21). Interestingly, only XX mice developed pronounced fatty liver with large triglyceride droplets, whereas XY mice accumulated much less triglyceride and only very small lipid droplets (**Figure 5**). The XX versus XY effect occurred regardless of whether the original gonads were male or female and occurred in the absence of gonadal secretions, indicating that gonad-independent sex differences are involved. However, although both XX and XY mice were obese, the XX mice had greater adiposity and hyperinsulinemia, making it difficult to determine whether the fatty liver in XX mice was independent or secondary to these other metabolic effects.

THE GUT MICROBIOME AND SEX DIFFERENCES IN METABOLIC SYNDROME

In the past several years, there has been a surge in studies investigating the connection between the gut microbiome and human health. Perturbations in the microbial community are related to a wide range of diseases, including bowel disorders, autoimmune diseases, and metabolic disorders (27). Indeed, a direct effect of gut microbiota on obesity was elegantly demonstrated by transplanting gut microbiota from lean or obese mice into germ-free mice. Mice that received microbiota from diet-induced or genetically obese mice gained weight compared with recipients of microbiota from lean donors (109, 110). These findings suggest that gut flora have the capacity to dramatically influence host metabolism, including effects on energy acquisition and storage.

Although the field is still young, studies demonstrate that diet, sex, and genetic background may each influence the composition of the gut microflora and that the microbial community in turn impacts hormone levels, immunity, and metabolic homeostasis in the host. Compared with chow-fed mice, mice fed a high-fat/high-sucrose diet for 8 weeks dramatically changed their microbial makeup, particularly the levels of Bacteroidetes and Firmicutes (92). These changes were dependent on the specific mouse strain, indicating that genetic background influences the gut microbiota composition. Furthermore, some genera (*Akkermansia*, *Lactococcus*, and *Allobaculum*) were correlated with the gain in body fat due to diet, highlighting the connection of gut microbes with metabolic health. In addition to diet, gut microbiota respond to the diurnal cycle, with fluctuations in major phyla such as Firmicutes, Bacteroidetes, and Verrucomicrobia (123). Feeding a high-fat diet (61% calories from fat) for 8 weeks suppressed diurnal fluctuations in microbial composition and reduced species, which may be detrimental to overall health (72). One hypothesis is that metabolites produced by gut flora, including catabolized complex carbohydrates and bile acids, may signal to and alter host metabolism.

A recent study investigated the effects of sex on gut microbial communities (89). Of 89 strains of mice examined, 7 strains had significant sex differences in abundance of microbial taxa. To determine how nutritional excess affected these sex differences, the authors (89) examined male and female C57BL/6J, C3H/HeJ, and DBA/2J mice on a high-fat/high-sucrose diet for 8 weeks. They identified a sex-by-diet interaction in microbiota composition, suggesting that specific taxa respond to diet in a sex-dependent manner. For example, in DBA/2J mice, members of the Firmicutes phylum increased in females but decreased in males in response to high-fat diet. In addition, gonadectomy revealed that circulating sex hormones regulate the gut microbiome in a strain- and diet-dependent manner.

Although many factors influence microbial composition, the gut flora remarkably affects testosterone levels in both males and females (76). Germ-free females had higher levels of testosterone compared with females housed in specific pathogen-free facilities, whereas germ-free males had

reduced levels of testosterone compared with males housed under specific pathogen-free conditions. Perhaps most strikingly, transplantation of microbiota from adult male cecum to female weanlings resulted in a significant increase of testosterone at 7 and 14 weeks of age (76). At 34 weeks of age, the microbial community reverted to a population resembling female gut flora, and testosterone changes were no longer apparent. The implications of this manipulation were reflected in protection from type 1 diabetes in nonobese diabetic mice. Female mice that had received male cecal bacteria were protected from autoimmune destruction of pancreatic β cells, whereas those that had received female cecal bacteria were not. In addition, treatment of an androgen receptor antagonist abrogated the protective effects of male cecal bacteria, suggesting that transfer-mediated-enhanced testosterone levels were required for protection from type 1 diabetes (76). Though these and other data showcase a critical role of the gut microbiome in regulating sex hormones and diabetes, future studies are needed to fully understand the underlying mechanisms (94).

Sex differences inherent in the host may also affect processing of metabolites generated by the gut microbiome. The hepatic enzyme flavin monooxygenase 3 (FMO3) is expressed more than 1,000 times higher in females compared with males (114). FMO3 catalyzes the last step in the gut-flora-dependent metabolism of dietary phosphatidylcholine to trimethylamine *N*-oxide (TMAO), which is a significant contributor to atherosclerosis (65, 107, 114). Aortic lesion size was positively correlated with *Fmo3* expression levels in *Apoe*$^{-/-}$ mice (114), and increased levels of TMAO were associated with thrombosis risk and with a fourfold increased mortality risk (101, 125). As more attention is focused on the impact of TMAO on cardiovascular disease risk, investigators should keep in mind the innate sex difference in TMAO levels and *Fmo3* expression (105). Taken together, these studies indicate that complex relationships exist among sex, gut microbiota, and metabolic health and underscore the importance of further mechanistic studies.

FUTURE PERSPECTIVES

Increased Emphasis on Studying Sex Differences

Although one of the most profound biological determinants, it is unfortunate that sex has not always received attention in studies of normo- and pathophysiology. Both preclinical and clinical studies have traditionally focused on a single sex, and more often than not, this has been male (for examples, see References 78, 83, 112). For studies in experimental animals, a frequent justification was that measurements made in male animals are less variable owing to the hormonal fluctuations that occur during the female reproductive cycle. These concerns about variation in female rodent models have been refuted by analysis of published data involving behavior, electrophysiology, neurochemistry, histology, and gene expression (10, 55). In recent years, the need for inclusion of both sexes in preclinical studies has been recognized and publicized (26, 32, 79, 100). We hope these discussions will lead to greater transparency in reporting the sexes used in preclinical studies and to increased inclusion of both sexes in both preclinical and clinical studies.

Tools to Investigate Sex Differences

To enhance our understanding of sex differences, it is imperative to continue to develop experimental tools and strategies. As described here, tools such as mouse models that disentangle the genetic basis of sex determination from the gonadal type are valuable to begin to interrogate the mechanisms that underlie observed sex differences. Additionally, manipulation of genes that respond to estrogens and androgens has provided insights into the many facets of gonadal hormone action in metabolism. In the future, greater exploitation of this technology to dissect tissue- and

temporal-specific effects of estrogen and androgen signaling would be valuable. A resource to explore sex-specific gene expression in humans is now available in the form of the Genotype-Tissue Expression (GTEx) data set (46). GTEx was developed primarily to identify loci that regulate gene expression in specific tissues. This has wide applicability in the interpretation of variants that are identified in GWAS and for understanding disease mechanisms. GTEx includes RNA-seq data from approximately 50 human tissues, with hundreds of individual samples for many of the tissues. Fortunately, the architects of the GTEx project strived to include tissues from both men and women, such that these data will be useful for comparing gene expression levels and signals for expression quantitative trait loci in men and women. These data have not yet been tapped extensively to analyze sex differences but will undoubtedly be a valuable resource in the future. Along the same lines, we hope more investigators will include the X chromosome in GWAS analyses, as tools are available for these analyses and recommendations have been made to promote their use (116).

SUMMARY

Despite decades of recognizing sex differences in obesity and cardiovascular disease, emphasis has seldom been placed on rigorously investigating the regulation, interaction, and effects of sex

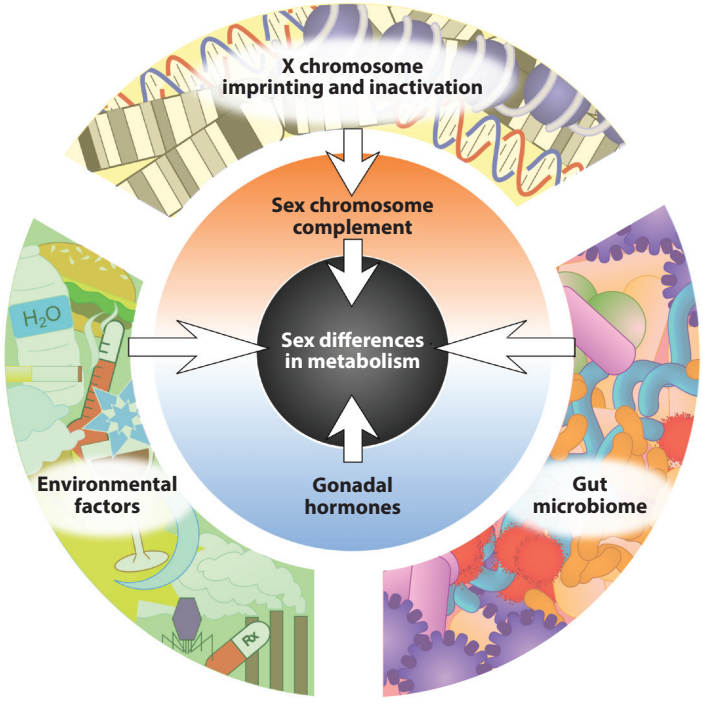

Figure 6

Sex differences in metabolism are influenced by many factors. Two primary sex-biasing sources are the sex chromosome complement and gonadal hormones, which in turn are influenced by other factors. White arrows represent mediators of regulation, including gene expression changes and altered protein signaling pathways. In addition to the number of X chromosomes and the presence of a Y chromosome, XX and XY cells differ by the occurrence of X chromosome inactivation (exclusively in XX cells) and by the parent-of-origin X chromosome imprinting (only XX cells have X chromosome imprints from both parents). Gut microbiome and environmental factors such as diet, pollutants, and circadian cycle may interact with the sex chromosome complement and gonadal hormones to affect metabolism differently between males and females.

differences on cellular mechanisms and whole-body physiology. In this review, we highlight several key studies that investigate how sex affects obesity and its comorbidities, including insulin resistance, dyslipidemia, and fatty liver. For many years, sex differences were attributed to gonadal secretions. We and others have begun to reveal sex chromosome complement as another determinant of sex differences in health and disease (**Figure 5**). However, gonadal hormones and sex chromosome complement are not mutually exclusive and do not act in isolation. Other factors, including X chromosome imprinting and inactivation, environmental stimulants, and the gut microbiome may interact with gonadal hormones and the sex chromosome complement to exert changes in gene expression and signaling pathways (**Figure 6**). Diet and circadian rhythm are two of the many external cues that influence metabolic homeostasis, and both interact with the gut microbiome. Although X chromosome imprinting and inactivation and other environmental factors are beyond the scope of this review, they may have important roles in determining sex differences in metabolism. There is undoubtedly a complex network that involves many components of sex differences and metabolism, which will be an exciting area of future research.

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