



# Serum testosterone level at the age of 12 is an important determinant of the following gain of bone mineral apparent density in 18-year-old males: a longitudinal study from puberty



Reeli Tamme<sup>1,3</sup>, Jaak Jürimäe<sup>2</sup>, Liina Remmel<sup>2</sup>, Evelin Mäestu<sup>2</sup>, Priit Purge<sup>2</sup>, Eva Mengel<sup>3</sup>, Vallo Tillmann<sup>1,3</sup>

<sup>1</sup>Institute of Clinical Medicine, Faculty of Medicine, University of Tartu, Tartu, Estonia, <sup>2</sup>Institute of Sports Sciences and Physiotherapy, Faculty of Medicine, University of Tartu, Tartu, Estonia  
<sup>3</sup>Childrens Clinic of Tartu University Hospital, Tartu, Estonia

The authors declare that there is no conflict of interest

This study was supported by the Estonian Ministry of Education and Science Institutional Grant IUT 20-58 and Personal Grant PUT 1382

## Background

Increased concentration of sex steroids plays a key role in the augmented bone growth and bone mass accumulation [1]. In our previous cross-sectional study we found that serum testosterone concentration was the most important biochemical predictor of BMD in 60 healthy non-obese schoolboys at the age of 10–18 years [2].

## Aim

to examine the associations between serum testosterone concentration at the age of 12 and the following gain in BMD in boys until the age of 18 years.

## Subjects

- 88 boys were studied at baseline (T1) at the mean age of 12.1 years (range 10.6–13.4) when 78 boys were at pubertal stage 2 or 3 and after 6 years (T2) when the mean age was 18.0 (16.5–19.2) years.
- The pubertal stage was determined at T1 according to self-assessment using the illustrated questionnaire of the pubertal stage by Tanner classification.
- Bone age was determined by the method of Greulich and Pyle at T1 using an X-ray of the left hand and wrist.

## Methods

### Physical activity

Physical activity (PA) was measured objectively by Actigraph accelerometer (GT1M ActiGraph, Monrovia, CA, USA) for seven consecutive days during the wake up time. Total daily physical activity (tot PA) was calculated as the total number of counts divided by the registered time (counts/min).

### Bone Mineral Density (BMD)

Total body (TB) BMD (g/cm<sup>2</sup>) and lumbar spine (LS) BMD (g/cm<sup>2</sup>) and bone area (BA) were measured by DEXA scan aDPX-IQ (Lunar Corporation, Madison, WI) at T1 and by DEXA scan Discovery (Hologic QDR Series, Waltham, MA, USA) at T2.

Bone mineral apparent density (BMAD) (g/cm<sup>3</sup>), an estimate of volumetric bone density, was calculated using a formula of TB BMAD=TB BMC/(TB BA<sup>2</sup>/height) and a formula of LS BMAD=LS BMC/(BA<sup>1.5</sup>). The precision of measurement expressed as coefficient of variation was <2% for all bone mineral measurements.

To adjust the bone mineral characteristics measured by different DEXA scans at different study points, standard deviation scores (SDS) were calculated for TB BMAD and LS BMAD at T1 as well at T2 using a formula: SDS BMAD = [an individual BMAD at T1 (or T2) – mean BMAD of total group at T1 (or T2)]/ standard deviation (SD) of total group at T1 (or T2). The change in SDS ( $\Delta$ ) of BMAD from T2 to T1 was calculated by subtracting BMAD SDS at T1 from BMAD SDS at T2. At both timepoints TB BMAD and LS BMAD were normally distributed.

### Blood Analyses

Venous blood samples were obtained after an overnight fast between 8:00 AM and 9:00 AM, the blood serum was separated and then frozen at -80 °C for further analysis. Serum testosterone (nmol/L) was determined using Immulite® 2000 (DPC, Los Angeles, USA) with the inter- and intra-assay coefficients of variation (CVs) of less than 5%.

### Statistical Analyses

Statistical analyses was performed using SPSS software version (17.0 for Windows; SPSS Inc., Chicago, IL). Normally distributed continuous variables are described as a mean and +/- 1 SD, not normally distributed variables as a median and 25<sup>th</sup> and 75<sup>th</sup> percentile.

Spearman correlation coefficients were calculated to explore the associations between baseline serum testosterone concentration and bone mineral characteristics at T2 and the change in BMD SDS ( $\Delta$ ).

Partial correlation analysis was performed to assess the relationships of bone mineral characteristics with testosterone after controlling for baseline bone age and tot PA. A P-value less than 0.05 was considered significant for both analyses.

## Results

Table 1. Clinical characteristics, serum testosterone concentration and physical activity data of subjects (n = 88) at T1 and T2. Median with 25<sup>th</sup> and 75<sup>th</sup> percentile for tot PA and mean with +/- 1SD for all other characteristics are shown.

Variable	Timepoint	
	T1	T2
Age (y)	12.1 (11.4-12.8)	18.0 (17.3-18.7)
Body mass (kg)	47.2 (34.5-59.8)	73.9 (61.8-86.0)
Body height (m)	1.55 (1.47-1.63)	1.81 (1.74-1.88)
BMI (kg/m <sup>2</sup> )	19.54 (15.58-23.5)	22.41 (19.13-25.69)
Tanner (No at stages 1/2/3/4/5)	(1/33/45/9/0)	
Bone age (y)	11.9 (10.75-13.05)	
Testosterone (nmol/L)	4.81 (0.85-10.47)	20.23 (14.99-25.47)
Tot PA (counts/min)	434 (359;573)	380 (303;498)

Table 2. Bone mineral characteristics at T1 (DPX-IQ Lunar densitometer) and T2 (Discovery Hologic densitometer). Median with 25<sup>th</sup> and 75<sup>th</sup> percentile are shown for TB BMD and LS BMD, and mean with +/- 1SD are shown for TB BMAD and LS BMAD.

Variable	Timepoint	
	T1	T2
TB BMD (g/cm <sup>2</sup> )	0.98 (0.94;1.03)	1.23 (1.21;1.25)
LS BMD (g/cm <sup>2</sup> )	0.82 (0.76;0.90)	1.07 (0.99;1.13)
TB BMAD (g/cm <sup>3</sup> )	0.088 (0.082-0.094)	0.095 (0.090-0.100)
LS BMAD (g/cm <sup>3</sup> )	0.147(0.134-0.160)	0.143 (0.130-0.156)

## MAIN FINDING

Serum testosterone concentration at T1 was positively correlated with:

- TB BMD at T2 ( $r = 0.28$ ;  $P < 0.01$ ),
- $\Delta$  TB BMAD SDS ( $r = 0.47$ ;  $P < 0.0001$ ),
- $\Delta$  LS BMAD SDS ( $r = 0.23$ ;  $P < 0.05$ ).

When controlling for bone age and tot PA at T1, the correlation between testosterone at T1 and  $\Delta$  TB BMAD SDS remained significant ( $r = 0.32$ ;  $P < 0.05$ ).

## References

1. Yilmaz D, Ersoy B, Bilgin E, Gümüşer G, Onur E, Pinar ED: Bone mineral density in girls and boys at different pubertal stages: relation with gonadal steroids, bone formation markers, and growth parameters. J Bone Miner Metab 2005;23:476–482.
2. Pomerants T, Tillmann V, Jürimäe J, Jürimäe T: The influence of serum ghrelin, IGF axis and testosterone on bone mineral density in boys at different stages of sexual maturity. J Bone Miner Metab 2007;25:193–197.

## Conclusions

Serum testosterone concentration at the age of 12 was associated with the following relative gain in total body BMAD in 18-year-old males suggesting that testosterone already at early pubertal stage is associated with the following bone mineral accrual in males.