



Published in final edited form as:

Clin Endocrinol (Oxf). 2012 September ; 77(3): 379–384. doi:10.1111/j.1365-2265.2011.04263.x.

The Consequences of GHRH-R Haplo-Insufficiency for Bone Quality and Insulin resistance

Miburge B. Gois-Jr^a, Roberto Salvatori^b, Manuel H. Aguiar-Oliveira^c, Francisco A. Pereira^a, Carla R. P. Oliveira^c, Luiz A. Oliveira-Neto^c, Rossana M. C. Pereira^c, Anita H.O. Souza^c, Enaldo V. Melo^c, and Francisco J. A. de Paula^a

^aDepartment of Internal Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil, 14049-900

^bDivision of Endocrinology, The Johns Hopkins University School of Medicine, Baltimore, Baltimore, MD, USA 21287

^cDepartment of Medicine, School of Medicine, Federal University of Sergipe, Aracaju, SE, Brazil 49060-100

Abstract

OBJECTIVE—Growth hormone (GH)/insulin like growth factor (IGF) axis and insulin are key determinants of bone remodeling. Homozygous mutations in the GH releasing hormone receptor (*GHRHR*) gene (*GHRHR*) are a frequent cause of genetic isolated GHD (IGHD). Heterozygosity for *GHRHR* mutation causes changes in body composition and possibly an increase in insulin sensitivity, but its effects on bone quality are still unknown. The objective of this study was to assess the bone quality and metabolism and its correlation with insulin sensitivity in subjects heterozygous for a *null* mutation in the *GHRHR*.

PATIENTS AND METHODS—A cross-sectional study was performed on 76 normal subjects (68.4% females) (N/N) and 64 individuals (64.1% females) heterozygous for a mutation in the *GHRHR* (MUT/N). Anthropometric features, quantitative ultrasound (QUS) of the heel, bone markers (osteocalcin and CrossLaps), IGF-I, glucose, and insulin were measured and homeostasis model assessment of insulin resistance (HOMA_{IR}) was calculated.

RESULTS—There were no differences in age or height between the two groups, but weight ($p = 0.007$) and BMI ($p = 0.001$) were lower in MUT/N. There were no differences in serum levels of IGF-I, glucose, *T* score, or absolute values of stiffness and osteocalcin, but insulin ($p = 0.01$), HOMA_{IR} ($p = 0.01$) and CrossLaps ($p = 0.01$) were lower in MUT/N. There was no correlation between osteocalcin and glucose, osteocalcin and HOMA_{IR} in the 140 individuals as a whole or in the separate MUT/N or N/N groups.

CONCLUSIONS—The present study suggests that one allele mutation in the *GHRHR* gene has a greater impact on energy metabolism than on bone quality.

Keywords

Growth Hormone; Growth Hormone Deficiency; Osteoporosis; Bone Ultrasound; Bone Remodeling

Correspondence to: Manuel H Aguiar-Oliveira, MD PhD, Rua Cláudio Batista s/n, 49069-100 Bairro Sanatório Aracaju-SE-Brasil, Phone/Fax: (+55) 79 2105-1805, herminio@infonet.com.br.

The authors declare no conflict of interest.

Introduction

Bone mass is influenced by several factors including body composition, bone remodeling activity, insulin sensitivity and growth hormone (GH)/insulin like growth factor I (IGF-I) secretion.^{1–8} Previous studies have shown that acquired GH deficiency (GHD) associated with multiple pituitary deficits is linked to low BMD and increased fracture risk compared to the general population⁹. The incidence of isolated GHD (IGHD) is estimated to be 1/3,480–1/10,000 live births¹⁰. Albeit rare, homozygous mutations in the GH releasing hormone receptor (*GHRHR*) gene represents an exceptional clinical model for the investigation of growth hormone deficiency and its consequences in human health.¹¹ On the other hand, the prevalence of heterozygous carriers in the normal population is unknown. Since the major determinants of peak bone mass and strength are genetic it would be worthwhile to determine if this genetic trait can cause lower bone quality.

We have identified large kindred of patients with familial isolated growth hormone deficiency (IGHD), with 105 affected individuals over 8 generations, residing in Itabaianinha County, in the Northeastern Brazilian state of Sergipe. IGHD is caused by a homozygous mutation in the splice donor site of intron 1 (c.104+1G>A) of the *GHRHR* gene.¹² Adult IGHD individuals present severely short stature, reduction of lean mass, and increase in percent fat mass,^{13–14} They have normal IGF binding protein 1 and 2¹⁵ and leptin,¹⁴ high adiponectin levels,¹⁴ and obvious reduction of serum IGF-I, IGF-II, IGF binding protein 3,¹⁵ and insulin.^{13,14} They also have a reduced T-Score of bone stiffness, assessed by heel quantitative ultrasound (QUS), when compared to age-matched normal controls.¹⁶

We have recently reported that heterozygous carriers of this mutation have a phenotype that includes changes in body composition but not in serum IGF-I or stature.¹⁷ The aim of the present study was to extend the study of heterozygous carriers to their bone phenotype, by assessing bone quality and biochemical markers of bone formation and resorption.

Subjects and Methods

Subjects

The study comprised adult individuals with normal stature, aged 25–75 years from the Itabaianinha community who were first-, second-, or third-degree relatives of IGHD individuals, recruited by advertisement on a local bulletin board and by word of mouth. We collected buccal cells from 240 adult individuals and DNA was extracted by alkaline lysis. Genotyping for the c.104+1G>A mutation was carried out as previously described.¹⁷ Subjects with a history of childhood disease known to influence bone density such as hepatic or renal failure, or with a history of exposure to chronic steroids, medications for the treatment of osteoporosis, anticonvulsants, any use of alcohol and tobacco (current or previous), physical inactivity (bedridden or walking difficulties) and vigorous physical activity (athletes) were excluded. We identified 76 subjects heterozygous for the c.104+1G>A *GHRHR* mutation (MUT/N) and 77 sex-matched genotype-proven normal subjects (N/N), as previously reported.¹⁷ Twelve MUT/N subjects and one individual in the N/N group were excluded due to the lack of data regarding one or more variables of the protocol. Therefore, 64 MUT/N (64.1 % female) and 75 N/N (68.4 % female) individuals were included in the study. The protocol was approved by the Johns Hopkins University, CONEP and the Federal University of Sergipe Ethics Committees. All subjects gave written informed consent. Height (m) and body weight (kg) were measured with a portable stadiometer and a portable scale. Body mass index (BMI) was calculated using the formula: weight in kg/height m².

Laboratory assessment

IGF-I was measured in duplicate by DSL-5600 immunoradiometric assay (IRMA; Diagnostic Systems Laboratories, Webster, TX). To pool IGF-I measurements in both genders and in different ages, results were normalized by standard deviation scores (SDS). SDS for serum IGF-I was calculated by subtracting the mean IGF-I level for age from the individual value and dividing this value by the standard deviation of the respective mean age given by the manufacturer. The normal SDS distribution has a mean of 0 and a standard deviation of ± 1 . Values of SDS above +2 or below -2 are by definition abnormal. Glucose was measured by Accu-Check Advantage[®], Roche, San Francisco, CA. Serum levels of insulin was measured by a solid-phase, two-site chemiluminescent assay DPC-5210 (Diagnostic Products Corporation, Los Angeles, CA) and insulin resistance was estimated using the Homeostasis Model Assessment of Insulin Resistance (HOMA_{IR}) with the formula: fasting serum insulin ($\mu\text{U/ml}$) \times fasting plasma glucose (mmol/l)/22.5.¹⁸ IGF-I and insulin assays were performed at the hormone laboratory of the University Hospital, Federal University of Sergipe. Osteocalcin (OC) was measured by DSL-7600 IRMA and serum levels of the C-terminal cross-linking telopeptide of type I collagen (CrossLaps) were measured by DK-2730 ELISA immunoradiometric assay (IRMA; Orion Diagnostica, Webster, TX) in the Metabolism Laboratory of the General Hospital, School of Medicine of Ribeirão Preto, University of São Paulo. The intra-assay coefficients of variation (CVs) for the determination of IGF-I, insulin, OC and CrossLaps were 6.5%, 4.2%, 8.3%, and 7.2%, respectively. All the assays were performed in a single run.

Bone status measurements

We determined the bone stiffness index by heel QUS. QUS is a water-based system that uses transmission of an ultrasound wave in a temperature-controlled water bath (37°C) through the heel. This measurement was performed with rigorous standardization of subject positioning using the Achilles Insight device (Lunar/GE, Madison, WI). QUS measures the stiffness index by the equation: stiffness = (0.67 \times coefficient of ultrasound broadband attenuation in dB/MHz + 0.28 \times speed of sound in m/s) - 420. Bone stiffness was expressed as the percentage of the values obtained by the manufacturer for a young adult population. For the determined bone stiffness index we chose a normal South American population. The CV of bone stiffness measurement was 2.23%.

Statistical analysis

Values for continuous variables were expressed as mean \pm standard deviation. Physical characteristics (weight, height and BMI), were analyzed by the Student *t*-test. Insulin and HOMA_{IR} data were transformed into decimal logarithm before analysis. A general linear model, encompassing both analysis of variance (ANOVA) and regression, using HOMA_{IR} as a dependent variable and ANOVA with two factors (sex and group) adjusted for age and BMI was subsequently used. The regression model was used to examine possible associations between weight and BMI with bone markers, and between bone markers with glucose, insulin and HOMA_{IR}, by estimating their square R (R^2). To test the hypothesis of any correlation between OC and glucose, insulin and HOMA_{IR} we used Spearman's rank correlation. The Pearson correlation coefficient was used to analyze the correlation between stiffness and OC and between stiffness and CrossLaps. *P* values of 0.05 or less were considered to be statistically significant. SAS software (SAS 9.1, Cary, NC) was used for the analysis.

Results

Anthropometric, biochemical and bone marker data (mean \pm SD) for homozygous normal subjects (N/N) and individuals heterozygous for the *GHRHR* mutation (MUT/N) are shown

in table 1. The statistical analysis did not change when individuals aged more than 50 years (37%) were excluded. There were no differences in age or height between groups but weight ($p = 0.007$) and BMI $p = 0.001$ were lower in MUT/N.

There were no differences in IGF-I or glucose between groups, but insulin and HOMA_{IR} ($p < 0.01$) were significantly lower in MUT/N. For the HOMA_{IR} the mean magnitude of difference was 0.23 ± 0.11 , 95% confidence interval from 0.12 to 0.448, $p = 0.03$. Table 2 shows the data of the general linear model using HOMA IR as a dependent variable and ANOVA with two factors (sex and group) adjusted for age and BMI. The model was able to explain 23 % of the variability of the HOMA_{IR}, being in the model BMI the principal determinant of this variability, with an adjusted R square of 0.134 (explaining 13.4% of the HOMA_{IR} variability). The only other significant covariate was group, with an adjusted R square of 0.134 (explaining only 3% of HOMA_{IR} variability). There was not effect of age, sex, and interaction between group and sex in HOMA_{IR}.

While serum OC did not differ between groups, CrossLaps levels were significantly lower in the MUT/N group. The regression model showed no association between weight with OC and CrossLaps in MUT/N ($R^2 = 0.00001$, $p = 0.98$ and $R^2 = 0.0000001$, $p = 0.99$, respectively) and N/N ($R^2 = 0.00003$, $p = 0.96$ and $R^2 = 0.02$, $p = 0.18$, respectively) or BMI with OC and CrossLaps in MUT/N ($R^2 = 0.02$, $p = 0.3$ and $R^2 = 0.00001$, $p = 0.98$ respectively) or N/N ($R^2 = 0.02$, $p = 0.28$ and $R^2 = 0.02$, $p = 0.19$ respectively). There was no correlation between OC and glucose ($r = 0.12$; $p = 0.693$), OC and insulin ($r = -0.05$; $p = 0.673$) and OC and HOMA_{IR} ($r = -0.06$; $p = 0.603$), between stiffness and OC ($r = 0.06$ $p = 0.83$), or between stiffness and CrossLaps ($r = 0.08$; $p = 0.59$) when all 140 individuals were analyzed as a whole or when the individual groups were analyzed. Similarly, there was no correlation between age and any bone marker in all individuals, or when the groups were analyzed separately (figure 1).

The parameter values obtained by quantitative ultrasound T-Score (N/N = 0.09 ± 1.53 vs MUT/N = 0.07 ± 1.32) and stiffness (N/N = 96.63 ± 23.83 vs MUT/N = $96.32 \pm 19.32\%$) did not differ significantly between groups.

Discussion

The GH-IGF-I axis plays a pivotal role in maintaining bone health and GHD may predispose to loss of bone¹⁹ and to fracture.²⁰ Additionally, previous studies have shown that a mild decline in the GH/IGF-I axis is associated with bone loss during aging. Our results show that heterozygosity for a *GHRHR* mutation is associated with reduced body weight, BMI, serum insulin and HOMA_{IR}, all factors that could potentially influence bone strength. The present study confirms data obtained previously when by using infrared interactance, we showed that absolute lean mass was reduced in MUT/N, while there was significant difference in percentage of fat mass but a trend of reduction in absolute fat.¹⁷ It is necessary to taken into account a critical period for fat mass development take place during childhood. Therefore, the trend in reduction of fat mass and BMI may reflect impairment of preadipocytes differentiation, which have high IGF-I receptor expression.²¹ Although both BMI and group are significantly associated to HOMA_{IR}, the general linear model showed that BMI explains the reduction of HOMA_{IR} more than the group effect in MUT/N shown in this protocol with a good power. However, the mechanism responsible for these alterations remains to be determined; one may hypothesize that they reflect a reduction in GH secretion caused by *GHRHR* haploinsufficiency which may not be unveiled by a reduction in serum IGF-I To test this hypothesis it will be necessary to compare the 24-h GH secretion profile of MUT/N to N/N individuals. The lack of difference in serum IGF-I between WT/MT and N/N suggests that these effects in adulthood are not modulated by circulating serum IGF-I,

although the expression of IGF-I receptor in muscle or fat tissue in MUT/N is unknown. Particularly, the low BMI has been reported to influence the risk of osteoporosis.²² However, in our study, T-score and absolute value of stiffness as measured by heel QUS were similar in both groups, demonstrating that bone quality is not impaired in heterozygous *GHRHR* individuals. However, while homozygosity for the mutation leads to decreased bone quality, heterozygosity has no impact on bone ultrasound parameters. This condition is not exclusive of *GHRHR* deficiency. The genetically engineered mouse knockout for the vitamin D receptor (VDR) shows bone disorder as well as lean phenotype. On the other hand, VDR heterozygote male mouse has a lean phenotype but normal bone structure.²³

The use of QUS rather than DXA in this study was dictated by logistic reasons. Although the WHO has objectively defined osteoporosis based on BMD assessment (i.e., T-Score -2.5 standard deviations below normal peak bone mass) osteoporotic fracture can occur across a wide spectrum of BMDs, suggesting an important role not only of bone mass, but also of bone quality. Additionally, in several conditions such as diabetes mellitus and obesity, increased bone fragility occurs in spite of normal or even increased BMD. Heel ultrasound has some advantages over other more expensive options such as computed tomography, quantitative peripheral tomography, magnetic resonance and DXA. Particularly, QUS is the only technique suitable for use in field studies and correlates with both fracture risk and bone quality.^{24–28}

MUT/N individuals exhibited a predominantly anabolic profile of biochemical markers of bone remodeling: while serum OC levels were similar to those of control individuals, the parameter of bone resorption was significantly decreased in MUT/N patients. Therefore, it can be hypothesized that the conserved bone formation associated with lower resorption activity is a mechanism for the maintenance of bone quality in MUT/N individuals in comparison to the previous MUT/MUT group.¹⁶ There are few studies dedicated to the investigation of BMI effect on bone remodeling markers. BMI has been shown to have a negative effect in both types of bone markers, i.e formation and resorption with women after menopause exhibited the strongest negative association.²⁹ In the present study, however this association was not found.

We did not find any correlation between serum levels of OC and glucose, insulin and HOMA_{IR}. Lee et al.,³⁰ and Fernández-real et al.² have recently demonstrated that bone regulates the insulin/glucose axis and energy metabolism in mice. Osteoblast cells and uncarboxylated OC have a pivotal role in this network.³¹ In parallel, Pitta et al.³ showed that both carboxylated and undercarboxylated OC was significantly correlated with insulin and HOMA_{IR}. In the BMI range of our patients (25 to 30 kg/m²), the reference HOMA_{IR} of Brazilian adult individuals ranges between 0.4 and 4.3, with a mean of 1.7, and a threshold value for insulin resistance of 2.71.³² This value is above our HOMA_{IR} values in both MUT/N and N/N. Therefore, the low values of both MUT/N and N/N might have extinguished a possible correlation between OC and HOMA_{IR}. In addition, recently reduced serum total OC was associated with metabolic syndrome in older men as shown by waist circumference, hyperglycemia, and triglyceride levels. The associations between serum total OC and these parameters were most apparent at the levels of total OC below a threshold of 20 µg/L,³¹ while the mean OC value of our MUT/N individuals was 25.5 ± 11.32 µg/L. Differently from individuals with metabolic syndrome, the MUT/N individuals presented lower lean mass, waist and hip circumferences, and glucose and triglyceride levels similar to those of N/N.¹⁷ These data suggest that only reduced serum total OC can identify individuals at increased risk of metabolic syndrome.

Our study has some limitations. We did not measure serum levels of vitamin D, calcium and alkaline phosphatase, but both groups live in the same environmental condition (equatorial

area, with abundant year-around sunlight exposure) and have similar diet habit. Additionally, the normal result obtained in bone evaluation suggests no major impairment on vitamin D supply. Another one is that only one third of all individuals were older than 50 years. In control individuals, bone loss and decreased IGF-I production are coincident factors of the aging process. It will be important to evaluate if aging in MUT/N individuals is associated with accelerated bone loss by studying a larger number of older individuals.

In conclusion, the present study shows that a monoallelic mutation in the *GHRHR* gene does not impact skeletal growth and bone ultrasonometry parameters in middle-aged individuals. However, these individuals exhibit a lean phenotype and an advantage in insulin sensitivity, assessed by HOMA_{IR}.

Acknowledgments

Research supported by grants from NIH 1 R01 DK065718, National Institutes of Health USA to R.S., from FAPITEC/SE, Brazil to R.M.C.P and from CAPES and FAEPA. We are grateful to Ms. Ivanilde Santana de Sousa, Mr. Sebastião Lázaro Brandão Filho, and Mr. Davi C. Aragon for secretarial, laboratory and statistical assistance.

References

1. Götherström G, Bengtsson BA, Bosaeus I, et al. Ten-year GH replacement increases bone mineral density in hypopituitary patients with adult onset GH deficiency. *European Journal of Endocrinology*. 2007; 156:55–64. [PubMed: 17218726]
2. Fernández-Real JM, Izquierdo M, Ortega F, et al. The relationship of serum osteocalcin concentration to insulin secretion, sensitivity and disposal with hypocaloric diet and resistance training. *Journal of Clinical Endocrinology and Metabolism*. 2009; 94:237–245. [PubMed: 18854399]
3. Pitta AG, Harris SS, Eliades M, et al. Association between serum osteocalcin and markers of metabolic phenotype. *Journal of Clinical Endocrinology and Metabolism*. 2009; 94:827–832. [PubMed: 19088165]
4. Petel MB, Arden NK, Masterson L, et al. Investigating the role of the growth hormone–insulin-like growth factor (GH–IGF) axis as a determinant of male bone mineral density (BMD). *Bone*. 2005; 37:833–841. [PubMed: 16153900]
5. de Albuquerque-Taveira AT, Fernandes MI, Galvão LC, et al. Impairment of bone mass development in children with chronic cholestatic liver disease. *Clinical Endocrinology (Oxf)*. 2007; 66:518–523.
6. Hedbacker K, Brisoy K, Wysocki RW, et al. Antidiabetic effects of IGFBP2, a leptin-regulates gene. *Cell Metabolism*. 2010; 11:11–22. [PubMed: 20074524]
7. Takeda S, Eleftriou F, Levasseur R, et al. Leptin regulates bone formations via sympathetic nervous system. *Cell*. 2002; 111:305–317. [PubMed: 12419242]
8. Jürimäe J, Jürimäe T. Plasma adiponectin concentration in healthy pre- and postmenopausal women: relationship with body composition, bone mineral and metabolic variables. *American Journal of Physiology Endocrinology and Metabolism*. 2007; 293:42–47.
9. Gillberg P, Olofsson H, Mallmin H, et al. Bone mineral density in femoral neck is positively correlated to circulating insulin-like growth factor (IGF-I) and IGF-binding protein (IGFBP)-3 in Swedish men. *Calcified Tissue International*. 2002; 70:22–29. [PubMed: 11907704]
10. Lindsay R, Feldkamp M, Harris D, et al. Utah Growth Study: Growth standards and the prevalence of growth hormone deficiency. *Journal of Pediatrics*. 1994; 125:29–35. [PubMed: 8021781]
11. Salvatori R, Fan X, Phillips JA III, et al. Three new mutations in the gene for the growth hormone (GH)-releasing hormone receptor in familial isolated GH deficiency type IB. *Journal of Clinical Endocrinology and Metabolism*. 2001; 86:273–279. [PubMed: 11232012]
12. Salvatori R, Hayashida CH, Aguiar-Oliveira MH, et al. Familial dwarfism due to a novel mutation in the growth hormone-releasing hormone receptor gene. *Journal of Clinical Endocrinology and Metabolism*. 1999; 84:917–923. [PubMed: 10084571]

13. Barreto-Filho JA, Alcantara MR, Salvatori R, et al. Familial isolated growth hormone deficiency is associated with increased systolic blood pressure, central obesity, and dyslipidemia. *Journal of Clinical Endocrinology and Metabolism*. 2002; 87:2018–2023. [PubMed: 11994335]
14. Oliveira CR, Salvatori R, Manegues-Moreno RA, et al. Adipokine profile and urinary albumin excretion in isolated growth hormone deficiency. *Journal of Clinical Endocrinology and Metabolism*. 2010; 95:693–698. [PubMed: 20016047]
15. Aguiar-Oliveira MH, Gill MS, Barreto ES, et al. Effect of severe growth hormone (GH) deficiency due to a mutation in the GH-releasing hormone receptor on insulin-like growth factors (IGFs), IGF-binding proteins, and ternary complex formation throughout life. *Journal of Clinical Endocrinology and Metabolism*. 1999; 84:4118–4126. [PubMed: 10566659]
16. de Paula FJ, Gois-Junior MB, Aguiar-Oliveira MH, et al. Consequences of lifetime isolated growth hormone (GH) deficiency and effects of short-term GH treatment on bone in adults with a mutation in the GHRH-receptor gene. *Clinical Endocrinology (oxf)*. 2009; 70:35–40.
17. Pereira RM, Aguiar-Oliveira MH, Sagazio A, et al. Heterozygosity for a mutation in the growth hormone releasing hormone receptor gene does not influence adult stature, but affects body composition. *Journal of Clinical Endocrinology and Metabolism*. 2007; 92:2353–2357. [PubMed: 17356054]
18. Lansang MC, Williams GH, Carroll JS. Correlation between the glucose clamp technique and the homeostasis model assessment in hypertension. *American Journal of Hypertension*. 2001; 14:51–53. [PubMed: 11206679]
19. Colao A, di Somma C, Pivonello R, et al. Bone loss is correlated to the severity of growth hormone deficiency in adult patients with hypopituitarism. *Journal of Clinical Endocrinology and Metabolism*. 1999; 84:1919–1924. [PubMed: 10372687]
20. Holmer H, Svensson J, Rylander L, et al. Fracture incidence in GH-deficient patients on complete hormone replacement including GH. *Journal of Bone Mineral Research*. 2007; 22:1842–1850.
21. Scavo LM, Karas M, Murray M, et al. Insulin-like growth factor-I stimulates both cell growth and lipogenesis during differentiation of human mesenchymal stem cells into adipocytes. *Journal of Clinical Endocrinology and Metabolism*. 2004; 89:3543–3553. [PubMed: 15240644]
22. de Paula FJ, Rosen C. Obesity, diabetes mellitus and osteoporosis. *Arquivos Brasileiros de Endocrinologia & Metabologia*. 2010; 54:150–157.
23. de Paula FJ, Dick-de-Paula I, Bornstein S, et al. VDR Haploinsufficiency Impacts Body Composition and Skeletal Acquisition in a Gender-Specific Manner. *Calcified Tissue International*. 2011; 89:179–191. [PubMed: 21637996]
24. Hans D, Dargent-molina P, Schott AM. Ultrasonographic heel measurements to predict hip fracture in elderly women: the EPIDOS prospective study. *Lancet*. 1996; 348:511–514. [PubMed: 8757153]
25. Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. Study of osteoporosis fracture research group. *New England Journal of Medicine*. 1995; 332:767–773. [PubMed: 7862179]
26. Kazakia GJ, Majumdar S. New Imaging technologies in the diagnosis of osteoporosis. *Reviews in Endocrinology et Metabolic Disorders*. 2006; 7:67–74.
27. Petrantonaki T, Maris T, Damilakis J. MRI techniques for the examination of trabecular bone structure. *Curr Med Imag Rev*. 2005; 1:35–41.
28. Maggi S, Noale M, Giannini S, et al. Quantitative heel ultrasound in population-based study in Italy and its relationship with fracture history: the ESOPO study. *Osteoporosis International*. 2010; 29:1083–1092.
29. Puntus T, Schneider B, Meran J, et al. Influence of age and gender on associations of body mass index with bone mineral density, bone turnover markers and circulating calcium-regulating and bone-active sex hormones. *Bone*. 2011 Jun 12. ahead of print. 10.1016/j.bone.2011.06.003
30. Lee NA, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell*. 2007; 130:456–469. [PubMed: 17693256]
31. Yeap BB, Chubb SA, Flicker L, et al. Reduced serum total osteocalcin is associated with metabolic syndrome in older men via waist circumference, hyperglycemia and triglyceride levels. *European Journal of Endocrinology*. 2010; 163:265–272. [PubMed: 20501596]

32. Ghiringhello MT, Vieira JG, Tachibana TT, et al. Distribution of HOMA_{1R} in Brazilian subjects with different body mass indexes. *Arquivos Brasileiros de Endocrinologia & Metabologia*. 2006; 50:573–574.

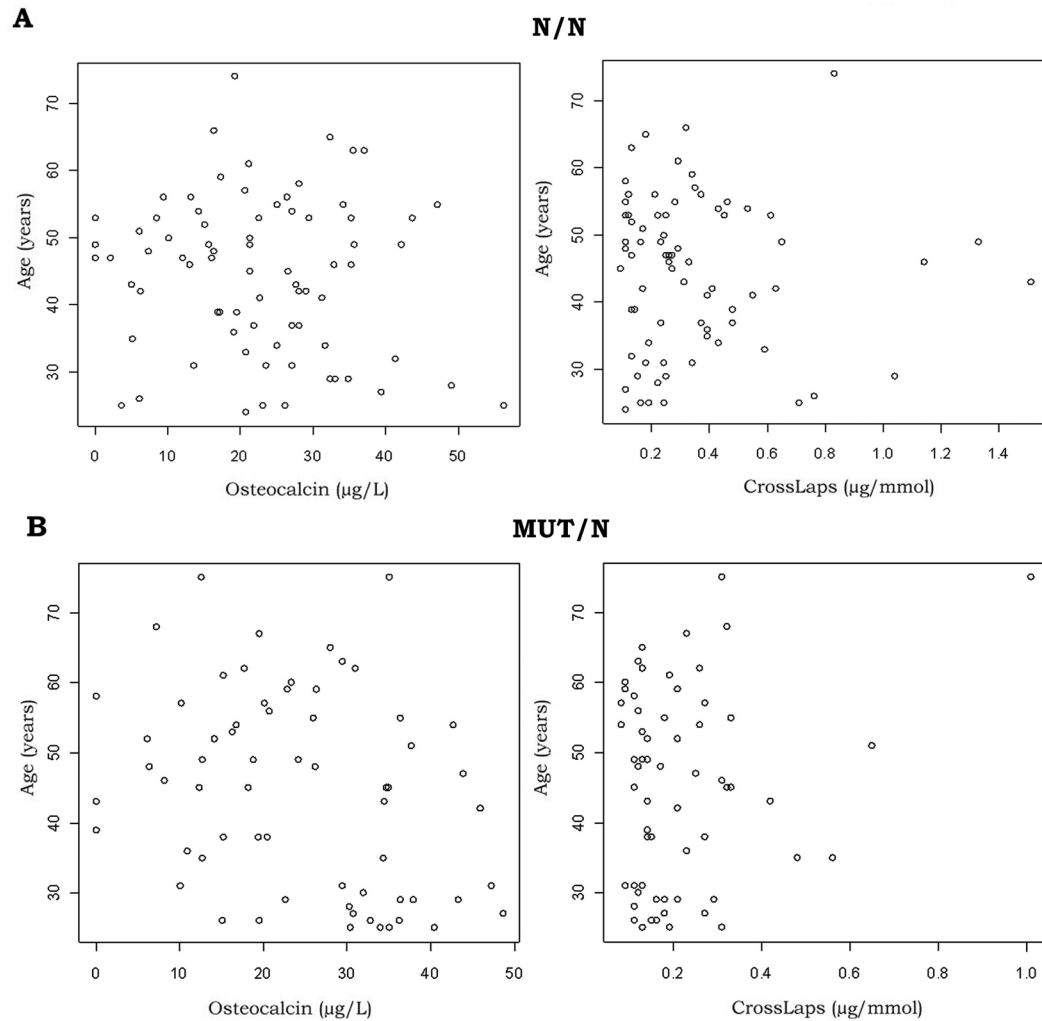


Figure 1.

A) Spearman correlations between age to osteocalcin ($r = -0.08$, $p = 0.617$) and age to crosslaps ($r = -0.06$, $p = 0.761$) in control (N/N) group. B Spearman correlations between age to osteocalcin ($r = -0.31$, $p = 0.342$) and age to crosslaps ($r = -0.02$, $p = 0.866$) in *GHRHR* heterozygote MUT/N group.

Table 1

Anthropometric data, SDS of serum IGF-I levels (SDS IGF- I), biochemical and heel QUS (mean \pm SD) in 76 normal homozygous and 64 heterozygous (MUT/N) subjects.

	N/N	MUT/N	<i>p</i>
Age (yr)	44.71 \pm 11.5	46.61 \pm 14.11	N.S.
Weight (kg)	65.71 \pm 14.12	59.72 \pm 11.61	0.007
Height (m)	1.57 \pm 0.098	1.57 \pm 1.200	N.S.
BMI (kg/m ²)	26.32 \pm 4.91	24.02 \pm 4.43	0.001
SDS IGF-I	-0.73 \pm 1.36	-0.98 \pm 1.54	N.S.
Glucose (mmol/L)	5.20 \pm 0.54	5.19 \pm 0.65	N.S.
Insulin (pmol/L)	28.77 \pm 19.95	19.52 \pm 17.00	0.01
HOMA _{IR}	1.00 \pm 0.68	0.69 \pm 0.59	0.01
Osteocalcin (μ g/L)	23.61 \pm 11.52	25.53 \pm 11.32	N.S.
CrossLaps (μ g/mmol)	350.5 \pm 280.12	220.16 \pm 150.23	0.01
<i>T</i> -score	0.09 \pm 1.53	0.07 \pm 1.35	N.S.
<i>Z</i> -score	0.61 \pm 1.42	0.61 \pm 1.32	N.S.
Stiffness (%)	96.63 \pm 23.84	93.34 \pm 19.31	N.S.

Homeostasis Model Assessment of Insulin Resistance (HOMA_{IR}) was calculated by the formula: fasting serum insulin (μ U/ml) \times fasting plasma glucose (mmol/l)/22.5.

Table 2

General linear model using HOMAIR as a dependent variable and ANOVA with Two factors (sex and group) adjusted for age and BMI.

Source	Type III Sum of Squares	Degrees of freedom	Mean Square	F	Sig.	Partial Eta Squared	Observed Power ^b
Corrected Model	13.82 ^a	5	2.76	7.90	0.0001	0.23	0.999
Intercept	0.97	1	0.97	2.78	0.098	0.020	0.38
Age	0.003	1	0.003	0.009	0.923	0.0001	0.05
BMI	7.26	1	7.26	20.75	0.00001	0.134	0.995
Group	1.53	1	1.53	4.37	0.03	0.03	0.55
Sex	0.93	1	0.93	2.6	0.10	0.019	0.367
Group* Sex	0.44	1	0.44	1.25	0.26	0.009	0.199
Error	46.89	134	0.35				
Total	160.87	140					
Corrected Total	60.73	139					

Partial Eta squared = Effect dimension; Group*Sex: Interaction group and sex

^aR Squared = 0.23 (Adjusted R Squared = 0.199)

^bComputed using alpha = 0.05