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Prepubertal Impact of Protein Intake and Physical Activity on Weight Bearing Peak Bone Mass and Strength in Males

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Abstract

Context: Peak bone mass (PBM) and strength are important determinants of fragility fracture risk in later life. During growth bone is responsive to changes in nutrition and physical activity (PA), particularly when occuring before pubertal maturation.

Objective: In prepubertal healthy boys, protein intake (Prot-Int) enhances the impact of PA on weight-bearing bone. We hypothesized that the synergism between Prot-Int and PA on proximal femur as recorded at mean age of 7.4 years would track until PBM.

Methods: 124 boys were followed from 7.4 to 15.2 and 22.6 years. At 7.4 years they were dichotomized according to the median (Med) of both PA and Prot-Int.

Results: In boys with PA > Med (310 vs 169 kcal.d^{-1}), higher vs low Prot-Int (57.7 vs 38.0 g.d^{-1}) was associated with +9.8% greater femoral neck (FN) BMC (*P=0.027*) at 7.4 years. At 15.2 and 22.6 years, this difference was maintained: FN BMC: +12.7% (*P=0.012*) and +11.3% (*P=0.016*), respectively. With PA > Med, in Prot-Int > vs < Med, the differences in FN BMC Z-scores were +0.60, +0.70 and +0.68 at 7.4, 15.2 and 22.6 years, respectively, and also associated with greater FN Width. Micro-finite element analysis of distal tibia at 15.2 and 22.6 years indicated that in the two groups with PA > Med, CSA, stiffness and failure load were greater in Prot-Int > vs < Med.

Conclusions: This study demonstrates the crucial influence of Prot-Int on the response to enhanced PA and the importance of prepubertal years for modifiying, by environmental factors, the bone growth trajectory and, thereby, for achieving higher PBM and strength in healthy male subjects.

Introduction

Bone structure and strength acquired by the end of the growth period is an important determinant of the risk of fragility or osteoporotic fracture in later life (1-4). For the sake of simplicity, the term "Peak Bone Mass" (PBM) is still used since it implicitly means the maximal values attained for both bone structure and stength in the first years of the third decade (3).

The objective of numerous studies was to identify the main determinants of PBM. Twin studies revealed that about 60 to 80 % of PBM variance were due to genetically or hereditary factors (5). Thus, the 20 to 40 % remaining variance appears to be determined by environmental factors among which there is evidence that both physical activity and nutrition play a major role (3).

Besides the relative contributions of the main determinants to PBM, an important issue was to identify when during growth bone would be most reactive to the impact of environmental factors. Counter-intuitively, peripuberty, i.e. during the years of accelerated bone acquistion, was not shown to be the most sensitive period. In contrast, during infancy and childhood, in other words before the onset of pubertal maturation, bone development was observed to be particularly responsive to either variations in physical activity or changes in nutrient consumption (for review see (3)).

Such an assessment would be of little practical interest if bone gain induced by modifiable environmental factors would only be transient, resulting in no protracted increase in skeletal structure and strength. However, follow-up studies have shown that bone gain acquired by modification of environmental factors before pubertal maturation can be maintained during several years. Thus we, in an eight-year follow-up study in healthy boys, recently reported that the positive impact of increased protein intake on physical activity tracks from prepuberty to mid-late adolescence (6,7).

In the present study, we further report on the tracking over a 15 years follow-up of the positive impact of protein intake on physical activity from prepuberty to young adulthood on both bone structure and strength of healthy males. This analysis emphasizes that early life alteration in environmental factors can affect key structural and mechanical characteristics of young adult weight-bearing bones.

Materials and Methods

Participants

The analysis was performed on data obtained in 124 healthy males with mean age of 22.6 ± 0.8 (± SD) years. These subjects belong to an initial cohort of 235 healthy prepubertal Caucasian boys recruited at a mean age of 7.4 ± 0.4 years (range 6.5-8.5 years) through the Public Health Youth Service of the Geneva district from September 1999 to September 2000 (8). Between 7.4 and 8.5 years, half of the cohort received a calcium supplementation as previously reported (8). Exclusion criteria were: ratio of weight to height below 3rd and above 97th percentile according to the Geneva reference values, presence of physical signs of puberty, chronic disease, gastro-intestinal disease with malabsorption, congenital or acquired bone diseases and regular use of medication. The 22.6 year old subjects presented in this report (n=124) were included in the group examined at the age of 7.4 years (n=232) (6) and 15.2 years (n=174) (7) regarding the relation between protein intake and physical activity. Their inclusion in the present analysis was merely determined by the fact these 124 subjects accepted the invitation to be examined at the age of 7.4, 15.2 and 22.6 years, thus allowing to calculate for each individual the progressive changes in their anthropometric, dietary and bone variables from prepuberty to mid-adolescence and to young adulthood. The Ethics Committees of both the Department of Pediatrics and the Department of Internal Medicine, Rehabilitation and Geriatrics of the Geneva University Hospitals approved the protocol. Informed consent was obtained from the parents and the children.

Clinical assessment

Body weight and standing height were measured using a Harpenden (Holtain, Crymych, UK) stadiometer and body mass index (kg/m^2) was calculated. All participants who were still sexually immature (63%) at the age of 15.2 years (7) became normally mature (Tanner stage 5) at the age of 22.6 years. From 7.4 to 22.6 years no disorder susceptible to affect the skeleton was detected among the 124 participants.

Intake of protein and calcium intake

At each visit, the consumption of protein and calcium was assessed by food frequency questionnaire as previously described (6,7). The total protein intake was computed from dairy products, meat, fish and egg. The calcium intake was essentially determined from dairy sources.

Physical activity assessment

To assess physical activity, we used a questionnaire based on self-reported time spent in physical education classes, organized sports, recreational activity, and usual walking and cycling (9). The data were then converted and expressed as physical activity energy expenditure (PAEE kcal/d) using established conversion formulae (10).

Bone measurements

A Hologic QDR 4500 instrument (Waltham, MA, USA) was used to measure by dual-energy x-ray absorptiometry (DXA) the areal bone mineral density (aBMD) and content (BMC) at the level of the femoral neck as previously reported (6,7). At this site, the coefficient of varation (CV) of repeated aBMD measurements determined in young healthy adults varied from 1.0-1.6 % (8).

Volumetric bone density (vBMD) and microstructure were measured at the distal tibia by high resolution-peripheral computerized tomography (HR-pQCT). The operated XtremCT instrument (Scanco Medical AG, Brütittsellen, Switzerland) acquired a stack of 110 parallel computerized tomography slices (9-mm length) with an isotropic voxel size of 82 µm, as previously described (11,12). The first CT slice at the distal tibia was 22.5 mm proximal to the reference line as described in a previous adult study (11). The following variables were measured: total, cortical, and trabecular volumetric bone density expressed as milligrams hydroxyapatite per cubic centimeter; trabecular bone volume fraction (BV/TV); trabecular number, thickness (micrometers), and spacing (micrometers); mean cortical thickness (micrometers); and cross-sectional area (CSA) (square millimeters). The in vivo short-term reproducibility of HR-pQCT at the distal tibia assessed in 15 subjects with repositioning varied by 0.6–1.0% and by 2.8–4.9% for bone density and for trabecular architecture, respectively. These reproducibility ranges are similar to those previously published (13). DXA and HR-pQCT measurements were usually performed in nondominant limb. One technician per device

performed all the scans. A daily quality control using a phantom was performed to check for possible drifts in the x-ray sources.

Finite element models of the tibia were created directly from the segmented HR-pQCT images using a procedure similar to that used in previous clinical studies (14-16). In summary, a voxel-conversion procedure was used to convert each voxel of bone tissue into an equally sized brick element (17), thus creating micro-finite element (μFE) models that can represent the actual trabecular architecture in detail. The models contained approximately 5 million elements for the tibia and could be solved in approximately 5 h. Material properties were chosen: isotropic and elastic. Both cortical and trabecular elements were assigned a Young's modulus of 10 GPa and a Poisson's ratio of 0.3 (15,18). A compression test was simulated to represent loading conditions during a fall from standing height (19). The estimated bone failure load was calculated as the force for which 2% of the bone tissue would be loaded beyond 0.7% strain (18,20). In addition to estimated failure load (N), μFEA-derived variables used in our study also included stiffness (kilo-Newtons per millimeter). In addition, an "apparent modulus" $\{N.mm^{-2}\}$ was calculated and defined as the stiffness multiplied by the height of the model (9 mm in all cases) and divided by the projected cross-sectional area. This measure thus provides information about the stiffness corrected for differences in height and cross-sectional area. All FEA were done using the FE solver integrated in the IPL software version 1.15 (Scanco Medical AG).

Expression of the results and statistical analysis

The various anthropometric, dietary, osteodensitometric, microstructural and mechanical variables are given as mean ± SD. All variables were also analyzed after segregation according to the median of both physical activity and protein intake assessed at 7.4 years by ANOVA analysis.

Anthropometric variables and the values of BMC, area, width and aBMD at the femoral neck as well as cross-sectional area, stiffness and failure load at the distal tibia were also expressed in standard deviation score (Z-scores) as computed from the measurements made in the whole cohort.

A two-way ANOVA was used to analyze the interaction between protein intake and physical activity on bone variables. The significance level for two-sided P-values was 0.05 for all tests. The data were analyzed using STATA software, version 9.2. (StataCorp LP, College Station, TX, USA).

Results

The normal age-related progression from prepuberty to mid-late adolescence and to young adulthood of anthropometric variables, dietary calcium and protein intakes, and DXA-acquired proximal femur measurements are presented in Table 1. At the age of 15.2 years, standing height and total FN BMC were 96.1 % and 86.9 % of the respective adult values measured at the age of 22.6 years (Table 1). Between mid-adolescence and young adulthood, the relative greater increase in body weight (+23.8%) than in standing height (+4.0%) was expressed by a 15.0 % progression in BMI (Table 1). At the age of 7.4 and 15.2 years, the total protein intake corresponded to 62.6 and 84.3 %, respectively, of the adult value (75.5 $g.d^{-1}$) (Table 1).

When aged 7.4 years, the cohort was dichotomized, according to the median (Med) of both physical activity (PA) and protein intake (Prot-Int). At 7.4 years, in the two groups with PA > Med (297 and 319 vs 170 and 166 $kcal.d^{-1}$), the impact of Prot-Int above vs below Med (57.7 vs 38.0 $g.d^{-1}$) was associated with +9.8 % greater FN BMC (*P=0.027*) (Table 2). At 15.2 and 22.6 y, this difference was maintained: FN BMC: +12.7% (*P=0.012*) and +11.3% (*P=0.016*) (Table 2), respectively. The impact of Prot-Int at PA>Med on FN bone mineral content was structurally combined with an increase in the width of the femoral neck (Table 2).

The impact of Prot-Int above vs below Med in groups with PA>Med was still associated with some gain in FN BMC from 7.4 to 15.2 years (*P=0.057*) but not from 15.2 to 22.6 years (*P=0.721*) (Supplemental Table 1). In contrast, the corresponding difference in FN width observed at 7.4 years was not further enhanced from 7.4 to 15.2 years and from 15.2 to 22.6 years (Supplemental Table 1). In PA > Med, the difference in FN BMC gain from 7.4 to 22.6 y (Δ = 354 mg) between Prot-Int above and below Med virtually equates that recorded between 7.4 and 15.2 (Δ = 363 mg) (Supplemental Table 1). Thus, in PA > Med, the influence of Prot-Int on FN BMC gain from prepuberty to young adulthood is already achieved by midpuberty.

In PA>Med, the differences in Z-score for FN BMC between Prot-Int above and below Med were 0.60, 0.70 and 0.68 at 7.4, 15.2 and 22.6 years respectively (Figure 1A). The corresponding differences in Z-score for FN width were 0.52, 0.45 and 0.52, respectively (Figure 1B). The

anthropometric Z-scores of the 4 groups distributed according to the median of both PA and Prot-Int are available online (Supplemental Table 2).

Most HR-pQCT measured microstructure variables in distal tibia increase from 15.2 to 22.6, except trabecular number and space, and CSA that remain stable (Table 3). Cortical thickness that increases by 67.5% from 15.2 to 22.6 years was the greatest relative change recorded among the measured microstructural components (Table 3). Strength variables generated by μFEA indicate that stiffness, estimated failure load and apparent modulus significantly increased by 26.7, 24.1 and 29.2 % from 15.2 to 22.6 years (Table 3).

At the age of 15.2 years, in the two groups with PA>Med, the impact of Prot-Int above rather than below Med was associated with greater trabecular number and CSA, with trend toward increase in both stiffness and estimated failure load (Table 4). From15.2 to 22.6 years in the two groups with PA > Med, the absolute differences in CSA, stiffness and estimated failure load between Prot-Int above and below Med did not increase further (Table 4). These results are illustrated by showing that the differences in Z-score of these three distal tibia strength-related variables observed at 15.2 years were maintained until young adulthood (Figures 2A, 2B, 2C).

Discussion

Main reported results

This study shows that, before pubertal maturation, environmental factors impacting on skeletal development can modify structural and strength components of young adult bone. This over 15 year prospective study, from 7.4 to 22.6 years, shows the tracking of interactive envionmental determinants of peak bone mass and strength in an homogenous cohort of healthy males. It complements and corroborates findings of two previous reports that describe the positive effect of protein intake on the bone response to higher physical activity (6,7). In these two previous analyses (6,7) made in boys from the same cohort as those of the foregoing report, the protein but not the calcium intake influenced the bone impact of increased physical activity.

Tracking of bone development

Bone structure tracks during growth until PBM (3,4,21), a phenomenon most often ascribed to the well-known genetic/heritabiliy determinant of PBM (5,22). Twin or parent-offspring studies show a predominant genetic influence on PBM. Familial resemblance in bone traits between premenopausal mothers and their daughters was shown to be expressed before the onset of pubertal maturation (23). Nevertheless, the trajectory of bone development can be influenced by factors other than those related to heritability.

Protracted influence of environmental factors impacting on bone development

Mechanical factors. The protracted bone impact until adulthood of enhanced mechanical loading during childhood was reported, particularly in young athletes involved in various competitive sports (for review see (3,24,25)).

Nutritional factors. A retrospective study suggests that milk intake during childhood and adolescence is positively associated with hip BMC in women aged 20-49 years (26).

There is evidence that before the onset of pubertal maturation, childhood years represent an opportune time to modify by lifestyle factors, including mechanichal loading and nutrition, the trajectory of bone development and thereby to favourably influence the structural and strength components of the so-called “peak bone mass“.

Biomechanical development of bone strength during growth

During childhood and adolescence, bone structure is altered in length and width. The main alteration in bone mass acquisition is an increase in size with relatively small change in volumetric density (see for review (25,27,28)). Longitudinal bone growth is determined by the rate of endochondral ossification, a process that continues throughout childhood and undergoes an acceleration during the pubertal growth spurt. In this crucial growth period, the change in the velocity of bone mass accumulation lags behind that of standing height (29). This asynchrony is clearly demonstrated in the foregoing prospective cohort study since, at 15.2 years, the percent of values measured at 22.6 years was about 10 % less for FN BMC (86.9 %) than for standing height (96.1%) (Table 1).

Variability in the individual responses to environmental factors

During growth, the bone response to either mechanical or nutritional intervention markedly varies from one child to another (for review see (30)). This variability suggests the involvement of genetic factors that might modulate the magnitude of the response to changes in environmental factors. This modulation could be related to bone gene polymorphisms, as suggested for the effects of either calcium supplement or exercise interventions (31-35). To our current knowledge, whether the bone response to protein intake might be associated to some genetic variants has not been reported.

Implication of increased bone mass and strength in early adulthood

The substantial upward shift in the bone development trajectory by amendable environmental factors is of special interest since its outcome is an increased PBM and strength. Such an effect may reduce the risk of osteporosis in later life, as bone features track not only during development, but also over several decades during adult life (2,4).

Protein vs calcium intake interaction with physical activity

As documented in two previous analyses made in subjects belonging to the same cohort at mean age of 7.4 and 15.2 years (6,7), the positive effect of protein on bone traits contrasts with the non influence of dietary calcium (from 546 to 971 $mg.d^{-1}$) in subjects with a relatively high degree of physical activity (310 vs. 169 $kcal.\ d^{-1}$). The positive effect of protein intake in healthy prepuberal children who are regularly engaged in relatively high physical activity is in keeping with several observational

or interventional studies carried out in adults of various ages combining resistance exercise and dietary protein ingestion (for reviews see (36,37)).

Structural modication and possible mechanism in response to protein intake

In subjects with relatively high physical activity, the bone structural changes with protein intake above vs below the median are detected at the proximal femur: an increased mineral content and width; and, in the distal tibia: an enhanced cross-sectional area and its derived perimeter without a reduction in the volumetric density. Therefore, on weight-bearing bone, the main consequence of combining relatively high physical activity with high protein intake is on bone size. Such structural alterations should confer greater mechanical resistance to loading (38). These structural/macroarchitectural "modeling" changes might be more stable throughout adult life than other modifications of bone variables undergoing more active "remodeling" such as the trabecular framework. Results from experimental intervention studies on the effects of either protein intake or physical exercise are compatible with the changes here reported. Protein intake increases bone growth in both longitudinal and cross-sectional dimensions. These effects could be mediated by IGF-I that stimulates bone growth in these two axial directions (for review see (3,39)). The effects of physical activity on several bone components (24) as well as the relationship between exercise training and IGF-I during chidlhood and adolescence (40) are also compatible with the bone structural alterations we report here.

Data generated by HR-pQCT and µFEA indicates that in the two groups with relatively high physical activity, the greater structural (increased CSA) and mechanical resistance (increased Stiffness and Failure Load) measured in the group with protein intake above than below the median at 15.2 years tend to be maintained at 22.6 years. Since these two environmental factors were recorded at a mean age of 7.4 years, it strongly suggests that key bone characteristics measured in young adulthood can be modified by environmental factors when applied early during skeleton development. These results recorded in this homogenous cohort of healthy males that was followed for more than 15 years are in keeping with the notion that prepuberty is an optimal time to intervene for improving bone health in the long term.

Strengths of the study

Several strengths can be mentioned: i) a cohort of more than 120 healthy boys followed from mean age 7.4 to 22.6 years with an intermediate examination at mid-adolescence; ii) an homogenenous group of participants as documented by a standard deviation of less than one year at 7.4, 15.2 and 22.6 years; iii) a 15 years follow-up including crucial years of bone development of weight-bearing bone from prepuberty to young adulthood once peak bone mass was attained; iv) measurements by DXA of proximal femur macrostructure combined with estimates of microstructure and strength by HR-pQCT and µFEA of distal tibia; v) highlighting of the interaction of two environmental factors, physical activity and protein intake, that positively modify the trajectory of bone development from childhood to young adulthood; vi) a plausible mechanistic hypothesis based on preclinical and clinical studies showing that protein intake enhances the impact of physical exercise on skeletal structure and strength.

Limitations of the study

Weaknessess which can be underlined are: i) both physical activity and protein intake were evaluated by frequency questionnaires, methods that cannot accurately measure the absolute energy expenditure due to physical activity or the actual amount of protein consumed. Nevertheless this method enables to compare intergroup differences in either physical activity or protein intake; ii) energy intake was not assessed in this cohort, therefore, the impact of protein intake on bone variables could be related to difference in energy intake. As previously discussed (7), the energy expenditure was measured by taking into account all kinds of physical activity and expressed in kcal/day. This measurement can be considered as reflecting the energy intake. Since the impact of relatively high vs low protein intake on bone mass, structure and strength was revealed in subjects spending the same amount of energy, it may be inferred that it was not related to difference in the overall energy intake but rather to difference in the protein intake; iii) another limitation is the non availability when this cohort was 7.4 years of the technology that was used later on at 15.2 and 22.6 years, that enables to assess microstructure and mechanical resistance characteristics of a weight-bearing long bone. Therefore it is not established that at 7.4 years of age the impact of protein intake on distal tibia variables was already present.

Conclusions

Structural and mechanical characteristics were measured by DXA, HR-pQCT and μFEA. Using these tools, the reported study carried out in a cohort of 124 healthy males prospectively followed from 7.4 to 22.6 years old demonstrates the crucial influence of protein intake in the response to enhanced physical activity on weigth-bearing bones such as proximal femur and distal tibia. The results further pinpoint the importance of prepubertal years for modifiying, through environmental factors, bone growth trajectory and, thereby, for achieving higher peak bone mass and strength in healthy male subjects.

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References

1. **Hernandez CJ, Beaupre GS, Carter DR** A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. *Osteoporos Int* 2003; 14(10)**:**843-847.
2. **Melton LJ, 3rd, Atkinson EJ, Khosla S, Oberg AL, Riggs BL** Evaluation of a prediction model for long-term fracture risk. *J Bone Miner Res* 2005; 20(4)**:**551-556.
3. **Bonjour JP, Chevalley T, Ferrari S, Rizzoli R** Peak Bone Mass and Its Regulation In: Glorieux FH, Pettifor FM, Jüppner H (eds.) Pediatric Bone,2012 2nd ed. Elsevier Inc, Amsterdam, pp 189-221.
4. **Farr JN, Khosla S** Skeletal changes through the lifespan--from growth to senescence. *Nat Rev Endocrinol* 2015; 11(9)**:**513-521.
5. **Eisman JA** Genetics of osteoporosis. *Endocr Rev* 1999; 20(6)**:**788-804.
6. **Chevalley T, Bonjour JP, Ferrari S, Rizzoli R** High-protein intake enhances the positive impact of physical activity on BMC in prepubertal boys. *J Bone Miner Res* 2008; 23(1)**:**131-142.
7. **Chevalley T, Bonjour JP, van Rietbergen B, Ferrari S, Rizzoli R** Tracking of environmental determinants of bone structure and strength development in healthy boys: an eight-year follow up study on the positive interaction between physical activity and protein intake from prepuberty to mid-late adolescence. *J Bone Miner Res* 2014; 29(10)**:**2182-2192.
8. **Chevalley T, Bonjour JP, Ferrari S, Hans D, Rizzoli R** Skeletal site selectivity in the effects of calcium supplementation on areal bone mineral density gain: a randomized, double-blind, placebo-controlled trial in prepubertal boys. *J Clin Endocrinol Metab* 2005; 90(6)**:**3342-3349.
9. **Boot AM, de Ridder MA, Pols HA, Krenning EP, de Muinck Keizer-Schrama SM** Bone mineral density in children and adolescents: relation to puberty, calcium intake, and physical activity. *J Clin Endocrinol Metab* 1997; 82(1)**:**57-62.
10. **Ainsworth BE, Haskell WL, Leon AS, Jacobs DR, Jr., Montoye HJ, Sallis JF, Paffenbarger RS, Jr.** Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* 1993; 25(1)**:**71-80.
11. **Boutroy S, Bouxsein ML, Munoz F, Delmas PD** In vivo assessment of trabecular bone microarchitecture by high-resolution peripheral quantitative computed tomography. *J Clin Endocrinol Metab* 2005; 90(12)**:**6508-6515.
12. **Chevalley T, Bonjour JP, van Rietbergen B, Ferrari S, Rizzoli R** Fractures during childhood and adolescence in healthy boys: relation with bone mass, microstructure, and strength. *J Clin Endocrinol Metab* 2011; 96(10)**:**3134-3142.
13. **MacNeil JA, Boyd SK** Accuracy of high-resolution peripheral quantitative computed tomography for measurement of bone quality. *Med Eng Phys* 2007; 29(10)**:**1096-1105.
14. **Boutroy S, Van Rietbergen B, Sornay-Rendu E, Munoz F, Bouxsein ML, Delmas PD** Finite element analysis based on in vivo HR-pQCT images of the distal radius is associated with wrist fracture in postmenopausal women. *J Bone Miner Res* 2008; 23(3)**:**392-399.
15. **Dalzell N, Kaptoge S, Morris N, Berthier A, Koller B, Braak L, van Rietbergen B, Reeve J** Bone micro-architecture and determinants of strength in the radius and tibia: age-related changes in a population-based study of normal adults measured with high-resolution pQCT. *Osteoporos Int* 2009; 20(10)**:**1683-1694.
16. **Vilayphiou N, Boutroy S, Sornay-Rendu E, Van Rietbergen B, Munoz F, Delmas PD, Chapurlat R** Finite element analysis performed on radius and tibia HR-pQCT images and fragility fractures at all sites in postmenopausal women. *Bone* 2010; 46(4)**:**1030-1037.
17. **van Rietbergen B, Weinans H, Huiskes R, Odgaard A** A new method to determine trabecular bone elastic properties and loading using micromechanical finite-element models. *J Biomech* 1995; 28(1)**:**69-81.
18. **Pistoia W, van Rietbergen B, Lochmuller EM, Lill CA, Eckstein F, Ruegsegger P** Estimation of distal radius failure load with micro-finite element analysis models based on

three-dimensional peripheral quantitative computed tomography images. *Bone* 2002; 30(6)**:**842-848.
19. **Chiu J, Robinovitch SN** Prediction of upper extremity impact forces during falls on the outstretched hand. *J Biomech* 1998; 31(12)**:**1169-1176.
20. **Mueller TL, Christen D, Sandercott S, Boyd SK, van Rietbergen B, Eckstein F, Lochmuller EM, Muller R, van Lenthe GH** Computational finite element bone mechanics accurately predicts mechanical competence in the human radius of an elderly population. *Bone* 2011; 48(6)**:**1232-1238.
21. **Wren TA, Kalkwarf HJ, Zemel BS, Lappe JM, Oberfield S, Shepherd JA, Winer KK, Gilsanz V, Bone Mineral Density in Childhood Study G** Longitudinal tracking of dual-energy X-ray absorptiometry bone measures over 6 years in children and adolescents: persistence of low bone mass to maturity. *J Pediatr* 2014; 164(6)**:**1280-1285 e1282.
22. **Ferrari S** Human genetics of osteoporosis. *Best Pract Res Clin Endocrinol Metab* 2008; 22(5)**:**723-735.
23. **Ferrari S, Rizzoli R, Slosman D, Bonjour JP** Familial resemblance for bone mineral mass is expressed before puberty. *J Clin Endocrinol Metab* 1998; 83(2)**:**358-361.
24. **Daly RM** The effect of exercise on bone mass and structural geometry during growth. *Med Sport Sci* 2007; 51**:**33-49.
25. **Kontulainen SA, Hughes JM, Macdonald HM, Johnston JD** The biomechanical basis of bone strength development during growth. *Med Sport Sci* 2007; 51**:**13-32.
26. **Kalkwarf HJ, Khoury JC, Lanphear BP** Milk intake during childhood and adolescence, adult bone density, and osteoporotic fractures in US women. *Am J Clin Nutr* 2003; 77(1)**:**257-265.
27. **Bonjour JP, Theintz G, Law F, Slosman D, Rizzoli R** Peak bone mass. *Osteoporos Int* 1994; 4 Suppl 1**:**7-13.
28. **Rizzoli R, Bonjour JP** Determinants of peak bone mass and mechanisms of bone loss. *Osteoporos Int* 1999; 9 Suppl 2**:**S17-23.
29. **Fournier PE, Rizzoli R, Slosman DO, Theintz G, Bonjour JP** Asynchrony between the rates of standing height gain and bone mass accumulation during puberty. *Osteoporos Int* 1997; 7(6)**:**525-532.
30. **Bonjour JP, Chevalley T, Rizzoli R, Ferrari S** Gene-environment interactions in the skeletal response to nutrition and exercise during growth. *Med Sport Sci* 2007; 51**:**64-80.
31. **Ferrari SL, Rizzoli R, Slosman DO, Bonjour JP** Do dietary calcium and age explain the controversy surrounding the relationship between bone mineral density and vitamin D receptor gene polymorphisms? *J Bone Miner Res* 1998; 13(3)**:**363-370.
32. **Kiel DP, Ferrari SL, Cupples LA, Karasik D, Manen D, Imamovic A, Herbert AG, Dupuis J** Genetic variation at the low-density lipoprotein receptor-related protein 5 (LRP5) locus modulates Wnt signaling and the relationship of physical activity with bone mineral density in men. *Bone* 2007; 40(3)**:**587-596.
33. **Lorentzon M, Eriksson AL, Nilsson S, Mellstrom D, Ohlsson C** Association between physical activity and BMD in young men is modulated by catechol-O-methyltransferase (COMT) genotype: the GOOD study. *J Bone Miner Res* 2007; 22(8)**:**1165-1172.
34. **Remes T, Vaisanen SB, Mahonen A, Huuskonen J, Kroger H, Jurvelin JS, Penttila IM, Rauramaa R** Aerobic exercise and bone mineral density in middle-aged finnish men: a controlled randomized trial with reference to androgen receptor, aromatase, and estrogen receptor alpha gene polymorphisms small star, filled. *Bone* 2003; 32(4)**:**412-420.
35. **Suuriniemi M, Mahonen A, Kovanen V, Alen M, Lyytikainen A, Wang Q, Kroger H, Cheng S** Association between exercise and pubertal BMD is modulated by estrogen receptor alpha genotype. *J Bone Miner Res* 2004; 19(11)**:**1758-1765.
36. **Phillips SM** A brief review of critical processes in exercise-induced muscular hypertrophy. *Sports Med* 2014; 44 Suppl 1**:**S71-77.
37. **Wall BT, Cermak NM, van Loon LJ** Dietary protein considerations to support active aging. *Sports Med* 2014; 44 Suppl 2**:**S185-194.

38. **Bouxsein ML** Determinants of skeletal fragility. *Best Pract Res Clin Rheumatol* 2005; 19(6)**:**897-911.
39. **Bikle DD, Tahimic C, Chang W, Wang Y, Philippou A, Barton ER** Role of IGF-I signaling in muscle bone interactions. *Bone* 2015; 80**:**79-88.
40. **Eliakim A, Nemet D** The endocrine response to exercise and training in young athletes. *Pediatr Exerc Sci* 2013; 25(4)**:**605-615.

Legends to Figures

Figure 1. Femoral neck (FN) BMC (A) and Femoral neck width (B) Z-scores measured at 7.4, 15.2 and 22.6 years according to physical activity (PA) and protein intake (Prot-Int) determined at 7.4 years. The cohort of 124 healthy boys was dichotomized according to the median of both PA and Prot-Int. The absolute values of FN BMC and FN Width of the 4 groups in relation to both PA and Prot-Int are presented in Table 2. The Z-score differences (Δ) with *P* levels for FN BMC and FN Width between the two groups with high PA and relatively either low or high Prot-Int are indicated above the brackets connecting the two corresponding bars.

Figure 2. Cross Sectional Area (CSA) (A), Stiffness (B) and Failure load (C) Z-scores of distal tibia measured at 15.2 and 22.6 years according to the physical activity (PA) and protein intake (Prot-Int) determined at 7.4 years. The cohort of 124 healthy boys was dichotomized according to the medians of both PA and Prot-Int. The absolute values of CSA, Stiffness and Failure load of the 4 groups in relation to both PA and Prot-Int are presented in Table 4. The Z-score difference (Δ) with *P* levels for CSA, Stiffness and Failure load between the two groups with high PA and relatively either low or high Prot-Int are indicated above the brackets connecting the two corresponding bars.

Table 1. Characteristics Assessed in 124 Healthy Boys at a Mean Age of 7.4, 15.2 ans 22.6 Years

Age (years)	**7.4 ± 0.4**	**15.2 ± 0.4**	**22.6 ± 0.8**	**Δ 7.4 to 15.2**	**Δ 15.2 to22.6**	**Δ 7.4 to 22.6**
Standing Height (cm)	125.7 ± 5.9	171.8 ± 8.4	178.7 ± 6.2	46.3 ± 4.7	6.9 ± 5.5	53.2 ± 4.3
Body Weight (kg)	25.3 ± 4.6	59.3 ± 11.1	73.4 ± 12.8	34.3 ± 8.2	14.1 ± 8.9	48.4 ± 10.3
BMI (kg/m^2)	15.9 ± 1.9	20.0 ± 2.7	23.0 ± 3.6	4.2 ± 2.0	3.0 ± 2.4	7.2 ± 3.0
Calcium Intake ($mg.d^{-1}$)	752 ± 263	1031 ± 569	949 ± 501	276 ± 531	-85 ± 513	194 ± 515
Proteins Intake ($g.d^{-1}$)	47.3 ± 11.6	63.7± 24.6	75.5 ± 27.0	16.6 ± 22.5	11.7 ± 30.7	28.5 ± 27.7
Total PA ($kcal.d^{-1}$)	242 ± 94	722 ± 378	738 ± 530	487 ± 359	15 ± 525	498 ± 516
FN BMC (mg)	2052 ± 337	4600 ± 803	5296 ± 839	2562 ± 662	680 ± 563	3250 ± 707
FN Area (cm^2)	3.00 ± 0.32	5.27 ± 0.43	5.65 ± 0.36	2.27 ± 0.37	0.38 ± 0.32	2.65 ± 0.35
FN Width (cm)	1.98 ± 0.21	3.49 ± 0.28	3.73 ± 0.24	1.50 ± 0.25	0.25 ± 0.21	1.75 ± 0.23
FN aBMD (mg/cm^2)	675 ± 72	872 ± 122	938 ± 135*	192 ± 92	63 ± 83	256 ± 108

All values are means ± SD. FN: Femoral neck variables assessed by DXA.
*Corresponds to a T-score = 0.06 ± 0.99, which is not significantly different from that used as for the clinical diagnosis of osteoporosis in the DXA unit of the University Hospitals of Geneva.

Table 2. Impact of Physical Activity (PA) and Protein Intake (Prot) Recorded at 7.4 Years on Anthropometric and Femoral Neck (FN) Characteristics Measured at 7.4, 15.2 and 22.6 Years

	(A) PA < Med Prot < Med (n = 40)	**(B) PA > Med Prot < Med (n = 26)**	**(C) PA < Med Prot > Med (n = 24)**	**(D) PA >Med Prot > Med (n = 34)**	***P***	****P***	***$^{+}$P***	***$^{\#}$P***
			7.4 YEARS					
Height (cm)	123.5 ± 5.2	123.9 ± 5.7	126.2 ± 5.3	128.5 ± 6.3	0.001	0.052	0.005	0.338
Weight (kg)	23.5 ± 3.1	24.1 ± 3.2	24.5 ± 3.1	27.9 ± 5.9	0.0001	0.241	0.005	0.064
BMI (kg/m^2)	15.4 ± 1.3	15.7 ± 1.6	15.3 ± 1.3	16.7 ± 2.2	0.003	0.912	0.054	0.086
FN BMC (mg)	1989 ± 303	1969 ± 233	2051 ± 314	2162 ± 384	0.066	0.435	0.027	0.262
FN AREA (cm^2)	2.96 ± 0.32	2.95 ± 0.33	2.95 ± 0.33	3.12 ± 0.30	0.088	0.877	0.045	0.125
FN WIDTH (cm)	1.96 ± 0.21	1.95 ± 0.33	1.95 ± 0.22	2.06 ± 0.20	0.088	0.877	0.045	0.125
FN aBMD (mg/cm^2)	672 ± 72	668 ± 62	696 ± 70	690 ± 79	0.384	0.194	0.249	0.931
			15.2 YEARS					
Height (cm)	169.4 ± 8.8	169.9 ± 9.4	172.1 ± 6.9	175.9 ± 6.7	0.005	0.212	0.006	0.264
Weight (kg)	55.3 ± 9.3	57.8 ± 9.9	59.5 ± 8.6	65.0 ± 13.2	0.002	0.077	0.023	0.443
BMI (kg/m^2)	19.1 ± 1.9	19.9 ± 2.3	20.2 ± 3.2	20.9 ± 3.2	0.049	0.112	0.192	0.978
FN BMC (mg)	4412 ± 769	4394 ± 837	4674 ± 683	4950 ± 803	0.013	0.174	0.012	0.305
FN AREA (cm^2)	5.16 ± 0.44	5.24 ± 0.47	5.25 ± 0.40	5.44 ± 0.38	0.045	0.433	0.080	0.483
FN WIDTH (cm)	3.41 ± 0.29	3.47 ± 0.31	3.47 ± 0.26	3.60 ± 0.25	0.045	0.433	0.080	0.483
FN aBMD (mg/cm^2)	850 ± 110	836 ± 124	892 ± 108	916 ± 134	0.032	0.141	0.021	0.381
			22.6 YEARS					
Height (cm)	177.6 ± 5.7	176.6 ± 5.5	178.2 ± 6.0	181.7 ± 6.6	0.006	0.717	0.003	0.041
Weight (kg)	69.8 ± 9.4	70.7 ± 8.0	74.0 ± 13.4	79.8 ± 16.4	0.005	0.154	0.014	0.279
BMI (kg/m^2)	22.1 ± 2.5	22.7 ± 2.7	23.3 ± 4.3	24.1 ± 4.6	0.110	0.152	0.172	0.872
FN BMC (mg)	5049 ± 750	5118 ± 768	5363 ± 738	5694 ± 940	0.006	0.108	0.016	0.383
FN AREA (cm^2)	5.61 ± 0.36	5.59 ± 0.31	5.59 ± 0.34	5.77 ± 0.38	0.127	0.762	0.055	0.109
FN WIDTH (cm)	3.71 ± 0.24	3.70 ± 0.21	3.69 ± 0.23	3.82 ± 0.25	0.127	0.762	0.055	0.109
FN aBMD (mg/cm^2)	901 ± 129	916 ± 128	960 ± 117	986 ± 148	0.037	0.071	0.066	0.824

Values are means ± SD. *P* by ANOVA analysis among all groups**P* comparison among groups A and C; ^{+}P comparison among groups B and D; $^{\#}P$ interaction between physical activity and protein intake

Table 3. Microstructure and Strength of Distal Tibia Measured at 15.2 and 22.6 Years in 124 Healthy Boys

	15.2 Years	**22.6 Years**	**Δ 15.2 to 22.6 Years**	***P***
Total vBMD (mg HA/cm^2)	267.5 ± 45.3	347.2 ± 46.3	79.3 ± 31.3	<0.0001
Cortical vBMD (mg HA/cm^2)	731.8 ± 55.0	884.2 ± 29.1	152.4 ± 50.5	<0.0001
Trabecular vBMD (mg HA/cm^2)	201.0 ± 27.4	223.3 ± 28.8	22.2 ± 13.5	<0.0001
BV/TV (%)	16.7 ± 2.3	18.6 ± 2.4	1.9 ± 1.1	<0.0001
Tb.N (mm^{-1})	2.09 ± 0.28	2.12 ± 0.30	0.03 ± 0.17	0.441
Tb.Th (μm)	80.8 ± 11.0	88.7 ± 10.8	7.6 ± 8.0	<0.0001
Tb.Sp (μm)	406.0 ± 59.1	393.0 ± 63.9	-13.7 ± 38.4	0.098
Ct.Th (μm)	825.0 ± 323.6	1384.2 ± 292.1	557.4 ± 264.3	<0.0001
CSA (mm^2)	873.5 ± 144.1	853.4 ± 135.4	-16.5 ± 56.1	0.261
Stiffness (KN/mm)	251.4 ± 51.9	317.9 ± 45.6	66.7 ± 32.9	<0.0001
Estimated failure load (N)	12035 ± 2396	14930 ± 2132	2916 ± 1429	<0.0001
Apparent modulus (N/mm^2)	2160 ± 424	2790 ± 391	630 ± 307	<0.0001

Values are means ± SD. BV/TV, trabecular bone volume fraction; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; Ct.Th, cortical thickness; CSA, cross sectional area.;

Δ: Difference between values at 15.2 and 22.6 years;

P: Comparison among values at 15.2 and 22.6 years.

Table 4. Impact of Physical Activity (PA) and Protein Intake (Prot) Recorded at a Mean Age of 7.4 Years on Microstructure and Strength of Distal Tibia Measured at 15.2 and 22.6 Years in 124 Healthy Boys

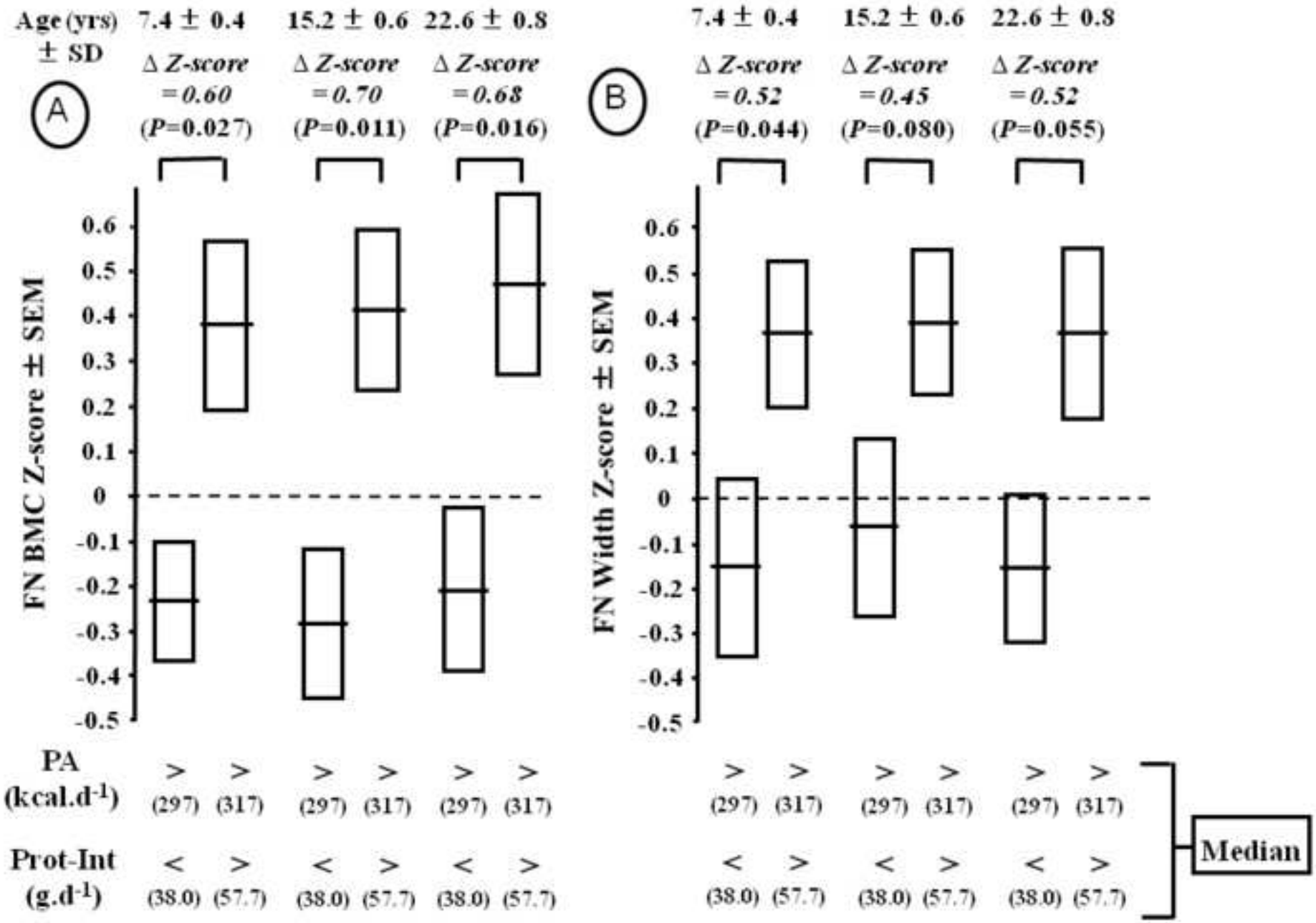
	Group A PA< PROT< (N=40)	**Group B PA > PROT < (N=26)**	**Group C PA < PROT > (N=24)**	**Group D PA> PROT > (N=34)**	***P***	****P***	***$^{+}$P***	***$^{\#}$P***
				15.2 YEARS				
Total vBMD (mg HA/cm^2)	245 ± 46	264 ± 54	276 ± 43	267 ± 40	0.747	0.326	0.746	0.652
Cortical vBMD (mg HA/cm^2)	726 ± 56	736 ± 54	739 ± 54	731 ± 56	0.807	0.370	0.765	0.397
Trabecular vBMD (mg HA/cm^2)	199 ± 27	195 ± 31	205 ± 25	205 ± 27	0.413	0.318	0.187	0.745
BV/TV (%)	16.6 ± 2.2	16.3 ± 2.6	17.1 ± 2.1	17.1 ± 2.2	0.415	0.320	0.188	0.748
Tb.N (mm^{-1})	2.04 ± 0.23	2.00 ± 0.23	2.10 ± 0.25	2.21 ± 0.35	0.015	0.312	0.012	0.150
Tb.Th (μm)	81.6 ± 10.5	81.6 ± 12.0	82.3 ± 10.9	78.3 ± 10.9	0.474	0.795	0.262	0.314
Tb.Sp (μm)	415 ± 50	424 ± 57	402 ± 61	385 ± 65	0.044	0.344	0.016	0.219
Ct.Th (μm)	798 ± 324	832 ± 367	878 ± 321	814 ± 298	0.809	0.339	0.828	0.409
CSA (mm^2)	833 ± 126	854 ± 120	847 ± 111	955 ± 172	0.001	0.664	0.013	0.083
Stiffness (KN/mm)	240 ± 52	246 ± 51	255 ± 50	267 ± 53	0.153	0.271	0.118	0.712
Estimated failure load (N)	11497 ± 2364	11711 ± 2268	12153 ± 2233	12883 ± 2512	0.085	0.277	0.070	0.554
Appar. modulus (N/mm^2)	2159 ± 439	2146 ± 467	2239 ± 415	2113 ± 384	0.747	0.478	0.769	0.478
				22.6 YEARS				
Total vBMD (mg HA/cm^2)	342 ± 51	342 ± 45	360 ± 41	348 ± 46	0.439	0.146	0.616	0.485
Cortical vBMD (mg HA/cm^2)	883 ± 24	883 ± 29	894 ± 27	879 ± 36	0.252	0.103	0.573	0.140
Trabecular vBMD (mg HA/cm^2)	219 ± 28	219 ± 30	228 ± 27	228 ± 30	0.405	0.218	0.260	0.985
BV/TV (%)	18.3 ± 2.3	18.3 ± 2.5	19.0 ± 2.2	19.0 ± 2.5	0.406	0.218	0.261	0.989
Tb.N (mm^{-1})	2.06 ± 0.26	2.06 ± 0.21	2.18 ± 0.27	2.19 ± 0.40	0.128	0.083	0.118	0.869
Tb.Th (μm)	89.4 ± 11.8	89.0 ± 9.7	87.8 ± 8.8	88.2 ± 11.9	0.929	0.552	0.780	0.830
Tb.Sp (μm)	403 ± 56	403 ± 51	379 ± 64	383 ± 79	0.304	0.115	0.270	0.851
Ct.Th (μm)	1346 ± 282	1372 ± 266	1439 ± 272	1401 ± 339	0.644	0.199	0.725	0.550
CSA (mm^2)	834 ± 131	845 ± 110	821 ± 104	907 ± 166	0.055	0.666	0.104	0.122
Stiffness (KN/mm)	308 ± 37	314 ± 39	318 ± 49	333 ± 54	0.123	0.344	0.131	0.593
Estimated failure load (N)	14487 ± 1767	14686 ± 1741	14915 ± 2196	15654 ± 2611	0.119	0.399	0.109	0.488
Appar. modulus (N/mm^2)	2759 ± 440	2786 ± 372	2887 ± 368	2761 ± 366	0.598	0.239	0.798	0.294

Values are means ± SD. BV/TV, trabecular bone volume fraction; Tb.N, trabecular number; Tb.Sp, trabecular separation; Tb.Th, trabecular thickness; Ct.Th, cortical thickness; CSA, cross sectional area.

P by ANOVA analysis among all groups; **P* comparison among groups A and C; $^{+}$*P* comparison among groups B and D; $^{\#}$*P* interaction between physical activity and protein intake

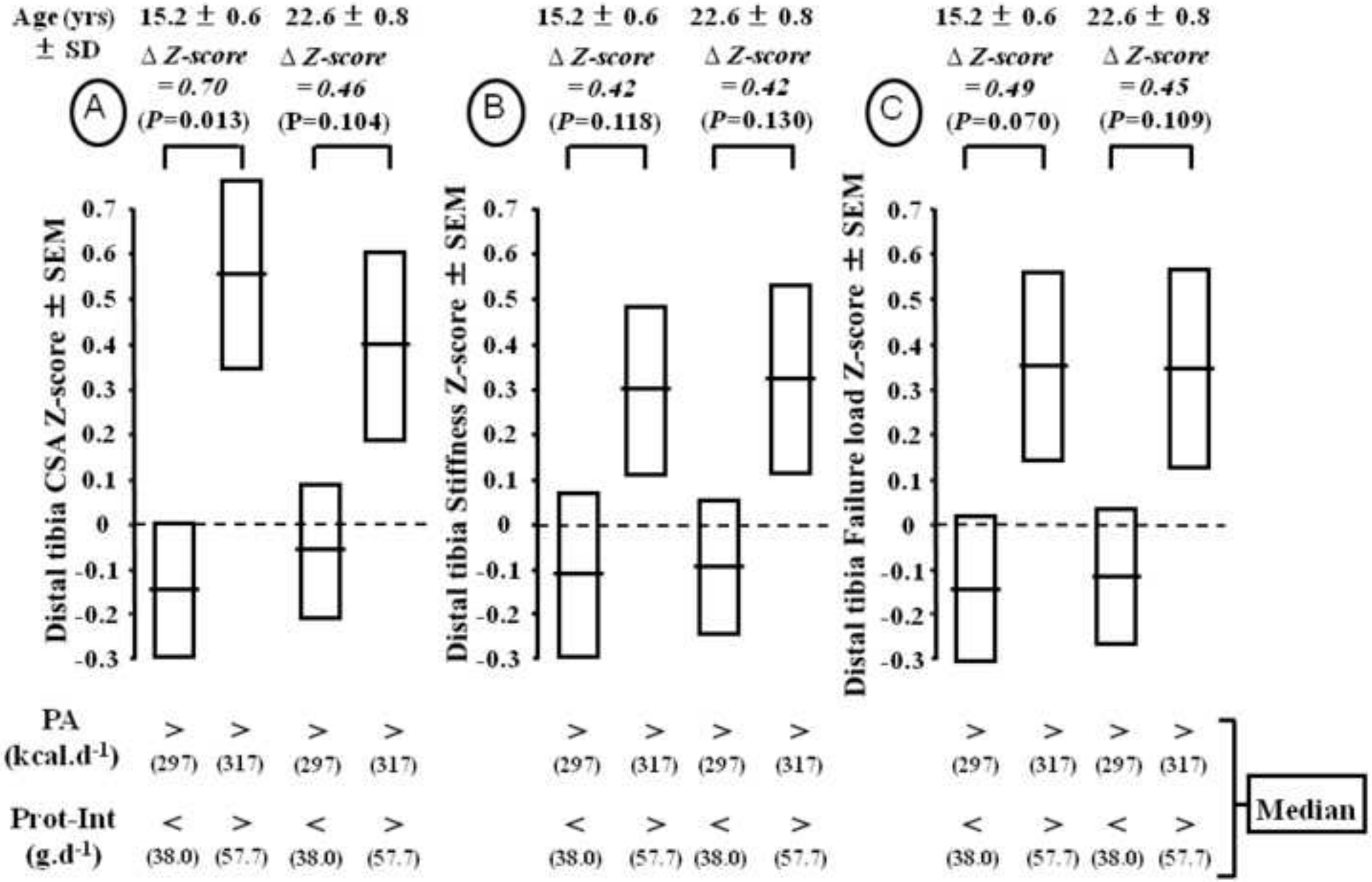
Figure

Fig. 1


Age (yrs) ± SD
7.4 ± 0.4
15.2 ± 0.6
22.6 ± 0.8
A
Δ Z-score = 0.60 (P=0.027)
Δ Z-score = 0.70 (P=0.011)
Δ Z-score = 0.68 (P=0.016)
FN BMC Z-score ± SEM
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
B
Δ Z-score = 0.52 (P=0.044)
Δ Z-score = 0.45 (P=0.080)
Δ Z-score = 0.52 (P=0.055)
FN Width Z-score ± SEM
PA (kcal.d-1)
Prot-Int (g.d-1)
(297)
(317)
(38.0)
(57.7)
Median

Figure

Fig. 2


Age (yrs) ± SD
15.2 ± 0.6
22.6 ± 0.8
Δ Z-score = 0.70 (P=0.013)
Δ Z-score = 0.46 (P=0.104)
Δ Z-score = 0.42 (P=0.118)
Δ Z-score = 0.42 (P=0.130)
Δ Z-score = 0.49 (P=0.070)
Δ Z-score = 0.45 (P=0.109)
A
B
C
Distal tibia CSA Z-score ± SEM
Distal tibia Stiffness Z-score ± SEM
Distal tibia Failure load Z-score ± SEM
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
PA (kcal.d-1)
Prot-Int (g.d-1)
(297) (317) (297) (317)
(38.0) (57.7) (38.0) (57.7)
Median