Relation of Physical Activity and Sex Steroid Hormones to Total Body Bone Area and Mass in Premenarcheal Girls

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Master's Thesis in Sports Medicine

Department of Health Sciences

University of Jyväskylä

December 2003

Manuscript

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Running title: Physical activity, sex hormones and bone

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Abstract

Associations of physical activity and sex hormones with total body bone area, mass, and density were studied in 10-12 year-old, premenarcheal, Finnish girls (n=216). Physical activity (PA) level was evaluated by self-reported questionnaire. Serum estradiol (E2) and testosterone (Ts) concentrations were analysed using immunofluorometry. Bone mass (BMC), bone area and bone mineral density (BMD) were measured with a dual energy x-ray absorptiometry (Prodigy, GE Lunar). Based on the PA scores and levels of hormone concentrations, subjects were divided into low, moderate and high groups. The results showed that, at Tanner stage I, the high active girls had higher BMC and BMD than the low active girls ($p=0.003$ and $p=0.001$). At Tanner stage II, PA was not associated with bone properties. Associations of sex hormones with bone properties were seen, at both maturational stages. However, when body weight and height were controlled, associations diminished markedly, especially at Tanner stage II. An interaction was found between the PA and serum free estradiol (fE2) concentration in BMD ($p=0.048$) at Tanner stage I. The high active girls with high fE2 had higher BMD than high active girls with low or moderate fE2, and the low active girls. In conclusion, there seemed to be a synergistic relation of fE2 and PA to BMD in prepubertal girls. The relation of sex steroid hormones to total body bone properties seemed to be covered by the strong relationship between sex steroid hormones and body weight and height, especially with early pubertal girls.

Key words:
Physical Activity, Sex Steroid Hormones, Premenarcheal Girls, Bone, Dual-energy X-ray absorptiometry

Introduction

Bone growth is very active during adolescence. Bone mass is accumulated by periosteal expansion and linear growth, [8, 35] thus about 37 % of adult total body bone mineral mass is achieved during the adolescent growth spurt [14]. During this period, the neuroendocrine function of the hypotalamus, pituitary gland and gonads accelerate and the restrictive effect of sex hormones on the production of gonadotropins reduces which leads to the increased production of sex hormones in the gonads. Increasing serum sex hormone concentrations with increased growth hormone production play a crucial role in bone growth [6, 30].

In girls during childhood, serum estradiol concentrations are low, usually at or below the detection level. Between the sixth and ninth year of life, hypothalamic action increases slightly effecting the serum estradiol concentration. In prepuberty, serum estradiol concentrations are usually <26-73 pmol/l, increasing at Tanner stage II to <26-129 pmol/l. After the onset of puberty, serum estradiol concentrations increase rapidly until they reach the values of adult women (44-918 pmol/l) at Tanner stage V. Before puberty, serum estradiol concentrations have a diurnal rhythm according to the fluctuation of the gonadotropins. Approximately one year after menarche, the regular menstrual cycle controls the fluctuation of gonadotropins and furthermore, estradiol production [11, 29]. In girls, serum testosterone concentrations during childhood are also at or below the detection limit. Prepubertal girls at Tanner stage I usually have serum testosterone concentrations 100-300 pmol/l. At Tanner stage II, serum concentrations values are 200-1000 pmol/l rising thereafter up to the values of adult women (600-2400 pmol/l) at Tanner stage V [2, 18, 39]. The secretion of testosterone follows a diurnal rhythm [1].

It is generally believed that the most important role of estradiol on bone growth is to reduce the rate of bone turnover by inhibiting osteoclastic resorption[8] while the primary function of testosterone is to induce the growth of long bones at the beginning of the growing years[32]. Testosterone is also a source for β-estradiol through the entzymatic aromatization in peripheral tissues [27, 36]. At the end of the growing period, the interrelationships between growth hormone and sex steroid hormones lead to the ossification of long bone epiphyses[32].

Prepubertal and early pubertal years are the most effective time to increase bone mass by exercising [13, 15, 25]. It is shown that, in particular, bone loading types of exercise lead to the osteogenic accumulation and gain of bone mass [5, 12, 20, 28]. However, intensive exercise and training require increases in energy expenditure and metabolism. Overtraining and/or eating disorders may lead to low body weight and low calcium intake, and consequently to average, low sex hormone production. Furthermore this would lead to delay in physical maturation and low bone mineral density [3, 22, 34].

The present cross-sectional investigation aimed to study the association of physical activity and serum sex steroid hormones to total body bone area, mass, and density in the two phases of bone development according to Tanner stages in premenarcheal girls. Additionally, possible interactions between physical activity and serum sex hormones to total body bone area, mass, and density were studied.

Materials and methods

Subjects

Study subjects were 216 healthy Finnish girls aged 10-12 years who enrolled in an intervention trial to study the effect of exercise, calcium, vitamin D, and milk product supplementation on bone growth during puberty (Calex-study). To be eligible for the study, subjects had to be at maturational stage I-II (Tanner stage), have no medical history of diseases or be taking any medication that could influence bone metabolism. All the subjects provided an informed consent form signed by themselves and their parent or legal guardian, in accordance with the Ethical Committees of the University of Jyväskylä, the Central Hospital of Central Finland and the Finnish National Agency of Medicines.

Measurements of physical characteristics

Weight and height were determined with the subject lightly clothed and shoeless. Weight was measured using an electronic scale and recorded to the 0.1 kg, and height with the standardized wall mounted stadiometer to the nearest 0.5 cm. The body mass index (BMI) was then calculated as the ratio of body weight (kg) / height (m^2). Maturational stage was evaluated together by the girl and the study nurse, using Tanner's criteria [23]. To make the evaluation easier for the subjects, the 5 stages of development of pubic hair and breasts were shown to them pictorially. If there was a disagreement between pubic hair and breasts, the decision was made according to the development of breasts.

Assessment of physical activity

A self-administered physical activity questionnaire was used to evaluate the intensity, type, duration and frequency of leisure-time physical activity. The questionnaire was modified from

the questions used in a WHO study [16]. Girls filled in the questionnaire with their parents' help at the research laboratory. The questionnaire asked the girls what were the first, second, and third favourite exercises they were practising, the duration of exercise in each session, and the frequency of exercises per week. The intensity of exercise was calculated on the basis of energy expenditure [24], and all of the sports were classified as either weight bearing or non-weight bearing exercise to determine bone loading. A score for physical activity was calculated as follows:

Score of physical activity = $\Sigma_{1\text{-}3}$ (frequency*intensity index*duration*loading)

Where frequency = times/week, intensity index = energy expenditure,

Duration = hours/session, loading: non-weight bearing = 1 and weight bearing = 2.

Assessments of serum sex steroid hormones and sex hormone binding globulin

Blood samples were taken from 7 to 9 a.m. after 12 hours fasting. Samples were centrifugated and divided to aliquots, which were stored at -70 degrees C. Serum testosterone (Ts), 17β-estradiol (E2) and sex hormone binding globulin (SHBG) were assessed using time resolved fluoroimmunoassay (Delfia^{R}, Wallac Oy, Turku, Finland). Before the SHBG assessments, samples were diluted 10 times. The precision of repeated measurements was expressed as coefficient of variance (CV %). An interassay CV was 5.21 % for E2, 9.4 % for Ts, 1.11 % for SHBG, and an intra-assay CV 5.13 %, 9.2 %, and 1.12 % respectively. Free estradiol (fE2) was calculated using the following formula:

Free estradiol (pmol/l) = estradiol(nmol/l) * 1000/(0.68 * SHBG(nmol/l) + 1) [1].

Free testosterone (fTs) was estimated using the formulas:

proportion of free testosterone (fT%) = 2.28-1.38 * log (SHBG (nmol/l)/10), and

serum free testosterone (pmol/l) = fT% * testosterone(nmol/l) * 10 [31].

Total body bone measurements

Bone projectional area (cm^2), bone mineral content (BMC, g), and areal bone mineral density (BMD, g/cm^2), were measured using a dual-energy X-ray absorptiometry (DXA, Prodigy, GE Lunar Corp., Madison, WI, USA). The precision of repeated measurements expressed as CV % were 1.2 % for area, 0.72 % for BMC, and 0.86 % for BMD.

Calcium intake

The daily calcium intake (mg/day) of each subject was evaluated from a self-reported three-day food record. The food diary included two weekdays and one weekend day.

Statistical analysis

The means and standard deviations were used as descriptive statistics for physical characteristics, bone properties and calcium intake. Independent samples t-test was used to assess the differences in physical characteristics, bone properties and calcium intake at different physical development stages. Sex hormone concentrations were described with means, standard errors and 95% confidence intervals. The non-parametric Kruskal Wallis test was used to detect differences between the development stages in sex hormone concentrations because they were not normally distributed among groups. Study subjects were divided into three groups (low, moderate and high) according to their levels of physical activity. Similarly, they were divided into low, moderate and high concentration groups by serum E2, fE2, Ts, and fTs concentration separately in Tanner stage I and II. One-way analysis of variance (ANOVA) was used to detect differences in bone variables between three physical activity groups and between different hormone concentration groups. Two-way ANOVA was used to test the interactions in bone area, BMC and BMD between three physical activity groups and different sex hormone groups. Body size index, calculated by principal component analysis

for weight and height was used as a covariate. The least square difference (LSD) test was used for multiple comparisons and a p-value of less than 0.05 was considered statistically significant in all of the analyses. Statistical analyses were carried out using SPSS version 11.0 for Windows.

Results

Physical characteristics

Body composition and calcium-intake of the whole study group are given in Table 1. Girls at Tanner stage II were older, heavier and taller and they had higher BMI, and more lean and fat mass compared to girls at Tanner stage I ($p<0.001$). Also, total body bone area was larger and total body BMC and BMD were higher in girls at Tanner stage II compared to the girls at Tanner stage I ($p<0.001$). Calcium-intake was similar between the groups ($p=0.814$).

Insert Table 1 here

Serum concentrations of sex hormones and SHBG for the study groups are given in Table 2. Sex hormone concentrations were higher in girls at Tanner stage II compared to the girls at Tanner stage I ($p<0.001$). On the contrary, the concentration of SHBG was higher at Tanner stage I compared to stage II ($p<0.001$).

Insert Table 2 here

Physical activity

The most common physical activities in which the girls participated in summer were swimming (29,7%), bicycling (25,3%), and walking (5,8%) and in winter ice-skating (25.8%), cross-country skiing (21.8%), and downhill skiing (11.7%). When detecting the girls in the three PA groups at Tanner stage I, it was seen that the high PA group had significantly higher BMC and BMD compared to the low PA group (p=0.003 and p=0.001, respectively) (Table 3). At Tanner stage II, no significant differences were found in any of the measured bone variables between different PA groups. Age, weight, height and BMI were similar in the PA groups in both Tanner stages.

Insert Table 3 here

Estradiol and free estradiol

At Tanner stage I there were no significant differences between the three E2 groups in any of the measured bone variables (Table 4). While at Tanner Stage II girls in the high E2 concentration group had significantly higher BMD compared to girls in the low E2 concentration group (p=0.006). After controlling analyses for the body size and age, no significant differences remained between the E2 groups.

When girls were divided into three groups by the level of fE2, it was found that at Tanner stage I the high fE2 group had significantly larger bone area (p=0.014, p<0.001), and higher BMC (p=0.002, p<0.001) and BMD (p=0.003, p<0.001) than the girls in the moderate and low fE2 groups. At Tanner stage II the high fE2 group had significantly larger bone area and higher BMC and BMD values than girls in the low fE2 group (p=0.006, 0.002 and 0.006 respectively), and higher BMC (p=0.049) and BMD (p=0.038) than girls in the moderate

fE2 group. After controlling the body size and age in the analyses at Tanner stage I the significant differences between the low and high fE2 groups and moderate and high fE2 groups in BMC (p=0.020, p=0.017,) and BMD (p=0.005, p=0.020) remained. On the contrary, at Tanner stage II the significant differences between the fE2 groups had disappeared.

Insert Table 4 here

Testosterone and free testosterone

When differences in total body bone variables were detected between three Ts groups it was seen at both Tanner stages that the low Ts group had significantly lower values in bone area, BMC and BMD compared to the moderate and high Ts groups (Table 4). Results were similar in the fTs groups compared with Ts groups. After controlling analyses with the body size at Tanner stage I, the significant differences in bone area, BMC and BMD remained only between the low and moderate Ts groups (p=0.001, p<0.001, p=0.002, respectively) and fTs groups (p=0.014, p=0.004, p=0.005, respectively), but disappeared between the low and high Ts and fTs groups. Unexpectedly, after adjusting analyses for the body size, the estimated BMC and BMD values were higher in the moderate Ts group compared to the high Ts group (1297g versus 1243g, p=0.021, and 0.943g/cm^2 versus 0.919g/cm^2, p=0.024, respectively). At Tanner stage II, after adjusting analyses for body size, the only significant difference that remained was between low and moderate fTs groups in BMC (p=0.013).

Combined relations of sex hormones and physical activity

When two-way ANOVA was used to detect possible interactions between PA and hormone concentrations to bone area, BMC and BMD, no interactions were found between E2, Ts or fTs and PA groups. However, there was an interaction between PA and fE2 in BMD at

Tanner stage I (p=0.048, Fig 1). Girls in the high PA group with high fE2 concentrations had significantly higher BMD than high active girls in moderate (p=0.02) or low (p=0.026) fE2 groups. High active girls with high fE2 concentration also had higher BMD than low active girls with high fE2 concentration (p=0.010). The same trend was seen in BMC between fE2 and PA, but significant interaction was not found.

There was an opposite trend between Tanner stages I and II in respect of PA and fTs to BMD. Girls with high fTs tended to have higher BMD values with increases in PA levels at Tanner stage I. On the contrary, girls with high fTs tended to have lower BMD in high PA levels at the Tanner stage II (results not shown).

Insert Figure 1 here

Discussion

Our purpose was to study the association of physical activity and serum sex steroid hormones to total body bone area, mass, and density in the two phases of bone development according to Tanner stages in premenarcheal girls. In our study, physical activity appeared to have a positive relation to total body BMC and a real BMD in prepubertal girls. On the other hand, a similar relation was not found in early pubertal girls. Serum sex hormone concentrations were associated with bone area, BMC and BMD at both Tanner stages. However, after controlling the effect of body size and age, the associations were not as regular as expected. Significant differences at Tanner stage I remained only for fE2 groups in BMC and BMD and for low and moderate serum concentration groups of Ts and fTs in bone area, BMC, and BMD. At Tanner stage II, the only significant difference between serum sex hormone concentrations

was in BMC between moderate and low Ts groups. An interesting finding was that at Tanner stage I there was also an interaction between physical activity and free estradiol on BMD. Girls with high activity level and high fE2 concentration showed higher BMD values than high active girls with either low or moderate fE2 concentrations and higher BMD than low active girls.

Previous studies of associations between exercise and bone showed positive associations between exercise training and bone mass in premenarcheal girls [15, 25]. Studies of Haapasalo et al and Kannus et al [12, 17] also indicated that if tennis and squash players started their training before menarche, the side-to-side differences between the playing extremity and the non-dominant extremity in BMC and BMD were much greater than those who started their training after the onset of menarche. Bone gain especially in prepubertal girls was detected in the study of Bass et al [4], in which female gymnasts had higher total body BMD compared to the controls. This study also suggested that in prepuberty, bones are more responsive to the anabolic effect of physical activity than later during pubertal growth, because the effect of sex hormones is still minor in prepuberty. In our study the independent relation of physical activity was only seen in prepubertal girls. This could indicate that the anabolic association of physical activity on bone growth is concealed by the effect of rapid growth of weight and height which is associated with elevated sex hormone production in early pubertal years.

A few previous studies have reported the relations between serum sex steroid concentrations and total body bone mass, size and density [9, 10, 21]. Some of them showed that body weight and height together with pubertal development were the strongest predictors for total body BMC and BMD, while urinary or serum estradiol had only a minor role or even no

association with predicting BMC and BMD [10, 21]. In our study we found that after adjustment for the effects of age and body size on bone area, BMD, and BMC, the relation of free estradiol to total body BMC and BMD remained the same at Tanner stage I, while the significant relationship between serum estradiol and free estradiol concentrations to bone variables vanished at Tanner stage II. Furthermore, there is a high correlation between body size, sex steroid hormones and bone variables. This may indicate that the strong relationship between estradiol and body weight and height, could mask the relationship between estradiol and bone properties in rapidly growing girls. The explanation for the observation that in particular serum free estradiol concentrations had a significant association with total body BMC and BMC may be due to relatively high SHBG concentrations in pre- and early pubertal girls, which is regulated by sex steroid hormones and growth hormone. The bioavailable estradiol fraction could be more important for bone metabolism compared to total estradiol concentration in pre- and early pubertal girls.

A positive association between bone mass and androgens in prepubertal and adolescent females and also in pre- and perimenopausal women were indicated in the studies of Dhuper et al, Buchanan et al, Steinberg et al, and Zborowski et al [7, 10, 38, 40]. In our study, the relation of testosterone and free testosterone to bone size, mineral content and density seemed to come mainly across the body size. However, the girls with moderate testosterone concentration seemed to have the highest BMC and BMD at Tanner stage I. It could be that the interaction between androgens, growth hormone, and SHBG limits the positive association of testosterone to bone mass with high serum testosterone concentrations. It is suggested that there are connections between adrenal androgens primarily dehydroepiandrostreone sulfate (DHEAS) and testosterone in early pubertal years between adrenarche and menarche [1, 33]. DHEAS is also converted to testosterone in peripheral

tissues [19]. Furthermore, the complicated physiology behind entzymatic aromatization of testosterone and other androgens, to their metabolites such as β-estradiol, in peripheral tissues [19, 27, 36] makes it impossible to evidently separate the effect of testosterone from estradiol on bone properties in statistical analyses.

In our study free estradiol concentration seemed to be related to the relationship between physical activity and total body BMD at Tanner stage I. The high active girls with high free estradiol concentration had significantly higher total body BMD compared to others, which supports earlier observations of the synergistic effect of physical activity and estradiol during growing years [26]. The results may indicate that the relation of physical activity is mediated through free estradiol to bone metabolism, or free estradiol could strengthen the association of physical activity on bone mineral density in prepubertal girls. It is interesting that the interaction of physical activity and sex steroid hormones was observed before the beginning of the pubertal growth since growth before puberty is mainly dependent on growth hormone and thyroid hormones [33].

No interaction between physical activity and free testosterone was found in this study. However, there was a tendency at Tanner stage I that the high physical activity group with high free testosterone levels had higher BMC and BMD compared to the others. On the contrary, at Tanner stage II girls with high free testosterone did not benefit from the high level of physical activity for their BMC or BMD. In other words, high active early pubertal girls with high serum free estradiol concentrations seemed more likely to have low or moderate than high serum free testosterone levels. It could be speculated, that the tendency for low BMC and BMD in the group with high testosterone concentration and high physical activity

at Tanner stage II suggests that in the early pubertal years high physical activity level could be related to the aromization of testosterone to estradiol in peripheral tissues.

In our study, serum sex hormone concentrations in pre- and early pubertal girls were measured only once for every girl. Thus, it is possible that a single measurement did not give us a representative indicator of sex steroid exposure. Nevertheless, in clinical settings a single measurement for serum sample in the early morning is commonly used and acceptable. The morning concentrations represent the highest concentrations in serum during diurnal rhythm of testosterone and estradiol secretion [1, 29]. Furthermore, it is worth attention that during growth spurt sex steroid hormones and growth hormone may have independent and additive effect on bone growth. These complicated interactions are difficult to study because of the pulsating rhythm of hormone production and simultaneous central, peripheral and paracrine actions of different hormones.

These findings support the idea that physical activity is a useful way for ordinary schoolchildren to improve the bone mineral gain during prepuberty. Since our study was a cross sectional inspection, we could only see differences in two separate phases of bone development, and further speculations of the effect of physical activity and sex steroid hormones on peak bone mass are not possible. However, the results were encouraging and support the framework for further longitudinal study with the same matter.

In perspective, our results indicate that in prepubertal girls physical activity and free estradiol concentrations seem to be connected to total body BMC and BMD. The interaction between physical activity, free estradiol and BMD, support earlier observations of the synergistic effect of physical activity and estradiol during the growing years. The crucial significance of

sex steroid hormones on the skeletal maturation and growth is known [37], but in our study the connection of sex steroid hormones to total body bone properties seemed to be covered by the strong relationship between sex steroid hormones and body weight and height, especially with early pubertal girls.

Acknowledgements

The authors acknowledge Pia-Leena Salo, Miia Kemikangas, Leila Vilkki and Erkki Helkala for their valuable work and assistance on this project. The authors would also like to extend their appreciation to Mrs. Ruth Lehtomäki for linguistic revision. Additionally, Miia Kemppinen wishes to thank Ph.D. student Petro Moilanen for his great assistance in preparing the manuscript. Financial support was provided by Academy of Finland, Cadia Institute, and Peurunka-Medical Rehabilitation Foundation.

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Table 1: Subjects' physical characteristics, bone properties, and calcium intake [mean and standard deviation (sd), with T-test for paired comparison].

	Whole group n = 216	**Tanner stage I n = 120**	**Tanner stage II n = 96**	**T-test (p-value)**
Age (year)	11.1 (0.7)	10.8 (0.6)	11.5 (0.7)	<0.001
Weight (kg)	38.3 (8.4)	33.9 (5.7)	43.8 (8.0)	<0.001
Height (cm)	144.8 (7.6)	140.8 (6.1)	149.7 (6.3)	<0.001
BMI	18.1 (2.9)	17.0 (2.1)	19.5 (3.1)	<0.001
Area (cm^2)	1458 (205)	1346 (151)	1598 (174)	<0.001
BMC (g)	1379 (254)	1249 (196)	1541 (224)	<0.001
BMD (g/cm^2)	0.941 (0.056)	0.924 (0.053)	0.961 (0.052)	<0.001
Fat mass (g)	10368 (5596)	8071 (3910)	13238 (6061)	<0.001
Lean mass (g)	26765 (3973)	24631 (2870)	29432 (3531)	<0.001
Ca-intake (mg/d)	760 (312)	756 (322)	766 (300)	0.814

Table 2: Sex steroid hormone and sex hormone binding globulin (SHBG) concentrations in study groups [mean, standard error (SE), and 95% confidence intervals (CI), with non-parametric Kruskal Wallis test for paired comparison].

	Tanner stage I n = 120		**Tanner stage II n = 96**		**Kruskal Wallis**
	Mean (SE)	95% CI	Mean (SE)	95% CI	p
Estradiol (nmol/l)	0.092 (0.004)	0.083-0.101	0.124 (0.006)	0.111-0.136	<0.001
Free estradiol (pmol/l)	1.6 (0.1)	1.4-1.8	3.0 (0.2)	2.6-3.3	<0.001
Testosterone (nmol/l)*	0.343 (0.314)	0.280-0.405	0.574 (0.042)	0.491-0.658	<0.001
Free testosterone (pmol/l)*	3.3 (0.3)	2.7-3.9	7.0 (0.6)	5.9-8.2	<0.001
SHBG (nmol/l)	94.7 (3.1)	88.5-100.9	67.8 (2.7)	62.3-73.3	<0.001

*26 cases have been omitted from the analysis because of undetectable testosterone concentrations.

Table 3: Comparison of bone area and mass measured by DXA among low (L), moderate (M) and high (H) physical activity groups [mean, and standard error (SE) with LSD justify for multiple comparison].

Tanner Stage I	**Physical activity group**			**ANOVA**
	L (n=47)	M (n=31)	H (n=42)	
	Mean (SE)	Mean (SE)	Mean (SE)	p-value
Area (cm^2)	1308 (24)	1353 (20)	1384 (23)	NS
BMC (g)	1191 (30)	1251 (24)	1313 (32) [a]	0.013
BMD (g/cm^2)	0.907 (0.008)	0.924 (0.007)	0.944 (0.009)[b]	0.004

Tanner Stage II	**Physical activity group**			**ANOVA**
	L (n=25)	M (n=41)	H (n=30)	
	Mean (SE)	Mean (SE)	Mean (SE)	p-value
Area (cm^2)	1585 (33)	1618 (28)	1583 (32)	NS
BMC (g)	1531 (43)	1557 (37)	1526 (40)	NS
BMD (g/cm^2)	0.963 (0.010)	0.959 (0.008)	0.961 (0.010)	NS

NS= not significant
[a] p=0.003 compared to low activity group
[b] p=0.001 compared to low activity group

Table 4: Comparison of bone area and mass measured by DXA among low (L), moderate (M) and high (H) estradiol and testosterone tertiles [mean, and standard error (SE), with LSD justify for multiple comparison].

Tanner Stage I	**Tertiles of estradiol**			**ANOVA**	**Paired comparison**			**Tertiles of testosterone**			**ANOVA**	**Paired comparison**		
	L (n=41) Mean (SE)	M (n=39) Mean (SE)	H (n=39) Mean (SE)	p-value	L-M	L-H p-value	M-H	L (n=40) Mean (SE)	M (n=40) Mean (SE)	H (n=39) Mean (SE)	p-value	L-M	L-H p-value	M-H
Area (cm^2)	1337 (23)	1317 (26)	1387 (22)	NS	NS	NS	NS	1285 (21)	1366 (23)	1391 (25)	0.004	0.042	0.005	NS
BMC (g)	1224 (29)	1227 (34)	1300 (30)	NS	NS	NS	NS	1165 (27)	1292 (30)	1295 (33)	0.003	0.009	0.008	NS
BMD (g/cm^2)	0.913 (0.008)	0.927 (0.008)	0.934 (0.009)	NS	NS	NS	NS	0.904 (0.008)	0.943 (0.008)	0.927 (0.009)	0.004	0.003	NS	NS

Tanner Stage II	**Tertiles of estradiol**			**ANOVA**	**Paired comparison**			**Tertiles of testosterone**			**ANOVA**	**Paired comparison**		
	L (n=32) Mean (SE)	M (n=32) Mean (SE)	H (n=31) Mean (SE)	p-value	L-M	L-H p-value	M-H	L (n=31) Mean (SE)	M (n=32) Mean (SE)	H (n=31) Mean (SE)	p-value	L-M	L-H p-value	M-H
Area (cm^2)	1572 (31)	1572 (26)	1642 (33)	NS	NS	NS	NS	1507 (32)	1609 (21)	1673 (31)	<0.001	0.041	<0.001	NS
BMC (g)	1486 (37)	1514 (35)	1614 (45)	NS	NS	NS	NS	1413 (36)	1562 (26)	1642 (45)	<0.001	0.014	<0.001	NS
BMD (g/cm^2)	0.943 (0.008)	0.961 (0.009)	0.979 (0.010)	0.024	NS	0.006	NS	0.936 (0.008)	0.971 (0.009)	0.978 (0.010)	0.002	0.019	0.004	NS

NS= not significant

Figure 1.

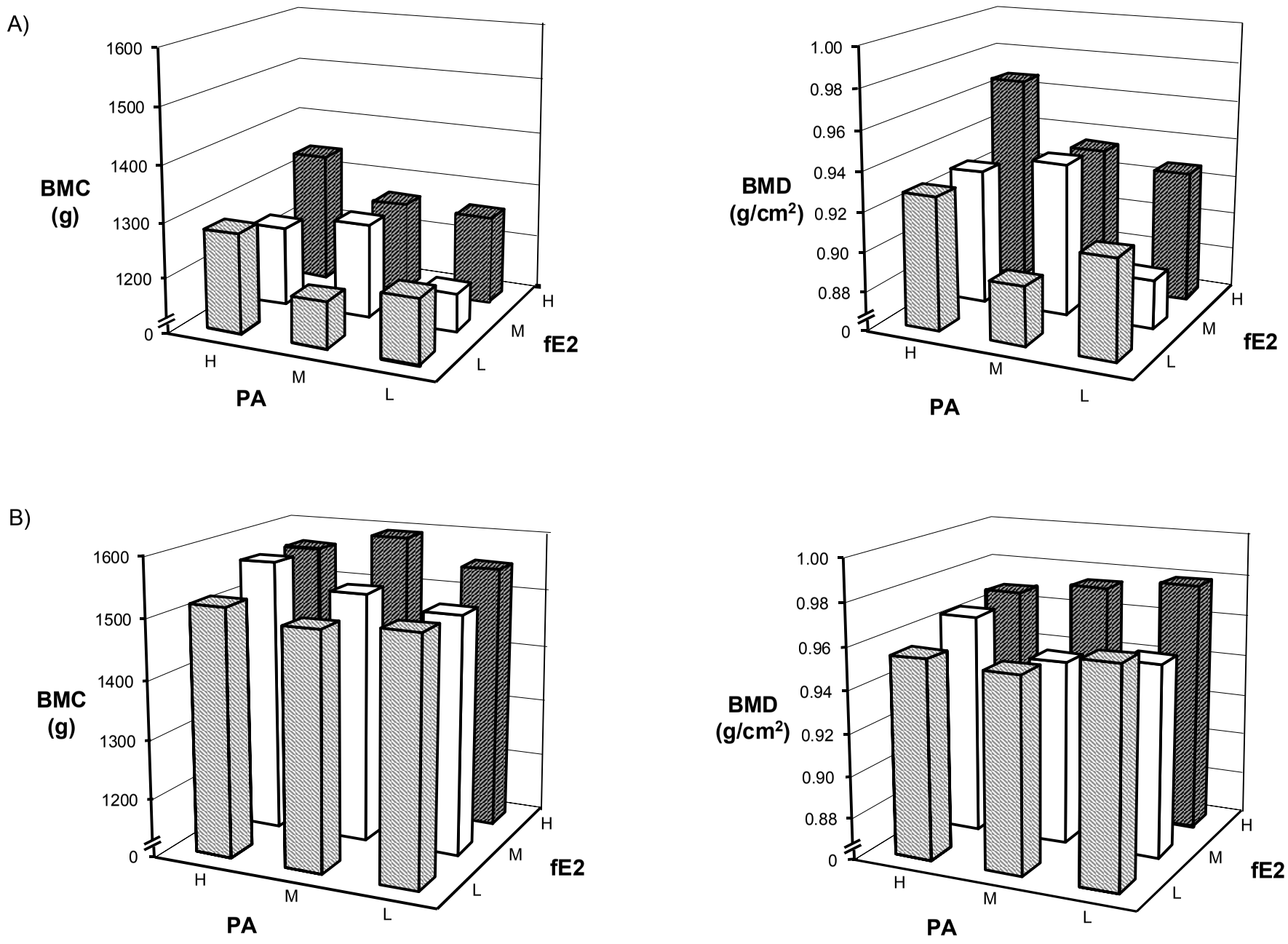
A)
BMC (g)
1600
1500
1400
1300
1200
0
H
M
L
PA
fE2
BMD (g/cm²)
1.00
0.98
0.96
0.94
0.92
0.90
0.88
B)

Figure captions:

Figure 1:

Combined relation of physical activity (PA) and free estradiol (fE2) on total body bone mineral content (BMC) and bone mineral density (BMD). For PA, L = low activity, M = moderate activity and H = high activity and for fE2, L = low serum concentration, M = moderate serum concentration and H = high serum concentration.

A) At Tanner stage I: (L_{fE2}=0.29-1.05 pmol/l, M_{fE2}=1.06-1.68 pmol/l, H_{fE2}=1.69-5.59 pmol/l; $L_{PA}L_{fE2}$ (n=18), $L_{PA}M_{fE2}$ (n=12), $L_{PA}H_{fE2}$ (n=17), $M_{PA}L_{fE2}$ (n=9), $M_{PA}M_{fE2}$ (n=13), $M_{PA}H_{fE2}$ (n=8), $H_{PA}L_{fE2}$ (n=12), $H_{PA}M_{fE2}$ (n=15), $H_{PA}H_{fE2}$ (n=15)) The two-way analysis of variance followed by LSD test for multiple comparison showed no interaction between PA, fE2 and BMC. Girls in the L_{PA}-group had significantly lower BMC compared to H_{PA}-group (p=0.004) and girls in the L_{fE2}-group had lower BMC compared to H_{fE2}-group (p=0.027).
When detected with one-way analysis of variance it was seen that H_{PA}-H_{fE2} group had significantly higher BMC than girls in L_{PA}-L_{fE2} (p=0.002), L_{PA}-M_{fE2} (p<0.001), L_{PA}-H_{fE2} (p=0.028) and M_{PA}-L_{fE2} (p=0.001) groups. The interaction was found (p=0.048) between PA, fE2 and BMD. Girls in H_{PA}-H_{fE2} group had significantly higher BMD compared to H_{PA}-L_{fE2} (p=0.026), and H_{PA}-M_{fE2} (p=0.02) groups, they had also higher BMD than girls in all of the low activity groups: L_{PA}-L_{fE2} (p=0.001), L_{PA}-M_{fE2} (p<0.001) L_{PA}-H_{fE2} (p=0.01) and M_{PA}-L_{fE2} (p<0.001) group.

B) At Tanner stage II: (L_{fE2}=0.04-2.15 pmol/l, M_{fE2}=2.16-3.24 pmol/l, H_{fE2}=3.25-8.62 pmol/l; $L_{PA}L_{fE2}$ (n=8), $L_{PA}M_{fE2}$ (n=7), $L_{PA}H_{fE2}$ (n=9), $M_{PA}L_{fE2}$ (n=13), $M_{PA}M_{fE2}$ (n=13), $M_{PA}H_{fE2}$ (n=14), $H_{PA}L_{fE2}$ (n=10), $H_{PA}M_{fE2}$ (n=12), $H_{PA}H_{fE2}$ (n=8)) no significant differences were found between groups in BMC or in BMD.