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# A New Condition in McArdle Disease: Poor Bone Health—Benefits of an Active Lifestyle

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**Abstract**

**Introduction-Purpose**: McArdle disease (muscle glycogen phosphorylase deficiency) is a genetic condition associated with exercise intolerance, but how it affects lean mass (LM) and bone mineral content (BMC) and density (BMD) in patients is unknown. We compared these variables between McArdle patients and age/sex-matched healthy controls and assessed their potential association with physical activity (PA) levels in patients.

**Methods**: A case-control, cross-sectional design was used to examine LM, BMC and BMD by dual-energy X-ray absorptiometry in 136 young adults of both sexes [36 McArdle patients (33±15y) and 103 controls (34±11y)]. PA was assessed with the International Physical Activity Questionnaire (IPAQ).

**Results**: McArdle patients had significantly lower LM values in whole-body and regional sites than their corresponding controls, whereas no differences were found (except for the trunk) when physically active patients (n=23) were compared with controls. All bone-related variables were significantly lower in patients than in controls (average difference of 13% for BMC and 7.6% for BMD). By contrast, no significant differences at the lumbar spine, pelvis and femur sites were found between physically active patients and controls.

**Conclusion**: We report on a previously undescribed condition in McArdle patients, poor bone health, which warrants further attention as it can occur in relatively young adults. An active

lifestyle can at least partly alleviate this disorder presumably because of its beneficial effect on LM.

**Key words:** DXA; Bone-muscle interactions; Exercise; Physical activity

## Introduction

McArdle disease (or glycogen storage disease type V) is an inherited metabolic disorder caused by deficiency of myophosphorylase, the enzyme that catalyses the first step of glycogen breakdown in muscle tissue (1). The disorder is arguably the paradigm of exercise intolerance (2) and manifests functionally as acute ‘‘crises’’ of fatigue, myalgia and muscle contractures (especially during the first minutes of exercise) that are often accompanied by myoglobinuria (dark urine) resulting from skeletal muscle damage, with the latter typically reflected in elevated serum levels of creatine kinase (CK). For these reasons, physical activity (PA) has long been contraindicated in these patients. That said, an inactive lifestyle further exacerbates the exercise intolerance of patients with McArdle disease (3) and could potentially increase the risk of other comorbidities associated with physical inactivity. Accordingly, an effective intervention for alleviating exercise intolerance is regular, light-moderate ‘aerobic’ exercise (e.g., cycling or brisk walking) (4-7) and also supervised muscle resistance (‘strength’) training, with carbohydrate ingestion prior to each session (8).

Because PA and muscular development are major determinants of bone mass acquisition (9), we thought it would be worthwhile to assess the lean mass (LM) and the bone mass (BM) of McArdle patients, and to ascertain to what extent these two important variables are associated with PA levels. To the best of our knowledge, aside from our previous preliminary, low sample-sized study reporting LM in McArdle disease (8), no study has assessed LM and BM objectively using dual-x-ray absorptiometry (DXA). The present study was therefore designed: 1) to determine LM and BM in McArdle patients and compare the results with those of healthy subjects stratified by age and sex, and 2) to examine whether an active lifestyle contributes to

increase LM and thus improve bone health in these patients. Our hypothesis was that LM and BM would be lower in patients with McArdle disease than in healthy individuals, and an active lifestyle could contribute to reverse these features.

## Materials and methods

### *Subjects and procedure*

We followed a case-control, cross-sectional design. The study protocol was approved by the ethics committee of the Research Institute of the *Hospital 12 de Octubre* (Madrid, Spain; reference # 16/081) and adhered to the tenets of the Declaration of Helsinki 1961 (fifth revision, Edinburgh, 2000). All participants were informed of the aims and procedures of the study, as well as the possible risks and benefits. Patients were recruited for the study if they met the following criteria: (i) genetic diagnosis of McArdle disease, that is, identification of the two mutant alleles in the *PYGM* gene encoding myophosphorylase (or, in those in whom only one mutant allele has been identified to date, biopsy diagnosis or alternatively laboratory confirmation of the 'pathognomonic' second wind phenomenon) (1); (ii) age 16–55 years; and (iii) having no condition contraindicating DXA (e.g., pregnancy). A total of 36 patients with McArdle disease (17 men and 19 women) who met all inclusion criteria and provided informed consent were evaluated during the period 06/2015–10/2016 (see flow diagram, **Figure 1**).

Age- and sex-matched healthy subjects with previous DXA data collected by us using the same instrument (see below) during the period 2012–2016, such as to meet a patient-control ratio as close as possible to ~1:3 [i.e., n=103 individuals (49 men and 54 women)], were contacted and

agreed to serve as controls for the current study. Written informed consent was obtained from each participant.

Sample size was determined from pilot data by an *a priori* power analysis for analysis of covariance [ANCOVA, $\alpha = 0.05$, $1\text{-}\beta = 0.90$, fz = 0.80, 2 groups and 3 covariates], based on expected differences in bone mineral density (BMD) between McArdle patients and controls. Thus, a minimum of 12 subjects per group was needed to detect significant differences in the contrast of the null hypothesis $H_0$:$\mu 1 = \mu 2$.

The following measurements were also obtained during the same week of DXA assessment: anthropometric (patients and controls), serum CK (patients) and PA levels (patients).

*Genetic diagnosis.* Mutant *PYGM* alleles were identified in muscle or blood samples using SNaPShot mini-sequencing (ThermoFisher) or polymerase chain reaction and restriction fragment length polymorphism methods (10), followed by Sanger sequencing of the entire coding region and intron/exon boundaries of the myophosphorylase *PYGM* gene (11). Alternatively, we used a next generation sequencing customised gene panel on a PGM-IonTorrent platform (ThermoFisher), consisting of 35 genes (including *PYGM*) associated with metabolic myopathies. In some cases, analysis of muscle or blood mRNA/cDNA was needed to demonstrate the molecular pathogenicity of a presumed mutant allele, particularly when an alteration of the splicing mechanism was suspected (12, 13).

***Anthropometry***

Anthropometric measurements were obtained on each subject immediately before DXA assessment. Height was measured in the upright position, in underwear and barefoot on a stadiometer with a precision of 1 mm (Seca 711, Hamburg, Germany). Body mass was determined with the same requirements using a balance with a 100 g precision (Seca 711, Hamburg, Germany). Body mass index (BMI) was calculated as body mass divided by height squared ($kg/m^2$).

***Body composition: lean and bone mass***

Total and regional body composition and BM were assessed in all the study participants using the same DXA instrument (Hologic QDR Discovery, Bedford, USA), in the Sports Science Department of the *Universidad de Castilla-La Mancha* (Toledo, Spain). The instrument was calibrated using a lumbar spine phantom following the Hologic guidelines. All scans were analysed using Physician's Viewer, APEX System Software version 3.1.2. (Bedford, USA). LM (kg), bone mineral content (BMC, in g) and BMD (in $g \cdot cm^{-2}$) were calculated from total and regional analysis of the whole-body scan. Whole-body scans were submitted to a regional analysis to determine the composition of the arm, leg and trunk regions. The arm region included the hand, forearm and arm, and was separated from the trunk by an inclined line crossing the scapulohumeral joint, such that the humeral head was located in the arm region. The leg region included the foot, the lower leg and the upper leg. It was separated from the trunk by an inclined line passing just below the pelvis, which crossed the neck of the femur. The trunk region included the rest of the body excluding the arms, legs and head regions. The head region comprised all skeletal parts of the skull and cervical vertebra above a horizontal line passing just

below the jawbone. BMC and BMD were also reported for the lumbar spine (L1–L4) and proximal region of the femur (total hip, greater trochanter, inter-trochanter, Ward´s triangle and femoral neck). Scans were made with subjects in the supine position, wearing light clothing with no metal and no shoes or jewellery.

***PA measures***

PA was assessed using the Spanish version of the International Physical Activity Questionnaire (IPAQ, long version) (14). The methods used to score the long IPAQ can be found at the IPAQ Web site (www.ipaq.ki.se). The IPAQ has been validated against accelerometry and is widely used to evaluate PA patterns (15, 16). It has been shown to have satisfactory psychometric properties (15, 17) and is suitable for patient populations (15) as it is divided in specific parts, with each addressing the types of PA that patients with chronic diseases are most likely to perform (18).

The IPAQ was used to categorise patients into two subgroups according to their leisure time PA levels in the previous 7 days. Accordingly, participants were categorised as ‘‘active’’ (n=23) if they completed ≥600 metabolic equivalents (MET)·min·week$^{-1}$ (where 1 MET is the resting energy expenditure, equivalent to ~3.5 mL $O_2$/kg$^{-1}$·min$^{-1}$, and 600 MET·min·week$^{-1}$ reflects the minimum of 150 min/week of moderate-vigorous PA (MVPA) recommended by the World Health Organisation for all adults (19). Participants were considered ‘‘inactive’’ (n=13) if their PA was below this threshold (20, 21). The IPAQ was also used to report the frequency, intensity, and duration of occupational, transport, home, and leisure/sport PA that the patients had performed in the previous 7 days.

### *Statistical analysis*

Statistical analyses were performed with the IBM SPSS Statistics package version 24 (SPSS, Inc., Chicago, IL, USA). Differences in DXA-derived variables between groups and also between active and inactive patient subgroups in LM, BM and PA were determined using Student's *t* test. An ANCOVA was used to test for differences in LM and BM between patients and controls using body mass, height, age, age at symptoms onset and number of episodes of rhabdomyolysis in the last year (as reported by number of episodes of myoglobinuria) as covariates, with Bonferroni post hoc tests. Differences between men and women related to the percentage of body fat were also studied; nevertheless, both were categorised in the overweight range (22) (i.e., 24.5 ± 9.3 % body fat for men and 32.8 ± 6.0 % for women). Given that both had the same categorisation, the influence of fat mass on BM or LM would be very similar, and as such fat mass was not included in the analyses. Statistical size and power were reported with Cohen's d test for comparisons of equal sample size and Hedge's g test for comparisons of unequal sample size. Additionally, bivariate analysis was applied to identify the association between LM and BM. The level of statistical significance was set at $p \leq 0.05$. Unless otherwise stated results are reported as mean ± SEM.

## Results

All the patients had previously-described pathogenic mutations (that are known to cause McArdle disease) in both alleles of the *PYGM* gene, except for one case in which only one mutant allele was identified (see Table, **Supplemental Digital Content 1**, *PYGM* mutations identified in the study patients, http://links.lww.com/MSS/B25). However, this patient showed the pathognomonic 'second wind' phenomenon in our laboratory. **Table 1** summarises

anthropometric and descriptive data for the patient and control groups. Whereas both groups had similar age, body mass and BMI, the control group had a lower percentage of body fat than the patient group ($p<0.05$).

***Body composition***

**Table 2** summarises total and regional LM values after controlling for the effect of body mass, height, age, age at symptoms onset and number of episodes of rhabdomyolysis in the last year. Whole-body and appendicular LM was significantly greater in patients than in controls (from 5.2 to 9.9%, except for leg LM, all $p<0.05$). Effect sizes of these differences were large for the trunk (Hedge's g = 1.18) and medium for the whole body and arms (0.70 and 0.67, respectively). The comparison between controls and non-active patients revealed a similar trend, with large effect sizes for the whole body, trunk and arms (Hedge's g from 1.10 to 1.62; 9.4% to 17.9% of difference, $p<0.05$), and a small effect size and difference for the legs (Hedge's g = 0.26 and 2.9%, $p>0.05$). By contrast, significant differences between controls and active patients were found only for the trunk LM (Hedge's g = 0.89 and 6.9%, $p<0.05$), with small effect sizes in the whole body, arms and legs (Hedge's g from 0.16 to 0.43). Finally, the comparison between active and non-active patients revealed significant differences in the whole-body and trunk LM (Cohen's d = 0.87 and 0.89, 6.2% of difference, $p<0.05$), and medium effect sizes for LM of arms and legs (0.72 and 0.51; 6.7 and -1.8% of difference, respectively).

**Table 3** and **Figure 2** show BMC and BMD values after controlling for the effect of body mass, height, age, age at symptoms onset and number of episodes of rhabdomyolysis in the last year. Significant differences were found between patients and controls in all the bone-related

variables, with large effect sizes in the BMC of the pelvis and legs (Hedge's g = 1.11 and 1.10; 24.0 and 12.2%, respectively) and in the BMD of the whole body, pelvis and legs (Hedge's g from 0.84 to 1.29; from 7.5 to 9.7%). However, when comparing BMD between controls and active patients, these differences only remained significant in the whole body, arms and legs (Hedge's g = 0.95, 0.53 and 0.99, 7.7, 6.3 and 8.1%, respectively), and were not observed in the pelvis, Ward's triangle, intertrochanteric zone, trochanter, femoral neck and lumbar spine. BMD values were on average 7.6% higher in controls (Hedge's g from 0.26 to 1.07; from 5.6 to 9.7%) than in patients, and these differences were even greater (Hedge's g from 0.23 to 1.51 and mean difference of 10.1%, range 5.8–12.8%) when controls were compared with active patients. These differences were significant for all BMD variables except for Ward's triangle, femoral neck and lumbar spine. BMC variables showed a similar trend with, on average, 13% greater values in controls than in patients (Hedge's g from 0.13 to 0.68, range 3.9–24%), with a 17.1% mean difference between controls and non-active patients (Hedge's g from 0.16 to 1.42, range 5.2–29.2%) and smaller differences in BMC values between controls and active patients (Hedge's g from 0.11 to 1.05, range 3.3–20.9% and 11.2%, mean difference). A representative image of a DXA scan comparing the different groups is shown in **Supplemental Digital Content 2** (see Figure, Supplemental Digital Content 2, Representative DXA image comparison between the different groups, http://links.lww.com/MSS/B26). No significant correlations were found between BMC, BMD or LM with age at symptoms onset or frequency of rhabdomyolysis.

***Association between whole body lean mass and bone mass***

In the patient group, the strongest associations were found between whole-body LM and either whole-body BMD (r = 0.45, $p<0.01$, **Figure 3a**) or leg BMD (r=0.74, $p<0.001$, **Figure 3b**).

These associations remained in the control group (r = 0.78, $p<0.001$ and r = 0.54, $p<0.001$, respectively).

***PA measures***

The following significant differences were found between active (n=23) and non-active patients (n=13), with greater values in the former (all $p<0.01$): "walk" category (1 948 ± 468 vs. 482 ± 115 MET·min·week$^{-1}$), MVPA (4 290 ± 903 vs. 1 578 ± 389 MET·min·week$^{-1}$), "leisure time" PA (2 031 ± 422 vs. 212 ± 65 MET·min·week$^{-1}$) and "total time" PA (5 465 ± 1 536 vs. 2 492 ± 769 MET·min·week$^{-1}$).

Sex comparisons within the patient group are included as Table, **Supplemental Digital Content 3**, soft-tissue composition from the total and regional body in McArdle patients by sex, http://links.lww.com/MSS/B27, and Table, Supplemental Digital Content 4, Bone mineral content (BMC) and density (BMD), from the whole-body, femoral and lumbar scans in the McArdle patients by sex, http://links.lww.com/MSS/B28. Men had greater LM values than women after controlling for the effect of body mass, height and age. Likewise, men had significantly higher BMC and BMD than women at all sites except for the proximal femur (BMD, $p=0.08$).

**Discussion**

This study examined the effects of McArdle disease on LM and BM, in particular in relation to patients' lifestyle (active vs. inactive). Our main novel finding is that LM and BM was significantly lower in McArdle patients than in age- and sex-matched control subjects. However,

active patients could mitigate the losses in LM and BM associated with an inactive lifestyle through the maintenance of their LM component, which remained similar to that of healthy controls.

An inactive lifestyle may attenuate the accrual of BM during growth, leading to a lower peak BM. The latter is usually attained in early adulthood both in men and women, and a high peak BM is crucial to prevent osteoporotic fractures later in life (23). In our study, whole-body, spine and femoral neck BMC values in McArdle patients were 12%, 4% and 12% lower and BMD values were 9%, 7% and 6% lower, respectively, than in age- and sex-matched healthy peers. This indicates that, on average, the BMC and BMD of McArdle patients are low from a young adult age (mean age of the patients was 33 ± 15 years). Indeed, when we compare our patients' data with those of an elderly Spanish population (≥65 years) assessed by us with the same instrument (unpublished data), we find that patients' BMD values are only 4% and 5% higher for the whole body, and spine, respectively. Thus, BMC and BMD values from young adult McArdle patients are clearly below the established reference values for their age (24), indicating that they are at high risk of bone diseases such as osteoporosis or osteopenia later in life. In this regard, a low BMD and a high incidence of fractures have been observed in other myopathies (25). Adult patients with Pompe disease or glycogenosis type II also seem to have lower-than-normal BMD values, especially for the femoral neck (26). Further, the McArdle patients in the present study had BMD values 5%, 17% and 4% lower in the whole body, spine and femoral neck areas, respectively, when compared with the values previously reported in adult Pompe patients (27). The whole-body BMD values of our patients were similar to those previously reported in other adult patients with glycogenosis type I and type III (28).

There is a dearth of knowledge about the effects that regular PA may have on bone content and density in McArdle patients, a group that has been traditionally advised by physicians to refrain from exercise. Notwithstanding that BMC and BMD levels were significantly lower in our McArdle patients than in healthy subjects in many of the whole-body areas, an active lifestyle had a positive effect in the femoral regions. Thus, active patients had similar proximal femur, trochanter and intertrochanteric BMD values to their healthy peers, whereas non-active patients showed significantly lower values in these areas.

Even though this positive exercise effect does not seem to be present in the spine, some studies have found similar results in other glycogenoses, showing that muscle strength and weight-bearing positively affect BMD, which is supported by the greater involvement of the femoral neck over the lumbar spine (26). Whereas trabecular bone (e.g., lumbar spine) is mainly influenced by general systemic factors such as hormone status (29), cortical bone (at the femur) is more subject to regional, mechanical influences, such as gravity, LM, and muscle strength (29). For these reasons, PA is essential to increase BMD, not only in healthy individuals but also in individuals with various pathological conditions (30).

Regular PA has an important osteogenic effect (31, 32), and land-based weight-bearing activities increase BM more than do non-weight bearing activities (e.g., swimming) in weight-loaded skeletal regions (33, 34). Although weight-supported land-based activities associated with relatively low-magnitude ground-reaction forces such as brisk walking do not always have a substantial osteogenic effect (35), McArdle patients can safely perform this type of exercise and

obtain, at least partially, indirect benefits for their bone health through the increase of muscle mass.

Since skeletal muscle is the primary component of LM, participation in sport and PA could have not only a direct osteogenic effect, but also an indirect effect by increasing muscle mass and hence the tensions generated on bones during prepubertal years (36). The muscle-bone relationship is presumably explained by the mechanostat theory, which proposes that bone strength is regulated by modelling and remodelling processes depending on the forces acting on the bones (37, 38); bigger muscles exert higher tensile forces on the bones they attach to. Moreover, a close relationship between whole-body LM and whole-body and leg BM was found in McArdle patients, suggesting that a higher whole-body LM will likely result in a higher osteogenic effect. Interestingly, a recent preliminary report from our laboratory indicated the feasibility and safety of an 8-week supervised, moderate-intensity resistance (weight lifting) training programme in McArdle patients (8). This is an encouraging finding given the greater potential of this exercise modality to increase muscle mass and subsequently BM (39). Indeed, despite its short duration, the resistance programme resulted in a total LM gain of nearly 1 kg in the studied McArdle patients.

Our McArdle patient group had 5% lower whole-body LM than the control group. However, the subgroup of active patients presented similar values of whole-body LM to the control group, and higher values than their sedentary patient peers. The low values of whole-body LM of the non-active McArdle patients were similar to those previously found in Pompe patients (40). Thus, in

contrast to Pompe or non-active McArdle patients, active McArdle patients can reach normal values of total LM.

Our study is not without limitations. We did not report dietary information, although several diet factors, especially calcium and vitamin D intake, have a potentially important impact on bone mass acquisition. It would have also been useful to analyse blood variables that are known to be associated with bone mass (e.g., levels of parathyroid hormone or vitamin D). Furthermore, the self-reported nature of the PA questionnaires makes these tools less accurate than more objective methods for PA assessment, notably triaxial accelerometry. Nonetheless, a main strength of our study stems from the fact that it is the first to objectively assess a crucial health phenotype, body composition, in a relatively large cohort of McArdle patients using the gold standard DXA method. Moreover, when considering that McArdle disease is a rare disease (prevalence of ~1/167 000 people (1), our study was well powered, with the sample size of the patient group sufficiently large to detect significant changes in bone variables, which allows extrapolation of our data to other patients with the same disease.

In conclusion, we have identified poor bone mass starting in early adulthood as a previously undescribed condition associated with McArdle disease. Although both active and non-active McArdle patients have compromised bone health, there seems to be a consistent association between higher PA levels and a healthier body composition phenotype (i.e., higher lean and bone mass) among young adult patients with McArdle disease, with active patients showing higher LM than their sedentary patient peers. Because there will likely be no genetic cure for McArdle disease in the foreseeable future, our study provides an additional rationale to support

implementation of PA intervention in these patients. Future research might explore whether specific dietary interventions (e.g., calcium supplement intake) could maximise the benefits of PA on the bone health of McArdle patients and what type of safe, feasible PA modality is most suitable to enhance the muscle and bone mass of these patients.

**Acknowledgments and Conflicts of interest**

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Supplemental File 1. *PYGM* mutations identified in the study patients (N=36).

Supplemental File 2. Representative DXA image comparison between the different groups.

Supplemental File 3. Soft-tissue composition from the total and regional body in McArdle patients by sex.

Supplemental File 4. Bone mineral content (BMC) and density (BMD) from the whole-body, femoral and lumbar scans in the McArdle patients by sex.

**Figure legends**

**Figure 1.** Flow diagram of McArdle patients. Symbol: * death due in both cases to causes independent from McArdle disease (i.e., cardiovascular disease).

**Figure 2.** Bone mineral density (BMD) adjusted for body mass, height and age at the whole-body (a) and regional level (b). Data are mean±SEM.

† $p<0.05$ for Control group vs. McArdle active subgroup.

‡ $p<0.05$ for Control group vs. McArdle non-active subgroup.

**Figure 3.** Association between whole body lean mass and whole body (a) and legs (b) bone mineral density (BMD), in the McArdle patients (n = 36).

**Supplementary figure legends**

**Supplementary File 2.** Representative DXA image comparison between the different groups.

Figure 1

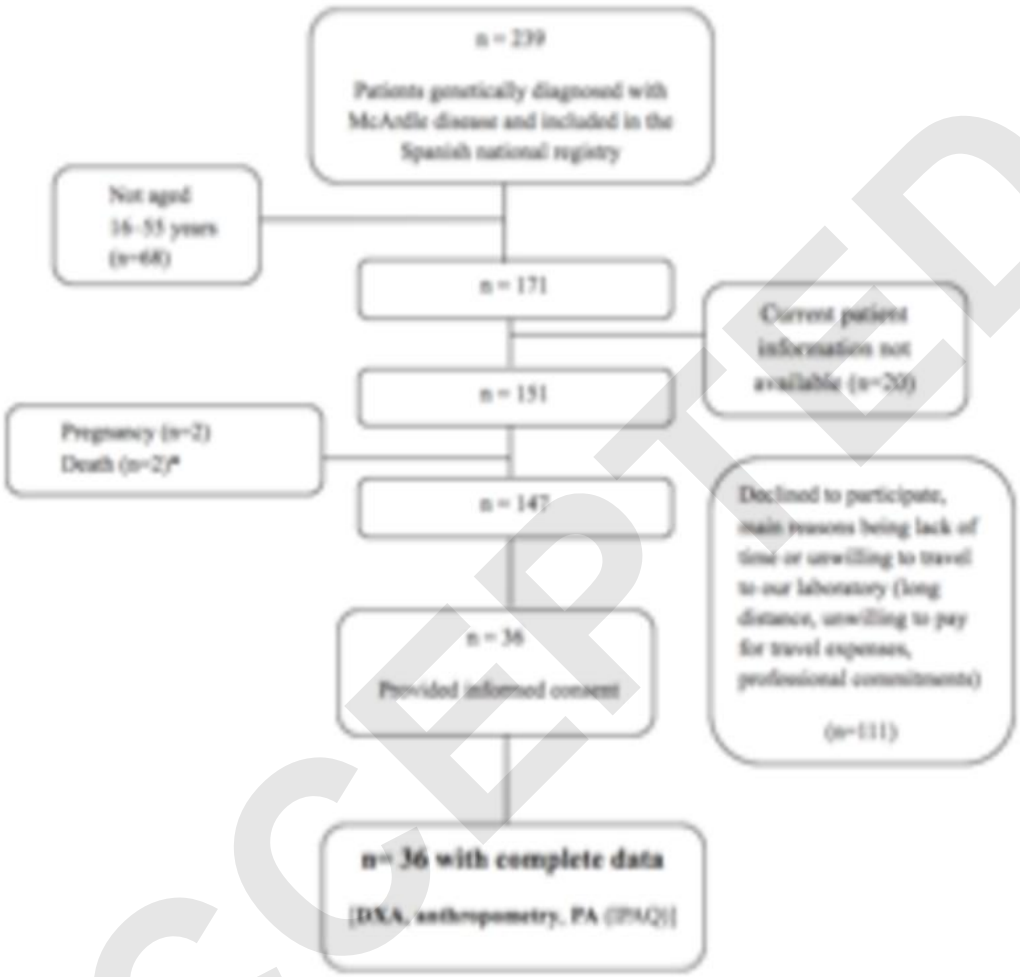


Figure 2

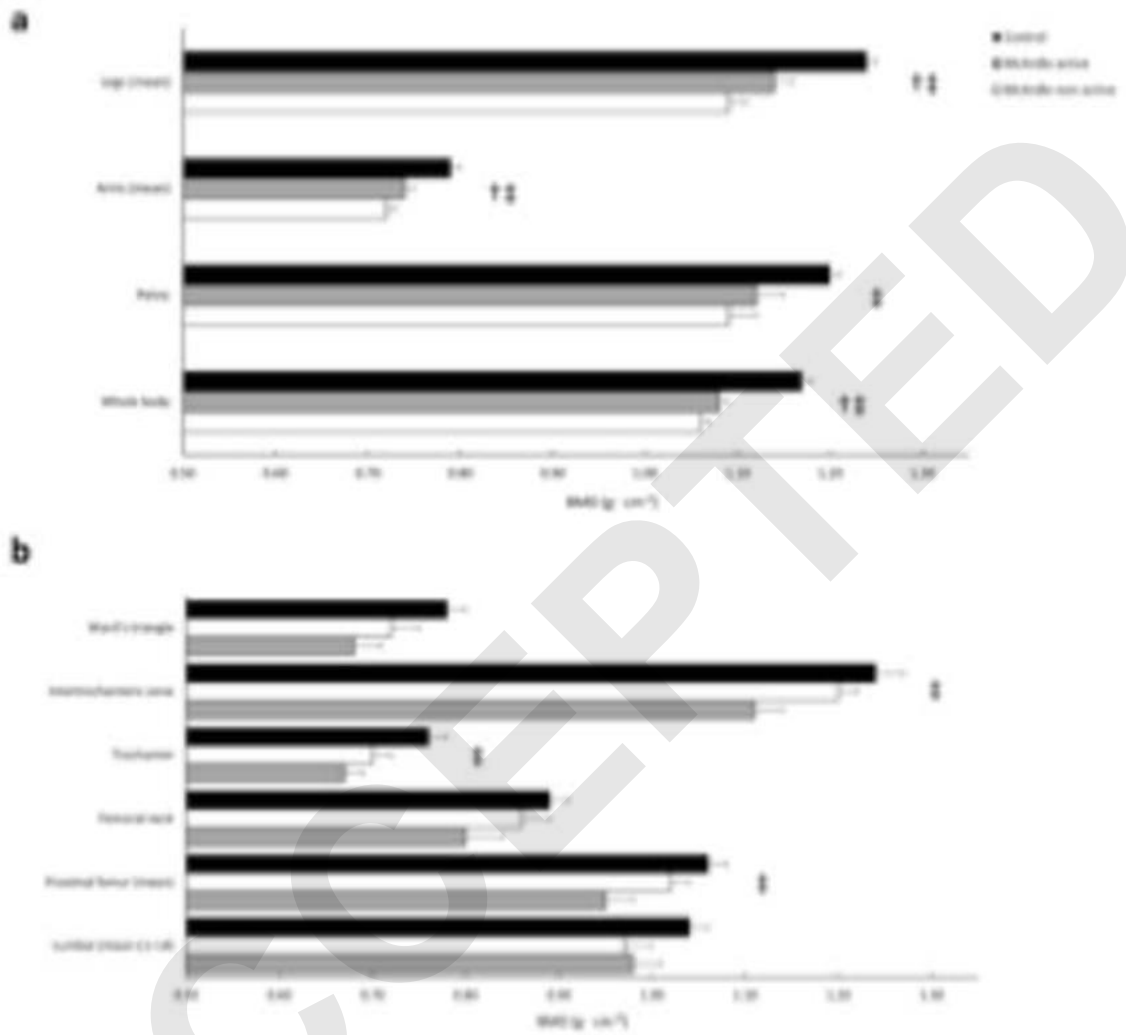


Figure 3

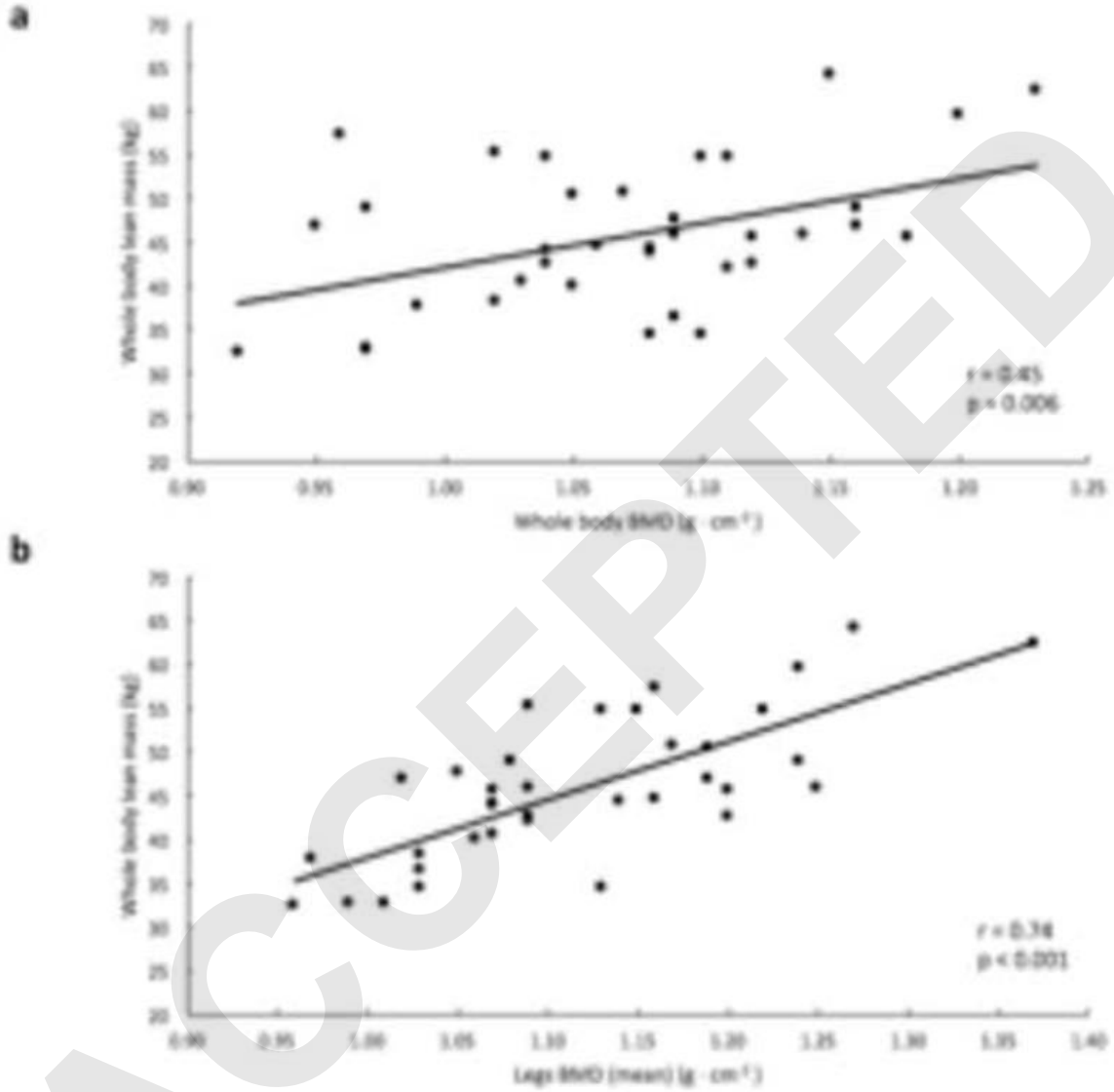


**Table 1.** Anthropometric and descriptive data.

| Variables | McArdle (n=36) | Control (n=103) |
|---|---|---|
| Age (years) | 35 ± 11 | 34 ± 11 |
| Body mass (kg) | 69.6 ± 16.1 | 69.2 ± 14.3 |
| Height (cm) | 169 ± 7 | 169 ± 15 |
| BMI ($kg/m^2$) | 24.3 ± 5.1 | 24.2 ± 7.2 |
| %body fat | 28.9 ± 8.7 | 24.8 ± 9.2* |
| CK (U/L) | 2 147 ± 2 033 | |

Data are mean ± SD. BMI, body mass index; CK, creatine kinase; * $p<0.05$ for McArdle vs. control group.

**Table 2.** Soft-tissue composition from the total and regional body scans corrected by body weight, height, age, and age of symptom onset and frequency of rhabdomyolysis episodes.

| | Lean Mass (kg) | | | |
|---|---|---|---|---|
| | McArdle (n=36) | Control (n=103) | McArdle, active only (n=23) | McArdle, non-active only (n=13) |
| Whole Body | 45.9 ± 0.4 | 48.5 ± 0.4* | 46.8 ± 0.7 | 43.9 ± 0.9‡ § |
| Trunk | 22.3 ± 0.2 | 24.4 ± 0.2* | 22.8 ± 0.3† | 21.3 ± 0.5‡ § |
| Arms (mean) | 2.3 ± 0.0 | 2.5 ± 0.0* | 2.4 ± 0.1 | 2.1 ± 0.1‡ |
| Legs (mean) | 7.9 ± 0.2 | 7.9 ± 0.1 | 8.0 ± 0.2 | 7.7 ± 0.2 |

Data are mean ± SEM.

* $p<0.05$ for Control vs. McArdle group.

† $p<0.05$ for Control group vs. McArdle active subgroup.

‡ $p<0.05$ for Control group vs. McArdle non-active subgroup.

§ $p<0.05$ for McArdle active vs. non-active subgroup, $p<0.05$.

**Table 3.** Bone mineral content (BMC) and density (BMD) from the whole-body, spinal and femoral regions, corrected by body weight, height, age, age, and age of symptom onset and frequency of rhabdomyolysis episodes.

| | BMC (g) | | BMD ($g \cdot cm^{-2}$) | |
|---|---|---|---|---|
| | McArdle (n=36) | Control (n=103) | McArdle (n=36) | Control (n=103) |
| Whole Scan | | | | |
| Whole body | 2 158.2 ± 315.5 | 2 442.8 ± 25.5* | 1.07 ± 0.01 | 1.17 ± 0.01* |
| Head | 494.4 ± 76.6 | 529.4 ± 7.8* | 2.14 ± 0.05 | 2.25 ± 0.03* |
| Pelvis | 227.1 ± 11.8 | 298.8 ± 6.1* | 1.11 ± 0.02 | 1.20 ± 0.01* |
| Arms (mean) | 143.4 ± 7.5 | 162.9 ± 2.0* | 0.73 ± 0.01 | 0.79 ± 0.01* |
| Legs (mean) | 403.3 ± 3.1 | 459.5 ± 5.7* | 1.12 ± 0.01 | 1.24 ± 0.01* |
| Spine | | | | |
| Lumbar (mean $L_1$–$L_4$) | 14.7 ± 0.4 | 15.3 ± 0.5* | 0.97 ± 0.02 | 1.04 ± 0.02* |
| Femoral Regions | | | | |
| Proximal femur (mean) | 39.8 ± 1.2 | 45.6 ± 1.5* | 0.99 ± 0.02 | 1.06 ± 0.02* |
| Femoral neck | 4.6 ± 0.2 | 5.2 ± 0.3* | 0.84 ± 0.02 | 0.89 ± 0.02* |
| Trochanter | 7.4 ± 0.2 | 9.3 ± 0.6* | 0.69 ± 0.02 | 0.76 ± 0.02* |
| Intertrochanteric zone | 27.8 ± 1.0 | 31.2 ± 1.1* | 1.17 ± 0.02 | 1.24 ± 0.03* |
| Ward's triangle | 0.8 ± 0.0 | 0.9 ± 0.0* | 0.71 ± 0.02 | 0.78 ± 0.02* |

Data are mean ± SEM. * $p<0.05$ for McArdle vs. Control group.

**Supplementary File 1.** *PYGM* mutations identified in the study patients (N=36).

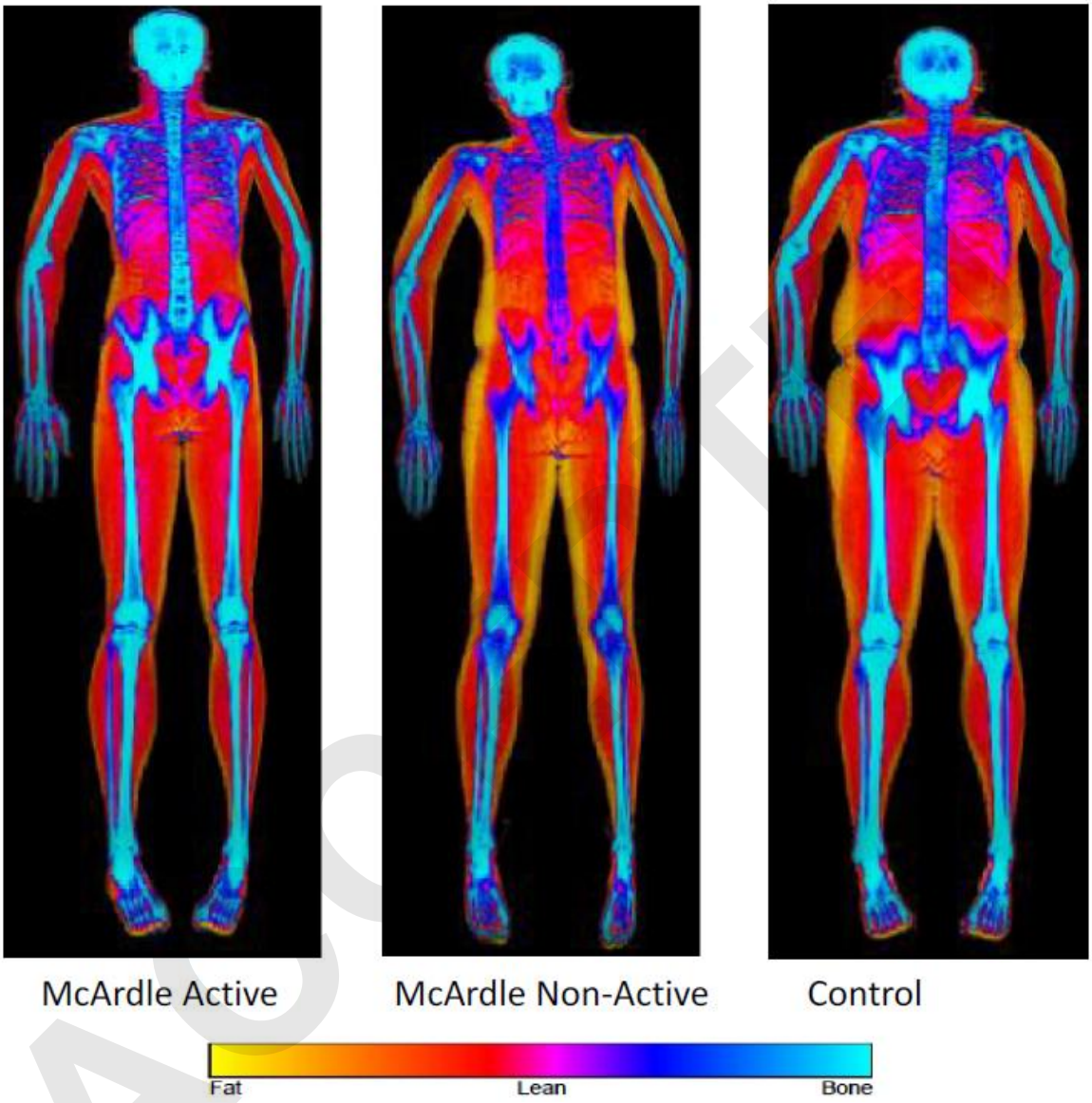
| Type of mutation | N |
|---|---|
| p.R50X (c.148C>T) / p.R50X (c.148C>T) | 13 |
| p.R50X (c.148C>T) / p.W798R (c.2392T>C) | 3 |
| p.G205S (c.613G>A) / c.1768+1G>A | 2 |
| p.R50X (c.148C>T) / c.1768+1G>A | 2 |
| p.R50X (c.148C>T)/ p.Q577R (c.1730A>G) | 2 |
| p.R50X (c.148C>T) / p.L5VfsX22 (c.13_14delCT) | 2 |
| p.R771PfsX33 (c.2310_2311dupCC) / p.R771PfsX33 (c.2310_2311dupCC) | 1 |
| p.G205S (c.613G>A) / p.G205S (c.613G>A) | 1 |
| p.G205S (c.613G>A) / p.Q176_M177insVQ (c.528-8g>a) | 1 |
| p.K754NfsX49 (c.2262delA) / p.K754NfsX49 (c.2262delA) | 1 |
| p.L5VfsX22 (c.13_14delCT) / p.K754fsx49 (c.2262delA) | 1 |
| p.R50X (c.148C>T) / p.L5VfsX22 (c.13_14delCT) + p.R324G (c.970C>G) | 1 |
| p.R50X (c.148C>T) / p.V456M (c.1366G>A) | 1 |
| p.R50X (c.148C>T) / p.E27AfsX50 ( c.78_79delTG) | 1 |
| p.R50X (c.148C>T) / * | 1 |
| c.1092-1G>T / c.244-3_244-2delCA | 1 |
| p.W388SfsX34 (c.1162_1169delTGGCCGGTinsA) / p.W388SfsX34 (c.1162_1169delTGGCCGGTinsA) | 1 |
| p.G205S (c.613G>A) / p.R590H (c.1769G>A) | 1 |

*Unidentified mutation in one allele.

References supporting the pathogenic nature of all the genotypes:

PMID: 25914343
Authors: Santalla A., et al. Title: Genotypic and phenotypic features of all Spanish patients with McArdle disease: A 2016 update. BMC Genomics (in press)

**Supplemental File 2**



**Supplemental file 3.** Soft-tissue composition from the total and regional body in McArdle patients by sex.

| | Lean mass (kg) | |
|---|---|---|
| | Men (n=17) | Women (n=19) |
| Whole Body | 49.2 ± 0.7 | 42.7 ± 0.6* |
| Trunk | 23.7 ± 0.4 | 21.0 ± 0.3* |
| Arms (mean) | 2.7 ± 0.1 | 1.9 ± 0.1* |
| Right Arm | 2.8 ± 0.1 | 2.0 ± 0.1* |
| Left Arm | 2.6 ± 0.1 | 1.8 ± 0.1* |
| Legs (mean) | 8.4 ± 0.2 | 7.5 ± 0.2* |
| Right Leg | 8.4 ± 0.2 | 7.5 ± 0.2* |
| Left Leg | 8.4 ± 0.2 | 7.4 ± 0.2* |

* $p<0.05$ for men vs. women with an ANCOVA test (adjusted by body mass, height and age).

**Supplemental file 4.** Bone mineral content (BMC) and density (BMD) from the whole-body, femoral and lumbar scans in the McArdle patients by sex.

| | BMC (g) | | | | | | BMD ($g \cdot cm^{-2}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Men (n=17) | | | Women (n=19) | | | Men (n=17) | | | Women (n=19) | | |
| Whole Scan | | | | | | | | | | | | |
| Whole body | 2169.3 | ± | 53.7 | 2147.1 | ± | 50.1* | 1.07 | ± | 0.02 | 1.07 | ± | 0.02 |
| Head | 473.8 | ± | 19.9 | 515.0 | ± | 18.6$^{p=0.06}$ | 1.99 | ± | 0.08 | 2.29 | ± | 0.07 |
| Pelvis | 220.8 | ± | 12.7 | 233.4 | ± | 11.8* | 1.10 | ± | 0.03 | 1.11 | ± | 0.03† |
| Arms (mean) | 152.5 | ± | 4.5 | 134.3 | ± | 4.2* | 0.76 | ± | 0.01 | 0.71 | ± | 0.01† |
| Legs (mean) | 414.6 | ± | 9.5 | 392.0 | ± | 8.9* | 1.16 | ± | 0.02 | 1.08 | ± | 0.02† |
| Spine | | | | | | | | | | | | |
| Lumbar (mean $L_1$–$L_4$) | 14.2 | ± | 0.6 | 15.1 | ± | 0.6* | 0.95 | ± | 0.03 | 1.00 | ± | 0.03† |
| Femoral Regions | | | | | | | | | | | | |
| Proximal femur (mean) | 41.9 | ± | 1.9 | 37.7 | ± | 1.8* | 1.00 | ± | 0.03 | 0.99 | ± | 0.03$^{p=0.08}$ |
| Femoral neck | 4.8 | ± | 0.3 | 4.3 | ± | 0.3* | 0.84 | ± | 0.04 | 0.83 | ± | 0.03† |
| Trochanter | 7.7 | ± | 0.3 | 7.1 | ± | 0.3* | 0.68 | ± | 0.03 | 0.69 | ± | 0.02 |
| Intertrochanteric zone | 29.4 | ± | 1.6 | 26.2 | ± | 1.5* | 1.18 | ± | 0.03 | 1.15 | ± | 0.03† |
| Ward's triangle | 0.8 | ± | 0.0 | 0.8 | ± | 0.0* | 0.70 | ± | 0.03 | 0.72 | ± | 0.03† |

* $p<0.05$ for men vs. women in BMC with an ANCOVA test (adjusted by body mass, height and age)

† $p<0.05$ for men vs. women in BMD with an ANCOVA test (adjusted by body mass, height and age)