Neuroendocrine regulation of human bone metabolism
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Chapter 8

SUMMARY AND DISCUSSION

In the first part of this thesis we investigated the role of the sympathetic nervous system in human bone metabolism and hematopoiesis and in the second part we investigated the hormonal control of bone and bone marrow. Chapter 1 presents an introduction to human bone metabolism, bone marrow physiology and the sympathetic nervous system.

To study the role of the sympathetic nervous system in human bone metabolism and hematopoiesis, we used three different approaches: a clinical approach (chapter 2), a genetic approach (chapter 3), and a pharmacological approach (chapter 4 and 5).

In chapter 2 we compared bone turnover markers in pheochromocytoma and control patients. A pheochromocytoma is a catecholamine-secreting tumor of the adrenal gland. Catecholamines are the neurotransmitters of the sympathetic nervous system; therefore pheochromocytoma can serve as a model of sympathetic overstimulation. Adrenalectomy (removal of the affected adrenal gland) is the recommended therapy for pheochromocytoma patients and, if successful, normalizes catecholamine concentrations. We showed that CTx, a bone resorption marker, is increased in pheochromocytoma patients and we showed that CTx concentrations return to normal following adrenalectomy. We conclude that excess of catecholamines increase bone resorption suggesting that the sympathetic nervous system influences human bone metabolism. Since the source of the catecholamines in this study was the adrenal gland and not the sympathetic nervous system, the physiological role of the sympathetic nervous system in bone metabolism remains to be determined. In addition, it is as yet unknown whether this change in bone turnover markers is clinically relevant, i.e., leading to changes in bone mineral density and fracture risk.

In chapter 3 we determined the association between polymorphisms in the beta-2 adrenergic receptor and fracture risk in two large, Dutch cohorts and we determined the association between these polymorphisms and bone mineral density in a large international consortium. A single nucleotide polymorphism is a DNA sequence variation occurring commonly (>1%) within a population, in which a single nucleotide differs between two alleles. When this results in a change in amino acids, it is called a nonsynonymous SNP. In the beta-2 adrenergic receptor (B2AR) gene, several polymorphisms exist and three are nonsynonymous. Previous research has shown these three polymorphisms to result in clinically relevant changes in receptor function. We did not find an association between these polymorphisms with fracture risk or bone mineral density. This lack of association could mean that either the B2AR is not involved in human bone metabolism, or that the polymorphisms in the B2AR gene do
not significantly influence receptor function in bone metabolism, or that fracture risk and bone mineral density are not reflecting the changes in bone metabolism induced by B2AR function. Whichever of these explanations are true remains to be investigated.

In **chapter 4** we conducted a randomized controlled trial to investigate the effects of a beta-adrenergic agonist and an antagonist on bone turnover in healthy postmenopausal women. Beta-adrenergic agonists mimic increased sympathetic signaling in target tissues by binding and activating adrenergic receptors. Beta-adrenergic antagonists compete with neurotransmitters to bind with the beta-adrenergic receptor, but instead of activating the receptor, they deactivate the receptor. We hypothesized that beta-adrenergic agonists would increase bone turnover whereas antagonists would decrease bone turnover, but the study did not show any effect of these medications on bone turnover. In this study, we used propranolol to block the beta-2 adrenergic receptor. Since propranolol is a non-selective beta-blocker, it also blocks the beta-1 adrenergic receptor. Studies in mice have shown that beta-1 adrenergic receptor knockout mice have a lower bone mass than wildtype mice and combined beta-1 and -2 adrenergic receptor knockout mice show the same [1]. Therefore targeting both the beta-1 and -2 receptor by propranolol could explain the lack of effect on bone turnover. Unfortunately, a beta-2 adrenergic antagonist does not exist for human use. We used terbutaline, a beta-2 adrenergic agonist and this has shown in mice to increase bone turnover although these studies used supraphysiological doses [2], which is impossible to do in human research. Although we cannot conclude from this study whether or not sympathetic control of bone metabolism exists, this study does show the difficulty of specifically targeting the sympathetic nervous system in bone and therefore this is not a feasible therapeutic option to treat human bone disease.

As a prespecified substudy to the study described in chapter 4, we studied in **chapter 5** the effects of a beta-adrenergic agonist and antagonist on circulating CD34+ hematopoietic stem cells. Hematopoietic stem cells reside in the bone marrow and migrate to the circulation to maintain hematopoiesis. This migration is among others regulated by the sympathetic nervous system. We hypothesized that beta-adrenergic agonists or antagonists could be used to influence hematopoietic stem cell migration. However, we did not find any effect on circulating hematopoietic stem cell numbers during the beta-adrenergic agonist or antagonist treatment. During hematopoietic stem cell transplantation, the hematopoietic stem cell migration is pharmacologically enhanced by G-CSF and recently, G-CSF was found to target the beta-adrenergic receptor pathway as well [3]. This opens up the possibility that beta-adrenergic treatment could be a synergistic treatment to G-CSF, but this remains to be investigated.
In the second part of this thesis, we studied the role of two hormones in bone and bone marrow metabolism, i.e., estrogen and insulin.

Estrogen has long been recognized as an important hormone controlling bone metabolism. Its role in bone marrow physiology in contrast, is unknown. Bone marrow consists of hematopoietic marrow and adipose marrow. During aging, caloric restriction and diseases such as osteoporosis, the amount of adipose bone marrow increases. These states are all accompanied by decreasing estrogen concentrations. Therefore we hypothesized that estrogen could be a regulator of adipose bone marrow. Adipose marrow used to be measured by bone biopsies, but nowadays magnetic resonance imaging (MRI) techniques offer a validated, noninvasive approach. In chapter 6, we investigated the variation in adipose bone marrow measured by quantitative chemical shift imaging (QCSI) MRI during the menstrual cycle in healthy women. We observed an increase in the fat fraction during the follicular phase and a decrease during the luteal phase. To show that this variation in adipose bone marrow is mediated by estrogen, we measured adipose bone marrow with QCSI MRI before, during, and after two weeks of estrogen therapy in postmenopausal women. Indeed, in all women adipose bone marrow decreased during estrogen treatment and normalized after cessation. This study clearly shows that estrogen can regulate adipose bone marrow in the short term. The mechanism of this regulation remains to be determined, as well as its potential pathophysiological role.

Insulin is a major anabolic hormone and it is well recognized that diabetes patients have an increased fracture risk. Recently, it was proposed that bone metabolism, through the secretion of osteocalcin, can also influence insulin secretion and glucose metabolism. In chapter 7 we reviewed the evidence concerning the regulation of glucose metabolism by bone from a clinical perspective. We concluded that the observational evidence seems promising, but that the interventional data do not completely support this hypothesis. Especially vitamin K levels and the carboxylation status of osteocalcin could have biased the results. Notwithstanding, the hypothesis is exciting considering the burden of obesity and diabetes and therefore we believe further studies are worthwhile.

GENERAL DISCUSSION

The ob/ob (obese) mouse is a naturally occurring mutant, which lacks the gene encoding the hormone leptin [4, 5]. The most striking feature of this mouse is its profound obesity caused by hyperphagia and its hypogonadotropic hypogonadism. For these reasons, it was expected to have a low bone mass. But when its bones were actually examined by the Karsenty group, it turned out to have a high bone mass [6]. Subsequently, it was discovered that leptin signals in the brainstem to decrease serotonin production
which increases sympathetic signaling via the ventromedial hypothalamus. This signal is mediated to the osteoblast via the beta-2 adrenergic receptor, inducing decreased bone formation and increased RANK-L production and thereby increased bone resorption [7, 8]. So ultimately, leptin signaling decreases bone mass via sympathetic nervous system signaling to bone and thus the ob/ob mouse, lacking leptin, has a high bone mass. The overall hypothesis from this group is that bone remodeling, extremely important for vertebrate species, consumes large amounts of energy and that therefore energy and bone metabolism are coupled via leptin. Although these experiments are elegantly conducted and convincing, there has been considerable debate on the exact role of leptin and the sympathetic nervous system in bone remodeling (reviewed in [9]). The debate focuses on multiple issues, including the direct versus the indirect effect of leptin on bone remodeling, the differential effects of leptin on cortical versus trabecular bone and the axial versus the appendicular skeleton, the possibly confounding effect of body mass and obesity with accompanying leptin resistance, and finally the effect of other adrenergic (beta-1 and -3, alpha) receptors involved in sympathetic signaling. Notwithstanding these issues, the purpose of the first part of this thesis was to translate the findings from the rodent studies on the sympathetic nervous system control of bone remodeling to the human situation. Unfortunately, it has proven difficult to do so. Mice are from the same genetic background and are born and raised under standard living conditions whereas humans are genetically diverse and have completely different lifestyles. In mice, it is possible to knockout or knockin a single gene, even tissue-specifically, to study its effect and this level of precision will never be met in human research. In addition, it is uncertain whether or not the interaction between leptin, the sympathetic nervous system and bone are exactly similar in mice and humans, especially under the specific study circumstances. Therefore it remains uncertain whether leptin only significantly influences bone metabolism at the extremes of the spectrum, or whether it also plays a role in ‘normal’ bone physiology. Taken together, our studies suggest that the first option is more plausible.

Adipose tissue has received a lot of attention over the last two decades. Historically, it was considered an inert tissue; this view has now changed as adipose tissue has been identified as the source of several hormones, i.e. adipokines that are crucial in the regulation of energy metabolism. In addition, several types (white, beige and brown) and depots (subcutaneous and visceral) of adipose tissue are recognized, all with different properties. Bones contain adipose tissue and this bone marrow adipose tissue was viewed as passive filling of the vacant bone marrow space. Recent studies however suggest that bone marrow adipose tissue is metabolically active and involved in energy homeostasis, hematopoiesis and bone metabolism (reviewed in [10, 11]). The exact interaction or balance between bone and adipose tissue in the bone marrow cavity is not elucidated yet. During skeletal growth there is an increase in both bone and adipose tissue, suggesting a
positive relation. However, during aging and as a result of diseases including osteoporosis and anorexia nervosa, there is an inverse relation between bone mass and adiposity. In addition, it is uncertain what progenitor cell type the bone marrow adipocyte is coming from and what factors regulate its differentiation and proliferation. Since anorexia nervosa and osteoporosis are accompanied by decreased estrogen concentrations, estrogen could be an important regulator of bone marrow adiposity. In the second part of this thesis we showed that estrogen rapidly decreases bone marrow adiposity, accompanied by an increase in bone formation. We did not investigate the molecular mechanism behind this effect, however we hypothesize that estrogen influences the differentiation of the mesenchymal stem cell away from the adipocyte and towards the osteoblast lineage. Alternatively, it may induce the transdifferentiation of the pre-adipocyte to the osteoblast lineage. This will be the subject of future studies.

Finally, a better understanding of bone marrow adipose tissue physiology as well as the interaction between adipose and bone cells in the bone marrow niche, may ultimately lead to new therapeutic options to treat both bone and metabolic diseases.
REFERENCES


