sensitivity. In contrast, low ghrelin level inhibited while high levels stimulated insulin from mouse islets (Salehi et al., 2004). Furthermore, Heijboer et al. (2006) reported that ghrelin treatment hampered insulin’s ability to suppress glucose production and increased glucose disposal, actions opposite to the current study. These discrepancies may arise from additional effects of ghrelin treatment on endogenous ghrelin levels, glucocorticoids, and various hormones. Overall, the studies provide compelling evidence that ghrelin has unique dual effects on glucose homeostasis, at least in a genetic model. Ghrelin antagonism may be a new approach for treating type 2 diabetes by increasing insulin secretion and enhancing peripheral insulin action. The challenge is to ascertain if these results in rodents can be translated to patients.

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FSH versus estrogen: Who’s guilty of breaking bones?

Bone loss after menopause or gonadectomy has been attributed to the drop in estrogen levels. A recent paper (Sun et al., 2006) challenges this view by showing that the pituitary hormone FSH, previously thought to target only the gonads, also acts on osteoclasts to activate bone resorption. In conjunction with genetic studies, these data raise the possibility that FSH, independent of estrogen, causes hypogonadal bone loss.

In a provocative paper recently published in Cell (Sun et al., 2006), Drs. Zaidi and Blair’s groups report observations from which they conclude that high-circulating follicular-stimulating hormone (FSH), not declining levels of estrogen, causes

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Selected reading


hypogonadal bone loss. This happens because FSH directly regulates osteoclasts and bone resorption. These two conclusions represent a major shift in two paradigms (Figure 1): first, FSH, like LH, was thought to target exclusively cells in the gonads, and second, decreasing estrogen levels have always been thought to be the cause for postmenopausal bone loss.

Indeed, it is considered self-evident that postmenopausal osteoporosis is directly linked to the declining levels of estrogen (Riggs et al., 2002). After all, acute estrogen depletion following gonadectomy in women or in animal models rapidly induces bone loss; this can be prevented or reversed by treatment with estrogen (Riggs et al., 2002). Estrogen’s role is not limited to females: in males, some androgen effects on bone mass are dependent on aromatization to estrogens, such that estrogen receptor or aromatase deficiency in men leads to bone loss (Smith et al., 1994; Hermann et al., 2002).

Few in the bone field paid particular attention to another aspect of menopause that accompanies or even precedes the decline in ovarian function and the drop in the levels of estrogen: FSH, the pituitary hormone responsible for the stimulation of granulosa cells in the ovary (or Sertoli cells in the testis) to produce sufficient amounts of sex hormones, reaches sky-high levels (Figure 2). This may possibly be in an attempt to compensate the declining hormonal production by less-responsive ovary (or testicular) cells.

The current work was prompted by earlier observations that, while ovariectomy causes profound bone loss, the response is blunted in hypophysectomized rats (Yeh, 1996, 1997). Furthermore, recent clinical studies have shown a stronger correlation for FSH levels than estrogen with bone turnover or bone mass in postmenopausal or amenorrheic women (Devleta et al., 2004; Sowers et al., 2006), or with bone mass gains after estrogen therapy (Kawai et al., 2004), leading to the hypothesis that FSH may directly affect bone metabolism.

The latest paper includes observations made in FSH or FSH receptor null mice. Deletion of FSH receptors (FSHR) leads, as expected, to an absence of endogenous stimulation of estrogen production by the ovary and an atrophy of the reproductive organs (hypogonadism). This lack of target organ response leads to a marked increase in FSH levels. These changes in FSH, together with low estrogen, mimic in part the situation at menopause or after gonadectomy. According to the existing paradigm (Figure 1), one would have therefore expected a rapid decrease in bone mass. Unexpectedly, however, bone mass remained unaffected in these mice, despite low estrogen levels, although measurement of these is not reported in the manuscript.

Moreover, whereas low estrogen levels are associated with a marked increase in bone turnover and specifically an increase in bone resorption (Figure 1B), this was not apparent in the absence of FSHR in vivo. This lack of osteoclast response could explain the lack of induced bone loss. Indeed, ex vivo studies indicated that osteoclastogenesis (the formation of bone resorbing cells) and bone resorption were markedly decreased in the absence of a receptor for FSH. Thus, in the absence of FSHR, low estrogen levels are not sufficient to induce the increase in bone turnover and bone resorption, which is characteristic of early menopause and leads to bone loss and osteoporosis.

The authors then went on to examine the changes in bone homeostasis that would occur in the absence of the hormone (FSH) rather than its receptor. Surprisingly, again, despite severe hypogonadism, FSH−/− null homozygous mice also failed to lose bone and even gained some (Figure 3A), despite low levels of estrogen (data not shown). The most compelling set of data was however obtained in mice haploinsufficient for FSH−/−. In these mice, the circulating levels of FSH are, as expected, decreased by about 50% but this is high enough to ensure a maintenance of normal levels of circulating estrogen. Yet, despite normal estrogen levels, these mice showed a decrease in bone resorption and a moderate increase, not decrease, in bone mass.

Taken together, these data suggested that FSH, acting via its cognate receptor, was capable of regulating osteoclastogenesis and bone resorption, independent of estrogen levels. But is it a direct effect of FSH on osteoclasts or their precursors? To answer this crucial question, the authors then tested osteoclast...
A marked decrease in bone mass.

Under decreased gonadal function (menopause), estrogen production is reduced, with reduced net-bone formation and increased bone resorption because of direct effects of FSH on osteoclasts; this leads to a marked decrease in bone mass.

First, it bears repeating that simply correcting estrogen levels is sufficient to prevent or treat low bone mass in patients and in animal models. It is therefore possible that residual, albeit low, levels of estrogen in the FSHR null mice were sufficient to protect against bone resorption and maintain bone mass. As the levels of estrogen (and androgen) in these mice are not reported, this possibility is difficult to verify.

Indeed, estrogen receptor (ER) knockout mice provide evidence that sex hormones can regulate bone mass independently of FSH: double ER null female mice have a low bone mass (Sims et al., 2002), despite normal levels of FSH (Couse et al., 2003). Furthermore, the surprisingly normal bone mass maintained by ERα null mice does not reflect an irrelevance of ERα; rather, in the absence of ERα circulating levels of androgen are markedly increased in both males and females (Couse and Korach, 1999; Sims et al., 2002), which prevent bone loss via the androgen receptor (Sims et al., 2003). These studies therefore show a critical role for estrogen and androgen receptors in the maintenance of bone mass, despite unchanged FSH levels. Furthermore, luteinizing hormone (LH) levels, not reported in Sun et al. (2006), could well have induced an increase in androgens. Indeed, Danilovich et al. (2000) measured androgen levels in FSHR knockout mice and found them markedly increased; this could potentially explain most of the changes in bone mass reported by Zaidi’s and Blair’s groups.

It should also be noted that showing that a decrease in FSH increases (modestly) bone mass does not automatically imply the converse, i.e., that an increase in FSH would lead to a decrease in bone mass. It will be interesting to test this in vivo, combining increased FSH with antiestrogen, for example, to more definitively address whether FSH has “estrogen-independent” effects on bone. Moreover, gonadectomies in female and male FSH mutant mice, and treatment with estrogen, androgen, and their respective antagonists will be necessary to answer the essential question of whether ovariectomy still leads to an increase in bone turnover and bone loss in the absence of FSH or FSHR. After all, a decrease in resorption in the absence of FSH with normal estrogen levels, as in the FSH haploinsufficient mice, does not directly demonstrate that the increase in bone resorption that follows ovariectomy is FSH dependent.

Until such experiments are performed, it would be safer to conclude from this exciting report that both FSH and estrogen play key roles in the response of bone to menopause or gonadectomy (Figure 3B). The paper by Zaidi and Blair’s groups goes a long way in establishing that FSH may contribute directly to the regulation of bone resorption and, thereby, bone mass, and this paper will doubtless trigger a myriad of follow-on investigations testing the hypothesis. The study certainly suggests that FSH may participate in the pathophysiological process of bone loss in osteoporosis, a thought-provoking finding. There is still some way to go, however, before definitively concluding that high-circulating FSH causes hypogonadal bone loss.

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Selected reading
Fat breakdown: A function for CGI-58 (ABHD5) provides a new piece of the puzzle

The hydrolysis of fat stored in adipose tissues is crucial for providing energy during fasting and exercise, and dysregulation of fat breakdown may contribute to metabolic disease. In this issue of *Cell Metabolism*, Lass et al. [2006] report that CGI-58/ABHD5, a lipid-droplet-associated protein that is mutated in a rare disease characterized by excess lipid storage, activates adipose triglyceride lipase and thus may regulate fat mobilization.

Obesity is a disorder of energy balance in which excess energy, in the form of triacylglycerols (triglycerides, TG), accumulates in white adipose tissue (WAT). In adipocytes, TG are stored in large, often solitary cytoplasmic organelles called lipid droplets, or adiposomes. Their TG content is determined by the balance of anabolic and catabolic pathways (Figure 1). The main anabolic pathway is the esterification of glycerol with fatty acids derived from cellular uptake, de novo fatty acid synthesis, or intracellular lipid hydrolysis (lipolysis). The catabolic pathway involves sequential lipolytic reactions that ultimately hydrolyze TG to its simple components, glycerol and fatty acids. Lipolysis is crucial for mobilizing these molecules as energy sources during times of demand such as during fasting or exercise.

With the discovery of molecules involved in lipolysis and esterification during the past several years, a clearer understanding of the molecular processes governing TG storage has emerged (reviewed in Zechner et al. [2005]). In particular, several newly identified molecules have illuminated the lipolytic pathways in WAT and other tissues. Until recently, hormone-sensitive lipase (HSL) was considered to be the sole lipase of significance in WAT, and its regulation is well understood. Catecholamines, which increase during a fast, stimulate HSL activity by binding to β-adrenergic receptors, which in turn activate stimulatory G proteins, increase adenyl cyclase activity and cAMP level, and activate protein kinase A. This kinase phosphorylates HSL, thereby activating the enzyme, and perilipin, an adiposome-associated protein. Perilipin phosphorylation allows HSL to access neutral lipids in the adiposome core.

For years, it was thought that HSL mediated most of the breakdown of TG to monoacylglycerol and fatty acids. However, gene knockout studies showed that mice lacking HSL do not accumulate TG in tissues and are not obese (Roduit et al., 2001; Haemmerle et al., 2002). Instead, they accumulate diacylglycerol (DG) in tissues, suggesting the major function of HSL is to hydrolyze DG, and they have significant residual lipase activity in tissues (Haemmerle et al., 2002). Subsequently, another lipase, adipose triglyceride lipase (ATGL, also called desnutrin and iPLA2c), was discovered by three laboratories (Jenkins et al., 2004; Villena et al., 2004; Zimmermann et al., 2004). ATGL is expressed in WAT and other tissues (Villena et al., 2004; Zimmermann et al., 2004) and hydrolyzes TG to yield DG and fatty acids (Zimmermann et al., 2004). Evidence suggested that ATGL accounts for the remaining lipolytic activity in HSL knockout mice (Zimmermann et al., 2004). ATGL is upregulated during fasting (Villena et al., 2004), consistent with a role in fasting-induced lipolysis, and is expressed in the cytoplasm and in association with adiposomes. Unlike HSL, ATGL does not translocate to the adiposome with adrenergic stimulation, suggesting that it is activated by another mechanism (Zimmermann et al., 2004).

In this issue of *Cell Metabolism*, Lass et al. [2006] provide another piece of the lipolysis puzzle. They report that comparative gene identification-58 (CGI-58)—a human gene transcript that is evolutionarily conserved with C. elegans and is also known as α/β-hydrolase domain containing 5 (ABHD5)—activates ATGL in vitro and may therefore play an important role as an activator of the enzyme. They also show that mutant forms of CGI-58 fail to activate ATGL and posit that this biochemical defect contributes to a rare autosomal recessive disease