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Longitudinal changes in bone-testis axis and their associations with insulin resistance in 11- to 12-year-old boys

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ABSTRACT

Purpose: Associations between osteocalcin (OCN), an osteoblast-specific hormone, and different markers of energy metabolism and insulin resistance have been reported in adults, but few studies have investigated this in children. The aim of the current study was to investigate serum OCN levels during pubertal development in normal weight (NW) and overweight (OW) boys, and to evaluate possible associations of OCN with body composition, testosterone, insulin resistance and adipocytokine values during puberty.

Methods: Ninety 11- to 12-year-old boys were investigated at 12-month intervals over the next 2 years. Boys were divided by their BMI into NW ($n = 60$) and OW ($n = 30$) groups. Serum OCN, testosterone, leptin, adiponectin, insulin, HOMA-IR score, and body composition were measured.

Results: Pubertal development over the 2-year period was similar in both groups. Serum OCN was not different at the beginning of the study and increased similarly in both groups. However, at the end of the study, NW had higher OCN than OW (142.9 ± 5.2 vs. 124.0 ± 7.4 ng/ml; $p < 0.05$). OW had higher leptin, insulin and HOMA-IR compared to NW, and these differences remained significant through the 2-year period. Testosterone, insulin and HOMA-IR increased through the study period in both groups. In multiple regression analyses increment in OCN was associated with the increase in testosterone in NW ($p < 0.001$) and OW ($p = 0.049$) boys. Increment in OCN was also associated with the increase in insulin ($p = 0.019$) and HOMA-IR ($p = 0.012$) over the 2-year period in NW boys.

Conclusion: Serum OCN concentration increases in puberty and the increment is positively associated with the rise in testosterone level in both NW and OW boys. The positive association between the rise in OCN and insulin in NW boys would suggest that OCN may have a role in the development of insulin resistance.

1. Introduction

The skeleton has been regarded as an endocrine organ that affects energy metabolism through its interactions with other tissues [1,2]. Emerging evidence suggests that skeletal, adipose and energy metabolisms are interlinked [3–5]. The bone-specific hormone osteocalcin (OCN) is produced by osteoblasts and positively correlates with osteoblast activity in the process of new bone formation [3,6]. Constant bone remodelling is an energy consuming process [1] and OCN has a capacity to regulate energy metabolism and maintain glucose homeostasis by stimulating insulin secretion and sensitivity [7–9]. In humans, reduced serum OCN levels have been reported in overweight and obese adults [3,10]. In healthy adults with various age, sex and adiposity levels, circulating OCN concentration has been found to be inversely associated with blood glucose level [3,11], serum insulin concentration and insulin resistance index assessed by homeostatic model assessment of insulin resistance (HOMA-IR) [3,4], but also with body mass index (BMI) [5,12,13], visceral fat [12,13] and total body fat mass (FM) [5,14,15]. However, the mechanisms explaining these associations are not fully understood [13]. There are some experimental studies to suggest that adipose tissue could influence bone remodelling by acting on osteoblasts through leptin [16] and/or adiponectin [17]. Animal studies have also shown that OCN regulates insulin sensitivity through adiponectin [8]. In humans, different cross-sectional studies demonstrate negative associations between OCN and leptin [1,18] and positive associations with adiponectin [12,19], while other studies have not found any relationship between OCN and different adipocytokine values [3,5].

It appears that the majority of published studies about the possible associations between OCN and metabolic health to date have focused on middle-aged and older adults [1,5,10,11,13–15,20], while relatively few studies have been published in growing children [21,22]. There are cross-sectional studies to suggest that circulating OCN levels are negatively associated with adiposity [21,22] and insulin resistance [2,19] also in children and adolescents. In contrast, there are also studies

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that have failed to demonstrate such relationships [18,23]. However, most of these investigations have been done in heterogeneous groups of children with different age, maturation and adiposity values. It could be suggested that the discrepancy among the results in children may be due to the fact that the effect of OCN on glucose metabolism is age dependent [1] as the OCN increases during growth and reaches the highest level during pubertal maturation [6,18]. Accordingly, it can be argued that if circulating OCN plays a significant role in glucose metabolism during growth and maturation, the age-dependent increase in OCN should be reflected in corresponding changes in circulating glucose and insulin levels [1]. However, to our best knowledge, no longitudinal studies have been conducted to examine the possible role of circulating OCN concentration on adiposity-related factors in boys during pubertal development. To date, the two longitudinal studies performed in children with obesity have demonstrated that weight loss has been associated with an increase in OCN, and an increase in OCN was correlated with the decrease in insulin resistance [2,19]. Therefore, there are some data suggesting that the regulatory role of the skeleton on glucose and energy metabolism in children may be mediated by circulating OCN.

The aim of this 2-year prospective study was to investigate changes in circulating OCN levels during pubertal development in a homogeneous groups of normal weight (NW) and overweight (OW) boys, and to evaluate possible associations of OCN with testosterone, insulin resistance index, body composition and serum adipocytokine levels. Our hypotheses were: 1) serum OCN in early stage of pubertal development is lower in OW boys compared with NW boys; 2) serum OCN increases further during pubertal maturation in both groups of boys; and 3) changes in OCN are associated with changes in testosterone, insulin resistance index, FM and serum adipocytokine levels.

2. Materials and methods

2.1. Study subjects

In total, 90 healthy Caucasian boys aged 11–12 years at the beginning of the study were recruited from different schools in Tartu, Estonia and were followed annually for two years. The boys were divided into NW ($n = 60$) and OW ($n = 30$) groups according to their body mass index (BMI) value using the overweight centile of BMI chart in boys by Cole et al. [24]. Accordingly, the BMI values being overweight were 19.84, 20.20, 20.55, 20.89, 21.22, 21.56 and 21.91 kg/m^2 for 10-, 10.5-, 11-, 11.5-, 12-, 12.5- and 13-year old boys, respectively [24]. Boys in OW group had to remain overweight at all measurement points. Throughout the study period, no restrictions were placed on dietary intake and all subjects consumed their ordinary everyday diet [25,26]. Participants were studied annually at 12-month intervals, and three measurement sessions were performed: at baseline (T0), after 12 (T1) and after 24 (T2) months. Height, body mass, pubertal stage, BMI, body composition and fasting blood samples were obtained at each measurement session. Both absolute values as well as changes (Δ scores) between T2 and T0 were used in the analysis [27].

At every time-point, the participants and their parents completed a questionnaire about the child's everyday physical activity (PA), general health and development, and family's socioeconomic status (SES) [28,29]. According to parental questionnaires, there were no differences in SES groups between NW and OW boys, the majority of them belonging to the middle-class SES [28,29]. The inclusion criteria for the current study were that a boy had to be healthy with no any chronic illnesses. None of the participants was receiving any medications during or prior to the study or had a history of any bone or renal diseases. Children with chronic illness or developmental delay were excluded from the study. Boys were matched by everyday PA level as all boys took part in obligatory physical education classes [28,29].

All procedures were approved by the Medical Ethics Committee of the University of Tartu, Estonia, and were explained to the children and their parents, who signed a consent form.

2.2. Anthropometry and sexual maturation

Body height was measured using Martin's metal anthropometer to the nearest 0.1 cm, body mass (kg) was measured to the nearest 0.05 kg using medical scales (A&D Instruments, Abingdon, UK). Body height and body mass data were used to calculate BMI (kg/m^2). Pubertal development of the participants was assessed by self-report using an illustrated questionnaire of pubertal stages by Tanner [30], which has been previously validated [31–34] and used successfully in our previous longitudinal studies with boys during pubertal development [25,28,29,35–37]. The boys were provided with line drawings, pictures and descriptions representing genitalia and pubic hair development stages. The subjects were asked to choose the appropriate development stage by themselves. In the case of discrepancies between the two variables, greater emphasis for the determination of the Tanner stage was placed on the degree of genitalia development [32].

2.3. Body composition

Whole-body FM, lean mass (LBM), bone mineral content (BMC), trunk FM, and body fat percentage were measured by dual-energy X-ray absorptiometry using the DPX-IQ densitometer (Lunar Corporation, Madison, WI, USA) equipped with proprietary software, version 3.6. The densitometry procedure has been described in our previous studies [26,38].

2.4. Blood analysis

A 10 ml blood sample was obtained from an antecubital vein with the participant sitting in the upright position after an overnight fast between 07.30 and 08.30 h. The blood serum was separated and then frozen at −80 °C for further analysis. Total OCN, testosterone and insulin were analysed using Immulite 2000 (DPC, Los Angeles, USA). The intra- and interassay coefficients of variations (CVs) for OCN and testosterone were <5%. The intra- and interassay CVs for insulin were 4.5% and 12.2% at an insulin concentration of 6.6 μIU/ml, respectively. Leptin was determined by radioimmunoassay (RIA) (Mediagnost, Reutlingen, Germany). This assay has intra- and interassay CVs < 5%, and the least detection limit was 0.01 ng/ml. Adiponectin was also determined with a commercially available RIA kit (Linco Research, St. Charles, MO, USA). The intra- and interassay CVs were <7%, and the least detection limit was 1 μg/ml. Glucose was measured with a commercial kit (Boehringer, Manheim, Germany). Insulin resistance index was calculated using homeostasis model assessment (HOMA-IR): fasting insulin (μIU/ml) × fasting glucose (mmol/l) / 22.5 [39].

2.5. Statistical analyses

All statistics procedures were performed using SPSS version 20.0 package for Windows (Chicago, IL, USA). Data are presented as mean ± standard error (SE). All variables were checked for normality of distribution before the analysis and not normally distributed values were log-transformed. Differences between NW and OW boys in age and pubertal stage and the changes over time (Δ scores) in age and pubertal stage were calculated using independent *t*-tests. In other measured variables, differences between NW and OW boys were analysed using analysis of covariance (ANCOVA) after adjustment for age and pubertal stage or after adjustment for changes (Δ scores) in age and pubertal stage over the 2-year period. Partial correlation analyses were applied to examine relationships between baseline OCN with baseline body composition and blood biochemical variables after adjustment for baseline age,