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Review

Obesity-Related Hypogonadism in Women

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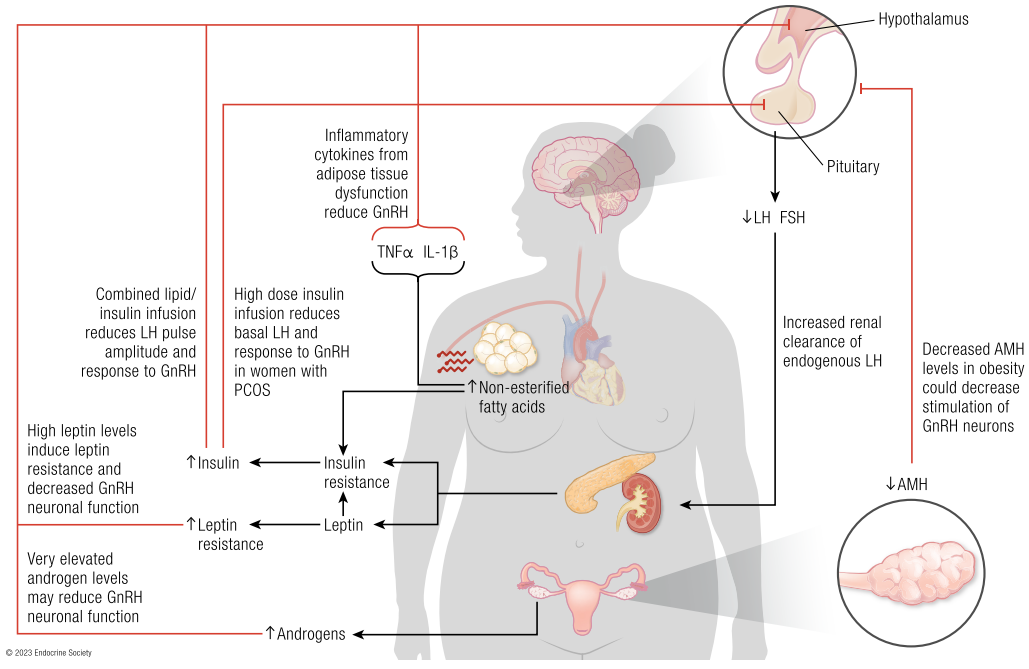
Abstract

Obesity-related hypogonadotropic hypogonadism is a well-characterized condition in men (termed male obesity-related secondary hypogonadism; MOSH); however, an equivalent condition has not been as clearly described in women. The prevalence of polycystic ovary syndrome (PCOS) is known to increase with obesity, but PCOS is more typically characterized by increased gonadotropin-releasing hormone (GnRH) (and by proxy luteinizing hormone; LH) pulsatility, rather than by the reduced gonadotropin levels observed in MOSH. Notably, LH levels and LH pulse amplitude are reduced with obesity, both in women with and without PCOS, suggesting that an obesity-related secondary hypogonadism may also exist in women akin to MOSH in men. Herein, we examine the evidence for the existence of a putative non-PCOS "female obesity-related secondary hypogonadism" (FOSH). We précis possible underlying mechanisms for the occurrence of hypogonadism in this context and consider how such mechanisms differ from MOSH in men, and from PCOS in women without obesity. In this review, we consider relevant etiological factors that are altered in obesity and that could impact on GnRH pulsatility to ascertain whether they could contribute to obesity-related secondary hypogonadism including: anti-Müllerian hormone, androgen, insulin, fatty acid, adiponectin, and leptin. More precise phenotyping of hypogonadism in women with obesity could provide further validation for non-PCOS FOSH and preface the ability to define/investigate such a condition.

Graphical Abstract



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Abbreviations: AMH, anti-Müllerian hormone; AUC, area under the curve; BMI, body mass index; DIO, diet-induced obese; FOSH, female obesity-related secondary hypogonadism; FSH, follicle-stimulating hormone; GC, granulosa cell; GnRH, gonadotropin-releasing hormone; HFD, high-fat diet; HPG, hypothalamic–pituitary–gonadal; IL, interleukin; IR, insulin resistance; LH, luteinizing hormone; MOSH, male obesity-related secondary hypogonadism; NEFA, nonesterified fatty acid; NPY, neuropeptide Y; PCOS, polycystic ovary syndrome; SHBG, sex hormone–binding globulin; TLR, toll-like receptor; TNF, tumor necrosis factor.

ESSENTIAL POINTS

- This article proposes that women with obesity can have reduced luteinizing hormone (LH) levels due to non-polycystic ovary syndrome (PCOS) "female obesity-related secondary hypogonadism," akin to "male obesity-related secondary hypogonadism" in men with obesity
- Obesity is associated with a decrease in LH levels both in women with and without PCOS.
- Lower LH levels in women with obesity are in part due to increased clearance of endogenous LH, as well as reduced pituitary response to gonadotropin-releasing hormone (GnRH)
- In women with PCOS, obesity is associated with a decrease in LH pulse amplitude rather than LH pulse frequency
- Women with obesity can have increased androgen production even in the absence of PCOS, and markedly elevated androgen levels can contribute to reduced LH levels
- Increased leptin levels in women with obesity can result in hypothalamic leptin resistance and a reduction in GnRH pulsatility and LH levels
- Anti-Müllerian hormone levels are unaltered or reduced in women with obesity, and can fall further following weight loss

Background

Obesity represents a significant burden to public health across the globe, with its prevalence having almost trebled over the past 40 years (1). According to World Health Organization, more than 1.9 billion adults are overweight (39% men and 40% women) and 13% of the adult population (11% men and 15% women) had obesity in 2016 (2). The global prevalence of obesity is projected to increase by at least a further 10% by 2030 (3).

Excess bodyweight is an established risk factor for comorbidities including a high prevalence of hypogonadism (4). Male obesity-related secondary hypogonadism (MOSH) is relatively well-characterized (Fig. 1) with the prevalence of hypogonadism secondary to obesity estimated at ~40% (5). The pathophysiology of secondary hypogonadism in men involves a complex interplay between visceral adiposity, leptin, and insulin resistance (IR), resulting in hypothalamic dysfunction (6, 7). Estradiol is also increased in proportion to bodyweight as a consequence of greater aromatase activity in adipose tissue, which is proposed to further inhibit gonadotropin-releasing hormone (GnRH)/luteinizing hormone (LH) secretion from the hypothalamus and pituitary gland via negative feedback (8). Furthermore, high leptin levels are proposed to lead to hypothalamic leptin resistance and reduced GnRH secretion, with a resultant decrease in LH levels (7, 9). Hyperinsulinemia secondary to increased free fatty acid delivery to the liver and increased levels of inflammatory markers (eg, tumor necrosis factor [TNF]-α and interleukin [IL]-6) from adipose tissue can further impair hypothalamic kisspeptin neuronal function and downstream testosterone production (7, 9). Overall, there is a bidirectional relationship between androgen deficiency and visceral fat accumulation/IR creating a self-perpetuating cycle inhibiting GnRH secretion and exacerbating hypogonadism.

In women, obesity is associated with a dramatic increase in the frequency of menstrual irregularity. Women with obesity at the age of 23 years have a 1.97-fold (95% CI 1.19-2.57) increased odds of menstrual disturbance, independent of their previous body mass index (BMI) during childhood (10). Further, Santoro et al estimated that the prevalence of menstrual disturbance increased from 23% if BMI <29.9 kg/m^2 to 27% if BMI >30 kg/m^2 (11). There is a J-shaped curve with menstrual irregularity, which occurs both in women with insufficient as well as excess bodyweight (12). Menstrual irregularity was present in 26% of women with BMI >30 kg/m^2 compared with a prevalence of ~14% in women with BMI of 20 to 24.9 kg/m^2 (12). The risk of menstrual disturbance is 18% at a nadir BMI of ~22 kg/m^2 and increases linearly with BMI to reach over 60% in women with a BMI of 60 kg/m^2 (12). Likewise, the risk of menstrual disturbance increases linearly beyond a waist circumference of 70 cm (12).

Evidence for Secondary Hypogonadism in Women (ie, Reduced LH Levels) With Obesity

Mean LH levels during 10-minutely LH measures over 12 hours were reduced in women with obesity compared with lean women (BMI 21 kg/m^2: LH 3.4 ± 0.2 IU/L; vs BMI 49 kg/m^2: LH 2.0 ± 0.3 IU/L) (13). Furthermore, there was a more than 50% reduction in LH pulse amplitude in the follicular phase (LH pulse amplitude in BMI 21 kg/m^2: 1.6 ± 0.2 IU/L; vs BMI 49 kg/m^2: 0.8 ± 0.1 IU/L) (13). Although women with obesity and lower LH levels had similar estradiol levels during the follicular phase, the amplitude of the midcycle LH surge was reduced, resulting in lower levels of a urinary metabolite of progesterone in the midluteal phase (BMI 21 kg/m^2: urinary progesterone 181 µg/mg Cr vs BMI 49 kg/m^2: urinary progesterone 38 µg/kg Cr) (13). However, no change in LH pulse frequency was observed in women with obesity compared with controls (controls: LH pulse frequency 3.0 ± 0.3 vs obese: LH pulse frequency 3.2 ± 0.3 pulses per 12 hours) (13). The impact of obesity on GnRH/LH pulsatility in women has been studied by several research groups including those of Janet Hall and Jeffrey Chang. Overall, obesity negatively impacts on LH pulse amplitude rather than pulse frequency, indicating that the increased pulse frequency often detected in women with polycystic ovary syndrome (PCOS) is still expected to be present in women who also have obesity (14, 15). Moreover, the tonic estradiol-induced negative feedback on the hypothalamic–

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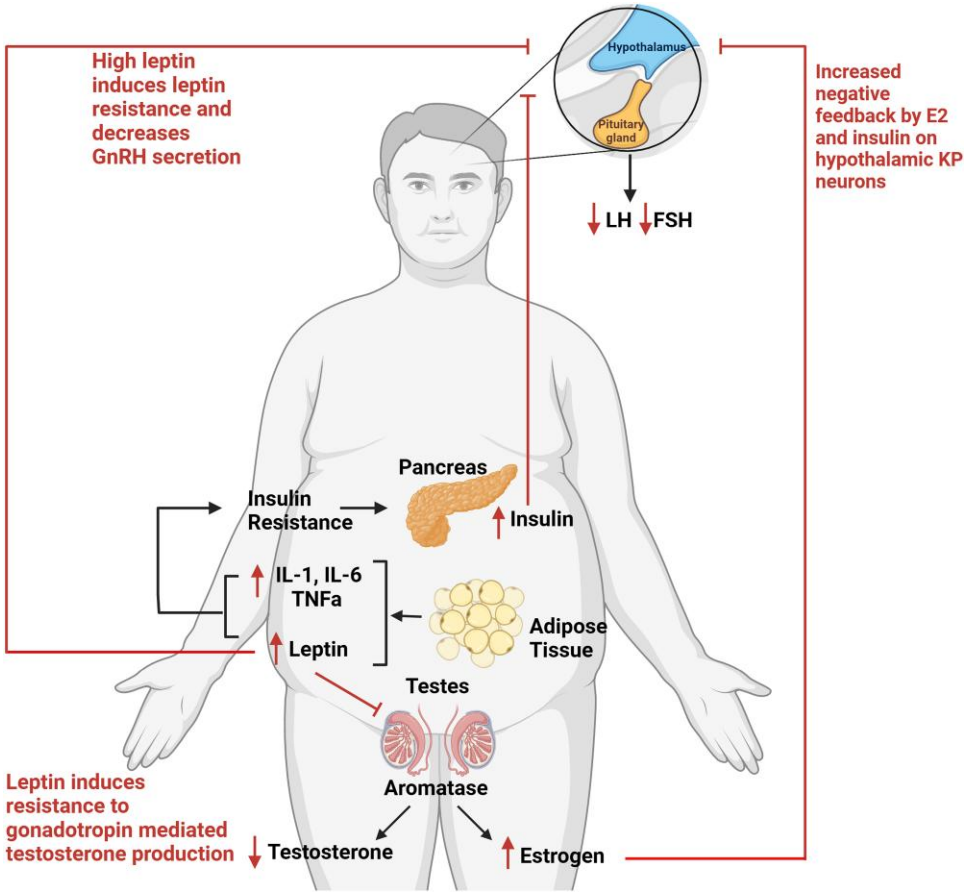

Figure 1. Mechanisms of hypogonadism in male obesity-related secondary hypogonadism (MOSH). Abbreviations: GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin; NEFA, Non-esterified fatty acids; IL, interleukin; TNF-α, tumor necrosis factor.

pituitary–gonadal (HPG) axis is greater in women with obesity, as evidenced by an aromatase inhibitor doubling LH pulse amplitude by 2.54-fold in women with obesity, but not in lean women (16, 17). In summary, obesity is associated with impaired LH pulse amplitude and LH levels, resulting in reduced stimulation of corpora lutea, and lower luteal progesterone levels (13, 17), with increased tonic estradiol-induced negative feedback.

Impact of Obesity on Diagnosis of PCOS

PCOS is the commonest endocrine disorder affecting between 8% to 13% of women of reproductive age (18-20). Approximately half of women with PCOS have obesity (21), though the reported prevalence of obesity in women with PCOS varies across countries from 38% to 88% (19, 22, 23). Using Mendelian randomization, a systematic large scale genetic-based analysis reported a moderate increased risk of PCOS (odds ratio 1.01-1.25) for each 1 SD increase in genetically-predicted BMI in women (24). While women with obesity have an increased odds of PCOS (~2.77-fold, 95% CI 1.88-4.10) (25), this increased risk may not necessarily account for all menstrual disturbance observed in women with obesity (which is increased linearly with BMI reaching 60% at a BMI of 60 kg/m^2 (12)). In lean women, PCOS is typically associated with increased GnRH pulsatility and high LH levels (26); however, mean LH levels and LH pulse amplitude are reduced in women with obesity, suggesting the additional presence of a distinct pathophysiological mechanism exacerbating the occurrence of hypogonadism in women with obesity (11).

PCOS is a leading cause of subfertility; however, its diagnosis can be challenging as some of its diagnostic criteria can be nonspecific, and none are requisite. Waldstreicher et al found that increased GnRH pulse frequency (reflected by LH levels) is a key pathophysiological feature that underpins PCOS (26), but is not required for diagnosis in current guidelines (27). Recently, polymorphisms within the genes for LH and the LH receptor have been recognized to associate with PCOS, indicating the significant role of gonadotropins in the pathophysiology of PCOS (28). Moreover, increased LH levels in PCOS promote ovarian hyperandrogenism and ovulatory dysregulation (27), but hyperandrogenism can occur in women with obesity even in the absence of PCOS (29). Furthermore, lean women with PCOS are more likely to have an increase in adrenal androgens (29), and obesity can also result in an increase in androgens produced through non-classical pathways (30, 31). Importantly, polycystic ovarian morphology is harder to assess in women with obesity for technical reasons (32). Thus, it is conceivable that menstrual disturbance in women with obesity often could be inaccurately attributed to PCOS.

Obesity negatively impacts on gonadotropin secretion in women with PCOS (33). Morales and colleagues showed that LH pulse amplitude in lean women with PCOS is over

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2.5-fold greater than that in lean controls (lean PCOS: 13.3 vs lean controls: 5.0 IU/L) (34). By contrast, this increase in LH pulse amplitude is tempered in women with obesity and PCOS who display relatively normal LH pulse amplitude (obese PCOS: 6.4 IU/L vs lean PCOS: 13.3 IU/L) (34). Mean serum LH was also lower in women with obesity and PCOS (lean PCOS 31.5 vs lean controls 10.4 IU/L; obese PCOS: 20.8 vs obese controls: 10.7 IU/L) (34) and mean LH and LH pulse amplitude were inversely correlated with BMI in eumenorrheic women and anovulatory women with PCOS (35). However, LH pulse frequency remains increased in women with PCOS even if obesity is present (number of LH pulses per 24 hours: obese PCOS 23.9; lean PCOS 21.9; obese controls 15.9; lean controls 15.9) (34). Similarly, in another cross-sectional study in women with PCOS by Arroyo and colleagues, spontaneous LH pulse frequency was increased (women with PCOS: 22.8 per 24 hours vs normal cycling women: 16.5 per 24 hours), but 24-hour mean LH levels were negatively correlated with BMI ($n = 33$, $r = -0.63$), attributed to a fall in LH pulse amplitude ($r = -0.53$) (36). Stimulated LH levels after GnRH (dose 75 μg/kg) ($r = -0.42$) in women with PCOS (15), or GnRH agonist in women with polycystic ovaries ($r = -0.386$) (37), were inversely related to BMI, indicating an impaired pituitary response in women with obesity.

In summary, LH pulse amplitude, but not the increased pulse frequency, is reduced with obesity in women with PCOS (13, 38). Likewise, obesity is associated with a decrease in LH pulse amplitude even in the absence of PCOS (13, 38). Thus, obesity can reduce LH levels both in women with PCOS as well as those without (39), suggesting that it induces a pathophysiological process that can affect reproductive hormones in all women with obesity.

Sexual dimorphism in the effect of obesity on hypogonadism

In this section, we consider how endocrine dysfunction due to obesity affects the HPG axis differentially in men (Fig. 1) and women (Figs. 2 and 3). Sexual dimorphism impacts on the effect of obesity on gonadal status, for instance with respect to androgen levels. While MOSH is manifested by androgen deficiency, androgen concentrations are increased in women with obesity, both in the presence (40) or absence (41) of PCOS. Body fat composition with different distributions of adiposity can additionally influence androgen production and metabolism (42, 43). Women with a shift in fat distribution from a gynoid to android pattern typically have lower sex hormone–binding globulin (SHBG) concentrations, higher testosterone production, with higher aromatization to estradiol (42, 44). Consequently, relative hyperandrogenism occurs in women with obesity, predisposing them to IR and metabolic dysfunction, whereas obesity results in reduced androgen levels in men.

The combination of insulin and elevated lipids had a greater effect on gonadotropin levels in women than in men. A 6-hour lipid infusion in combination with a hyperinsulinemic euglycemic clamp in 10 women resulted in a greater reduction in

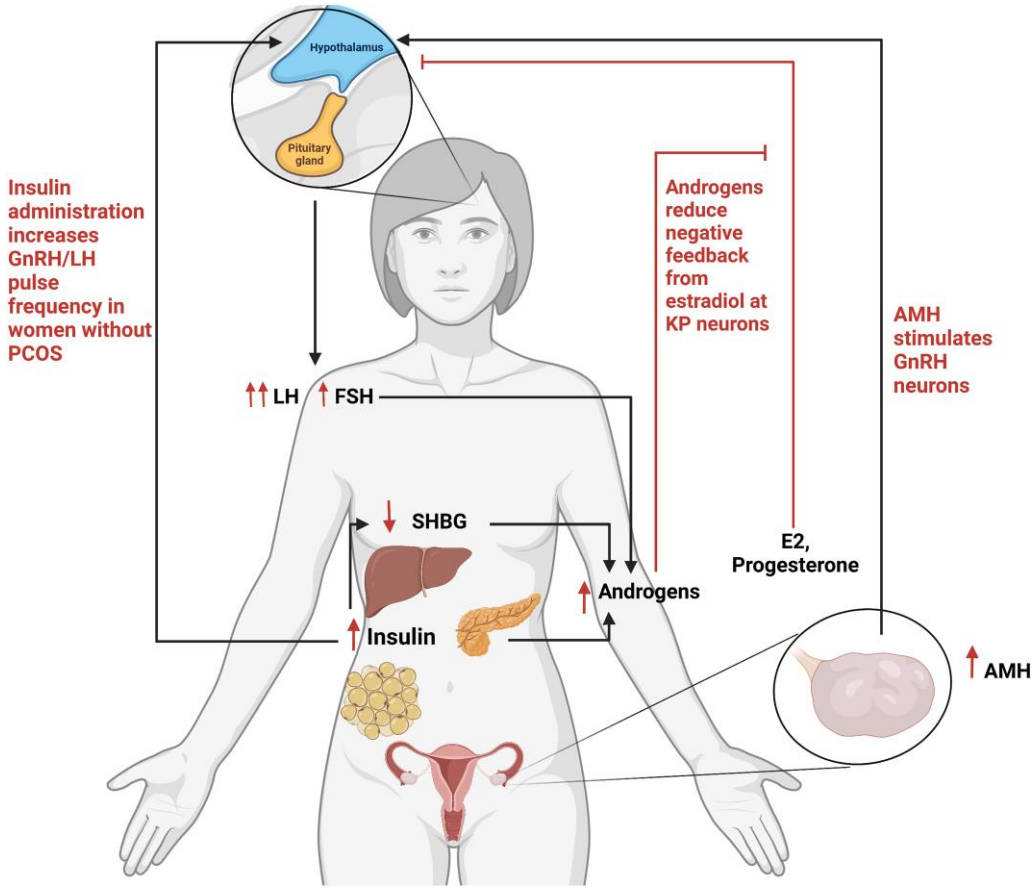

Figure 2. Mechanisms of polycystic ovary syndrome (PCOS) in lean women. Abbreviations: GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone–binding globulin; E2, estradiol; AMH, anti-Müllerian hormone; PCOS, polycystic ovary syndrome.

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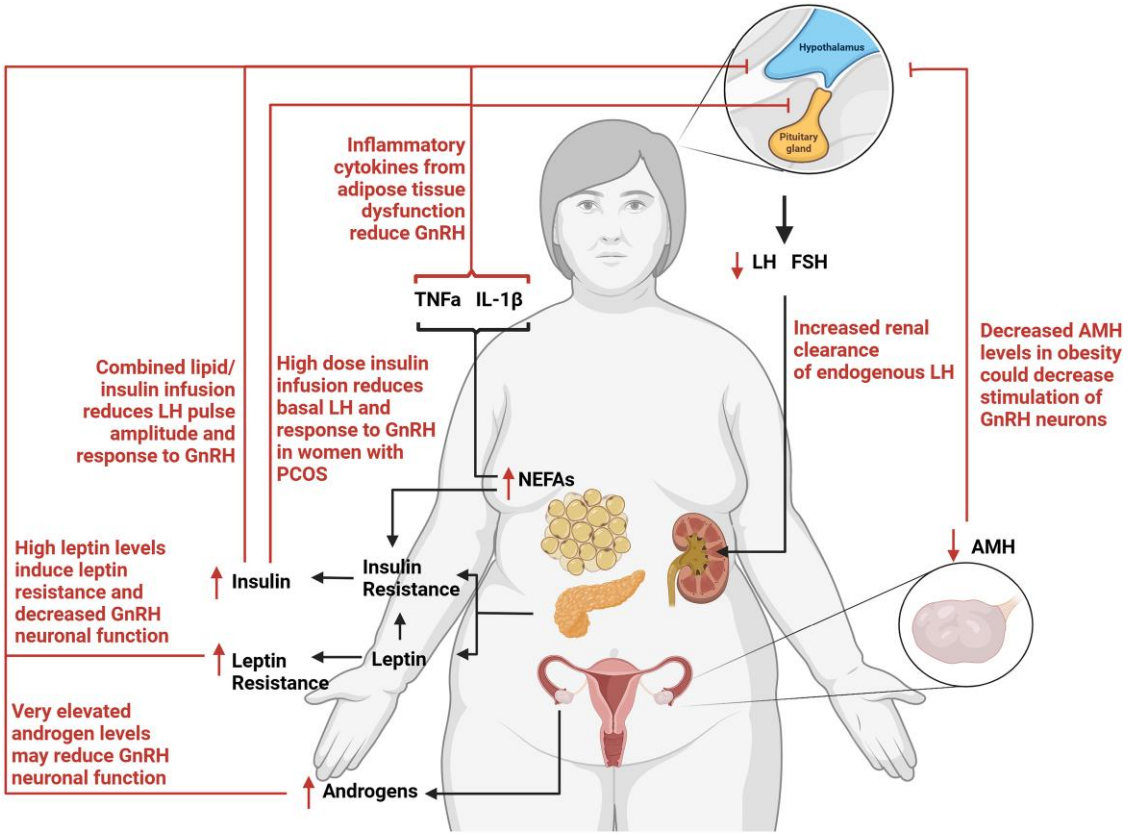

Figure 3. Putative mechanisms of female obesity-related secondary hypogonadism in women with obesity. Abbreviations: GnRH, gonadotrophin releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone–binding globulin; E2, estradiol; AMH, anti-Müllerian hormone; PCOS, polycystic ovarian syndrome; NEFA, nonesterified fatty acids; IL, interleukin; TNF-α, tumor necrosis factor.

gonadotropins (change in LH −1.24 IU/L, change in follicle-stimulating hormone [FSH] −0.82 IU/L) than in 10 men (change in serum LH −0.37 IU/L, change in FSH −0.40 IU/L) (45), which partly could be attributed to a blunting of pituitary sensitivity to GnRH (45).

Preclinical Evidence of Obesity-related Hypogonadism From Female Rodent Models

Obese animal models including the leptin-deficient (ob/ob) mice, diet-induced obese (DIO) mouse models, and transgenic female mouse model, have been used to investigate effect of obesity on the reproductive axis. Leptin deficient (ob/ob) mice exhibit hyperphagia, morbid obesity, and infertility (46, 47). Infertility in leptin deficient mice is thought to be due to hypothalamic dysfunction as administration of exogenous GnRH can restore the LH levels (48). Exogenous administration of leptin reversed the metabolic and hypothalamic dysfunction in male and female ob/ob mice (47, 49) highlighting the role of leptin as an endocrine signal reflecting sufficiency of energy stores for fertility. Similarly, female mice with central nervous system–wide neuron insulin receptor knockout have increased plasma insulin and leptin concentrations and exhibit a reduction in serum LH compared with wild-type mice (50). In another study, female melanocortin-4 receptor knockout mice initially displayed normal pulsatile LH in early adulthood (8 weeks of age) despite an increase in bodyweight (51). However, as bodyweight increased further, insulin and glucose levels continued to rise, and female MC4R KO mice developed reduced GnRH and LH levels, irregular estrus cycles, and reduced corpora lutea at 16 weeks (51), signifying the negative impact of obesity and hyperinsulinemia on LH concentrations.

In addition to transgenic mouse models, high-fat DIO rodents can be more aptly used to study the effect of obesity on reproduction as they develop obesity-induced leptin resistance (52), as in humans (53). Indeed, DBA/2J female mice (DIO) who received 45% high-fat diet (HFD) for 16 weeks had weight gain, hyperleptinemia, and a 60% reduction in pregnancy rates (54). These mice had a 50% reduction in hypothalamic GnRH expression, a 95% reduction in expression of the leptin receptor (LepR-B), and a 100% increase in expression of neuropeptide Y (NPY) compared with lean mice (54). Under physiological conditions, the absence of leptin increases hypothalamic NPY, which in turn inhibits GnRH pulsatility leading to hypogonadotropic hypogonadism (54). Furthermore, compared with chow-fed mice, 16 weeks of HFD markedly decreased kisspeptin gene expression in the rostral periventricular region and the arcuate nucleus leading to a decrease in number of kisspeptin-immunoreactive neurons in DBA/2J mice (55). As rostral periventricular region kisspeptin neurons are known to mediate the preovulatory LH surge, mice deficient in leptin signaling were unable to generate an LH surge (56), thus indicating that central resistance to leptin underpins DIO-related infertility in rodents.

To simulate human obesity more closely, Balasubramanian et al compared the effect of chow and HFD on the HPG axis in DIO and dietary resistant (selectively bred over generations to be lean and glucose tolerant) Sprague–Dawley rats (57). When

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fed a 45% HFD for 6 weeks, DIO rodents gained more weight (349.3 ± 17.1 g vs 271.6 ± 5.5 g) and had higher leptin levels compared to dietary resistant animals (57). Chow-fed DIO rats exhibit a 50% reduction in estrus cycle regularity whereas HFD-fed DIO rats display an 80% reduction in estrus cyclicity with loss of the LH surge at proestrus (equivalent to preovulatory phase in women) (57). By contrast, chow-fed dietary resistant rats retained the increase in LH at proestrus (rising from 1.7 ± 0.41 to 8.4 ± 1.96 ng/mL at proestrus) (57); however, LH levels did not rise as much in HFD-fed rats (rising to only 2.79 ± 0.73 ng/mL). The degree of impairment in LH and estradiol levels appears to relate to the amount of bodyweight gained, with an inverse relationship between estradiol and leptin levels (57). In summary, hypothalamic leptin resistance is postulated to result in a reduction in hypothalamic kisspeptin expression (indirectly) and a resultant decrease in hypothalamic GnRH, and in turn LH secretion.

Psychosocial factors, including exposure to external psychological stress, affect feeding behaviors and can increase the propensity to obesity, as well as activating the hypothalamic–pituitary–adrenal axis leading to raised cortisol levels, which can suppress the reproductive endocrine axis (58, 59). For instance, stress reduced GnRH pulse amplitude in ewes, albeit a glucocorticoid receptor antagonist was unable to reverse this effect (60). However, stress (and increased cortisol levels) enhanced estradiol-induced negative feedback inhibition of serum LH, leading to anovulation (59). Thus, stress can affect sex steroid feedback and modulate the sensitivity of the HPG axis to feedback signals in the setting of obesity.

Impact of Bariatric Surgery on Gonadotropin Secretion in Women With Hypogonadism

Bariatric surgery is an effective long-term therapy for obesity. As obesity influences the regulation of the HPG axis through multiple mechanisms, weight loss is hypothesized to have a favorable impact on menstrual cycle regularity and subfertility in women with obesity.

Several studies have evaluated the effect of bariatric surgery on the restoration of menstrual cyclicity in women with obesity. A cross-sectional study of 515 women with obesity (BMI 42.2 ± 7.5 kg/m^2) who underwent sleeve gastrectomy or Roux-en-Y gastric bypass surgery and lost 35.3 ± 17.9 kg of bodyweight (postsurgery BMI 29.8 ± 6.3 kg/m^2) had a reduction in the proportion of women with irregular cycles (>35 days) from 38% to 25% (61). In another study, 195 of 410 women (age <40 years) responded to a questionnaire on menstrual cycle regularity after bariatric surgery (62). Of the 195 women, 98 (50.2%) were oligomenorrheic (>35 days) before surgery, but this improved to 28 out of 195 (14.4%) women after surgery (62). This was associated with a decrease in BMI from 52.0 to 31.9 kg/m^2 postoperatively and menstrual regularity was restored at an average of 1.6 months postoperatively (range 0-28 months) (62). In another clinical study, prevalence of menstrual irregularity decreased from 40.4% preoperatively to 4.6% postoperatively in 138 women who achieved at least 50% of excess weight loss through bariatric surgery (63).

A number of studies have looked at sex hormone profiles before and after weight loss achieved through bariatric surgery. Sarwer et al examined sexual function and reproductive hormones in 106 women aged 25-60 years old at 1 and 2 years after bariatric surgery (64). The average weight loss was 32.7% at 1 year and 33.5% at 2 years after surgery (64). LH levels increased from 9.4 IU/L to 13.3 at 1 year, and to 15.9 IU/L at 2 years, whereas FSH increased from 15.3 to 22.0 IU/L at 1 year, and 29.9 IU/L at 2 years (64). The timing of blood-sampling with regards to the menstrual cycle was unclear, and some women may have become peri/postmenopausal during the follow-up period to explain the marked increase in gonadotropin levels (64). However, total testosterone fell from 47.8 ng/dL to 30.4 ng/dL at 1 year and 23.4 ng/dL at 2 years; dehydroepiandrosterone sulfate decreased from 118.6 µg/dL to 106.1 µg/dL at 1 year, and 92.6 µg/dL at 2 years, indicating an improvement in hyperandrogenism after bariatric surgery (64). Overall, this is in keeping with obesity being associated with increased androgen levels, which are reversed on weight loss.

Interestingly, levels of urinary LH and progesterone metabolites in the luteal phase also increase with weight loss after bariatric surgery in eumenorrheic women with obesity (65). Following 25% weight loss at 6 months after bariatric surgery, there was an increase in urinary LH from 27.6 ± 12.4 to 43.7 ± 10.6 mIU/mg Cr (65). Bastounis et al prospectively evaluated changes in reproductive hormones in 38 premenopausal women (aged <40 years) in whom PCOS was excluded prior to vertical band gastroplasty (66). Of these 38 women, 9 were oligomenorrheic and 4 had hirsutism. Women lost an average of 59 ± 11 kg in bodyweight (66). All 9 women who were oligo/amenorrheic before surgery regained menstrual cyclicity by 12 months after surgery (66). Moreover, FSH levels increased from 5.26 ± 2.40 IU/L to 6.77 ± 2.95 IU/L, whereas LH remained similar at 3.29 ± 1.76 IU/L vs 3.20 ± 1.42 IU/L (66). SHBG increased from 32.8 ± 10.8 to 63.4 ± 20.7 ng/mL, total testosterone decreased from 0.49 ± 0.25 ng/mL to 0.34 ± 0.17 ng/mL, androstenedione decreased from 1.78 ± 0.90 to 1.49 ± 0.40 ng/mL, and dehydroepiandrosterone sulfate from 222 ± 147 µg/dL to 202 ± 150 µg/dL (66). Thus, in addition to a fall in androgens, it is possible that the quality of ovulation and resultant luteal phase progesterone secretion could also be impacted by obesity.

Not all studies of weight loss have observed a change in gonadotropin levels. In a prospective study involving 31 non-PCOS women with obesity (n = 31) undergoing Roux-en-Y gastric bypass surgery, a mean weight loss of 39.6 kg was achieved in 1 year, but this was not associated with any change in follicular phase gonadotropin levels (67). However, SHBG doubled at 12 months after weight loss surgery (from 41.5 ± 21.1 nmol/L to 85.6 ± 24.6 nmol/L at 12 months after surgery) (67). Parallel to the rise in SHBG, total testosterone decreased from 1.14 ± 0.58 nmol/L preoperatively to 0.92 ± 0.29 nmol/L, and DHEA declined from 5405.5 ± 2455.5 nmol/L to 3262.5 ± 1687.1 nmol/L at 12 months after surgery (67). Of the 13 oligo/amenorrhoeic women, 11/13 (85%) regained regular menstrual cycles (67). Paul et al investigated the hormonal profile before and 12 months after surgery in 68 women who underwent Roux-en-Y gastric bypass (68). Women in the study had a median weight loss of 31% (68). Median SHBG increased from 33 to 71 nmol/L, and median testosterone levels reduced from 1.0 to 0.75 nmol/L ($P < .001$) (68), but there were no significant changes in serum gonadotropin, estradiol, and progesterone levels. In summary, weight loss after bariatric surgery improved menstrual irregularity in women with obesity. Following weight loss, SHBG is increased, and androgen levels are reduced, suggesting a reduction in bioavailable

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androgens, although not all studies have shown consistent changes in gonadotropin levels.

Mechanisms that Could Contribute to Secondary Hypogonadism in Women With Obesity

Reduced Pituitary Gonadotropin Response to GnRH in Women With Obesity

Eumenorrheic women with BMI ≥ 30 kg/m^2 (n = 11) had lower baseline LH levels (with obesity 2.5 ± 0.2 IU/L, without obesity 5.2 ± 0.8 IU/L) and mean LH pulse amplitude (with obesity 1.12 ± 0.19 IU/L, without obesity 2.73 ± 0.46 IU/L) compared with women with BMI 18-25 kg/m^2 (n = 10) (17). Women with obesity had a reduced pituitary response to exogeneous GnRH (17). Mean LH, area under the curve (AUC) for LH over 2 hours, and maximum stimulated LH after GnRH (75 ng/kg) were all significantly lower in women with obesity (AUC for LH after GnRH stimulation over 2 hours: with obesity 581.6 ± 69.9 IU h/L vs without obesity 1203.5 ± 160.0 IU h/L). Serum FSH parameters (baseline FSH, AUC over 2 hours, and peak stimulated FSH following GnRH) were also lower in women with obesity (17). Transdermal estradiol treatment (0.1 mg/day) over 1 menstrual cycle length did not change mean basal LH or FSH levels; however, LH pulse amplitude was increased in women with obesity (17). Furthermore, 1 month of estradiol treatment resulted in an increase in the peak LH after GnRH from 6.81 IU/L to 10.6 IU/L in women with obesity, but decreased the peak LH after GnRH from 14.6 IU/L to 11.9 IU/L in women without obesity (17). Likewise, estradiol treatment resulted in the maximal change in FSH following GnRH to increase from 6.14 IU/L to 7.46 IU/L in women with obesity, but to decrease from 7.09 IU/L to 4.99 IU/L in women without obesity (17). These findings suggest an attenuation of pituitary response to GnRH stimulation in non-PCOS women with obesity (17). Thus, whilst estrogen treatment increased the gonadotropin response in women with obesity, women without obesity had a reduction in gonadotropin response. Indeed, estrogen treatment increased the urinary progesterone excretion from 17.6 to 21.8 µg/mg of creatinine in women with obesity but not in women without obesity (17). The improvement in pituitary responsiveness to GnRH with estrogen treatment suggests that feedback to estrogen is altered in women with obesity, in keeping with a differential effect to an aromatase inhibitor in women in the presence of obesity (16, 17).

Increased Clearance of Endogenous LH Resulting in Reduced LH Levels in Women With Obesity

BMI is inversely related to endogenous LH in women with PCOS, in part due to increased renal clearance of LH (69). In a study of 21 healthy women with PCOS who received a GnRH antagonist to suppress endogenous LH, serum LH was quantified after GnRH (75 ng/kg), and after a bolus of 300 IU of recombinant human LH (69). Srouji et al showed that women with higher BMI (35.4 kg/m^2) displayed a blunted rise in LH after GnRH (change in serum LH of ~30 IU/L) compared with women with lower BMI (18 kg/m^2; change in serum LH of ~160 IU/L) (69). The half-life of endogenous LH exhibited a significant negative correlation with BMI in women with PCOS ($r = -0.46$, $P = .037$), and the estimated renal clearance of LH positively correlated with BMI ($r = 0.53$, $P = .014$) (69). Increased renal clearance with BMI was not observed after recombinant LH (rhLH), which was attributed to alternative isoforms of endogenous LH being present in women with obesity and PCOS (70). Sulphonated isoforms of LH are cleared faster than sialylated isoforms (70). Wide et al collected serum samples from 71 women (12 of whom had PCOS) and used electrophoretic analysis to quantify the number of sulphonated and sialylated forms of LH and FSH (70). Although sialyation of LH molecules is increased in PCOS, the proportion of sulphonated LH isoforms (which are cleared faster) positively correlated with BMI ($r = 0.74$, $P = .008$), which could contribute to the reduced LH levels seen in obese vs lean women with PCOS (70). This relationship was not observed in lean women during the follicular phase; however, there were only a few women without PCOS or obesity in this study (70). Overall, increased clearance of endogenous LH is likely to contribute to lower LH levels in women with obesity.

Insulin Resistance-impact of Raised Insulin on LH Secretion

Insulin has a well-established role as a central metabolic regulator and may also influence reproductive function. Obesity increases the risk of IR; however, this resistance may be tissue specific such that organs, including the ovaries, can remain sensitive to the effects of elevated insulin levels with respect to steroidogenesis (71). Preincubation of granulosa cells (GCs) with insulin synergistically amplified the steroidogenic response to LH (72, 73). Moreover, IR in the ovary is likely to be a postreceptor abnormality and a signaling pathway-specific defect (74, 75). While insulin-mediated glucose uptake and lactate production were both attenuated in GCs from women with anovulatory PCOS, steroidogenic (progesterone) response to insulin remained undiminished (74, 75). The implication is that the ovaries in women with PCOS remains sensitive to the effects of insulin with respect to steroidogenesis in insulin resistant states (73, 75).

IR occurs secondary to an increase in circulating nonesterified fatty acids (NEFAs), inflammatory cytokines, and abdominal fat deposits (76, 77). Insulin receptors have been reported in the hypothalamus including in the arcuate nucleus, suprachiasmatic nucleus, and in the median eminence (78). In overweight individuals, the brain, in particular the hypothalamus, can develop impaired insulin response, referred to as "brain insulin resistance" (79). Brain IR in humans can vary widely, as seen during imaging studies by magneto-encephalography following intranasal insulin administration (80).

In vitro studies using perfused rat hypothalamic fragments, or murine hypothalamic neurons, showed a dose-dependent increase in GnRH secretion following insulin administration (81, 82). In a rat GnV-3 cell line (hypothalamic neuronal cell line), insulin induced an increase in c-fos and GnRH gene expression via the mitogen-activated protein kinase and extracellular signal–regulated kinase 1/2 pathway (83). Correspondingly, streptozotocin-induced diabetic female rats with hypoinsulinemia had reduced GnRH pulsatility and secretion (84, 85), altered feedback to estradiol (80), and delayed or absent LH surges (84-87). The hypothalamic effect of a lack of insulin could be reversed partially with peripheral insulin administration in male rats (88). Furthermore, female neuron insulin receptor knockout mice had a 90%

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reduction in circulating LH levels coupled with a 15% weight gain, a 120% increase in leptin, and a 30% increase in triglycerides (50). Thus, in addition to a direct insulin effect on GnRH/LH; changes in energy balance could, in part, be induced indirectly via a central insulin–leptin effect (50).

Kisspeptin has been identified as an upstream regulator of GnRH neurons (89, 90). Arcuate and periventricular kisspeptin neurons express the insulin receptor (78). Moreover, a subpopulation of *Kiss1* neurons (~26%) hyperpolarized in response to insulin through activation of K_{ATP} channels (91). Streptozotocin-induced diabetic rats with hypoinsulinemia showed reduced hypothalamic *Kiss1* mRNA expression in the arcuate nucleus (92, 93). Kisspeptin-specific insulin receptor knockout in female *Kiss1*-Cre transgenic mouse showed delayed pubertal development but preserved fertility (91). Intriguingly, high-fat feeding to these female mice resulted in disrupted estrus cycles but GnRH expression and LH levels remain unchanged (91). In contrast, kisspeptin-specific insulin receptor knockout in C57BL/6J mice had normal estrus cyclicity and fertility (94).

There are few studies investigating effect of insulin on gonadotropin response in humans. Moret and colleagues administered insulin in 5 lean women and 5 women with PCOS and measured LH levels at 10-minute intervals (95). Interestingly, insulin increased LH pulse frequency (but not amplitude) in healthy women, but not in women with PCOS (95), which could be attributed to LH pulse frequency already being increased in women with PCOS. Conversely, Lawson et al infused insulin and assessed the gonadotropin response to GnRH in 18 women with PCOS and 21 control women (96). Although, basal LH and LH responses to GnRH were unaltered by insulin infusion in control women, these were reduced in women with PCOS (96). There was a negative correlation between AUC of insulin and LH levels after controlling for BMI in 21 eumenorrheic women without PCOS ($r = -0.674$; $P = .016$) (96). However, diazoxide, which reduces insulin levels, did not alter basal or GnRH-stimulated gonadotropin levels in women with PCOS (97). The action of insulin could occur via hypothalamic GnRH neurons (97), or via the pituitary (98) or via agouti-related protein/proopiomelanocortin (AgRP/POMC) neurons (78). Thus, the precise site of action of insulin on hypothalamic regulation of GnRH remains unclear (95, 99).

In summary, mean LH over 12 hours was negatively correlated with BMI, but not with insulin levels, in women with PCOS (95). High-dose insulin infusion reduced basal LH, and the response to GnRH in women with PCOS, but not significantly in healthy women without PCOS (96). Conversely, another report found that insulin infusion increased LH pulse frequency in healthy women without PCOS (95). Furthermore, the effect of insulin on LH can differ if present in the context of elevated leptin and fatty acid levels, as found in obesity, which is discussed further below. In women with PCOS, diazoxide treatment failed to alter basal LH or GnRH-stimulated LH or FSH levels (97).

Effect of Nonesterified Fatty Acids on LH Secretion

NEFAs are metabolic substrates of lipolysis (100). In women with obesity, dysregulated lipolysis results in increased circulating NEFAs (101), whereas women with PCOS do not consistently show altered NEFA levels (102-104). Elevated circulating NEFAs accumulate in the follicular fluid of developing ovarian follicles (105). Exposure of murine or bovine ovarian follicles to elevated levels of NEFAs in vitro caused impaired fertilization and reduced production of estradiol (105, 106). An increase in GC estradiol levels is needed for induction of the ovulatory LH surge (107). Therefore, elevated NEFAs in obesity could lead to decreased estradiol levels and contribute to anovulation.

In in vitro rodent studies, the type of NEFA appears to influence the effect of fatty acids on GCs; saturated fatty acids had a proapoptotic action (108), whereas unsaturated fatty acids and estradiol had antiapoptotic (109) effects on GCs (110). Some unsaturated fatty acids can diminish GnRH-induced LH release (111, 112). For instance, linoleic acid stimulates Lhβ and suppresses Fshβ expression in rodents (112). A 24-hour intracarotid lipid infusion increased the gene transcripts for Lhβ but did not increase serum LH concentrations, GnRH or *Kiss1* transcripts, implying a translational block (112). Li et al also showed that physiological concentrations of Oleic acid activated unfolded protein response that suppressed translation of Lhβ mRNA and reduced LH secretion from LβT2 cells (111). However, concentrations of NEFA in in vitro studies may exceed that observed in humans. Thus, to better evaluate the metabolic profile in obese women, Chosich and colleagues conducted a crossover study administering infusions of saline (control), lipid solution, insulin alone, and a combination of lipid and insulin, over 6 hours in lean eumenorrheic women in the follicular phase (45). Neither lipid nor insulin infusions led to significant changes in gonadotropin levels, but the combined lipid/insulin infusion reduced basal gonadotropins after 2 hours (serum LH reduced from 4.6 IU/L to 3.3 IU/L, FSH reduced from 3.9 to 3.1 IU/L compared with saline control) (45). Santoro et al showed that LH pulse amplitude was reduced after infusion of lipid plus insulin compared to saline (mean LH pulse amplitude 1.49 IU/L after lipid/insulin vs 2.33 IU/L after saline) but LH pulse frequency did not change (113). In addition, peak gonadotropin levels were blunted following GnRH stimulation, indicating reduced pituitary sensitivity (peak LH and peak FSH were ~12 IU/L and 22 IU/L in the lipid/infusion group vs 16 IU/L and 14 IU/L in saline groups) (113). The same protocol did not alter proinflammatory cytokine levels (114) or other pituitary hormone levels such as TSH, prolactin, and or cortisol (115), suggesting an effect specific to gonadotropins.

In summary, the metabolic milieu seen in women with obesity can contribute to the reduced LH levels observed in women with obesity. Indeed, the combination of elevated fatty acid and insulin levels can reduce basal and GnRH-stimulated LH levels (113, 116).

Inflammation and its Impact on Reduced GnRH/LH Secretion

Obesity is a chronic disease characterized by a proinflammatory state (117). IL-1β, for instance, is a potent inhibitor of GnRH secretion and has been shown to reduce c-fos expression of GnRH neurons during the preovulatory gonadotropin surge in rats (118-120). Central infusion of IL-1β inhibits secretion of hypothalamic GnRH and LH in gonadectomized female rats (121) but not if administered peripherally (118). In HFD-induced obese rabbits, high circulating levels of cytokines (eg, IL-6, IL-8) were associated with reduced expression of hypothalamic *Kiss1r* and treatment with an anti-TNFα inhibitor attenuated gene expression of cytokines (122).

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Moreover, saturated fatty acid in fat-rich foods bind to toll-like receptor 2 (TLR2) and TLR4 during in vitro rat cultures of astrocytes, glial cells and neurons, and initiate inflammation through increased TNFα expression (123, 124). Indeed, mice with either genetically engineered mutations of TLR2 or TLR4 are protected from obesity-associated IR (125-127). Female C57BL/6J mice were also found to have higher levels of hypothalamic IL-10 and the anti-inflammatory effect of IL-10 has been proposed to be responsible for the resistance of the HPG axis to inflammatory damage compared with male rodents (128).

Women with obesity are reported to have higher baseline proinflammatory cytokine levels such as IL-6 and IL-12 (17). These cytokines may act directly through their receptors on GnRH neurons or through intermediary neurons to affect GnRH neuronal function (129). However, acute infusion of lipid/insulin to healthy women in the follicular phase did not produce an increase in inflammatory markers except for a small increase in macrophage inflammatory protein 1β, which is an inflammatory signal implicated in disrupting folliculogenesis (114). Estradiol could potentially exert a protective anti-inflammatory effect as transdermal estradiol replacement was shown to decrease levels of IL-1β in obese patients (17). In summary, NEFAs, insulin, and inflammatory cytokines are potential mediators for obesity-related hypogonadism in women (Fig. 3).

Contribution of Hypothalamic Leptin Resistance to Reduced GnRH/LH Secretion

Leptin is a satiety-signaling adipokine responsible for reducing appetite and increasing energy expenditure via its action in the hypothalamus (130). Circulating levels of leptin are associated with fat mass ($r = 0.66$-0.68) (131). Leptin is regarded as a permissive factor for GnRH secretion; thus low bodyweight and leptin levels are associated with decreased hypothalamic function and subfertility (132, 133). Humans with congenital leptin deficiency are hypogonadal (134), and healthy reproductive endocrine function can be restored via recombinant leptin administration (134). Likewise, women with hypothalamic amenorrhea have low leptin levels and LH pulsatility can be restored with recombinant leptin treatment (132).

Human obesity is associated with leptin resistance from either receptor downregulation or postreceptor defects (135, 136). Consistent with this, Tortoriello et al showed that female DBA/2J mice given a 20-week HFD became obese, hyperleptinemic, and had 60% reduction in pregnancy rates, which could be restored using gonadotropin administration, consistent with a central reproductive defect (54). Hypothalamic GnRH and leptin receptor mRNA expression were reduced by 50% and 95% in female DIO DBA/2J mice respectively, whereas NPY expression was increased by 100% (51). Leptin normally suppresses inhibitory influences by NPY and AgRP on GnRH and *Kiss1* neurons (54, 137). Thus, lack of leptin signaling enhances NPY expression and is associated with reduced GnRH secretion, as observed in ob/ob mice (138). Indeed, AgRP-leptin receptor knockout mice exhibit low LH, prolonged estrus cycles and reduced fertility (139).

A phase of enhanced leptin expression can be seen before an ultimate leptin receptor downregulation in obesity. Indeed, administration of HFD to C57BL/6J female mice manifested augmented leptin receptor mRNA expression at 8 weeks but a 33% decline in leptin receptor expression was observed in the arcuate nucleus at 19 weeks (135). This is coupled with a decline in *NPY* mRNA by ~32% and in *POMC* mRNA by ~55% after 19 weeks (135), indicating a profound dysregulation of neuropeptide control systems of energy balance on prolonged HFD exposure. Interestingly, induction of chronic hyperleptinemia uncoupled from obesity-induced leptin resistance may cause late-onset reproductive failure in transgenic "skinny" mice overexpressing leptin (140). Hyperleptinemia induces early puberty in these female mice but beyond 21 to 22 weeks of age, they showed reduced fertility (140). Hypothalamic GnRH contents were lower than the nontransgenic littermates (21-week-old leptin overexpressing mice: 0.52 ng vs control mice: 0.92 ng of GnRH per hypothalamus; $n = 4$) and impacted on the amplitude of LH at proestrus (leptin overexpressing mice: 3.3 ± 1.1 vs control mice: 29.4 ± 6.8 ng/mL; $n = 4$-6), reduced the response to GnRH, and lengthened the duration of estrus cycles (transgenic vs non transgenic mice: 10.2 vs 5.5 days; $n = 5$), suggesting a direct negative impact of leptin on hypothalamic function (140). Thus, chronic hyperleptinemia, as found in obesity, can downregulate hypothalamic leptin signaling and lead to decreased hypothalamic function.

Leptin could also impact on fertility via an action at the ovaries. Leptin enters the brain via a saturable transport system (141), and cerebrospinal fluid:serum leptin ratio is decreased in obesity (142). In women with obesity, tissues such as the ovaries are exposed to higher concentrations of leptin (143). In vitro studies reveal that high physiological levels of leptin (~100 ng/mL) counteract the stimulating effects of insulin-like growth factor-1 on the development of dominant follicles and ovarian steroidogenesis (144). In another in vitro study, application of physiological leptin concentrations (10 ng/mL to 50 ng/mL) suppressed progesterone production from GCs at 4 hours (145). Similarly, leptin inhibited FSH stimulated steroid production by bovine GCs (46). In humans, incubation of human lutein-GCs with low leptin levels (1 and 10 ng/mL) stimulated estradiol and progesterone secretion (146), whereas higher concentrations (50-200 ng/mL) could no longer do so (146). In another study, an inhibitory effect of leptin on progesterone production was observed if GCs were cocultured in the presence of insulin (3-4 mg/mL) (46, 145) by antagonizing insulin-supported steroidogenesis (147, 148). Together, these data highlight that leptin can impair reproductive function through both a central and peripheral effect. However, some researchers in the field feel that leptin resistance is associative rather than causative to the decreased GnRH function observed with obesity.

Impact of Adiponectin on LH Secretion

Adiponectin is an adipokine hormone derived from white adipose tissue that exerts its insulin sensitizing and anti-inflammatory activity (149) via actions on its 2 receptors, AdipoR1 and AdipoR2, as well as a third receptor, T-cadherin (150). Adiponectin circulates in human blood in various oligomeric complexes and crosses the blood–brain barrier via a regulated receptor-mediated transcytosis (151, 152). Adiponectin receptors are expressed in peripheral tissues including muscle, liver, bone, ovaries, as well as the hypothalamus and pituitary gland (153). Central administration of adiponectin induced distinct c-fos activity and

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dose-dependently decreased the body weight of male C57Bl/6J mice (154). Adiponectin is also involved in glucose and lipid metabolism (155) and potentiates the effect of leptin on thermogenesis and lipid levels. While both hormones increased expression of hypothalamic corticotropin-releasing hormone, adiponectin had no substantial effect on other neuropeptide targets of leptin.

Adiponectin reduced the transcription and promoter activity of *Kiss1* in GT1-7 cells via activation of AMPK (156). In rat pituitary cell cultures, exposure to adiponectin for 4 hours reduced GnRH receptor expression by 50% (157). Lu et al confirmed that adiponectin reduced basal and GnRH-stimulated-LH release in an AMPK-dependent manner in an LβT2 pituitary cells line and decreased plasma LH in vivo in male mice (158).

Compared with wild-type mice, adiponectin knockout C56BL/6J female mice had similar weight and insulin sensitivity but fewer GnRH immunoreactive neurons (adiponectin knock out: 117, wild type: 158), reduced ovulatory LH (6.8 vs 9.5 ng/mL), and a decreased number of ovulated oocytes (5.4 vs 28.2) (159). In vitro studies indicate that adiponectin directly impairs follicle growth in the ovaries as apoptosis-related factors were increased in GCs in adiponectin knockout mice (159). However, fertility is retained, suggesting only a modulatory action of adiponectin (158, 160).

Adiponectin levels are at least 2 to 3 times higher in females and are reduced in individuals with type 2 diabetes, IR, androgens, or high BMI (161-163). In women with PCOS, adiponectin is inversely related to BMI (164). Women with PCOS and BMI ≥ 25 kg/m^2 had lower levels of adiponectin and higher levels of insulin than women with PCOS and BMI < 25 kg/m^2 (adiponectin in women with BMI ≥ 25 kg/m^2: 3.7 mg/L; women with BMI < 25 kg/m^2: 35.5 mg/L) (164). Although emerging human data suggest an association between obesity and adiponectin levels, the extent to which altered adiponectin levels directly modulates peripheral or central reproductive tissues and contributes to reproductive capability in women with obesity remains unclear.

Anti-Müllerian Hormone in Women With Obesity

Anti-Müllerian hormone (AMH) is produced by GCs of ovarian follicles and is regarded as a surrogate marker of ovarian reserve and fertility (165). Locally, AMH reduces follicular sensitivity to FSH and so inhibits follicular recruitment (165). In women with PCOS, AMH corresponds to the number of PCOS features (31) and the degree of severity of the PCOS phenotype (166). Increased AMH levels negatively correlate with serum FSH levels ($R = -0.3$, $P = .018$) potentially leading to defective follicular maturation and follicular arrest (167). Additionally, metformin treatment results in improvements in PCOS clinical parameters and a decrease in AMH levels (168).

The impact of obesity on AMH remains unclear. Some studies have reported that AMH negatively correlates with BMI (169, 170); however, others have showed no change (171, 172). Park and colleagues found that AMH was inversely correlated with BMI, and women with obesity had 1.5 fold lower AMH levels than lean women (173). A recent meta-analysis of 26 studies found that AMH was significantly reduced in obese populations by 1.08 ng/mL compared with nonobese populations (95% CI −1.52 to −0.63) (174). This was in contrast to Oldfield et al, who reviewed 13 studies of AMH in women and found no impact of obesity on AMH levels (175).

If AMH is indeed reduced by obesity, this could be explained by increased GC apoptosis, as evidenced by increased apoptotic markers (176) and impaired Akt/FoxO3a signaling (177) in HFD-induced obese mice. Leptin levels from the follicular fluid of infertile women positively correlated with body fat mass and fat mass ($r = 0.55$ and $r = 0.58$; both $P < .0005$) (178) and in vitro treatment of GCs with recombinant leptin suppresses AMH expression and reduces AMH levels in follicular fluid (179).

IR is amplified by obesity; some studies have reported a negative correlation of serum AMH with IR in lean women with PCOS (180), whereas others have found no correlation (181), and no difference in AMH levels amongst women with different PCOS phenotypes or across different BMIs (182, 183). In contrast, La Marca et al showed that 14 women with PCOS with mean BMI of 25 kg/m^2 had higher AMH levels than non-PCOS controls, and their AMH levels positively correlated with homeostatic model assessment of IR scores (184). AMH levels are correlated with fasting insulin levels in women with PCOS; women with PCOS and BMI $\geq$ 25 kg/m^2 had higher AMH levels than lean women with PCOS (185). Treatment with metformin for 8 months results in a 20% reduction in AMH in women with PCOS, while BMI was also reduced from 37.1 to 35.7 kg/m^2 (186). This suggests that AMH could correlate with IR in women with PCOS and improve with treatment to reduce IR.

AMH also has an additional action on GnRH neuronal migration during development. In vitro studies have shown that migratory GnRH neurons in mouse and human fetuses express AMH and AMH receptor 2 (187). AMH also exerts a direct central action on GnRH neurons in vitro to increase GnRH-dependent pulsatility and secretion (188). Thus, a reduction in AMH with obesity could theoretically result in a reduction in hypothalamic GnRH pulsatility as seen in obesity-related hypogonadism. Conversely, GnRH can acutely lower serum AMH (189); in a study by Van Helden et al, a GnRH bolus (2.5 mg/kg) was injected into both female and male children ($n = 31$) and adults ($n = 78$), and AMH was observed to be significantly decreased at 30 minutes after injection in all groups (189).

If AMH is reduced by obesity, one could expect an increase in AMH following weight loss. However, a prospective cohort study of 183 women with obesity (BMI 39.6 kg/m^2; AMH 2.66 ± 3.71 µg/L) and 63 women with PCOS and obesity (BMI 39.6 kg/m^2; 5.47 ± 4.89 µg/L), found there was no change in serum AMH levels at 12 months after weight loss of 10% to 12% achieved with dietary intervention (190).

Further, AMH could even be reduced following more substantial weight loss after bariatric surgery. In women with obesity (BMI ~45 kg/m^2), AMH was decreased in 14 women with PCOS (5.44 to 4.25 ng/mL) and in 18 non-PCOS women (1.83 to 1.36 ng/mL) at 12 months after bariatric surgery after a mean weight loss of 65% (170). In 10 adolescent girls aged 13-16 years with PCOS and obesity, AMH was reduced by 1.4 ± 1.8 ng/mL after a 1-year lifestyle intervention only in those who lost weight (change in BMI −3.8 kg/m^2), but increased by 0.4 ± 0.8 ng/mL in those who gained weight (BMI increase by 1.9 ± 1.7 kg/m^2) (191). Thus, overall AMH is either unaltered or reduced in women with PCOS and obesity and is either unchanged or could even be

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decreased further after weight loss intervention, potentially consistent with some resolution of the PCOS-associated raised AMH levels.

Impact of Obesity on Androgen Levels, and Their Effect on LH Secretion

Levels of testosterone and dihydrotestosterone in women are one-fifteenth of that seen in men, yet they play an important role in regulation of the HPG axis in women (192). Control of androgen secretion in women is multifaceted and occurs partly in response to stimulation by LH as well as modulation by intraovarian regulatory factors (eg, inhibin B) that coordinate thecal androgen production with conversion to estrogen in GCs (193). Both global and hypothalamic neuron-specific androgen receptor knockout female mice have a reduction in hypothalamic *Kiss1* mRNA with associated mistimed/diminished GnRH/LH surges (194, 195).

Dysregulation of androgens is regarded as being central to the pathophysiology of PCOS, with 80% to 90% of patients having clinical or biochemical hyperandrogenism (193). Prenatal androgen treated female mice have increased androgen receptor mRNA expression but reduced progesterone and dynorphin mRNA expression in kisspeptin neurons within the arcuate nucleus (196). Androgens are hypothesized to cause a reduction in sex steroid–mediated negative feedback on GnRH neurons (197), contributing to the abnormally high basal levels of LH and pulsatility seen in PCOS.

Androgens are also elevated in women with obesity who do not have PCOS. In a cross-sectional study comprising 1900 premenopausal women with severe obesity, hyperandrogenemia was present in 32% (n = 616) of women with obesity without PCOS and 45% (n = 845) of women with obesity and PCOS (198). Several studies have shown a positive association between free testosterone level with visceral adipose tissue and abdominal fat (199-201). Increased androgen production is additionally due to increased conversion of androstenedione to testosterone via 17β-hydroxysteroid dehydrogenase 5 in subcutaneous fat, expression of which has been found to positively correlate with BMI (r = 0.506) (201).

Supraphysiological testosterone levels (at least 2 times physiological level) reduce serum LH in female rats (202) and in lean healthy women (203). In another study, increasing testosterone levels by 3-fold over a 12-hour period in healthy women (to 120 ng/dL = 4.1 nmol/L) resulted in increased LH pulsatile secretion by ∼50%, but when testosterone levels were raised by 6-fold (to 245 ng/dL = 8.5 nmol/L), mean LH fell by 22% due to a fall in basal LH (204). Women with PCOS had higher pulse frequency and were more impervious to the effect of testosterone and only exhibit a reduction in basal LH when testosterone levels were raised to 10.4 nmol/L (204). These studies indicate that hyperandrogenism may affect LH and contribute to a decrease in gonadotropin levels in non-PCOS women with obesity, who have not been exposed to androgens prenatally. Pre-existing exposure to higher androgen levels and already heightened GnRH pulsatility in women with PCOS could lead to a higher threshold for androgen-induced inhibition of GnRH-LH (204). In summary, lower levels of androgens could be stimulatory to GnRH pulsatility in women without PCOS, but higher levels could potentially contribute to a reduction in the LH levels as observed in obesity related hypogonadism.

Furthermore, obesity can lead to androgen production directly in adipose tissue in both women with and without PCOS. 17β-Hydroxysteroid dehydrogenase 5 (also known as aldo-keto reductase family 1 member C3, AKR1C3), catalyzes the conversion of androstenedione to testosterone (which in turn can be activated to dihydrotestosterone, by 5α reductase type 1, SRD5A1, which is also abundantly present in adipose tissue). Expression of AKR1C3 in buttock subcutaneous adipose tissue was decreased in 6 women with obesity without PCOS at a median duration of 10 weeks who achieved weight loss of more than 10% of initial body weight on a very low–calorie diet (425 kcal/day) (205). IR and resultant hyperinsulinemia are also strongly linked to increased androgens in a positive feedback loop (193). Insulin stimulates ovarian androgen production by binding to the insulin receptor using inositol-glycan as mediators (206). In adipocytes, insulin can induce conversion of classical and 11-oxygenated androgens via stimulating expression of AKR1C3 resulting in potent androgen production (207). Increased *KLF15* expression in the ovary also leads to upregulation of 17β-hydroxysteroid dehydrogenase leading to increased testosterone biosynthesis (208). Additionally, hyperinsulinemia decreases SHBG in both PCOS and non-PCOS populations by decreasing its synthesis in the liver (209). Decreases in SHBG increase availability of free androgens. Treatment with liraglutide 3 mg daily in women with obesity (BMI > 30 kg/m^2) and PCOS (n = 55) for 32 weeks increased SHBG and decreased the free androgen index from 6.9 ± 0.6 to 5.98 ± 0.6 compared with placebo, where it increased from 5.6 ± 0.4 to 6.4 ± 0.75 (210). In another study, treatment with liraglutide 1.8 mg/day for 26 weeks reduced free testosterone by 0.005 nmol/L (95% CI −0.009 to −0.001) and free androgen index by 1.34 (−2.19 to −0.48) in women with PCOS and a BMI > 25 kg/m^2 (211). Together, these data suggest that hyperinsulinemia due to IR could also contribute to the pathophysiology of obesity-related hypogonadism by directly and indirectly increasing androgen levels.

Hyperandrogenemia increases uric acid by inducing hepatic metabolism of purine nucleotides and increasing purine renewal in kidney (212, 213). Hyperuricemia in turn induces IR through suppression of both basal and glucose-stimulated insulin secretion (214). Consequently, serum uric acid was positively associated with visceral obesity, dyslipidemia, and hypertension (215). Interestingly, uric acid to creatinine ratio strongly correlated with free androgen index (r = 0.81) (216). Women with obesity and PCOS had significantly higher uric acid to creatinine ratio than those without PCOS (216). The combination of uric acid to creatinine ratio and free androgen index increased the odds of PCOS in women with obesity by 4.3-fold (95% CI 3.4-7.6) (216).

Obesity is associated with both inflammation and hyperandrogenism. Recently, inflammation has been suggested to directly increase ovarian androgen production (217). An inflammatory stimulus increased androstenedione production from rat ovarian theca interstitial cells, and this effect could be blocked by a nonsteroidal anti-inflammatory drug (218). Furthermore, a 3-week pilot trial in women with PCOS showed that ibuprofen (400 mg twice daily in women with bodyweight <70 kg; or 400 mg 3 times daily if >70 kg) reduced total testosterone from 0.75 ± 0.06 ng/mL to 0.59 ± 0.05 ng/mL (P = .008) (219). These studies support the

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concept of inhibiting ovarian hyperandrogenism by suppressing inflammatory pathways.

Gut microbiome

Gut microbiota could influence reproductive health and cause disrupted sex steroid homeostasis, and conversely sex steroids can influence the composition of the microbiome (220). Metabolic health is associated with broader microbial diversity in the gut microbiome. A greater systemic burden of lipopolysaccharides owing to diet and altered gut microbial permeability induced a greater inflammatory response (221). Intracerebroventricular administration of lipopolysaccharides to ovariectomized rats and female sheep reduced expression of GnRH in the hypothalamus, leading to a subsequent reduction in gonadotropins and sex steroids (222, 223).

The gut microbiome of women with PCOS had markedly elevated levels of *Bacteroides vulgatus*, accompanied by reduced glycodeoxycholic acid and tauroursodeoxycholic acid levels (224). Another report found that women with PCOS had lower relative abundance of Tenericutes phylum in their gut microbiota (225). Furthermore, serum testosterone correlated with the alpha diversity of the gut microbiome in women with PCOS (226). Transplantation of fecal microbiota from women with PCOS into female prepubertal mice resulted in infertility and metabolic impairment including altered bile acid metabolism, and reduced levels of the anti-inflammatory IL-22 (as observed in women with PCOS) (224). This suggests that alteration in the gut microbiome could contribute to changes associated with the PCOS-like phenotype.

Letrozole-treated pubertal female rodents results in elevation of testosterone, elevated luteinizing hormone levels, unaltered diestrous levels of estradiol, and a metabolic phenotype like PCOS including weight gain, dysglycemia, hyperinsulinemia, and IR (227). Additionally, these letrozole-treated mice display a shift in gut bacterial alpha and beta diversity (228). Arroyo et al showed that certain species of the gut bacterial genera such as *Lactobacillus*, *Dorea*, *Lachnospiraceae*, *Ruminococcus*, *Roseburia*, *Sutterella*, *Bifidobacterium*, *Parabacteroides*, and *Blautia* were altered with letrozole treatment (229). Thus, letrozole-treated mice were observed to have reduced bacterial species richness (alpha diversity) (229).

Furthermore, a normal microbiome appeared to be protective against developing a PCOS-like phenotype in response to letrozole. A cohousing study showed that letrozole-treated mice had significant improvement in the reproductive and metabolic PCOS-like phenotypes after exposure to placebo-treated mice (230), which was associated with corresponding changes in bile acid levels (231). Thus, dysbiosis of the gut microbiome in women with PCOS represents an additional possible mechanism linking metabolic dysfunction, inflammation, and reproductive health.

Summary of Evidence for non-PCOS Female Obesity-Related Secondary Hypogonadism

While women with obesity and hypogonadism are often labelled as having PCOS, there are mechanistic differences causing the increased activation of hypothalamic GnRH neurons observed in lean PCOS, as opposed to the reduction in GnRH neuronal activity in obesity-related secondary hypogonadism.

Our review highlights that the following features are more associated with obesity-related secondary hypogonadism than PCOS in lean women. LH pulse amplitude is reduced with obesity, both with and without PCOS, whereas raised LH levels are more typically found in lean women with PCOS. The diagnosis of PCOS can therefore be less clearcut in women with obesity, as raised androgens can occur even in the absence of PCOS, menstrual disturbance can occur due to obesity-related secondary hypogonadism, and imaging of the ovaries to identify polycystic ovarian morphology can be more challenging. Notably, LH pulse frequency remains elevated in women with PCOS despite obesity, suggesting that assessment of LH pulse frequency could be informative as to the presence of underlying PCOS in women with obesity, although there are only limited number of studies investigating this to date.

In this review, we have discussed the various mechanisms that can contribute to "female obesity-related secondary hypogonadism" (FOSH). Obesity is associated with an increase in leptin levels, which can result in hypothalamic leptin resistance and a reduction in GnRH pulsatility and LH levels. Lower LH levels can occur due to increased clearance of endogenous LH in women with obesity as well as reduced pituitary response to GnRH. AMH levels are reduced in some women with obesity, and theoretically a reduction in AMH levels with obesity could lead to reduced stimulation of GnRH neurons and thus LH levels. Androgens are increased in women with obesity, and markedly elevated levels could contribute to a reduction in LH levels (205). Obesity is associated with an increase in inflammatory markers that can also contribute to the reduction in LH levels and hypogonadism observed in women with obesity.

Thus, thorough evaluation of reproduction endocrine function in women with obesity and hypogonadism is needed to differentiate those with PCOS from those with obesity-related secondary hypogonadism. It is possible that persistence of increased LH pulse frequency can be used to identify underlying PCOS rather than obesity-related secondary hypogonadism. However, assessment of LH levels does not form a major part of current assessment of women with possible PCOS. Although unsupported by data, it is attractive to believe that reaching a more precise phenotyping of women with hypogonadism and identification of the specific mechanisms causing hypogonadism in an individual woman could lead to more tailored treatments and better outcomes. Indeed, altered feedback to estradiol (which underpins many ovulation induction treatments) is recognized to be present in women with obesity.

Conclusion

In conclusion, we have summarized evidence in support of the concept of a distinct FOSH that is distinct from PCOS. Further dedicated research is needed to confirm the existence of FOSH and to specify diagnostic criteria for it, to deepen our understanding of reproductive dysfunction in women with obesity.

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Author Contributions

P.C.E., M.P., A.Q., C.W., H.L., S.A., B.M., A.A. contributed to writing the first draft of the manuscript. P.C.E., A.M., A.N.C., T.T., S.F., W.S.D., A.A. reviewed and edited the manuscript. A.A. takes final responsibility for this article.

Disclosures

The authors have nothing to disclose and no conflict of interest.

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