Sex differences in obesity and the regulation of energy homeostasis

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Summary

Obesity prevalence is generally higher in women than in men, and there is also a sex difference in body fat distribution. Sex differences in obesity can be explained in part by the influence of gonadal steroids on body composition and appetite; however, behavioural, socio-cultural and chromosomal factors may also play a role. This review, which evolved from the 2008 Stock Conference on sex differences in obesity, summarizes current research and recommendations related to hormonal and neuroendocrine influences on energy balance and fat distribution. A number of important gaps in the research are identified, including a need for more studies on chromosomal sex effects on energy balance, the role of socio-cultural (i.e. gender) factors in obesity and the potential deleterious effects of high-fat diets during pregnancy on the foetus. Furthermore, there is a paucity of clinical trials examining sex-specific approaches and outcomes of obesity treatment (lifestyle-based or pharmacological), and research is urgently needed to determine whether current weight loss programmes, largely developed and tested on women, are appropriate for men. Last, it is important that both animal and clinical research on obesity be designed and analysed in such a way that data can be separately examined in both men and women.

Introduction

The prevalence of obesity is higher in women than in men in most countries around the world (http://www. IOTF.org; accessed July, 2008). Although it has been suggested that evolutionary pressures predispose women to store excess fat for reproduction and lactation, the factors driving the greater propensity for excess body weight in women are not well understood. One line of research has focused on gonadal hormones, their influence on peripheral and central mechanisms that control appetite and body weight. Another line of research has focused on behavioural and social differences between men and women that relate to eating or activity behaviours. Pregnancy and menopause also have physiological and behavioural consequences on appetite and weight regulation that confer elevated obesity risk in many women.

Despite the generally lower population prevalence of obesity in men, obese men are at substantial risk of obesity-related chronic diseases because of fat accumulation in abdominal, visceral depots. It has long been recognized that men and premenopausal women differ in their fat distribution, the so-called 'gynoid' and 'android' fat distribution. Because of the significantly increased cardiometabolic risk associated with abdominal fat in men (and postmenopausal women), it is important to understand mechanisms that determine where fat is accumulated. Recent research using molecular approaches and animal models has provided greater understanding of the role of sex hormones and other molecules on fat partitioning.

Despite burgeoning research in this field, a number of important questions remain unanswered. What is the role of chromosomal sex and *in utero* effects on obesity? What specific effects do male and female sex steroids have on central and peripheral regulation of appetite? How do men and women differ in the hypothalamic centres that control appetite, body weight and body composition? How can we effectively translate laboratory findings on sex differences and effects of sex hormones on energy metabolism to clinical practice to best assist both men and women in maintaining healthy body weights?

In March 2008, the International Association for the Study of Obesity (IASO) convened a

3-d Stock Conference in Bangkok, Thailand, to address these important questions. The conference was co-chaired by the authors and sponsored by Weight Watchers International. Twenty-five speakers and invited participants attended (Table 1), each bringing unique clinical or basic research expertise to the question of sex and obesity. The 2008 Stock Conference was the seventh in a series of annual conferences initiated by IASO to commemorate the lifetime obesity research contributions of Dr Mike Stock, whose life was cut short by cancer. This article is a summary of the work conducted by the 25 individuals at this conference and the conclusions and recommendations they reached.

Table 1 Participants in the 2008 Stock Conference on sex differences in energy homeostasis and fat metabolism

Jennifer Lovejoy	Chair	Free and Clear Inc. and University of Washington, Seattle, WA, USA
Amanda Sainsbury-Salis	Chair	Garvan Institute of Medical Research, Sydney, NSW, Australia
Kristy Brown	Speaker	Prince Hentry's Institute of Medical Research Monash Medical Centre, Clayton, VIC, Australia
Lesley Campbell	Speaker	Garvan Institute of Medical Research, Sydney, NSW, Australia
Loredana Asarian (for Deborah Clegg)	Speaker	Swiss Federal Institute of Technology Zurich (ETHZ), Zurich, Switzerland
Susan Fried	Speaker	University of Maryland Department of Medicine, Baltimore, MD, USA
Nori Geary	Speaker	Swiss Federal Institute of Technology Zurich (ETHZ), Zurich, Switzerland
Daniel Marks	Speaker	Oregon Health and Science University, Portland, OR, USA
Renato Pasquali	Speaker	University Alma Mater Studiorum, Bologna, Italy
François P Pralong	Speaker	University of Lausanne, Lausanne, Switzerland
Margriet Westerterp-Plantenga	Speaker	Maastricht University, Maastricht, the Netherlands
Rekia Belahsen	Participant	Chouaib Doukkali University, Jadida, Morocco
Berit Christofersen	Participant	Novo Nordisk, Maaloev, Denmark
Rachel Colley	Participant	Children's Hospital of Eastern Ontario – Research Institute, Ottawa, ON, Canada
Gal Dubnov-Raz	Participant	Hebrew University – Hadassah Medical School, Jerusalem, Israel
Christopher Gasteyger	Participant	University of Copenhagen, Frederiksberg, Denmark
Berit Heitmann	Participant	Institute of Preventative Medicine, Centre for Health and Society, Copenhagen, Denmark
Sherry Marts	Participant	Society for Women's Health Research, Washington, DC, USA
Magdalena Pasarica	Participant	Pennington Biomedical Research Center, Baton Rouge, LA, USA
Emma Stevenson	Participant	Northumbria University, Newcastle Upon Tyne, UK
Magdalena Szopa	Participant	Jagiellonian University, Krakow, Poland
Klaus Westerterp	Participant	Maastricht University, Maastricht, The Netherlands
Erwin Van Der Veken	Participant	Jolimont-Lobbes Hospital, Haine-Sait-Paul, Belgium
Karen Miller-Kovach	Participant	Weight Watchers International Inc, New York, NY, USA
David York	Conference Convenor	Utah State University, Logan, UT, USA

It should be noted that the term 'sex' refers to differences between men and women that result from the chromosomal complement and the effects of hormones, whereas the term 'gender' refers to an individual's identity as a man or a woman and the cultural and behavioural expectations associated with being a man or a woman. With regard to obesity and the regulation of energy homeostasis, little is known about gender differences, thus this review focuses primarily on sex differences.

Sex differences in energy metabolism

It is widely recognized that at any given body mass index (BMI) women tend to be shorter, weigh less and have less fat-free mass and more fat mass than men. Because body size and fat-free mass are such strong determinants of energy expenditure (EE), it is therefore not surprising that in absolute terms women have lower EE than men **(1,2)**.

It is less clear whether there are sex differences in basal metabolic rate or total daily EE independent of body composition. A number of studies have failed to find any sex difference in EE after adjustment for body composition differences **(1,3,4)**. However, several studies have found that even after adjusting for differences in fat-free mass women have lower daily EE than men **(5,6)**. Furthermore, the decline in resting EE with age has been found to be greater in women (−80.3 kJ d−1 year−1) than in men (−46.9 kJ d−1 year−1) **(7)**, suggesting that women may be at greater risk for obesity with aging. As sex differences in EE have also been found in pre-pubertal children matched for body composition **(8,9)**, the difference may be due to sex chromosome gene effects or *in utero* organizational effects of gonadal hormones.

More consistent sex differences have been observed in response to physical activity EE. In men, higher levels of physical activity are associated with reduced percent body fat, but this relationship is not observed in women **(10,11)**. Moreover, physical training programmes result in smaller reductions in body weight and fat loss in women than in men **(12)**. The relative lack of impact of exercise on body weight or body fat in women may be due to sex differences in

metabolic response to exercise training as well as dietary compensation, as energy intake increases in women but not men after exercise **(12)**. Meijer *et al.***(13)** compared men and women training for a half-marathon event for 5 months and found that men increased their total daily EE by the end of training because of increases in both exercise and non-exercise (habitual) activities. Women, on the other hand, did not significantly increase their total EE after endurance training since, unlike the men, they did not experience increases in non-exercise-associated or dietary thermogenesis.

Sex differences in body fat distribution

It is well recognized that there are sex differences in body fat distribution, with men and oestrogen-deficient postmenopausal women tending to accumulate more abdominal and visceral fat and premenopausal women more lower body (gluteo-femoral) fat. Visceral fat has been associated with increased cardiometabolic risk in numerous studies (recently reviewed by Despres *et al.*, **Ref. 14**), thus men and postmenopausal women typically have increased cardiometabolic risk relative to premenopausal women.

What is less well-recognized is mounting evidence suggesting that larger gluteo-femoral fat stores are protective. Several epidemiological studies suggest that a high waist-to-hip ratio is a better predictor of all-cause and cardiovascular disease (CVD) mortality than a large waist circumference alone **(15,16)**. Moreover, a larger hip circumference has been shown to be protective against both CVD and metabolic risk in multiple ethnic groups, independent of waist circumference or abdominal fat **(17,18)**. Intriguingly, transplantation of subcutaneous fat from the inguinal region of male donor mice into the intra-abdominal compartment of male recipient mice on a high-fat diet resulted in significantly protective effects on adiposity, insulin sensitivity and glucose tolerance **(19)**. It would be interesting to see if similar or even greater protective effects could be conferred from transplanting female subcutaneous (inguinal) fat, because in humans protective effect of a large hip circumference with regard to CVD morbidity and mortality was significant only in women, with only a borderline significant effect on total mortality in men **(20)**.

Increases in thigh fat mass in women are related to increased fat cell hyperplasia rather than increased fat cell size **(21)**. Nonetheless, the sex difference in abdominal vs. gluteo-femoral fat cell size persists even in extreme obesity. Because of the differential effects on health risk of upper and lower body fat depots, it is important to understand how fat distribution is differentially regulated in in men and women.

The question of sex differences in fat distribution can be examined in animal models. Several animal species, including pigs and some rodents, show sex-specific differences in fat distribution, with males having more intra-abdominal fat and less subcutaneous fat than females. As in humans, oestrogen appears to be key in maintaining the sex-specific fat distribution pattern. In rats, ovariectomy leads to an increase in visceral fat and a loss of subcutaneous fat in females and oestrogen treatment reverses this effect **(22,23)**. Exogenous oestradiol administration to male rats also decreases visceral and increases subcutaneous fat relative to males not given oestrogen **(23)**. In addition, research has shown that both male and female aromatase-knockout (ArKO) mice, which are oestrogen-deficient and have elevated testosterone, accumulate more intra-abdominal adipose tissue with increased adipocyte size in the gonadal and infrarenal depots **(24)**. Male ArKO mice also develop fatty liver, as do aromatase-deficient men, and this is reversible with oestradiol treatment **(25)**.

Considerable research has assessed the role of sex hormones on adipose tissue lipolysis and fat uptake. Sex differences in alpha-2 adrenergic control of lipolysis have been demonstrated **(26)**. In addition, rodent studies show that oestrogen treatment in mice increases lipolysis in abdominal fat cells **(27)**. Lactation and menopausal status also affect the lipolytic responsiveness of cells. Over 20 years ago, Rebuffe-Scrive *et al.* conducted a series of elegant studies examining the impact of menopause and lactation on regional fat cell lipolysis and Lipoprotein lipase (LPL) activity. These investigators found that higher adipose tissue LPL activity (promoting fat storage) occurs in femoral adipocytes compared with abdominal adipocytes in premenopausal women but not in postmenopausal, oestrogen-deficient women and that lipolysis increases specifically in femoral fat during lactation **(28)**. Furthermore, treatment with oestrogen in postmenopausal women restores the lipoprotein lipase activity of the femoral adipocytes and attenuates lipolytic response in subcutaneous adipocytes but not in abdominal adipocytes **(29)**. More recently, Johnson *et al.* observed that gluteal adipocytes from premenopausal women are more sensitive

to the anti-lipolytic effects of insulin than abdominal adipocytes **(30)**, but this difference between fat depots disappears in postmenopausal women (SK Fried, personal communication). These differences between premenopausal and postmenopausal women suggest a role of oestrogen in controlling regional fat distribution in the periphery. Indeed, both direct genomic and non-genomic effects of oestrogen on adipose tissue metabolism have been observed **(27)**.

Oestrogen may also influence fat distribution centrally by modulating leptin responsiveness, as leptin not only influences total body fat in rodents but also favours loss of visceral fat via Stat3 signalling in the hypothalamus **(31)**. Female mice are more responsive than male mice to the effects of centrally administered leptin to decrease food intake and body weight and to increase c-fos and Stat3 expression in the arcuate nucleus (DJ Clegg, personal communication). The sex difference in central leptin responsiveness appears to be due to oestrogen as ovariectomy reduces central leptin responsiveness, oestradiol treatment reverses this leptin resistance, and responsiveness to central leptin in male mice is enhanced by peripheral oestradiol administration **(23)**. These effects of oestrogen appear centrally mediated as centrally administered oestradiol (E2) does not increase plasma E2 concentrations yet restores body fat distribution and leptin sensitivity in ovariectomized female rats **(23)**. Oestrogen could conceivably influence leptin responsiveness in several ways, including increasing leptin transport across the blood–brain barrier or increasing leptin receptor expression in the arcuate nucleus **(32)**.

Of the two types of nuclear oestrogen receptors (ERα and ERβ), ERα appears to be the one involved in regulation of fat distribution. Bilateral knock-down of ERα in the ventromedial nucleus of the hypothalamus in mice with siRNA results in increased body weight, decreased EE, increased visceral adiposity and decreased leptin sensitivity **(33)**. In male ER knockout mice, the obesity-promoting effect of oestrogen deficiency appears to be mediated specifically through ERα, as ERα knockouts exhibit increased fat mass while ERβ knockout mice do not **(34)**. Clinically, ERα gene polymorphisms predict abdominal obesity in women, but not in men, suggesting a possible sexual dimorphism in the ERα effects **(35)**.

Androgens also impact body fat distribution. In men, the observed gradual decline in circulating androgens with aging (the so-called 'andropause') is accompanied by increased total and abdominal fat **(36)**. Several studies have shown that administration of aromatizable androgens such as testosterone reduces both total and abdominal fat in older men **(37,38)**, although administration of non-aromatizable dihydrotestostone does not **(37)**. In contrast, androgen administration to ovariectomized female mice results in a significant increase in body weight and visceral adipose tissue **(39)**. These changes were associated with decreased phosphorylation of Adenosine Monophosphate (AMP)-activated protein kinase and acetyl-CoA carboxylase in visceral fat, suggesting decreased fatty acid oxidation **(39)**. As in female mice, in women both higher circulating androgen levels **(40)** and exogenous androgen administration **(41,42)** increase visceral, abdominal fat. These findings raise significant clinical concern about the use of testosterone as a hormone replacement therapy in postmenopausal women.

In summary, sex differences in body fat distribution appear to be largely a result of differences in sex hormones between men and women. Oestrogens reduce visceral fat in both men and women, an effect that is likely mediated by both central and peripheral mechanisms. In contrast, opposite effects of androgens on fat distribution in men and women are seen, with aromatizable androgens decreasing visceral fat in men but increasing it in women.

Sex hormones and obesity in women

Menopause and oestrogen deficiency: effects on central control of energy balance

Oestrogen deficiency in female animals is clearly associated with hyperphagia and increased body weight and adiposity, especially visceral adiposity **(22,23,43)**. Similarly, in women some studies (although not all) report that menopause is associated with weight gain independent of age **(44)**, and the majority of studies observe increased abdominal or visceral adiposity at menopause **(45–47)**. In both animals and humans, exogenous oestrogens reduce body weight and prevent abdominal fat gain **(23,48)**.

Studies in ovariectomized rodents have elucidated possible mechanisms by which changes in oestrogen levels may impact body weight. In rodents, ovariectomy induces an increase in food intake, and concomitantly increases body weight **(22,49)**. These effects of oestrogen deficiency may be mediated by increases in hypothalamic expression of the orexigenic peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP) **(49,50)**, both of which

increase food intake and inhibit physical activity in rodents as previously reviewed **(51)**. Effects of oestrogen deficiency may also be mediated by decreased expression of proopiomelancortin (POMC), the precursor of alpha melanocyte stimulating hormone and corticotropin releasing hormone **(50)**, both of which are anorexigenic **(51)**.

The effects of oestrogen deficiency and oestradiol treatment on daily food intake in rats are due to effects on meal size rather than meal frequency **(52,53)**. Of potential satiety mechanisms that may mediate E2's effects on meal size, cholecystokinin (CCK) shows particular promise. CCK is released from the proximal gut during meals and acts locally on CCK-1 receptors to bring about satiety via activation of vagal afferent fibres to the nucleus tractus solitarius (NTS) in the brainstem. The NTS is a major processing area for information coming from the gut, and it also has bidirectional inputs from the hypothalamus. The decrease in meal size that occurs in oestrus is blocked by antagonism of CCK-1 receptors in rodents **(54)**, suggesting that the surge in oestrogen secretion that immediately precedes oestrus, when ovulation occurs, influences CCK-1 receptor-mediated neural processing to bring about heightened meal-induced satiety. Moreover, E2 administration in rodents increases the satiating effect of intraduodenal infusion of the CCK secretagogue intralipid **(55)**. It is likely that these effects of E2 to enhance satiety via CCK-1 receptor mediated mechanisms are due to direct action of E2 on ERα in the NTS as direct stereotaxic administration of E2 to the NTS is sufficient to reduce food intake **(56)**, and ERα- but not ERβ-knockout mice gain more weight after ovariectomy than wild-type mice **(57,58)**.

In addition to satiating effects via CCK, E2 also decreases the hyperphagic effect of ghrelin in rats **(49)**. Ghrelin is a stomach-derived orexigenic hormone that peaks prior to meals and may be a trigger for the onset of eating. In rats, intracerebroventricular or intraperitoneal injection of ghrelin stimulates eating in females with low-oestrogen levels (i.e. a 'postmenopausal' model) but not in females during oestrus **(49)**. It is likely that oestrogens tonically inhibit ghrelin signalling and release from this inhibition contributes to the early hyperphagia and lasting weight gain seen after ovariectomy. Indeed, ghrelin receptor knockout mice failed to show any increase in food intake or body weight in response to ovariectomy **(49)**.

Menopause or oestrogen deficiency may also contribute to weight gain via mechanisms related to EE. ERα knockout mice show perturbations in physical activity **(59)**. Additionally, on the night of oestrous, female rats and mice show a dramatic increase in physical activity, which is abolished by removal of oestrogens (e.g. ovariectomy) **(59)**. In humans, Lovejoy *et al.***(47)** recently showed that the onset of menopause is associated with a significant reduction in 24-h EE and physical activity EE, although dietary intake did not change. The relatively sudden drop in physical activity at menopause onset may be related to effects of lack of oestrogen on the hypothalamus, which is an important regulator of physical activity particularly via expression of NPY and AgRP, which are increased in rodents after ovariectomy **(49–51)**.

As oestrogens and androgens promote accrual of lean mass in addition to affecting fat mass, the sudden drop in oestrogens that occur with menopause and the gradual decline in androgens that occur in older men are associated with a decline in lean body mass, including bone mass. This is likely due to a combination of direct effects of sex hormones on lean tissues and bone, as well as indirect effects via the hypothalamus. For instance, the sudden drop in oestrogens after ovariectomy in rodents increases hypothalamic expression of NPY **(49,50)**, which reduces lean mass via inhibition of the growth hormone axis and circulating IGF-1 levels **(60)**. In contrast, orchidectomy in male rodents decreases NPY expression in the arcuate nucleus of the hypothalamus and reduces adiposity **(61)**. These effects can be abolished by testosterone replacement **(61)**. In addition to the negative effects of reductions in oestrogens or androgens on muscle mass, circulating levels of IGF-1 decrease with age, and this further contributes to the gradual loss of lean body mass in ageing women and men **(62)**. These reductions in lean body mass may not only promote weight gain by reducing 24-h EE, as lean mass is a major determinant of EE as discussed above but may also promote the development of insulin resistance through the loss of insulin sensitive muscle mass.

Effects of the menstrual cycle and oral contraceptives on energy homeostasis

Many women experience a heightened desire to eat around the time of menses (when oestrogen levels are low). When actual food intake and the cycle are accurately recorded, variations in spontaneous food intake over the course of the menstrual cycle are reliably detected. In a review of 21 data sets, Buffenstein *et al.***(63)** found that food intake was 1.00 ± 0.16 MJ d−1 less during the follicular phase of the cycle that the luteal phase, with the nadir consistently occurring around the time of ovulation when circulating oestrogen levels peak. Similar results have been

shown in rodents, which show a significant drop in food intake at the time of oestrus **(22,43)**. These changes in food intake over the course of the menstrual or oestrus cycle are likely due to cyclical variations in circulating oestrogen levels, as these cyclical changes in spontaneous food intake can be mimicked in ovariectomized rats with the use of cyclical oestradiol (E2) but not progesterone replacement **(53)**.

It is important to note that although the menstrual cycle is associated with measurable changes in food intake and some (but not all) studies have also shown variations in EE in individual women over the course of the menstrual cycle **(64)**, most women remain in energy balance from 1 month to the next. Therefore, whereas the permanent reduction in oestrogen levels in menopause or total hysterectomy incurs heightened risk of excessive weight gain and obesity in women, the fluctuations in sex hormone levels over the course of the normal menstrual cycle *per se* are not a risk factor for weight gain.

It is commonly perceived that oral contraceptives contribute to weight gain; however, review of the literature shows limited evidence of this **(65)**. Although one study reported that oral progestins actually increase EE and decrease body weight and fat relative to placebo in perimenopausal women **(66)**, most studies show a neutral effect of progestin-containing oral contraceptives on weight. On the other hand, several studies show that injected progestin contraceptives result in significant weight gain, particularly among adolescent girls **(67,68)**.

Polycystic ovarian syndrome – insights into androgens and obesity in women

A clinical condition with important connections to obesity is polycystic ovarian syndrome (PCOS), which affects 6–7% of women. A recent consensus conference identified oligo- or amenorrhea and chronic anovulation, hyperandrogenism and polycystic morphology of the ovaries as defining criteria, resulting in the recognition of a broad clinical spectrum of the disease **(69)**.

There is a strong connection between obesity and PCOS: 40–60% of women with PCOS are obese, and PCOS has a higher prevalence in women with higher BMI **(70)**. While the direction of this relationship is not clear (i.e. does having PCOS increase the propensity for being obese, does obesity increase the risk of PCOS, or both), several lines of evidence point to a possible causal role of obesity in the development of PCOS. First, a significant portion of women with PCOS had high birth weights and were born to obese mothers **(71)**, suggesting a possible effect of inter-generational effect of maternal obesity. Second, excess weight gain in childhood has been associated with menstrual disorders and hyperandrogenism **(72)**, potentially increasing risk for PCOS. Abdominal obesity in particular may be a driving factor for functional hyperandrogenism (reviewed in **Ref. 73**).

In addition to playing a possible causal role in PCOS onset, once PCOS is diagnosed the presence of obesity is clearly detrimental to the progression and prognosis of the disease. Obese women with PCOS have a greater degree of insulin resistance and glucose intolerance than lean women with PCOS and are at greater risk of developing diabetes **(74)**. Even in lean women with PCOS, there tends to be a more central fat distribution and increased metabolic risk, presumably because of increased androgen levels.

While obesity has an adverse effect on PCOS pathophysiology and prognosis, weight loss through lifestyle intervention significantly improves hyperandrogenism and insulin resistance in women with PCOS and also improves fertility **(75,76)**. Bariatric surgery has also been shown to completely normalize PCOS symptoms in many women **(77)**. Given the known role of physical activity in increasing EE, favouring fat loss and improving insulin action, regular exercise is an important component in the management of PCOS. Exercise training has been shown to decrease insulin resistance and lead to the onset of menstruation in women with PCOS **(78)**, and in some countries (e.g. Switzerland) assisted exercise programmes for women with PCOS are covered by health insurance policies.

It is possible that decreased insulin sensitivity and hyperinsulinemia in PCOS not only contribute to the well-characterized dys-regulation of the hypothalamo-pituitary gonadal axis **(79)** but also exacerbate weight gain. Neuron-specific insulin receptor knockout mice – particularly females – have increased body weight and adiposity, and both male and female knockouts have low-circulating levels of luteinizing hormone (LH) and impaired reproductive capacity **(80)**. Moreover, insulin administration in mice leads to a 30% increase in circulating LH, possibly because of direct effects on gonadotropin releasing hormone (GnRH) secretion **(81)**. In PCOS, these effects of insulin on the gonadotropic axis appear to be blunted. For example, in normal women, insulin administration increases the number of LH pulses, but this effect is not

seen in women with PCOS **(82)**. As peripheral LH pulses are a surrogate marker of hypothalamic GnRH neuronal activity, these data suggest that peripheral insulin may modulate activity of GnRH neurons and that this regulation is blunted in women with PCOS. Consistent with this idea, treatment with the insulin-sensitizer metformin has been shown to normalize LH secretion profiles in PCOS **(83)**, and prolonged insulin infusion suppresses pituitary response to GnRH in women with PCOS but not normal controls **(84)**.

In summary, while the contribution of peripheral hyperinsulinemia to the abnormalities in LH secretion in PCOS is not entirely clear, it remains possible that long-lasting elevations in insulin levels accompanying reduced insulin sensitivity may account for the altered LH pulse frequency and reproductive abnormalities observed in PCOS.

Pregnancy and in utero effects

The prevalence of maternal obesity has increased along with the obesity epidemic worldwide. In the USA, ~50% of women of childbearing age are overweight or obese **(85)**. Furthermore, the majority of women in developed countries gain more weight than are recommended during pregnancy. Excess weight gain in pregnancy is of concern in part because it is associated with significantly increased long-term obesity risk for the mother **(86)**. However, equally as concerning is the fact that maternal obesity dramatically increases complications of pregnancy, labour and delivery and is associated with adverse fetal and neonatal outcomes (reviewed in **Ref. 87**). In addition, maternal obesity and excess weight gain during pregnancy increase the risk of later obesity and metabolic complications in the offspring. Surkan *et al.***(88)** reported that the prevalence of large-for-gestational-age (LGA) infants increased by 23% between 1991 and 2001, largely attributable to the increase in maternal BMI over time. LGA infants typically have a much greater increase in fat mass than in lean mass, and this increase in neonatal fat mass is exaggerated in pregnancies complicated by gestational diabetes **(89)**. LGA infants are also at increased risk for developing diabetes as adolescents and young adults **(90)**.

Studies have begun to examine the mechanisms by which maternal obesity produces adverse metabolic outcomes in the offspring. A major factor may be increased systemic inflammation associated with obesity as well as consumption of high-fat diets, which are pro-inflammatory. Both normal pregnancy and obesity are conditions associated with increased production of inflammatory cytokines **(91,92)**. When female rhesus macaques are exposed to a high-fat diet during pregnancy, greater inflammatory response occurs relative to control animals fed a low-fat diet. Moreover, foetuses of the high-fat diet-fed mothers exhibited increased liver fat and markers of oxidative stress (D Marks and K Grove, personal communication). These changes were observed even in those mothers who didn't become obese on the high-fat diet, indicating that the effect is diet-specific rather than obesity-specific. Offspring of female monkeys exposed to a high-fat diet in pregnancy also show changes in neurons in the arcuate nucleus that synthesize POMC and AgRP, suggesting that dietary composition may programme the fetal brain to develop an obese state (BE Grayson, SM Williams, MS Smith and KL Grove, manuscript in preparation). Although it is not known whether these findings in non-human primates are directly applicable to humans, the human ancestral dietary pattern was typically much lower in fat (particularly saturated fat) than modern diets, and it is plausible that humans have limited physiological ability to adapt to high-fat diets without adverse long-term health consequences.

Hypothalamic regulators of energy homeostasis and the reproductive axis

In situations of negative energy balance, when energy requirements exceed energy intake, organisms respond with activation of pathways that increase energy intake (e.g. increased appetite) and inhibition of pathways that consume energy (e.g. non-obligate physical activity, basal metabolic rate and reproductive functions) **(93,94)**. Leptin, which inhibits appetite and promotes fat loss via central and peripheral effects, is a key component in the coordinated regulation of energy homeostasis and reproduction **(60)**. Prior to puberty or in sexually mature adults in negative energy balance, reproductive adequacy is inhibited but can be enhanced by administration of exogenous leptin. Moreover, leptin-deficient *ob*/*ob* mice not only show hyperphagia, decreased EE and massive obesity but also are hypogonadal with dramatically impaired fertility. Situations of negative energy balance or low leptin action are associated with increased hypothalamic expression of NPY, in keeping with the inhibitory action of leptin on hypothalamic NPY expression.

This increase in NPY expression during negative energy balance contributes significantly

to coordinate regulation of energy homeostasis and reproduction, because *ob*/*ob* mice that lack NPY are fertile, eat less and have significantly less body fat than *ob*/*ob* controls **(95)**. The dual effect of NPY to promote fat gain and inhibit fertility is likely mediated by Y1 receptors, at least in the male, because *ob*/*ob* mice deficient in Y1 receptors, like *ob*/*ob*NPY−/− mice, also show significant reductions in body weight and adiposity as well as improvements in function of the gonadotropic axis **(96)**. Double mutant *ob*/*ob*Y4−/− mice also show enhanced fertility relative to *ob*/*ob* animals, in the absence on any effects on adiposity **(97)**. Moreover, whereas wild-type mice subjected to food restriction show delayed or absent sexual maturation unless exogenous leptin is administered, juvenile Y1−/− mice can still proceed through sexual maturation **(98)**. These data demonstrate the importance of Y1 as well as Y4 receptors in sensing decreased energy stores (or decreased or absent leptin action) by the neuroendocrine reproductive axis, as well as an important role of Y1 receptors in mediating restoration of energy homeostasis.

The effect of Y1 receptor deficiency to inhibit adiposity under situations, when leptin levels are low, may be mediated via hypothalamic and/or extrahypothalamic pathways. In support of non-hypothalamic pathways, hypothalamus-specific deletion of Y1 receptors in adult mice via hypothalamic injection of a cre-recombinase-expressing adeno-associated viral vector did not affect body weight, adiposity or food intake in males or females **(99)**. In contrast, lack of Y1 receptors on adipocytes could conceivably reduce adipocyte proliferation and may also stimulate lipolysis, because NPY is produced in preadipocytes and not only promotes proliferation of adipocyte precursors via the Y1 receptor **(100)** but is also strongly anti-lipolytic **(101)**. While ablation of Y1 receptors in white adipose tissue may promote weight loss, any novel weight loss agents based on Y1 receptor antagonism would need to circumvent the hyperinsulinemic effects of Y1 receptor ablation, hyperinsulinemia being a condition that promotes increased adiposity. Germline Y1 receptor knockout mice on a lean background are obese and hyperinsulinemic, and these effects are particularly pronounced in female Y1−/− mice **(99)**. Other research suggests that the hyperinsulinemia in Y1−/− mice possibly mediated by release of NPY's inhibition of insulin secretion via Y1 receptors on pancreatic β cells **(102)** contributes to the obesity in this model. Therefore, novel anti-obesity drugs based on Y1 receptor antagonism would ideally circumvent Y1 receptors in the pancreas.

Weight loss treatment – do we need a sex-specific approach?

Maintaining a healthy body weight for height is widely recognized as critical for reducing the risk of chronic diseases in both men and women. Weight loss in both sexes is related to successful dietary restraint and for long-term weight maintenance, sparing of fat-free mass.

In general, there do not appear to be major sex differences in the weight loss response to dietary restriction. Although it is not uncommon clinically to observe greater weight losses in men than in women, this is typically because of greater initial body weights or a greater degree of caloric restriction, rather than an inherent sex difference. Nonetheless, some studies do suggest that the female lose less weight with a comparable degree of energy restriction than the male even after matching for initial body weight **(103)**. Women have also been shown to lose less weight than men after bariatric surgery **(104)**. Furthermore, studies consistently find that men lose more abdominal, visceral fat during weight loss than do women **(105,106)**, an effect also seen in mice subjected to caloric restriction or lipectomy **(107)**. Postmenopausal women lose less visceral fat during weight reduction than premenopausal women **(108)**, a finding likely related to the important role of oestrogen in regulating abdominal fat stores in women as discussed previously.

As discussed above, physical exercise as an approach to weight loss may be less effective in women than in men **(12,13)**. The sex difference in the response to exercise was recently confirmed by data from the initial 6-month weight loss period of the Weight Loss Maintenance trial **(109)**. In this study, both African–American and Caucasian men lost more weight than race-matched women, and this difference was largely because of the fact that any given increase in physical activity had a significantly greater impact on weight loss in men relative to women. Regardless of sex differences in weight loss with exercise, it does not negate the significant benefits of physical activity to increase lean mass and reduce risk of cardiovascular and metabolic disease in both men and women.

With regard to long-term regulation of body weight and energy balance, some studies have suggested that meal frequency may play an important role. For example, population studies have suggested that consuming more frequent, smaller meal is associated with lower body

weight **(110)**. It appears, however, that the effect of meal frequency on regulation of energy balance differs between men and women. Carefully controlled studies have found that, while the relationship between increased meal frequency and reduced appetite/body weight is strong in men, it is absent in women **(111,112)**. Westerterp-Plantenga **(112)** observed that this sex difference in the effect of meal frequency could be explained by sex differences in fat-free mass, such that meal frequency is a function of EE only in those individuals with high fat-free mass (i.e. men). These data imply that adopting a strategy of consuming more frequent small meals throughout the day may be effective for weight regulation in men, but could be less effective in women. Further research is needed, however, in order to determine whether this finding from epidemiological observations can be translated into clinical interventions. Indeed, a recent weight loss intervention has shown no difference in weight loss, reduction in waist circumference, fat or lean mass when the same number of kilojoules are consumed as frequent meals or less frequent meals **(113)**.

Sex differences have also been reported in the reduction of health risk factors with weight loss. In the Diabetes Prevention Program, a large randomized trial of lifestyle vs. metformin in adults at high risk for developing diabetes, weight loss of >3% of body weight produced a greater reduction in serum glucose, insulin and lipids in men than in women **(114)**. In the Stanislas Family Study conducted in France, weight gain over 5 years was related to cardiometabolic risk factors in a sex-specific way. While weight gain worsened blood pressure, serum lipids and uric acid in both men and women, apolipoprotein A1 and several liver enzymes were worsened only in men, and serum high-sensitivity C-reactive protein and haptoglobin (inflammatory markers) were worsened only in women **(115)**. Sex differences in markers of inflammation in relation to body weight have been found in other studies as well **(116)**.

Clinical implications of central obesity in women and men

Weight loss is particularly important for both men and women who have increased abdominal adiposity, because of the well-known health risks associated with an upper body fat distribution. However, the negative implications of central obesity may be greater in women than in men. The prospective, population-based MONICA/KORA Augsburg cohort study suggested that increasing central fat is more predictive of diabetes in women than in men, and diabetes increases coronary risk more in women than in men **(117)**. Excess central adiposity is frequently associated with other risk factors for chronic disease, namely hypertension, glucose intolerance and elevated circulating triglyceride levels, a cluster of risk factors initially termed 'Syndome X' by GM Reaven in his 1988 Banting lecture, now more commonly called 'metabolic syndrome'.

Notably, women with risk factors of the metabolic syndrome are at significantly greater risk of subsequent development of diabetes or CVD than men with the same risk factors. For instance, compared with men, women with type 2 diabetes have a twofold greater risk of death because of coronary disease and a two- to threefold greater risk of hypertension. Moreover, reduced circulating high-density lipoprotein cholesterol levels and elevated circulating triglyceride levels are better predictors of CVD in women then in men **(118)**. In men with diabetes, mortality rates dropped in the years 1971 to 2000, whereas this is not the case in women **(119)**. In fact, CVD now kills more women than men in Europe (55% vs. 43%) **(120)**. However, women with risk factors for chronic disease are less likely to be offered treatment than men. Clearly, more clinical research is needed in women as well as in men in order to determine optimum treatment strategies, such as the effects of lipid-lowering drugs on the subsequent risk of CVD.

Taken together, these data suggest that people – particularly women – with central obesity should be given top priority for medically assisted weight loss programmes, especially in light of the potentially protective effect of larger hip circumference. On the other hand, weight loss in women in the upper end of the normal weight range who have a gynoid distribution of body fat might be considered a 'cosmetic' intervention, the benefits or adverse consequences of which require further investigation.

Recommendations and conclusions

It is clear that the questions related to sex differences in obesity, energy homeostasis and central regulation of appetite have important physiological and clinical relevance. While research has grown in this field, there is need for much more work to be done to fully understand the impact

of obesity-related sex differences in health and disease. Based on the findings discussed at the 2008 Stock Conference on Sex and Obesity and the research reviewed here, a number of recommendations emerge:

- Further research is needed to identify the relative roles of chromosomal sex (XX, XY) and sex hormone influences on obesity-related physiology and pathophysiology. Sex differences can be due to direct effects of sex chromosome genes on the brain or other tissues or organizational/activational effects of gonadal hormones. To date, most of the research has focused on activational effects of gonadal hormones, and much less is known about organizational effects of these hormones or direct sex chromosome effects. One way to gain insight into the mechanisms of sex differences in obesity is to study animals or humans prior to the onset of puberty or to experimentally manipulate sex chromosomes in animal models.
- Little is known about gender differences (as opposed to sex differences) in energy homeostasis and appetite. Clearly, men and women experience different social pressures around thinness, and more women than men engage in dieting behaviour. Additionally, some research suggests that body image and emotional factors play a more significant role in the development and maintenance of obesity in women than in men. Understanding these socio-cultural differences related to self-defined gender perception has important clinical ramifications for developing effective obesity treatment and prevention approaches.
- Pregnancy and early life appear to be critical times for determining long-term body weight and metabolic risk. Although some research was presented describing the deleterious effects of high-fat diet in pregnancy on the offspring, considerably more research is needed. Epigenetic studies to determine the effects of diet and exercise during pregnancy on the offspring continue to be an important need. Research on the effects of obesity and/or diabetes during pregnancy on the health of the mother and baby has huge potential public health ramifications and should be a high priority for funding.
- Stress is an important area for future focus in relation to obesity and sex differences. Stress has been implicated in overeating and the development of obesity and abdominal fat accumulation. Stress hormones interact with sex hormones and there are reported sex differences in the response to stress, as well as differences in stress response between pre- and postmenopausal women. More research is needed in the area of stress-obesity interactions, as well as on obesity treatment programmes that incorporate stress management as a major treatment objective.
- The weight loss industry is largely geared towards women and is based on clinical science mostly done in women. Thus, when men choose to lose weight using commercial programmes, they have to rely on strategies largely designed for and tested in women. There are also limited studies on sex differences in response to anti-obesity drugs and sex differences in pharmacokinetics or clinical response to these drugs need to be evaluated. Given the sex differences in response to physical activity for weight loss and in socio-cultural factors related to appetite and body image, it is likely that sex-specific treatment approaches to weight loss, and maintenance would be beneficial. There is a strong need for clinical trials to test this hypothesis.
- In order to facilitate the study of sex differences, it is important for studies (especially large population studies) to stop statistically adjusting for sex and to instead report the results separately for men and women. As certain journals have policies that require authors to statistically adjust for sex, thereby potentially obscuring sex differences that may exist in the population, this recommendation may need to be addressed at multiple levels.
- As both peripheral and central mechanisms that regulate appetite and energy homeostasis are significantly influenced by sex and sex hormones, studies investigating these mechanisms need to account for sex differences. This would ultimately entail studying people or animals of *both* sexes and – for studies done in females – specifying or controlling for when in the ovarian cycle the studies were carried out.
- Many animal studies investigating pathways that regulate body fat mass only look at total fat mass and do not investigate regional body fat distribution. Given that many animal species, like humans, show sex differences in body fat distribution, it would be advantageous if animal studies investigating body fat mass (e.g. by dual-energy X-ray

absorptiometry (DXA) or fat pad dissection) would report fat mass in different anatomically defined depots in addition to total fat mass. This would add considerably to the body of knowledge on the mechanisms controlling fat distribution.

- Body mass index is an inaccurate estimate of body composition, and use of other methods that reflect body fat and lean content is to be encouraged, especially when comparing men and women whose body composition differs at the same absolute weight and height.
- Ovariectomy in rodents leads to acute effects that tend to promote weight gain (i.e. increased food intake), but this hyperphagic effect does not persist beyond about 5 weeks after surgery nor is hyperphagia reported in women going through menopause. Therefore, an outstanding question is whether the acute effects of menopause are more obesogenic than the longer-term effects of oestrogen deficiency, and if so, would interventions that focus on helping women to manage their weight during the menopausal transition help to reduce the burden of obesity in older postmenopausal women.
- Given the mounting evidence that larger hip circumference is protective against chronic disease morbidity and mortality, more research is needed to understand the pathophysiology of subcutaneous fat in different body regions as well as the effects of alterations in lower body muscle mass and intramuscular lipid on metabolic health.
- As the prevalence of PCOS increases along with the growing obesity epidemic, studies are needed to determine optimal approaches for weight loss and improvement of insulin sensitivity in women with this condition.

In summary, the study of sex and gender differences in obesity and the regulation of appetite and EE is an area rich with basic and clinical research opportunities for years to come. As countries around the world struggle to deal with increasing population overweight and obesity, it will be important to continue to focus on sex differences in prevalence, pathophysiology, mechanisms and treatment in order to achieve improved health and economic outcomes.

Conflict of Interest Statement

No conflict of interest was declared.

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