Original article

MOLECULAR METABOLISM

Osteoglycin, a novel coordinator of bone and glucose homeostasis

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ABSTRACT

Objective: The skeleton, which is strongly controlled by endocrine factors, has recently been shown to also play an active endocrine role itself, specifically influencing energy metabolism. However, much less is known about this role. Therefore, we sought to identify novel endocrine factors involved in the regulation of both bone mass and whole-body glucose homeostasis.

Methods: We used transcriptomic and proteomic analysis of Y1 receptor deficient osteoblasts combined with the generation of a novel osteoglycin deficient mouse model and performed comprehensive *in vivo* phenotype profiling, combined with osteoglycin administration in wildtype mice and human studies.

Results: Here we identify a novel role for osteoglycin, a secreted proteoglycan, in coordinating bone accretion with changes in energy balance. Using an osteoglycin knockout mouse model, we show that at a whole body level, osteoglycin acts to suppress bone formation and modulate whole body energy supplies by altering glucose uptake through changes in insulin secretion and sensitivity, as well as by altering food intake through central signaling. Examining humans following gastric surgery as a model of negative energy balance, we show that osteoglycin is associated with BMI and lean mass as well as changes in weight, BMI, and glucose levels.

Conclusions: Thus, we identify osteoglycin as a novel factor involved in the regulation of energy homeostasis and identify a role for it in facilitating the matching of bone acquisition to alterations in energy status.

Keywords Bone; Osteoglycin; Neuropeptide Y; Osteoblasts; Glucose homeostasis

1. INTRODUCTION

The regulation of energy homeostasis is a complex process that must guarantee whole body energy requirements while ensuring adequate energy supply to individual tissues. In addition, changes in energy balance often reflect changes in body weight, and this presents an additional layer of complexity for weight-bearing tissues such as the skeleton. In particular, where positive energy balance precedes an increase in weight, bone must increase its strength to match the increasing mechanical demand. Inadequate adaptation would result in fracture and uncertain survival. While it is known that bone adapts to increasing body weight through mechanical stimuli, the mechanism whereby bone cells ensure adequate energy for adaptation during weight gain and whether non-mechanical triggers for adaptation exist are unknown.

NPY is an important regulator of energy homeostasis and has developed considerable complexity in order to coordinate the demands of energy balance at the organism- and tissue-specific level, playing a pivotal role within the central nervous system as well as being directly involved in numerous organ systems [1]. NPY circuits originating from the hypothalamus not only control energy intake but also play a particularly important role in coordinating whole body energy expenditure through peripheral energy partitioning, ensuring that specific organ systems receive the energy they require to function without risk to the organism as a whole. Bone is one system under substantial control by NPY signaling, acting from the brain as well as locally within the cells of bone itself [2,3], consistent with its necessary ties to overall energy balance and body weight. Under conditions of negative energy balance, when hypothalamic NPY levels are high, NPY exerts a strong inhibitory tone on bone formation [4]. This suppression of bone production during periods of starvation provides a mechanism to match skeletal homeostasis to available energy stores and body weight, thereby protecting whole body energy reserves from depletion. Conversely, during positive energy balance NPY signaling decreases, releasing the inhibition of bone formation and increasing bone mass as body weight and its associated mechanical demand on the skeleton increases [4]. The central NPY axis acts through its Y2 receptors in the hypothalamus [2]. However, in addition to the actions of central NPY/Y2 receptors, in recent years the actions of peripheral Y1 receptors has suggested the existence of a parallel axis, involving local production of

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NPY and direct Y1 receptor signaling within bone forming osteoblasts. This was confirmed by the demonstration of increased bone formation and bone mass in osteoblast-specific Y1 receptor deletion models [3,5] and decreased bone formation and mass in an osteoblast-specific NPY over expression model [6]. The local production and signaling of NPY in the osteoblast now suggests that, in parallel to the central circuit, a local energy-responsive circuit also exists that is critically involved in the coordination of bone-specific energy requirements.

Work from our laboratory has now progressed these investigations to show that NPY signaling via osteoblastic Y1 receptors not only controls bone mass but also contributes significantly to the control of whole-body insulin secretion and glucose homeostasis in mice through the release of novel factor(s), which are different from the previously implicated osteocalcin [7]. Specifically, a mouse model with conditional deletion of Y1 receptors in early osteoblasts, while displaying the expected high bone mass phenotype, also revealed impaired glucose tolerance, associated with reduced pancreatic islet volume and insulin secretion [7]. These glycemic changes were not evident when Y1 receptors were deleted from more mature osteoblasts [7], indicating a pathway confined to early cells of the osteoblast lineage. Analysis of bone marrow chimeras as well as *in vitro* experiments confirmed that the alterations in glucose and insulin homeostasis were the result of a direct endocrine signaling pathway originating in the osteoblast [7]. This represented the first mechanistic evidence for peripheral co-ordination of energy utilisation within bone tissue via an NPY/energy balance-dependent pathway; however, the capabilities and mechanism of such an axis were not known.

Here, we identify osteoglycin as a down-stream mediator of Y1 receptor signaling in early osteoblasts and its involvement in the communication between bone, energy and glucose homeostasis. We define novel actions for osteoglycin in the control of bone production, as well as in the modulation of whole body energy balance through the control of food intake and glucose uptake. To determine the exact role of osteoglycin in the co-ordination of bone, glucose, and whole body energy homeostasis, we have generated a novel osteoglycin deficient mouse model and performed comprehensive *in vivo* phenotype profiling, combined with osteoglycin administration in wildtype mice, human studies, and transcriptomic and proteomic analysis of Y1 receptor deficient osteoblasts.

2. METHODS

2.1. Mice

All animal experiments were approved by the Garvan Institute/St Vincent's Hospital Animal Experimentation Ethics Committee and conducted in accordance with relevant guidelines and regulations. All data presented are for male mice randomly assigned to experimental groups. Mice were group housed unless otherwise stated under a controlled temperature of 22 °C and a 12 h light cycle (lights on from 07:00 to 19:00 h) with ad libitum access to water. Mice were fed either a standard chow diet (6% calories from fat, 21% calories from protein, 71% calories from carbohydrate, 2.6 kcal/g, Gordon's Specialty Stock Feeds, Australia) or a high fat diet (HFD; 43% kilojoules from fat, 17% kilojoules from protein, 40% kilojoules from carbohydrate, 4.8 kcal/g, Specialty Feeds, Australia) starting from 7 weeks of age.

To isolate purified populations of osteoblasts, mice with green fluorescent protein (GFP) expression under control of a 3.6 kb fragment of the rat α1(I)-collagen promoter [8] were bred with previously described germline Y1 receptor knockout mice ($Y1^{-/-}$) [9]. GFP expression was confirmed using fluorescent microscopy of tails as well as PCR using oligos to detect the GFP transgene (forward oligo: 5′-GTTCTGCTGGTAGTGGTCG-3'; reverse oligo: 5′-CTGCACCACCGG-CAAGCT GC-3′). PCR was performed with 40 cycles with an annealing temperature of 58 °C.

Mice with osteoblastic-specific deletion of the Y1 receptor ($Y1^{lox/lox}3.6Cre$) were generated and bred as previously described [7]. C57BL/6JAusb mice were sourced from the Mouse Engineering Garvan/ABR (MEGA) Facility (Moss Vale and Sydney, Australia). Mice expressing GFP under control of the NPY promotor (Tg (Npy-hrGFP)1Lowl) were obtained from The Jackson Laboratory (Bar Harbor, Maine, USA).

$Ogn^{-/-}$ mice were produced by the Mouse Engineering Garvan/ABR (MEGA) Facility (Moss Vale and Sydney, Australia) by CRISPR/Cas9 gene targeting in C57BL/6J mouse embryos following established molecular and animal husbandry techniques [10]. To minimize the possibility of off-target genome modifications, the double-nicking approach utilizing the single strand-cleaving mutant of the Cas9 endonuclease (Asp10Ala = Cas9n) was employed [11]. For this purpose, paired single guide RNAs (sgRNAs) were designed to target within the first coding exon (Exon 2) of *Ogn*. The sites used were TGTGAGTC-CAGCTGCGACTGTGG and GTTAACTATGAGTATGCAACAGG (proto-spacer-associated motifs = PAMs underlined). To target *Ogn*, a solution consisting of the two sgRNAs (15 ng/μl each) and full length, polyadenylated Cas9n mRNA (30 ng/μl) was prepared and micro-injected into the nucleus and cytoplasm of C57BL/6J zygotes. Micro-injected embryos were cultured overnight and those that underwent cleavage introduced into pseudo-pregnant foster mothers. Pups were screened by PCR across the two target sites and Sanger sequencing to detect those with modifications to *Ogn*. A founder male carrying a 21bp deletion within Exon 2 (Supplementary Figure 1) was identified, backcrossed to a wildtype C57BL/6J male, and progeny heterozygous for the deletion were inter-crossed to obtain homozygous $Ogn^{-/-}$ mice.

2.2. Human studies

We conducted a prospective, non-randomized study of severely obese male and female patients undergoing three different weight loss interventions: dietary program, sleeve gastrectomy, and Roux-en-Y gastric bypass. The study was approved by the St Vincent's Hospital (Sydney, NSW) Human Research Ethics Committee in accordance with national guidance and all study participants signed written consent. The subjects were recruited in Sydney from Obesity Clinics at Royal Prince Alfred and Royal North Shore Hospitals as well as from private bariatric clinics at St George Private and St Vincent's Hospitals between October 2009 and May 2012. Inclusion criteria for the study were age between 18 and 70 years, a body mass index (BMI) > 35 or BMI > 30 with medical complications from obesity. Women were either premenopausal or at least 5 years post menopause to avoid confounding by menopausal bone loss. Exclusion criteria were pregnancy and/or use of bone active (e.g. anti-resorptive) therapy. 22 patients who fulfilled the inclusion criteria were included in this study. The diet group underwent a weight loss program (Optifast® VLCD™ Program) under the care of their treating physician and dietician. Surgical procedures were performed according to currently accepted surgical techniques [12–14].

2.3. Cell culture

Bone marrow stromal cells (BMSCs) were isolated from male mice as previously described [3] using osteogenic media (control media supplemented with 50 mg/l ascorbic acid and 10 mM β-glycerophosphate). Briefly, following sacrifice by cervical dislocation, marrow was flushed from femurs and tibias with osteogenic media and cells were plated at a density of 1.9×10^6 cells/cm^2 in 50 cm^2 plastic tissue

