UNIVERSIDADE DE LISBOA
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SKELETON GEOMETRY, PHYSICAL ACTIVITY AND PROXIMAL FEMUR BONE MASS DISTRIBUTION IN 8-12 YEAR OLD CHILDREN

Dissertação elaborada com vista à obtenção do Grau de Doutor no Ramo de Motricidade Humana, Especialidade em Atividade Física e Saúde

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To my family, friends and colleagues.

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Abstract

In the context of bone health promotion, the aim of this Ph.D dissertation was to analyze potential explanatory factors of the effects of physical activity and of bone geometry on bone mass distribution at the proximal femur in 8-12 year old children. Four studies were undertaken to compare the bone mineral density (BMD) between: (a) the sub-regions of the proximal femur – the neck and its superolateral and inferomedial aspects, the trochanter and the intertrochanter; (b) sexes, concerning the associations/effects of non-targeted physical activity and bone geometry. Sex and regional specific effects of non-targeted physical activity on bone mass distribution at the proximal femur in children were observed. The geometry of the pelvis and the proximal femur, namely the pelvis width and the abductor lever arm, emerged as predictors of bone mass distribution at the proximal femur, therefore as explanatory factors of both the regional and the sex specific patterns. These geometric features might mediate the physical activity effects on bone mineralization at the proximal femur, as long as, when they are considered, the power of physical activity to explain the distribution of bone mass at this skeletal site seems limited.

Keywords: Proximal Femur; Bone Geometry; Pelvis; BMD; Physical Activity; Children; Sex; DXA.

Resumo

No contexto da promoção da saúde óssea, o objetivo desta dissertação de doutoramento foi analisar potenciais fatores explicativos dos efeitos da atividade física habitual e da geometria óssea na distribuição da massa óssea do fémur proximal, em crianças de 8-12 anos de idade. Para o efeito foram realizados quatro estudos comparando a densidade mineral óssea (DMO) entre: (a) as diversas sub-regiões do fémur proximal - o colo do fémur e os seus aspetos supero-lateral e infero-medial, o grande trocanter e a sub-região intertrocantérica; (b) os sexos, relativamente às associações/efeitos da atividade física habitual e da geometria óssea. Foram observadas associações/efeitos da atividade física habitual na massa óssea do fémur proximal diferenciados quanto ao sexo e sub-região. A geometria da pélvis e do femur proximal, nomeadamente a largura da pélvis e o braço de momento de força dos abdutores, surgiram como preditores da distribuição de massa óssea no fémur proximal e consequentemente como fatores explicativos de diferenciação da distribuição de massa óssea de acordo com o sexo e sub-região. Estas caraterísticas geométricas poderão mediar os efeitos da atividade física na mineralização do femur proximal uma vez que quando consideradas parecem limitar a capacidade explicativa da atividade física na distribuição de massa óssea no fémur proximal

Palavras-chave: Proximal Fémur; Pelvis; BMD; Geometria Óssea; Atividade Física; Crianças; Sexo; DXA.

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1 Introduction

This dissertation presents my Ph.D research project's main outputs. It's core is the collection of the four research articles developed during this investigation period. This research is broadly motivated by a public health issue – the risk of bone fracture in the elderly phase of life.

It is now widely accepted that the risk of fracture in that phase, particularly at the spine and proximal femur, which is strongly associated to osteoporosis disease, constitutes a public health problem due to: its widespread incidence among adults; its dramatic consequences for the quality of life of those sustaining a fracture and due to the challenges it brings to national health care systems, namely those concerning resource allocation.

Being that there is no cure to osteoporosis and given the widespread belief that some of its origins may lay in childhood and adolescence, a large emphasis has been put on prevention strategies in these earlier phases of life, to such an extent that some authors consider that osteoporosis is a pediatric issue [1]. In fact, the development of bone mass during growth years is extensively described and documented in the literature [2], as are the effects of loads induced by physical activity, the main modifiable lifestyle factor.

Without disregarding the paramount importance of non-modifiable factors, physical activity during pediatric ages is considered to be the most effective strategy to promote bone mass accrual, not only because this is the phase across all lifespan in which the positive effects of mechanical loading are most evident [3], but also because entering adulthood with a higher peak bone mass is considered to significantly reduce the risk of fracture in old age [4].

Nevertheless, the effects of physical activity on bone mass vary considerably. The benefits of high-impact activities via specially designed intervention programs is a consolidated result in the literature, but increasing evidence of skeletal development benefits from lifestyle physical activity during childhood [5, 6, 7] should support the promotion of regular physical activity in youth - specially weight-bearing activity (as body weight increases the magnitude of loading) - as a strategy to reduce the risk of bone fracture later in life.

The understanding of the factors determining the specific effects on bone mass caused by physical activity induced loads is essential for the appropriate design of those prevention policies. But whereas the effects of intervention programs, or even very high intensity (and frequency) activities that some elite athletes engage in, are rather consolidated in the literature, the understanding of the effects of the habitual volunteer physical activity of non-athletes is less developed. Whether there is a distinct bone responsiveness to mechanical loading induced by non-targeted physical activity in boys and girls, or whether there are skeletal sex differentiating aspects that may influence the impact of physical activity on bone mineralization are among the questions that have arisen in this research branch.

In this context, the general aim of the present Ph.D dissertation was to analyze and explore the factors that explain the effects of physical activity induced mechanical loads on bone mass distribution at the proximal femur in children. To pursuit this general objective, several specific objectives were defined:

- To analyze the potentially differentiated effects of physical activity at the proximal femur neck, trochanter and intertrochanter sub-regions;
- To analyze eventual sex specificities in response to mechanical loading;

- To analyze the role of the pelvis and hip geometry on physical activity induced effects on the mineralization of the proximal femur.

To accomplish these tasks, body composition, bone mineral parameters and bone geometry features obtained from DXA scanning, and physical activity data from accelerometry and questionnaires were statistically treated in several regression models. To analyze the bone mass distribution at all three sub-regions of the proximal femur, intra-individual bone mineral density ratios of proximal femur sub-regions were defined.

This dissertation's structure is composed of an initial introductory chapter (chapter 2) where a literature review and theoretical background is presented, not as a substitute for the specific literature review that is part of each of the articles that constitute the core of the document, but solely with a unifying purpose of all the work developed. The methodological aspects are not treated in this chapter as they are instrumental for the research project objectives and are described in detail in each of the four studies presented in chapter 3. The fourth chapter, in a brief general discussion, attempts to integrate the main results of the studies produced, highlighting those that seem to be the most interesting aspects of the overall research project. Once more, they must not be considered without the studies' specific discussion sections presented in the precedent chapter.

Chapter 5 outlines the main conclusions and reflections for further research on the issues dealt with in this dissertation, and that the candidate believes deserve additional research.

Finally, the annex includes presentations given in international congresses which were direct outputs of the research work behind the four studies present in chapter 3.

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2 Literature overview and theoretical background

Bone fracture in old age, usually associated to osteoporosis and often caused by falls, represents a serious public health problem, as it broadly affects the population and can significantly reduce individual wellbeing or even anticipate death [1, 2]. Among the adult population the risk of suffering any major fracture at the hip, the distal forearm, the proximal humerus and the spine after the age of 50 is estimated to be up to 20% for men and up to 50% for women [3- 7]. Given the population aging trend, an increase of the incidence of this type of fracture on the society as a whole is expected [8, 9]. This is also a relevant challenge for actual and future health-care systems, namely via the corresponding burden on their budgets [10, 11]. Bone fracture is also experienced in younger ages but its implications are not as dramatic from a public health policy perspective.

Bone fracture occurs whenever a bone is subjected to stress and it is not strong enough to hold it, originating a structure failure [12]. Stresses on bone are usually caused by the impact on an external object - in the event of a fall, a car accident or alike – or, in some more extreme cases, by the action of the individual’s muscles. Therefore the study of bone strength – its ability to resist fracture –and the circumstances in which forces exerted over bones become abnormal are at the core of the prevention of osteoporosis-related fractures. The prevention approach may include both interventions to enhance bone strength and to reduce falls.

The three main determinants of bone strength are bone mass, bone structure (i.e. distribution of the bone mass and shape), and bone material properties (intrinsic properties of the materials that comprise the bone) [12, 13]. The latter is associated with the porosity, collagen traits, matrix mineralization and other micro intrinsic aspects of bone material, that despite being non negligible in the overall perspective, were assumed as exogenous in the present dissertation.

The mechanical properties of bones, as any other object, are also dependent on the object structure. This is why bone shape and geometric attributes, bone mass distribution and the microarchitecture of bone matter [12, 13]. Consequently bones with different bone structure may not be equally strong, given the same bone mass. But, conversely to other objects, bone structure cannot easily be studied in a lab, unless in rare postmortem situations, requiring, instead, sophisticated techniques that have not been available for long and are very expensive. This may justify the relatively lower maturity in the research of bone structure when compared to bone mass.

Bone mass is the most studied of the bone strength factors and it probably is the single most important determinant as it may explain over 50% of the mechanical strength [14-17]; the higher the bone mass, *ceteris paribus*, the strongest the bone. Bone mass accrual is directly associated to the net effect of modeling and remodeling activity that naturally occurs on the bone during the first two decades of live, and determines the peak bone mass (PBM) - maximum bone mass. — After the growth period, a slow and steady net loss is observed during the remaining lifetime [18]. With aging less new bone is formed than resorbed in each bone site remodeled, resulting in bone mass loss and structural damage, particularly in postmenopausal women. In cases of more intense loss in old ages or of malfunctioning of the modeling and remodeling processes, the risk of bone fracture may be exceptionally increased due to bone fragility, configuring a process of osteoporosis disease [19].

In order to design any policy to contribute to the prevention of the risk of fractures through the improvement of bone strength, one has to identify the determinants of bone mass accrual and loss and their respective relationship with bone mass, as well as their interactions. Beyond age and sex, a great variety of factors have been indicated in the literature [20-24] which, irrespective of alternative aggregation proposals, can be grouped as follows: genetic, hormonal and metabolic, nutritional; mechanical; and risk factors (Figure 1).

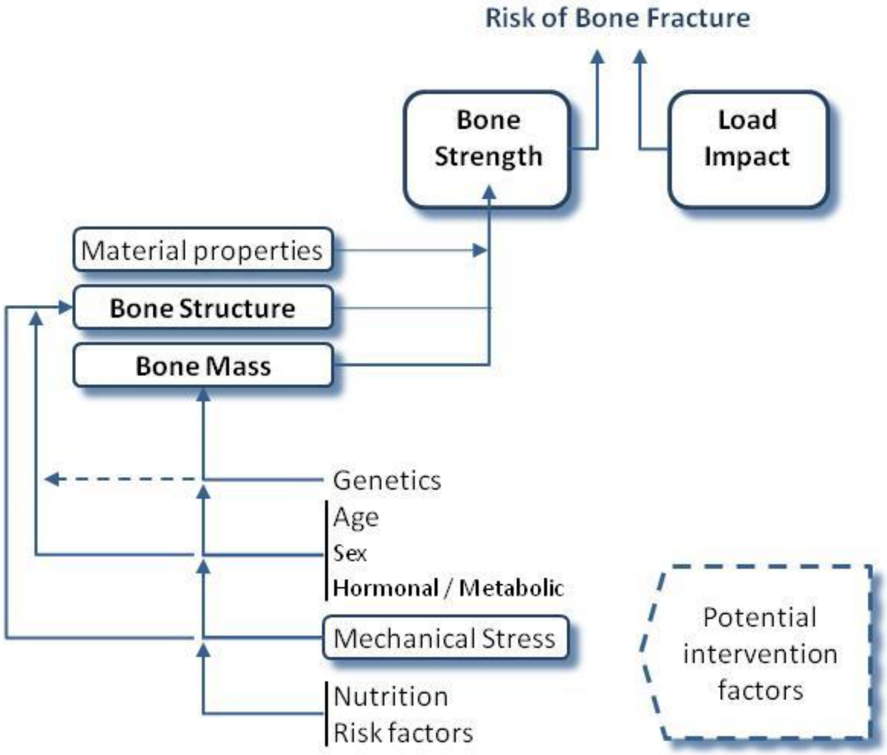

Figure 1 - Main known factors influencing bone mass, in the context of the risk of bone fracture problem.

Genetics has been reported as explaining 60-80% of the variance in PBM [21, 22, 25], but unfortunately it is not influenced by any public health policy's decision variable. As described above, there is an age-related pattern of evolution of the bone mass, which is also influenced by sex. Women reach the PBM earlier but at a lower level than men and experience a more intense bone loss in older ages [18, 26]. On its turn the hormonal and metabolic profile of each individual is determinant of bone modeling and remodeling activity influencing bone mass accrual and loss; the influence of hormonal and metabolic risk factors can be restricted by external intervention, namely through appropriate medication to mitigate and prevent the impact of osteoporosis disease. All the remaining factors constitute potential vehicles to influence the bone mass through appropriate external intervention. Adequate diet may provide the necessary diary intake of calcium, proteins and vitamin D for a healthy bone development, whereas smoking and excessive alcohol intake are risk factors among the environmental factors [20, 23, 24]. The mechanical stress on bone, given its stimulus of the (re)modeling activity, is the intervention vehicle that allows the most powerful preventive strategies in promoting bone mass accrual and fighting osteoporosis, namely through appropriate physical activity in childhood and adolescence [27-35].

Despite de importance of the partial or direct relationship of each of the above described determining factors with the bone mass, it must not be disregarded their interrelation, given the complexity of the human biological system. Additionally, keeping in mind that bone mass accrual and loss do not necessarily happen homogeneously in the existing bone structure, those determining factors of bone mass are also, indirectly, determining factors of the bone structure [27].

Summing up, bone strength is at the core of the public health issue of bone fracture and is mainly determined by bone mass and structure, which are also the result of exogenous factors. Particularly relevant to bone mass and structure seems to be the mechanical stress stimulus that is singled out in the present dissertation.

Understanding the effects of the different types of physical activity and the corresponding mechanical forces exerted on specific bone sites of the skeleton is crucial from a public policy perspective. Despite some areas of still open controversy, the literature has described the impact of physical activity on the bone mass in the main skeletal sites with highest risk of fractures – hip, spine and forearm – [27]. However, the underlying reasoning for the diverse rate of fractures among skeletal sites is at a lower research maturity stage. Assuming that it is due to the corresponding mechanical loads that physical activity benefits bone mass and bone structure, the role of the skeletal site specific biomechanics cannot be disregarded, as it might provide a better understanding of susceptibility to bone fracture.

The number of studies that integrates biomechanical models and physical activity are scarce, and we know none also adding the association/effect on bone mass distribution. Though with respect to the focus of this research project – the proximal femur –two biomechanical models were identified [27]: the biomechanics of the proximal femur, especially on the profile of forces observed at the femoral neck; and the biomechanics of the pelvis-hip system [36, 37]. The addition of the physical activity and the biomechanics topics to the previously identified ones, closes the setting up for the theoretical macro framework of this Ph.D dissertation (Figure 2).

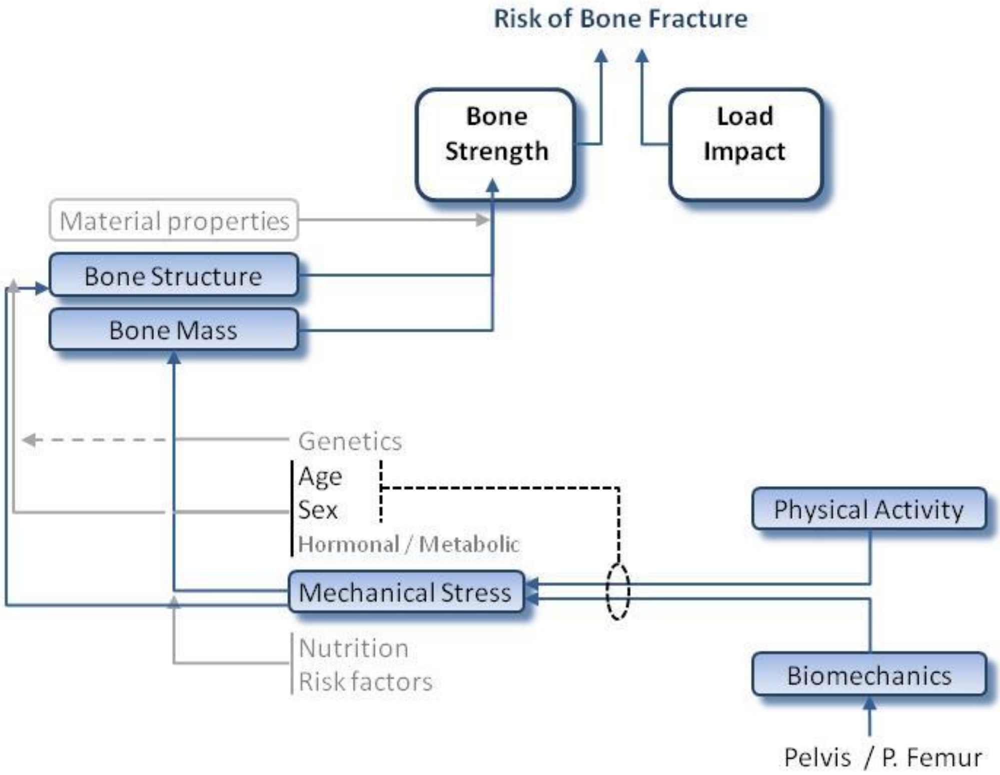

Figure 2 – Theoretical macro framework of this Ph.D thesis

The next sections present a literature overview of the four main topics that were linked and highlighted by the above framework.

2.1.1 The risk of bone fracture

Bone fractures are more frequent in the adolescence and in the older age periods, but may occur throughout the whole lifetime, with a site- and sex-specific profile, as suggested by several epidemiological studies [38-41]. The fracture of the distal forearm, hand and foot are among the most common during the adolescence, a period at which the incidence of a fracture is 27–40% in girls and 42–64% in boys [42, 43]. The reason for this predominance in boys is probably a combination of biological factors and social, sex-related differences associated to activity and risk taking, namely with boys showing a greater sports participation [32]. Sports are responsible for almost 40% of fractures at this age period, and soccer - mostly played by boys - contributes with the greatest number of cases [44]. Maturational factors also provide important explanations to understand fractures in adolescents, given that it is precisely during the pubertal growth spurt - when there is a relative decrease in bone mineral density due to bone expansion and insufficient mineralization - that their incidence is maximum [45].

Despite around one in two children in the adolescence experiencing a bone fracture, this is not considered a public health issue such as fractures in old age, since its consequences do not affect the individuals wellbeing so deeply [46]. During the old age period, low bone mineral density, low physical activity, low muscle mass and overweight are the principal risk factors for fractures [47, 48], that occur mainly at the hip, the distal forearm, the proximal humerus and the spine, with an epidemiologic pattern quite different between sexes [49, 50]. Women’s fracture risk more than doubles men’s, a disadvantage that starts as soon in life as the prepubertal period of skeletal growth, that by being 1-2 years longer in boys generates significant differences in bones size, geometry and strength, benefiting boys [51-53]. But also during the last decades of life, women are also at a disadvantage position because of the accelerated bone mass loss caused by the increased bone remodeling as a consequence of the menopause-related estrogene deficiency. Menopause is a phenomena that is women specific and reduces the ability of bones to adapt to ageing by the natural periosteal apposition [51] through which bone size increases and partially compensates (concerning strength) bone mass loss [54, 55]. As a result, the worsening in bone strength parameters – such as size, bone mineral density, cross-sectional area, etc. – to levels that endanger bone structural ability to hold expected loads affects a higher proportion of elderly women than elderly men [56, 57].

Nevertheless, the difference in fracture incidence between sex described above has been narrowing, namely in hip fractures, probably as a combined consequence of women biased timely diagnosis programs, preventive measures, and therapeutic interventions [58-61].

Among all types of fracture at old age, the fractures of the proximal femur are the most serious ones; not only they are around ten times more frequent than any other femur fracture, but, in particular, their consequences are much more severe for the deterioration of patients quality of life, higher morbility, mortality and disability, as well as for their subsequent health care system costs [62-65]. Proximal femur fractures can be divided in two main groups – cervical (or neck) fractures, and trochanteric fractures – and alike fractures in other skeletal, the incidence of femur fractures is higher in women, in particular at the femoral neck, where it is over 2.5 higher than in men, while in the trochanter it “only” overcomes men’s by about 25% [63-66, 68]. Apparently these types of fracture are much more associated to intrinsic bone- and structure-related properties of the proximal femur than to any external event causality [69]. Furthermore, the literature suggests that both the fracture mechanisms and the risk factors are different between the two types of fracture [69-72] and that the relative incidence of trochanteric fractures compared to cervicals' increases moderately with aging [63-66].

The geometry of the proximal femur - the hip axis length, the neck-shaft angle and the neck width, included - and of the pelvis seem to be specially associated to cervical fractures, whereas trochanteric fractures particularly affect subjects with low bone density and osteoporosis mainly in the trabecular region [69-72, 74-79].

However the relative importance of proximal femur fracture risk factors is not consensual among researchers. Some support that the low level of bone mineral density (BMD), namely, areal density obtained by dual x-ray absorptiometry (DXA) scanning, is one of the best fracture predictors as the bone mass and its spatial distribution are strong determinants of bone strength [80-83]. Others question this predictive ability of BMD on the grounds that around half of supposedly osteoporotic fractures occur in individuals who are not diagnosed as suffering from osteoporosis [82, 84-88]. The fact that both bone mass and bone structure interact to simultaneously determine bone strength is probably the basis for this disagreement. Irrespective of this debate, research has focused on factors that influence bone mass, either its loss during aging or its accrual during growth.

2.1.2 Peak Bone Mass and bone health

Despite the fact bone consolidation can take place during the third decade of life, mainly in males' peripheral skeleton, the maximum amount of bone mass is achieved by the end of the second decade, at the end of the maturation process [20, 26, 89]. In fact about 95% of the adult skeleton is formed by the end of the adolescence and it is estimated that during the 3 to 5 years around peak height velocity, about 20% to 40% of total young adult bone mass is obtained [26, 90, 91]. Furthermore, the normal bone mass lifetime evolution shows that the bone mass gain in the two years around the time of peak bone gain approximately equals all the amount of bone mass loss in the three decades from 50 years on [92, 93].

A 10% increase above average (1 standard deviation) in bone mineral density during growth, delays postmenopausal osteoporosis in 13 years and reduces the fracture risk in 50% while a ~6% lower PBM seems to double the risk of fracture; [47, 94, 95].The pattern of bone mass gains has been reported to be site specific, with the peak bone mass occurring in the hip and the spine earlier than in the whole body, for example [96]. Concerning the bone mass accrual at the hip, in the 3 to 5 period around the peak height velocity girls and boys gain 25%-46% and 28%-43%, respectively, of the PBM observable at this site. At the femoral neck region and in the same period, the corresponding figures (here for both sexes) are 22% to 33% [90].

All this evidence supports the importance of promoting the maximization of the PBM as a prevention strategy to reduce the risk of bone fractures related to bone mass loss in old age [20, 94, 97-100], and drive the notion that osteoporosis is a pediatric issue. This widely accepted policy “prescription” is based on the assumption that the intervention programs at childhood have long-lasting benefits on the bone. In fact the evidence seems to give ground to that assumption, as both animal and human based studies observed better bone indicators for a long period after a physical activity stimuli induced during the growth phase of skeletal. By comparing retrospectively athletes and non-athletes or adults with different levels of physical activity, it was observed that benefits obtained on bone during the growth period persist at least to young adulthood in athletes or in those who were more physically active [30, 31, 35, 101, 102]. Nevertheless, if not for fracture prevention in old age, the optimization of bone mass during the growing age might contribute to reduce the fracture risk until the early adulthood.

Several equipments and technologies have been used to assess bone parameters particularly the bone mineral as areal or volumetric BMD. Despite the 2D nature of its measurements disregarding the 3D nature of bones, DXA is the widely used equipment for assessing areal BMD. DXA is easily available, has internationally accepted standardized measures and analysis protocols, has short scanning times, and relatively low radiation exposure when compared to alternative methods [4, 53, 103]. These two latter features are of paramount importance in pediatric applications, not only because of the side effects of x-ray radiation but also because children might find it difficult to keep still even for short periods. The computed tomography (CT) is another x-ray based technique, but with the advantage of returning volumetric BMD and 3D measurements. However it is not as widely used due to its higher cost and complexity. It isn't either recommended for pediatric purposes. Radiography is also x-ray based but, even though it provides greater accuracy and precision than DXA or CT concerning geometric 2D measurements, it is not recommended for osteoporosis diagnosis due to its low sensitivity for bone mass. Using nonionizing radiation the magnetic resonation imaging (MRI) techniques return accurate 3D measurements and has a great potential for use in osteoporosis research, but it high cost has limited its large scale application and the long scanning periods are not compatible with studies involving children. Finally, the quantitative ultrasound (QUS) is a non-radiographic method to assess bone , but due to the low quality of its measurements it is not used in research work, despite its safety and low cost.

Entering adulthood with greater bone mass (i.e., higher PBM) may reduce the proportion of fractures suffered in old age [35, 104]. Given that, one third of adolescents do not participate in vigorous activity three days per week for at least 20 minutes per session, the population-attributable risk of inactivity as a factor in adult fracture risk is likely to be considerable [105].

2.1.3 Effects of Physical Activity on bone mass in children

The mechanical stress or load exerted over bone is one of the main factors determining bone mass and structure. The underlying reason for this causality effect is the fact that bone responds to mechanical stress by getting stronger, just up to the required level (within certain limits), and to disuse by losing strength capabilities [106], as first suggested by Julius Wolff in the last quarter of the XIX century. According to the Wolff's Law, as it became known, when mechanical loads reach a certain magnitude, rate, and frequency they stimulate bone osteogenic activity [107, 108]. Apparently this osteogenic potential is particularly induced by the peak force reached during impact loading. Physical activity – comprising all movements from light leisure activities to the more vigorous ones like practicing organized sports or participating in target exercise – is the main source of mechanical loads on the skeleton and has been considered to be the best intervention tool to efficiently and safely reduce osteoporosis and the associated fractures risk in old ages [109].

It has been extensively demonstrated that, either through the gravitational body mass forces or through the action of muscles, physical activity may have beneficial effects on bone parameters – mineral content and density – associated to bone strength and health during the course of life [110, 111]. This physical activity-bone health association is the result of the combined effect of physical activity induced loading forces, on one hand, and the ability of bone to respond to those forces, on the other, therefore these two aspects deserve some additional consideration. Since the effects on bone loading depend on the nature and intensity of the concrete physical activity a subject is engaged in, great attention has been given by researchers on determining the type of physical activity with the best osteogenic effect. The evidence suggests that the participation in weight-bearing activities, such as jumping, gymnastics, football or handball, that impose high strains on bones, delivered quickly, convey the greatest benefit in the promotion of bone acquisition [110-113].

Concerning the responsiveness of bone to mechanical loads, there is a wide consensus that it is in the period prior to puberty, up to five years around the peak height velocity, that bone is especially adaptable to physical activity induced loads [3, 114]. Studies comparing athletes that initiated sports training prior to and after the puberty, support the idea of that “golden period”, as well as a vast number of trials in youth whose results for the percentage of bone mass gains due to physical activity range, between 1% and 6% prior to puberty but only 0.3% to 2% afterwards [91, 115]. However, looking for the pattern of physical activity participation through life, it is possible to fully understand its importance when fracture risk prevention is at stake. In fact pre-pubertal children are among the most active groups of the population and activity levels are reported to be lower in successive age groups, declining significantly during the adolescence [116]. This combination of high bone responsiveness to loads and of natural predisposition to engage in physical activity during the pre-pubertal period is the basis for considering this period to be an opportunity window for bone health prevention programs and for the belief that physical activity in childhood is its most powerful tool [3, 35, 114],

Unfortunately, it has been reported that a great number of children and youth (around 50% in the 6 to 11 year old group and around 90% in the 12 to 19 year old group) are not active enough to fully benefit from physical activity [116], reinforcing the importance of physical activity promotion among children in the context of public health policy strategies.

The literature results strongly support this policy recommendation as several intervention studies with children, typically applying vigorous activities, including some sports, dancing aerobics or high impact exercises, three or more times per week during school days, for seven to twenty four months. In some cases, very low time intervention per session (12 minutes), have reported significant increases in bone mass parameters [3, 28, 67, 114, 117-121, 122-124]. Physical activity induced bone gains appear to last for several years, irrespectively of resulting from interventions programs, from sports participation or non-targeted physical activity. In fact it was possible to find bone mineral benefits at the hip 8 years after a 7 month intervention controlled trial [35] as well as in young adults [23-30 year old) that were more active at the 8-15 year old period (after controlling for their adult physical activity levels) [30]. Other longitudinal and retrospective studies involving subjects with sport participation in their peripubertal and pubertal ages have also showed sustained bone content and density benefits up to 10 years after sports retirement, namely at the lumbar spine and the femoral neck [101, 125-128].

The response of the skeleton to physical activity stimulus is site specific, as would be expected as long as there is a very wide range of different types of physical activity and, consequently, of mechanical load profiles and muscle pulling forces. Globally loading activities affect positively weight- bearing regions of the skeletal [31, 35], such as the lumbar spine and the femoral neck, whereas non-weight bearing activities mostly affect the skeletal sites, or bones, directly stimulated by muscles contractions, as, for instance, the studies comparing bone parameters in playing and non-playing arms in racquet sports have demonstrated [129-131]. Even at the micro level of the proximal femur, bone response appears to be site (region) specific, as different results have been obtained for the impact on the three proximal femur sub-regions (neck, trochanter and intertrochanter), the neck and the trochanter being reported as the most positively affected – with up to 14% higher bone mineral content or density scores than less active peers – by children physical activity [31, 32, 132]. These results were observed in transversal, longitudinal, and observational studies, with targeted intervention in non-athletes, but also with young athletes increasing their robustness. However, in the perspective of policy intervention to improve population bone health, especially in children, particular attention shall be paid to the observational studies of habitual physical activity that children voluntarily engage in. The high loads imposed during an intervention program or experienced by (competition) athletes are not possible to generalize to a large population, irrespective of eventually configuring the best possible conditions for bone health promotion through physical activity. Here, the use of objective measures of physical activity, namely accelerometry based approaches, is a most valuable tool [31], without disregarding the contribution of questionnaire based approaches [133].

Another issue concerning the effects of physical activity on bone health is whether there is a sex specific bone response to mechanical loading. Objectively, a variety of studies observed significantly “better” physical activity induced results on boys’ proximal femur, in particular on the femoral neck, than on girls’ (some with no observable effect at all) suggesting that boys’ bones could be more sensitive to loading than girls’ [134-137], despite the fact that many studies have not been designed to explore those sex differences [33-138,139]. But the possible explanations for these results sustain an open debate on this issue among the scientific community [119, 28, 136, 138], as besides any biological, metabolic or hormonal reasons [140-142], there are other plausible explanations that are also, but indirectly, related to sex.

Girls lower participation is sports and vigorous activities, or physical activity intensities below some threshold level to effectively stimulate the osteogenic bone activity, constitutes one of the possible reasons behind those results, as in almost all of them girls were observed to be less active then boys [134, 135, 116, 143-146]. In fact the evidence shows that girls are consistently less active than boys at the same age group, a difference that widens during the adolescence because the observed decrease in activity level is lower in boys [146]. A second important explanation lies on the differences between sexes concerning the skeletal morphology and, consequently, the biomechanical and kinematic aspects of the whole loads transmitting mechanisms, as both the physical activity pattern (for the same type of activity) and the way the correspondent weight and muscle associated forces are transmitted through bone structures differ, as described in the following section.

Whatever the reasons for the observed sex differences in relation to the effective effects of physical activity, this issue shall be taken in consideration when designing any policy initiative intended to prevent fractures or osteoporosis among the population, eventually justifying different approaches for both sexes, concerning the type, timing, intensity and duration of proposed physical activity [147].

2.1.4 Geometry and biomechanics of the proximal femur and pelvis

Bone structure – essentially its geometry (shape) and bone mass distribution – along with the quantity of bone mass and the intrinsic properties of the material that compose bone are determinant factors of bone strength [148]. But, concerning the risk of fracture, the geometry of the bone also plays a role in case of external impact (a fall, an accident, or alike) as it determines how forces are transmitted from the point of impact through the bone and whether those forces exceed (or not) bone strength, resulting in a fracture [149].

In the case of hip fracture, a great variety of studies have identified geometric properties of the proximal femur as predictors of the most common types of fracture in this skeletal site. It has been reported that longer hip axis length (HAL) [150-153], wider femoral neck width [73, 74] and larger neck-shaft angles [74, 154] are associated to increased risk of proximal femur fractures. These proximal femur variables contribute to determining the way forces are exerted on bone (for example, a longer HAL result in a longer lever arm between the hip joint center and the femur shaft), but to better understanding the hip fractures geometry-related variables of the pelvis, such as inner and outer pelvis diameter or the pelvis width, have to be also included in the analysis [14, 155, 72-79].

Bone size is positively associated to bone strength as it improves bone mechanical properties to support loads, as has been suggested [156-162]. From a biomechanical perspective, the loads over a bone are a combination of compressive and tension forces as well as bending or torsional moments that are influenced by the geometric properties of the bone itself and of the system to which it belongs [12].

Concerning the focus of this dissertation, two biomechanical models seem to help understanding the bone mass distribution on the proximal femur and, consequently its structure and strength, given that, consistent with Wolff's law, the trabecular architecture of the proximal femur is aligned with stress trajectories facilitating the transmission of loads from joints to diaphyseal cortical bone [163].

The first model explains, in a quite consolidated manner, which are the main forces in the femoral neck. From the bending moment on the proximal femur neck, caused by both the bodyweight downward force on the pelvis and the upward ground reaction force on the femur, results a compression force on the inferior part of the neck whereas a tension force in the superior part, as represented in panel a) in Figure 3. However, the contraction of the abductor muscles, in order to balance the pelvis during gait, will cause a compression force over both parts of the neck, compensating partially the previous distinction of the nature of forces on the superior and inferior parts of the femoral neck.

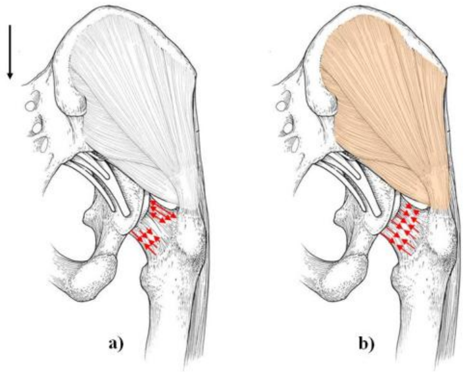

Figure 3 Forces exerted on the femoral neck through the action of body weight and abductor muscle forces (adapted from Lovejoy, 1988 [164]).

It is based on this simple model that researchers have explained the fact that natural femoral neck bone loss with aging occurs mainly at the superior part, causing the thinning of this cortical region and resulting on a biased bone mass distribution towards the region most subjected to compression forces, the inferior part of the neck [165]. In fact these mechanical effects are particularly relevant during walking, the main physical activity of elderly people [35, 105].

This biomechanical contribute to the explanation of the neck's bone mass distribution ageing pattern, is particularly relevant to understand the increased risk of cervical fractures in this population group, as in the event of a side fall, the regions of compression and tension on the femoral neck are inverted, as represented by the blue (compression) and red (tension) areas in Figure 4 [166-168]. Therefore the probability of structural failure beginning at the superior part of the neck, which is thinner and more fragile, is particularly augmented.

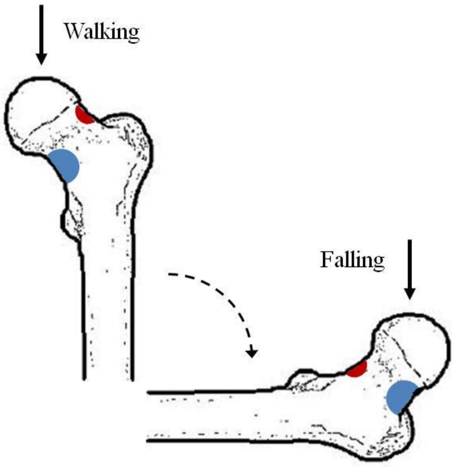

Figure 4 - Stress and tension forces on the femoral neck (adapted from Tunner, 2005 [167])

The second biomechanical model adds some pelvis features in an integrated model for the pelvis-hip system. It was first proposed by Pauwels [36] and has been extensively used in the field of hip arthroplasty.

This model explains the intensity of the force exerted by the abductor muscles during the moments of single leg stance when walking or running, as a balanced system between the torque associated to this muscle force, given its lever arm, and the torque resulting from the bodyweight over the center of rotation of the femoral head (Figure 5).

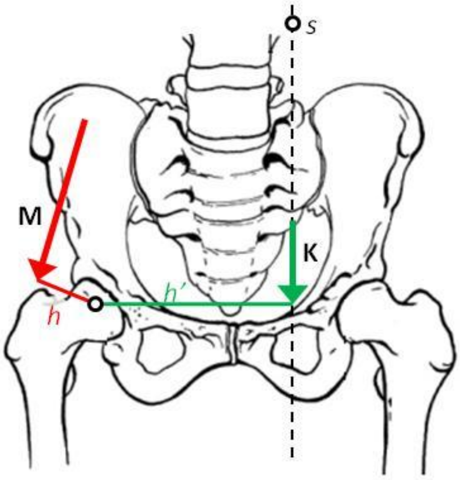

Figure 5 - Biomechanical model of the pelvis-hip system (adapted from Maquet, 1999 [169])

In fact, during locomotion each hip alternatively carries the bodyweight without the opposite leg's weight. That bodyweight-related force (***K***, in Figure 5) acts on the hip joint multiplied by the lever arm ***h'*** resulting in a force that is counterbalanced by the abductor muscles force **M** times it's lever arm ***h*** [207]. In the analytical general form the model goes as follows:

$$M \,.\, h = \mathrm{K} \,.\, h'$$

As bodyweight associated lever arm is longer than the abductors related one (***h'*** > ***h***) the force exerted by the abductor muscles has to proportionally exceed the bodyweight related force (***M*** > ***K***) as represented in Figure 6. Depending on the data used to estimate those forces and lever arms, the analytical formulation may suffer some minor adaptations. The lever arm ***h*** can be estimated by a proximal femur geometric measure represented by the perpendicular distance between the tangent of great trochanter (a line tangential to the lateral margin of the great trochanter which represent the path of abductor muscle) and the center of rotation of the femoral head [170] - the abductor lever arm (ALA) – but the hip offset can also be used. In this latter case the analytical formulation of the model has to consider some relevant angles [171]. In relation to the estimation of the arm ***h'*** there is no concrete proposal in the literature, but it is for sure linearly correlated with the pelvis width assessed by the inter-acetabular distance or any related measure.

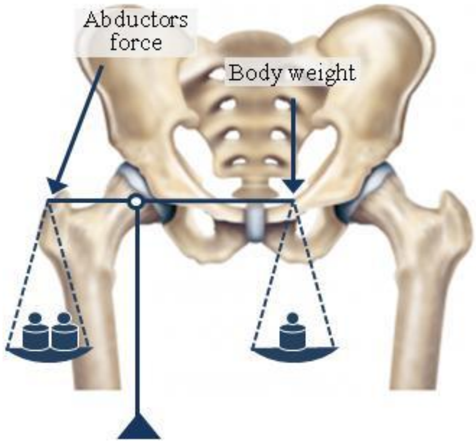

Figure 6 - “Disadvantaged” positioning of the abductor muscles in the pelvis-hip system (adapted from Traiana et al., 2009 [77])

Based on this model, a study designed to simulate the load stresses on the proximal femur reported the relevance of the pelvis width on the load pattern [171].

The two models presented, added to the empirical evidence suggesting that the anatomy of the hip and pelvis may play a role in the risk factor patterns of hip fracture, give ground to the relevance of exploring the effects of the complex loads and forces exerted in the different regions of the proximal femur on the mineralization of those specific regions, as it has already been done for the neck region (as described above).

The detailed analysis of the distribution of bone mass in the proximal femur (even using DXA images) has revealed to be an interesting way of studying and understanding the risk of fracture as the identification of small spots of lower bone density (statistically associated to some type fracture) corresponds to those regions that are less mechanically stimulated during normal load bearing [172].

Finally, the geometry and biomechanics of the hip and pelvis is also a sex differentiating issue. In fact, several of the geometric referred variables are significantly different between sexes, namely girls and women have shorter hip axis lengths, lower neck-shaft angles, lower hip offsets and wider pelvis [77]. These geometric differences may directly affect the forces and loading pattern on the proximal femur for biomechanical reasons, but also because they influence the pattern of locomotion and muscle activity, therefore the consequent weight loading forces on the femur [142, 173-175]. Consequently, these sex specific forces and loads may also help to understand the different proximal femur mineralization profile between sexes.

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3 Experimental work

3.1 Ward's area location, Physical Activity and body composition in 8 and 9 years old boys and girls[1]

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[1] Cardadeiro G, Baptista F, Zymbal V, Rodrigues L, Sardinha L 2010 Ward's area location, Physical Activity and body composition in 8 and 9 years old boys and girls. J Bone Miner Res 25:1-10.

WARD'S AREA LOCATION, PHYSICAL ACTIVITY, AND BODY COMPOSITION IN 8- AND 9-YEAR-OLD BOYS AND GIRLS

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Abstract

Bone strength is the result of its material composition and structural design, particularly bone mass distribution. The purpose of this study was to analyze femoral neck bone mass distribution by Ward's area location and its relationship with physical activity (PA) and body composition in children 8 and 9 years of age. The proximal femur shape was defined by geometric morphometric analysis in 88 participants (48 boys and 40 girls). Using dual-energy X-ray absorptiometry (DXA) images, 18 landmarks were digitized to define the proximal femur shape and to identify Ward's area position. Body weight, lean and fat mass, and bone mineral were assessed by DXA, PA by accelerometry, and bone age by the Tanner-Whitehouse III method. Warps analysis with Thin-Plate Spline software showed that the first axis explained 63% of proximal femur shape variation in boys and 58% in girls. Most of this variation was associated with differences in Ward's area location, from the central zone to the superior aspect of the femoral neck in both genders. Regression analysis demonstrated that body composition explained 4% to 7% of the proximal femur shape variation in girls. In boys, body composition variables explained a similar amount of variance, but moderate plus vigorous PA (MVPA) also accounted for 6% of proximal femur shape variation. In conclusion, proximal femur shape variation in children ages 8 and 9 was due mainly to differences in Ward's area position determined, in part, by body composition in both genders and by MVPA in boys. These variables were positively associated with a central Ward's area and thus with a more balanced femoral neck bone mass distribution.

Key words: Ward's Area; Proximal femur; Physical activity; Body composition; Children

Introduction

Ward’s triangle is a space formed near the center of femoral neck by the intersection of three trabecular bundles, namely, the principal compressive, the secondary compressive, and the tensile trabecular (1). This central region, containing some thin and loosely arranged trabeculae, defines a neutral axis where tensile and compressive forces balances each other (2). Changes in the appearances of these groups of trabeculae with aging are the basis of the grading scheme proposed by Singh that ranges from grade 6 (normal) to grade 1 (severe osteoporosis) (3). In grade 6, the compressive and tensile trabeculae cross each other, and the upper end of the femur is seen to be completely occupied by cancellous tissue. In this grade, Ward’s triangle shows some thin trabeculae and is not completely delimited. In lower grades, Ward’s triangle looks empty and more prominent (grade 5) and opens up laterally, with resorption proceeding outward from the center of the bone (grade 4, borderline between osteoporotic and normal skeleton). Thus Ward’s triangle is a region of initial bone loss in the femoral neck that can be identified on standard radiographs once bone loss becomes significant.

When assessed using densitometry, Ward’s triangle is the femoral neck region with the lowest bone mineral density (BMD) rather than a specific anatomic region and is identified as a square, generally called Ward’s area (4). Since its location is not standardized between subjects, that is, is not always in the same position, usually Ward’s area is not considered useful for skeletal health evaluation by dual X-ray absorptiometry (DXA) or for bone research because BMD of this region cannot be compared with a reference database or control group. However, determination of the location of Ward’s area contributes to an understanding of femoral neck bone mass distribution, given that its position, when it is not central, suggests a bone mass distribution imbalance. This imbalance may be particularly important to evaluate the risk of bone fragility.

Trabecular architecture of the proximal femur is aligned with stress trajectories facilitating the transmission of loads from joints to diaphyseal cortical bone (2). Variation in the trabecular pattern is a consequence of stress-force changes. During gait, the superior part of the femoral neck incurs a tension, whereas the inferior part incurs a compression stress, causing the latter to be thicker than the former and suggesting that bone mass distribution tends to become biased toward the compression (as opposed to tension) regions. With aging, bone loss seems to occur mainly on the superior aspect of the femoral neck (superolateral cortex), a specific location that is not loaded adequately during walking, the main physical activity of elderly people (5,6), who are also the most vulnerable segment of the population to fragility-related fractures (7). However, femoral neck bone mass distribution is not only a function of the amount of bone lost during aging. Bone accrued during growth also seems to be an important determinant of bone mass in old age (8). The effect of mechanical loading on the acquisition/ optimization of bone mass, geometry, or derived structural measures of bone strength is a relevant research outcome.

In general, studies support a positive association between mechanical loading and these skeletal parameters (9–11). These works used, however, global assessments of bone, and patterns of skeletal adaptation induced by loads seem to be consistent with regional variation (12), with some areas of bone under tension and others under compression (13). In this context, the main purpose of this study was to analyze relationships between the distribution of bone mass, habitual physical activity, and body composition (as surrogates for weight-bearing forces) through an approach based on Ward’s area (femoral neck area with the lowest BMD) location using a landmark-based morphometric methodology to quantify shape variations. Since prepubertal children are one of the most active groups of the population (14) and one of those in which the response to mechanical loading appears to be maximized (9), this study was conducted in 8- and 9-year-old boys and girls. The assumption is that more active boys and girls have a higher mineralization of the superolateral region of femoral neck owing to the action of weight-bearing forces and thus a more central Ward's area.

The morphometric evaluation of shape variations has been used for about a decade in biology and palaeontology and allows scientists to use advanced statistical methods to analyze anatomic features in order to classify organisms taxonomically and understand life diversity. However, as far as we know, it has not yet been used in the area of bone health; still, its use is promising and may overcome some geometric measurement limitations currently used to describe bone shape, which, as properties of the same object, are highly correlated, limiting their usefulness in statistical analysis (15,16).

Materials and Methods

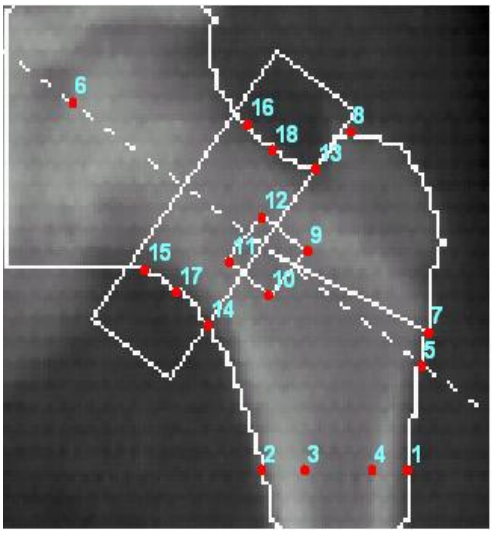
Participants

The subjects for this study were drawn from 18 schools (third grade) and 20 sports clubs. The sample consisted of 88 children (48 boys and 40 girls) with ages between 8 and 9 years. None of the subjects was taking any medication affecting bone. None of the children reported a history of hip fracture. The university's internal review approved the study, and parents or legal guardians provided written informed consent.

Proximal femur analysis

Bone mineral content (BMC) and BMD of left leg proximal femur were measured with DXA (QDR 1500, pencil beam mode, Version 12.4, Hologic, Inc., Waltham, MA, USA). Identification of Ward's area with Hologic equipment was made automatically by searching a specific portion of the femoral neck and intertrochanter (2.7 x 3.0 cm) for the minimum point of bone density, and then a fixed area box (10.5 x 10.5 mm) was centered around this point. In case of system difficulty in locating an area of minimum density, Ward's area was centered automatically at the intersection of the femoral midline and the initial position of the bottom edge of the femoral neck box. The coefficient of variation for Ward's area BMD was 0.3%.

In order to identify the femoral neck Ward's area position, several 2D landmarks (x, y coordinates) were digitized on proximal femur DXA images, specifically, 14 landmarks to characterize the proximal femur shape (PFS) and 4 additional landmarks to identify Ward's area position (Fig. 1).



Anatomical Landmarks	**Description**
1-2	Femoral shaft width*
3-4	Femoral intramedulary width **
5-6	Femoral axis length
7	Lower tip of greater trocanter
8	Upper tip of greater trocanter
9-12	Ward's area
13-14	Lower limit of femoral neck
15-16	Upper limit of femoral neck
17-18	Average distance of lower and upper femoral neck limits

* 1.5cm under the lower limit of femoral neck axis.
** Width of medullary canal at the same level of 1-2 landmarks

Fig. 1. Proximal femur DXA image showing the location of the landmarks used to identify proximal femur shape and position of Ward's area. Four landmarks (9–12) were digitized manually at the corners of the square shape automatically defined by the DXA software.

The same number of landmarks was digitized in each image, and each point always refers to the same feature in the shape. The 18 x and y coordinates for the 18 landmarks were obtained by using the Thin-Plate Spline Digitize (tpsDig) software Version 2.10(17).

The morphometric analyses assigned generally as thin-plate spline can be described as an interpolation function that computes the bending of an idealized thin steel plate that can be used as well to compute deformed grid pictures in order to illustrate differences between shapes. With Thin-Plate Spline Relative Warps Analysis (tpsRelw) software Version 1.45 (17), a consensus landmark configuration was imposed through the generalized Procrustes analysis after translating, scaling, and rotating landmark configurations to minimize the squared differences between corresponding landmarks (18). This process means that all data about proximal femur shape and Ward's area location were stored proportionally rather than absolutely, with elimination of the overall size effects on femur measurements.

Relative warps analysis corresponds to a principal-components analysis of the covariance matrix of the partial warp scores, which are different scales of a thin-plate spline transformation of landmarks. Relative warp scores (ie, the principal-component scores with respect to bending energy) may point out clusters or trends in the collection of shapes.

Each relative warp is plotted as a deformation of the space of the reference configuration of landmarks. Consequently, all clusters within a pair of relative warp plots must be interpreted as a group of specimens revealing identical shape deformation from the consensus.

In general, partial warps are components that result from the decomposition of a nonuniform deformation based on the thin-plate spline interpolation function (19). Each partial warp describes a pattern of relative landmark displacement grounded on the spacing and location of the reference form landmarks.

The shape variables (ie, partial warps) obtained by the thinplate spline function were determined and decomposed in shape descriptors along the x axis (stretching or shortening) and the y axis (dilation or compression) to analyze changes that occurred at specific regions. The partial warps are the eigenvectors of the matrix of the deformation energy, and each one describes a possible change in the shape applicable to the main configuration.

The superimposed configuration then was projected on the principal warps, describing the differences in shape as deviations of the main configuration (20). The projections or scores generated indicated how much of each principal warp was needed to accomplish the deformations. The scores described each proximal femur shape as a linear combination between principal warps and the standardized coordinates x and y for each anatomic landmark. These shape variables, that is, partial warps, were used in a multivariate analysis to quantify shape differences.

Body size and body composition

Standing height (centimeters) was measured on a stadiometer (Secca 770, Hamburg, Germany) with subjects in underwear and barefoot. Body weight (kilograms), total fat (kilograms), and total lean mass (kilograms) were determined from a total-body scan using DXA (QDR 1500, pencil beam mode, Version 12.4, Hologic, Waltham, MA, USA) with subjects in a fasting state. Body mass index (BMI) was calculated as body weight in kilograms divided by height (in meters) squared.

Physical activity

Physical activity was assessed with the Actigraph accelerometer (Model GT1M, Pensacola, FL, USA). Participants wore the monitor over 7 days, and the activity data were summed on a minute-by-minute basis. Subjects were excluded if they failed to provide a minimum of 3 days of at least 600 minutes per day of accelerometer data. The accelerometer was secured on the right hip, and the subjects were asked to wear the accelerometer during the daytime, except during water activities. Activity data were analyzed and processed using the MAHUffe Analysis Program (www.mrc-epid.cam.ac.uk). The output from the program included accumulated time spent at sedentary, light, moderate, and vigorous physical activity intensity in minutes per day. The total amount of physical activity was expressed as total counts divided by registered time, that is, counts per minutes, which is an indicator of the total volume of physical activity and the numbers of minutes the child engaged in activity of different intensities. Cut points of 100, 1952, and 5724 counts/min were used to identify sedentary, light, moderate, and vigorous physical activity. These cut points have been validated previously in children under free-living conditions using the doubly labeled water method as the criterion (21).

Maturation level and calcium intake

Skeletal age, assessed with a portable X-ray (Ascott Model, Belgium; Kodak Chassis 20 x 15), and the Tanner-Whitehouse III method (22) was used to define the level of maturity. The energy (kilocalories) and calcium intakes (milligrams) were calculated from a semiquantitative food frequency questionnaire assessing regular intake of a wide set of a typical Portuguese foods.

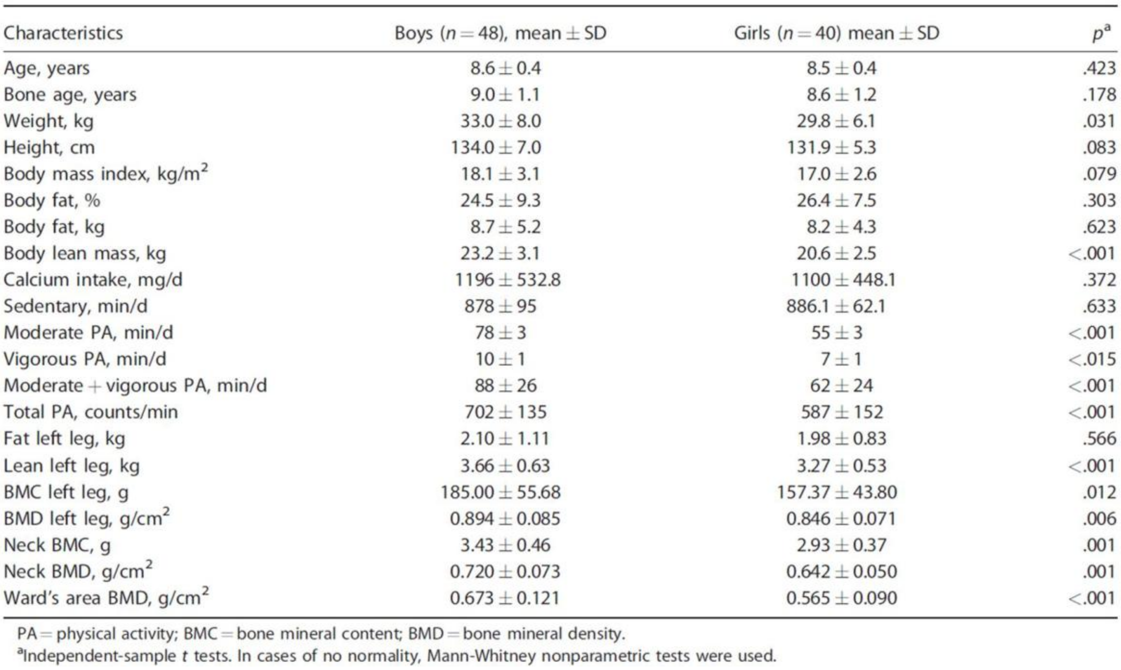
Statistical analysis

Data were analyzed using the SPSS Statistical Software Package (Version 16.0 for Windows, SPSS, Inc., Chicago, IL, USA). Distribution properties of all variables were examined, and appropriate measures of central tendency and variability were selected. Then differences between groups were analyzed by independent-samples t tests or by Mann Whitney nonparametric tests (in case of no normality). A principal-components analysis method on the partial warps—relative warps—was used to estimate each relative warp's ability to explain the shape variations between individuals' landmark configurations through calculation of the singular values and corresponding determination coefficients. This analysis was made with tpsRelw Version 1.45 (23), with the parameter a=0, in order to have all the partial warps in the same scale of variation, the most appropriate option for exploratory studies (24). Finally, relationships between shape variations (computed in previous landmark data analyses) and each of the body composition and physical activity variables alone were tracked by linear regression analysis with tpsRegr Version 1.35 (23). The dependent variable for this analysis was the quantified deviation from the consensus shape image of the proximal femur, accessed by the thin-plate function. The global quality of each model was assessed by using the generalized Goodall F test. Associations between independent variables were examined by bivariate correlation because tpsRegr does not allow multicollinearity analysis. Statistical significance was set at the 5% level. All the analyses were performed separately for boys and girls.

Results

Table 1 presents the characteristics of the sample, including chronological age, bone age, body weight, height, body composition, calcium intake, physical activity, and bone outcome variables. Boys had higher values than girls for body weight, lean mass of total and left leg, BMC and BMD of left leg and femoral neck, and BMD of Ward's area. Means also were higher in boys than in girls for moderate, vigorous, moderate plus vigorous, and total physical activity. There were no gender differences for chronological age, bone age, body height, BMI, body fat, calcium intake, or inactivity.

Table 1. Subject Characteristics



Characteristics	Boys ($n = 48$), mean ± SD	Girls ($n = 40$) mean ± SD	p^a
Age, years	8.6 ± 0.4	8.5 ± 0.4	.423
Bone age, years	9.0 ± 1.1	8.6 ± 1.2	.178
Weight, kg	33.0 ± 8.0	29.8 ± 6.1	.031
Height, cm	134.0 ± 7.0	131.9 ± 5.3	.083
Body mass index, kg/m^2	18.1 ± 3.1	17.0 ± 2.6	.079
Body fat, %	24.5 ± 9.3	26.4 ± 7.5	.303
Body fat, kg	8.7 ± 5.2	8.2 ± 4.3	.623
Body lean mass, kg	23.2 ± 3.1	20.6 ± 2.5	<.001
Calcium intake, mg/d	1196 ± 532.8	1100 ± 448.1	.372
Sedentary, min/d	878 ± 95	886.1 ± 62.1	.633
Moderate PA, min/d	78 ± 3	55 ± 3	<.001
Vigorous PA, min/d	10 ± 1	7 ± 1	<.015
Moderate + vigorous PA, min/d	88 ± 26	62 ± 24	<.001
Total PA, counts/min	702 ± 135	587 ± 152	<.001
Fat left leg, kg	2.10 ± 1.11	1.98 ± 0.83	.566
Lean left leg, kg	3.66 ± 0.63	3.27 ± 0.53	<.001
BMC left leg, g	185.00 ± 55.68	157.37 ± 43.80	.012
BMD left leg, g/cm^2	0.894 ± 0.085	0.846 ± 0.071	.006
Neck BMC, g	3.43 ± 0.46	2.93 ± 0.37	.001
Neck BMD, g/cm^2	0.720 ± 0.073	0.642 ± 0.050	.001
Ward's area BMD, g/cm^2	0.673 ± 0.121	0.565 ± 0.090	<.001

PA = physical activity; BMC = bone mineral content; BMD = bone mineral density.

[a] Independent-sample *t* tests. In cases of no normality, Mann-Whitney nonparametric tests were used.

The principal-components analysis results [relative warps (RWs)] showed that 91% of the total variation of proximal femur shape was explained by the first five relative warps (ie, the first five principal components). For both boys and girls, the first two RW axes accounted for approximately 70% of that variation (73% for boys and 70% for girls). RW$_1$ explained approximately 60% (63% for boys and 58% for girls) of the total shape variation (Table 2).

Table 2. Relative Warps Analysis

	Boys			Girls		
	SV	R^2 (%)	Cum%	SV	R^2 (%)	Cum%
RW$_1$	0.58328	63	63	0.57137	58	58
RW$_2$	0.23710	10	73	0.25339	12	70
RW$_3$	0.20734	8	81	0.22683	9	79
RW$_4$	0.16234	5	86	0.18392	6	85
RW$_5$	0.14816	5	91	0.17391	5	91

RW = relative warps; SV = singular values; R^2 = determination coefficient; Cum = cumulative percentage.

Plots for the first two principal components (RW$_1$ and RW$_2$) are displayed in Fig. 2.

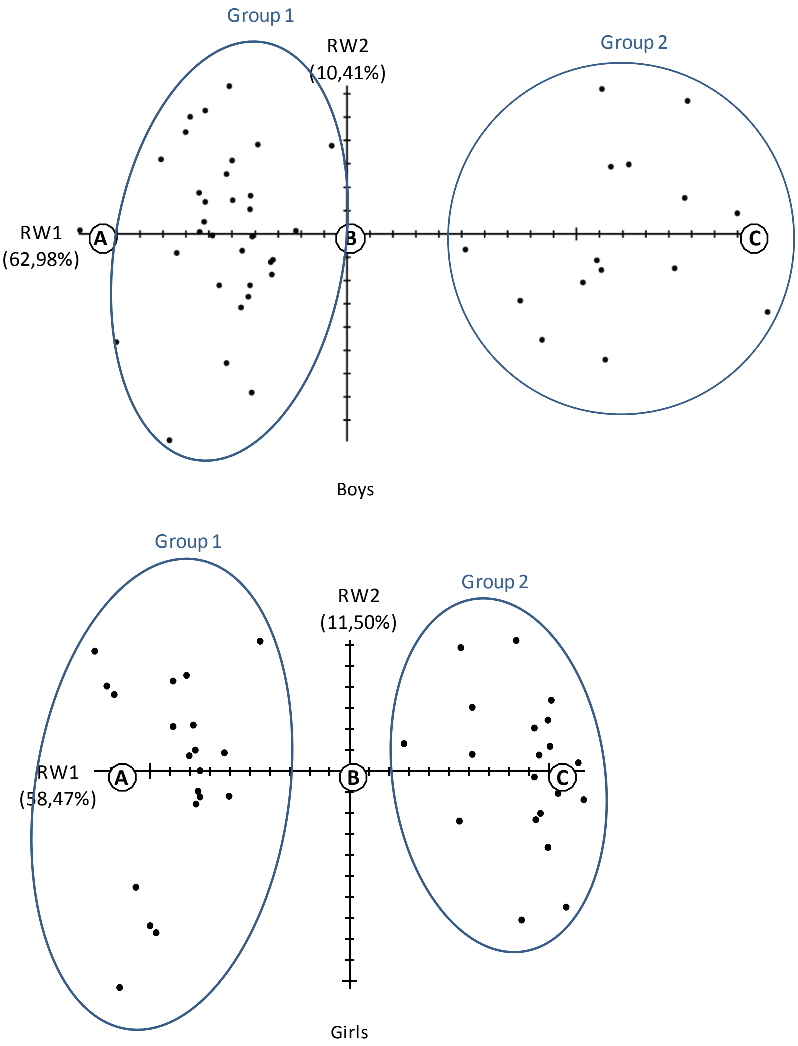


Fig. 2. Relative warp (RW) plots for RW$_1$ and RW$_2$ in boys and girls.

Two morphologic clusters along the RW_1 axis were positioned around the left-end side (group 1) and around the opposite side (group 2), with a spatial distribution pattern common to both boys and girls. Regarding the RW_2 axis, the placement of individuals was more dispersed.

Figure 3 presents the shape of the proximal femur in three different positions along the first principal-component axis (RW_1) of Fig. 2. From one extreme (point A in Fig. 2) to the other (point C in Fig. 2), Ward's area moves from a central position to the superolateral limit of the femoral neck, showing that most of the shape variation from the consensus was due to displacement of Ward's area. This observation was similar for both boys and girls.

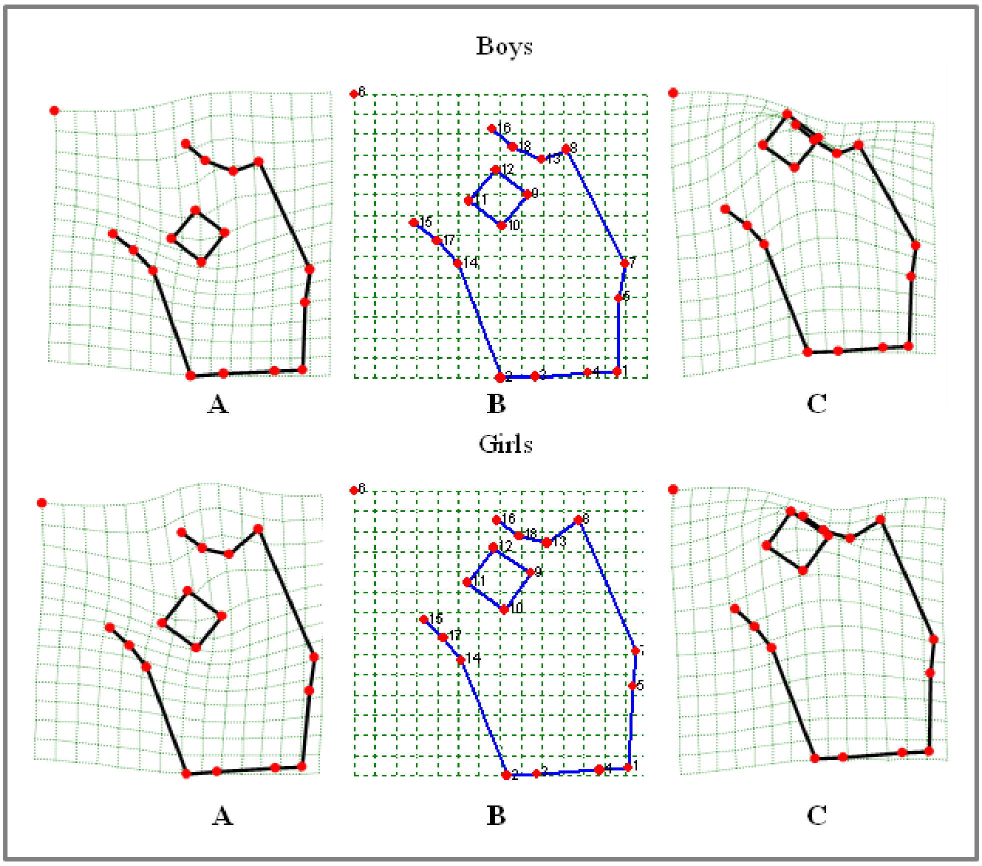
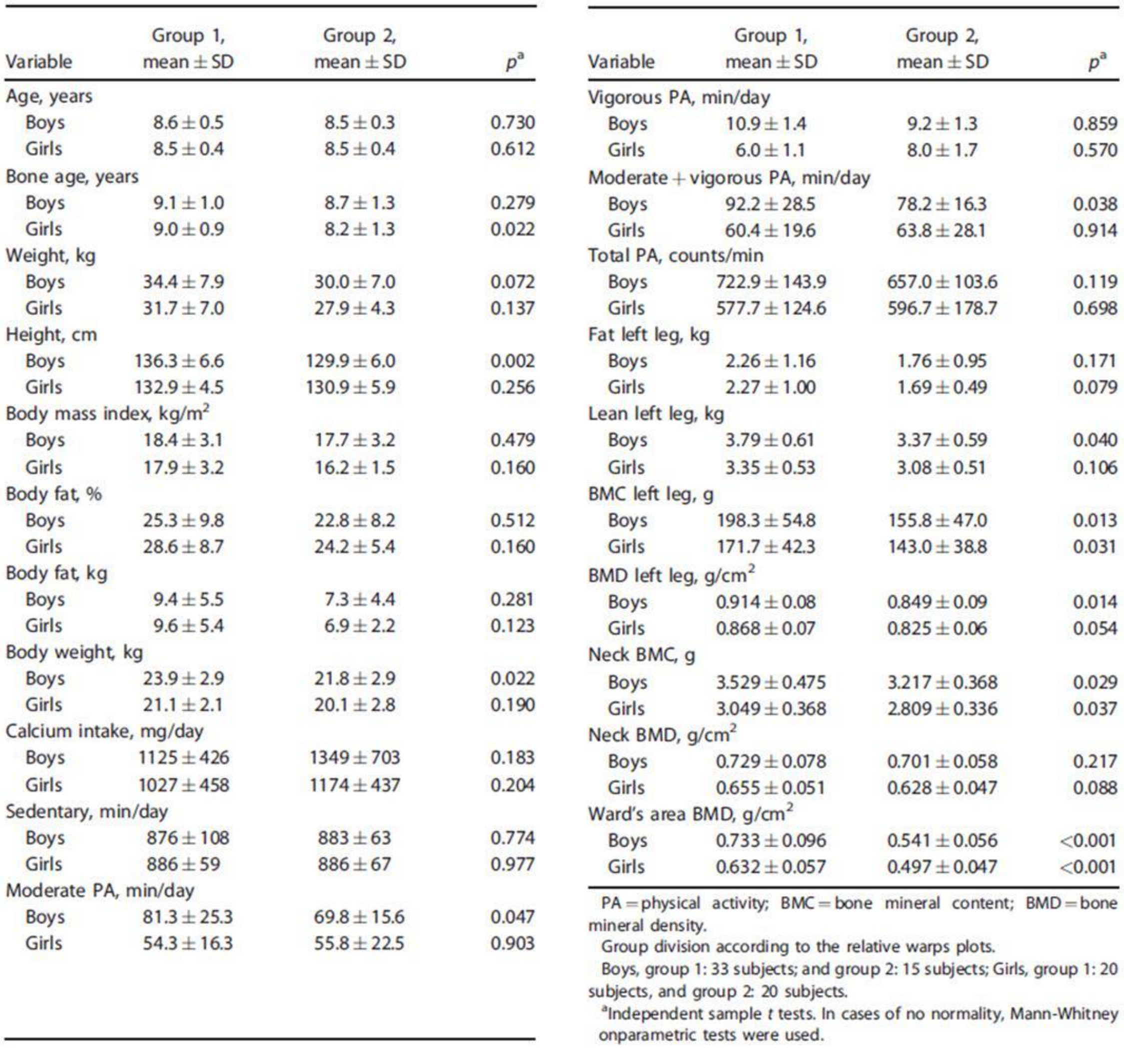


Fig. 3. Femoral neck Ward's area position changes in boys and girls depicted by RW_1. (A) Shape configuration at point A with a deformation relative to the mean shape (consensus shape) toward the negative direction of RW_1. (B) Consensus shape. (C) Shape configuration at point C with a deformation relative to the mean shape (consensus shape) toward the positive direction of RW_1.

Concerning the second principal-component axis (RW_2), the main shape deformation from the consensus was related to the distance between landmarks 13 and 14 (lower limit of the femoral neck) and landmarks 15 and 16 (upper limit of the femoral neck) from the bottom-end side of the axis to the upperend side, representing a change in femoral neck length. However, groups were not identified with reference to the individuals' positioning in relation to RW_2 or to the other RWs, given the much lower singular values of these RWs compared with RW_1.

Table 3 presents the subject characteristics of groups 1 and 2 within each gender clustered according to Ward's area position, that is, their relative position along the axis of the first principal component (RW_1). In girls, there was no difference between groups, except for bone age (9.0 versus 8.2 years, $p<.022$), BMC of the left leg (20% difference, $p<.031$), BMC of the femoral neck (8.5% difference, $p<.037$), and BMD of Ward's area (27% difference, $p<.01$), with group 1 demonstrating higher values for these variables. In boys, differences were found for body height (4.9% difference, $p<.002$), total lean mass (12.3% difference, $p<.022$), leg lean mass (12.3% difference, $p<.040$), leg BMC (27.2% difference, $p<.013$), leg BMD (12.5% difference, $p<.014$), femoral neck BMC (9.7% difference, $p<.029$), and Ward's area BMD (35% difference, $p<.01$). Values were always higher for group 1. While physical activity variables showed no significant differences among the girls' groups, moderate physical activity (16.5% difference, $p<.047$) and moderate plus vigorous physical activity (17.9% difference, $p<.038$) were higher for group 1 in boys. In both genders, individuals in group 1 showed a more homogeneous femoral neck bone mass distribution, expressed by the absence of significant differences between the BMD of the femoral neck and BMD of Ward's area.

Table 3. Comparison of Age, Body Composition, Calcium Intake, Physical Activity, and Bone Variables Between Group 1 and Group 2



Variable	Group 1, mean ± SD	Group 2, mean ± SD	p^a
Age, years			
Boys	8.6 ± 0.5	8.5 ± 0.3	0.730
Girls	8.5 ± 0.4	8.5 ± 0.4	0.612
Bone age, years			
Boys	9.1 ± 1.0	8.7 ± 1.3	0.279
Girls	9.0 ± 0.9	8.2 ± 1.3	0.022
Weight, kg			
Boys	34.4 ± 7.9	30.0 ± 7.0	0.072
Girls	31.7 ± 7.0	27.9 ± 4.3	0.137
Height, cm			
Boys	136.3 ± 6.6	129.9 ± 6.0	0.002
Girls	132.9 ± 4.5	130.9 ± 5.9	0.256
Body mass index, kg/m^2			
Boys	18.4 ± 3.1	17.7 ± 3.2	0.479
Girls	17.9 ± 3.2	16.2 ± 1.5	0.160
Body fat, %			
Boys	25.3 ± 9.8	22.8 ± 8.2	0.512
Girls	28.6 ± 8.7	24.2 ± 5.4	0.160
Body fat, kg			
Boys	9.4 ± 5.5	7.3 ± 4.4	0.281
Girls	9.6 ± 5.4	6.9 ± 2.2	0.123
Body weight, kg			
Boys	23.9 ± 2.9	21.8 ± 2.9	0.022
Girls	21.1 ± 2.1	20.1 ± 2.8	0.190
Calcium intake, mg/day			
Boys	1125 ± 426	1349 ± 703	0.183
Girls	1027 ± 458	1174 ± 437	0.204
Sedentary, min/day			
Boys	876 ± 108	883 ± 63	0.774
Girls	886 ± 59	886 ± 67	0.977
Moderate PA, min/day			
Boys	81.3 ± 25.3	69.8 ± 15.6	0.047
Girls	54.3 ± 16.3	55.8 ± 22.5	0.903
Vigorous PA, min/day			
Boys	10.9 ± 1.4	9.2 ± 1.3	0.859
Girls	6.0 ± 1.1	8.0 ± 1.7	0.570
Moderate + vigorous PA, min/day			
Boys	92.2 ± 28.5	78.2 ± 16.3	0.038
Girls	60.4 ± 19.6	63.8 ± 28.1	0.914
Total PA, counts/min			
Boys	722.9 ± 143.9	657.0 ± 103.6	0.119
Girls	577.7 ± 124.6	596.7 ± 178.7	0.698
Fat left leg, kg			
Boys	2.26 ± 1.16	1.76 ± 0.95	0.171
Girls	2.27 ± 1.00	1.69 ± 0.49	0.079
Lean left leg, kg			
Boys	3.79 ± 0.61	3.37 ± 0.59	0.040
Girls	3.35 ± 0.53	3.08 ± 0.51	0.106
BMC left leg, g			
Boys	198.3 ± 54.8	155.8 ± 47.0	0.013
Girls	171.7 ± 42.3	143.0 ± 38.8	0.031
BMD left leg, g/cm^2			
Boys	0.914 ± 0.08	0.849 ± 0.09	0.014
Girls	0.868 ± 0.07	0.825 ± 0.06	0.054
Neck BMC, g			
Boys	3.529 ± 0.475	3.217 ± 0.368	0.029
Girls	3.049 ± 0.368	2.809 ± 0.336	0.037
Neck BMD, g/cm^2			
Boys	0.729 ± 0.078	0.701 ± 0.058	0.217
Girls	0.655 ± 0.051	0.628 ± 0.047	0.088
Ward's area BMD, g/cm^2			
Boys	0.733 ± 0.096	0.541 ± 0.056	<0.001
Girls	0.632 ± 0.057	0.497 ± 0.047	<0.001

PA = physical activity; BMC = bone mineral content; BMD = bone mineral density.

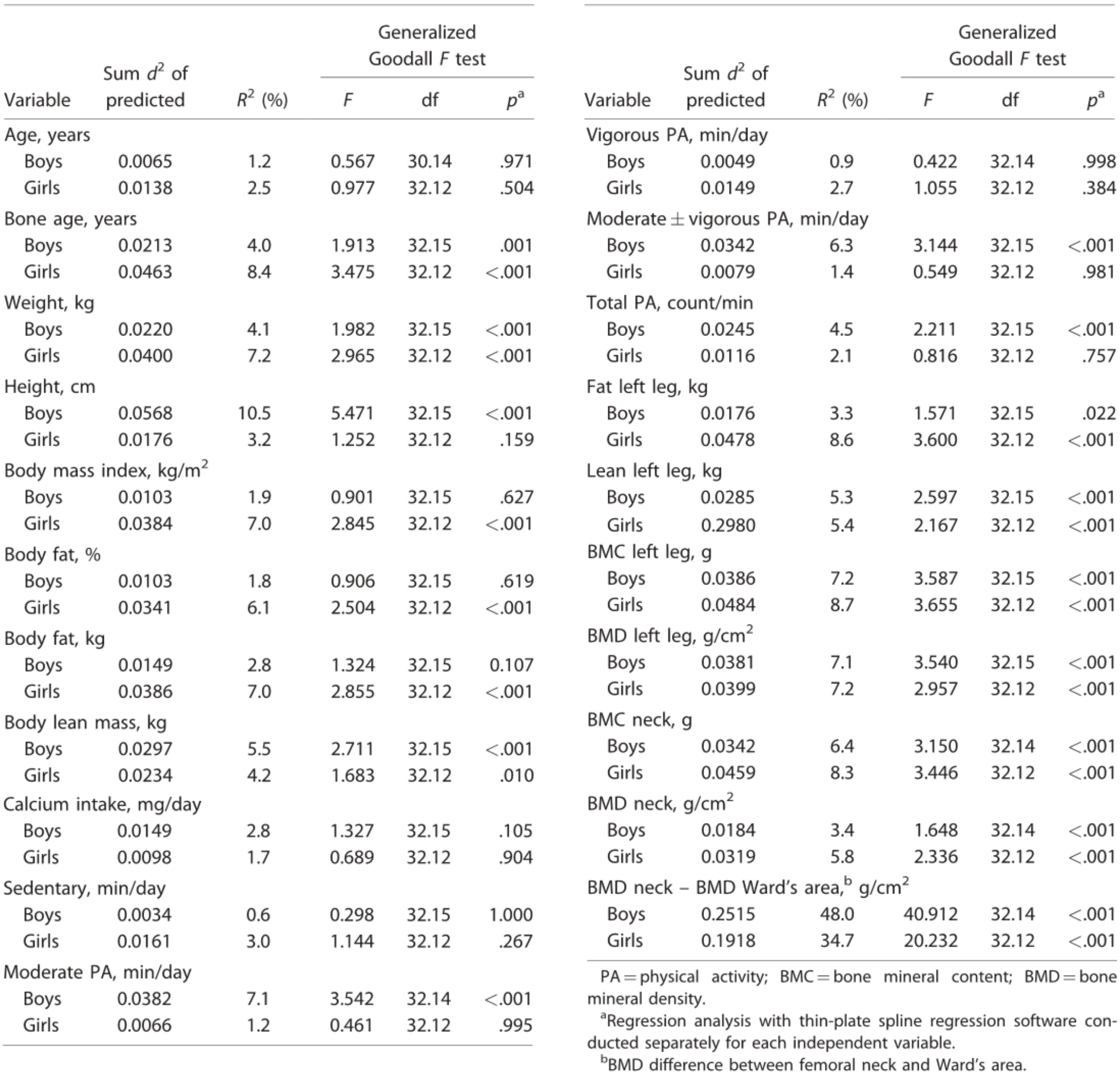
Group division according to the relative warps plots.

Boys, group 1: 33 subjects; and group 2: 15 subjects; Girls, group 1: 20 subjects, and group 2: 20 subjects.

[a] Independent sample *t* tests. In cases of no normality, Mann-Whitney onparametric tests were used.

Regression analysis, using the shape variables (partial warps) of the proximal femur as the dependent variable and each of the other variables as independent variables, demonstrated that body weight and body composition variables (body fat mass and lean mass) explained 4% to 9% (p<.01) of the observed variation in both genders, except fat mass, which was a significant predictor only in girls (R^2=7%, p<.001; Table 4).

Table 4. Regression Analysis: prediction of proximal shape variation



Variable	Sum d^2 of predicted	R^2 (%)	Generalized Goodall F test F	 df	 p^a
Age, years					
Boys	0.0065	1.2	0.567	30.14	.971
Girls	0.0138	2.5	0.977	32.12	.504
Bone age, years					
Boys	0.0213	4.0	1.913	32.15	.001
Girls	0.0463	8.4	3.475	32.12	<.001
Weight, kg					
Boys	0.0220	4.1	1.982	32.15	<.001
Girls	0.0400	7.2	2.965	32.12	<.001
Height, cm					
Boys	0.0568	10.5	5.471	32.15	<.001
Girls	0.0176	3.2	1.252	32.12	.159
Body mass index, kg/m^2					
Boys	0.0103	1.9	0.901	32.15	.627
Girls	0.0384	7.0	2.845	32.12	<.001
Body fat, %					
Boys	0.0103	1.8	0.906	32.15	.619
Girls	0.0341	6.1	2.504	32.12	<.001
Body fat, kg					
Boys	0.0149	2.8	1.324	32.15	0.107
Girls	0.0386	7.0	2.855	32.12	<.001
Body lean mass, kg					
Boys	0.0297	5.5	2.711	32.15	<.001
Girls	0.0234	4.2	1.683	32.12	.010
Calcium intake, mg/day					
Boys	0.0149	2.8	1.327	32.15	.105
Girls	0.0098	1.7	0.689	32.12	.904
Sedentary, min/day					
Boys	0.0034	0.6	0.298	32.15	1.000
Girls	0.0161	3.0	1.144	32.12	.267
Moderate PA, min/day					
Boys	0.0382	7.1	3.542	32.14	<.001
Girls	0.0066	1.2	0.461	32.12	.995
Vigorous PA, min/day					
Boys	0.0049	0.9	0.422	32.14	.998
Girls	0.0149	2.7	1.055	32.12	.384
Moderate ± vigorous PA, min/day					
Boys	0.0342	6.3	3.144	32.15	<.001
Girls	0.0079	1.4	0.549	32.12	.981
Total PA, count/min					
Boys	0.0245	4.5	2.211	32.15	<.001
Girls	0.0116	2.1	0.816	32.12	.757
Fat left leg, kg					
Boys	0.0176	3.3	1.571	32.15	.022
Girls	0.0478	8.6	3.600	32.12	<.001
Lean left leg, kg					
Boys	0.0285	5.3	2.597	32.15	<.001
Girls	0.2980	5.4	2.167	32.12	<.001
BMC left leg, g					
Boys	0.0386	7.2	3.587	32.15	<.001
Girls	0.0484	8.7	3.655	32.12	<.001
BMD left leg, g/cm^2					
Boys	0.0381	7.1	3.540	32.15	<.001
Girls	0.0399	7.2	2.957	32.12	<.001
BMC neck, g					
Boys	0.0342	6.4	3.150	32.14	<.001
Girls	0.0459	8.3	3.446	32.12	<.001
BMD neck, g/cm^2					
Boys	0.0184	3.4	1.648	32.14	<.001
Girls	0.0319	5.8	2.336	32.12	<.001
BMD neck – BMD Ward's area,[b] g/cm^2					
Boys	0.2515	48.0	40.912	32.14	<.001
Girls	0.1918	34.7	20.232	32.12	<.001

PA = physical activity; BMC = bone mineral content; BMD = bone mineral density.

[a] Regression analysis with thin-plate spline regression software conducted separately for each independent variable.

[b] BMD difference between femoral neck and Ward's area.

Total physical activity, particularly moderate and moderate plus vigorous physical activity, explained approximately 5% to 7% (p<.001) of proximal femur shape variation in boys. Bone age and height explained 4% and 11% of proximal femur shape variation in boys, respectively, and bone age explained 8% of the variation in girls. The difference between the BMD values for the femoral neck and Ward's area was the variable that best explained the variation in proximal femur shape, namely, 37% in girls and 48% in boys (p<.001).

Discussion

The main purpose of this study was to analyze femoral neck bone mass distribution by Ward's area location and its relationship with physical activity and body composition in prepubertal children. Ward's area, the region of the femoral neck with the lowest density, is structurally important because if it is not centred, it could reveal a diminution of tensile trabeculae and thus a reduction in strength for impact loading. The influence of neuromuscular anatomy and function on the control of the biologic mechanism that determines the strength of loadbearing bones in children provided the rationale to analyze the relationship between mechanical loading (physical activity and body composition) and Ward's area location.

We found that about 30% of proximal femur shape variation was related to its external configuration, expressed by the second to fifth principal-components axes (RW_2 to RW_5). However, most of the shape variation was related to the location of Ward's area, controlled by the first principalcomponent axis (RW1), which explains approximately 60% of the shape variance. Consequently, the location of Ward's area was considered the single main cause of form variation, with similar patterns of positioning for boys and girls, between the center and the superolateral cortex of the femoral neck.

Comparison of subject characteristics between the previously identified Ward's area location groups (central versus superolateral) showed differences in skeletal and body composition variables, denoted in girls by bone age and BMC and in boys by height, lean mass, BMC, and BMD. Boys and girls with higher values of these variables tended to present with Ward's area in the center of the femoral neck and thus had a more balanced bone mass distribution. Additionally, regression analysis showed that skeletal and body composition variables accounted for 3% to 9% of proximal femur shape variation. In boys, physical activity, principally moderate plus vigorous physical activity, accounted for 6% of the shape variation of the proximal femur. Boys with more time spent in moderate plus vigorous physical activity (~92 min/day) were likely to have Ward's area located in the middle of the femoral neck, whereas boys with lower moderate plus vigorous physical activity (~78 min/day) mostly had Ward's area located in the superior aspect of the femoral neck. The explained variability was similar to and independent of the effect of skeletal and body composition variables (no associations were found between physical activity and these variables).

Physical activity was assessed with epochs of 1 minute, which underestimated the physical activity of moderate to vigorous intensity because the high-intensity activity usually occurs at shorter intervals. This estimation did not influence interpretation of the results, however, and allowed comparison of the physical activity levels. The mean intensity of physical activity of the children in our study was slightly lower than that of the 9-yearold children in the European Youth Heart Study (boys 702 versus 784 counts/min; girls 587 versus 649 counts/min) (25) and higher than that of US 6- to 11-year-old children (boys 647 counts/min; girls 568 counts/min) (14).

Consistent with other studies, measures of physical activity showed that boys spent more time in moderate and vigorous physical activity than girls (25,26). These results could mean that girls' physical activity did not reach a sufficiently high threshold to influence proximal femur shape. However, in a previous investigation, girls demonstrated lower femoral neck strength measures than boys despite a higher cumulative participation in vigorous intensity physical activity by some girls. This previous study suggests the possibility of a gender-specific responsiveness to mechanical stimulus (11).

Among body composition variables (ie, weight, BMI, fat mass, and lean mass), fat mass (total or left leg) was the strongest predictor of proximal femur shape in girls, whereas lean mass (total or left leg) was the strongest in boys. Fat mass may stimulate bone growth in prepubertal children (27), but lean mass is seen as the major contributor to bone mass and geometry accrual, particularly during pubertal years. Peak lean mass accrual velocity precedes by some months the peak of total-body BMC, proximal femur cross-sectional area, and section modulus accrual velocity. This growth pattern is consistent with the Mechanost theory, which suggests that the increase in lean mass (and consequently muscle mass and strength) stimulates bone formation (28,29).

Physical activity and body composition are important determinants of bone mass, geometry, and strength of the femoral neck (30–34). It is reasonable to assume that mechanisms connecting physical activity and body composition to bone mass are also linked to structural adaptation, that is, redistribution of bone mass. As a weight-bearing bone, the femur is particularly stimulated during locomotion movements that affect different diverse areas of the bone. Habitual physical activity during childhood is frequent and intense but also varies widely among children. The bending strength of bone increases with physical activity and is gained primarily through apposition of relatively small amounts of bone at specific sites on the periosteal surface rather than through a general increase in bone mass (10,12,35,36). This may be a potential explanation for the observed association between physical activity and the location of Ward's area in our study, with more active boys demonstrating a Ward's area in a more central position of the femoral neck.

Comparison of the differences between femoral neck and Ward's area BMDs, according to the location of the Ward area groups (central, group 1 versus superolateral, group 2), suggests a more homogeneous bone mass distribution associated with a central location of Ward's area (group 1: difference of 0.023 g/ cm2 among BMDs in girls, $p<.025$, and no BMD differences in boys) and a more heterogeneous bone mass distribution associated with a superolateral location (group 2: difference of 0.131 g/cm2 among BMDs in girls, p<.003, and difference of 0.160 g/cm2 among BMDs in boys, p<.001). On the other hand, more than 33% of proximal femur shape variation in girls and almost 50% in boys was explained by the BMD difference between the femoral neck (which includes Ward's area) and Ward's area itself. A diminished bone mass at the superior aspect of the femoral neck could make the bone susceptible to failure by buckling owing to large compressive stresses caused by a fall, particularly in the elderly (6).

In this study, we examined regional patterns of bone mass distribution in children using DXA, the most accepted quantitative measurement technique for assessing skeletal status. BMD of Ward's area is usually measured in a DXA exam of the proximal femur but is not used to make any interpretation of bone health because this area does not have a standardized localization, as do the other regions of the hip, and thus the bone density result cannot be compared with a reference database.

However, this site is important because its position denotes the internal architecture of the proximal femur composed of two major trabecular systems arranged along the lines of compressive and tensile stresses produced during weight bearing (2). A central region defines a neutral axis where tensile and compressive forces balance each other. Two femoral necks with the same bone density may diverge concerning the location of Ward's area, that is, with different distributions of bone mineral mass, and should behave differently when submitted to a side fall. Despite the impossibility of an analysis of the bone section by quadrants, with DXA, we were able to obtain information about the most critical femoral neck region to fracture risk—the superolateral—using the geometric morphometric analyses of DXA images of the proximal femur. This is a novel aspect of our work.

In summary, proximal femur shape variation in 8- and 9-year old boys and girls was explained primarily by the position of Ward's area. Skeletal and body composition variables explained most of the variability, and for boys, moderate plus vigorous physical activity also was an important predictor variable. These exposure variables were positively associated with a more balanced bone mass distribution and thus with enhanced femoral neck stability, expressed by a central location of Ward's area.

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Disclosures

All the authors state that they have no conflicts of interest.

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3.2 Sex specific association of physical activity on proximal femur BMD in 9 to 10 years-old children[2]

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SEX SPECIFIC ASSOCIATION OF PHYSICAL ACTIVITY ON PROXIMAL FEMUR BMD IN 9 TO 10 YEARS-OLD CHILDREN

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Abstract

The results of physical activity (PA) intervention studies suggest that adaptation to mechanical loading at the femoral neck (FN) is weaker in girls than in boys. Less is known about gender differences associated with non-targeted PA levels at the FN or other clinically relevant regions of the proximal femur. Understanding sex-specific relationships between proximal femur sensitivity and mechanical loading during non-targeted PA is critical to planning appropriate public health interventions. We examined sex-specific associations between non-target PA and bone mineral density (BMD) of three sub-regions of the proximal femur in pre- and early-pubertal boys and girls. BMD at the FN, trochanter (TR) and intertrochanter (IT) regions, and lean mass of the whole body were assessed using dual-energy x-ray absorptiometry in 161 girls (age: 9.7±0.3 yrs) and 164 boys (age: 9.7±0.3 yrs). PA was measured using accelerometry. Multiple linear regression analyses (adjusted for body height, total lean mass and pubertal status) revealed that vigorous PA explained 3–5% of the variability in BMD at all three sub-regions in boys. In girls, vigorous PA explained 4% of the variability in IT BMD and

6% in TR BMD. PA did not contribute to the variance in FN BMD in girls. An additional 10 minutes per day of vigorous PA would be expected to result in a ~1% higher FN, TR, and IT BMD in boys ($p<0.05$) and a ~2% higher IT and TR BMD in girls. In conclusion, vigorous PA can be expected to contribute positively to bone health outcomes for boys and girls. However, the association of vigorous PA to sub-regions of the proximal femur varies by sex, such that girlś associations are heterogeneous and the lowest at the FN, but stronger at the TR and the IT, when compared to boys.

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Introduction

Mechanical loading by impact or muscle forces is a contributing factor to skeletal health throughout the life course; however, mechanical loading is particularly important during the transition period from childhood to adolescence. This may be due to the efficient response of bone to loading during middle childhood (elementary school years), since the magnitude of bone accrual associated with mechanical loading is reported to be greater when compared to early childhood and adulthood [1]–[6]. In addition, the evidence shows that the amount and intensity of PA levels are highest during middle childhood compared to other time points across childhood and adolescence [7]. The amount and intensity of PA during middle childhood is important since PA levels dramatically decrease during adolescence [7]–[9]. Consequently, a timely intervention in children’s activity habits when bone appears to be most responsive to activity’s effect and PA is more easily accepted could be an important public health strategy for optimal bone development.

Studies conducted with pre- and early-pubertal children have shown augmented bone mineral accrual in several skeletal sites in both girls [10]–[17] and boys [15]–[17] after high-impact mechanical loading (running and jumping) interventions. The effect of high-impact loading exercise on bone has been shown to be site specific, with accrual occurring at weight bearing sites such as lumbar spine [11] and proximal femur [18]–[22]. The loads imposed during targeted exercise are likely to represent the “best case scenario” and might not generalize to the spontaneous PA choices of children.

Population-based observational studies provide important information on the relationship of bone accrual to the type and amount of PA children voluntarily choose to do. Twenty-five to forty minutes of vigorous PA per day has been suggested as a minimum daily dose for optimal bone growth [23]–[26] but the relationship between bone mineral accrual and PA during the growing years has not been thoroughly examined. Of the studies that exist, there is a lack of consensus on whether sex moderates the association between mechanical loading and bone accrual at the proximal femur [18], [19], [27], [28]. However, it has been suggested that the proximal femur of boys' is more sensitive to mechanical loading than girls' [27]. This idea was not derived from work specifically powered to examine sex differences [23], [27]–[31]. Furthermore, most studies reporting a lower responsiveness of PA in girls (when compared to boys) analysed only the femoral neck and not other sub-regions of the proximal femur (i.e. trochanter and intertrochanter).

A potential explanation for sex-specific bone response at the proximal femur is the lower intensity mechanical loading on the skeleton by muscle or impact forces in girls due to their lower lean body mass and less weight-bearing PA. However, given sex differences in body composition [32], [33], in PA [7], [8], maturation timings [34], morphology and gait kinematic parameters [35], it is plausible that bone response sensitivity to PA differs among the proximal femur sub-regions for both boys and girls. This possibility has not been examined and is clinically relevant because women suffer more fragility fractures in old age [36]–[38] and have a higher incidence of fractures at the femoral neck region compared to men who have higher incidence of trochanteric femoral fractures [39], [40]. Therefore, the purpose of this cross-sectional study was to investigate the sex-specific association between non-targeted PA and bone mineral density (BMD) of sub-regions of the proximal femur in boys and girls.

Methods

Sample

This cross sectional cohort study included 325 pre and early pubertal subjects (Tanner stage 1 and 2), aged 9–10 years (164 boys and 161 girls) living in the island of Madeira (Portugal) and drawn from the European Youth Heart Study (EYHS). Selection procedures and methods are described in detail elsewhere [24]. None of the subjects were taking any medication affecting bone and none reported a history of bone fracture in lower limbs. The research protocol was in accordance with the Helsinki Declaration. Parents or legal guardians provided written informed consent and the study was approved by the Ethics Committee attached to the scientific board of the Faculty of Human Movement.

Physical Activity.

PA was assessed with a uniaxial accelerometer (model WAM 6471, Manufacturing Technology Incorporated, Fort Walton Beach, FL), over two weekdays and two weekend days. The subjects were asked to wear the accelerometer all day except during water activities, in a representative week of their normal activity, and the procedure was repeated in all cases in which any abnormal event was reported. MAHUffe software (www.mrc-epid.cam.ac.uk) was used to analyze and process activity data. Outcome variables were time (minute/day) spent in light, moderate and vigorous intensity of PA. The intensity of PA was defined according the counts per minute (cpm) as follows: light intensity from 501 to 1999 cpm; moderate intensity from 2000 to 2999 cpm; and vigorous intensity over 2999 cpm. All of the activity data were averaged over the 4-day period and subjects who failed to provide a minimum of 3 days of ≥600 minutes of accelerometer data were excluded. PA procedures are detailed in previous report [41].

Clinic Measures

Standing height (to the nearest millimetre) was measured on a stadiometer (Secca 770, Hamburg, Germany) without shoes. Body weight (kilograms), total fat (kilograms), and lean mass without bone (kilograms) were determined from a total body scan by dual x-ray absorptiometry (DXA) (QDR-1500, high-speed performance mode, software 5.7) (Hologic, Waltham, MA; pencil beam, software 5.73). Sexual maturity was assessed using self-report and Tanner's 5-stage scale for breast development in girls and pubic hair in boys. Children were stratified as prepubertal (Tanner stage 1) or having started puberty (Tanner stage 2) [42].

BMD from three proximal femur sub-regions, i.e., femoral neck, trochanter and intertrochanter, were measured with DXA (QDR-1500, high-speed performance mode, software 4.76). Quality assurance tests were performed each morning. Precision errors were estimated from 2 measurements in 14 subjects [43]. The coefficients of variation of femoral neck, trochanter and intertrochanter BMD ranged from 1.2% to 1.5%. From the DXA scans, BMD ratios among sub-regions were calculated as indicators of BMD homogeneity in the proximal femur as follows [40]: FNTR is the ratio between femoral neck BMD and trochanter BMD (FNBMD/TRBMD); FNIT is the ratio between femoral neck BMD and intertrochanter BMD (FNBMD/ITBMD); TRIT is the ratio between trochanter BMD and intertrochanter BMD (TRBMD/ITBMD).

Calcium intake were calculated from a semi-quantitative Food Frequency Questionnaire assessing regular intake of a wide set of typical Portuguese foods using the Food Processor SQL software (ESHA Research, Salem OR).

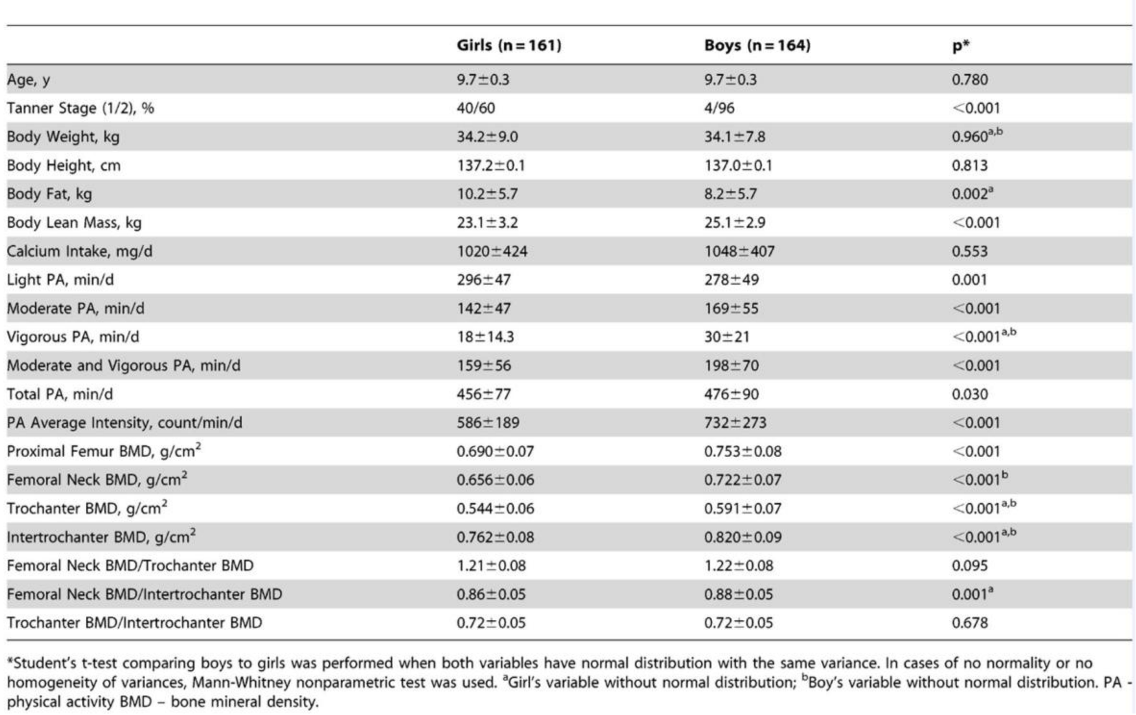
Statistical Analysis

Data were analysed using the SPSS statistical software package (Version 18.0 for Windows; SPSS, Chicago, IL, USA). Distribution properties of all variables were examined using the Kolmogorov-Smirnov test and appropriate measures of central tendency and variability were selected. Differences between groups (girls and boys) were analysed by Independent-samples T-tests in case of normality and equally of variance and Mann-Whitney nonparametric test otherwise. The Chi-square test of homogeneity was used to compare Tanner stage distributions across sexes. Stepwise regressions were used to analyse associations between PA variables, (i.e., time spent at moderate intensity, vigorous intensity, and moderate-through-vigorous PA - MVPA) and BMD or BMD ratios of proximal femur sub-regions, adjusted for Tanner stage, body height, and total body lean mass. Data were initially analysed with boys and girls pooled together to test the significance of sex as predictor variable and then separately for each sex. All the assumptions for the linear regression analysis were verified (normality and linearity of the residuals, multicollinearity and homoscedasticity). The hypothetical effect of PA intensity on the BMD of the proximal femur sub-regions was estimated by regression analyses (enter approach, $p<0.05$) calculating the percentage of BMD change associated with an additional 10 minutes per day of PA at two different intensities (MVPA and vigorous PA) by multiplying the unstandardized regression coefficients by 10 and dividing by the correspondent BMD mean at each sub-region of proximal femur. Significance level was set at $p<0.05$.

Results

The characteristics of the children are presented in Table 1. There were no differences in age, body weight and height between boys and girls. However, lean mass was higher and fat mass was lower in boys, who were also more active than girls. Boys spent more time in moderate and vigorous PA than girls, whereas girls spent more time in light activities. The proportion of participants in early puberty (Tanner 2) was higher in boys than in girls (40% of the girls and 4% of the boys were in the Tanner stage 1). The BMD of the proximal femur and of its three sub-regions was higher in boys than in girls, but statistically significant sex differences in BMDs ratios were not found, with the exception of the FNIT, with boys revealing a higher ratio than girls.

Table 1. Characteristics of participants as mean±standard deviation.



	Girls (n = 161)	Boys (n = 164)	p*
Age, y	9.7±0.3	9.7±0.3	0.780
Tanner Stage (1/2), %	40/60	4/96	<0.001
Body Weight, kg	34.2±9.0	34.1±7.8	0.960[a,b]
Body Height, cm	137.2±0.1	137.0±0.1	0.813
Body Fat, kg	10.2±5.7	8.2±5.7	0.002[a]
Body Lean Mass, kg	23.1±3.2	25.1±2.9	<0.001
Calcium Intake, mg/d	1020±424	1048±407	0.553
Light PA, min/d	296±47	278±49	0.001
Moderate PA, min/d	142±47	169±55	<0.001
Vigorous PA, min/d	18±14.3	30±21	<0.001[a,b]
Moderate and Vigorous PA, min/d	159±56	198±70	<0.001
Total PA, min/d	456±77	476±90	0.030
PA Average Intensity, count/min/d	586±189	732±273	<0.001
Proximal Femur BMD, g/cm^2	0.690±0.07	0.753±0.08	<0.001
Femoral Neck BMD, g/cm^2	0.656±0.06	0.722±0.07	<0.001[b]
Trochanter BMD, g/cm^2	0.544±0.06	0.591±0.07	<0.001[a,b]
Intertrochanter BMD, g/cm^2	0.762±0.08	0.820±0.09	<0.001[a,b]
Femoral Neck BMD/Trochanter BMD	1.21±0.08	1.22±0.08	0.095
Femoral Neck BMD/Intertrochanter BMD	0.86±0.05	0.88±0.05	0.001[a]
Trochanter BMD/Intertrochanter BMD	0.72±0.05	0.72±0.05	0.678

*Student's t-test comparing boys to girls was performed when both variables have normal distribution with the same variance. In cases of no normality or no homogeneity of variances, Mann-Whitney nonparametric test was used. [a]Girl's variable without normal distribution; [b]Boy's variable without normal distribution. PA - physical activity BMD – bone mineral density.

Associations between PA and BMD of the proximal femur sub-regions were analysed using multiple regression models, first with boys and girls polled together (Table 2) and after separated (Table 3). Among PA variables (time spent at moderate, MVPA, and vigorous PA), vigorous PA was the one with the highest contribution to the R squared of each model (3–7%, p<0.001) (Table 2). None of the other two PA variables showed additional explanatory power once vigorous PA had entered the model. In the same table, body lean mass explained 20–24% of variance in all BMD models ($p<0.001$) while Tanner stage was responsible for ~1% variability of femoral neck BMD ($p = 0.038$). In all the three regression models ran with boys' and girls' data pooled together (Table 2), sex turned out to be a significant predictor variable, giving empirical ground for subsequent separated data treatment.

Table 2. Standardized regression coefficients (β), level of significance (p) and coefficient of determination (R^2) for proximal femur sub-region models, adjusted for sex, Tanner stage, body height and body lean mass, with data for boys and girls pooled together.

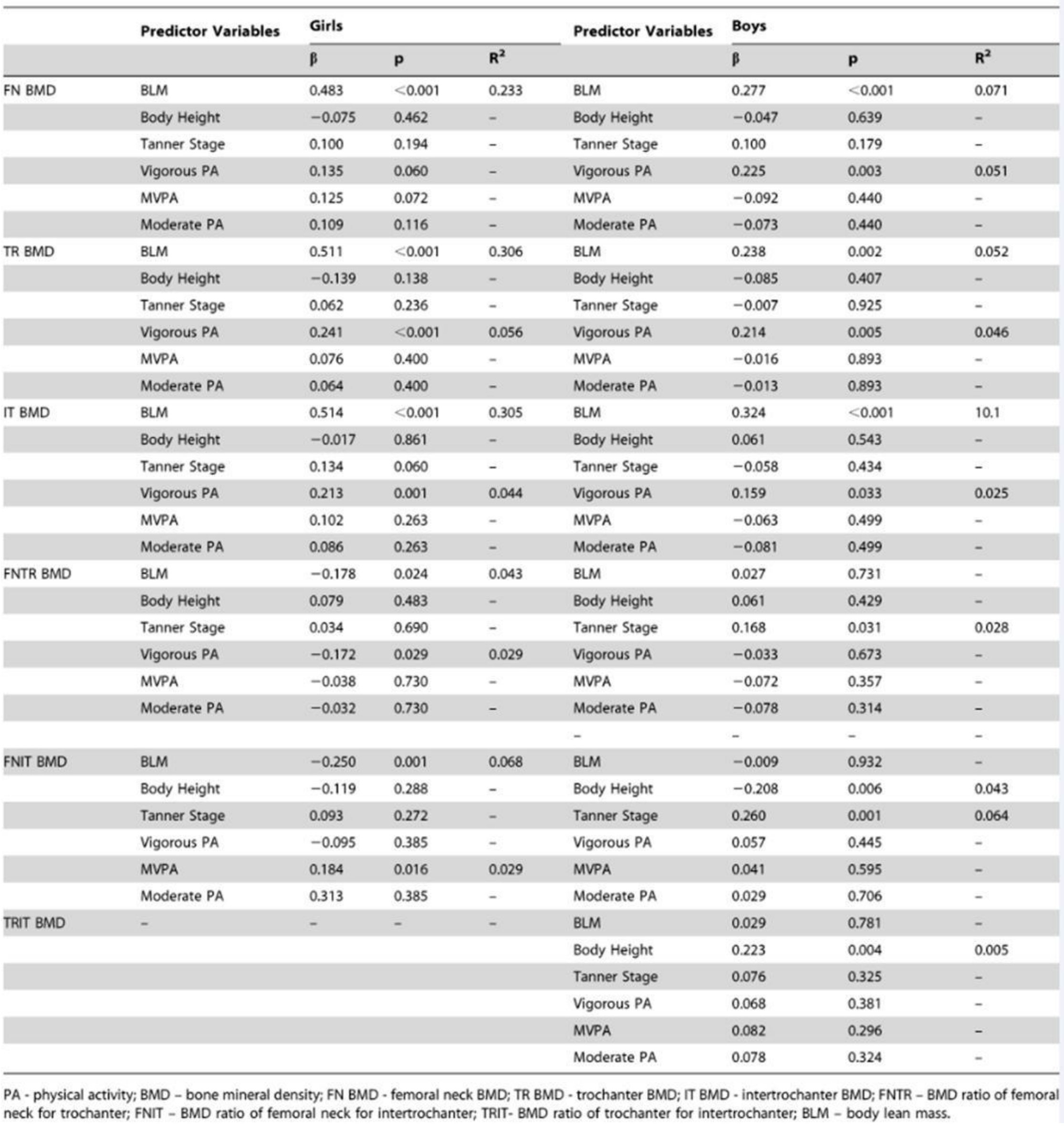
	Predictor Variables	β	p	R^2
FN BMD	BLM	0.308	<0.001	0.204
	Sex	0.239	<0.001	0.100
	Body Height	−0.064	0.311	–
	Tanner Stage	0.110	0.038	0.009
	Vigorous PA	0.191	<0.001	0.033
	MVPA	−0.004	0.955	–
	Moderate PA	−0.003	0.955	–
TR BMD	BLM	0.372	<0.001	0.209
	Sex	0.170	0.001	0.024
	Body Height	−0.096	0.179	–
	Tanner Stage	0.077	0.156	–
	Vigorous PA	0.227	0.001	0.073
	MVPA	0.039	0.604	–
	Moderate PA	0.031	0.604	–
IT BMD	BLM	0.426	<0.001	0.242
	Sex	0.129	0.012	0.014
	Body Height	0.030	0.634	–
	Tanner Stage	0.082	0.030	–
	Vigorous PA	0.182	<0.001	0.046
	MVPA	0.019	0.798	–
	Moderate PA	0.015	0.798	–

BMD – bone mineral density; FN BMD - femoral neck BMD; TR BMD - trochanter BMD; IT BMD - intertrochanter BMD; PA - physical activity; MVPA – moderate-through-vigorous PA; BLM – body lean mass.

Three other similar models were run for the proximal femur BMD ratios with boys and girls together but none of them complied with the assumptions for regression analysis, having therefore been rejected. Conversely, the models of proximal femur BMD ratios were added to the initial three ones when data was considered separately for boys and girls to analyse associations between BMD and PA variables adjusted for Tanner stage, lean body mass and body height (Table 3). Among all the PA intensity variables examined, vigorous PA was the best predictor: it explained ~3–5% of the BMD variance ($p<0.05$) in boy's femoral neck, trochanter and intertrochanter. However, none of the variation of BMD ratios in boys was predicted by PA intensity variables. In girls, vigorous PA was also the best PA predictor variable explaining 6% of the trochanter BMD and 4% of the intertrochanter BMD variance; a 3% variation in the FNTR and FNIT was also associated with vigorous and MVPA, respectively. In girls, with exception of femoral neck BMD, PA (vigorous and MVPA) explained 3–6% of all BMD variances. Unlike boys, in girls there was a negative association between PA variables and FNTR and FNIT ($p<0.05$).

Table 3 also shows that body lean mass was a significant predictor variable in all girĺs and boy's models for the proximal femur's regional BMDs, except in the girĺs TRIT BMD model.

Table 3. Standardized regression coefficients (β), level of significance (p) and coefficient of determination (R^2) for proximal femur sub-region models, adjusted for Tanner stage, body height, and body lean mass, with data for boys and girls treated separately.

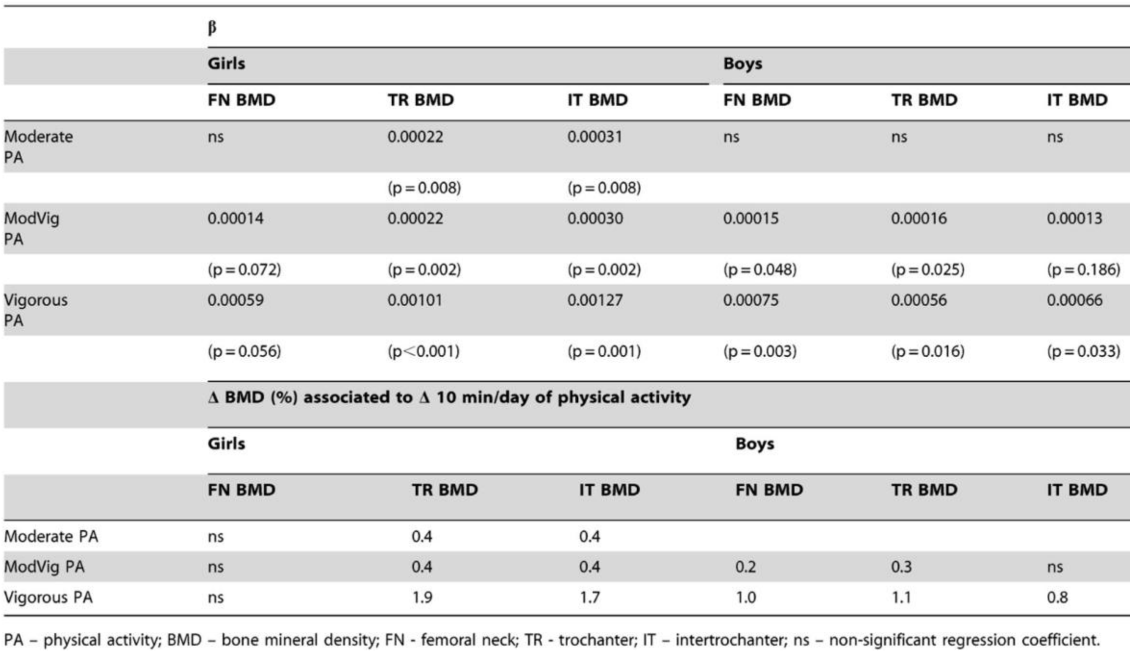


	Predictor Variables	Girls			Predictor Variables	Boys		
		β	p	R^2		β	p	R^2
FN BMD	BLM	0.483	<0.001	0.233	BLM	0.277	<0.001	0.071
	Body Height	−0.075	0.462	–	Body Height	−0.047	0.639	–
	Tanner Stage	0.100	0.194	–	Tanner Stage	0.100	0.179	–
	Vigorous PA	0.135	0.060	–	Vigorous PA	0.225	0.003	0.051
	MVPA	0.125	0.072	–	MVPA	−0.092	0.440	–
	Moderate PA	0.109	0.116	–	Moderate PA	−0.073	0.440	–
TR BMD	BLM	0.511	<0.001	0.306	BLM	0.238	0.002	0.052
	Body Height	−0.139	0.138	–	Body Height	−0.085	0.407	–
	Tanner Stage	0.062	0.236	–	Tanner Stage	−0.007	0.925	–
	Vigorous PA	0.241	<0.001	0.056	Vigorous PA	0.214	0.005	0.046
	MVPA	0.076	0.400	–	MVPA	−0.016	0.893	–
	Moderate PA	0.064	0.400	–	Moderate PA	−0.013	0.893	–
IT BMD	BLM	0.514	<0.001	0.305	BLM	0.324	<0.001	10.1
	Body Height	−0.017	0.861	–	Body Height	0.061	0.543	–
	Tanner Stage	0.134	0.060	–	Tanner Stage	−0.058	0.434	–
	Vigorous PA	0.213	0.001	0.044	Vigorous PA	0.159	0.033	0.025
	MVPA	0.102	0.263	–	MVPA	−0.063	0.499	–
	Moderate PA	0.086	0.263	–	Moderate PA	−0.081	0.499	–
FNTR BMD	BLM	−0.178	0.024	0.043	BLM	0.027	0.731	–
	Body Height	0.079	0.483	–	Body Height	0.061	0.429	–
	Tanner Stage	0.034	0.690	–	Tanner Stage	0.168	0.031	0.028
	Vigorous PA	−0.172	0.029	0.029	Vigorous PA	−0.033	0.673	–
	MVPA	−0.038	0.730	–	MVPA	−0.072	0.357	–
	Moderate PA	−0.032	0.730	–	Moderate PA	−0.078	0.314	–
					–	–	–	–
FNIT BMD	BLM	−0.250	0.001	0.068	BLM	−0.009	0.932	–
	Body Height	−0.119	0.288	–	Body Height	−0.208	0.006	0.043
	Tanner Stage	0.093	0.272	–	Tanner Stage	0.260	0.001	0.064
	Vigorous PA	−0.095	0.385	–	Vigorous PA	0.057	0.445	–
	MVPA	0.184	0.016	0.029	MVPA	0.041	0.595	–
	Moderate PA	0.313	0.385	–	Moderate PA	0.029	0.706	–
TRIT BMD	–	–	–	–	BLM	0.029	0.781	–
					Body Height	0.223	0.004	0.005
					Tanner Stage	0.076	0.325	–
					Vigorous PA	0.068	0.381	–
					MVPA	0.082	0.296	–
					Moderate PA	0.078	0.324	–

PA - physical activity; BMD – bone mineral density; FN BMD - femoral neck BMD; TR BMD - trochanter BMD; IT BMD - intertrochanter BMD; FNTR – BMD ratio of femoral neck for trochanter; FNIT – BMD ratio of femoral neck for intertrochanter; TRIT- BMD ratio of trochanter for intertrochanter; BLM – body lean mass.

Table 4 presents regression models using the three PA intensity variables mostly widely used in the literature, moderate PA, vigorous PA and MVPA. In our analysis, there was a higher absolute effect (estimated by unstandardized regression coefficients) of one minute per day of vigorous PA on the BMD than one minute per day of MVPA or moderate PA (only in girls). The effect of PA was not homogeneous for all proximal femur sub-regions and was dissimilar between boys and girls. For example, in girls the hypothetical BMD increase associated with an additional 10 min/day of PA was comparable (~2%) for trochanter and intertrochanter regions (with no effect on femoral neck). The effect for boys was lower (~1%) but the response was similar among all three sub-regions of proximal femur.

Table 4. Effects of 10 minutes per day of additional physical activity on femoral neck, trochanter, and intertrochanter BMD, adjusted for Tanner stage, body height, and body lean mass.



	β					
	Girls			**Boys**		
	FN BMD	**TR BMD**	**IT BMD**	**FN BMD**	**TR BMD**	**IT BMD**
Moderate PA	ns	0.00022	0.00031	ns	ns	ns
		(p = 0.008)	(p = 0.008)			
ModVig PA	0.00014	0.00022	0.00030	0.00015	0.00016	0.00013
	(p = 0.072)	(p = 0.002)	(p = 0.002)	(p = 0.048)	(p = 0.025)	(p = 0.186)
Vigorous PA	0.00059	0.00101	0.00127	0.00075	0.00056	0.00066
	(p = 0.056)	(p<0.001)	(p = 0.001)	(p = 0.003)	(p = 0.016)	(p = 0.033)

	Δ BMD (%) associated to Δ 10 min/day of physical activity					
	Girls			**Boys**		
	FN BMD	**TR BMD**	**IT BMD**	**FN BMD**	**TR BMD**	**IT BMD**
Moderate PA	ns	0.4	0.4			
ModVig PA	ns	0.4	0.4	0.2	0.3	ns
Vigorous PA	ns	1.9	1.7	1.0	1.1	0.8

PA – physical activity; BMD – bone mineral density; FN - femoral neck; TR - trochanter; IT – intertrochanter; ns – non-significant regression coefficient.

Discussion

PA showed a positive contribution to the BMD variation of the three sub-regions of the proximal femur in boys but in girls PA did not help to explain femoral neck BMD variance. For the same duration of PA, the regression coefficients of more intense PA (vigorous PA) were always higher than those corresponding to a less intense PA (MVPA) in boys and girls. The extrapolation of our results suggest a ~2% higher BMD in the trochanter and intertrochanter regions in girls at the studied age range if an additional 10 minutes per day of vigorous PA is achieved. In boys, the corresponding gain is a ~1% higher femoral neck, trochanter and intertrochanteric BMDs.

The higher regression coefficients for PA of highest intensity – vigorous PA –compared to lower levels of intensity – MVPA or moderate PA – when regional BMDs of femoral neck, trochanter and intertrochanter are in stake underline the relevance of the PA intensity to bone mineral accrual during the studied pediatric years. The PA threshold under which the effects on bone mass could be modest has been proposed [24]–[26]. Given that boys are usually more active than girls [7]–[9], this could partially explain BMD differences between sexes at proximal femur sub-regions. However this difference seems not be homogeneous among sub-regions. Our results are consistent with studies that reported a positive response of girls' proximal femur BMD (or bone mineral content - BMC) to PA but also with studies that revealed a response of femoral neck BMD or BMC to PA only in boys. Particularly, our site specific response of girls' proximal femur in the trochanteric region is in line with the Iowa Bone Development Study which reported 5% and 14% more BMC at the total body and trochanteric region in the most active pre- and early pubertal boys and girls, when compared to inactive peers [29]. Similar effects regarding skeletal regions were also found by Stear et al., who reported greater BMC accrual at the trochanter (4.8%) than in the whole body

(0.8%) or lumbar spine (1.9%) in 144 adolescent girls enrolled in a 45-min exercise-to-music classes programme, three times per week, after 15.5-month [44]. Witzke et al. reached analogous findings at the trochanter BMC in adolescent girls using a plyometric jump training programme with no significant differences for the femoral neck, spine or whole body BMC [45]. McKay et al. who examined the effect of an 8-month school-based jumping programme in pre and early pubescent girls, found that the intervention group showed a significantly greater change in trochanter BMD than the control group [16], [18]. Additionally, increments (4.3%) for femoral neck BMC of 8 to 12 years old boys (compared to controls) were reported after 2 years of a high-impact circuit intervention [19]. These observations contradict the idea that girĺs proximal femur is not responsive to PA, although, notably none of these studies reported a positive effect of PA on girl's femoral neck.

The positive associations that we found between PA and the BMD of the three proximal femur sub-regions in boys and only at the trochanter and at the intertrochanter region in girls is similar with the results of those studies that suggested no effect of PA in girls' proximal femur, whose analyses were focused in the femoral neck region [6], [10], [11]. The exception, seems to be the study conducted by Petit et al. [17] that showed significant gains in the BMD at the intertrochanter (1.7%) and at the femoral neck (2.6%) region in early pubertal girls (Tanner stages 2 and 3) when compared to controls after a 10-minute jumping programme, 3 times per week during 7 months.

The analysis of all sub-regions of proximal femur in both sexes was a distinctive aspect of our study that provided a more comprehensive examination of bone's response to PA. Compared to boys, girls showed inferior BMD in the different sub-regions of the proximal femur, which is not new. However, we observed a lower or a tendency to a lower BMD in the femoral neck relative to other sub-regions (FNIT, girls: 0.86 vs.

boys: 0.88, p = 0.001; FNTR, girls: 1.21 vs. boys: 1.22, p = 0.095), i.e. the proximal femur sub-region where we did not find any positive association with MVPA or vigorous PA in girls. Our study showed that the pattern of proximal femur responsiveness to PA was more homogeneous in boys, when compared to girls. Conversely, in girls, there were negative relationships between PA and FNTR and FNIT, suggesting a heterogeneous responsiveness favoring the trochanteric and intertrochanteric sub-regions of the proximal femur. If our response pattern findings were generalizable, it is not surprising that researchers using the neck region to represent the entire proximal femur suggest that boys' femur is more responsive to mechanical loading than girls' at this age.

In addition to the well-known limitations of DXA technology in the assessment of bone, our study may have an additional limitation due to the self-report of children's maturity status. The sample selection was based on chronological age (910 yrs) and not to assure a representative maturational profile. At these ages, girls usually demonstrate a more advanced biological maturity than boys which did not happen in our study. However we conducted our analyses with and without adjustments for maturational status obtaining similar results (data not shown).

In conclusion, although a large proportion of bone mineralization is attributable to growth during late childhood, MVPA and especially vigorous PA can have an additional osteogenic effect in the proximal femur. The effect is not homogeneous throughout all bone regions in girls. Our work was not designed to detect why the femoral neck appears non responsive to PA in girls. Further research designed to simultaneously compare site-specific bone responses to PA in boys and girls at a wider age range and level of sexual maturity is needed. In addition, sample sizes should be large enough to allow investigators to test interactions among PA and hip biomechanical factors (as opposed to systemic factors as nutritional, hormonal or sun exposure factors) that can affect differently the BMD of specific regions of proximal femur. Our study shows a region-specific bone response to vigorous PA in pre and early pubertal girls and boys. More active girls have greater BMD in the trochanter and intertrochanter while more active boys have greater BMD in all sub-regions of the proximal femur.

Author Contributions

Conceived and designed the experiments: FB LBS GC. Performed the experiments: RO GC FB. Analyzed the data: GC RO LBS FB KFJ. Contributed reagents/materials/analysis tools: RO LBS FB GC. Wrote the paper: GC FB LBS KFJ.

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3.3 Pelvis width associated with bone mass distribution at the proximal femur in 10-11 yr old children[3]

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[3] Cardadeiro G, Baptista F, Janz K, Rodrigues L, Sardinha L B. Pelvis width associated with bone mass distribution at the proximal femur in 10-11 yr old children. J Bone Miner Metab 2013 (in press)

PELVIS WIDTH ASSOCIATED WITH BONE MASS DISTRIBUTION AT THE PROXIMAL FEMUR IN 10-11 YR OLD CHILDREN.

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Abstract

Differences in skeletal geometry may generate different patterns of mechanical loading to bone. Impact and muscle loading during physical activity has been shown to influence skeletal geometry. The purpose of this study was to compare geometric measures of the pelvis and proximal femur (PF) of young children and to analyze the contribution and potential interaction of these geometric measures with physical activity on PF bone mass distribution. Participants were 149 girls and 145 boys, age 10-11 yr. Total body and left hip DXA scans were used to derive pelvic and PF geometric measures and PF bone mineral density (BMD) at the femoral neck (FN), trochanter (TR) and intertrochanter (IT). These sub regions were used to represent bone mass distribution via three BMD ratios – FN:PF, TR:PF, and IT:PF. Physical activity was objectively measured using accelerometry and maturity was estimated as the years of distance from peak height velocity. When compared to boys, girls had a wider pelvic diameter and greater inter-acetabular distances ($p<0.001$), lower BMD at FN, TR, and

IT ($p<0.05$), and higher TR:PF ($p<0.001$). After controlling for maturity, body height, and lean body mass, the inter-acetabular distance in girls explained 21.1% ($\beta=0.713$, $p<0.001$) in TR:PF and 3.1% ($\beta=-0.179$, $p=0.028$) in the IT:PF. Neck-shaft angle explained 5.6% ($\beta=-0.260$, $p=0.002$) of the IT:PF and 3.1% ($\beta=-0.194$, $p=0.018$) of the FN:PF. In boys, FN axis length explained 2.9% ($\beta=0.195$, $p=0.040$) of TR:PF. There was no main effect of physical activity or interaction effect with pelvic geometry in explaining BMD differences among the sub-regions of the PF. Even prior to sexual dimorphism, girls have a wider pelvis than boys which accounted for proportionally greater BMD of the TR than other sub-regions of the PF.

Keywords: Bone; Children; Pelvis; Physical Activity; Proximal Femur.

1. Introduction

Intervention programmes to maximize bone mineral accrual during late childhood and early adolescence are a promising approach to offset the age-related bone loss and the risk of fracture in later life [1, 2]. Peri-pubertal growth is a critical period for skeletal mineralization [3, 4], and better adaptive bone responses are expected during this time with adequate mechanical loading via physical activity [5-7]. The hypothesis of a sex specific bone response to physical activity has been raised suggesting that the skeleton is less sensitive to mechanical loading in girls than in boys [8-10]. Recently a study with 325 boys and girls aged 9-10 years shows a girls' heterogeneous response to vigorous physical activity in the three sub-regions of the proximal femur but an homogeneous response in boys [11].This hypothesis of different skeletal sensitivity to site-specific mechanical loading during late childhood and early adolescence may partly explain why women face a greater risk of fragility fractures in old age [12-16] and stress fractures in early adulthood [17, 18].

In addition, there is a known association between bone geometry of the pelvis and the proximal femur with bone fracture risk [19-21]. Larger neck shaft angles (NSA) and longer hip axis lengths or femoral neck axis lengths (FNAL) have a greater association with cervical fractures than trochanteric fractures [19]. These associations could result from (only) the effect of bone geometry on bone strength or alternatively might be influenced by the interaction between bone geometry and mechanical loading on bone mineralization and ultimately strength.

Due to the biomechanics of the hip, it is plausible that the geometry of the pelvisproximal femur structure might moderate mechanical forces exerted on the proximal femur and, therefore, result in different degrees of mineralization among proximal femur sub-regions. Accordingly, the objectives of this study were to 1.)

compare geometric measures of the pelvis and proximal femur – the inter-acetabular distance, femoral neck axis length, and neck-shaft angle, among others – between girls and boys age 10-11 yrs and 2.) analyze the contribution of these geometric measures to the variance of bone mass distribution at the proximal femur. For this purpose BMD ratios between each proximal femur sub-region (femoral neck, trochanter and intertrochanter) and the integral proximal femur were used as surrogates of proximal femur bone mass distribution. As a third aim, we investigated interactions between these geometric measures and objectively measured habitual physical activity on proximal femur bone mass distribution. The latter aim is important since physical activity is one of the few known modifiable factors to reduce fracture risk.

2. Materials and methods

2.1 Sample

Study participants were drawn from Oeiras schools (fifth grade). The total sample was composed of 294 children (139 boys and 146 girls), after excluding 22 who were over 11 years in age. All participants were healthy Caucasian students not taking any medication known to influence bone metabolism. The Ethics Committee of the Faculty of Human Movement, Technical University of Lisbon approved the study and parents or legal guardians of each child provided written informed consent.

2.2 Proximal femur bone mass distribution

Integral BMD of the left proximal femur and BMD of each proximal femur sub-region were evaluated using dual x-ray absorptiometry (DXA) (QDR Explorer; Hologic, Waltham, MA, USA) and standard measurement routines. Three BMD ratios were calculated as indicators of BMD homogeneity of the proximal femur:

$$FN{:}PF=\frac{Neck\ BMD}{Proximal\ femur\ BMD}\quad TR{:}PF=\frac{Trochanter\ BMD}{Proximal\ femur\ BMD}\quad IT{:}PF=\frac{Intertrochanter\ BMD}{Proximal\ Femur\ BMD}$$

Where FN:PF is the femoral neck to proximal femur BMD ratio, TR:PF is the trochanter to proximal femur BMD ratio, and IT:PF is the intertrochanter to proximal femur BMD ratio.

All measurements were made by the same technician and a spine phantom was scanned daily to maintain quality assurance. The coefficients of variation of the femoral neck, the trochanter and the intertrochanter BMDs, estimated from 2 measurements by repositioning and scanning 28 subjects, were 1.6%, 1.7% and 1.3% respectively.

2.3 Pelvis' and femur geometry analysis

Linear geometric measures of pelvis and proximal femur geometry were determined by DXA for each subject from images of whole body and left hip scans, respectively. All images were saved and appropriately transformed in grayscale (using *Adobe Photoshop Elements*) in order to be used as an input for the geometric morphometric software – the Thin-Plate Spline Digitize (tpsDigQ1) software Version 2.10 [22]. This program and its procedures are described in detail elsewhere [22]. With tpsDigQ1 several 2D landmarks (x, y coordinates) were digitized in every single image, namely eight 2D landmarks in the pelvis image (Fig. 1) and twenty 2D landmarks in the proximal femur image (Fig. 2), with each point referring always to the same shape feature. To comply with tpsDigQ1 requirements, the scale of the scanned images was done at the femur using the Ward's area, alike previous literature [23]. At the pelvis, a metal object (4.4cm x 5.6cm) was scanned and merged with the pelvis images (Fig. 1). Estimation of linear geometric measures in the pelvis included the largest inner diameter of the pelvic bone (LIDP); the lower inter-acetabular distance (LIAD), defined as the distance between the left and the right lower points of the acetabular opening $\left(\overline{\mathrm{AA}'}\text{ in Fig. 1}\right)$; the upper *inter-acetabular distance* (UIAD), defined as the distance between the left and the right upper points of the acetabular opening $\left(\overline{\mathrm{BB}'}\text{ in Fig. 1}\right)$; and the *inter-acetabular distance* (IAD), calculated as the distance between left and right middle points of the previous references $\left(\overline{\mathrm{CC}'}\text{ in Fig. 1}\right)$.

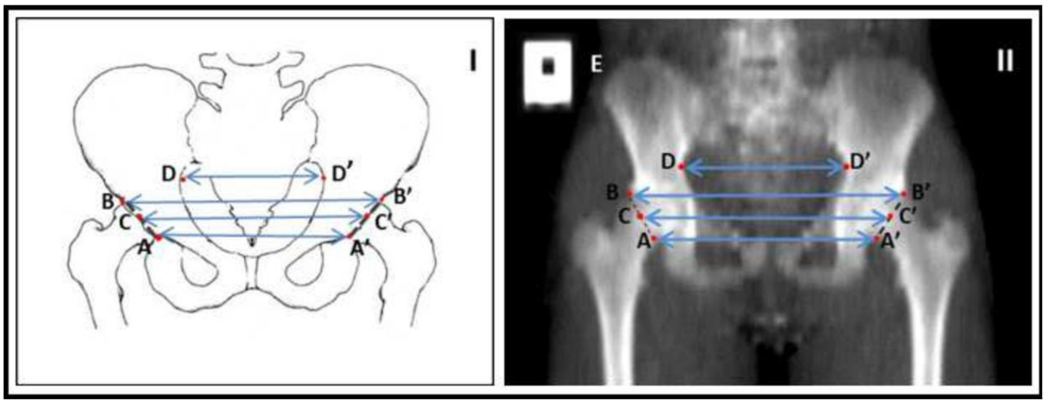


Fig. 1 – Geometric measures of the pelvic bone
(I) Diagram illustrating the pelvic measures; [AA'] – lower inter-acetabular distance (LIAD); [BB'] - upper inter-acetabular distance (UIAD); [CC'] – inter-acetabular distance (IAD); [DD'] - largest inner diameter of the pelvic bone (LIDP). (II) DXA image of one of our subject with metal object (E) for the scale of the scanned images.

Estimation of geometric measures in proximal femur included the FNAL, defined as the linear distance measured from the base of the greater trochanter to the apex of the femoral head [24] ($\overline{AB}$ in Fig. 2), the NSA, defined as the angle between the femoral neck axis ($\overline{AB}$ in Fig. 2), and the femoral shaft axis ($\overline{CD}$ in Fig. 2). The femoral neck width (FNW), defined as the distance across the femoral neck, often constrained to being perpendicular to the neck axis ($\overline{EF}$ in Fig. 2) was also measured.

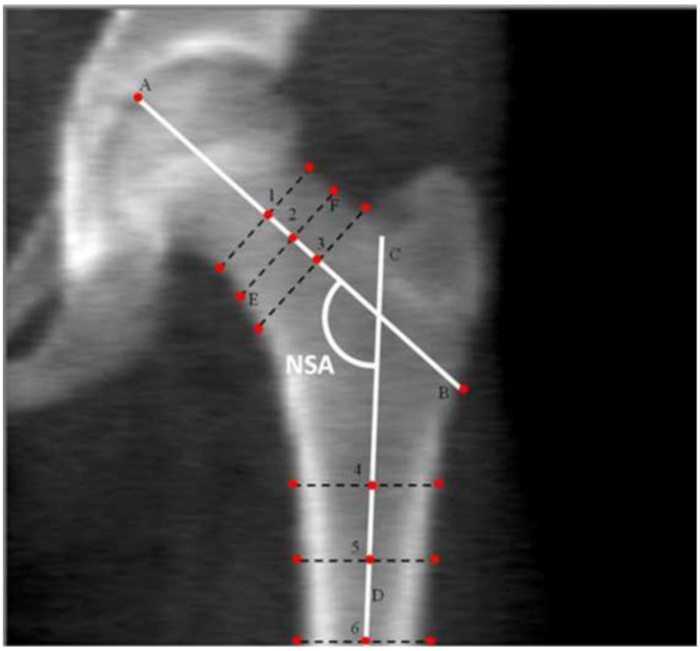


Fig. 2 – Measurements of the Geometric measures of the proximal femur geometry

[A-B] Femoral neck axis length (FNAL); [C-D] femoral shaft axis; [EF] femoral neck width (FNW); neck–shaft angle (NSA); (1,2,3) landmarks representing the middle distance of neck width; (4,5,6) landmarks representing the middle distance of shaft width.

Since the accuracy of DXA scans in measuring bone geometry is quite sensitive to the patient's position during imaging, strictly standard protocols for positioning and analysis of DXA scans were followed. The reproducibility of the measurements was calculated by three blind repeated measurements of the geometric measures from DXA images. The root mean square of coefficient of variations was between 0.4% and 0.5% for LIAD, IAD, UIAD, and NSA, 0.8% for LID, and 2.2% for FNAL.

2.4 Physical Activity

Habitual (non-targeted) physical activity was assessed using the GT1M accelerometer (Actigraph, Fort Walton Beach, Florida, USA) with 15 second epochs. Participants wore the monitor for 4 days (two weekdays and two weekend days) providing at least

600 minutes per day of accelerometer data. Those not complying with these requirements were excluded from the sample. The accelerometer was secured on the right hip and the children were asked to wear the accelerometer all day, except during water activities and sleeping. Activity data were analyzed and processed using the MAHUffe analysis program (www.mrc-epid.cam.ac.uk). The output from the program included accumulated time spent at sedentary-, light-, moderate-, and vigorous-intensity physical activity in minutes per day. The intensity of physical activity was defined according to the counts per minute (cpm) as follows: sedentary activity, up to 100 cpm; light-intensity (LPA) from 101 to 2295 cpm; moderate-intensity (MPA) from 2296 to 4011 cpm; and vigorous-intensity (VPA) over 4012 cpm [25].

Historical sport activity participation relevant to the musculoskeletal system was assessed with the bone-specific physical activity questionnaire (BPAQ), a validated tool that accounts for bone-relevant loading [26] and records current and historical activity. Under the supervision of a researcher, participants were asked to record all regular sport activities practice, including approximate duration, throughout their lives (historical activity) as well as over the previous 12 months, including frequency of participation (current activity). Using the evidence-based osteogenic index described by Turner and Robling [27], an algorithm was applied in order to convert the raw BPAQ data into an individual score that reflects total bone-relevant sport activity history.

2.5 Body size and body composition

Standing and sitting height were measured to the nearest 0.1 cm using a stadiometer (Secca 770, Hamburg, Germany) with children in underwear and barefoot. Body mass (kg), total fat (kg), and total lean mass without bone (kg) were determined from a totalbody scan using DXA (QDR Explorer; Hologic, Waltham, MA, USA) with children in a fasting state. Body mass index (BMI) was calculated as body mass in kilograms divided by height (in meters) squared.

2.6 Maturity and calcium intake

Maturity (+ yr) was estimated as the years of distance positive or negative from the age of peak height velocity using sex-specific prediction equations that include age, height, and sitting height [28]. The calcium intake (mg) was calculated from a semi-quantitative Food Frequency Questionnaire, assessing regular intake of a wide set of a typical Portuguese foods.

2.7 Statistical Analysis

Data were analysed using the SPSS statistical software package (Version 18.0 for Windows; SPSS, Chicago, IL, USA). Distribution properties of all variables were examined and appropriate measures of central tendency and variability were selected. Differences between groups (girls and boys) were analysed by Independent-samples T-tests in case of normality and Mann-Whitney nonparametric tests otherwise. Stepwise linear regressions models were used to analyse the associations between geometric related variables of the pelvis and proximal femur (as independent variables), firstly with each of the sub-regional BMD's and then with the three BMD ratios (as dependent variables of bone mass distribution). All regression models were controlled for maturity, body height, and body lean mass. Initial models pooled boys and girls to test the significance of sex as predictor variable and then separately. In every case, the assumptions for the linear regression analysis were verified (normality and linearity of the residuals, multicollinearity and homocedasticity).

Sex-specific multivariate analysis of covariance (MANCOVA) was used to analyse main and interaction effects of moderate- and vigorous-intensity physical activity and geometric measures on the three BMD ratios of the proximal femur. The physical activity and geometric variables were set as fixed factors and grouped as below (group 1) or above (group 2) the mean (when variable distribution was normal) or below and above the median (in case of no normality). Maturity, body height, and body lean mass were covariates. Normality of distribution and homogeneity of variance assumptions required to conduct MANCOVA were verified using the Kolgomonov-Sminov test and the Levene's test, respectively. Statistical significance was set at $P<0.05$.

3. Results

Sample characteristics including body composition and physical activity are presented in Table 1. Compared to boys, girls were more mature, taller and had greater fat mass. Weight, lean body mass, and light-intensity physical activity were similar between boys and girls. Boys had lower sedentary time and greater levels of sedentary-, moderate-, and vigorous-intensity physical activity, as assessed by accelerometry, when compared to girls. However, there were no statistically significant differences between sexes concerning BPAQ variables.

Table 1 – Participants characteristics: age, maturity, body composition and physical activity as mean ± standard deviation.

	Girls (n=146)	Boys (n=139)	P*
Age, y	10.3±0.5	10.3±0.5	0.315$^{a)}$
Maturity Offset, y	-1.19±0.6	-2.87±0.5	<0.001
Peak High Velocity, y	11.5±0.5	13.2±0.6	<0.001
Height, cm	145.8±7.1	143.5±6.6	0.005
Weight, kg	40.6±9.2	38.4±9.1	0.008$^{a)}$
Body Mass Index, kg/m^2	19.0±3.5	18.6±3.6	0.186$^{a)}$
Body Fat, kg	12.3±5.5	10.3±5.7	<0.001$^{a)}$
Body Lean Mass, kg	28.3±4.7	28.1±4.2	0.808
Body Fat, %	29.3±7.1	25.4±7.7	<0.001$^{a)}$
Calcium Intake, mg/d	1062.4±58.2	1277.5±92.5	0.105^{a}
Sedentary, min/d	1076±69.5	1056±70.2	0.020$^{a)}$
Light PA, min/d	245±38	243±39	0.587
Moderate PA, min/d	27.9±9.6	34.9±13	<0.001
Vigorous PA, min/d	11.2±7.0	15.5±9	<0.001$^{a)}$
Moderate and Vigorous PA, min/d	39.1±14	50.3±21	<0.001$^{a)}$
PA Average Intensity, count/min/d	400±108	452±132	0.001$^{a)}$
Past BPAQ, score	9.86±16.42	8.15±12.87	0.121
Current BPAQ, score	13.58±26.59	10.74±15.02	0.183
Total BPAQ, score	23.45±36.16	18.88±20.59	0.096

PA - physical activity; BPAQ – bone specific physical activity questionnaire; * Student's t-test comparing boys to girls. $^{a)}$ In cases of no normality, Mann-Whitney nonparametric tests were used.

Bone variables are presented in Table 2. BMD was greater in boys at the integral proximal femur as well as at all three sub-regions. There were no differences between boys and girls for bone mass distribution (as defined using ratios) at the femoral neck and intertrochanter. However, girls' TR:PF was greater ($p<0.001$) than boys'. All measured geometric variables of the pelvis were wider in girls when compared with boys. The narrowest neck width (NW) of the proximal femur was wider in boys than in girls ($p<0.001$). The proximal femur NSA and the FNAL means did not differ between girls and boys ($p>0.05$).

Table 2 – Participants characteristics: BMD and bone geometric measures as mean ± standard deviation.

	Girls (n=146)	Boys (n=139)	P*
Proximal Femur BMD, g/cm^2	0.731 ± 0.08	0.773 ± 0.08	<0.001
Neck BMD, g/cm^2	0.694 ± 0.08	0.740 ± 0.08	<0.001
Trochanter BMD, g/cm^2	0.592 ± 0.08	0.611 ± 0.07	0.028 a)
Intertrochanter BMD, g/cm^2	0.812 ± 0.09	0.856 ± 0.10	<0.001 a)
Neck / Proximal Femur BMD	0.950 ± 0.05	0.957 ± 0.05	0.198
Trochanter / Proximal Femur BMD	0.809 ± 0.05	0.790 ± 0.04	<0.001
Intertrochanter / Proximal Femur BMD	1.110 ± 0.03	1.107 ± 0.03	0.532 a)
Neck Shaft Angle, °	134.9 ± 5.2	134.7 ± 5.7	0.642
Femoral Neck Axis Length, cm	7.18 ± 0.54	7.22 ± 0.5	0.591
Femoral Neck Width, cm	2.56 ± 0.20	2.62 ± 0.20	0.005
Inter-Acetabulum Distance, cm	12.7 ± 0.9	12.3 ± 0.7	<0.001
Upper Inter-Acetabulum Distance, cm	13.9 ± 1.0	13.6 ± 0.8	0.007
Lower Inter-Acetabulum Distance, cm	11.5 ± 0.9	11.0 ± 0.7	<0.001
Largest Inner Distance of Pelvic Bone, cm	8.4 ± 0.9	8.0 ± 0.6	<0.001

BMD – Bone mineral density; *Student's t-test comparing boys to girls. a) In cases of no normality, Mann-Whitney non parametric tests were used.

Positive associations of both lean body mass and physical activity with BMD at all proximal femur sub-regions were observed in linear regressions analyses (Table 3). However, the physical activity variable that entered each model (with the best explanatory contribution) was not always the same: moderate- and vigorous-intensity physical activity entered in all the three boys' models whereas in girls vigorous-intensity physical activity entered in the femoral neck BMD model, vigorous-intensity physical activity entered in the trochanter BMD model, and moderate-intensity physical activity in the intertrochanter BMD model.

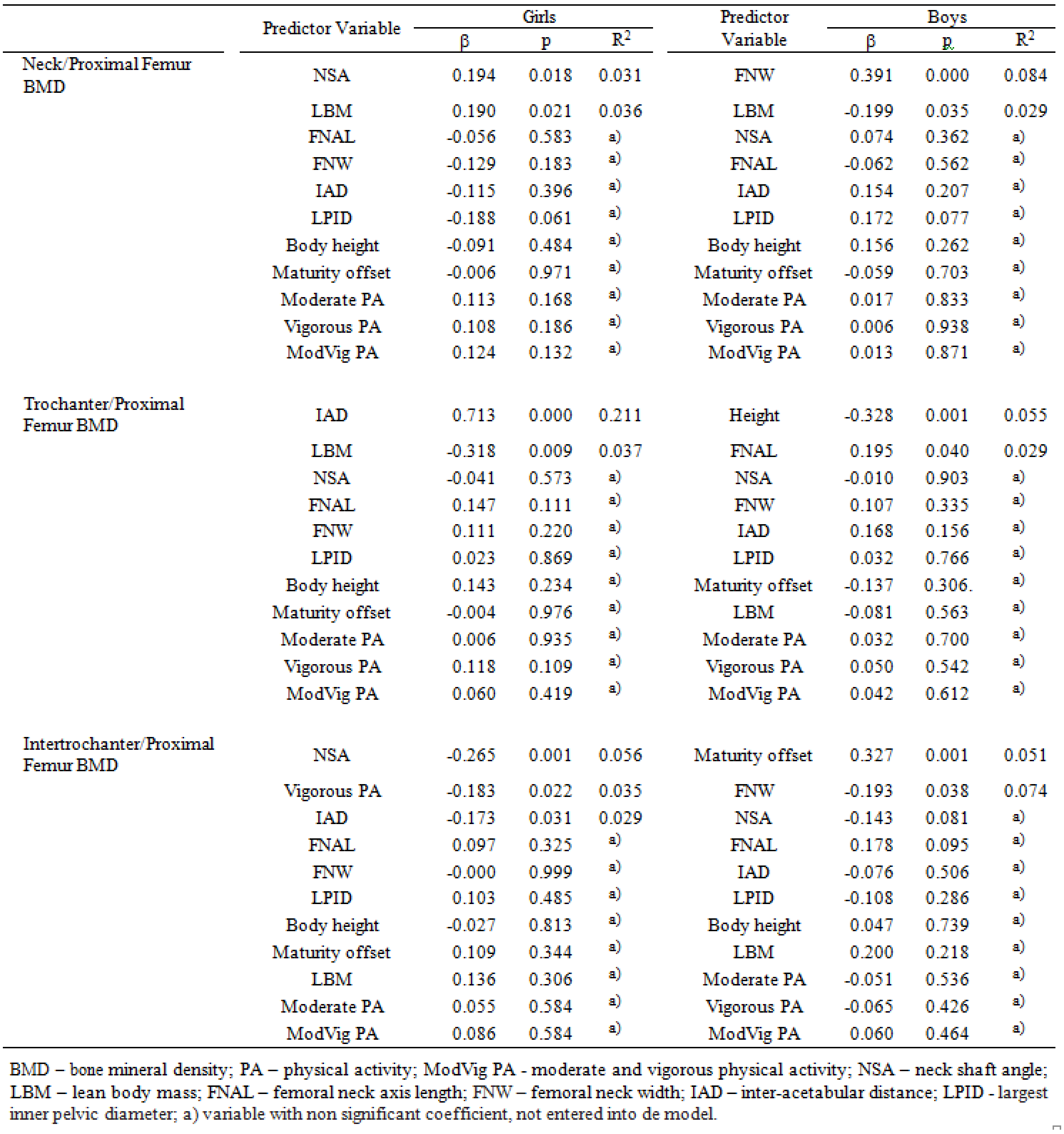
Table 3 – Regression coefficients (β), level of significance (p) and coefficient of determination (R^2) of the associations between physical activity, geometric measures of pelvis and proximal femur, and proximal femur BMD, adjusted for maturity, body height, and lean body mass.

	Predictor Variable	Girls			Predictor Variable	Boys		
		β	p	R^2		β	p	R^2
Neck BMD	LBM	0.657	0.000	0.467	LBM	0.405	0.000	0.173
	ModVig PA	0.211	0.001	0.044	ModVig PA	0.393	0.000	0.155
	FNAL	-0.118	0.104	a)	FNAL	-0.069	0.404	a)
	FNW	-0.136	0.056	a)	FNW	-0.004	0.962	a)
	IAD	-0.128	0.200	a)	IAD	-0.071	0.511	a)
	Moderate PA	0.050	0.753	a)	Moderate PA	-0.116	0.668	a)
	Vigorous PA	-0.037	0.753	a)	Vigorous PA	0.085	0.430	a)
	Total PA	-0.045	0.752	a)	Total PA	0.175	0.483	a)
	Past BPAQ	0.040	0.505	a)	Past BPAQ	0.038	0.615	a)
	Current BPAQ	0.003	0.959	a)	Current BPAQ	0.041	0.578	a)
	Total BPAQ	0.021	0.730	a)	Total BPAQ	0.054	0.473	a)
	Body height	-0.133	0.160	a)	Body height	-0.166	0.181	a)
	Maturity offset	0.029	0.801	a)	Maturity offset	-0.180	0.213	a)
Trochanter BMD	LBM	0.434	0.000	0.427	LBM	0.552	0.000	0.156
	Vigorous PA	0.161	0.011	0.021	ModVig PA	0.387	0.000	0.087
	IAD	0.250	0.018	0.022	Body height	-0.316	0.016	0.033
	FNAL	-0.011	0.890	a)	FNAL	0.003	0.972	a)
	FNW	-0.018	0.813	a)	FNW	-0.047	0.605	a)
	Moderate PA	0.056	0.479	a)	IAD	0.006	0.962	a)
	ModVig PA	0.088	0.479	a)	Moderate PA	-0.257	0.369	a)
	Total PA	0.085	0.460	a)	Vigorous PA	0.187	0.369	a)
	Past BPAQ	0.026	0.682	a)	Total PA	0.135	0.604	a)
	Current BPAQ	-0.001	0.982	a)	Past BPAQ	0.086	0.269	a)
	Total BPAQ	0.011	0.863	a)	Current BPAQ	0.096	0.210	a)
	Body height	-0.003	0.974	a)	Total BPAQ	0.128	0.106	a)
	Maturity offset	0.087	0.474	a)	Maturity offset	-0.316	0.724	a)
Intertrochanter BMD	LBM	0.591	0.000	0.372	LBM	0.569	0.000	0.227
	Moderate PA	0.142	0.035	0.020	ModVig PA	0.374	0.000	0.136
	FNAL	-0.045	0.584	a)	FNW	-0.202	0.012	0.030
	FNW	-0.095	0.232	a)	FNAL	0.015	0.865	a)
	IAD	-0.143	0.199	a)	IAD	-0.160	0.123	a)
	Vigorous PA	0.010	0.909	a)	Moderate PA	-0.129	0.616	a)
	ModVig PA	0.020	0.909	a)	Vigorous PA	0.094	0.616	a)
	Total PA	-0.060	0.585	a)	Total PA	-0.003	0.989	a)
	Past BPAQ	-0.047	0.481	a)	Past BPAQ	0.117	0.109	a)
	Current BPAQ	-0.031	0.645	a)	Current BPAQ	0.036	0.603	a)
	Total BPAQ	-0.045	0.508	a)	Total BPAQ	0.097	0.176	a)
	Body height	-0.071	0.509	a)	Body height	-0.214	0.079	a)
	Maturity offset	0.081	0.529	a)	Maturity offset	-0.099	0.471	a)

BMD – bone mineral density; LBM – lean body mass; FNAL – femoral neck axis length; FNW – femoral neck width; IAD – inter-acetabular distance; PA - physical activity; ModVig PA - moderate and vigorous physical activity; BPAQ - bone-specific physical activity questionnaire; a) variable with non significant coefficient, not entered into the model.

The results of linear regression models for the three BMD ratios – FN:PF, TR:PF and IT:PF – are presented in Table 4. In girls, the NSA was positively associated with the FN:PF (β=0.194, *p*<0.05). The NSA explained 3.1% of FN:PF suggesting that a larger NSA contributes to a greater relative mineralization of the femoral neck region.

Table 4 – Regression coefficients (β), level of significance (p) and coefficient of determination (R^2) of the associations between physical activity, geometric measures of pelvis and proximal femur, and proximal femur BMD ratios, adjusted for maturity, body height, and lean body mass.



	Predictor Variable	Girls			Predictor Variable	Boys		
		β	p	R^2		β	p	R^2
Neck/Proximal Femur BMD	NSA	0.194	0.018	0.031	FNW	0.391	0.000	0.084
	LBM	0.190	0.021	0.036	LBM	-0.199	0.035	0.029
	FNAL	-0.056	0.583	a)	NSA	0.074	0.362	a)
	FNW	-0.129	0.183	a)	FNAL	-0.062	0.562	a)
	IAD	-0.115	0.396	a)	IAD	0.154	0.207	a)
	LPID	-0.188	0.061	a)	LPID	0.172	0.077	a)
	Body height	-0.091	0.484	a)	Body height	0.156	0.262	a)
	Maturity offset	-0.006	0.971	a)	Maturity offset	-0.059	0.703	a)
	Moderate PA	0.113	0.168	a)	Moderate PA	0.017	0.833	a)
	Vigorous PA	0.108	0.186	a)	Vigorous PA	0.006	0.938	a)
	ModVig PA	0.124	0.132	a)	ModVig PA	0.013	0.871	a)
Trochanter/Proximal Femur BMD	IAD	0.713	0.000	0.211	Height	-0.328	0.001	0.055
	LBM	-0.318	0.009	0.037	FNAL	0.195	0.040	0.029
	NSA	-0.041	0.573	a)	NSA	-0.010	0.903	a)
	FNAL	0.147	0.111	a)	FNW	0.107	0.335	a)
	FNW	0.111	0.220	a)	IAD	0.168	0.156	a)
	LPID	0.023	0.869	a)	LPID	0.032	0.766	a)
	Body height	0.143	0.234	a)	Maturity offset	-0.137	0.306.	a)
	Maturity offset	-0.004	0.976	a)	LBM	-0.081	0.563	a)
	Moderate PA	0.006	0.935	a)	Moderate PA	0.032	0.700	a)
	Vigorous PA	0.118	0.109	a)	Vigorous PA	0.050	0.542	a)
	ModVig PA	0.060	0.419	a)	ModVig PA	0.042	0.612	a)
Intertrochanter/Proximal Femur BMD	NSA	-0.265	0.001	0.056	Maturity offset	0.327	0.001	0.051
	Vigorous PA	-0.183	0.022	0.035	FNW	-0.193	0.038	0.074
	IAD	-0.173	0.031	0.029	NSA	-0.143	0.081	a)
	FNAL	0.097	0.325	a)	FNAL	0.178	0.095	a)
	FNW	-0.000	0.999	a)	IAD	-0.076	0.506	a)
	LPID	0.103	0.485	a)	LPID	-0.108	0.286	a)
	Body height	-0.027	0.813	a)	Body height	0.047	0.739	a)
	Maturity offset	0.109	0.344	a)	LBM	0.200	0.218	a)
	LBM	0.136	0.306	a)	Moderate PA	-0.051	0.536	a)
	Moderate PA	0.055	0.584	a)	Vigorous PA	-0.065	0.426	a)
	ModVig PA	0.086	0.584	a)	ModVig PA	0.060	0.464	a)

BMD – bone mineral density; PA – physical activity; ModVig PA - moderate and vigorous physical activity; NSA – neck shaft angle; LBM – lean body mass; FNAL – femoral neck axis length; FNW – femoral neck width; IAD – inter-acetabular distance; LPID - largest inner pelvic diameter; a) variable with non significant coefficient, not entered into de model.

Inter-acetabular distance was positively associated with TR:PF, (β=0.713, p<0.001) explaining 21.1% of the variance. The NSA (β=-0.260, p=0.002) and the IAD (β=-0.179, p=0.028) were inversely associated with the IT:PF. In boys, the FNAL was positively associated with the TR:PF (β=0.195, p=0.040) explaining 2.9% of the variance. With the exception of the IT:PF model in girls, no physical activity variable was associated to the relative mineralization of the sub-regions of the proximal femur.

As a follow-up, MANCOVA analysis of BMD ratios was conducted for girls and is presented in Table 5. In these models, the inter-acetabular distance and moderate- and vigorous-intensity physical activity were set as fixed factors and body height, maturity and body lean mass used as covariates. Moderate- and vigorous-intensity physical activity was not a main effect nor did it interact with inter-acetabular distance for any BMD ratios. (The lack of main effect and lack of interaction was true for all other physical activity intensities substituted in these models, data not shown).

Consistently with the regression analysis results, a wider pelvis in girls was associated with a higher mean TR:PF (0.826 vs. 0.791, p<0.01) and lower mean IT:PF (1.104 vs. 1.117, p<0.05).

Table 5 – Main and interaction effects of inter-acetabular distance and physical activity on proximal femur BMD ratios in girls, adjusted for maturity, body height, and body lean mass.

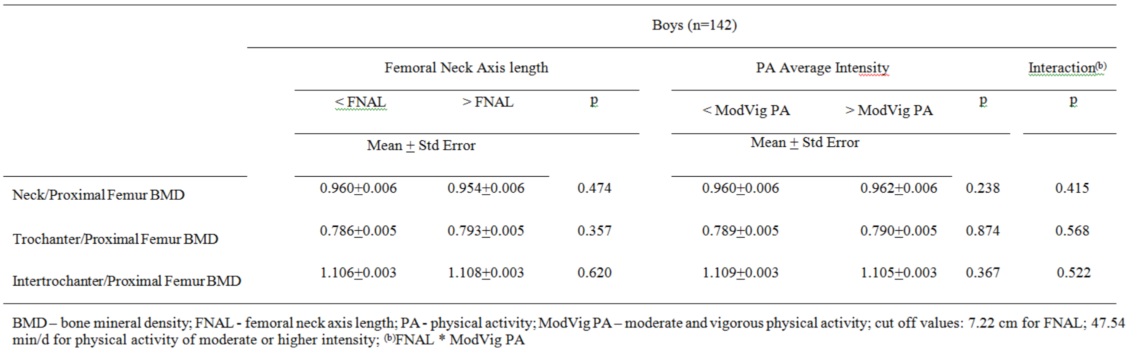
	Girls (n= 149)						
	Inter-acetabular distance			Physical Activity (Moderate+Vigorous)			Interaction$^{(a)}$
	< IAD	> IAD	P	< ModVig PA	> ModVig PA	P	P
	Mean ± Std Error			Mean ± Std Error			
Neck/Proximal Femur BMD	0.952±0.007	0.948±0.007	0.726	0.946±0.005	0.954±0.006	0.281	0.292
Trochanter/Proximal Femur BMD	0.791±0.006	0.826±0.006	0.001	0.806±0.005	0.811±0.005	0.470	0.829
Intertrochanter/Proximal Femur BMD	1.117±0.004	1.104±0.004	0.039	1.114±0.003	1.107±0.003	0.162	0.466

BMD – bone mineral density; IAD - Inter-acetabular distance; PA – physical activity; ModVig PA – moderate and vigorous physical activity; Cut off values: 12.70 cm for IAD; 37.28 min/d for physical activity of moderate or higher intensity; $^{(a)}$IAD * ModVig PA

The analysis conducted for boys was similar, but IAD was replaced by the FNAL as fixed factor, given that the IAD didn’t turn out to be significant in any of the previous boys’ regressions (table 6).

Based on the linear regression models, we used FNAL as the geometric fixed factor in the MANCOVA analysis for boys. None of the fixed factors – the geometry related variable, here the FNAL, or the moderate- and vigorous-intensity physical activity – was a main effect nor did they interact with each other, for any BMD ratios.

Table 6 – Main and interaction effects of inter-acetabular distance and physical activity on proximal femur BMD ratios in girls, adjusted for maturity, body height, and body lean mass.



	Boys (n=142)						
	Femoral Neck Axis length			PA Average Intensity			Interaction$^{(b)}$
	< FNAL	> FNAL	p	< ModVig PA	> ModVig PA	p	p
	Mean ± Std Error			Mean ± Std Error			
Neck/Proximal Femur BMD	0.960±0.006	0.954±0.006	0.474	0.960±0.006	0.962±0.006	0.238	0.415
Trochanter/Proximal Femur BMD	0.786±0.005	0.793±0.005	0.357	0.789±0.005	0.790±0.005	0.874	0.568
Intertrochanter/Proximal Femur BMD	1.106±0.003	1.108±0.003	0.620	1.109±0.003	1.105±0.003	0.367	0.522

BMD – bone mineral density; FNAL - femoral neck axis length; PA - physical activity; ModVig PA – moderate and vigorous physical activity; cut off values: 7.22 cm for FNAL; 47.54 min/d for physical activity of moderate or higher intensity; $^{(b)}$FNAL * ModVig PA

4. Discussion

Available data have shown that women and men tend toward differing pelvic types [29-33] and sex-related differences are evident early in life even during the gestation period [34, 35]. However, there is a lack of research addressing geometric differences of the pelvis in children. The objectives of this research were to 1.) compare linear geometric measures of the pelvis and proximal femur between girls and boys aged 10-11 yrs, 2.) analyze the contribution of these geometric measures to the variance of the bone mass distribution at the proximal femur, and 3.) investigate interaction effects between geometric measures and physical activity on bone mass distribution at the proximal femur. Three BMD ratios – FN:PF, IT:PF, and TR:PF – were used as surrogates of the bone mass distribution at the proximal femur. This is a novel approach which controlled for inter-subject variability in timing and tempo of mineralization by creating measures of intra-individual relative difference of mineralization at the proximal femur. The main findings indicated that girls have a wider pelvis than boys which helps to explain their proportionally greater trochanter BMD when compared to other sub-regions of the proximal femur. Our findings also indicated that distinctive features of the pelvis are associated with proximal femur bone mass distribution.

In the present study, a wider pelvis in girls was associated with proportionally greater BMD in the trochanter (compared with neck and intertrochanter sub-regions) and lower BMD in the intertrochanter (compared with the neck and trochanter proximal femur sub-regions). However, this latter contribution was small (R2=3.1%) compared to the contribution of a wide pelvis in explaining the variance of the TR:PF (R2=21.1%). Similar relationships were not found in boys, perhaps as consequence of a much lower inter-acetabular distance variation coefficient (0.056) in boys when compared to girls (0.074). But when boys and girls were analyzed together, (data not showed), the relationship between inter-acetabular distance and the TR:PF was also significant (β=0.494, R2=8.8%, $p<0.001$).

The importance of the width of pelvis in girls for a proportionally greater BMD of the trochanter compared to other sub-regions of the proximal femur might be explained by the biomechanics of the hip [36, 37]. Mechanical loads exerted on the proximal femur are a consequence of a complex system of vector forces, axis, and levers which depend on the geometry of both the pelvis and the proximal femur [38]. A wider pelvis increases the torque exerted by the body mass on the pelvis-proximal femur joint and, as long as it promotes a valgus thigh type of posture, requires a stronger action of the abduction muscles on the proximal femur to counterbalance the body mass forces [36, 37]. As the attachment of these muscles into the proximal femur is done at the great trochanter, the corresponding (re)modelling effects would be observable at this region [39, 40]. During childhood, the largest physiologic bone loads are from muscles forces and not from gravity forces. This is due to the disadvantageous positioning of muscles insertions on bony lever [41]. Therefore, our result associating a wider pelvis (expressed by inter-acetabular distances) to a relatively more mineralized trochanter is consistent with running and jumping intervention studies that report the greatest intervention effect on the trochanter [42, 43]. Of key importance is the fact that a wider pelvis and running/jumping interventions engage the same muscles [44]. The biomechanics of the hip should apply to both girls and boys. However we did not see this effect in our sample of boys. Shwarzkopf et al., nevertheless, observed that the wideness of the pelvis was an important factor in determining how forces are exerted on the different regions of the proximal femur [45]. Their work simulated forces and stresses transmitted through the proximal femur via a finite element analysis model developed from the basic model of the pelvis-hip biomechanics: $\alpha.B \times a = ALA \times A$, where $\alpha.B$ is the proportion (α) of the body weight (B) supported by the standing leg while the opposite leg swings during single leg stance, ALA is the abductor lever arm and A is the abductor force needed to balance the pelvis. They simulated the stresses for a wide and a narrow pelvis, concluding that the maximum principal stress on the femoral neck is 1.7 times lower in the narrower pelvis model.

In our study, the NSA in girls and the FNAL in boys were explanatory variables for the bone mass distribution of the proximal femur, and, as far as these variables integrate the geometric characteristics of the proximal femur, corroborate the hypothesis that the geometry of the proximal femur may influence the relative mineralization of the three sub-regions of the proximal femur. However, unlike the inter-acetabular distance, these two variables were not geometric features that differed between boys and girls, then could not justify a possible sex-specific response to physical activity. We are aware that the pelvis-hip biomechanics suggest other more (theoretically) relevant geometric measures variables of the proximal femur geometry, such as the abductor lever arm, but this data was not available for the present study.

We expected a main effect and perhaps an interaction effect between pelvis geometry and physical activity in the BMD of a specific bone region, particularly in the region of maximum stress [45]. This was not clearly observed suggesting that physical activity has equivalent effects on the three sub-regions in boys and girls.

The use of DXA in our study provided low exposure to radiation and a clinically relevant site (proximal femur). However, DXA use brought along a set of well known limitations not only associated to its 2D nature but also our quantitative measurement technique. An image technique such as plain radiography would have provided greater resolution and improved the location of land marks. In addition, we used the whole body scan images for the pelvis, making this task even more challenging. To overcome these limitations, Photoshop options to enhance images’ contrast and quality were essential. The young age of our participants was another limitation since until the end of puberty there are only small sex differences in body shape and composition. Finally, cross-sectional studies are vulnerable to confounding by unrecognized determinants and cohort effects are also present. A longitudinal study including additional biomechanical related variables would mitigate some of these drawbacks.

In conclusion, children ages 10-11 years have sex-specific pelvic geometry differences, with girls having a wider pelvis than boys. In girls, this geometric aspect accounted for a proportionally greater BMD of the trochanter than the other sub-regions of the proximal femur. Physical activity showed a positive association with the absolute BMD for all the three regions of the proximal femur in both sexes, but did not have any effect nor did it interact with pelvic geometry to explain relative BMD of a particular sub-region of the proximal femur. The findings support the hypothesis that differences between boys and girls in bone mass distribution among neck, trochanter and intertrochanter proximal femur sub-regions may be better explained by pelvis width than by differences in gender-specific physical activity responsiveness.

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3.4 Influence of physical activity and skeleton geometry on bone mass at the proximal femur in 10-12 year old children – a longitudinal study[4]

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INFLUENCE OF PHYSICAL ACTIVITY AND SKELETON GEOMETRY ON BONE MASS AT THE PROXIMAL FEMUR IN 10-12 YEAR OLD CHILDREN – A LONGITUDINAL STUDY

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Abstract

Introduction Using a longitudinal observational study with two evaluations and a one year follow-up interval, we investigated the influence of physical activity (PA) and skeletal geometry in bone mineral density (BMD) and bone mass distribution at the proximal femur (PF) in 96 girls and 81 boys (10-12 yr). It is plausible that the geometry of the pelvis-PF structure moderates mechanical forces exerted at the hip and therefore creates different degrees of mineralization among PF sub-regions.

Methods Whole body and left hip DXA scans were used to derive geometric measures of the pelvis (inter acetabular distance-IAD) and PF (abductor lever arm-ALA). BMD was measured at the integral, superolateral (SL), and inferomedial (IM) femoral neck (FN), and at the trochanter (TR). These sub-regions were used to represent bone mass distribution via three BMD ratios-FN:PF, IM:SL, and TR:PF. PA was measured using accelerometry and a bone-specific PA questionnaire (BPAQ).

Results A longitudinal panel data approach revealed BPAQ as a positive predictor for all BMD variables ($p<0.05$) except TR BMD in girls and FN BMD in boys. In girls, the IAD was a positive predictor of TR:PF ($p<0.001$) and ALA was a negative predictor of FN:PF. In boys, the IAD was a positive predictor of FN:PF ($p<0.01$) and IM:SL ($p<0.05$) and ALA was a negative predictor of IM:SL ($p<0.001$).

Conclusion Geometric measures of IAD and ALA seem to play a role in the relative mineralization of the PF sub-regions. On the other hand, absolute BMD levels appear to be determined by mechanical loading.

Keywords: Pelvis; Proximal Femur; BMD; Bone Geometry; Physical Activity; Children; Sex.

1. Introduction

Osteoporosis is an underlying etiological factor in most hip fractures in elderly people with sex distinction in hip fracture risk attributed largely to a lower peak adult bone mass in females and women's accelerated bone loss following the menopause [1]. However sex-specificities in bone morphology and mechanical competence may also contribute to rate differences in two main types of hip fracture. Until age 70 yr, femoral neck (cervical) fractures are more common than trochanteric fractures in women, when compared to men, while men are at greater risk for trochanteric fractures [2, 3]. After age 70 yr, both women and men are more likely to incur trochanteric fractures (rather than femoral neck fractures) [2, 3]. Some research suggests that these two types of fractures reveal dissimilar etiologies, i.e., trochanteric fractures are associated with bone fragility or reduced bone mineral density (BMD) [4, 5], and femoral neck fractures are determined by proximal femur geometry [6, 7]. BMD and proximal femur geometry might, therefore, play distinctive roles as risk factors for hip fractures.

Geometric measures of the proximal femur, particularly the proximal femur axis length [6-8] and the neck shaft angle [9], and geometric measures of the pelvis structure have been associated with hip fracture risk in adults [9-11]. These observations suggest the anatomy of the proximal femur and the pelvis are potential determinants of the type of hip fracture. For example, geometry influences the combination of bending and axial compression at the proximal femur in the event of a (side) fall in elderly people [12]. Geometry may also influence the distribution of bone mass throughout the life course and especially during growth.

The geometry of the pelvis–hip and mechanical forces caused by abductor muscles during physical activity (PA) create stresses at different regions of the proximal femur [13, 14]. The abductor lever arm (ALA), the hip offset, and the length of the femoral neck are determinants of the abductors' contraction forces that stabilize the pelvis during single leg stance. This stance is essential for locomotion as it allows the other leg to swing while the body weight is balanced (on the contra lateral leg) [15]. The body weight lever arm is linearly related to the pelvis width [15]; therefore it is plausible that the inter-acetabular distance (IAD) also plays a role in the forces exerted on the proximal femur during locomotion. Concerning the geometry of the pelvis-hip system, dissimilarities between sexes are well documented, in particular the wider female pelvis and the female pro-valgus hip type [16-18], compared to male individuals. This sex-specific morphology of the pelvis and thigh, observable as early as the gestation period [19], along with the sex discrepancies in muscle activity during locomotion may contribute to different motion patterns [20]. As clear sex differences in hip kinematics and muscle activity during walking and running have been observed [21, 22], and as PA (including locomotion) is one of the determinants of the loads exerted on the proximal femur, it is reasonable to formulate the hypothesis that the geometry of the pelvis and the hip may be associated to sex-specific mineralization patterns of the proximal femur.

It has been widely accepted that entering adulthood with greater bone mass may reduce fractures experienced in old age [23]. Nearly 40% of total young adult bone mass is achieved during the four years around peak high velocity (approximately11-12 yr for girls and 13-14 yr for boys) [24]. This peri-pubertal period is considered crucial for skeletal mineralization and a time when bone adapts in a particularly efficient way to loading [25]. This is also an opportune period during which physical activities that generate impact to the skeleton are naturally integrated into day-to-day life. Recent studies have suggested that sex moderates the association between mechanical loading from PA and bone accrual at some weight-bearing bones [26-28]. The studies report that boys' proximal femur appears to be more sensitive to mechanical loading than girls' [27, 28]. The hypothesis of a higher responsiveness of the proximal femur to PA in boys (when compared to girls) has mainly been based on studies focused only on the femoral neck and not on other sub-regions of the proximal femur (i.e., trochanter and intertrochanter). In addition, these studies have not been designed to specifically analyse sex differences.

Using a longitudinal design with boys and girls aged 10 to 12 yr, the aims of our study were to: a) analyse the effects of PA and pelvis - proximal femur geometry on bone mass distribution at the proximal femur; and b) investigate whether sex distinctive geometric variables influence sex-specific bone mass distribution patterns. We hypothesized that higher responsiveness might be an artefact of sex-related biomechanical differences that influence loading at different regions of the proximal femur.

2 Materials and methods

2.1 Sample

Participants were 10 to 12 yr children recruited from schools in Oeiras municipality, in the greater Lisbon area, Portugal. DXA scans, PA, and maturity measures were obtained twice at baseline and one-year follow-up. All participants were healthy Caucasian students not taking any medication known to influence bone metabolism. The Ethics Committee of the Faculty of Human Kinetics, Technical University of Lisbon approved the study and parents or legal guardians of each child provided written informed consent.

2.2 Proximal femur bone mass distribution

Using standard measurement routines, integral BMD of the left proximal femur and BMD of each proximal femur sub-region were evaluated using dual x-ray absorptiometry (DXA) (QDR Explorer, Hologic, Waltham, MA, USA). Three BMD ratios were calculated as indicators of bone mass distribution of the proximal femur [29, 30]:

$$FN\!:\!PF = \frac{Femoral\ neck\ BMD}{Proximal\ femur\ BMD}\ ;\ TR\!:\!PF = \frac{Trochanter\ BMD}{Proximal\ femur\ BMD}\ ;\ IM\!:\!SL = \frac{Inferomedial\ femoral\ neck\ BMD}{Superolateral\ femoral\ neck\ BMD}$$

where FN:PF is the femoral neck to proximal femur BMD ratio, TR:PF is the trochanter to proximal femur BMD ratio, and IM:SL is the inferomedial to superolateral femoral neck BMD ratio.

All measurements were made by the same technician and a spine phantom was scanned daily to maintain quality assurance. The coefficients of variation of the femoral neck and the trochanter BMDs, estimated from 2 measurements by repositioning and scanning 28 subjects, were 1.6% and 1.7%, respectively, in the baseline evaluation. In the follow-up evaluation, they were 1.5% and 1.6%, respectively. After verification and saving of the results of the standard regions of interest, a manual analysis of each hip scan was performed. We reused every image to reanalyze the femoral neck region. In order to get the superolateral femoral neck BMD, we dragged the inferomedial neck box line towards the proximal femur axis length (which is defined automatically by the DXA analysis software and represent the midline between the two sides of the femoral neck) (Fig.1A). A symmetric procedure, using the DXA-defined superolateral box line, was followed to determine the inferomedial femoral neck BMD (Fig.1B).

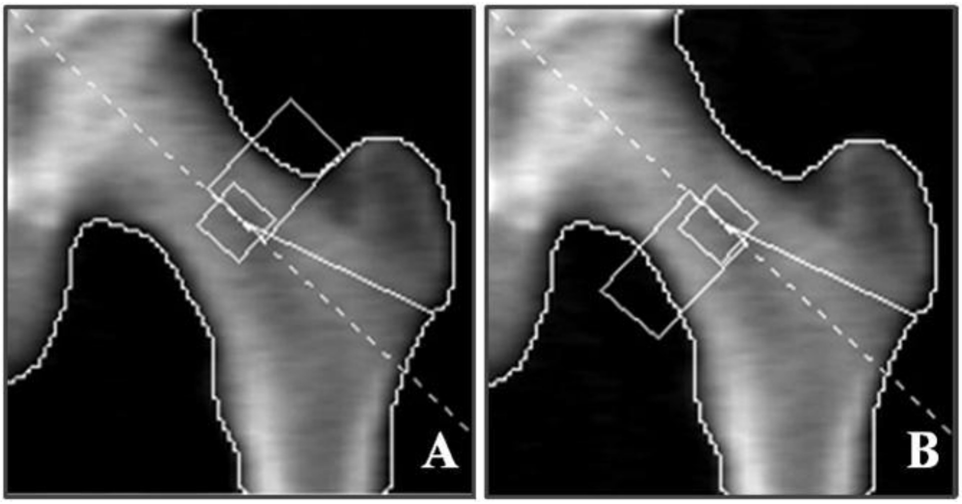

Fig. 1 – Hip image from Hologic DXA scanner showing the region of interest of the superolateral femoral neck (A) and the inferomedial femoral neck (B).

2.3 Inter-acetabular distance and abductor lever arm

Images of whole body and left hip were obtained for all children using DXA to determine the inter-acetabular distance and abductor lever arm, respectively. Since the accuracy of DXA images to determine geometric measures of the skeleton is quite sensitive to the patient’s position during scanning, strict protocols for positioning and analysis of DXA scans were followed. From images, linear measures of the inter-acetabular distance and the abductor lever arm were made for each subject using the CorelDRAW X6 software (Coral Corporation, Ottawa, Ontario, Canada). All measurements were performed by the same technician. Linear geometric measures of the pelvis included: the lower *inter-acetabular distance* (LIAD), defined as the distance between the left and the right lower points of the acetabular opening ($\overline{\mathrm{CD}}$ in Fig. 2); the upper *inter-acetabular distance* (UIAD), defined as the distance between the left and the right upper points of the acetabular opening ($\overline{\mathrm{AB}}$ in Fig. 2); and the *inter-acetabular* *distance* (IAD), calculated as the distance between left and right middle points of the previous references $(\overline{\mathrm{EF}}\ \text{in Fig.}\ 2)$.

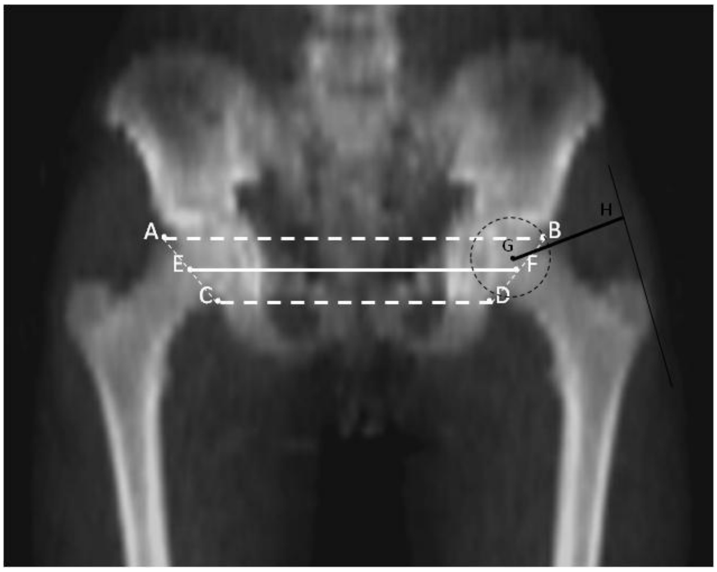

Fig 2 - Geometric measures of the pelvic bone
[AB] – upper inter-acetabular distance (UIAD); [CD] - lower inter-acetabular distance (LIAD); [EF] – inter-acetabular distance (IAD); [GH] – abductor lever arm.

The path of the abductor muscles was represented by drawing a tangential line to the lateral margin of the greater trochanter which was parallel to the line between the highest point of great trochanter (point B in Fig. 3) and the inferior limit of this sub-region (point C in Fig. 3). The abductor lever arm $(\overline{\mathrm{AD}}\ \text{in Fig.}\ 3)$ is represented by the perpendicular distance between that tangent of the greater trochanter and the center of rotation of the femoral head [31].

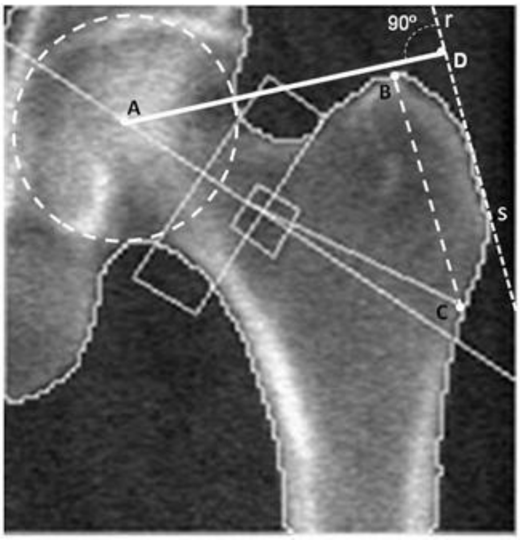

Fig 3 - DXA image illustrating the abductor lever arm determination [AD] – abdutor lever arm (ALA); [BC] - line between the higher point of great trochanter and the inferior limit of this sub-region; rs – Line tangential to the lateral margin of the greater trochanter.

All parameters were measured three times in a random sub-sample of fifteen participants in order to determine measurement precision. The root mean square of coefficient of variations was 0.8%, 0.6%, 0.5% and 0.8% for IAD, UIAD, LID and ALA, respectively.

2.4 Physical Activity

2.4.1 Accelerometry

PA was assessed at baseline and follow-up using the GT1M accelerometer (Actigraph, Fort Walton Beach, Florida, USA) with 15 second epochs. Children were instructed to wear the accelerometers for four consecutive days (two weekdays and two weekend days) providing at least 600 minutes per day of accelerometer data. Those not complying with these requirements were excluded from the sample The accelerometer was secured on the right hip and the children were asked to wear the accelerometer all day, except during water activities and sleeping They were also asked to put them on as soon as they got out of bed in the morning and take them off when they went to bed at night. Accelerometers were programmed to start recording in the morning of the first day and measure continuously for 4 days. Time with over 30 min of continuous zero values was assumed to represent non-wear time and was not analyzed.

Activity data were analyzed and processed using the MAHUffe analysis program (www.mrc-epid.cam.ac.uk). The output from the program included accumulated time spent at sedentary-, light-, moderate-, and vigorous-intensity PA in minutes per day. The intensity of PA was defined according to the counts per minute (cpm) as follows: sedentary activity, up to 100 cpm; light-intensity (LPA) from 101 to 2295 cpm; moderate-intensity (MPA) from 2296 to 4011 cpm; and vigorous-intensity (VPA) over 4012 cpm [32]. MVPA was calculated as the sum of moderate and vigorous activity.

2.4.2 Bone-specific physical activity questionnaire

The Bone-Specific Physical Activity Questionnaire (BPAQ) was used to quantify current and historical PA participation relevant to the musculoskeletal system [33]. This questionnaire uses ground reaction forces (GRF) loading reference values. An algorithm was developed to weight the factors of load intensity, years of participation, and frequency of current and historical activity according to the principles of the osteogenic index [34]. This algorithm was used to convert the raw BPAQ data into a score that reflects total bone-relevant PA history. The original BPAQ was validated for young adults, however, it was designed to be applied to a wide age range (including children) via a specific age weighting factor in the algorithm. Recent analyses indicate that, in contrast of the inability of traditional measures of PA to reflect bone loading history, BPAQ score predicts up to 60% of the variance in indices of bone strength at the femoral neck and lumbar spine [35]. The questionnaire was administered to each participant by a trained interviewer. Participants were asked to record (a) all regular physical activities performed throughout their life and the approximate number of years of participation; and (b) all activities practiced on a regular basis over the previous 12 months, including frequency of participation. A PA score was derived for each individual. We assumed the following GRF equivalences for popular sports reported that were not included in GRF original database [33]. Handball ≈ Basketball; Canoeing ≈ Rowing; Rhythmic gymnastic ≈ Dance.

2.5 Body size and body composition

Hip BMC (g), bone area (cm^2), and areal bone mineral density (g/cm^2) were derived from the scan images. Quality control scans were performed daily using the Hologic phantom. To minimize operator-related variability, all measurements were conducted by the same technician. Our error for BMC measurements is low, with a coefficient of variation < 1% for quality control scans.

Standing and sitting height were measured to the nearest 0.1 cm using a stadiometer (Secca 770, Hamburg, Germany) with children in underwear and barefoot. Body mass (kg), total fat (kg), and total lean mass without bone (kg) were determined from a total-body scan using DXA (QDR Explorer; Hologic, Waltham, MA, USA) with children in a fasting state. Body mass index (BMI) was calculated as body mass in kilograms divided by height (in meters) squared.

2.6 Maturity and calcium intake

Maturity was estimated as the years of distance positive or negative from the age of peak height velocity using sex-specific prediction equations that include age, height, and sitting height [36]. Calcium intake (mg) was calculated from a semi-quantitative Food Frequency Questionnaire, assessing regular intake of a wide set of a typical Portuguese foods.

2.7 Statistical Analysis

Data were analysed using the STATA statistics and data analysis software package (Version 12.0 for Windows; StataCorp LP, Texas, USA). The sample distribution of the survey variables was examined using appropriate measures of central tendency and variability. Differences between girls and boys were analysed by comparing sample means using independent-samples t-tests (or parametric t-tests for proportions) when variables were normally distributed and Mann-Whitney nonparametric tests when not. A longitudinal data approach was adopted to control for unobservable individual effects, which reflect heterogeneity between subjects related to genetics, environment, family, etc. Several linear regression models were considered to analyse the effect of the explanatory variables of PA and geometry on proximal femur sub-regional BMDs and each of the three BMD ratios (response variables). Models were adjusted for maturity, body height, and body lean mass. The F-test for overall significance of the regression was used to confirm the joint significance of the chosen explanatory variables, $p<0.001$. A test for the presence of unobservable individual effects confirmed significant heterogeneity across individuals which were accounted for. Estimation of the regression parameters was performed under the hypothesis of random effects (i.e., supposing that the unobservable individual effects are not correlated with the observed explanatory variables) and the Hausman test used to confirm that the random effects hypothesis fit the current sample. All models were initially estimated for boys and girls together and then repeated separately.

3. Results

Participant characteristics are provided in table 1. The mean body height, body mass index, and body lean mass were similar between boys and girls at baseline and follow-up. Body weight was similar at baseline, but at follow-up, girls were heavier. At both measurement periods, girls had greater mean fat mass than boys. At baseline, but not at follow-up, boys had higher BMD values at the integral proximal femur and at the neck region when compared to girls. Bone mass distribution at the neck region, expressed by FN:PF ratio mean, did not differ between boys and girls at baseline nor follow-up. Girls' TR:PF ratio was greater than that of the boys at baseline and follow-up. IAD was wider and ALA was greater in girls when compared to boys at both measurement periods.

When assessed by accelerometry, the means of all four PA variables – MPA, VPA, MVPA, and total PA – were not significantly different for girls and boys at base line. At follow-up, PA levels for girls decreased while boys became more active. In contrast, the PA estimated through BPAQ was similar between boys and girls at both baseline and follow-up.

Table 1 – Descriptive characteristics of participants at baseline and one year follow-up

	Baseline				One-year follow-up			
	Girls		Boys		Girls		Boys	
	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)
Age, y	10.7	(0.4)	10.7	(0.3)	11.8	(0.4)	11.8	(0.3)
Maturity Offset, y	-1.26	(0.5)	-2.87	(0.5) [a, b]	-0.03	(0.5)	-1.88	(0.6)[a, b]
Peak High Velocity, y	11.5	(0.5)	13.1	(0.7) [a, b]	11.8	(0.5)	13.6	(0.7)[a]
Height, cm	145.1	(6.8)	143.5	(6.8)	152.4	(6.9)	149.9	(8.1)
Weight, kg	39.9	(8.1)	38.2	(8.6)	45.8	(8.9)	43.0	(9.8)[a, b]
Body Mass Index, kg/m^2	18.9	(3.3)	18.4	(3.2)	19.6	(3.0)	19.0	(3.2)
Body Fat, kg	11.8	(4.7)	9.92	(5.1) [a, b]	13.53	(5.2)	11.0	(5.3)[a, b]
Body Lean Mass, kg	26.88	(4.2)	27.12	(4.1)	30.7	(4.9)	30.58	(5.5)
Body Fat, %	28.8	(6.8)	24.73	(7.3) [c]	28.9	(6.6)	24.7	(6.8)[a]
Moderate PA, min/d	32.5	(11.5)	31.0	(10.9)	28.5	(11.3)	39.7	(11.2)[a, b]
Vigorous PA, min/d	13.7	(8.5)	13.3	(7.5)	11.6	(7.4)	18.9	(9.7)[a, b]
Moderate and Vigorous PA, min/d	46.1	(18.3)	44.3	(17.5)	40.1	(17.2)	58.6	(19.2)[a, b]
PA Average Intensity, count/min/d	441.1	(109.2)	419.6	(111.0)	387.9	(117.2)	481.3	(118.6)[a, b]
Past BPAQ	9.0	(14.1)	6.6	(14.2)	12.8	(19.2)	8.1	(15.4)
Current BPAQ	18.6	(34.2)	16.1	(20.4)	17.1	(28.5)	17.2	(18.8)
Total BPAQ	27.7	(41.2)	22.7	(31.8)	29.9	(42.7)	25.3	(25.5)
Proximal Femur BMD, g/cm^2	0.729	(0.86)	0.774	(0.78) [a]	0.801	(0.11)	0.807	(0.09)
Neck BMD, g/cm^2	0.699	(0.09)	0.744	(0.08) [a]	0.754	(0.103)	0.771	(0.09)
Trochanter BMD, g/cm^2	0.592	(0.08)	0.609	(0.07)	0.655	(0.09)	0.638	(0.08)
Neck / Proximal Femur BMD	0.96	(0.05)	0.96	(0.05)	0.94	(0.05)	0.95	(0.04)
Trochanter / Proximal Femur BMD	0.81	(0.04)	0.79	(0.04) [a, b]	0.82	(0.04)	0.79	(0.03)[a, b]
SL Neck BMD, g/cm^2	0.602	(0.09)	0.638	(0.08) [a]	0.665	(0.11)	0.678	(0.10)
IM Neck BMD, g/cm^2	0.775	(0.09)	0.831	(0.09) [a]	0.825	(0.11)	0.845	(0.10)
IM Neck BMD / SL Neck BMD	1.297	(0.13)	1.308	(0.10)	1.253	(0.13)	1.255	(0.11)
Inter-Acetabulum Distance, cm	12.59	(0.8)	12.31	(0.6) [a]	13.49	(1.0)	12.77	(0.8)[a, b]
Abdutor Lever Arm, cm	4.20	(0.4)	3.68	(0.5) [a, b]	4.66	(0.3)	4.22	(0.5)[a]

PA, physical activity; BPAQ, bone physical activity questionnaire; BMD, bone mineral density; SL, superolateral; IM, inferomedial; [a] $p < 0.05$ difference between boys and girls within each examination; [b] Non parametric test ; [c] Parametric T-Test for proportions

Table 2 shows the estimated parameters for the random effects GLS regression models for BMD outcomes. Body lean mass was significant in all sex-specific and combined models (FN BMD, SLFN BMD, IMFN BMD, and TR BMD). Body height was inversely and total BPAQ was positively associated with the FN BMD in the combined model and in the girls only model. In the TR BMD models, maturity was positively associated in the girl's and combined models.

Table 2 - Random-effects GLS regression models for femoral neck (total, superolateral, and inferomedial) and trochanter BMDs testing for the effects of physical activity and geometric measures of the pelvis and hip, controlling for maturity, body height and body lean mass.

	FN BMD		SLFN BMD		IMFN BMD		TR BMD	
	Coef. estimate	Robust SE	Coef. estimate	Robust SE	Coef. estimate	Robust SE	Coef. estimate	Robust SE
Boys and Girls								
Sex	0.0327	0.0102 ^{b}	0.0293	0.0121 ^{c}	0.0389	0.0108 ^{a}		
Height, cm	-0.0016	0.0005 ^{b}						
Lean mass, kg	0.0139	0.0011 ^{a}	0.0129	0.0008 ^{a}	0.0140	0.0013 ^{a}	0.0105	0.0010 ^{a}
Maturity, yrs					-0.0244	0.0076 ^{b}	0.0129	0.0045 ^{b}
Total BPAQ	0.0003	0.0001 ^{b}	0.0004	0.0002 ^{c}	0.0002	0.0001 ^{b}		
Constant	0.5547	0.0566 ^{a}	0.2503	0.0224 ^{a}	0.3192	0.0545 ^{a}	0.3527	0.0394 ^{a}
Model R^2								
within	0.65		0.67		0.46		0.75	
between	0.46		0.32		0.48		0.30	
overall	0.48		0.36		0.48		0.38	
Girls								
Height, cm	-0.0017	0.0006 ^{b}			-0.0030	0.0010 ^{b}		
Lean mass, kg	0.0170	0.0016 ^{a}	0.0154	0.001 ^{a}	0.0185	0.002 ^{a}	0.0125	0.0012 ^{a}
Maturity, yrs							0.0207	0.0052 ^{a}
Total BPAQ	0.0003	0.0001 ^{c}	0.0004	0.0002 ^{c}	0.0001	0.0001 ^{c}		
Constant	0.4790	0.0616 ^{a}	0.1776	0.0294 ^{a}	0.6983	0.1059 ^{a}	0.3182	0.0455 ^{a}
Model R^2								
within	0.74		0.73		0.61		0.87	
between	0.59		0.48		0.56		0.56	
overall	0.61		0.51		0.56		0.61	
Boys								
Height, cm					-0.0031	0.0013 ^{c}	-0.0034	0.0011 ^{b}
Lean mass, kg	0.0081	0.0009 ^{a}	0.0101	0.0011 ^{a}	0.0113	0.0021 ^{a}	0.0133	0.0020 ^{a}
Moderate PA							0.0005	0.0002 ^{c}
Total BPAQ							0.0003	0.0001 ^{b}
Constant	0.5241	0.0274 ^{a}	0.3665	0.0328 ^{a}	0.9732	0.1460 ^{a}	0.7131	0.1076 ^{a}
Model R^2								
within	0.56		0.59		0.30		0.67	
between	0.23		0.11		0.32		0.24	
overall	0.25		0.15		0.31		0.28	

FN) femoral neck; (SLFN) superolateral femoral neck; (IMFN) inferomedial femoral neck; (BMD) bone mineral density; (TR) trochanter; (SE) standard error; (BPAQ), bone physical activity questionnaire; (PA) physical activity.
^{a} $p < 0.001$; ^{b} $p < 0.01$; ^{c} $p < 0.05$

For boys, body height was negatively associated with TR BMD. Body height was also negatively associated with the IMFN BMD in the girls' and boys' models.-Total BPAQ was positively associated with the combined and girls-specific models for FN BMD, SLFN BMD and IMFN BMD. Total BPAQ was associated with TR BMD in the boys specific model. Moderate physical activity was the only accelerometry-based variable that entered one of the twelve models presented in table 2, with a positive effect in the boys' TR BMD model.

The results of the estimated parameters for the random effects GLS regression models for BMD ratios are presented in table 3. Maturity was inversely associated with the FN:PF BMD ratio in the combined model and the boys specific model. Total BPAQ was a significant predictor with a positive effect on the relative mineralization of the femoral neck in the combined and the girls' specific models. A similar (positive) effect of body lean mass on FN:PF was found in the same models but the estimated coefficient for this variable was negative in the regression model for IM:SL ratio in girls. The IAD and the ALA, as well as their ratio, were significant predictors of the FN:PF BMD ratio in the combined model. In the girls' model only the ALA was a negative predictor of FN:PF and in boys' only the IAD showed a significant positive prediction. The interpretation of the coefficients of IAD and ALA variables on the FNPF for boys and girls should not be done individually, but considering the partial effects, which are shown by

$$\frac{\partial FNPF}{\partial IAD} = \beta_1 + \frac{1}{ALA}\beta_3$$

and

$$\frac{\partial FNPF}{\partial ALA} = \beta_2 - IAD.ALA^{-2}.\beta_3$$

where β_1 represents the estimated coefficient associated to IAD, β_2 the coefficient associated to ALA and β_3 the coefficient associated to IAD*ALA^{-1}. Therefore, the sign and size of the partial effect depend not only on the estimated coefficients but also on the values of ALA and IAD.

We simulated the partial effects in the range of possible values for IAD and ALA in our sample, separately for boys and girls, and ended up with the following situation: the sign of the partial effect of IAD on boys and of the partial effect of ALA on girls was always negative; on the other hand, for the ALA effect on boys and the IAD effect on girls we obtained negative figures in the lower limits of the estimated intervals, and positive figures in the upper limits, showing that the sign of the partial effect depends on the individual's levels of IAD and ALA.

Concerning the models for the IM:SL BMD ratio (table 3), we find that in the boys' model the IAD, the ALA, and their ratio were also all significant predictors; in this case, the sign of the partial effect of IAD was clearly defined as positive for all the observations in the sample, and that of the partial effect of ALA was negative for 97,5% of the observations. In the model for boys and girls together only the ALA was negatively associated with the IM:SL BMD, while in the girls' model only IAD.ALA^{-1} was a significant predictor, with a positive effect, suggesting that IAD has a direct effect and ALA has an inverse effect on the girls' IM:SL BMD. The IAD was also positively associated to the girls' TR:FN ratio while none of the tested variable revealed any ability to explain this same BMD ratio in boys.

Table 3 - Random-effects GLS regression models, using panel data, for proximal femur BMD ratios, controlling for maturity, body height and body lean mass and testing for the effects of physical activity and geometric measures of the pelvis and hip.

	FN:PF BMD		IM:SL FN BMD		TR:PF BMD	
	Coef. estimate	Robust SE	Coef. estimate	Robust SE	Coef. estimate	Robust SE
Boys and Girls						
Sex					-0.0347	0.0107 [b]
Lean mass, kg	0.0020	0.0009 [c]			0.0014	0.0004 [b]
Maturity, yrs	-0.0218	0.0058 [a]				
Total BPAQ	0.0002	0.0001 [c]				
IAD, cm	0.0198	0.0098 [c]				
ALA, cm	-0.0753	0.0282 [b]	-0.0740	0.0090 [a]		
$IAD.ALA^{-1}$	-0.0863	0.0335 [b]				
Constant	1.1581	0.1271 [a]	1.5888	0.0385 [a]	0.7741	0.0134 [a]
Model R^2						
within	0.28		0.31		0.05	
between	0.03		0.01		0.07	
overall	0.05		0.03		0.08	
Girls						
Lean mass, kg	0.0022	0.0010 [c]	-0.0071	0.0015 [a]		
Total BPAQ	0.0002	0.0001 [b]				
IAD, cm					0.0089	0.0023 [a]
ALA, cm	-0.0563	0.0094 [a]				
$IAD.ALA^{-1}$			0.1157	0.0369 [b]		
Constant	1.1299	0.0306 [a]	1.1365	0.1271 [a]	0.6979	0.0301 [a]
Model R^2						
within	0.39		0.25		0.04	
between	0.06		0.06		0.18	
overall	0.11		0.09		0.15	
Boys						
Maturity, yrs	-0.0213	0.0055 [a]			--	--
IAD, cm	0.0161	0.0062 [b]	0.0531	0.0217 [c]	--	--
ALA, cm			-0.2007	0.0463 [a]	--	--
$IAD.ALA^{-1}$			-0.1361	0.0500 [b]	--	--
Constant	0.6932	0.0905 [a]	1.8461	0.2134 [a]	--	--
Model R^2						
within	0.14		0.39		--	
between	0.01		0.01		--	
overall	0.02		0.04		--	

(FNPF) Femoral neck to proximal femur BMD ratio; (SLFN) superolateral femoral neck; (IMFN) inferomedial femoral neck; (TR:PF) trochanter to proximal femur BMD ratio; (SD) standard deviations; (BPAQ) bone physical activity questionnaire; (IAD) inter-acetabular distance; (ALA) abductor lever arm; ($IAD.ALA^{-1}$); inter-acetabular distance to abductor lever arm ratio. [a] $p < 0.001$; [b] $p < 0.01$; [c] $p < 0.05$

4. Discussion

This longitudinal observational study examined the effect of PA and geometric-related variables of the pelvis and hip on proximal femur sub-regional BMDs and proximal femur relative mineralization. Differences in relative mineralization are important since it might provide additional understanding of the risk for two main hip fracture types (cervical and trochanteric fractures) [11]. The femoral neck was differentiated between the superolateral and the inferomedial femoral bone areas to provide additional insight of the risk for cervical fracture [7].

The results showed a positive influence of PA on the regional neck BMDs for boys and girls together as well as for girls, but not for boys. Concerning TR BMD this positive association was found only for boys. As expected, the body lean mass was significant in every BMD model and other control variables such as body height and maturity in some of them. Lean mass is frequently entered in models for proximal femur bone mass and its high explanatory power is occasionally referred as a cause for reduced contribution of other variables in the same models [37]. But above all, the geometric variables, ALA and IAD, were not significant in the BMD models. This was surprising since locomotion involves a single leg stance that is supported by the joint action of ALA and the abductor muscles. The seminal model of Pauwels [38] for the biomechanics of the pelvis-hip system suggests that since the length of the ALA is smaller than the bodyweight lever arm, the abductors must generate a force that is higher than the bodyweight (without the weight of the stance leg) to maintain the pelvis leveled [39]. Therefore, all other factors equal, an increase of the ALA, or the hip offset, will reduce the required force of the abduction muscles, while an opposite consequence is obtained with the increase of bodyweight lever arm indicators, such as IAD. This model of counterbalancing forces has been used to explain abduction muscles activity [13] and inform surgical strategies for total hip arthroplasty [40], using different variables as indicators of the body weight lever arm: the IAD; half of the distance measured between the centers of the femoral heads; or the bi-trochanteric width [20]. All these variables are linearly related to the bodyweight lever arm. However, we didn’t find any significant influence of either the IAD or the ALA on the BMD levels of the studied regions of the proximal femur.

Unlike the BMD models, IAD, ALA or their interaction was significant in most of the relative mineralization models (the exception was the combined model for TR:PF BMD ratio).

In these models, the estimated coefficients suggest a positive partial effect of larger IADs on the relative mineralization of the trochanteric region in girl’s, whereas the effect of larger ALAs seems to be negative on the neck. Regarding the IM:SL ratio, the confirmation of these results would mean that the narrower the IAD and the wider the ALA the more homogeneous the bone mass distribution at the proximal femur neck tends to be. The same applies to the levers ratio IAD:ALA.

According to the biomechanics theoretical model of the hip it was observed opposite effects of the IAD and ALA on the BMD ratios. The coefficient associated to the IAD.ALA-1 interaction in the model for the relative mineralization of the inferomedial to the superolateral femoral neck does not contradict it, but the interpretation of these positive/negative effects requires caution. In the FN:PF model for boys and girls combined as well as for the boys’ model for the IM:SL, the sign of the global effect remains undetermined taking in consideration the partial effects for the range of possible IAD and ALA values for both sexes.

The geometric variables we studied did not help to explain the relative mineralization of the trochanteric region with the exception of IAD in the girl's model. In general the geometric variables seem to better explain the variance of the BMD ratios within the individuals than between them (R^2 up to 39% versus R^2 up to 7%), which highlights the importance of longitudinal approaches when examining these effects.

Should there be a biomechanical reasoning behind the effects of IAD and ALA, as the theory suggests, one would expect to find similar effects on models for both sexes, being sex-specificities given by the significant differences in the IAD and ALA. These geometric differences were observed in our children sample, but sex-specific morphology of the pelvis and hip are also well documented in the literature for men and women, namely the hip offset and some pelvis width related variables [42-44]. Our results are compatible with the theoretical importance of the geometric variables in the relative mineralization of the studied regions of the proximal.

This study has some limitations, mostly intrinsic to the technology used to assess physical activity, body composition and bone. The ability to accurately measure physical activity in a free-living environment is among the many overarching strengths of accelerometry, but our use of a short time period (four days) may not be representative of the activity level during last months. To minimize this limitation, we applied the accelerometry in a regular week of the school calendar and explained to the subjects the importance of keeping their usual standards. The bone-specific questionnaire (BPAQ) may have provided more complete description of children's activity, as well as yearlong information and physical activity history. However, assessing physical activity through self-report questionnaires in children has a drawback associated with the children's recall skills. In order to mitigate this limitation, the interviews were carefully standardized and we contacted the parents whenever the responses provided did not seem to be valid. The use of DXA created additional limitations. Areal BMD does not represent a volumetric density; therefore the densitometric image provides no direct information about strength and bone material composition. However, the alternative use of pQCT also presents limitations including movement challenges and scans that are not clinically relevant. In general, DXA is preferable for pediatric application because it scans large regions of interest (ROIs) rapidly at low radiation doses and is robust to movement and positional variation. The latter factors improve scan quality and congruence of repeated measures within subjects—a critical issue in longitudinal pediatric applications.

Our use DXA images for geometric measures, rather than radiographic images, provided important insights without exposing the children to additional radiation, a critical issue in a pediatric research.

In conclusion, we made use of the commonly applied DXA technique and appropriate procedures to mitigate known limitation, to examine the potential importance of the hip and pelvis geometric features on the mineralization of the proximal femur. Our results suggest that the IAD and ALA, as indicators of the main lever arms of the biomechanics of the hip, may play a role in the relative mineralization of the proximal femur in peripubertal boys and girls. However unlike total lean body mass and PA, the same geometric variables don't seem to influence the absolute BMD levels at the proximal femur neck and trochanter. Further research is needed to better understand the effects of geometric variables on the relative mineralization of the proximal femur regions including the development of a specific biomechanical model to simulate the vector forces exerted on these regions.

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4 General discussion

The specific results' discussion of each of the studies that constitute this thesis is already done and theoretically framed in each one of them. The aim of this chapter is not the reproduction of those discussion sections, but solely to attempt a coherent linkage of the main results and set forth some possible global interpretations.

The four studies presented had the common broad objective of studying the bone mass distribution at the proximal femur in children from 9 to 12 years old and exploring possible explanatory factors for the observed patterns. The relevance of focusing on this age period in the context of bone research health, aimed at improving public policies' design in order to reduce the risk of later life fractures, was detailed in chapter 1.

The methodological approach used in pursuing this goal wasn't the same in all the submitted studies, particularly in the first, but is detailed in each under the methods' section, hence this chapter's focus is on the main findings and its overall interpretation.

The first study's main purpose was to analyze femoral neck bone mass distribution using the Ward’s area location as a distribution indicator, as a more central position reflects a less heterogeneous bone mineral composition between the superolateral and the inferomedial aspects of the femoral neck. Its relationship with physical activity and body composition was also explored.

It was found that the principal shape variation (as identified through the morphometric analysis) observed in all the boys and girls in the sample, was precisely the location of the Ward’s area. Additionally, the overall shape variation of the proximal femur was associated to body composition variables – particularly the lean mass in boys and the fat mass in girls – and with the moderate plus vigorous physical activity in boys. In concrete, boys with higher levels of vigorous physical activity tended to show a more central position of the Ward’s area, i.e. a more homogeneous 2D distribution of bone mass considering the superolateral and the inferomedial aspects of the femoral neck.

This physical activity effect seemed to be consistent with the expected tension and the compression forces in the femoral neck, explained by this region's biomechanics. The absence of a similar statistically significant effect on girls could be explained by lower levels of vigorous physical activity.

Given the relevant, but sex biased, association between physical activity and the distribution of bone mass at the femoral neck obtained in the first study, the second was designed with the purpose of investigating the sex-specific association between physical activity and bone mass distribution, but with a broader focus. This time, all the three proximal femur sub-regions were investigated and intra-individual bone mineral density ratio's between each pair of regions were defined as indicators of bone mass distribution at the proximal femur. The results of this second study not only showed that, within our sample, physical activity positively benefits the BMD of all three proximal femur sub-regions, with the exception of girls’ femoral neck, but also that the effect was higher the more intense the physical activity. Concerning the impact on bone mass distribution, the study revealed a sex-specific pattern, as physical activity showed an homogeneous effect among all three regions in boys, whereas in girls the effect seemed to benefit especially the trochanteric and inter-trochanteric sub-regions.

Besides other possible explanations for the identified regional and sex patterns, biomechanical reasons associated with the geometry of the proximal femur and pelvis were advanced. In fact, based on the pelvis-hip system's biomechanics, the abductors muscles' force applied to balance the pelvis during locomotion, depends on the relative lever arms of the body weight and the abductors muscles whose attachment is at the great trochanter.

This potential research path was followed, encouraged also by several published studies showing sex differences in kinematics and muscle activities during locomotion [1, 2] which has been attributed, in part, to differences in their structure, given that women have a larger hip width to femoral length ratio [3] and a greater bi-trochanteric width [2]. This structural difference is seen as leading to a combination of increased hip adduction, hip internal rotation, and *genu valgus* in women, therefore, different movement patterns [4]. In addition to muscle activity, it would also be expected finding distinct ground reaction forces, but while some have reported no differences [5] others found that women exhibited greater vertical ground reaction forces and free vertical moments compared to men [6, 7]. Nevertheless, these overall results seemed to give some support to the idea that the way loads spread through the proximal femur depends on the involved mechanical levers [8].

Therefore, the third study aimed at exploring the hypothesis that pelvic and proximal femur geometric-related variables were related to the distribution of bone mass in the proximal femur and could moderate the impact of physical activity. Another goal was to compare the available geometric variables between boys and girls. Again the results showed a positive effect of physical activity on the BMD of all three proximal femur sub-regions, for both sexes. However, apart from body composition variables, namely the lean mass, what turned out to be associated to the distribution of bone mass in the proximal femur were geometric-related variables of the proximal femur or the pelvis, rather than physical activity.

A particular significant result was the importance of the pelvis width in the relative mineralization of girls' trochanteric and inter-trochanteric regions, revealing the importance of the pelvis geometry. Probably due to a much lower dispersion of the boys' data on the pelvis width, a similar association was not found for this sex.

The third study also showed significant differences between sexes for all pelvic geometric variables and the femoral neck width, confirming the sex differentiating nature of some geometric aspects of the pelvis-hip system.

Despite the promising results concerning geometric-related variables, only part of the relevant variables of the Pauwels model for the biomechanics of the pelvis-hip system was considered in this third article. To overcome this limitation, a fourth study was setup with the inclusion of additional femoral neck geometric-related variables, namely the abductor lever arm. In this study the focus was on the two sub-regions with higher fracture incidence, the neck and the trochanter, including the distinction between the superolateral and the inferomedial parts of the femoral neck aspects that had already turned out to be relevant in the first study. The longitudinal nature of this study also contributed to improve the quality of the analysis and the use of more sophisticated statistical models.

The main results showed benefits of physical activity in the BMD of the integral femoral neck and at its two sub-regions, when data was analyzed with boys and girls pooled together or girls alone, and benefits on the trochanter in boys. But in relation to bone mass distribution once again the pelvic and proximal femur geometric-related variables, namely those associated with the balanced lever arm pelvic-hip system – the pelvis width and the abductor lever arm – appear to be statistically relevant, except for the relative mineralization of the trochanter when boys and girls are considered together and boys considered alone.

The overall results of these four studies suggest the following three considerations:

1. Physical activity effects on the BMD of the proximal femur seems to be region-specific and sex-specific.

2. In addition to different physical activity profiles, age, hormonal, maturational and body composition factors, some geometric features of the pelvis and proximal femur might contribute to explain both the sub-regional and the sex specific patterns of the physical activity effects on BMD at the proximal femur.

3. When the geometry of the pelvis and the proximal femur are considered, the power of physical activity to explain the distribution of bone mass at the proximal femur seems limited.

In fact, despite the natural difficulty of a straightforward comparison between the´studies' results, an interesting outcome was the sex-specific response of the proximal femur sub-regions to physical activity. This result is not common in the literature and might contribute to explain why some studies have reported a lower, or even inexistent, response of girls' proximal femur to physical activity. Concretely, some of those studies focused only on one proximal femur sub-region [9, 10] disregarding the effects on the other sub-regions. The different physical activity-related of determination coefficients observed in our last three studies clearly contradict the lower sensitiveness of bone to physical activity loading stimulus in girls, as some have suggested [10, 11]. Irrespectively of the underlying reasons, the fact is that in some cases and sub-regions, the impact of equivalent physical activity was estimated to be higher in girls than in boys.

Probably the present dissertation's studies most interesting consequence was the inclusion of geometric variables derived from biomechanical models into the scientific debate on the effects of physical activity on the mineralization of the proximal femur in children. Based on generic biomechanic models it would be expectable to find inverse effects of the pelvis' width and the abductor lever arm on regional mineralization of the proximal femur, but this observation was not absolutely consistent throughout the sub-regions and in both sexes. In some cases the partial estimated effects were inversed but the situation was not clearly manifested in others, therefore it was not possible to unequivocally generalize the effects signs of the inter-acetabular distance or of the abductor lever arm on the relative mineralization of some proximal femur sub-regions.

Assuming that the load profiles on the various sub-regions of the proximal femur are partially determined by those variables, as the biomechanical theory proposes and these empirical studies suggested, the reasoning for the regional specific pattern of physical activity effects might rest on the geometric aspects of the dynamic skeletal systems involving the pelvis. This is implicitly suggested when some authors include some pelvis variables to explain proximal femur risk of fractures [12-15].

Additionally, our results confirmed the significant geometric differences, particularly concerning the pelvis between sexes, giving empirical ground to the inclusion of geometric features in the factors explaining sex specific mineralization patterns, the same way they explain sex specific locomotion patterns.

However, this sex specificity should not justify the inability to find statistical evidence of geometric-related variables relevance in the mineralization of some proximal femur sub-regions, namely the pelvis width in boys, as long as the biomechanical reasoning shall apply irrespectively of sex. The lack of absolute consistence of biomechanical-related results across sub-regions and sexes is a challenging issue raised by this dissertation.

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5 Conclusions and further research

Bearing in mind that the ultimate goal of this dissertation's research is the improvement of public health policies design and implementation to deal with the problem of bone fracture in elderly populations, this final chapter should be able to answer the question: how did this research work contribute to that goal? Furthermore, the quality of the work developed should be judged by the degree of that (eventual) contribution. It would however be a too ambitious objective of this Ph.D dissertation, to find a positive link between its results and that ultimate goal. At most, the ambition was to contribute to a better understanding of the mechanisms governing the influence of physical activity on the proximal femur mineralization, as long as it is believed to benefit bone strength and, consequently, reduce the risk of fracture.

Concretely, the aim of this dissertation as a whole was to explore the effects of physical activity on BMD and bone mass distribution at the three sub-regions of the proximal femur, adding relevant geometric variables of the pelvis-hip system, always with a sex differentiating approach. Based on the results obtained, the main conclusion is the following: *There is evidence of sex and regional-specific effects of physical activity on bone mass distribution at the proximal femur in 9-12 year old children. The pelvis geometry, namely its width, and the proximal femur geometry, namely the abductor lever arm, emerged as significant predictors of bone mass distribution at the proximal femur. Furthermore, these geometric features might interfere with the physical activity effect on bone mineralization at this skeletal site, as the results confirmed the benefits of physical activity on BMD but not on bone mass distribution indicators.*

This conclusion and its supporting results could constitute useful information in the design of strategies promoting a healthy skeletal development of children 9 to 12 year old, for many authors the “gold period” for optimizing lifespan skeletal health. However this work was pioneer in narrowing the gap between the biomechanics theory and the physical activity induced bone mineralization and, consequently, guidance for future research is required.

Two non-mutually exclusive paths might deserve additional research:

One path consists in setting up a research study to provide robust theoretical support for a concrete regional loads and stresses connection to the local mineralization at the proximal femur. This objective might be pursued by starting with the development of a biomechanical model of the pelvis-hip system that could simulate the transmission of forces through the proximal femur structures, depending on individual relevant geometric parameters. Then, it would be possible to explore the association between the resulting regional stresses and loads individual profile and the mineralization parameters for the same individuals. The research would be particularly useful if effective accelerations of the body mass during physical activity periods were registered for each individual, in order to compute the forces effectively exerted on the skeleton, something that cannot be done with the traditional accelerometers as those available for the present work. This multidisciplinary approach involving the fields of physical activity and bone health, biomechanics and mechanical engineering would be an effective way of moving forward.

Another path could be to split a group of athletes with a prolonged and uninterrupted intensive practice of weight-bearing sports with high osteogenic potential, such as gymnastics, where running and drop jumping are frequent, in two sub-groups based on their pelvis-hip profile, followed by a comparison in bone mass distribution at the proximal femur sub-regions, in an attempt to obtain further evidence of the relevance of the geometric aspects of the pelvis-hip system.

Annex

Ward's Area Location, Body Composition and Physical Activity in 8-9 Years Boys and Girls

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INTRODUCTION

Ward´s triangle is a space formed near the center of femoral neck by the intersection of three trabecular bundles (1). This central region defines a neutral axis where tensile and compressive forces balance each other (2). Changes in the appearances of these groups of trabecular with ageing are the basis of the grading scheme proposed by Singh, from normal to severe osteoporosis (3). In densitometry Ward´s triangle is the femoral neck region (area) with the lowest BMD (4). Since its location is not standardized between subjects, (not always in the same position), Ward area is not considered for analysis. However, the determination of its location might contributes to the understanding of femoral neck bone mass distribution which is importante to the study of bone strength and the associated evaluation of the hip fracture risk. Although this risk is particularly relevant in elderly groups, there is a paediatric issue given bone mass acquisition and intensive physical activity during growth. Thus, the aim of the present study was to analyse: (a) the location of the femoral neck Ward area in pre-pubertal children of both genders; and (b) the relationship between mechanical factors, such as physical activity and body composition, and Ward area location, using a landmark-based morphometric methodology to quantify shape variations.

METHODS

Subjects. 48 pre-pubertal boys and 40 girls were drawn from schools (3rd grade) and sport clubs. None of the subjects were taking any medication affecting bone or reported a history of hip fracture.

Age and Body Composition

	Boys (n=48) Mean + SD	Girls (n=40) Mean + SD	P*
Age , y	8.6 ± 0.4	8.5 ± 0.4	0.423
Bone Age, y	9.0 ± 1.1	8.6 ± 1.2	0.178
Weight , kg	33.0 ± 8.0	29.8 ± 6.1	0.031
Height , cm	134.0 ± 7.0	131.9 ± 5.3	0.083
Body Mass Index, kg/m²	18.1 ± 3.1	17.0 ± 2.6	0.079
Body Fat , kg	8.7 ± 5.2	8.2 ± 4.3	0.623
Body Fat , %	24.5 ± 9.3	26.4 ± 7.5	0.303
Body Lean Mass , kg	23.2 ± 3.1	20.6 ± 2.5	<0.001

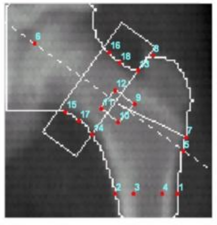
Diet and Physical Activity

	Boys (n=48) Mean + SD	Girls (n=40) Mean + SD	P*
Calcium Intake, mg/d	1196 ± 77.7	1100.6 ± 70.8	0.226
Sedentary , min/d	878 ± 95	886.1 ± 62.1	0.323
Moderate PA , min/d	77.5 ± 3.4	55 ± 3.1	<0.001
Vigorous PA , min/d	10.3 ± 1.1	7 ± 1	<0.015
Mod. + Vigor. PA , min/d	88 ± 26	62.1 ± 23.9	<0.001
Total PA , count/min	702 ± 135	587.2 ± 152.4	<0.001
Neck BMC, g	3.431 ± 0.46	2.929 ± 0.37	0.001
Neck BMD, g/cm²	0.720 ± 0.07	0.642 ± 0.50	0.001

*Independent Sample T-Tests. In cases of no normality, Mann-Whitney non-parametric tests were used; PA - physical activity; BMC - bone mineral content; BMD - bone mineral density

Proximal femur analysis. BMC and BMD of left leg proximal femur were measured with DXA (QDR 1500, pencil beam mode, version 12.4- Hologic, Waltham, MA, USA). In order to identify femoral neck Ward area position, 18 2D landmarks (x, y coordinates) were digitized on proximal femur DXA images using the Thin-plate spline digitize (tpsDig) software version 2.10 (5). The Relative Warp Analysis on this specimens was performed with TPSRelw 1.45 (6) with the following parameters: α=0; Scaling to centroid size = 1; Uniform component – complement; Projection method – orthogonal. The Regression analysis was performed with TPSRegr 1.34 (6) with the following parameters: PCA align reference; Include uniform; Do multivariate tests; Scale Method = 1; Projection method – orthogonal.

Proximal Femur DXA Image



Anatomical Landmarks	Description
1-2	Femoral shaft width*
3-4	Femoral intramedulary width **
5-6	Femoral axis length
7	Lower tip of greater trocanter
8	Upper tip of greater trocanter
9-12	Ward's area limits
13-14	Lower limit of femoral neck
15-16	Upper limit of femoral neck
17-18	Average distance of lower and upper femoral neck limits

* 1.5cm under the lower limit of femoral neck axis.
** Width of medullary canal at the same level of 1-2 landmarks

Body size and body composition. Standing height was measured on a stadiometer (Secca 770, Hamburg, Germany) with subjects in underwear and without shoes. Body weight, total fat and total lean mass evaluations were determined from a total body scan by DXA (QDR 1500, pencil beam mode, version 12.4 ,Hologic, Waltham, MA, USA) with subjects in a fasting state. Body mass index (BMI) was calculated as body weight in kilogram divided by height in meter squared.

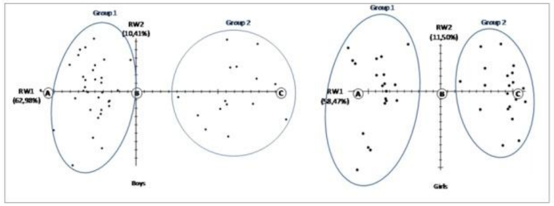
Physical activity was assessed with the Actigraph accelerometer (model GT1M) over seven days and the activity data were sampled on a minute-by-minute basis. Subjects were excluded if they failed to provide a minimum of 3 days of at least 600 minutes per day. The output from the programme included accumulated time spent at sedentary, light, moderate and vigorous physical activity intensity in minutes per day. The total amount of physical activity was expressed as counts per minutes. Cut points of 100, 1952, 5724 and 9498 counts/min were used to identify sedentary, light, moderate and vigorous physical activity.

Energy and calcium intake were calculated from a semi-quantitative Food Frequency Questionnaire assessing regular intake of a wide set of typical Portuguese foods.

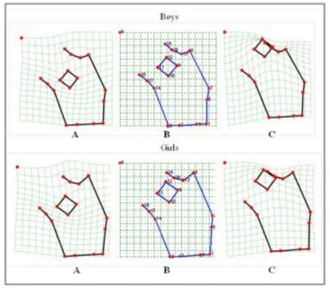
Maturation level consisted of a skeletal age assessed with a portable x-ray (Ascott model, Kodak chassis 20x15), and determined by the Tanner-Whitehouse III Method (7).

Relative Warp (RW) Analysis

Relative Warps plots for RW1 and RW2 (boys and girls)



Shape Changes Depicted by RW1



Femoral neck Ward's area position in boys and girls. A- group 1 shape configuration; B–consensus shape; C– group 2 shape configuration

Boys

	SV	R² (%)	Cum %
RW_1	0.58328	63	63
RW_2	0.23710	10	73
RW_3	0.20734	8	81
RW_4	0.16234	5	86
RW_5	0.14816	5	91

SV – Standard Deviation; R² – Explained variance in percentage; Cum – Comulative explained variance in percentage

Girls

	SV	R² (%)	Cum %
RW_1	0.57137	58	58
RW_2	0.25339	12	70
RW_3	0.22683	9	79
RW_4	0.18392	6	85
RW_5	0.17391	5	91

SV – Standard Deviation; R² – Explained variance in percentage; Cum – Comulative explained variance in percentage

Regression Analysis

Variable	Sum d² of predicted	R² (%)	Generalized Goodall F-Test F	df	P*
Age , y					
Boys	0.0065	1.2	0.567	30.14	0.971
Girls	0.0138	2.5	0.977	32.12	0.504
Bone Age, y					
Boys	0.0213	4.0	1.913	32.15	0.001
Girls	0.0463	8.4	3.475	32.12	<0.001
Weight , kg					
Boys	0.0220	4.1	1.982	32.15	<0.001
Girls	0.0400	7.2	2.965	32.12	<0.001
Height , cm					
Boys	0.0568	10.5	5.471	32.15	<0.001
Girls	0.0176	3.2	1.252	32.12	0.160
Body Mass Index, kg/m²					
Boys	0.0103	1.9	0.901	32.15	0.627
Girls	0.0384	7.0	2.845	32.12	<0.001
Body Fat , kg					
Boys	0.0149	2.8	1.324	32.15	0.107
Girls	0.0386	7.0	2.855	32.12	<0.001
Body Fat , %					
Boys	0.0103	1.8	0.906	32.15	0.619
Girls	0.0341	6.1	2.504	32.12	<0.001
Body Lean Mass , kg					
Boys	0.0297	5.5	2.711	32.15	<0.001
Girls	0.0234	4.2	1.683	32.12	0.010

Variable	Sum d² of predicted	R² (%)	Generalized Goodall F-Test F	df	P*
Calcium Intake, mg/d					
Boys	0.0149	2.8	1.327	32.15	0.105
Girls	0.0098	1.7	0.689	32.12	0.905
Sedentary , min/d					
Boys	0.0034	0.6	0.298	32.15	1.000
Girls	0.0161	3.0	1.144	32.12	0.268
Moderate PA , min/d					
Boys	0.0382	7.1	3.542	32, 14	<0.001
Girls	0.0066	1.2	0.461	32, 12	0.995
Vigorous PA , min/d					
Boys	0.0049	0.9	0.422	32, 14	0.998
Girls	0.0149	2.7	1.055	32, 12	0.384
Mod. + Vigor. PA , min/d					
Boys	0.0342	6.3	3.144	32.15	<0.001
Girls	0.0079	1.4	0.549	32.12	0.981
Total PA , count/min					
Boys	0.0245	4.5	2.211	32.15	<0.001
Girls	0.0116	2.1	0.816	32.12	0.757
Neck BMC, g					
Boys	0.0342	6.4	3.150	32.14	<0.001
Girls	0.0459	8.3	3.446	32.12	<0.001
Neck BMD g/cm²					
Boys	0.0184	3.4	1.648	32.14	<0.001
Girls	0.0319	5.8	2.336	32.12	<0.001

•Regression analysis with Thin Plate Spline regression software conducted separately for each independent variable.
PA - physical activity; BMC - bone mineral content; BMD - bone mineral density.

CONCLUSIONS

❖ The first two **RW axis** accounted for approximately 70% of the proximal femur shape variation (73% for boys and 70% for girls) and **RW1** explained approximately 60% (63% for boys and 58% for girls) of this variation.

❖ The main identified **shape variation** observed along the RW1 was associated to the location of the Ward's area, which moves from a central position to the superolateral limit of femoral neck in both genders.

❖ Advanced **somatic maturity** was associated with a more central Ward´s area position in femoral neck, either in both genders.

❖ **Body weight** and **body composition** variables (body fat mass and lean mass) explained 4-9% ($p \leq 0.01$) of the observed variation of the proximal femur shape in both genders, except fat mass that was only a significant predictor in girls ($R^2=7\%$, $p \leq 0.001$)

❖ Total **physical activity,** particularly moderate physical activity, explained 7-11% ($p \leq 0.001$) of proximal femur shape variation in boys.

✓ **Proximal femur shape variation in 8-9 yrs old boys and girls** was primarily explained by the position of the Ward's area, which could be in part determined by maturity level, expressed by bone age or body composition, but also by physical (in) activity. These exposure variables were positively associated with a more balanced bone mass distribution and thus, with enhanced femoral neck stability, expressed by a central location of the Ward's area.

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Proximal femur shape in pre-pubertal boys and girls: relationship with physical activity and body composition

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INTRODUCTION

Osteoporosis can be seen as a pediatric disorder, despite its geriatrics consequences, because most of bone mass and size is acquired during childhood and adolescence period. Investigations indicated that physical activity may increase bone mass and size (Bass et al., 2002; Sundberg et al., 2002), thereby increasing bone resistance, suggesting that bone changes are associated with mechanical loading. In the context of the evaluation of hip fracture risk, femoral neck geometry is an essential element in determining bone strength, and a number of recent studies have shown how it can affect the likelihood of fracture (Michelotti & Clark, 1999; Bergot et al., 2002; Crabtree et al., 2002). Considering that changes in geometric properties (linear measures and/or shape), happen particularly during growth and that childhood is also the most physically active period, the main purposes of this study were to analyse (1) the shape of proximal femur in pre-pubertal boys and girls and (2) the relationship between physical activity, body composition, and children´s proximal femur shape.

METHODS

Subjects. 48 pre-pubertal boys and 40 girls were drawn from schools (3rd grade) and sport clubs. None of the subjects were taking any medication affecting bone or reported a history of hip fracture. The University's internal Review approved the study.

Proximal femur analysis. Bone mineral content and BMD of left leg proximal femur were measured with DXA (Hologic, Waltham, MA, USA, pencil beam mode, version 4.76). In order to quantify shape differences among participants, 14 landmarks (x, y coordinates) were digitized on proximal femur DXA 2D images. The Relative Warp Analysis on this specimens was performed with TPSRelw 1.45 (Rohlf 2007) with the following parameters: $\alpha=0$; Scaling to centroid size = 1; Uniform component – complement; Projection method – orthogonal. The Regression analysis was performed with TPSRegr 1.34 (Rohlf 2007) with the following parameters: PCA align reference; Include uniform; Do multivariate tests; Scale Method = 1; Projection method – orthogonal. Data were analyzed using the SPSS statistical software package (Version 16.0 for Windows; SPSS, Chicago, IL, USA). Differences between groups were analysed by Independent-samples T-tests. In case of no normality comparisons were made by Mann-Whitney non-parametric tests.

Body size and body composition. Standing height was measured on a stadiometer (Secca 770, Hamburg, Germany) with subjects in underwear and without shoes. Body weight, total fat and total lean mass evaluations were determined from a total body scan by DXA (Hologic, Waltham, MA, USA, pencil beam mode, s version 5.73) with subjects fasted. Body mass index (BMI) was calculated as body weight in kilogram divided by height in meter squared.

Physical activity was assessed with the Actigraph accelerometer (model GT1M) over seven days and the activity data were sampled on a minute-by-minute basis. Subjects were excluded if they failed to provide a minimum of 3 days of at least 600 minutes per day of accelerometer data. The output from the programme included accumulated time spent at sedentary, light, moderate and vigorous physical activity intensity in minutes per day. The total amount of physical activity was expressed as total counts divided by registered time, that is, counts per minutes, which is an indicator of the total volume of physical activity and the numbers of minutes the child engaged in activity of different intensities. Cut points of 100, 1952, 5724 and 9498 counts/min were used to identify sedentary, light, moderate and vigorous physical activity.

Energy and calcium intake were calculated from a semi-quantitative Food Frequency Questionnaire assessing regular intake of a wide set of typical Portuguese foods.

Maturation level consisted of a skeletal age assessed with a portable x-ray (Ascott model, Kodak chassis 20x15), and determined by the Tanner-Whitehouse III Method (36).

Age and Body Composition

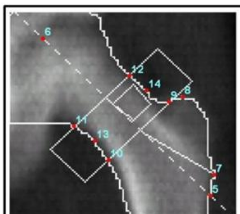
	Boys (n=48) Mean ± SD	Girls (n=40) Mean ± SD	P*
Age , y	8.6 ± 0.4	8.5 ± 0.4	0.423
Bone Age, y	9.0 ± 1.1	8.6 ± 1.2	0.178
Weight , kg	33.0 ± 8.0	29.8 ± 6.1	0.031
Height , cm	134.0 ± 7.0	131.9 ± 5.3	0.083
Body Mass Index, kg/m^2	18.1 ± 3.1	17.0 ± 2.6	0.079
Body Fat , kg	8.7 ± 5.2	8.2 ± 4.3	0.623
Body Lean Mass , kg	23.2 ± 3.1	20.6 ± 2.5	<0.001

Diet and Physical Activity

	Boys (n=48) Mean ± SD	Girls (n=40) Mean ± SD	P*
Calcium Intake, mg/d	1196 ± 77.7	1100.6 ± 70.8	0.226
Sedentary , min/d	878 ± 95	886.1 ± 62.1	0.323
Moderate PA , min/d	77.5 ± 3.4	55 ± 3.1	<0.001
Vigorous PA , min/d	10.3 ± 1.1	7 ± 1	<0.015
Total PA , count/min	702 ± 135	587.2 ± 152.4	<0.001
BMC Left Leg, g	185.0 ± 55.7	157.4 ± 43.8	0.012
BMD Left Leg, g/cm^2	0.894 ± 0.1	0.846 ± 0.1	0.006

*Independent Sample T-Tests. In cases of no normality, Mann-Whitney non-parametric tests were used; PA - physical activity; BMC - bone mineral content; BMD - bone mineral density

Proximal Femur DXA Image



Anatomical Landmarks	Description
1-2	Femoral shaft width*
3-4	Femoral intramedulary width **
5-6	Femoral axis length
7	Lower tip of greater trocanter
8	Upper tip of greater trocanter
9-10	Lower limit of femoral neck
11-12	Upper limit of femoral neck
13-14	Average distance of lower and upper femoral neck limits

*1.5cm under the lower limit of femoral neck axis.
** Width of medullary canal at the same level of 1-2 landmarks

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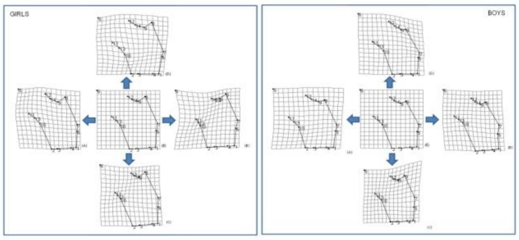
Relative Warp (RW) Analysis



Shape changes depicted by the RW1 and RW2. (A) Deformation relative to the mean shape toward the negative direction of RW1. (B) Deformation relative to the mean shape toward the positive direction of RW1. (C) Deformation relative to the mean shape toward the negative direction of RW2. (D) Deformation relative to the mean shape toward the positive direction of RW2. (E) consensus configuration.

Girls				Boys			
	SV	R^2 (%)	Cum %		SV	R^2 (%)	Cum %
RW$_1$	0.26442	30.38%	30.38%	RW$_1$	0.24658	32.89%	32.89%
RW$_2$	0.23432	23.86%	54.24%	RW$_2$	0.19815	21.24%	54.12%
RW$_3$	0.18952	15.61%	69.85%	RW$_3$	0.15568	13.11%	67.23%
RW$_4$	0.16342	11.61%	81.46%	RW$_4$	0.12456	8.39%	75.63%
RW$_5$	0.11183	5.43%	86.89%	RW$_5$	0.11432	7.07%	82.70%

S V: Singular Value ; R² (%): Percentage explained by each axis of shape variation; Cum %: Cumulative percentage of shape variation explained

Regression Analysis

Variable	Sum d^2 of predicted	R^2 (%)	Generalized Goodall F-Test		
			F	df	P
Age , y					
Boys	0.0058	3.1	1.484	24.11	0.063
Girls	0.0125	5.4	2.195	24.91	<0.001
Bone Age, y					
Boys	0.0173	9.4	4.757	24.11	<0.001
Girls	0.0149	6.5	2.653	24.91	<0.001
Weight , kg					
Boys	0.0074	4.0	1.928	24.11	0.005
Girls	0.0108	4.7	1.880	24.91	0.007
Height , cm					
Boys	0.0087	4.7	2.292	24.11	<0.001
Girls	0.0070	3.1	1.202	24.91	0.230
Body Mass Index, kg/m^2					
Boys	0.0089	4.8	2.328	24.11	<0.001
Girls	0.0106	4.6	1.840	24.91	0.008
Body Fat , kg					
Boys	0.0081	4.4	2.128	24.11	0.001
Girls	0.0094	4.1	1.631	24.91	0.029
Body Lean Mass , kg					
Boys	0.0055	3.0	1.411	24.11	0.091
Girls	0.0092	4.0	1.587	24.91	0.037

Variable	Sum d^2 of predicted	R^2 (%)	Generalized Goodall F-Test		
			F	df	P
Calcium Intake, mg/d					
Boys	0.0043	2.7	1.285	24.11	0.162
Girls	0.0040	1.8	0.682	24.91	0.872
Sedentary , min/d					
Boys	0.0035	1.9	0.900	24.11	0.603
Girls	0.0159	6.9	2.838	24.91	<0.001
Moderate PA , min/d					
Boys	0.0153	9.4	4.190	24.11	0.000
Girls	0.0069	3.1	1.195	24.91	0.236
Vigorous PA , min/d					
Boys	0.0026	1.4	0.672	24.11	0.881
Girls	0.0089	3.9	1.539	24.91	0.047
Total PA , count/min					
Boys	0.0044	2.4	1.132	24.11	0.299
Girls	0.0123	5.4	2.154	24.91	0.001
BMC Left Leg, g					
Boys	0.0088	4.8	2.321	24.11	<0.001
Girls	0.0134	5.8	2.366	24.91	<0.001
BMD Left Leg, g/cm^2					
Boys	0.0077	4.2	2.014	24.11	0.003
Girls	0.0126	5.5	2.221	24.91	<0.001

PA - physical activity; BMC - bone mineral content; BMD - bone mineral density

CONCLUSIONS

- **RW1 and RW2** accounted for 54% of the total variation of proximal femur shape in both genders.
- The main identified **shape variation** observed along the two relative warps was associated to the **width (mostly along RW1) and the hip axis length of femoral neck (mostly along RW2),** either for boys or girls.
- **Body composition variables** (body weight, fat and lean mass, and BMC or BMD), and bone age, explained 4-9% ($p\leq0.02$), of the proximal femur shape variation observed, also in both genders.
- Advanced **somatic maturity** was associated with a wider and longer proximal femur shape, either in both genders.
- Moderate **physical activity in boys and sedentary time in girls** were positively associated with a wider proximal femur, explaining 9% and 7% of proximal femur shape variation, respectively.

✓ **Proximal femur shape variation in 8-9 yrs old boys and girls** seemed to be mainly due to differences in femoral neck width and length, which could be in part determined by maturity level, expressed by bone age or body composition, but also by physical (in) activity.

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Sex Specific Associations of Physical Activity on Proximal Femur BMD in 9 to 10 Years Old Children

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INTRODUCTION

Studies conducted with pre and early pubertal subjects have reported augmented bone mineral accrual in several skeletal sites in both girls and boys after high-impact mechanical loading (running and jumping) interventions [1-7].

However, the loads imposed during sport and targeted exercise may represent the "best case scenario" and might not generalize to the everyday physical activity choices of children. There is evidence emerging to support the importance of physical activity during childhood independent of exercise interventions for achieving optimal bone mass in later life [8-9].

Yet, one of the most challenging issues concerning the proximal femur is the suspicion razed by some authors that boys' femoral neck is more sensitive to mechanical loading than girls' [10-16].

Thus, to provide rational for bone response differences to physical activity between boys and girls, the main purpose of this cross-sectional study was to investigate the gender specific association between everyday physical activity and mineralization of sub-regions of proximal femur, in boys and girls aged 9-10 years.

METHODS

Subjects. 325 children, aged 9–10 years (164 boys and 161 girls) living in the island of Madeira (Portugal) and drawn from the European Youth Heart Study (EYHS). Selection procedures were described previously [15]. None of the subjects were taking any medication affecting bone or reported a history of lower limbs fracture.

Physical activity was assessed with the Actigraph accelerometer (model WAM 6471) over 2 weekdays and 2 weekend days and the activity data were sampled on a minute-by-minute basis. Subjects were excluded if they failed to provide a minimum of 3 days of at least 600 minutes per day. Cut points of 500, 2000, and 3999 counts/min were used to identify accumulated time spent at sedentary, light, moderate and vigorous physical activity in minutes per day.

Proximal femur. BMD of femoral neck, trochanter and inter-trochanter were evaluated with DXA (QDR-1500, high-speed performance mode, software 4.76). The coefficient of variation of BMDs was < 2%. BMD ratios between these three sub-regions were also calculated as potential indicators of density homogeneity in the proximal femur.

Body size and composition. Standing height was measured on a stadiometer (Secca 770, Hamburg, Germany) with subjects in underwear and without shoes. Body weight, total fat and total lean mass evaluations were determined from a total body scan by DXA (QDR 1500, pencil beam mode, software 5.73, Hologic, Waltham, MA, USA) with subjects in a fasting state.

Maturation level was assessed using Tanner's 5-stage scale for breast development in girls and pubic hair in boys. Children were stratified as prepubertal (Tanner stage 1) or having started puberty (Tanner stage 2).

Age, Body Composition and PA

	Girls (n=161)	Boys (n=164)	P*
Age, y	9.7±0.3	9.7±0.3	0.780
Tanner stage (1/2), %	40 / 60	4 / 96	-
Weight, kg	34.2±9.0	34.1±7.8	0.960
Height, cm	137.2±0.1	137.0±0.1	0.813
Body Fat, kg	10.2±5.7	8.2±5.7	0.002
Body Lean Mass, kg	23.1±3.2	25.1±2.9	<0.001
Sedentary, min/d	984.1±77.3	963.9±89.8	0.030
Light PA, min/d	296.4±46.5	278.0±49.4	0.001
Moderate PA, min/d	141.5±47.3	168.5±55.1	< 0.001
Vigorous PA, min/d	17.9±14.3	29.5±21.3	< 0.001
Moderate and Vigorous PA, min/d	159.4±56.2	198.0±70.1	< 0.001
Total PA, min/d	455.8±77.3	476.1±89.8	0.030
PA Average Intensity, count/min/d	586.0±188.7	731.5±272.6	< 0.001

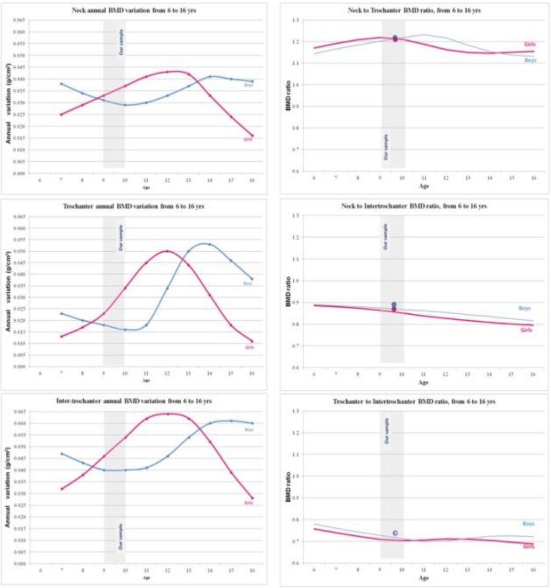
PA - physical activity *Student's t-test comparing boys to girls.

Bone Mineral Density

	Girls (n=161)	Boys (n=164)	P*
Proximal Femur BMD, g/cm^2	0.690 ± 0.070	0.753 ± 0.076	<0.001
Neck BMD, g/cm^2	0.656± 0.062	0.722 ± 0.071	<0.001
Trochanter BMD, g/cm^2	0.544 ± 0.060	0.591 ± 0.066	<0.001
Intertrochanter BMD, g/cm^2	0.762 ± 0.085	0.820 ± 0.089	<0.001
Neck / Trochanter BMD	1.210 ± 0.083	1.226 ± 0.081	0.095
Neck / Intertrochanter BMD	0.864 ± 0.052	0.883 ± 0.053	0.061
Trochanter / Intertrochanter BMD	0.716 ± 0.049	0.723 ± 0.052	0.678

BMD – bone mineral density * Student's t-test comparing boys to girls.

Proximal Femur Sub-Regions BMD and BMD Ratios (6-16 years old) *



*Our sample age range is represented by a grey area and BMD average values are plotted by dotted lines.

Partial Correlations*

	Neck		Trochanter		Intertrochanter		N/TR		N/IT		TR/IT	
	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
Sedentary Physical Activity	-0.158*	-0.065	-0.167*	-0.080	-0.170*	-0.053	0.025	0.051	0.053	0.001	0.024	-0.042
Light Physical Activity	0.063	-0.081	-0.046	-0.068	-0.028	-0.046	0.129	-0.008	0.125	-0.061	-0.034	-0.043
Moderate Physical Activity	0.147	0.091	0.230**	0.111	0.229**	0.062	-0.116	-0.067	-0.160*	0.030	-0.014	0.083
Vigorous Physical Activity	0.168*	0.228**	0.302**	0.212**	0.265**	0.172*	-0.174*	-0.026	-0.172*	0.061	0.031	0.068
Mod+Vig Physical Activity	0.166*	0.142	0.270**	0.153	0.260**	0.102	-0.142	-0.061	-0.178*	0.042	-0.005	0.086
Physical Activity Average Intensity	0.187*	0.188*	0.269**	0.182*	0.291**	0.126	-0.107	-0.038	-0.193*	0.073	-0.052	0.090

TR – trochanter; IT – intertrochanter; *p<0.05 ; **p<0.01 *Adjusted for maturity, body height, and lean body mass

Regression Analysis

	Physical Activity Predictor Variable	Girls			Physical Activity Predictor Variable	Boys		
		β	p	R^2 (%)		β	p	R^2 (%)
Neck BMD	-	-	-	-	Vigorous Physical Activity	0.225	0.003	5
Trochanter BMD	Vigorous Physical Activity	0.241	<0.001	6	Vigorous Physical Activity	0.214	0.005	5
Intertrochanter BMD	Vigorous Physical Activity	0.213	0.001	4	Vigorous Physical Activity	0.159	0.033	3
Neck/ Trochanter BMD	Vigorous Physical Activity	-0.172	0.029	3	-	-	-	-
Neck / Intertrochanter BMD	Moderate + Vigorous Physical Activity	-0.184	0.016	3	-	-	-	-

Note: only models where physical activity was significant are shown

Effects of 10 minutes PA on Proximal Femur Sub-Regions

	Δ BMD (%) associated with Δ 10 min/day Physical Activity					
	Girls			Boys		
	Neck	Trochanter	Intertrochanter	Neck	Trochanter	Intertrochanter
Moderate + Vigorous Physical Activity	ns	0.4	0.4	0.2	0.3	ns
Vigorous Physical Activity	ns	1.9	1.7	1.0	1.1	0.80

CONCLUSIONS

- ❖ **Vigorous physical activity** explained 3-5 % of the variability in BMD at all three regions in boys.
- ❖ In **girls**, vigorous physical activity explained 4% of the variability in intertrochanter BMD and 6% in trochanter BMD.
- ❖ Physical activity did not contribute to the variance in **femoral neck** differences in girls.
- ❖ An **additional 10 minutes per day of vigorous physical activity** was associated with a ~1% higher femoral neck, trochanter, and intertrochanteric BMD in boys (p<0.05) and a ~2% higher intertrochanteric and trochanteric BMD in girls.
- ✓ Everyday vigorous physical activity can be expected to contribute positively to **bone health** outcomes for boys and girls. However, the association of vigorous PA to regions of the proximal femur varies by gender with a greater homogeneous response in boys (when compared to girls). The lack of association between the femoral neck region and vigorous physical activity in girls may be related to gender-differences in the quality of everyday physical activity or the interaction of physical activity with hormonal or biomechanical factors may account for observed differences.

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