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NEWS

Skeletal regulation of glucose metabolism: challenges in translation from mouse to man

Neil A Andrews

International Bone and Mineral Society, Chicago, IL, USA.

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Human physiological relevance of mouse osteocalcin findings dismissed by some experts, while others say it is still too soon to tell; new findings on RANKL further complicate the picture

In 2007, new research from Gerard Karsenty and colleagues presented the bone field with an unexpected finding: the skeleton appeared to regulate glucose metabolism in mice via osteocalcin, an osteoblast-specific protein.[1] The finding was both surprising and exciting to bone experts, as a much-studied tissue was revealed to possess a brand new function. Along with follow-up mouse data, most notably from experiments by Professor Karsenty's group in 2010,[2] work by Thomas Clemens and colleagues that same year,[3] a late 2012 study implicating the osteoblast as the mediator of the adverse effects of glucocorticoids on glucose metabolism,[4] and more recent findings suggesting a role for RANKL signaling in hepatic insulin resistance and diabetes mellitus,[5] the idea that the skeleton has a role in regulating glucose metabolism in mice gained credence. 'Overall, the mouse data are fairly convincing,' said Sundeep Khosla, an osteoporosis expert and a professor of medicine and physiology at Mayo Clinic, Rochester, MN, USA. 'There's a reasonable body of evidence to suggest that, in mice, the factors that regulate bone turnover, such as osteocalcin and RANKL, also regulate glucose metabolism.'

Mouse physiology, however, does not always apply to human physiology, and the bone field is now grappling with that issue more than 5 years after the initial discovery from Dr Karsenty's laboratory.[1] Though the data on RANKL are still too new to make any firm conclusions, some investigators have already concluded that osteocalcin does not have any meaningful role in human glucose metabolism. However, others are more cautious, and emphasize that the crucial studies that could demonstrate translational relevance have yet to be performed. However, the task will not be easy: experts stress that species differences in osteocalcin, and challenges in measuring the protein and understanding what osteocalcin measurements actually mean, are complexities that have not always received the full consideration they deserve, but must be grappled with at this time. Most of all, there is a strong feeling in the bone field that the time is now to show what bearing the mouse has for man, and if it has little or no relevance, the bone research agenda will be better focused elsewhere.

Testing a Prediction

Since the first studies on osteocalcin, a working hypothesis emerged from the Karsenty group to explain the unanticipated role for the protein in mice. Insulin signaling in osteoblasts was posited to stimulate bone resorption, resulting in the release of undercarboxylated osteocalcin (ucOC)—osteocalcin contains three glutamic acid residues, each of which can be carboxylated—stored in bone matrix into the circulation. Circulating ucOC would then reach the pancreas, where it would promote the proliferation of β-cells and insulin secretion. If the hypothesis is correct—if it is bone resorption that stimulates the release of ucOC, with ensuing beneficial effects on glucose metabolism—one prediction that follows is that anti-resorptive treatments should have a detrimental impact on glucose metabolism by reducing levels of ucOC.

Putting that prediction to the test was the goal of Ann Schwartz, Ian Reid and colleagues in a recent study[6] published in the *Journal of Bone and Mineral Research*. The study was needed in large part because of limitations in existing clinical data; that data is mainly of the cross-sectional variety, thus carrying with it the inability to reveal causality. 'Many cross-sectional studies show a relationship between osteocalcin and diabetes, with type 2 diabetics exhibiting lower osteocalcin levels,' said Dr Schwarz, an epidemiologist and associate professor at the University of California, San Francisco, CA, USA, and first author on the paper. 'But those studies can't address if it is diabetes that affects bone, or if it is bone that affects diabetes. The studies are repeatedly cited as evidence supporting mouse findings on osteocalcin, but it's very flimsy evidence because it's not longitudinal,' Dr Schwarz told *BoneKEy*.

To look for answers in more compelling data, Dr Schwartz and colleagues turned to the bone field's seminal randomized placebo-controlled clinical trials of anti-resorptives for the treatment of postmenopausal osteoporosis, including the Fracture Intervention Trial (FIT) of alendronate, the Health Outcomes and Reduced Incidence with Zoledronic Acid Once

Yearly Pivotal Fracture Trial (HORIZON-PFT), and the Fracture REduction Evaluation of Denosumab in Osteoporosis Every 6 Months (FREEDOM) trial. For their *post-hoc* analysis, the investigators looked at fasting glucose levels and weight, as well as the incidence of diabetes, at the time the trials ended, and examined whether those measures changed compared with the time of trial randomization. In each of the trials, the investigators found no statistically significant differences between treatment and placebo groups in fasting glucose levels. They also found no statistically significant differences in weight in HORIZON-PFT, and while they did find differences in FIT and FREEDOM, those differences were small (a difference in average weight change of 0.32 kg at the end of FIT and of 0.31 kg at the end of FREEDOM) and likely not clinically relevant, according to the researchers. Finally, they found no differences in diabetes incidence in each of the trials, nor did they see differences in diabetes incidence when pooling the data from all three trials.

'I don't think osteocalcin is a critical regulator of blood sugar in human beings,' said Ian Reid, a professor of medicine and endocrinology at the University of Auckland in New Zealand and senior author of the *JBMR* paper. 'Our data indicate that the mouse work on osteocalcin probably has very little relevance to human physiology,' he said. Dr Reid stressed that the issue is not that the mouse findings are incorrect, but rather that a transgenic knockout mouse exhibits extreme physiology, as osteocalcin levels are completely suppressed throughout the life of the animal. That situation differs from the setting of anti-resorptive treatment for osteoporosis, where drugs are administered for a short time, later in life, and suppress osteocalcin, but not by nearly as much compared with a transgenic approach.

Dr Reid pointed to his study's findings on weight as further support of the idea that the transgenic mouse findings on osteocalcin have little bearing on human physiology.[6] The gain in weight over the FIT and FREEDOM trials, he noted, is in fact consistent with what the mouse data might predict—an increase in weight resulting from the adverse effects of osteocalcin-lowering anti-resorptives on glucose metabolism. Furthermore, the results from the HORIZON study are also in line: though the effect was not statistically significant in that trial, subjects in HORIZON did nonetheless show a weight gain. Yet, even though the direction of the weight effect was consistent, across all three trials, with the animal data, the size of the effect was still negligible. 'That it's such a small difference reinforces that in human physiology, osteocalcin regulation of glucose metabolism is not biologically significant,' Dr Reid said.

Dr Schwarz reached a similar conclusion. 'My takeaway from this research is that there are other factors that are more important in a normal, physiological setting. I don't think our work has ruled out that osteocalcin has an effect, but it's not a predominant effect.'

Osteocalcin: Species Differences and Challenges in Measurement—and Interpretation

Some experts emphasize that any arguments in support of or against the translational relevance of the mouse findings must carefully consider species differences in osteocalcin, a complicating factor that has not yet received the full attention it deserves. 'The carboxylation status of osteocalcin in humans is probably very different from that in mice and likely most other animal species,' said Caren Gundberg, a leading authority on osteocalcin and a professor of orthopedics at Yale University School of Medicine, New Haven, CT, USA. Indeed, in most species, osteocalcin is fully carboxylated, while in people, it is not. One of the main reasons for that difference is vitamin K, which is necessary to carboxylate osteocalcin: humans don't get enough vitamin K in the diet, while other species do. Lab animals in particular, Dr Gundberg said, receive diets very high in vitamin K, making the findings coming from animal experiments particularly difficult to parse.

Sarah Booth, a vitamin K nutrition expert and associate director of the Jean Mayer United States Department of Agriculture (USDA) Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA, further underscored the importance of these nutritional differences across species. 'Chow is not comparable to a human diet. The form of vitamin K in a mouse chow is different, it transforms to a form of vitamin K in the body that is different, and the doses are very high compared to what humans consume.'

Beyond species differences in osteocalcin and vitamin K, the very measurement of osteocalcin also poses a problem. Dr Gundberg and Dr Booth have emphasized[7] that most associations between serum osteocalcin and indices of glucose metabolism in people have come from studies that do not distinguish between total osteocalcin levels and levels of ucOC. One way to overcome that difficulty is to look to manipulations of vitamin K levels that alter the relative amount of ucOC to total osteocalcin without affecting bone turnover. If mice and people are in fact similar in skeletal regulation of glucose metabolism, one might expect that vitamin K supplementation would have adverse effects on glucose metabolism, but Dr Booth's work has found the opposite. 'In people, a high vitamin K level is associated with reduced insulin resistance,' she said. Dr Booth and her colleagues were the first to report that finding, but she said that other investigators have since confirmed it. Furthermore, another type of vitamin K manipulation, use of the anticoagulant warfarin, which decreases osteocalcin carboxylation through effects on vitamin K, might also be expected to have an impact on glucose metabolism. However, experts note there is no such signal from the millions of patients who take that drug.

Finally, an even more fundamental problem is that it's unclear what the measurement of osteocalcin actually means, in a mouse or in a person. 'It's very hard to separate osteocalcin as a mediator of changes in glucose homeostasis from osteocalcin as a marker of the normal changes that occur during bone turnover,' Dr Gundberg said. 'Unfortunately, most of the human data out there do not address this, and I'm not sure they really can, because most of the studies were not designed to look directly at osteocalcin effects on glucose metabolism. I think the only way that will ever happen is when there is a true clinical trial,' Dr Gundberg said. Until that occurs, whether osteocalcin is a marker or a mediator remains unclear.

RANKL Enters the Story

If the picture from mouse and human osteocalcin investigations wasn't already complicated or confusing enough, a recent *Nature Medicine*[5] study documenting a role for RANKL signaling in regulating glucose metabolism further muddied the

waters. In seemingly contradictory fashion to the predictions from the mouse osteocalcin work, the authors of that study reported that inhibiting bone resorption in mice actually had *beneficial* effects on glucose metabolism. The study first reported epidemiological data showing that high serum levels of soluble RANKL predicted the risk of type 2 diabetes mellitus in subjects from the Bruneck Study, a prospective population-based survey that examined the epidemiology and pathogenesis of atherosclerosis and related traits. The investigators next found that blocking RANKL signaling systemically or in the liver improved hepatic insulin sensitivity and prevented diabetes mellitus in a number of genetic and nutritional mouse models of type 2 diabetes mellitus.

'We think that if bone resorption prevails, it facilitates insulin resistance,' wrote first author Stefan Kiechl in remarks emailed to *BoneKEy*. 'In general we think that inhibition of resorption may have beneficial effects on insulin resistance rather than adverse effects,' said Kiechl, a professor of neurology at Medical University Innsbruck in Austria. 'We regard it as interesting,' Kiechl further said, 'that the opponents in bone metabolism, osteocalcin and RANKL, appear to be opponents in glucose metabolism as well, with osteocalcin ameliorating insulin sensitivity and RANKL, according to our study, exhibiting the opposite effect.'

Kiechl said that the development of diabetes would be expected in the setting of inflammation, for instance, where resorption is high and formation is low, but would also depend on other factors, especially adipose tissue. 'In a condition of high resorption but low bone formation such as chronic inflammatory disease, the prevalence of insulin resistance is high, which may reflect a condition with high RANKL but low osteocalcin concentrations. It is important in this context that obesity creates a pro-inflammatory state and the imbalance of bone turnover associated with inflammation could link obesity with insulin resistance,' he said.

When asked about the results from Reid *et al.*[6], Kiechl said that those findings have little bearing on the question of what the effects of inhibiting bone resorption on glucose metabolism will be, because the FREEDOM study of denosumab was not specifically designed to address it. 'Clinical studies need to be prospective, use type 2 diabetes mellitus patients, include a detailed assessment of insulin resistance and other outcomes used in type 2 diabetes mellitus trials and [study] different doses/intervals of RANKL blockers. *Post-hoc* analyses of studies designed for treatment of osteoporosis in postmenopausal women are not suitable to answer this question,' he said.

Some experts not involved with the RANKL study say that the idea that RANKL could have a role in insulin resistance and diabetes, in the setting of chronic inflammation for instance, is plausible. Nonetheless, as with the mouse work on osteocalcin, there are reasons to doubt whether the mouse work on RANKL will translate to humans. Yet again, species differences could be at play. For instance, in mice, far more glucose—5–10-fold more, in fact—is produced in the liver, compared with people.[8] Thus, the import for humans of manipulations that alter glucose metabolism in the mouse liver is unclear. Another criticism is that while the epidemiological data reported by Kiechl *et al.*[5] are consistent with those investigators' mouse findings, the epidemiological data are based on a measurement—soluble RANKL levels—that cannot yet be made in a dependable fashion. 'We've found it very hard to reliably measure soluble RANKL in humans, so there are some concerns with the epidemiological data, based on how difficult it is to make the measurement,' Dr Khosla said.

What the Future May Hold

For his part, Dr Khosla said he has an open mind about the potential translational relevance of mouse findings on osteocalcin, because the key investigation that could prove or disprove it has yet to be undertaken. 'In my view, the critical study that needs to be performed is to treat people [who have diabetes or glucose intolerance] with undercarboxylated osteocalcin, carefully measure indices of glucose metabolism with glucose tolerance tests and other means, and then see if the undercarboxylated osteocalcin really had an effect on any of those parameters. That study hasn't been done,' he said. Dr Khosla also said that clinical studies of RANKL inhibition should investigate diabetics or those with glucose intolerance, rather than the relatively normal population studied by Reid *et al.*[6]

However, in the case of the skeletal regulation of glucose metabolism, many experts have strong doubts that the mouse scenario will hold true in humans. 'When it comes to the skeleton, the mouse has been a wonderful model,' said Stavros Manolagas, an osteoporosis expert and professor of medicine at the University of Arkansas for Medical Sciences in Little Rock, USA. 'With hindsight, osteoporosis disease mechanisms in mice, and the effects of glucocorticoids, parathyroid hormone, estrogen, androgen—whatever you like—were absolutely reproducible in humans,' he said. Considering this impressive history of mouse models, it should give the bone field pause, Dr Manolagas believes, when clinical data, such as those from Schwartz *et al.*[6] on osteocalcin, do not support those models. But Dr Manolagas also said the fact that the mouse models themselves are inconsistent is even more troubling. For instance, the mouse work from Kiechl *et al.*[5] showing a beneficial rather than a detrimental effect of inhibiting bone resorption on glucose metabolism appears to contradict the predictions of the mouse work on osteocalcin from Karsenty and colleagues.[1] With all the talk of translational relevance, there is still much to work out in the rodent.

Conflict of Interest

The author declares no conflict of interest.

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