FSH, Bone Mass, Body Fat, and Biological Aging

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The Study of Women's Health Across the Nation has taught us that impending ovarian failure during late perimenopause is associated with a sharp rise in serum FSH, which coincides with the most rapid rate of bone loss and the onset of visceral adiposity. At this time in a woman's life, serum estrogen levels are largely unaltered, so the hypothesis that hypoestrogenemia is the sole cause of bone loss and visceral obesity does not offer a full explanation. An alternative explanation, arising from animal models and human data, is that both physiologic aberrations, obesity and osteoporosis, arise at least in part from rising FSH levels. Here, we discuss recent findings on the mechanism through which FSH exerts biological actions on bone and fat and review clinical data that support a role for FSH in causing osteoporosis and obesity. We will also provide a conceptual framework for using a single anti-FSH agent to prevent and treat both osteoporosis and obesity in women across the menopausal transition. (*Endocrinology* 159: 3503–3514, 2018)

It became clear as early as 1989 that women can lose bone between the ages of 35 and 50 in the presence of regular cycles and high serum FSH levels (1). Since then, multiple reports have documented not only early postmenopausal bone loss but also profound decrements in bone mineral density (BMD) before the onset of menopause, particularly during the latter third of the perimenopausal transition (2–11). This bone loss continues unabated for the next two decades (8, 10, 12, 13). The most well-studied population of perimenopausal women is the Study of Women's Health Across the Nation (SWAN), a longitudinal and cross-sectional study that examined several biological parameters, including bone mass, body fat, and physical activity, in an ethnically diverse cohort of >2000 perimenopausal women (9–11). Notably, there were profound reductions in BMD and high resorption rates ~2 to 3 years before menopause, irrespective of ethnicity. This phase of bone loss is associated with increases in body weight and visceral adiposity, as well as dysregulated energy homeostasis and reduced physical activity (14, 15). All these aberrations occurred in the face of rising serum FSH levels when serum estrogen levels were normal (Fig. 1A) (11, 16). This finding suggests alternative mechanisms in parallel with the well-characterized role of estrogen in the regulation of body composition.

In 2006, we published the first evidence that, by increasing bone resorption by osteoclasts, FSH could itself regulate bone mass in animal models (17). More recently, we found that FSH regulates body fat and that blocking FSH action on its receptor, expressed in both bone and fat cells (17, 18), not only increases bone mass but also reduces body fat profoundly and induces thermogenic "beige" adipose tissue (19). These data, replicated in C.J.R.'s laboratory (19, 20), have laid the foundation for using an anti-FSH agent, in our case a monoclonal FSH blocking antibody (18) to treat osteoporosis and obesity concurrently and, in particular, prevent bone loss and visceral adiposity during the later years of the perimenopausal transition.

Bone Loss and Fat Gain Occur Concurrently During Late Perimenopause

Postmenopause is formally defined as the permanent cessation of menstrual periods for 12 consecutive months

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Abbreviations: BMAT, bone marrow adipose tissue; BMD, bone mineral density; BMI, body mass index; CTX, C-terminal telopeptide of type I collagen; FSHR, FSH receptor; STRAW, Stages of Reproductive Aging Workshop; SWAN, Study of Women's Health Across the Nation.

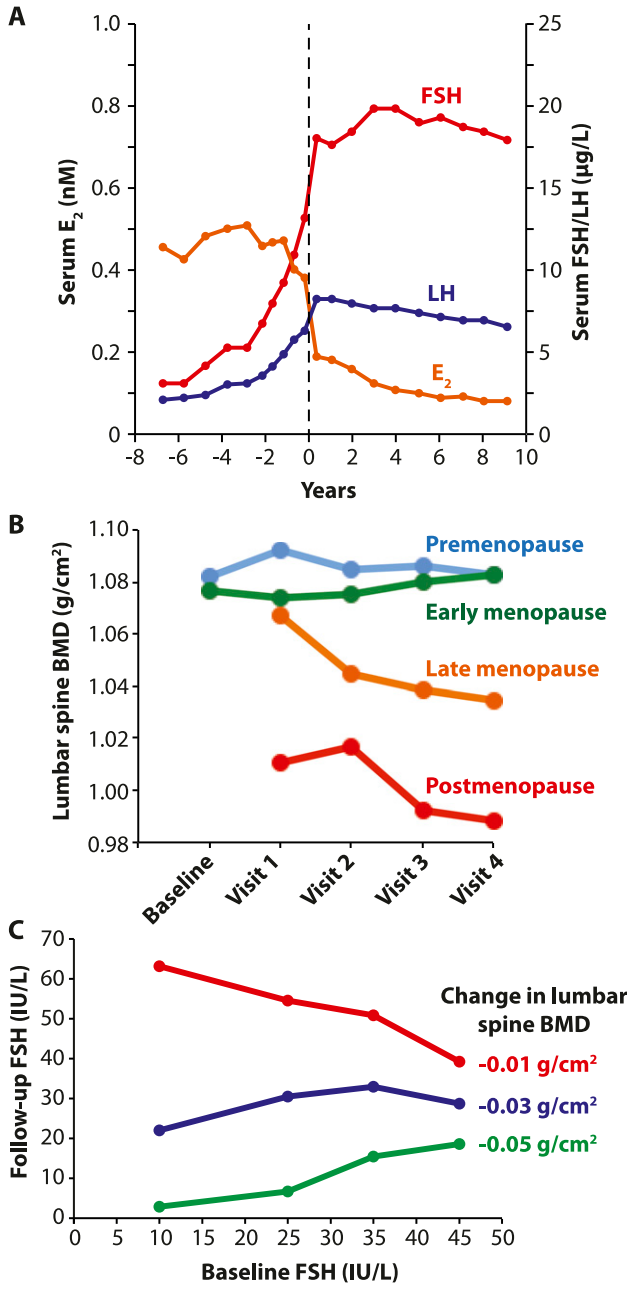

Figure 1. Relationship between serum FSH levels and BMD across the menopausal transition. (A) Hormonal profiles before and after menopause (dashed line) show that serum FSH levels begin to rise even when estrogen is within the normal range. (B) BMD changes over a 5-year timeframe in the SWAN. (C) Correlation between baseline and 4-year measurements of FSH at three levels of BMD change (noted). Figure 1A: Reproduced with permission from Rannevik G, Jeppsson S, Johnell O, et al. A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. Maturitas 1995; 21: 103–113. Figures 1B and 1C: Reproduced with permission from Sowers MR, Jannausch M, McConnell D, et al. Hormone predictors of bone mineral density changes during the menopausal transition. J Clin Endocrinol Metab 2006; 91(4):1261–1267. E_2, estradiol.

in the absence of any other obvious pathological or physiological cause. The median age of US women at the time of menopause is 51.4 years. The Stages of Reproductive Aging Workshop (STRAW) defined perimenopause, also known as the menopausal transition, as the transitory period from reproductive age through to menopause that includes the onset of irregular cycles together with profound endocrine changes (21). Perimenopause begins on average at age 47 years. Perimenopause and postmenopause are both divided into early and late stages (21).

Longitudinal and cross-sectional studies show that rates of bone loss are the highest during perimenopause and that these affect mainly trabecular bone (9–11). The ongoing SWAN has reported more than two decades of observations showing accelerated lumbar spine bone loss in a multiethnic cohort of 2375 eugonadal women between ages 42 and 52 years (Fig. 1B) (9–11, 22). The mean annual change in lumbar spine BMD was greatest 1 to 2 years after the last menstrual period (2.16% per year) (22); this drop occurred even while BMD values remained in the normal range (10, 22). The evidence for interim bone loss was confirmed through increased bone turnover markers during late perimenopause but when menstrual cycles were still regular (8). Bone biopsies in a subgroup of women provided direct evidence for accelerated activation frequency, a measure of osteoclast activity, 1 year after menopause (23). Notably, this acceleration begins during the final years of menopausal transition and continues over the next two decades (8, 10, 12, 13). These high levels of bone resorption and decrements in BMD in perimenopausal women occur even at normal serum estrogen levels (10). However, women in this phase are often not diagnosed by standard BMD assessments.

Furthermore, women undergoing the menopausal transition display a marked deterioration in bone microarchitecture that is synchronous with the rapidity of bone loss, as evidenced by histomorphometry and high-resolution microcomputed tomography of bone biopsies (24). About 13% of trabecular bone loss is attributable to the decreased trabecular number and increased trabecular perforation (24). Interestingly, women lose bone primarily by perforation of trabeculae, whereas men lose bone by trabecular thinning (25). Trabecular perforation causes a significantly greater reduction in bone strength than trabecular thinning, as elegantly demonstrated *via* three-dimensional spatial modeling and Voronoi tessellation (26). This trabecular perforation is an irreversible change, and lost trabeculae cannot be rebuilt.

Perimenopausal and early postmenopausal women generally undergo weight gain and changes in body composition. An increase in body mass index (BMI) among middle-aged women is typically attributable to aging, as multiple studies have demonstrated that women gain weight regardless of menopausal state (27–30). The Healthy Women Study reported an increase in weight of ~2 kg over the 3-year study period in women >40 years

of age. However, there was no difference in the extent of weight gain between premenopausal and early postmenopausal women (31). Likewise, in the SWAN participants, mean weight increased by 2.1 kg over a 3-year follow-up study, further substantiating the premise that menopausal status alone was not associated with a change in weight (32).

In contrast to BMI, climacteric deposition of visceral adipose tissue has been attributed to the menopausal state in several cross-sectional (33, 34) and longitudinal studies (35–38). For example, a higher percentage of fat was found in the trunk region in perimenopausal compared with premenopausal women, whereas fat deposits in the arm were higher in postmenopausal women (39). Moreover, the Michigan cohort in the SWAN showed a 6-year increase of 6 cm in waist circumference and a decrease in skeletal muscle mass of ~0.23 kg (40). All of these changes persisted despite adjustment for chronologic age (40). Importantly, the studies used objective measures such as CT, MRI, or dual-energy X-ray absorptiometry (35, 41–44) to confirm an increase in visceral fat in early perimenopausal and postmenopausal women.

Serum FSH and Bone Loss in Women

Epidemiological data suggest that decrements in bone mass during the perimenopausal transition and after menopause can be predicted by changes in serum FSH (10, 11). SWAN data showed that bone turnover markers and BMD in perimenopausal and early postmenopausal women are independent of serum estrogen, and they displayed a strong negative correlation with serum FSH. Importantly, 4-year changes in serum FSH levels predicted decrements in BMD (Fig. 1C) (10, 16). Furthermore, low serum FSH and high estrogen have been associated with lower rates of lumbar spine bone loss during menopausal transition (22).

The predictive value of serum FSH for bone loss has been confirmed in a number of clinical studies across the globe, including Europe (45–49). The Italian Bone Turnover Range of Normality study showed that women with serum FSH levels >30 IU/L displayed significantly higher bone turnover markers than age-matched women, despite having regular menstrual periods (45). Similarly, serum osteocalcin and C-terminal telopeptide of type I collagen (CTX) levels positively correlated with serum FSH but not with estradiol in a cross-sectional study of 92 postmenopausal women from Spain (47). Likewise, there were strong correlations between serum FSH and femoral neck BMD in the US National Health and Nutrition Examination Survey III cohort of women between 42 and 60 years of age (46). Univariate regression analyses further showed that BMD was inversely related to serum FSH in premenopausal and early menopausal women in a recent US cross-sectional study (50). One study did not show a relationship between bone mass and FSH or, indeed, estrogen (51).

Relationships between high serum FSH and bone loss have been extensively documented in Chinese cohorts. Strong correlations between high levels of bone loss, increased bone turnover markers, and high serum FSH levels have been noted (48, 49), such that, for example, women in the highest quartile of serum FSH lost bone at a rate 1.3 to 2.3 times higher than those in the lowest quartile (52). In another Chinese cohort, high serum FSH levels tracked with increased *ex vivo* expression of bone resorption genes, namely *RANK*, *ACP5*, *MMP9*, and *CTSK* (53). Likewise, a more recent analysis of perimenopausal women between 45 and 50 years of age revealed a strong correlation between serum levels of CTX and FSH (54). CTX levels were higher when serum FSH levels were >40 IU/L (54). Further substantiating this relationship, healthy women between 20 and 82 years of age showed a strong correlation between serum FSH and BMD at different skeletal sites and reported a higher prevalence of overt osteoporosis when serum FSH levels were in the upper quartile (49).

Although these studies are correlative, there are clinical indications that serum FSH may contribute to the bone loss that has hitherto been attributed solely to declining estrogen. Most notably, women with hypergonadotropic hypogonadism with mean serum FSH levels of ~35 IU/L displayed greater bone loss than those with hypogonadotropic hypogonadism with mean serum FSH ~8 IU/L (55). Concordant with this finding, women with functional hypothalamic amenorrhea have been found to develop only modest bone loss over time (56, 57).

Finally, and perhaps most importantly, evidence from genetic polymorphisms points to the function of the FSH receptor (FSHR) in human pathophysiology. An activating $FSHR^{N680S}$ polymorphism in women results in high resorption markers and low bone mass (58). Likewise, digenic combinations between wild-type genotypes of 3′ untranslated region or IVS4 markers for the *CYP19A1* gene and the *BMP15* and *FSHR* genes yield skeletal protection (59). Together, these data suggest a modulatory effect of FSH on human bone physiology and a role in the pathophysiology of postmenopausal osteoporosis.

Direct Action of FSH on Bone in Mice

Evidence for a direct effect of FSH on bone cells has accumulated since our discovery of a proresorptive action (17). In bone, FSH acts through a distinctly shorter FSHR isoform to increase osteoclast formation, function, and survival (17, 53, 60–62). The early failure to identify FSHR on osteoclasts was probably caused by the use of

PCR primers that targeted the full-length ovarian FSHR (63, 64). We and other have repeatedly detected FSHR in human $CD14^+$ cells and osteoclasts by using nested PCR to amplify regions containing an intron to avoid genomic DNA contamination, with Sanger sequencing to confirm the identity of the PCR products (60, 65). Indeed, FSH binding to bone has been unequivocally established *in vivo* (18, 66). The injection of FSH conjugated with a small near-infrared fluorophore CH1055 into live mice resulted in the capture of infrared signals not only in ovaries and testes but also in bone; the fluorophore was displaced when a 100-fold molar excess of unlabeled FSH was infused, establishing specificity (66). Recombinant FSH molecules with variable glycosylation levels should be used cautiously because it is now known that the fully glycosylated 24-kDa isoform is more active on the bone FSHR, whereas the partially glycosylated 21-kDa isoform best activates the ovarian receptor (67, 68). Indeed, the age-related decline in the abundance of partially glycosylated FSH may contribute to the decreased fertility in older women (69).

FSH acts directly on bone through direct and indirect mechanisms. First, FSH acts directly on FSHR on osteoclast precursors to increase osteoclastogenesis by sensitizing the MAPK, NFκB, and AKT pathways (17). Additionally, unlike coupling of the ovarian FSHR to $G\alpha_s$, the bone FSHR interacts with $G\alpha_{i2}$, thereby reducing rather than elevating cellular cAMP levels. The absence of $G\alpha_{i2}$ or abrogation of any downstream signaling cascade by chemical inhibitors, dominant negative molecules, or knockout cells abolishes FSH responsiveness (17). Interestingly, the response to FSH is abolished in mice lacking immunoreceptor tyrosine-based activation motif signaling mediated by DAP12 or Fc receptor γ chain. The latter finding suggests an interaction between FSH and immune receptor complexes (62). Second, FSH increases expression of the receptor activator of nuclear factor κB (70) and indirectly stimulates osteoclastogenesis by releasing cytokines, namely IL-1β, TNF-α, and IL-6 in proportion to the surface expression of FSHR (50, 71). Not unexpectedly, therefore, in a study of 36 women between the age of 20 and 50 years, serum FSH levels correlated with circulating cytokine concentrations (50).

Consistent with the proresorptive action of FSH *in vitro*, ovariectomy-induced bone loss in rats was augmented by the exogenous administration of FSH and was reduced by injection of an FSH antagonist (72, 73). Mice haploinsufficient or absent in *Fshβ* or *Fshr* showed reduced bone resorption and increased bone mass (17); however, we acknowledge that this increase in bone mass may be explained in part by the accompanying hyperandrogenemia caused by conserved luteal function (74). However, this effect cannot be solely responsible for the high bone mass, because mice lacking aromatase have an equivalent increase in serum testosterone but lose bone profoundly (75). Furthermore, we found that even in male mice, the inhibition of FSH action by a blocking antibody increases bone mass (19). Furthermore, a GST-FSHβ antigen was found to prevent trabecular bone loss and increased bone strength in ovariectomized rats (76).

It has indeed been difficult to separate the action of FSH from that of estrogens on bone *in vivo*. FSH promotes the secretion of estrogen from the ovaries to increase bone mass, whereas it has an opposite effect in directly stimulating osteoclastic bone resorption. Thus, the modulation of FSH action *in vivo*, such as with the use of recombinant FSH to treat mice with intact ovaries (64), or transgenic expression of human FSH (63), even in *hpg* mice, is unlikely to clearly demonstrate the proresorptive properties of FSH. In any of aforementioned cases, the direct effects of FSH on bone are confounded by the opposite actions of estrogen. At the same time, low FSH levels in women are associated with less bone loss (55). Therefore, the effectiveness of estrogen therapy is related to the degree of FSH suppression (77). In contrast, the use of GnRH agonists does not prevent bone resorption, wherein reduction of endogenous estrogen levels appears not to be compensated by the suppressed FSH (78). Thus, we believe and agree with others (79) that the selective inhibition of FSH action, with modest or no effects on serum estrogen, will be the best way forward for establishing the direct effects of FSH.

Thus, to specifically leverage the increase in FSH early in menopausal transition, as noted earlier, and to inhibit FSH action, we generated an antibody to a 13–amino acid peptide sequence within the receptor-binding domain of the FSH β-subunit (80, 81). This antibody blocked FSH action on osteoclast formation *in vitro* (18, 81). When injected into ovariectomized mice, the FSH antibody attenuated bone loss not only by inhibiting bone resorption but also by stimulating bone formation (17, 80, 81). Notably, mice treated with FSH antibody or mice deficient in *Fshr* showed more osteoblast precursors in stromal cells (80). This suggested that FSH also acted *via* FSHR present on mesenchymal stem cells to negatively regulate differentiation to the osteoblast lineage (80).

Clinical Associations Between Serum FSH and Body Fat

Several studies have characterized the relationship between serum FSH levels and body composition in perimenopausal and postmenopausal women. Longitudinal data from women in the Michigan cohort of SWAN displayed a positive correlation between changes in

serum FSH and fat mass over a 6-year period during the menopausal transition (40). Increasing FSH levels were associated with increases in waist circumference and fat mass, even after adjustments for baseline measures (40). Likewise, in the Penn Ovarian Aging Study, multivariable linear regression modeling revealed that serum FSH and estradiol were each associated with increased visceral fat volume (15). Specifically, FSH was inversely related to visceral fat tertiles (15). Consistent with these studies, the Oklahoma Postmenopausal Health Disparities Study, a large multiethnic cohort of postmenopausal women, reported that waist-to-hip ratio correlates positively with serum FSH and estrogen (82). Likewise, a study conducted in north India among infertile premenopausal women showed a positive correlation between serum FSH and indicators of central obesity, including waist circumference and waist/hip ratio (83).

There have also been interesting reports documenting a negative association between serum FSH and lean mass in women, and these findings are consistent with our observations that blocking FSH increases lean mass in mice (19). An independent association was noted between high serum FSH and lower lean mass, after adjusting for multiple confounders and covariates in postmenopausal US women (84). Likewise, the cross-sectional Study of Women Entering and in Endocrine Transition, conducted among Sub-Saharan African postmenopausal women, showed that lean mass was significantly lower in women with high FSH levels (85).

Despite the strong positive correlations observed between serum FSH and fat mass, the 11-year SWAN follow-up, the Pan Asia Menopause Study, and several other studies have shown paradoxically that low serum FSH levels occur in women with a high BMI (86–91). This paradox probably arises from feedback inhibitory effects of estrogen produced by aromatization in fat tissue on FSH production. In men there appears to be no clear association between serum FSH and BMI across age groups (92–95). With that said, there is compelling evidence for positive associations between serum FSH levels and metabolic syndrome. A cross-sectional study of 320 postmenopausal women from Poland showed that serum FSH was a better predictor of metabolic syndrome than serum SHBG, C-reactive protein, or leptin (96, 97).

Associations between serum FSH levels and bone marrow adipose tissue (BMAT) have not been studied. Nonetheless, ovariectomy induces both bone loss and bone marrow adiposity in mice and humans (98, 99). In postmenopausal women and in premenopausal women who undergo gonadotrophin agonist treatment, there is a rapid and substantial increase in vertebral BMAT, as measured by magnetic resonance spectroscopy, typically occurring within ~2 weeks. Aging humans similarly display marked increases in femoral and vertebral BMAT, primarily in the regulated marrow adipose tissue compartment. In some cases, BMAT increases can, at least in part, be reversed by estrogen replacement (100).

Evidence for an Action of FSH on Fat

We recently published evidence for direct effects of FSH on fat tissue, thus documenting FSH as a fat-stimulating hormone (19). We found that FSH acts *via* a pertussis toxin–sensitive $G\alpha_i$-coupled FSHR on adipocytes, which we cloned and Sanger sequenced from both primary adipocytes and differentiated 3T3.L1 cells (19). We found that FSHR activation opposes the β_3-adrenergic signaling cascade in reducing cAMP levels and in attenuating *Ucp1* activation in dedifferentiated brown adipocytic Thermo cells (Fig. 2). FSHR activation also increased the expression of the core fat genes *Lpl*, *Fas*, and *Pparg* (19). $G\alpha_i$-mediated FSHR signaling in human and mouse adipocytes is concordant with previous evidence, which shows that the FSHR activation is negatively coupled through protein kinase A to the inhibition of p38 phosphorylation and reduced transcriptional activation of CREB and ATF2 (Fig. 2) (19, 103, 104).

Through computational modeling, we identified a 13–amino acid epitope as the receptor-binding motif of FSHβ, which we have since fine mapped (18, 19). Modeling of the FSH-FSHR complex predicted that capping this FSHβ epitope with an IgG (antibody) would prevent the entry of FSHβ into the FSHR binding pocket, thus blocking its action. Using UCP1 as the reporter in Thermo cells, we showed that the FSH antibody at 1 μg/mL indeed completely reversed the FSH-mediated *Ucp1* inhibition. Pharmacokinetic studies showed a peak serum concentration of the FSH antibody of 20 μg/mL, with a half-life of 25.6 hours, after a single injection; this was far higher than the *in vitro* requirement (19). The FSH antibody dramatically reduced body fat in all compartments, namely viscera, subcutaneous tissue, and bone marrow, and in every mouse model tested: mice pair-fed on a high-fat diet, mice pair-fed on regular chow, mice allowed to consume regular chow *ad libitum*, and ovariectomized and sham-operated mice (Fig. 3) (19). The data sets were contemporaneously reproduced at several laboratories and replicated with different technologies, notably dual-energy X-ray absorptiometry, microcomputed tomography, quantitative nuclear magnetic resonance, and simple tissue weight measurements (19, 20). Important to note is that serum estrogen levels did not change after ≤8 weeks of daily antibody treatment (19). We believe that the concentration of "active" FSH (not bound to the antibody) in antibody-treated mice is enough to activate follicular cells of the ovaries,

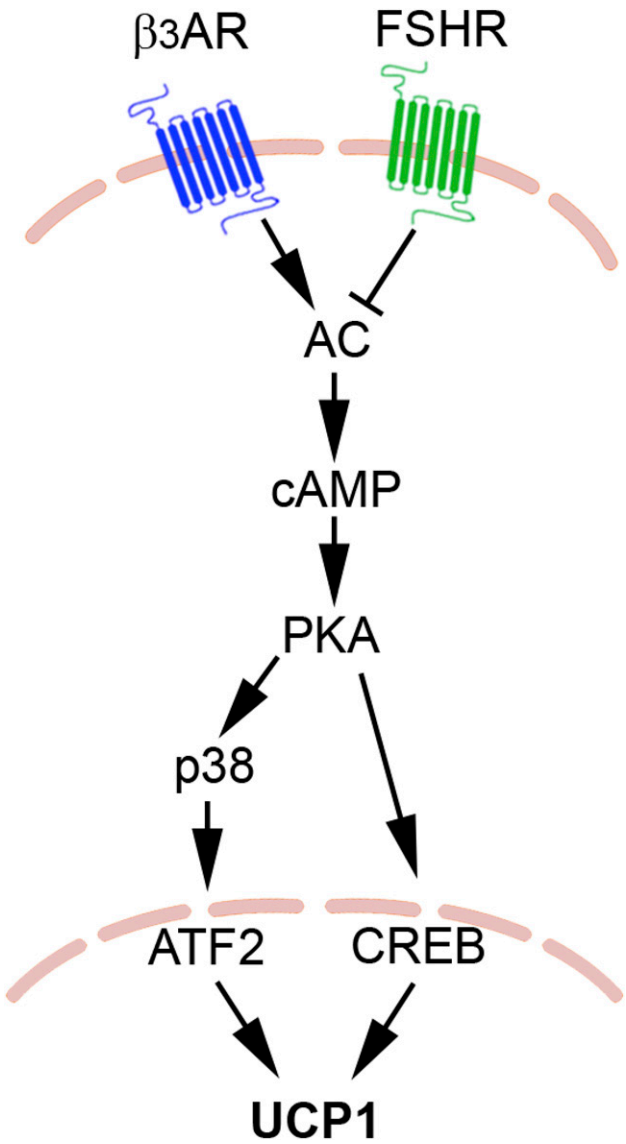

Figure 2. Mechanism of action of FSH on adipocytes. The newly described FSH signaling pathway opposes β_3 adrenergic signaling. The latter is known to cause "beiging" *via* interaction of the β_3 receptor with a $G\alpha_s$ protein that stimulates cAMP production and activates the MAPK p38 and the transcription factor ATF2, which then translocates to the nucleus to cause the transcriptional activation of the *Ucp1* gene (101, 102). FSH opposes this action by interacting with a $G\alpha_i$-coupled FSHR, also involving CREB-mediated pathway (19, 103).

but the concentration probably falls below that needed to activate nonovarian tissues, namely bone and fat. We have consistently found that inhibition of FSH action or its genetic haploinsufficiency does not alter serum estrogen (17, 80). Furthermore, the effect of the FSH blocking antibody was recapitulated in male *Fshr*$^{+/-}$ mice pair-fed on a high-fat diet. As would be expected, the FSH antibody did not reduce body fat in the *Fshr*$^{+/-}$ mice, thus establishing FSH specificity (Fig. 3) (19). And, concordant with earlier findings, there was a notable increase in bone mass (Fig. 3) (19, 80).

In addition to what would be considered a profound reduction of body fat, administration of the FSH blocking antibody also induced marked "beiging" of white adipose tissue, noted best in UCP1-labeled sections. Consistent with this phenotype, we documented a significant increase in the expression of brown fat genes in white adipose tissue, notably *Ucp1*, *Cox7*, *Cidea*, and *Cox8a* (19). This white adipose tissue beiging was coupled with brown adipose tissue activation, both of which were documented with the ThermoMouse, a transgenic line in which the *Ucp1* promoter drives *Luc2* expression (stock number 026690, Jackson Laboratory). *In vivo* IVIS-based imaging (Perkin Elmer) showed early increases in Luc2 (UCP1) radiance in brown fat–rich areas, followed by enhanced radiance in the inguinal, mainly white adipose tissue–containing, region at 8 weeks (Fig. 3) (19). Importantly, the beiging response remained intact under thermoneutral conditions and, as far as we can determine by measuring noradrenaline post–α-methyl-*p*-tyrosine, was not mediated by increased sympathetic tone.

With that said, and consistent with white adipose tissue beiging, indirect calorimetry with metabolic cages showed increased basal energy expenditure, together with increases in certain physical activity parameters. Overall, therefore, FSH inhibition, pharmacologically or genetically, yielded a phenotype of reduced body weight and thermogenic adipose tissue accrual. Surprisingly, insulin sensitivity and glucose tolerance were not improved in these mice, a finding that remains unexplained mechanistically, particularly because serum FSH levels in women predict the onset of metabolic syndrome (96, 97). Equally unclear is how the "beige" cell is derived, whether through the transdifferentiation of existing white adipocytes or from a committed precursor (105, 106).

Serum FSH Is Associated With Cardiovascular Risk

Several studies have reported the relationship between serum FSH levels and cardiovascular risk measures, such as carotid intima-media thickness and coronary artery calcium deposition. The Assessment of the Transition of Hormonal Evaluation With Noninvasive Imaging of Atherosclerosis study identified a direct association between serum FSH and aortic plaque number in perimenopausal women by using contrast-enhanced CT angiography and carotid ultrasound (107). Another cross-sectional study of premenopausal and postmenopausal women reported significant correlations between serum FSH and ultrasound measures of carotid intima-media thickness (108). In contrast, a population-based longitudinal Survey on Prevalence in East China for Metabolic Diseases and Risk Factors in 2658 postmenopausal

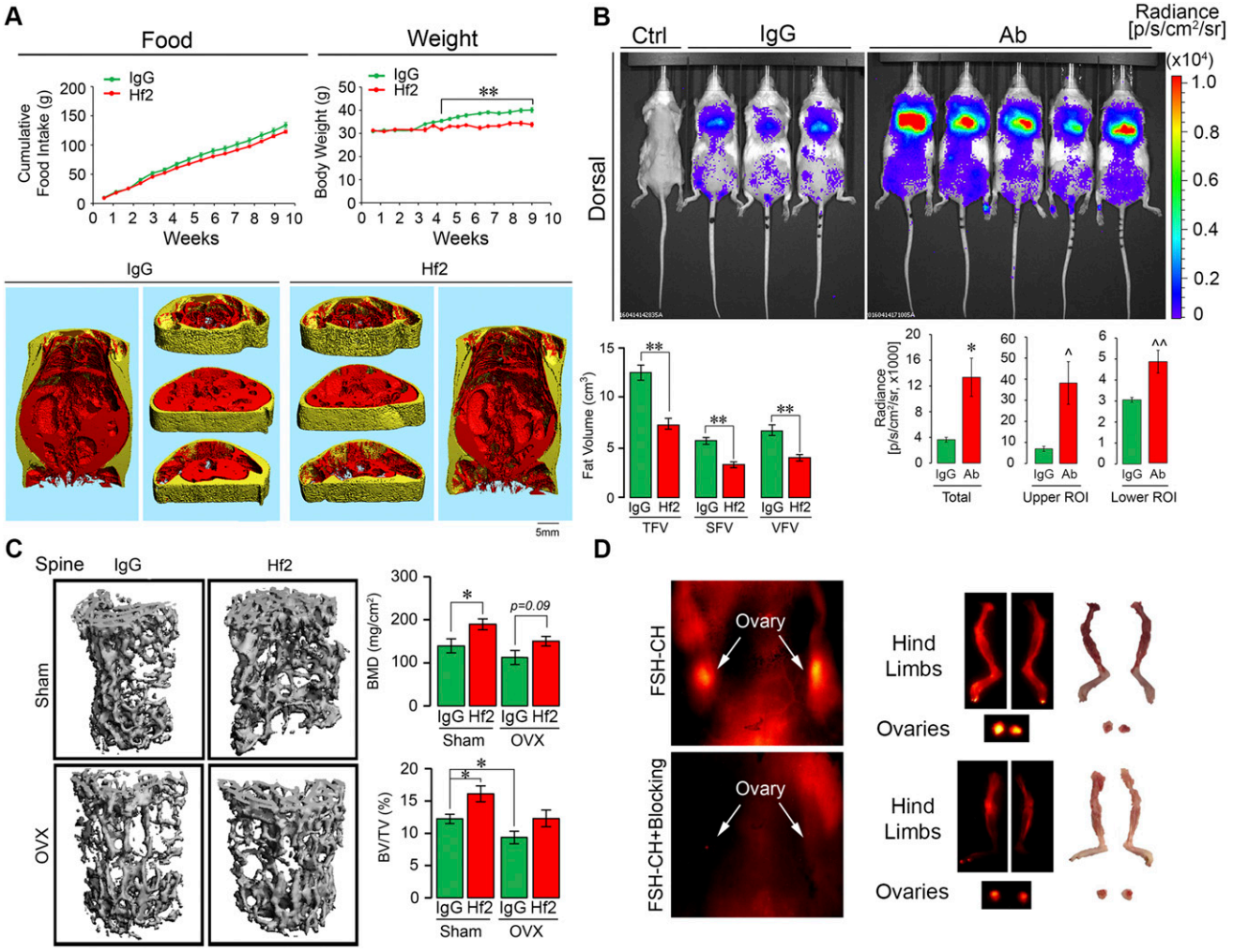

Figure 3. Antiobesity and bone-protective actions of FSH blockade. (A) Our monoclonal FSH blocking antibody, Hf2, raised to the FSHR-binding epitope of human FSHβ, triggered a sharp reduction in body weight and body fat, measured by microcomputed tomography in male mice pair-fed on a high-fat diet. Shown are total, subcutaneous (yellow), and visceral (red) fat volumes. Polyclonal FSH antibody (Ab) (and Hf2) also caused "beiging" and brown adipose tissue activation, which was confirmed in the ThermoMouse, a surrogate for UCP1 activation, after 8 weeks of daily IP injections. (B) Radiance in the upper and lower regions of interest (ROIs) is shown. Hf2 showed potent osteoprotective actions in preventing ovariectomy-induced bone loss after 4 weeks of daily injections. (C) Micro-CT images and microstructural parameters, namely BMD and fractional bone volume (BV/TV) are shown. (D) *In vivo* binding of fluorescently labeled human FSH (FSH-CH) to ovary and bone (upper panel), and its displacement by a 100× unlabeled FSH (bottom panel). *$P \leq 0.05$; **$P \leq 0.01$, or as shown; ^$P = 0.060$; ^^$P = 0.051$. These data collectively establish that interruption of Fshr signaling pharmacologically induces a lean thermogenic, high–bone mass phenotype. Figures 3A and 3B: Reproduced with permission from Liu P, Ji Y, Yuen T, et al. Blocking FSH induces thermogenic adipose tissue and reduces body fat. Nature 2017; 546:107–112. Figures 3C and 3D: Reproduced with permission from Ji Y, Liu P, Yuen T, et al. Epitope-specific monoclonal antibodies to FSHb increase bone mass. Proc Natl Acad Sci USA 2018; 115(9):2192–2197. Ctrl, control; OVX, ovariectomy; SFV, subcutaneous fat volume; TFV, total fat volume; VFV, visceral fat volume.

women showed that mean cardiovascular risk was decreased with increased serum FSH levels (109). However, longitudinal data derived from 856 women from SWAN documented that lower serum FSH levels were related to lower intima-media thickness compared with medium- and high-FSH groups (110).

FSH: An Aging Hormone?

Bartke *et al.* (111) recently surmised that suppressed FSH levels may contribute to longevity. They found that a 20% to 60% increase in longevity mirrors a 50% reduction in serum FSH both in *Ames* mice that are deficient in the pituitary transcription factor *Prop1* and in *Laron* mice that lack the growth hormone receptor (112, 113). In addition to their increased lifespan and reduced rates of aging, both *Ames* and *Laron* mice display a metabolic phenotype (114–117) that is surprisingly similar to that described by us in $Fshr^{+/-}$ mice and in wild-type mice treated with our blocking FSH antibody (19). Matching phenotypic features include reduced adiposity, beiging of white adipose tissue, brown adipose tissue activation, and increased energy expenditure (114–116). Furthermore, similarly to the sex-independent

action of blocking FSH that we noted (19), the metabolic phenotypes of *Laron* and *Ames* mice are equally prominent in both males and females (113). However, it has been difficult, if not impossible, to molecularly explain the increased lifespan, or indeed, the metabolic phenotype of the *Ames* and *Laron* mutants because of multiple hormonal perturbations, including decrements in serum TSH, LH, and prolactin levels and reduced or absent GH signaling (118, 119). The close phenotypic similarity nonetheless prompts further studies into whether reduced FSH signaling can contribute, even in part, to longevity, particularly when the primary reproductive function of FSH is no longer needed (111).

In fact, several clinical observations compel further studies into the action of FSH across the process of biological aging. First, serum FSH increases track with age in both women and men, at a rate of 3.5% per year in the latter (120). Second, high FSH levels have recently been implicated in the visceral obesity and cardiovascular risk in patients with prostate cancer on androgen deprivation therapy (121). This newly described action of FSH is in addition to its possible contribution to the acute rapid and severe bone loss in these patients (122). Third, as noted earlier, serum FSH levels rise sharply before the last menstrual period to compensate for reduced ovarian reserve but with estrogen remaining normal (10, 16, 123). This high-FSH, estrogen-replete period witnesses the most rapid rates of bone loss and the onset of visceral adiposity, weight gain, disrupted energy balance, and reduced physical activity (10, 14, 16, 123). And, importantly, visceral adiposity itself increases the long-term risk of diabetes, metabolic syndrome, cardiovascular disease, and cancer.

Treating Osteoporosis and Obesity With a Single Anti-FSH Agent?

In fertile women, FSH regulates follicular recruitment, supports follicle development and maturation, and participates in LH-triggered ovulation and luteinization (124, 125). Over the last decade, it has become increasingly clear that like other pituitary hormones (126–128), FSH has additional functions beyond reproduction, as evidenced by the expression of FSHR in bone and fat (17–19, 66) as well as in the placenta, endometrial tissue, monocytes, and several tumor tissues (60, 70, 129–133). Considering the notable detrimental effects of FSH on bone and body fat (17, 19, 87), we can deduce its global involvement in the physiology of aging and pathophysiology of age-related conditions, importantly osteoporosis and obesity. Equally intriguing is our therapeutic premise that the inhibition of FSH signaling, either genetically or with our polyclonal antibody, increases bone mass, reduces body fat, and induces thermogenic adipose tissue. We thus ask whether an anti-FSH agent can be used to prevent and treat both osteoporosis and obesity in women (without reducing estrogen levels) and in men across the lifespan.

The concept of a high-FSH, estrogen replete state during the perimenopausal transition has been clearly documented through the SWAN, in which bone loss and visceral obesity track together ~2 to 3 years before menopause (16). This finding was recapitulated faithfully in a rat model in which ovotoxin 4-vinylcyclohexene diepoxide, administered to mimic gradual ovarian failure, triggered notable decreases in bone density (5% to 13%) during periods of increased FSH (and decreased inhibins) in the face of a prolonged estrogen-replete period (134). To leverage this therapeutic window in particular, and to provide a means of treating osteoporosis and obesity more generally in both sexes, we developed two monoclonal antibodies, one of which, Hf2, was raised against the receptor-binding epitope of human FSHβ (18, 19). In contrast to our polyclonal antibody (IC_{50} ~100 nM) used in our proof-of-concept studies (19, 80, 81), Hf2 displayed an IC_{50} of ~6 nM. It caused impressive reductions in body fat, triggered the "beiging" of white adipose tissue, and increased cortical thickness and trabecular bone volume in mice (18, 19). Overall, these studies lend support to our approach for treating osteoporosis and obesity, diseases that affect millions of women and men worldwide.

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