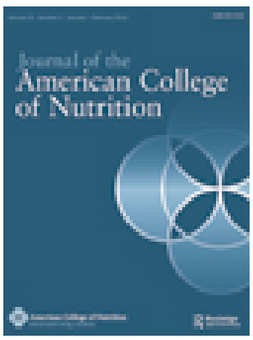

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Crosstalk Between Bone and Fat Tissue: Associations Between Vitamin D, Osteocalcin, Adipokines, and Markers of Glucose Metabolism Among Adolescents

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ABSTRACT

Objectives: This study aimed to investigate the relationship between 25-hydroxyvitamin D (25(OH)D), osteocalcin, markers of glucose metabolism, and obesity-related parameters among adolescents.

Methods: This was a cross-sectional study with 198 adolescents age 14–18 years. Weight, height, and waist and hip circumferences were measured, as well as the following biochemical parameters: serum 25(OH)D, parathyroid hormone (PTH), total (tOC) and undercarboxylated (ucOC) osteocalcin, adiponectin, leptin, glucose, and insulin. The homeostasis model of assessment estimate of insulin resistance (HOMA-IR) and β-cell function (HOMA-β) and quantitative insulin sensitivity check index (QUICKI) were calculated. Student's *t* test, analysis of variance (ANOVA), Pearson's correlation, and linear regression models were performed.

Results: Overweight was observed in 42.6% of the sample. This group presented significantly higher PTH, leptin, insulin, HOMA-IR, and HOMA-β and lower 25(OH)D, adiponectin, tOC, ucOC, and QUICKI than normal-weight subjects. 25(OH)D was positively correlated with ucOC and adiponectin and negatively associated with body mass index (BMI), weight, and waist circumference ($p < 0.05$ for all). The association between 25(OH)D and ucOC was also observed in the multiple regression analysis, adjusted for age, BMI, and season of the year (partial $r^2 = 0.071$, $p < 0.0001$). 25(OH)D and ucOC were not associated with markers of glucose metabolism. However, leptin was strongly correlated with insulin, HOMA-IR, HOMA-β, and QUICKI ($p < 0.0001$ for all).

Conclusion: The present study demonstrated that undercarboxylated osteocalcin is related to 25(OH)D and adiponectin concentrations. Both ucOC and 25(OH)D were lower in overweight and obese adolescents, reinforcing the importance of fighting obesity. Although a relationship of ucOC and 25(OH)D with markers of glucose metabolism was not observed, leptin has shown to be the hormone most related to energy homeostasis.

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ANOVA, analysis of variance; BMI, body mass index; cOC, carboxylated osteocalcin; ELISA, enzyme-linked immunosorbent assay; HbA1c, glycated hemoglobin A1c; HOMA-β, homeostasis model assessment estimate for β cell function; HOMA-IR, homeostasis model assessment estimate for insulin resistance; HPLC, high-performance liquid chromatography; ISA, Health Survey–São Paulo; LEPR, leptin receptor; NHANES, National Health and Nutrition Examination Survey; OC, osteocalcin; PTH, parathyroid hormone; QUICKI, quantitative insulin sensitivity check index; tOC, total osteocalcin; ucOC, undercarboxylated osteocalcin; VDR, vitamin D receptor; VDRE, vitamin D response element.

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Vitamin D; osteocalcin; glucose; insulin resistance; bone; leptin; obesity

Introduction

In the last decades, it has been increasingly recognized that vitamin D and bone interact with many tissues and impact several metabolic pathways, in both animal models and humans. The way bone and adipose tissue dialogue and how they affect glucose homeostasis depends on the production and interaction of their products, like osteocalcin—a protein produced by osteoblasts—and adipokines such as leptin and adiponectin [1–6]. Studies with rodents and findings from studies with humans have shown that, through hypothalamic stimulation, leptin plays an important role in bone formation and glucose homeostasis [1,5], and adiponectin is capable to attenuate insulin resistance by enhancing glucose utilization and fatty acid oxidation by muscle cells and increasing insulin sensitivity of the liver [7].

Osteocalcin in circulation can be carboxylated (cOC) and undercarboxylated (ucOC). Its carboxylation depends on vitamin K [8], and the carboxylated form has higher affinity for hydroxyapatite (a mineral compound that forms bone) and is believed to act in bone mineralization by regulating osteoblast and osteoclast activity [9]; the undercarboxylated form seem to have extra bone effects, enhancing insulin sensitivity and secretion, stimulating β-pancreatic cell proliferation, and promoting adiponectin expression in mice [2,4]. There is also evidence that osteocalcin carboxylation is controlled by the product of the expression of the *ESP* gene, the protein OST-PTP, because mice that did not express this gene (*ESP*−/−) had lower cOC and higher insulin sensitivity, whereas those overexpressing *ESP* in osteoblasts presented reduced β-cell proliferation and

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lower adiponectin concentrations [2]. It is important to note, however, that findings from experimental studies have to be carefully interpreted when considering human metabolism, because not all metabolic pathways function equally. However, further research has already established that multiple aspects of osteocalcin biology are similar in rodents and humans [10]. Although *ESP* is not expressed in humans, another phosphatase (called PTP1B) has shown to have effects analogous to those of OST-PTP [11].

Vitamin D is another factor influencing bone–fat–glucose metabolism relationships, because vitamin D receptor (VDR) response elements are found on the osteocalcin [3,12] and leptin genes [13]. Studies with humans have shown 25-hydroxyvitamin D (25(OH)D) concentrations to be positively associated with adiponectin [14] and negatively associated with parameters of insulin resistance [14,15], body mass index (BMI), fat mass, and leptin [16].

The increasing prevalence of overweight and vitamin D insufficiency observed among adults and elderly worldwide is now reflected among adolescents, due to shared genetic and environmental factors [17,18]. Being overweight when young is also known to increase the chance of becoming an obese adult [19], exposed to the full range of metabolic disorders for which obesity is a risk factor, such as diabetes mellitus and cardiovascular diseases. In addition, particular physiological characteristics from adolescence (as transient insulin resistance and higher growth hormone concentrations) may lead to faster development of type 2 diabetes in this age group compared to adults. This proposed pathophysiology would involve a lower capacity of pancreatic β cells to compensate for insulin resistance through insulin overproduction [20].

Considering that during adolescence the underlying mechanisms involved in the crosstalk between bone and fat mass are not completely evidenced, this study aimed to investigate the relationship between serum 25-hydroxyvitamin D, osteocalcin, adipokines, markers of glucose metabolism, and weight status among adolescents.

Materials and methods

Study design and population

This cross-sectional study included adolescents between 14 and 18 years old living in São Paulo, Brazil. They were participants in the Health Survey–São Paulo (ISA-SP; http://www.fsp.usp.br/isa-sp), a multicenter, population-based study with people from the city of São Paulo, Brazil, and those utilizing the Primary Care Center Horácio Geraldo de Paula Souza of the University of São Paulo or the League of Childhood Obesity from the School of Medicine of the University of São Paulo. Interviews and blood collection varied from 2011 to 2013. Individuals with chronic diseases besides obesity, such as diabetes mellitus and hypertension, were not included. Of the 437 individuals who fit the inclusion criteria, 241 agreed to join the study; 198 were interviewed and provided blood samples.

The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics in Research Committee from the School of Public Health of the University of São Paulo (protocol number 2307/2011). All participants (or their legal guardians, for those under 18 years old) signed an informed consent and were informed about the procedures and objectives of the research.

Study variables

Participants were interviewed by trained personnel and answered socioeconomic and demographic questionnaires. Height and weight were measured in duplicate, as well as waist and hip circumference, by trained nutritionists. Participants were weighed and height was measured with light clothes and wearing no shoes. Weight was determined within 0.1 kg for each subject using a digital scale (Filizola, São Paulo, Brazil). Height was determined using a fixed wall scale stadiometer (Seca Bodymeter 208, Chino, CA) to the nearest 0.1 cm. Waist and hip circumferences were measured with an inextensible 150-cm measuring tape. BMI was calculated as weight (kg)/height^2 (m^2).

Weight status was determined according to BMI-for-age growth charts [21]. Adolescents between the 3rd and 85th percentiles were considered normal weight; those between the 85th and 97th percentiles were considered overweight; and those above the 97th percentile were considered obese. One individual was not included in the analysis that involved BMI due to missing height information.

Blood collection after 12-hour fasting was done for biochemical analysis of 25-hydroxyvitamin D (measured by high-performance liquid chromatography; Immunodiagnostik AG, Bensheim, Germany), parathyroid hormone (enzyme-linked immunosorbent assay [ELISA]; IBL America, Minneapolis, MN), glucose (enzymatic colorimetric method; Quibasa, Belo Horizonte, Brazil), insulin (chemiluminescence; Beckman Coulter, Brea, CA), intact total osteocalcin (tOC; ELISA; IBL America), undercarboxylated osteocalcin (ELISA; Cusabio, Wuhan, China), leptin (ELISA; Enzo Life Sciences, Farmingdale, NY), and adiponectin (ELISA; Enzo Life Sciences). Glucose and insulin measures were available for 69 (49.3% male) and 63 (49.2% male) participants, respectively.

The homeostasis model of assessment estimate of insulin resistance (HOMA-IR) was calculated as fasting glucose (mg/dL) $\times$ fasting insulin (μU/mL)/405. The homeostasis model of assessment estimate of β-cell function (HOMA-β) was calculated as [360 $\times$ fasting insulin (μU/mL)]/[fasting glucose (mg/dL) -63]. The Quantitative Insulin Sensitivity Check Index (QUICKI) was determined as 1/(log insulin + log glucose).

Vitamin D status was determined according to the cutoffs proposed by Holick et al. [22], widely used in the literature, in which 25-hydroxyvitamin D concentrations $<$ 20 ng/mL were considered deficient and those between 20 and 30 ng/mL were considered insufficient.

Statistical analysis

Descriptive analyses were used for characterization of the sample, with results expressed as means (standard deviations) for continuous variables and as frequencies (percentages) for categorical variables. The normality of the distribution of each continuous

variable was tested by the Shapiro-Wilk test, and logarithmic transformation was used when needed. The comparison of means for quantitative variables was made by 2-tailed Student's *t*-test (2 means) and analysis of variance (ANOVA; more than 2 means). Equality of variances was assessed by Levene's test. Correlations between variables were tested using Pearson's correlation. Linear regression models were employed to investigate the relationship among 25(OH)D concentrations and other biochemical and anthropometrical variables.

Age, season in which blood was collected, and BMI were included in the model to adjust for their potentially confounding effects. Statistical power analysis was assessed for each tested regression model and, considering a probability level of 5% ($\alpha = 0.05$), found a power of >0.98, which confirms that our sample size was adequate for the analyses in the present study. Statistical analysis was performed using IBM SPSS Statistics Software, Version 22.0 (SPSS Inc., Chicago, IL). Statistical significance was set at $p < 0.05$.

Results

Of the 198 participants with a mean age of 16.3 years (SD = 1.4), 101 were male (51.0%), 72 (36.4%) were vitamin D deficient (concentrations < 20 ng/mL), and 70 (35.4%) were vitamin D insufficient (concentrations between 20 and 30 ng/mL).

Table 1 shows biochemical and anthropometric variables according to sex and weight status. Girls presented higher leptin (42.9 ng/mL, SD = 15.3 vs 16.6 ng/mL, SD = 18.3; $p < 0.0001$) and lower glucose concentrations (88.5 mg/dL, SD = 8.6 vs 93.1 mg/dL, SD = 9.5; $p = 0.042$) compared to boys.

Of the total sample, 42.6% ($n = 84$) were classified as overweight or obese. This group had significantly higher parathyroid hormone (PTH), leptin, waist and hip circumferences, insulin, HOMA-IR, and HOMA-β and lower 25(OH)D, adiponectin, ucOC, tOC, and QUICKI than normal-weight subjects (Table 1).

Correlation analysis demonstrated significant positive associations between 25(OH)D and both ucOC and adiponectin concentrations. 25(OH)D negatively correlated with BMI, weight, and waist circumference. In the multiple regression analysis, the associations between 25(OH)D with ucOC and with waist circumference remained significant, after adjusting for age, BMI, and season of the year (ucOC: partial $r^2 = 0.071$, $p < 0.0001$; waist: partial $r^2 = 0.005$, $p = 0.002$; Fig. 1). In addition, undercarboxylated osteocalcin was associated to adiponectin (partial $r^2 = 0.035$; $p = 0.005$), after adjusting for serum 25(OH)D, BMI, age, and season of the year (Fig. 2).

Compared to individuals not deficient in vitamin D, individuals with vitamin D deficiency presented lower ucOC (0.48 ng/mL, SD = 0.70 vs 0.87 ng/mL, SD = 1.10; $p < 0.0001$) and adiponectin concentrations (24.3 μg/mL, SD = 17.8 vs 28.9 μg/mL, SD = 17.1; $p = 0.016$). Furthermore, this group presented higher body weight (81.1 kg, SD = 31.5 vs 65.9 kg, SD = 21.0; $p < 0.0001$), BMI (28.6 kg/m^2, SD = 10.6 vs 23.3 kg/m^2, SD = 6.7; $p < 0.0001$) and waist circumference (89.1 cm, SD = 20.8 vs 81.3 cm, SD = 14.9; $p = 0.006$).

Correlations between osteocalcin or 25(OH)D with markers of glucose metabolism were not observed among our sample; however, leptin strongly correlated with insulin, HOMA-IR, HOMA-β, and QUICKI. Significant results were also observed among adiponectin and insulin, HOMA-IR, HOMA-β, and QUICKI (Table 2).

Fig. 3 shows markers of glucose metabolism according to leptin quartiles, with lower means for insulin, HOMA-IR, and HOMA-β in the first quartile (Q1) of serum leptin concentration and higher means in the fourth quartile (Q4; $p < 0.0001$

Table 1. Anthropometric and biochemical characterization of adolescents according to sex and weight status.

		Sex		Weight status	
	Total ($n = 198$) Mean (SD)	Male ($n = 101$) Mean (SD)	Female ($n = 97$) Mean (SD)	Normal weight ($n = 113$) Mean (SD)	Overweight/obese ($n = 84$) Mean (SD)
Age (years)	16.3 (1.4)	16.3 (1.4)	16.3 (1.4)	16.4 (1.4)	16.1 (1.4)
Anthropometric measures					
Weight (kg)	71.4 (26.3)	73.8 (28.2)	69.0 (24.1)	55.4 (7.2)	93.3 (27.0)†
Height (m) ($n = 197$)	1.68 (0.09)	1.74 (0.08)	1.62 (0.06)†	1.69 (0.08)	1.67 (0.10)
BMI (kg/m^2) ($n = 197$)	25.2 (8.7)	24.3 (8.4)	26.1 (8.9)	19.4 (1.9)	33.0 (8.1)†
Waist circumference (cm) ($n = 186$)	84.3 (17.8)	84.3 (18.6)	84.3 (16.9)	72.1 (5.3)	100.1 (15.6)†
Hip circumference (cm) ($n = 69$)	113.1 (20.8)	113.6 (23.4)	112.7 (18.3)	88.7 (6.6)	122.4 (16.4)†
Vitamin D and PTH					
25-Hydroxyvitamin D (ng/mL)	25.2 (13.4)	23.4 (11.1)	27.0 (15.2)	27.5 (12.9)	22.0 (13.5)†
PTH (pg/mL) ($n = 195$)	43.6 (27.9)	44.5 (29.2)	42.6 (26.5)	39.3 (25.3)	49.6 (30.3)†
Adipokines					
Leptin (ng/mL)	29.5 (21.4)	16.6 (18.3)	42.9 (15.3)†	18.1 (16.0)	45.1 (17.5)†
Adiponectin (μg/mL)	27.2 (17.5)	27.7 (17.6)	26.7 (17.4)	30.1 (17.5)	23.6 (16.9)†
Osteocalcin					
ucOC (ng/mL) ($n = 194$)	0.73 (1.00)	0.79 (0.88)	0.65 (1.10)	0.77 (0.94)	0.69 (1.07)†
tOC (ng/mL) ($n = 185$)	52.7 (65.9)	59.5 (74.5)	44.9 (53.7)	60.2 (75.4)	41.9 (47.6)†
Glucose metabolism					
Glucose (mg/dL) ($n = 69$)	90.7 (9.3)	93.1 (9.5)	88.5 (8.6)†	90.8 (6.5)	90.7 (10.2)
Insulin (mUI/L) ($n = 63$)	10.8 (7.1)	10.2 (7.2)	11.5 (7.0)	4.8 (2.1)	13.4 (6.9)†
HOMA-IR ($n = 63$)	2.4 (1.6)	2.4 (1.7)	2.5 (1.6)	1.1 (0.5)	3.0 (1.6)†
HOMA-β ($n = 63$)	165.8 (134.3)	135.7 (104.8)	194.9 (153.8)	66.3 (32.4)	208.7 (138.9)†
QUICKI ($n = 63$)	0.35 (0.04)	0.35 (0.04)	0.34 (0.03)	0.39 (0.03)	0.33 (0.03)†

BMI = body mass index, PTH = parathyroid hormone, ucOC = undercarboxylated osteocalcin, tOC = total osteocalcin, HOMA-IR = homeostatis model of assessment estimate of insulin resistance, HOMA-β = homeostatis model of assessment estimate of β cell function, QUICKI = quantitative insulin sensitivity check index.
†$p < 0.05$ (Student's *t* test).

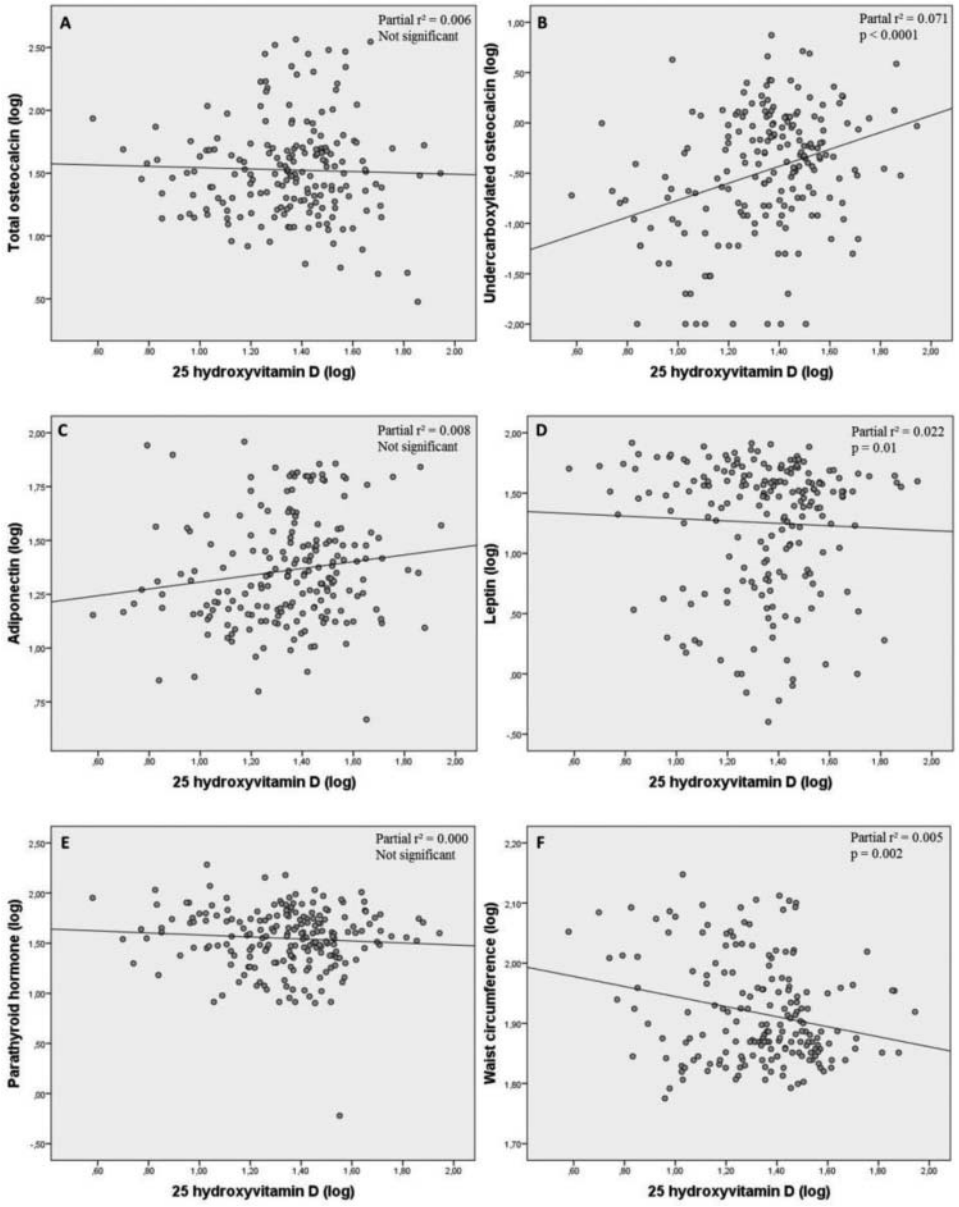

Figure 1. Linear regression analyses of 25-hydroxyvitamin D with (A) total osteocalcin, (B) undercarboxylated osteocalcin, (C) adiponectin, (D) leptin, (E) parathyroid hormone, and (F) waist circumference adjusted for body mass index, age, and season of the year among adolescents.

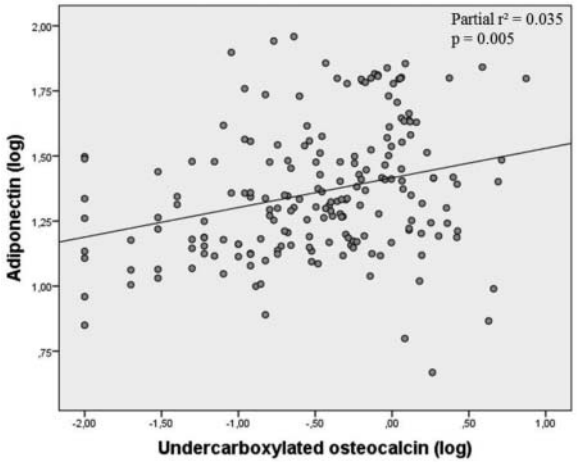

Figure 2. Linear regression analysis of undercarboxylated osteocalcin and adiponectin adjusted for serum 25-hydroxyvitamin D, body mass index, age, and season of the year among adolescents.

for all). Accordingly, an inverse relation was observed with QUICKI ($p < 0.0001$), with higher mean in Q1 and lower mean in Q4.

Discussion

Important findings were observed in the present study; that is, a positive correlation between ucOC and 25(OH)D concentrations and a positive correlation between ucOC and adiponectin. Vitamin D–deficient subjects presented lower ucOC and adiponectin concentrations and higher body weight, BMI, and waist circumference compared to those not deficient in vitamin D. The relationship between 25(OH)D with ucOC and with waist circumference persisted after adjusting for BMI, age, and season of the year in linear regression analysis. On the other hand, serum 25(OH)D was not associated with total osteocalcin in

Table 2. Pearson's correlation coefficients between markers of glucose metabolism and 25-Hydroxyvitamin D, osteocalcin, and adipokines among adolescents.

	Glucose ($n = 69$)		Insulin ($n = 63$)		HOMA-IR ($n = 63$)		HOMA-β ($n = 63$)		QUICKI ($n = 63$)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
25(OH)D	−0.041	NS	−0.160	NS	−0.160	NS	−0.118	NS	0.151	NS
tOC	0.197	NS	−0.029	NS	−0.005	NS	−0.143	NS	−0.012	NS
ucOC	0.148	NS	0.041	NS	0.046	NS	0.032	NS	−0.015	NS
Leptin	0.013	NS	0.746	<0.0001	0.720	<0.0001	0.703	<0.0001	−0.749	<0.0001
Adiponectin	0.174	NS	−0.318	0.011	−0.274	0.03	−0.410	0.001	0.282	0.025

HOMA-IR = homeostatis model of assessment estimate of insulin resistance, HOMA-β = homeostatis model of assessment estimate of β cell function, QUICKI = quantitative insulin sensitivity check index, 25(OH)D = 25-hydroxyvitamin D, tOC = total osteocalcin, ucOC = undercarboxylated osteocalcin, NS = not significant.

our study, which is contrary to the findings of a study of Polish obese children and adolescents [23].

Vitamin D acts—through its receptor—on the osteocalcin gene. The vitamin D response element located in the distal promoter of the osteocalcin gene leads to the activation of several mechanisms that increase its transcription, such as chromatin reorganization, facilitation of interactions between the proximal and distal regulatory domains, and recruitment of receptor coactivator molecules [9]. Moreover, vitamin D regulates osteocalcin gene expression at the posttranscriptional level by stabilizing osteocalcin mRNA, as demonstrated in clonal osteoblast cells [24]. Therefore, considering an unaltered vitamin K status (which controls osteocalcin carboxylation [8]), it is presumable that higher 25(OH)D concentrations would positively affect ucOC—but not cOC—circulating levels.

Undercarboxylated osteocalcin is known to increase pancreatic β-cell proliferation and insulin secretion and to improve insulin sensitivity by regulating the expression of adiponectin in adipocytes of mice [2]. Studies with rodents have shown that ucOC produced by osteoblasts increases adiponectin production and decreases fat mass accumulation and the risk of developing diabetes [25,26]. Not many studies have evaluated ucOC concentrations among adolescents and, similar to vitamin D, there are many different methods used to make this assessment, making it difficult to make comparisons. Polgreen et al. [27] found a mean ucOC of 12.7 ng/mL (SD = 0.6) among 137

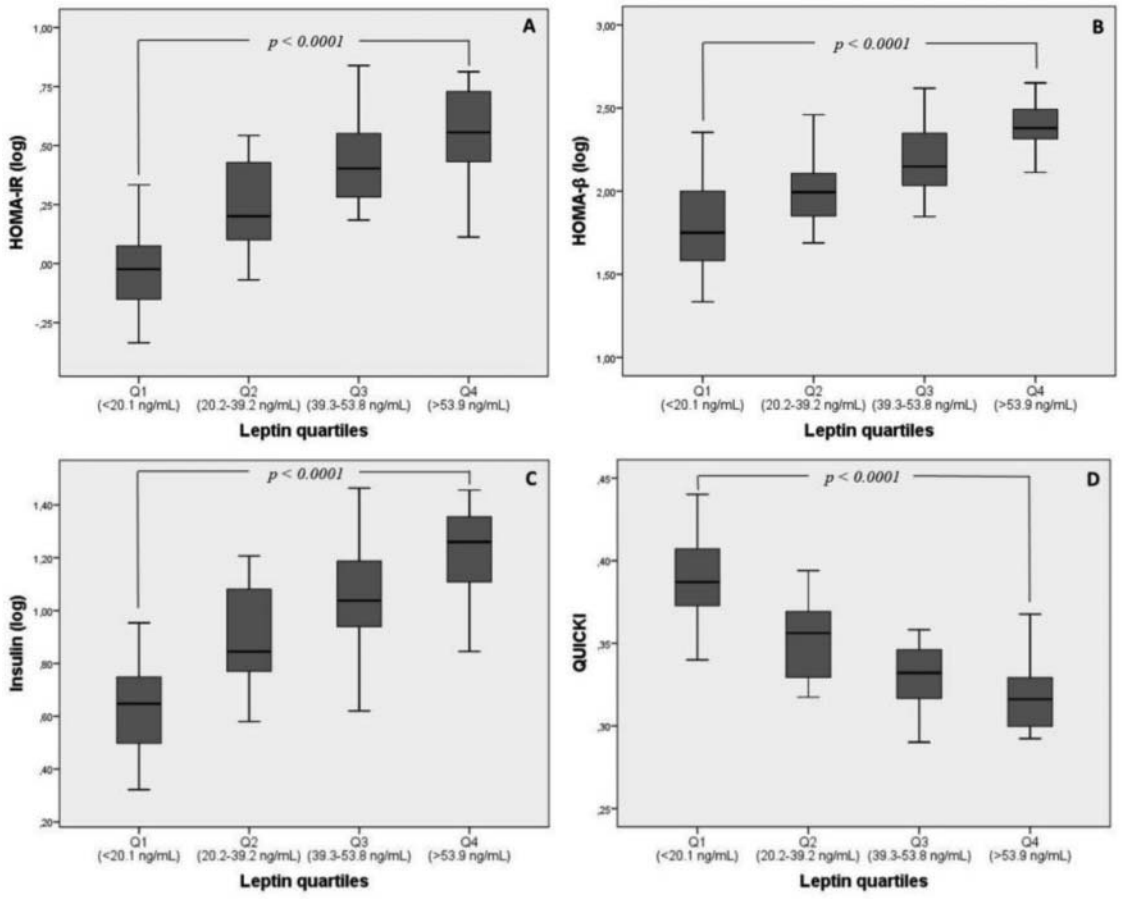

Figure 3. Boxplots with mean values for (A) HOMA-IR, (B) HOMA-β, (C) insulin, and (D) QUICKI according to leptin quartiles among adolescents. *p* Values for differences between first quartile (Q1) and fourth quartile (Q4; ANOVA).

youth with a mean age of 18.6 years. However, they did not observe significant associations between ucOC and any marker of glucose metabolism or BMI, nor did Rochefort et al. [28] among 13 normal-weight and 27 obese children age 9 to 12 years. They only found a negative relationship between ucOC and insulin confined to a subgroup of obese participants. In accordance, studies with adults observed that circulating ucOC was not associated with insulin resistance [29] or body composition [30]. On the other hand, a meta-analysis involving 39 studies and 23,381 subjects concluded that both tOC and ucOC negatively correlated with fasting plasma glucose and glycated hemoglobin A1c (HbA1c) [31].

Other studies that did not assess ucOC separately have shown low total osteocalcin to be related to insulin resistance and obesity among youth [23,32] and adults [33,34]. Garanty-Bogacka et al. [23] found that serum total osteocalcin was inversely associated with insulin resistance, systemic inflammation, and body fat among obese children and adolescents. Studies have also indicated an inverse correlation between serum osteocalcin and glucose concentrations [33–36]. Our study, however, failed to show a correlation between osteocalcin and insulin resistance or insulin secretion, similar to Abseyi et al. [6] in their study of 150 obese children and adolescents without diabetes. A possible explanation for our finding is that osteocalcin may have different effects among people with diabetes and euglycemic individuals, as shown by Rosato et al. [37] in a study of non-insulin-dependent subjects with diabetes and healthy controls. Thus, the few cases with abnormal serum glucose concentrations in our sample might be one of the factors responsible for this lack of relationship.

In addition, we found that adiponectin was negatively associated with insulin resistance, similar to other studies with children and adolescents [6,38]. In addition, positive correlations between adiponectin and both tOC and ucOC were observed in the present study, and the relationship between ucOC and adiponectin persisted after adjusting for serum 25(OH)D, age, BMI, and season of the year, which indicates that circulating adiponectin may play a significant role in the relationship between osteocalcin and glucose homeostasis among adolescents without diabetes. This hypothesis is supported by a study by Lee et al. [2], who created compound heterozygous mice for osteocalcin and adiponectin genes (Ocn+/−; Adipo+/−). They observed decreased insulin sensitivity and adiponectin concentrations in these animals (compared to wild-type or single-heterozygote mice), whereas glucose and insulin serum concentrations and insulin secretion remained within the normal range, and concluded that osteocalcin regulates insulin sensitivity independent of its effect on insulin secretion and that this regulation is, at least partly, mediated by adiponectin.

Other adipokine particularly involved in the interplay between bone and glucose metabolism is leptin, a hormone that is overproduced among overweight and obese people. It is capable of inhibiting bone formation and favoring bone resorption through the regulation of the central nervous system; an experimental study with mice has shown that the expression of its receptor (LEPR) leads to noradrenalin release and then to the activation of β-2 adrenergic receptor (β2R) in osteoblasts, reducing their activity [1]. Leptin also decreases the bioactivity of osteocalcin by stimulating expression of the *ESP* gene in osteoblasts in mice [39], which leads to reduced adiponectin expression and, as a consequence, to a decrease in its beneficial effects on glucose homeostasis. Leptin concentration was strongly correlated with insulin resistance and insulin secretion in the present study, even after controlling for age and BMI. Similar results were found among young women [40] and male adults [41]. These data reinforce the idea that obesity is a risk factor for metabolic disorders by affecting glucose homeostasis partly through the role of leptin.

We found important differences when comparing overweight/obese people with normal-weight subjects. Beyond presenting higher leptin and PTH levels and waist and hip circumferences, those with excess weight had lower tOC, ucOC, and adiponectin and worse indicators of glucose metabolism. Adiposity is a strong determinant of insulin sensitivity and secretion [20], and obesity seems to promote a hypersecretory pattern in pancreatic β-cells. This exacerbated insulin secretion (measured by HOMA-β) is probably a compensatory way in which metabolism acts in order to maintain a normal glycemia. The problem is that the overload in these cells could lead to cell exhaustion and a consequent loss of their ability to work properly, which is especially concerning among youth and makes it clear how they are exposed to a higher risk of developing glucose intolerance and type 2 diabetes.

In our sample of adolescents in which 42% were overweight or obese, we found 25(OH)D to be inversely correlated with weight and BMI. This is explained, in part, by the fact that adipocytes abduct vitamin D (a molecule soluble in fat) from circulation, storing it and making it unavailable [42,43]. On the other hand, some findings in the literature also suggest that vitamin D deficiency could promote greater adiposity, by elevating PTH release, which may promote calcium influx into adipocytes and thereby enhance lipogenesis [44]. Although we did not observe a correlation between 25(OH)D and PTH, overweight/obese individuals presented higher PTH concentrations than normal-weight subjects in our study.

25-Hydroxyvitamin D did not relate to insulin resistance or fasting insulin in our study, similar to the results found by others [45–47]. These findings disagree with some cross-sectional nationwide studies with young populations, which have consistently reported inverse associations between 25-hydroxyvitamin D levels and fasting hyperglycemia and/or increased HOMA-IR, even after adjusting for BMI [48,49]. The positive effect of vitamin D on glucose metabolism was also shown by Chung et al. [50], who observed a significant inverse relationship between vitamin D status and insulin resistance, independent of adiposity, among Korean adolescents.

The present study has some limitations. Because of the cross-sectional design, these results do not allow to indicate causality and temporal associations. Measures of biological maturation (as Tanner stages of pubertal development) were not available. In order to limit possible bias due to variation in stages of puberty, we only included adolescents older than 14 years and girls who reported already having their menarche. It is known that metabolic interactions are multifactorial, which may lead to weak, though statistically significant, associations. Thus, we cannot exclude the possibility that other variables not measured in this study may also play a role in these metabolic

pathways. Despite these limitations, our results clearly indicate the crosstalk between vitamin D, the bone-released protein osteocalcin, and weight excess among adolescents.

Conclusion

Our data confirm the link between obesity, vitamin D, osteocalcin, and leptin. Overweight/obese subjects presented lower 25 (OH)D, adiponectin, and total and undercarboxylated osteocalcin concentrations and higher risk of future development of diabetes. Taken together, these findings and the low 25(OH)D concentrations observed among people in the process of bone mass gain and body growth lead us to reinforce the importance of fighting obesity and stimulating safe sun exposure and the intake of food sources containing vitamin D. Although 25(OH) D and osteocalcin did not associate with glucose metabolism, adiponectin and leptin have shown to be part of these complex metabolic interactions. Further prospective or clinical trials are needed to assess the impact of vitamin D adequacy on osteocalcin levels in the risk for developing chronic diseases among this population.

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