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Bone mineral density in familial partial lipodystrophy

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Summary

Objective: Type 1 and type 2 familial partial lipodystrophies (FPLD) are characterized by the loss or increase of subcutaneous fat in certain body regions, as well as metabolic disorders. Higher muscle volume and mass have also been described. However, so far, possible bone involvement has not been studied. The aim of this study was to evaluate bone mineral density (BMD) in patients with type 1 and type 2 FPLD.

Methods: A total of 143 women were selected and distributed into three groups (17 women with FPLD2, 82 women with FPLD1 and 44 non-lipodystrophic obese female controls). A thorough history and physical examination were carried out on all subjects, as well as the measurement of anthropometric features. BMD along with fat and fat-free mass (FFM) were determined by DXA (dual-energy X-ray absorptiometry). Statistical analyses, primarily using the χ^2, ANOVA and ANCOVA tests, were performed, using age, height, fat and FFM as covariables.

Results: After eliminating the possible influences of age, height, fat and FFM, we observed that there were no significant differences in total BMD between patients with FPLD and the control group, showing total BMD values of 1.092 ± 0.037 g/cm^2 in the FPLD2 group, 1.158 ± 0.013 g/cm^2 in the FPLD1 group and 1.173 ± 0.018 g/cm^2 in the control group (p = 0.194). Similarly, no significant differences were found in segmental BMD.

Conclusions: Unlike in other types of laminopathy in which bone is affected, in the case of FPLD there are no differences in BMD compared to non-lipodystrophic subjects.

Introduction

Lipodystrophies are a heterogeneous group of rare diseases characterized by the selective loss of adipose tissue and, depending on their aetiology, can be congenital or acquired. They may be generalized (if the loss of adipose tissue affects the whole body) or partial (if only part of the body is affected) (1).

Among congenital lipodystrophies, familial partial lipodystrophy (FPLD) has been associated with the following genes: *LMNA*, *PPARG*, *AKT2*, *CIDEC*, *PLIN1* and *LIPE* (1), along with a seventh subtype, type 1 (Köbberling syndrome, FPLD1), which normally follows an autosomal dominant pattern of inheritance and for which, until now, no responsible gene has been found (2), although a polygenic pattern has recently been suggested (3) (Table S1).

The classical presentation of FPLD corresponds to type 2 or Dunnigan disease (FPLD2) (4). It is an autosomal dominant disorder associated with mutations in the *LMNA* gene, which codifies for lamin A/C protein (5). It is characterized by the selective loss of adipose tissue affecting the limbs, buttocks and hips, while an accumulation of fat can be observed in the face, neck, axillae and interscapular region (1, 6). A muscular appearance is common, particularly calf muscular hypertrophy. Patients show insulin resistance from an early stage (7) as well as hypertriglyceridaemia and non-alcoholic fatty liver, and may have an increased risk of cardiovascular disease (8). Other alterations may also appear, such as acanthosis nigricans, hirsutism, as well as menstrual alterations which may be associated with polycystic ovary syndrome (PCOS) (9).

FPLD1 is characterized by the loss of adipose tissue, usually from puberty, which is restricted to the hips, buttocks and lower limbs, and fat accumulation in face, neck, back and abdomen, frequently

associated with severe insulin resistance /diabetes mellitus and hypertriglyceridaemia (2, 10, 11). It is not infrequent to find members of the same pedigree with a similar phenotype.

Characteristically, other laminopathies such as Hutchinson-Gilford Progeria Syndrome (HGPS) and Mandibuloacral Dysplasia (MADA and MADB), in addition to lipodystrophy, also show osteolysis and other bone abnormalities (12, 13).

It has also been reported that patients with HIV-lipodystrophy, at least men, have reduced bone density associated with higher visceral fat (14). On the other hand, some putative mechanisms for HIV-lipoatrophy have been associated with prelamin A accumulation (15), which is one of the proposed causes for the loss of fat in FPLD2.

Therefore, it is appealing to hypothesize that bone mineral density (BMD) could be reduced in familial partial lipodystrophy.

Faced with a lack of data on how bone tissue is affected in this group of illnesses, the aim of this study was to evaluate bone mineral density in patients with familial partial lipodystrophy type 1 and type 2.

Materials and methods

Subjects

Three groups of subjects were studied: 17 women between 22 and 65 years of age with a molecular diagnosis of FPLD2 (6 patients were carriers of the p.Asn466Asp *LMNA* mutation, 4 carried the p.Arg482Try mutation, 3 carried the p.Ile299Val mutation, 2 carried the p.Arg482Gln mutation, one carried the p.Thr528Met and another the p.Cys591Phe mutation); 82 women between 31 and 89 years of age with FPLD1, and a control group composed of 44 non-lipodystrophic women between 20 and 81 years of age, who had consulted for obesity, diabetes and/or dyslipidaemia. Demographic characteristics are shown in Table 1. The diagnosis of FPLD1 was based on physical examination and

exclusion of mutations in the *LMNA*, *PPARG* and *LIPE* genes. Due to the difficulty of identifying Köbberling syndrome in men, only women were studied (2). The exclusion criteria were: the diagnosis of another type of genetic or acquired lipodystrophy; Cushing's syndrome or another uncontrolled endocrinopathy; nephrotic syndrome; coeliac disease; severe renal disease and/or liver disease; advanced malignancy; women who where being treated with aromatase inhibitors; women who had been diagnosed with osteoporosis or who were being treated with calcium, vitamin D, bisphosphonates or steroids administered by any route.

Methods

Study design and rationale

This was a retrospective observational study, in which the FPLD2 group was compared with a non-lipodystrophic group and with a lipodystrophic group phenotypically similar to Dunnigan disease but in which no mutations had been found in the *LMNA* gene (Köbberling syndrome).

History and physical examination were performed on all participants in the study. Their height and weight were verified with a digital balance and a stadiometer. The skinfolds of the patients with FPLD1 were measured using a Lange Skinfold Caliper (Cambridge Scientific Industries, Maryland, USA) on the dominant extremity of the subscapular and calf regions in order to determine the KöB index. This index is a skinfold-based ratio established by our group for the diagnosis of FPLD1, which is obtained by dividing the subscapular skinfold thickness by the calf skinfold thickness. It has high sensitivity (89%) and specificity (84%), and a value > 3.477 is highly suggestive of Köbberling syndrome (2).

LMNA, PPARG and LIPE mutation analysis

DNA was prepared from peripheral white blood cells using standard procedures (16). DNA from the Köbberling and Dunnigan groups was amplified with PCR to investigate *LMNA* exons 1-12, *PPAR*G exons 1-8, *LIPE* exons 1-10 and the surrounding intronic sequences. The PCR products were purified

with Illustra ExoStar 1-step (Ge Healthcare, Freiburg, Germany), then directly sequenced with the same primers used for amplification. Sequencing was performed with the BigDye Terminator v3.1 Cycle Sequencing Kit (PE Applied Biosystems, CA, USA). Electropherograms were produced with an ABI 3100 Automated Sequencing Analyzer (PE Applied Biosystems, CA, USA) and analysed with Sequence Navigator software (PE Applied Biosystems, CA, USA).

Bone mineral density and body composition

Determination of bone mineral density (BMD), total and segmental, as well as fat and fat-free mass (FFM) was performed using DXA (whole-body dual-energy X-ray absorptiometry) with a Lunar DPX model (GE Medical Systems, Pittsburgh, PA, USA); in accordance with the instructions provided by the manufacturer, we asked all of our patients to fast for 10-12 hours in order to avoid possible influences on body composition.

Biochemistry

Fasting serum samples were analysed for glucose and insulin, as described previously (7). Insulin resistance was evaluated with the HOMA method (17).

Statistical analysis

Data are expressed as the mean ± SD or SEM. For each continuous variable, the hypothesis of a normal distribution was verified by the Kolmogorov-Smirnov test. The χ^2 test was used to compare qualitative variables. The means of the quantitative variables with normal distribution were compared with the ANOVA test. The means of non-normally distributed, independent variables were compared with the Kruskal-Wallis test. T-test or Mann-Whitney test were used to compare a quantitative variable in two groups. For the post-hoc analysis, the Scheffe and Bonferroni tests were used. The analysis of covariance (ANCOVA) was used in the dependent variable BMD, in order to eliminate the possible modifying effect of certain covariates such as age, height, fat and FFM. The level of significance was set at $p < 0.05$. All statistical analyses were performed with the SPSS 22.0 program (Chicago, Illinois; USA).

Results

Demographic, anthropometric, genetic and clinical data for the different groups studied are shown in Table 1.

Regarding the DXA measurement of fat content (Table 1), it was observed that, in FPLD2 patients, this was significantly reduced both in total and in the limbs and trunk, compared to the other groups studied. In the FPLD1 group, lower extremity fat content was significantly lower than in the control group. FFM (Table 1) was found to be significantly increased in the FPLD2 group in the upper limbs with respect to the other two groups, and in the trunk with respect to the control group.

In this study, in which BMD adjusted for age, height, fat and FFM was analysed (Figure 1), we observed that there were no significant differences in total or segmental BMD between the three groups studied. Although FPLD2 patients showed lower mean BMD values (both total and in extremities), this finding was not statistically significant. When analysing the results of BMD for FPLD1 and FPLD2 subjects compared with 17 age and BMI-matched control subjects, there were still no significant differences (Figure S1).

On the other hand, in our sample, FPLD2 was linked to mutations in exon 8 of the *LMNA* gene in 70.6% of our patients. When comparing BMD among patients with mutations in exon 8 and those with mutations in other exons (5, 9 or 11), there were no significant differences (Figure 2).

Of all our patients with FPLD1, only 11 had a KöB index < 3.477. When comparing BMD among patients with a KöB index > 3.477 and those with a KöB index < 3.477, no significant differences were found (Figure 3).

Only 33.3% of FPLD2 patients were menopausal, compared with 68.4% of FPLD1 patients and 53.7% of the control patients. Lipodystrophic menopausal patients showed lower total and segmental unadjusted BMD, in comparison to lipodystrophic women of childbearing age. However, no significant differences were found in BMD, when adjusted for height, fat and FFM, between menopausal lipodystrophic patients and menopausal control patients (Figure 4). On the other hand,

6% of the lipodystrophic patients had polycystic ovary syndrome (PCOS). However, no statistically significant differences were found in the BMD of these patients and those without ovarian pathology (Figure S2).

Discussion

To the best of our knowledge, this is the first study to be carried out on bone metabolism in patients suffering from familial partial lipodystrophy. It shows that there are no significant differences in BMD between patients with familial partial lipodystrophy and control subjects, once possible distractors, such as age, height, fat and FFM have been eliminated. The observed fat loss, as well as the increase in FFM, are features previously described in these patients (4, 18).

Laminopathies are a group of diseases caused by mutations in the *LMNA* gene, or other genes that influence lamin processing such as *ZMPSTE24* or *BANF1* (19, 20). Lamins are intermediate filament proteins present in the nuclear lamina that are important determinants of nuclear and cellular architecture and are significant regulators of stem cell differentiation (19). Pluripotent mesenchymal stem cells are present in a variety of mature tissues, including bone marrow stroma, adipose depots, muscle and cartilage, and can differentiate into heterogeneous cell types, including osteoblasts, adipocytes, myocytes and chondrocytes (21, 22).

Thus, laminopathies mainly affect mesenchymal tissues: muscle, adipose tissue, and bone, which are affected in premature aging syndromes and related disorders (19). In this way, Hutchinson-Gilford progeria syndrome (HGPS) is characterized by bone alterations such as osteoporosis or deformities (12), as in the case of Mandibuloacral Dysplasia (23, 24) or Atypical Werner Syndrome (25), suggesting that lamin A/C could play an important role in the pathogenesis of the loss of bone mass related to aging.

With regard to this, it has been suggested that normal levels of lamin A/C are required in order to maintain the integrity of bone structure and that a decrease in the expression of lamin A/C inhibits osteoblastogenesis, whilst facilitating the adipogenic differentiation of mesenchymal stem cells

(MSC) both *in vitro* and *in vivo*. Therefore, enhanced adipocyte differentiation from MSC leads to a reduction in the number of stem cells available for osteoblast differentiation (26-28).

Dunnigan disease is characteried by the loss of fat in some parts of the body and its accumulation in others (4). In addition, higher muscle volume and mass have also been described (18). Thus, it is not unreasonable to think that bone could also be affected, showing decreased BMD, in relation to the role which lamin A/C plays in the pathogenesis of age-related bone loss, considering it therefore not only a model of lipodystrophy, but also of premature senescence in a mild degree. However, despite the fact that, to the best of our knowledge, there are no previous studies evaluating the BMD of FPLD through DXA, it is also true that no mention of an increase in the risk of fractures in these patients has been made. In fact, in our study, there were no significant differences in BMD between patients with familial partial lipodystrophy and control subjects. Nevertheless, the possibility that no significant differences were found due to the low sample size of the FPLD2 group should also be considered, which is why further studies with larger numbers of patients are needed. Moreover, the FPLD-linked *LMNA* mutations are mainly located within a highly conserved region in exon 8 of the gene. However, Dunnigan disease can be linked to mutations in other exons of the *LMNA* gene, as was the case with 29.4% of our FPLD2 patients, who had exons 5, 9 or 11 affected (29). Nevertheless, patients with the classical mutation in the *LMNA* gene did not show any difference in BMD compared to those with mutations in other exons.

In the case of FPLD1, which normally follows an autosomal dominant pattern of inheritance, no responsible gene has yet been found (2), although a polygenic pattern has recently been suggested (3). Despite the similarities in the phenotype of FPLD1 and FPLD2 patients, the fact that no mutations have been found in the *LMNA* gene in Köbberling syndrome makes it an ideal group of patients to compare BMD results with Dunnigan disease, taking into account the hypothesis described previously for the role of lamin A/C in the pathogenesis of age-related bone loss.

Moreover, factors such as age, height, fat and FFM are influential variables in bone metabolism, hence the need to eliminate them when studying BMD in our patients. As far as these factors are

concerned, the increase in FFM seen in the FPLD2 patients in our study is a feature that has previously been described in these subjects. This can be attributed to a combination of high insulin, myopathy and aberrant lipid deposition (18). The proposed mechanism for the association between FFM and BMD is the exertion of greater force by muscle on bone. In addition, these subjects have also been shown to be taller, which may influence BMD results, taking into account the larger bone size in individuals with higher stature. Indeed, in the case of patients with congenital generalised lipodystrophy (CGL), it has been found that patients have increased bone mineral content (BMC) partly due to their high lean mass and tall stature. Despite the fact that attempts have been made to find a relationship with their leptin-deficient state, leptin replacement did not change the BMC of these patients (30).

As has been mentioned above, another variable to be taken into consideration is age. Estrogen deficiency following the menopause has been demonstrated to be perhaps the major contributor to age-related bone loss in women, due to its effects on RANKL expression and its influence on the regulation of the Wnt inhibitor, sclerostin, among other aspects of bone metabolism. Reductions in progesterone and androgen levels during perimenopause, as well as reductions in serum inhibins, can also enhance the effects of estrogen deficiency on bone loss (31). In fact, in this study, we could observe, once again, how menopausal lipodystrophic patients had lower unadjusted BMD, both in total and segmentally, than lipodystrophic women of childbearing age. However, although age-related bone alterations are clearly related to hormonal changes, there are a number of additional changes that may contribute to bone loss, such as the previously mentioned enhancement of adipocyte differentiation from MSC, or even molecular mechanisms mediating age-related osteoblast/osteoclast dysfunctions, for example prelamin A accumulation, which can trigger hyperactivation of the Akt/mTOR pathway (32).

In relation to the influence of hormonal alterations on bone, it is also necessary to consider the possibility that some patients could have PCOS and, therefore, increased androgen levels, which can positively influence BMD. In fact, it has been reported that in both *LMNA*-linked lipodystrophic patients and in the case of FPLD1, the prevalence of PCOS is higher than in the general population (2,

9). Thus, in this study, 6% of the lipodystrophic patients had PCOS. However, we found no differences in BMD between these patients and women without ovarian disease (Figure S2).

Regarding the fat variable, it is known that obesity has been associated with greater BMD. Most of the available evidence supports a lower overall risk of fracture in obese adults, compared to adults with a normal BMI (33). However, when comparing the BMD of FPLD patients with age and BMI-matched control subjects, no significant differences were found. Furthermore, it is also known that fracture risk in obesity is not lower at all skeletal sites (33). In the case of HIV-infected men with lipodystrophy, it has been reported that there is reduced lumbar spine BMD, in association with increased abdominal visceral fat (14), which also leads us to consider the possibility that obesity may have different effects on bone depending on the type, deposition and distribution of adipose tissue. With regard to this, we wondered if there could be any difference between those patients with FPLD1 who showed a KöB index > 3.477 (and, therefore, had a marked difference between trunk and lower limb fat distribution) and those who, despite being diagnosed with Köbberling syndrome showed a KöB index < 3.477. However, once again, we have found no difference. On the other hand, obesity may induce insulin resistance (IR) with the resulting hyperinsulinaemia, the latter stimulating the production of hepatic insulin-like growth factor 1 and decreasing free insulin-like growth factor (IGF) by reducing IGF-binding protein levels (34), thus having positive effects on bone formation. It is known that FPLD1 and FPLD2 patients frequently show IR (7, 11). However, in this study, only FPLD1 subjects showed higher HOMA indices than controls (Table 1); even so, given that HOMA index is not the gold standard for IR measurement, we cannot rule out the possibility that this variable may have influenced our results.

Another limitation could be the lack of measurement of bone turnover markers. These were not evaluated because this was a retrospective study, and these data were not available for the majority of patients whose BMD was measured by DXA. However, this limitation would have been more relevant had we obtained results indicating a decrease in BMD.

In conclusion, we have demonstrated for the first time that, contrary to what might have been expected from the role of lamin A/C in the pathogenesis of bone loss in laminopathies, there are no significant differences in total or segmental BMD between patients with familial partial lipodystrophy and control subjects, once possible distractors such as age, height, fat and FFM have been eliminated. However, consideration should be given to the smaller sample size of the FPLD2 group, which is why further studies with a larger number of patients are needed.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Review Panel of the Consellería de Sanidade, Xunta de Galicia, and with the 2013 Helsinki declaration and its later amendments or comparable ethical standards. All subjects provided informed consent for participation in the study and for the publication of their clinical, biochemical and genetic information.

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Tables

Table 1. Demographic, anthropometric, genetic and clinical data for the different groups studied.

	Control (n = 44)	FPLD1 (n = 82)	FPLD2 (n = 17)
Age (years)	51.5 ± 13.6	55.0 ± 11.0	41.5 ± 14.3*†
Height (cm)	158.7 ± 6.4	153.9 ± 6.8*	162.4 ± 6.7†
Weight (kg)	84.5 ± 18.8	81.3 ± 12.5	66.5 ± 10.7*†
BMI (kg/m^2)	33.7 ± 7.9	34.2 ± 4.1	25.2 ± 4.0*†
Menopausal status (%)	53.7	68.4	33.3*†
PCOS (%)	0.0	5.1	13.3
KöB Index > 3.477 (%)	-	84.9	-
HOMA index	2.82 ± 2.19	4.80 ± 3.29*	3.09 ± 1.64
Exon mutations in *LMNA* (%)			
Exon 5	-	-	17.6
Exon 8			70.6
Exon 9			5.9
Exon 11			5.9
Total fat (kg)	37.4 ± 13.1	34.8 ± 7.6	17.0 ± 6.3*†
Upper-limb fat (kg)	3.9 ± 1.3	3.9 ± 1.0	1.6 ± 0.7*†
Lower-limb fat (kg)	11.7 ± 4.2	7.5 ± 2.2*	2.9 ± 0.8*†
Trunk fat (kg)	20.8 ± 8.8	22.3 ± 5.9	10.2 ± 4.5*†
Total FFM (kg)	42.9 ± 5.8	43.6 ± 6.2	46.9 ± 6.1
Upper-limb FFM (kg)	4.5 ± 0.8	4.7 ± 0.8	5.5 ± 1.1*†
Lower-limb FFM (kg)	14.4 ± 2.7	13.9 ± 2.3	14.9 ± 2.5
Trunk FFM (kg)	21.0 ± 2.9	22.1 ± 3.6	23.5 ± 2.8*

Data are mean ± SD or % values.

BMI: body mass index; PCOS: polycystic ovary syndrome; FFM: fat-free mass; FPLD1: Köbberling syndrome; FPLD2: Dunnigan disease.

* $p < 0.05$ vs. Control group; † $p < 0.05$ vs. FPLD1 group.

Figure legends

Figure 1. Adjusted BMD results obtained by DXA for the different groups studied. Data are mean ± SEM values. Age, height, fat and fat-free mass were used as covariables. BMD (g/cm^2): bone mineral density; Control: control group (n = 44); FPLD1: Köbberling syndrome (n = 82); FPLD2: Dunnigan disease (n = 17).

Figure 2. Adjusted BMD in FPLD2 patients according to exon mutation of the *LMNA* gene. Data are mean ± SEM values. Age, height, fat and fat-free mass were used as covariables. BMD (g/cm^2): bone mineral density.

Figure 3. Adjusted BMD in FPLD1 patients according to their KöB Index. Data are mean ± SEM values. Age, height, fat and fat-free mass were used as covariables. BMD (g/cm^2): bone mineral density.

Figure 4. BMD in relation to menopausal status. A, BMD in lipodystrophic patients according to their menopausal status. B, Adjusted BMD in menopausal patients of the different groups studied. Height, fat and fat-free mass were used as covariables. Data are mean ± SD/SEM values. BMD (g/cm^2): bone mineral density; FPLD1: Köbberling syndrome; FPLD2: Dunnigan disease.