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Glucose-dependent insulinotropic polypeptide receptor deficiency leads to modifications of trabecular bone volume and quality in mice

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ABSTRACT

A role for the gastro-intestinal tract in controlling bone remodeling is suspected since serum levels of bone remodeling markers are affected rapidly after a meal. Glucose-dependent insulinotropic polypeptide (GIP) represents a suitable candidate in mediating this effect. The aim of the present study was to investigate the effect of total inhibition of GIP signaling on trabecular bone volume, microarchitecture and quality. We used GIP receptor (GIPR) knockout mice and investigated trabecular bone volume and microarchitecture by microCT and histomorphometry. GIPR-deficient animals at 16 weeks of age presented with a significant (20%) increase in trabecular bone mass accompanied by an increase (17%) in trabecular number. In addition, the number of osteoclasts and bone formation rate was significantly reduced and augmented, respectively in these animals when compared with wild-type littermates. These modifications of trabecular bone microarchitecture are linked to a remodeling in the expression pattern of adipokines in the GIPR-deficient mice. On the other hand, despite significant enhancement in bone volume, intrinsic mechanical properties of the bone matrix was reduced as well as the distribution of bone mineral density and the ratio of mature/immature collagen cross-links. Taken together, these results indicate an increase in trabecular bone volume in GIPR KO animals associated with a reduction in bone quality.

Introduction

Bone is a living mineralized material, highly complex and constantly remodeled in mass and architecture to adapt and repair the damages induce by growth, ageing and mechanical stress. In order to maintain a constant bone mass, bone remodeling necessitates a spatio-temporal coupling between osteoclasts, the bone-resorbing cells, and osteoblasts, the bone-forming cells. Bone remodeling is under a complex regulation from various factors that may affect osteoclast and osteoblast physiologies. A role for gastro-intestinal hormones in controlling bone remodeling has been suggested as changes in the profile of serum markers of bone remodeling after a meal coincides with a peak in gastro-intestinal hormones releases [1,2].

Glucose-dependent insulinotropic polypeptide (GIP) is an important gastro-intestinal hormone synthesized and secreted into the blood stream by the duodenal endocrine K cells after ingestion of a mixed meal [3–5]. To induce a biological response, it binds to glucose-dependent insulinotropic polypeptide receptors (GIPRs), expressed in the endocrine pancreas, gastrointestinal tract, brain, immune and cardiovascular systems, testis, pituitary, lung, kidney, thyroid, several regions in the central nervous system and adipose tissue [6]. Despite a wide distribution of its receptor in the body, the most remarkable action of GIP is to potentiate glucose-stimulated insulin secretion from pancreatic β-cells [7]. Recently, the presence of a functional GIPR has been evidenced at the surface of osteoblasts and osteoclasts [8,9]. However, the role of GIP in bone physiology remains unclear. *In vitro*, it seems that GIP stimulates the synthesis of collagen type I and TGF-β by osteoblasts [10,11]. However, the possible role that GIP might have in controlling bone resorption and/or bone remodeling *in vivo* is more controversial. Indeed, although Zhong et al., reported that GIP directly reduced osteoclast activity in mature murine osteoclasts *in vitro* [12], Tsukiyama et al. observed no direct effects of GIP on the same cells [13]. By using a model of GIPR-deficient mice, Tsukiyama et al. described a decrease in trabecular thickness at 8 weeks of age, but this decrease was not observed in 6-week-old animals. Furthermore, 6 week-old GIPR-deficient mice did not present with a reduction of the trabecular bone volume. However, the number of osteoclasts was augmented at both ages [13]. On the other hand, using the same GIPR-deficient model, Xie et al. described a reduction in trabecular bone volume in younger animals (4-week-old) [14]. This reduction in BV/TV was accompanied by an increase in

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trabecular number with no differences in trabecular thickness and a reduction in trabecular separation [14]. These authors also reported a reduction in bone mineral content in older animals (3- and 5-month old) [14]. From these two studies, the overall conclusion is that the lack of GIPR signaling leads to a decrease, significant or not, in bone mass. More intriguing is the phenotype of GIP-overexpressing mice. In these mice, GIP expression is under the control of a metallothionein promoter and as such the supplementation in zinc led to high circulating GIP levels [15]. Although one could expect a high bone mass, induction of GIP over-expression led to a low bone mass phenotype with a drop in trabecular number [15]. These results support a negative regulation of bone mass under GIP stimulation.

The aims of the present study were to conduct a comprehensive investigation of trabecular bone mass and microarchitecture in GIPR knockout male mice and to assess other parameters of quality of the trabecular bone matrix [16]. Our results indicated that 16-week-old GIPR-deficient male mice present with a significant increase in BV/TV, associated with an increase in trabecular number and a decrease in osteoclast number. Bone quality of the trabecular bone matrix was also reduced in animals lacking a functional GIPR.

Material and methods

Animals

Male and female mice presenting a deletion of the GIPR were used in this study. The background and generation of GIPR-deficient mice used in this study has been previously described [17]. Age-matched wild-type (WT) mice with the same C57BL/6 genetic background were used as controls (Harlan Ltd., Oxon, UK). Animals were maintained on a 12 h:12 h light–dark cycle in a temperature-controlled room (21.5 ± 1 °C). Animals were individually caged and received food and water *ad libitum*. All experiments were conducted according to United Kingdom Office regulations (UK Animals Scientific Procedures Act 1986) and European Union laws. Animals were injected intraperitoneally with calcein (10 mg/kg) seven and two days before necropsy. Blood samples were collected from the cut tip of the tail vein (~200 μl) of conscious mice prior to sacrifice by lethal inhalation with CO_2 and tibias were then harvested and processed as previously described [18].

Body composition measurements

Lean and fat percentage were measured by dual energy X-ray absorptiometry using a PIXImus system (Inside Outside Sales, Wisconsin, U.S.A.). The PIXImus system allows accurate measurement of small laboratory animals using a relatively low X-ray energy and ultra high resolution (0.18×0.18 mm pixel size). Whole body weight was measured after overnight fasting and the nose-to-tail length was determined.

Intraperitoneal glucose tolerance tests

Drinking water and a standard rodent maintenance diet (Trouw Nutrition, Cheshire, UK) were freely available until 18 h before test. Mice received an intraperitoneal injection of glucose alone (18 mmol/kg body weight) in a final volume of 8 ml/kg body weight. Blood samples were collected from the cut tip on the tail vein of conscious mice into chilled fluoride/heparin glucose microcentrifuge tubes (Sarstedt, Nümbrecht, Germany) immediately prior to injection and at 15, 30 and 60 min post-injection. Plasma was aliquoted and stored at −20 °C prior to glucose determination. Plasma glucose and insulin were assayed by an automated glucose oxidase procedure using a Beckman Glucose Analyzer II (Beckman Instruments, Galway, Ireland) and a modified dextran-coated charcoal radioimmunoassay [19], respectively.

X-ray microcomputed tomography

MicroCT analysis was performed with a Skyscan 1172 microtomograph (Skyscan, Kontich, Belgium) equipped with an X-ray tube working at 69 kV/100 μA. The pixel size was fixed at 3.75 μm, the rotation step at 0.25° and exposure was done with a 0.5-mm aluminum filter. Bone mass and microarchitecture at the tibia metaphysis were determined with the CTan software (release 1.11.4.2, Skyscan). The volume of interest (VOI) was located 0.5 mm below the growth plate on a height of 2 mm (534 sections). Trabecular bone volume (BV/TV_{3D}, in %), trabecular thickness ($Tb.Th_{3D}$, in μm), trabecular number (Tb.N, in 1/μm), trabecular separation (Tb.Sp, in μm), trabecular pattern factor ($Tb.P_f$) and structure model index (SMI) were measured according to guidelines and nomenclature proposed by the American Society for Bone and Mineral Research [20].

Bone histomorphometry

Bone samples were embedded, undecalcified in poly (methylmethacrylate) (pMMA) at 4 °C to preserve enzyme activities. Sections (7-μm thickness) were performed on a heavy duty microtome equipped with a 50° tungsten carbide knife. For each animal, four non serial sections (~50 μm apart) were left unstained for the measurement of calcein-based parameters (original magnification ×400), four sections were stained with Goldner's trichrome for two-dimensional bone volume and marrow adiposity measurements (original magnification ×40), four sections were stained with toluidine blue for osteoblast counting (original magnification ×400) and four additional sections were stained for the osteoclastic tartrate resistant acid phosphatase (TRAcP). TRAcP identification is based on a histoenzymatic detection with a simultaneous azo-dye coupling method, bone being counterstained with phosphomolybdic aniline blue and was performed at an original magnification of ×200 [21]. Only TRAcP-positive nucleated cells in contact with bone were counted as osteoclasts. The region of interest (ROI) was located in the secondary spongiosa 0.5 mm below the growth plate on a height of 2 mm. Standard bone histomorphometrical nomenclatures, symbol and units were used as described in the report of the American Society for Bone and Mineral Research [22].

Ex vivo cell culture

Bone marrow cells were isolated from the long bones of 16 weeks-old male animals by flushing tibias and femurs with alpha-MEM as previously reported [23]. For osteoblast differentiation, bone marrow cells were plated into a 25 cm^2 culture flask until confluency (~3 days). At confluency, cells were detached with trypsin 1% and seeded into a 24-well tissue plate at a density of 2.5×10^4 cells/well in alpha-MEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 5 mM β-glycerophosphate and 50 μg/ml ascorbic acid. For osteoclast culture, bone marrow cells were cultured for 24 h into a 25 cm^2 flask in alpha-MEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin to allow stromal cells attachment. Non adherent cells were then collected and plated in 24 well-plate in alpha-MEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 25 ng/ml macrophage-colony stimulating factor (M-CSF-R&D systems, Abingdon, UK) and 50 ng/ml soluble RANKL (Peprotech Ltd, London, UK). After seven days of culture, TRAcP staining was performed as previously described [24]. TRAcP-positive cells with more than three nuclei were considered as osteoclasts.

Gene expression

Total RNA was extracted from osteoblast or osteoclast *ex vivo* cultures using TriZol, reversed transcribed using iScript cDNA synthesis kit (Bio-Rad) and amplified by real-time PCR using SYBR GREEN PCR