REVIEW

FSH-metabolic circuitry and menopause

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Abstract

FSH has a primary function in procreation, wherein it induces estrogen production in females and regulates spermatogenesis in males. However, in line with our discoveries over the past decade of non-unitary functions of pituitary hormones, we and others have described *hitherto* uncharacterized functions of FSH. Through high-affinity receptors, some of which are variants of the ovarian FSH receptor (FSHR), FSH regulates bone mass, adipose tissue function, energy metabolism, and cholesterol production in both sexes. These newly described actions of FSH may indeed be relevant to the pathogenesis of bone loss, dysregulated energy homeostasis, and disordered lipid metabolism that accompany the menopause in females and aging in both genders. We are therefore excited about the possibility of modulating circulating FSH levels toward a therapeutic benefit for a host of age-associated diseases, including osteoporosis, obesity and dyslipidemia, among other future possibilities.

Key Words

- ▶ FSH
- ▶ menopause
- ▶ osteoporosis
- ▶ obesity
- ▶ bone

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Introduction

The early menopausal transition is associated with a sharp rise in serum FSH levels, even when serum estrogen levels remain within normal limits (Randolph *et al.* 2003). The median age of menopause in the United States is 51.4 years, while the average age for the start of the perimenopausal transition is 47 years, as defined by The Stages of Reproductive Aging Workshop (STRAW) (Harlow *et al.* 2012). This transition is marked by changes in bone remodeling, body composition, and energy metabolism, all of which are most prominent during the late perimenopause (Perrone *et al.* 1995, Ebeling *et al.* 1996, Ito *et al.* 1999, Chapurlat *et al.* 2000, Recker *et al.* 2000, Seifert-Klauss *et al.* 2002, 2006). The Study of Women's Health Across the Nation (SWAN) studied a large cohort of perimenopausal women (42–52 years of age) and examined biological parameters, including bone mass and body fat, among others, in relation to endogenous hormone levels at various stages of the perimenopause and post-menopause (Sowers *et al.* 2003, 2006). The study,

performed longitudinally for over a decade, revealed a decline in bone mineral density (BMD), together with increased body weight, visceral adiposity, disrupted energy homeostasis and reduced physical activity (Thurston *et al.* 2009, Senapati *et al.* 2014). The occurrence of these metabolic aberrations in the face of rising serum FSH levels with relatively unchanged serum estrogen prompted our consideration of alternate mechanism(s) of menopausal bone loss and obesity rather than the generally accepted unitary attribution to estrogen deficiency.

We published the first evidence for a role of FSH in the regulation of bone mass in mice (Sun *et al.* 2006). FSH increased bone resorption by enhancing the genesis and function of osteoclasts and increasing survival (Sun *et al.* 2006, 2010). More recent studies have shown that FSH is a primary regulator of body fat and energy homeostasis (Liu *et al.* 2015, 2017). Notably, we found increases in bone mass and lowered body fat in mice treated with an anti-FSH antibody, as well as in mice genetically deficient in

FSH or the FSHR (Sun *et al.* 2006, Liu *et al.* 2017, Rosen & Zaidi 2017, Ji *et al.* 2018). A further interesting observation was the induction of thermogenic 'beige' adipose tissue and, as a consequence, increased energy expenditure in antibody-treated mice (Liu *et al.* 2017). Extending our premise for the existence of novel pituitary-metabolic circuits of physiological and medical significance (Zaidi 2007, Zaidi *et al.* 2018a,b), the link between serum FSH, osteoporosis and obesity lays a firm foundation for using a single FSH-blocking agent to prevent and/or treat both postmenopausal osteoporosis and obesity.

Clinical association between FSH and bone loss

The SWAN study reported that the rate of bone loss is highest during the perimenopause period despite normal estrogen levels. The mean annual decrement in lumbar spine BMD was highest between 1 year prior to and 2 years after the last menstrual period, although the BMD values remained within normal range at this time (Sowers *et al.* 2003, 2006, Crandall *et al.* 2013). This bone loss during the menopausal transition has been confirmed by increased bone turnover markers, including N-terminal telopeptide (NTX) (Seifert-Klauss *et al.* 2002). A bone biopsy cohort showed evidence of increased osteoclastic resorption, noted as accelerated activation frequency as early as one year after menopause (Recker *et al.* 2004). Bone loss was found mainly to occur in trabecular bone, characterized histologically by decreases in trabecular number and increased trabecular perforations. Marked changes in trabecular bone structure, noted on micro-CT (μ -CT) and histomorphometry, included decreased bone volume density and trabecular number, and increased trabecular spacing (Akhter *et al.* 2007).

A number of correlative studies have also confirmed a relationship between rising serum FSH levels and bone loss, independently of serum estrogen (Adami *et al.* 2008, Xu *et al.* 2009, Gallagher *et al.* 2010, Wu *et al.* 2010, Garcia-Martin *et al.* 2012, Crandall *et al.* 2013). Notably, the Italian Bone Turnover Range of Normality (BONTURNO) study and a study from Spain both showed a positive correlation between high serum FSH and bone turnover markers, including osteocalcin and C-terminal telopeptide of type 1 collagen (CTX), irrespective of estradiol levels (Adami *et al.* 2008, Garcia-Martin *et al.* 2012). Likewise, a study investigating the relationship between urinary cadmium, serum FSH and femoral BMD in a cohort of women from US NHANES III (aged 42–60 years) showed an independent inverse association between FSH and

BMD in certain groups (Gallagher *et al.* 2010). Similarly, Cannon and coworkers reported an inverse relation between FSH and BMD, independent of variables, such as serum estradiol, LH and inhibin B in 36 women between the ages of 20 and 50 years (Cannon *et al.* 2010). Multiple studies from China have also reported strong correlations between high serum FSH and bone loss, using bone turnover markers, BMD, and *ex vivo* bone resorption genes as surrogates (Xu *et al.* 2009, Wu *et al.* 2010, Cheung *et al.* 2011, Wang *et al.* 2015).

Studies investigating the patterns of bone loss in amenorrheic women have allowed a further delineation of the role of FSH on bone turnover independent of estrogen. Although women with either hypogonadotropic amenorrhea or hypergonadotropic amenorrhea were noted to have lower lumbar spine BMD compared with eumenorrheic controls, the hypergonadotropic group (FSH >40 IU/L) had significantly lower BMD compared with the hypogonadotropic amenorrheics (Devleta *et al.* 2004). In contrast, in a conflicting study FSH was found not to be an independent variable affecting such BMD differences in adolescent girls with hypergonadotropic or hypogonadotropic hypogonadism (Ozbek *et al.* 2016). These conflicting results could at least in part be explained by the difference in the mean age of females in the two studies (around 32 years vs 14 years), and may relate to the difference in the duration of excess FSH exposure between the two groups.

Molecular studies on FSH and bone loss

Starting with our discovery of the pro-resorptive action of FSH, there is now a body of incontrovertible evidence for a direct effect of FSH on bone both *in vitro* and *in vivo* (Sun *et al.* 2006, Liu *et al.* 2010a,b, Wang *et al.* 2015). Of note, ovariectomy-induced bone loss in rats was accentuated by the injection of FSH (Liu *et al.* 2010a). Furthermore, the same group reported a protective effect on bones of the injection of an FSH inhibitor (Liu *et al.* 2010b). As noted earlier, we have blocked FSH action using an epitope-specific anti-FSH antibody to find a rescue of post-ovariectomy bone loss (Zhu *et al.* 2012a, b, Ji *et al.* 2018).

This action of FSH on bone appears to be mediated primarily by a distinct isoform of the FSHR, which is shorter than the full-length ovarian FSHR. The presence of the FSHR isoform on human CD14⁺ cells and osteoclasts has been confirmed using nested PCR primers specific to the shorter isoform (Robinson *et al.* 2010, Tourkova *et al.* 2015). There is also compelling data using near-infrared spectroscopy for the binding of fluorophore-labeled

FSH (FSH-CH) to bone *in vivo*, in addition to ovarian and testicular tissues (Feng *et al.* 2017, Ji *et al.* 2018). Importantly, co-injection of a 100-fold molar excess of unlabeled FSH markedly attenuated these signals, demonstrating specificity (Feng *et al.* 2017, Ji *et al.* 2018).

Unlike their coupling to $G\alpha_s$ in ovarian follicular cells, FSHRs in bone are coupled to $G\alpha_{12\alpha}$ resulting in reduced cyclic AMP levels. This action is also associated with the sensitization of MAP kinase, NF κ B and AKT pathways to stimulate osteoclastogenesis (Sun *et al.* 2006). In addition, FSH increases osteoclast formation by enhancing the expression of RANK, along with an increased production of cytokines, including interleukin-1 β , tumor necrosis factor- α and interleukin-6 (Iqbal *et al.* 2006, Cannon *et al.* 2010, 2011).

Genetic studies investigating SNPs in the *Fshr* gene have further substantiated a link between FSH and bone loss. Specifically, an activating rs6166 SNP in the *Fshr* gene results in an increased risk of osteoporosis as seen by lower BMD and increased bone turnover markers in this group of women (Rendina *et al.* 2010). Moreover, digenic combinations resulting in skeletal protection were noted to have involvement of the *BMP15* and *Fshr* genes (Mendoza *et al.* 2012).

Development of highly specific blocking antibodies to FSH

The biggest challenge with studying FSH action on bone *in vivo* is the confounding effects of estrogen, which change simultaneously with altered FSH signaling. While FSH can cause bone loss, it also promotes the secretion of ovarian estrogen – its primary biological function – and this results in protection of the otherwise pro-resorptive action of FSH.

Our anti-FSH antibody was generated with the goal of inhibiting FSH with minimal effects on estrogen. For this, we selected a 13-amino-acid-long peptide sequence on FSH β , which, we knew from computational modeling, would prevent the access of FSH into the FSHR pocket (Zhu *et al.* 2012a,b, Ji *et al.* 2018). Thus, we were able to titrate circulating levels of the antibody to inhibit FSH action on osteoclasts, while sparing ovarian function (Zhu *et al.* 2012a). This level of specificity would not have, at least in principle, been obtainable with an anti-FSHR antibody. When the polyclonal antibody was injected into ovariectomized mice, there was not only a reduction in osteoclastic bone resorption, but also an increase in new bone formation, prompting the exploration of osteoblastic

FSHRs (Zhu *et al.* 2012a). Notably, we discovered that FSHRs were present on osteoblast precursors, rather than on mature bone-forming osteoblasts. We have now raised and tested the effect of monoclonal anti-FSH antibodies against the mouse and corresponding human sequences, which differ by two amino acids (Ji *et al.* 2018). Both antibodies, Mf4 and Hf2, effectively prevent bone loss post ovariectomy, with IC₅₀s of 5.4 and 6.1 nM for inhibition of osteoclastogenesis, respectively (Ji *et al.* 2018).

FSH effects on body fat and thermogenesis

Weight gain and changes in body composition are typically seen in women around the menopausal transition. Overall weight gain is prominent in perimenopausal and early postmenopausal women alike and has largely been attributed to aging independent of menstrual status (Sternfeld *et al.* 1999, Demerath *et al.* 2011, Trikanathan *et al.* 2013, Zsakai *et al.* 2015). However, changes in body composition, specifically those accruing from visceral adiposity, have been closely linked to the menopausal transition (Gambacciani *et al.* 1999, Toth *et al.* 2000, Sowers *et al.* 2007, Lovejoy *et al.* 2008, Franklin *et al.* 2009, Lee *et al.* 2009, Ho *et al.* 2010, Janssen *et al.* 2010). Notably, high FSH levels are associated with increases in waist circumference, waist-hip ratio and visceral fat volume (Gavaler & Rosenblum 2003, Sowers *et al.* 2007, Seth *et al.* 2013). Additionally, there seems to be a reduction in lean mass associated with higher FSH levels (Gourlay *et al.* 2012, Jaff *et al.* 2015, Liu *et al.* 2017). In contrast, the 11-year SWAN follow-up and the Pan Asia Menopause study report lower FSH levels in women with higher BMI. This discrepancy may be due to feedback inhibition of FSH secretion by estrogen produced from aromatization in fat tissue (De Pergola *et al.* 2006, Ausmanas *et al.* 2007, Tepper *et al.* 2012). Interestingly, there is no association between FSH and BMI in males (Bieniek *et al.* 2016, Yamacake *et al.* 2016), despite evidence for the reduction of body fat by FSH inhibition in male mice (Liu *et al.* 2017) (see below). However, there is limited evidence for a role for FSH in the pathophysiology of metabolic syndrome (Stefanska *et al.* 2014).

Our overall results attest to a role for FSH in promoting adiposity (Liu *et al.* 2017). Similar to bone, the adipocyte FSHR, which at least in 3T3.L1 cells is a variant of the ovarian FSHR, couples to $G\alpha_i$ – this results in reduced cAMP levels and a subsequent decrease in the activation of the mitochondrial protein, uncoupling protein-1 (UCP1) in de-differentiated brown adipocytes (or thermo cells).

This pathway, which opposes β_3 adrenergic stimulation, has been shown in previous studies to be linked downstream to the activation of cyclic AMP response element-binding protein (CREB) and lipogenesis. Expectedly, in differentiated adipocytes derived from 3T3.L1 cells, FSH increases the expression of genes related to lipid metabolism, namely *Lpl*, *Fas* and *Pparg* (Cui *et al.* 2012).

Our anti-FSH antibody, which blocks FSH action in the face of unperturbed estrogen levels, not only reduces adiposity in a variety of mouse models, but also induces the transition of white adipocytes to energy-producing beige adipocytes (Liu *et al.* 2017). These murine models include mature male and female mice fed pairwise or *ad libitum* on a high-fat diet or on normal chow, sham-operated and ovariectomized mice, 8-month-old mice, and *Fshr*^{+/-} mice (Liu *et al.* 2017). The data have been replicated and reproduced at multiple laboratories using multiple modalities, namely quantitative nuclear magnetic resonance (qNMR), dual-energy X-ray absorptiometry (DXA), μ -CT, and manual weighing of tissues (Liu *et al.* 2017, Rosen & Zaidi 2017). Equally impressive declines were noted in subcutaneous, visceral, perigonadal, inguinal and bone marrow fat compartments (Liu *et al.* 2017). The antibody reduced body fat to a level similar to that in haploinsufficient *Fshr*^{+/-} mice, indicating a dominant action of FSH signaling. Importantly, however, the antibody did not further reduce body fat in these mice, establishing *in vivo* specificity (Liu *et al.* 2017).

In addition to inducing leanness, the anti-FSH antibody also induced the production of thermogenic adipose tissue. We noted abundant beige cells in UCP1-stained sections of white adipose tissue. Beiging was contemporaneously documented by implanting Thermo cells into athymic nude mice, as well as through the use of the transgenic ThermoMouse, in which a luciferase (*Luc2*) gene construct is inserted into the *Ucp1* locus to report UCP1 activation. Upon blocking FSH, we found increased LUC2 radiance initially in the brown fat-rich interscapular area, which was followed at around 8 weeks by increased radiance in the inguinal fat, primarily containing white adipose tissue. Quantitative PCR also showed increased expression of brown fat genes in white adipose tissue, namely *Ucp1*, *Cox7*, *Cidea* and *Cox8a*. Finally, the induced beiging was confirmed by documenting an increase in mitochondrial density in the PhAM mouse, as well as through indirect calorimetry using metabolic cages, where increases in energy expenditure and oxygen consumption were noted (Liu *et al.* 2017).

FSH effects on cholesterol metabolism

There is an increased prevalence of dyslipidemia and cholesterol accumulation around the menopausal transition, which has traditionally been linked to estrogen deficiency (Rossouw *et al.* 2002). However, there has been recent evidence that FSH might play a role in increasing hepatic cholesterol production, independent of serum estrogen. A recent study of 278 pre- and perimenopausal women found that serum FSH, total cholesterol (TC) and LDL cholesterol (LDL-C) levels were higher in the perimenopausal group compared with pre-menopausal women, despite similar serum estrogen levels (Guo *et al.* 2019). Serum FSH levels displayed a positive correlation with TC and LDL-C after adjustment for estrogen. Similarly, another study of 588 postmenopausal women noted that subjects with higher serum FSH levels had higher levels of both TC and LDL-C (Serviente *et al.* 2019). In a cohort of 400 postmenopausal Chinese women with a similar relationship between serum FSH, TC and LDL-C, it was noted that significant improvement of lipid levels after hormone-replacement therapy was seen only in women who had $\geq 30\%$ reduction in serum FSH levels (Song *et al.* 2016).

To further substantiate a role for FSH in cholesterol metabolism, Guo *et al.* used ovariectomized mice in which estrogen was clamped by exogenous administration. These mice, when injected with recombinant FSH, showed higher levels of serum TC and LDL-C, and elevated hepatic cholesterol biosynthesis (Guo *et al.* 2019). Furthermore, in contrast to our results where we did not find decrements in serum cholesterol after 8 weeks of anti-FSH antibody treatment (Liu *et al.* 2017), the authors provide compelling evidence that blocking FSH action either through an anti-FSH β antibody or in *Fshr*^{-/-} mice significantly reduced serum and hepatic cholesterol content, without significantly altering estrogen levels (Guo *et al.* 2019). The presence of the FSHR in the liver was also established in both human and mouse liver samples using RT-PCR, *in situ* hybridization and immunofluorescent labeling (Guo *et al.* 2019). Finally, FSH was shown to upregulate liver HMG-CoA reductase, a rate-limiting enzyme for cholesterol biosynthesis; this effect appears to be regulated through the activation of the transcription factor sterol regulatory element-binding protein 2 (SREBP-2) (Pertusa *et al.* 2007, Guo *et al.* 2019). Another possible mechanism of elevated circulating LDL-C is the FSH-mediated reduction in hepatic LDL receptors which results in decreased endocytosis of LDL-C. This was shown both by reduced hepatic LDLR expression in ovariectomized mice, as well

as FSH-mediated LDLR inhibition in HepG2 cells (Song *et al.* 2016). Given the small number of studies on the topic, there is room for further exploration of the role of FSH on lipid and cholesterol metabolism and the underlying mechanisms.

Effects on cardiovascular risk

Although several studies have reported an association between serum FSH levels and markers of cardiovascular risk, it remains a controversial subject with lack of consistent findings. The Assessment of the Transition of Hormonal Evaluation and Noninvasive Imaging of Atherosclerosis study used contrast-enhanced CT angiography and carotid ultrasound to find that subclinical atherosclerosis is prevalent in perimenopausal women, with the prevalence of any coronary plaque being as high as 35.5% (Munir *et al.* 2012). Of note was that the number of aortic plaques was associated directly with serum FSH levels, but was unrelated to serum estrogen (Munir *et al.* 2012).

Similarly, a study from Brazil noted a significant positive correlation between serum FSH and carotid intima-media thickness, a surrogate for atherosclerosis (Celestino Catão Da Silva *et al.* 2013). Furthermore, data from a SWAN cohort showed lower serum FSH levels to be associated with a lower carotid intima-media thickness compared to the mid and high FSH groups, although the latter had a superior cardiovascular disease risk profile (El Khoudary *et al.* 2016). To the contrary, the SPECT-China study, a large, multi-center study on 2658 postmenopausal women, showed a negative association between serum FSH levels and atherosclerotic cardiovascular risk (Wang *et al.* 2017).

FSH and the biology of aging

Aside from what is now emerging as a critical function for high plasma FSH in causing physiologic perturbations of menopause, there has also been recent speculation for its role in the aging process *per se* in both sexes (Bartke 2017). Even in men, serum FSH levels rise by 3% per annum (Feldman *et al.* 2002). In murine models, enhancements in longevity are associated with reduced FSH levels (Bartke 2017). Namely, *Ames* and *Laron* dwarf mice, which are deficient in the pituitary transcription factor PROP1 and growth hormone receptor, respectively, display reduced serum FSH, in addition to other pituitary hormones. These mice display increases in brown adipose tissue, similar to what we have reported using our anti-FSH

antibody (Bartke *et al.* 2013, Bartke 2017, Liu *et al.* 2017). In addition, there is an impressive increase in lifespan and reduced biological aging in these mice. However, further studies are needed to decipher the precise mechanism(s) underscoring increased longevity. In addition to possible effects on lifespan *per se*, one might speculate that the relationship between FSH and obesity may confer a survival benefit when FSH is low, particularly as adiposity can independently affect metabolic and cardiovascular outcomes, thus decreasing longevity.

Conclusion

Extensively validated sets of data provide compelling evidence that FSH inhibition reduces body fat and serum cholesterol, induces beiging and thermogenesis, and increases bone mass. Most of these studies have been carried out in animal models, given the ability to study the effects of FSH, somewhat independent of estrogen. This prompts the question whether a single FSH-blocking agent can have the therapeutic novelty of simultaneously treating and/or preventing osteoporosis, obesity and dyslipidemia, diseases that affect millions of women and men worldwide. Highly targeted monoclonal anti-FSH antibodies are thus being developed currently as first-in-class agents for human use. Further studies in humans examining the effects of varying levels of FSH against the above-mentioned parameters would help delineate its exact role, and the potential use of these FSH-blocking antibodies in clinical practice.

Declaration of interest

M Z is a named inventor on a patent related to FSH and bone, owned by Icahn School of Medicine at Mount Sinai. M Z will receive royalties and/or licensing fees *per* Mount Sinai policies, in case the patent is commercialized. M Z also consults for Merck, Roche, and a number of financial consulting platforms. The other authors have nothing to disclose.

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