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Interrelationship between bone turnover markers, calciotropic hormones and leptin in obese Saudi children

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Abstract. – OBJECTIVE: Fat-bone relationship involves the interaction among endocrine, inflammatory, immune processes and bone turnover. We tried to assess the association between Leptin and bone turnover markers (OCN, β-CTx, ALP), calciotropic hormones PTH and 25(OH)D in obese Saudi children.

PATIENTS AND METHODS: A cross-sectional study performed with 60 obese children and 36 lean children. For all subjects, OCN, ALP, β-CTx, PTH, 25(OH)D, leptin, Ca and Pi were investigated. Levels of leptin were measured by [ELISA] method, and OCN, β-CTx, PTH and 25(OH)D by an electrochemiluminesce immunoassay.

RESULTS: Sixty obese Saudi children had means weight (38.3 *vs.* 13.8 kg), height (121.0 *vs.* 91.8 cm) leptin (23.04 vs.16.88 ng/ml), PTH (31.5 *vs.* 14.7 pg/ml), Pi (1.67 *vs.* 1.54 mmol/l) were significantly higher and 25(OH)D (21.02 *vs.* 29.45 ng/ml) was significantly lower than controls. There was no difference in serum OCN, β-CTx, ALP and calcium between groups ($p > 0.05$). In the correlation study, OCN were significantly positively correlated with height, ALP, age, PTH, and β-CTx (r = 0.347, 0.32, $p < 0.05$), (r = 0.35, 0.51, 0.66, $p < 0.01$ respectively), while serum 25(OH)D was negatively correlated with PTH, weight, height and BMI (r = -0.45, -0.55, -0.55, -0.47, $p < 0.01$ respectively). PTH was positively correlated with leptin and β-CTx (r = 0.41, 0.44, p < 0.01), but not to ALP and BMI percentile. β-CTx correlated significantly positive with Pi (r = 0.34 $p < 0.05$) and ALP with BMI percentile (r = 0.42, $p < 0.05$). Multiple regression analysis demonstrated that PTH was predicted by leptin and β- CTx ($R^2 = 0.55$); β-CTx by leptin and OCN ($R^2 = 0.498$); OCN by PTH and β-CTx ($R^2 = 0.47$); and 25(OH)D by PTH ($R^2 = 0.21$)

CONCLUSIONS: The obese children had increased levels of leptin and PTH with strong associated with bone turn over markers OCN, β-CTx and deficiency of 25(OH)D which may be playing an important role in the pathogenesis of obesity and related bone metabolic risk diseases as osteoporosis and fractures.

Key Words:
Obesity, Children, Leptin, PTH, Osteocalcin, β-CTx, 25(OH)D.

Introduction

Obesity is currently a worldwide pathological epidemic and a major public health concern. Medical disorders, health risks, increased risk of adult obesity, with its subsequent effects on morbidity and mortality rates are all related to childhood and adolescent overweight and obesity. The etiology is multifactorial, with influences of many factors included genetic, environmental, socioeconomic, and behavioral or psychological. Comorbid diseases as cardiovascular disease, Insulin resistance, type 2 diabetes, orthopedic problems, and several other chronic diseases are highly associated with obesity. The prevalence of obesity in childhood and adolescence observably increased in many countries. In 2010, evaluation of 144 studies in several countries considered overweight and obese preschool children to be around 43 million and around 35 million of them in developing countries. Furthermore around 92 million stood to be susceptible to be overweight. The global prevalence of childhood overweight and obesity rose from 4.2% in 1990 to 6.7% in

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2010. This trend is anticipated to become 9.1% which is around 60 million, in 2020[1].

Increased body weight with a higher fat mass appears to have a detrimental effect on bone leading to increase incident of fragility and fractures. How body fat has a negative effect on bone metabolism remains, however, unexplained. There are cumulative proofs of a cross-talk between adipose tissue and bone[2].

Either formation or resorption of bone can be assessed by biochemical measurements of bone turnover markers. Frequently used markers of bone formation are osteocalcin (OCN), alkaline phosphatase (ALP), bone specific alkaline phosphatase (BAP), and procollagen type I propeptides, PICP (carboxy terminal) and PINP (amino terminal), all indicative of various phases of osteoblast proliferation and differentiation. Markers of bone resorption are collagen crosslinks type I carboxyterminal telopeptide (β-CTx) measured in serum and the urinary marker deoxypyridinoline (DPD), both degradation products of type 1 collagen[3]. Markers of Ca and Pi metabolism, such as the calciotropic hormones, parathyroid hormone [PTH] and vitamin D metabolites have a role to play in evaluating pathophysiological changes in the processes of resorption following physical sedentariness. Vitamin D act directly on enterocytes to stimulate calcium (Ca) and inorganic phosphorus (Pi) absorption, in so doing protection bone from resorption. Calciotropic hormones may also influence OCN levels as revealed by *in vitro* and *in vivo* researches. OCN gene transcription is partially controlled by levels of $1,25(OH)_2D$. Also lower OCN levels have been stated among obese children and adolescents who were deficient in vitamin D[4].

Osteocalcin, is an osteoblast produced protein recognized as "*bone gamma-carboxyglutamic acid (Gla) protein* (BGP)," It is formed by mature osteoblasts, hypertrophic chondrocytes and odontoblasts through the course of formation of bone. OCN is the most abundant noncollagenous protein of bone matrix. Though, there was no evident association of OCN serum concentration to bone mass. OCN exhibits numerous characteristics of a hormone: cells specific molecule, discharge in a circadian pattern, formed as a pre-pro-molecule and it is liberating into the circulation, however till now its receptor is not recognized. Modification of OCN occurs by carboxylation on three glutamic acid residues (17, 21, and 24) posttranslational depending on vitamin K by the action of the enzyme γ glutamyl carboxylase. This carboxylation is essential for hydroxyapatite binding and deposition in the extracellular matrix of bone. Uncarboxylated fraction of OCN in adults is a fairly large fraction (20-30%) of circulating OCN, and this fraction is even higher (up to 60%) in children[5]. Uncarboxylated fraction of OCN may be accountable for vital endocrine function controlling glucose and lipid homeostasis and appears to exhibit a clear consequence on energy metabolism and probably also the production of testosterone by the testes. OCN deficient mice were obese and reported to have a decreased insulin levels with elevated blood glucose levels[6]. The processes connecting OCN to obesity and insulin resistance are not clear. OCN levels are proposed to be modified by leptin. Leptin is an important modulator of weight condition via well- recognized pathways in the hypothalamus[7].

Leptin, a marker of adipocyte activity is a 16 KD neurohormone and is important in the control of body weight and nutrition through decreasing food intake and stimulating thermogenesis. It regulate food intake via its receptors in the hypothalamus by decreasing the production of the neuropeptide Y (NPY) which stimulate food intake[7]. Leptin is a major regulator of bone remodeling through changes in local factors controlling osteoclastogenesis and its alterations in obese children predispose them to low bone mass and fracture. Leptin play its role via the central nervous system to decrease osteogenesis. In animals models intracerebroventricular administration of leptin produces not only weight reduction but also osteolysis. Leptin action facilitated by the sympathic nervous system; the β2 adrenergic receptor found on the osteoblasts accelerates osteoclast differentiation through the receptor activator of NF-κB (RANK) – RANK ligand pathway[8]. Leptin reduce the formation of serotonin in the brain. Serotonin inhibits the activity of the sympathetic nervous system. Leptin signaling therefore raises the sympathetic tone and decrease bioactivity of OCN and consequently bone resorption. However, leptin decrease osteoclast formation and increase osteoblast differentiation *in vitro* and it is also reported to decrease osteoclast differentiation through the central cocaine- and amphetamine-related transcript [CART] pathway[8]. The effect of leptin on bone is complex, but it appears to exert antiosteogenic effects via a hypothalamic relay system (primarily affecting the axial skeleton), while it may have direct proosteogenic effects at appendicular sites[9].

The complex effect of leptin on bone indicate that it could operate in collaboration with the calciotropic hormones accountable for mineral homeostasis and metabolism, specifically PTH and $1,25(OH)_2D$. Calcium and phosphate movement at the gut, bone and kidney is controlled by these hormones which form an incorporated system for regulating transportation and are essential for mineral retention by the skeleton. Of the mineral-controlling hormones, PTH is most constantly said to be raised in obesity[10]. PTH is powerfully anabolic hormone and if given as daily sc injections, producing increased bone turnover and enhanced bone mass in patients with osteoporosis[11]. In obesity, described serum levels of $1,25(OH)_2D$ are contradictory, with some researches reporting high levels and others low levels[12]. Hypovitaminosis D can be related to its sequestration by fat due to its liposolubility and was reported to be associated with low forearm bone mineral density and increased incidents of distal forearm fractures[13].

Thus, the aims of this study were to examine the effect of simple obesity in children on the serum levels of leptin, PTH, 25(OH)D, OCN, ALP and β-CTx and to correlate their levels to the degree of obesity. Also to investigate whether their interaction could clarify the impact of obesity on bone metabolism and to evaluate the predictors of the bone turn over marker OCN, β-CTx, and the calciotropic hormones 25(OH)D and PTH.

Patients and Methods

Patients

Between November and June 2014-2015 we assessed sixty overweight and obese children (body mass index [BMI] > 85th centile; 34 males, 26 females), chronological age 7.55 ± 3.34 years ranged from 3 to 13 years, who had been attending Medina pediatric outpatient clinic of maternity and children hospital (Al-Medina, KSA). They were all apparently healthy with no obvious endocrine disease. BMI were expressed as a standard deviation score (SDS) according to WHO Child Growth Standards[14]. Patients were classified as prepubertal by physical inspection, according to the standard Tanner's criteria. All children were diagnosed with simple obesity; other syndromic, organic, and hormonal causes were excluded. Thirty-six normal-weight, healthy children (21 males and 15 females) of the same chronological age acted as controls for the biochemical parameters and bone markers: All were prepubertal chronological age 5.64 ± 4.1 years ranged from 2 to 13 years. All subjects of the control group were healthy and did some physical activity appropriate for their age. Furthermore, none of them was taking drugs known to intervene with calcium metabolism. All control subjects were within 2 SD of the means for height and weight, with a BMI < 85th percentile. They were normal children undertaking a routine check.

Measurement of Anthropometric Parameters

Height measured using precision Harpenden stadiometer and weight using a Scale Tronix scale to the nearest of 0.1 cm and 0.1 kg, respectively.

Reference percentiles for children in Saudi Arabia were applied for standardization of height and weight. Referring to national reference data attached to every child file, the body mass index (BMI) was standardized. Underweight, normal weight and overweight were defined as BMI below the 10th, between 10th and 85th and above the 85th percentile, respectively.

Study Design

This was an observational cross-sectional, a case-control study carried out after appropriate approval by the Ethics Committee of the Hospital Institutional Board. All children, 60 patients and 36 controls, submitted to a full evaluation and serum collection after an overnight fast; sample were divided into two parts, ALP, Ca, albumin and Pi levels were done on the same day of collection and the second serum samples were stored at -20C until assayed for PTH, 25(OH)D, β-CTx, OCN, and leptin.

Assays

25(OH)D, PTH, N-MID Osteocalcin, and β-CTx kits (Roche Diagnostics, GmbH, Mannheim, German) used to estimate the serum levels of these parameters and were determined using the Cobas e 411 immunoassay analyzer (Roch Diagnostics, GmbH, German). All assays are based on chemiluminescence immunoassay technology. Intra-assay CVs in our laboratory were 3% for β-CTx, 3% OCN and 4.5% for PTH. Interassay CVs were 7% for OCN, β-CTx, and PTH. Serum 25(OH)D was with an intra-assay CV of 5% and an interassay CV of 7%.

Serum leptin was identified with commercially available test kits which were used to measure leptin (enzyme-linked immunosorbent assay [ELISA] method, ASSAYPRO, Saint Charles, MO, USA). Assay Max Human Leptin ELISA Kit, Catalog No. EL2001-1 that has a range of 0.00-32 ng/mL. The ASSAYPRO Leptin ELISA is an enzyme immunoassay for the measurement of leptin in serum and plasma.

By determining this bone resorption marker, Collagen type I fragments β-isomerized C-terminal telopeptides (β-CTx), the activity of osteoclasts can be detected. These isomerized telopeptides are highly specific for the degradation of type I collagen dominant in bone. Elevated serum levels of isomerized C-terminal telopeptides of type I collagen have been reported for patients with increased bone resorption.

ALP, Ca, inorganic phosphorous (Pi) and albumin were measured by commercially available kits using standard procedures on a clinical chemistry automated machine, Dimension X Pand, Siemens Healthcare Diagnostics Ltd., Frimley, Camberley, UK.

Exclusion Criteria

A detailed medical and family history was obtained from all subjects. At enrollment, obese and control children submitted to physical examination including weight, standing height, BMI, and blood pressure measurements. Exclusion criteria were: (1) The existence of endocrine disorders or genetic syndromes, (2) History of any chronic diseases or chronic medication use or children under special diets or use of mineral and/or vitamin supplements were not included in the study. (3) Taking medications that could influence growth, pubertal development. Pubertal stage was established according to the Tanner scale, and subjects who showed pubertal development were excluded.

Statistical Analysis

Statistical analysis was performed using SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). Quantitative data were normally distributed and expressed as mean ± SD. Differences between groups were evaluated by Student's *t*-test. Simple correlations were used to investigate the association between the different parameters. $p < 0.05$ indicated statistical significance. Calculation of Pearson correlation coefficients and linear regression analyses were performed to assess relationships between key variables.

Variables chosen for inclusion in the multiple regression analyses were established on physiologically expected outcomes. The following variables were included to measure to what extent: (1) weight, leptin, Ca, 25(OH)D, β-CTx, OCN and Pi predicted PTH; (2) age and weight, leptin, Ca, PTH, OCN, β-CTx and Pi predicted 25(OH)D; (3) leptin, Ca, 25(OH)D, Pi, β-CTx, and PTH predicted ALP; (4) weight, leptin, Ca, PTH, 25(OH)D, Pi and OCN predicted β- CTx; and (5) age, weight, leptin, Ca, PTH, 25(OH)D, β-CTx and Pi predicted OCN. For model selection stepwise and r^2 methods were used. Model R^2 and partial R^2 of the individual model parameters are specified, where R^2 (the square of r) is the coefficient of determination and represents the portion of total variation deducible to the variables in the model. Partial R^2 quantifies the distinctive contribution of each explanatory variable to the total R^2.

Results

Sixty overweight and obese Saudi children had means BMI percentiles (94.7 *vs*. 20.3), weight (38.3 *vs*. 13.8 kg), height (121.0 *vs*. 91.8 cm) serum leptin (23.04 *vs*. 16.88 ng/ml), PTH (31.5 *vs*. 14.7 pg/ml), phosphate (Pi) (1.67 *vs*. 1.54 mmol/l) were significantly higher and 25(OH)D (21.02 *vs*. 31.95 ng/ml) was significantly lower than controls (Tables I and II, Figure 1). There was no difference in serum OCN, β-CTx, calcium, and ALP between groups (Table II and Figure 2). Normal weight and obese children were insignificantly different regarding age, gender or pubertal stage. Mean BMI_{SDS} for OW/obese children was (2.31 ± 1, all pre-pubertal stage) and Mean BMI $_{SDS}$ for control children was (-1.85 ± 1.49, all pre-pubertal stage) (Table I).

PTH was significantly correlated with OCN ($r = 0.51$, $p < 0.001$; Figure 3), β-CTx ($r = 0.44$, $p < 0.001$; Figure 4), leptin ($r = 0.41$, $p < 0.01$; Figure 6) and 25(OH)D ($r = -0.45$, $p < 0.01$; Figure 5), but not to ALP and BMI percentile. Also a significant positive correlation between β-CTx and PTH, OCN or Pi (r = 0.44, 0.66, 0.34 $p < 0.05$ respectively) was detected. Further, s25(OH)D showed significant negative correlations with PTH, weight, height and BMI percentile (r = -0.45, -0.55, -0.55, -0.47, $p < 0.01$ respectively, Figures 5, 8) while OCN were positively correlated to height, ALP, age, PTH, and β-CTx (r = 0.347, 0.32, $p < 0.05$) and

Table I. Anthropomorphic data in overweight, obese children and control group.

Subject number	Normal weight (n = 36)	Overweight (n=9)/ obese children (n =51) Total (n =60)	*p*-value[a]
Age, years	5.65 ± 4.1	7.55 ± 3.34	0.07
Height (cm)	91.8 ± 33.23	121.02 ± 27.7	0.007
Weight (kg)	13.83 ± 12.5	38.36 ± 16.68	< 0.001
Sex:			
Male	21 (58.33%)	34 (6/28) (56.66%)	0.681
Female	15 (41.66%)	26 (3/23) (43.33%)	
BMI %	13.65 ± 2.64	25.34 ± 5.25	< 0.001
BMI_{SDS}	-1.85 ± 1.49	2.31 ± 1	< 0.001
Percentile	20.38 ±19.98	94.74 ± 4.16	< 0.001

Results are shown as mean ± SD (percentages). [a]Student's *t*-test.

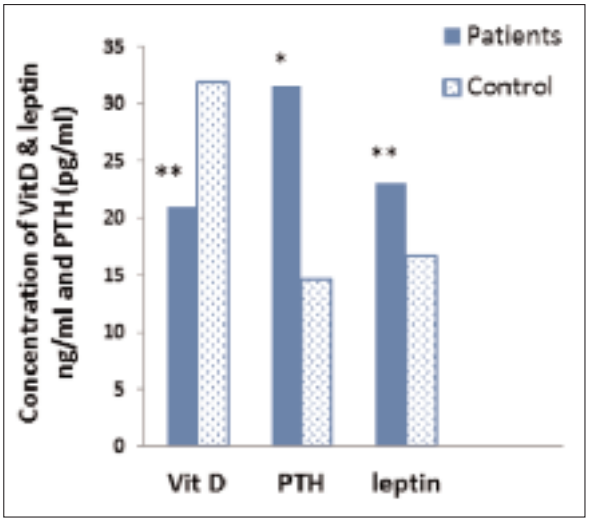

Figure 1. Concentration of 25(OH)D, leptin ng/ml and PTH pg/ml in overweight and obese children and control group. *$p < 0.05$ and **$p < 0.01$.

(r = 0.35, 0.51, 0.66, $p < 0.01$ respectively, Figure 7). In addition, OCN demonstrated no significant association with leptin or BMI. ALP demonstrated positive significant correlations with BMI percentile and OCN (r = 0.42, 0.32, $p < 0.05$ respectively).

We subdivided further the obese children according to sex, but no difference was found except in β-CTx which was lower significantly in obese girls comparing to boys (0.74 ± 0.29 *vs.* 1.04 ± 0.44, $p = 0.018$). This study did not show a clear linkage of OCN to obesity or any higher values of OCN in control group. The present study established no significant relationship between serum OCN levels and leptin, neither in the total study population nor in the subsets stratified for gender but as anticipated leptin serum levels were higher in over-weight and obese children.

Table II. Serum biochemical variables in overweight and obese children and control group.

Subject number	Normal weight (n = 36)	Overweight (n = 9)/ obese children (n = 51) Total (n =60)	*p*-value[a]
OCN (ng/ml)	63.43 ± 26.96	79.01± 37.37	0.063
β-CTx (ng/ml)	0.86 ± 0.31	0.90 ± 0.48	0.64
Leptin (ng/ml)	16.88 ± 6.88	23.04 ± 10.5 (n = 30)	0.014
25(OH)D (ng/ml)	29.45 ± 6.89	21.02 ± 10.07 (n = 41)	0.044
PTH (pg/ml)	14.72 ± 11.3	31.54 ± 26.2	0.001
ALP (U/L)	258.64 ± 56.6	301.86 ± 96.87	0.49
Ca (mmol/L)	2.32 ± 0.22	2.4 ± 0.14	0.109
Pi (mmol/L)	1.54 ± 0.19	1.67± 0.19	0.044

Results are shown as mean ± SD. [a]Student's *t*-test.

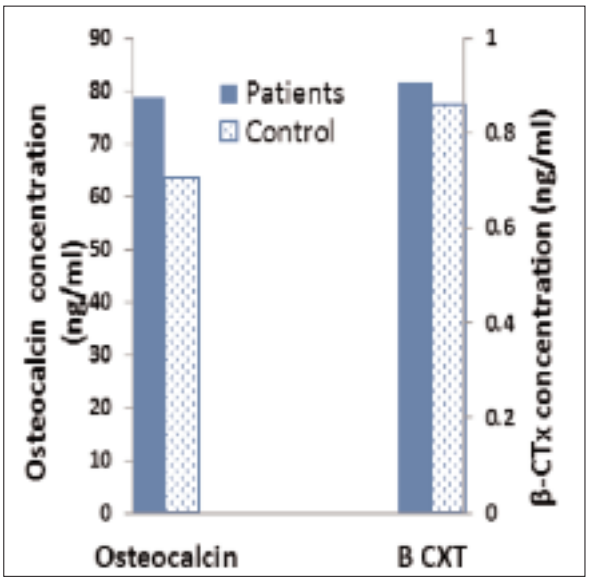

Figure 2. Concentration of β CTX and osteocalcin ng/ml in overweight and obese children and control group. $p > 0.05$.

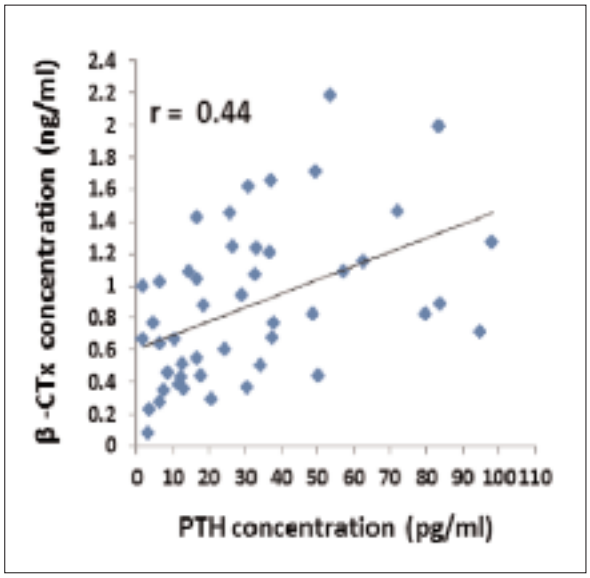

Figure 4. Correlation between β- CTx and PTH showing significant positive correlations, r = 0.44, $p < 0.001$.

Multiple regression analysis verified that PTH was predicted by leptin and β-CTx ($R^2 = 0.55$); β-CTx by leptin and OCN ($R^2 = 0.498$); OCN by PTH and β-CTx ($R^2 = 0.47$); 25(OH)D by PTH ($R^2 = 0.21$) (Table III).

Discussion

Many indicators suggest that obesity and bone metabolism are interdependent. Firstly, a common mesenchymal stem cell is a shared precursor for both adipocytes and osteoblasts and factors decreasing adipogenesis increasing osteoblast differentiation and also, those restraining osteoblastogenesis enhanced adipogenesis[15]. Nevertheless, there are inadequate data obtainable regarding the interactions between the osteoblast-derived hormone OCN, bone-resorption marker β-CTx, weight status, leptin and hormones of minerals metabolism, PTH and vitamin D metabolites in obese humans, particularly in children and adolescent.

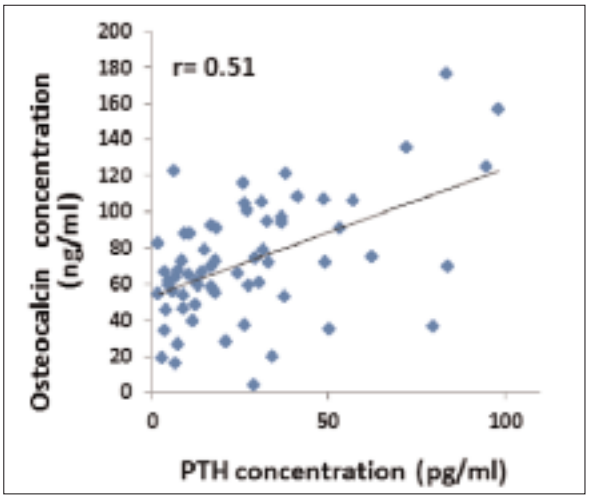

Figure 3. Correlation between osteocalcin and PTH showing significant positive correlations, r = 0.51, $p < 0.001$.

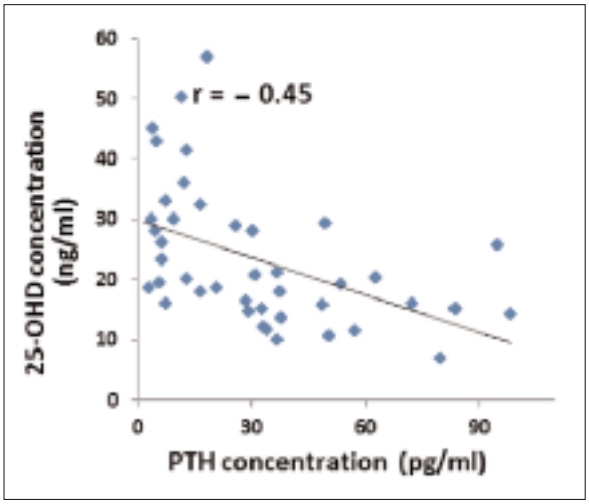

Figure 5. Correlation between PTH and 25(OH)D showing significant negative correlations, r = - 0.45, $p = 0.006$.

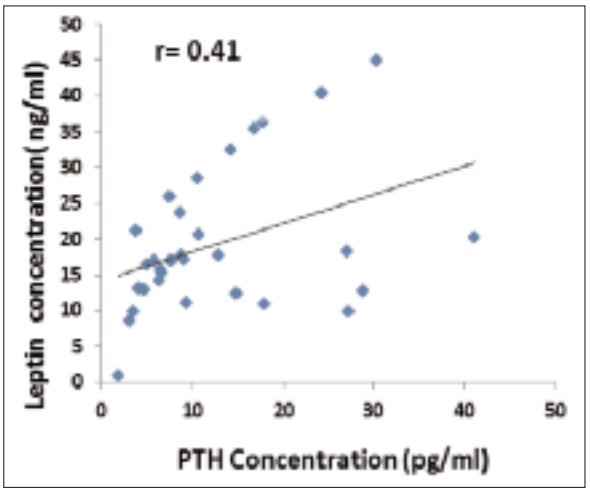

Figure 6. Correlation between leptin and PTH showing significant positive correlations, r = 0.41, $p = 0.01$.

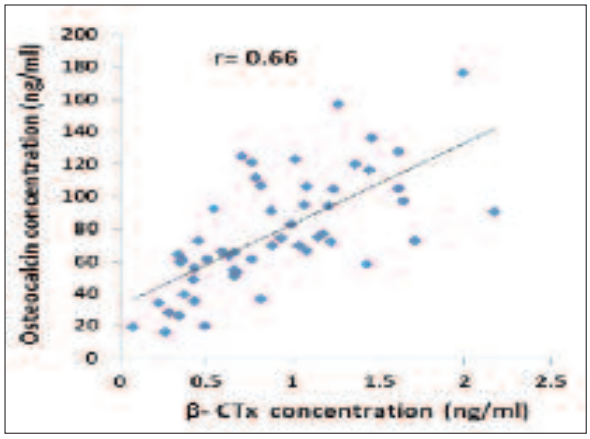

Figure 7. Correlation between osteocalcin and β-CTx showing significant positive correlations, r = 0.66, $p < 0.001$.

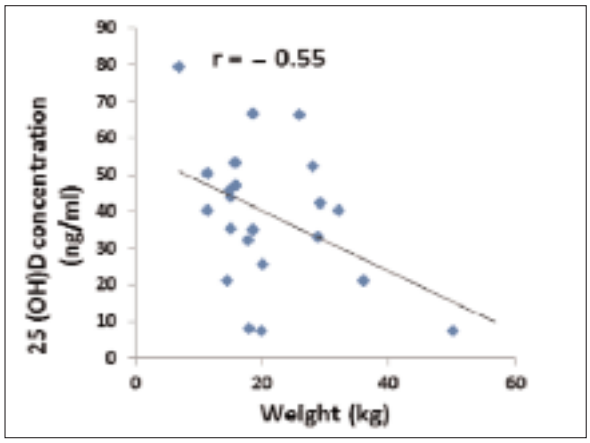

Figure 8. Correlation between 25(OH)D and weight showing significant negative correlations, r = -0.55, $p = 0.005$.

In this study, s25(OH)D was lower in overweight and obese Saudi children than control group. This result comes to an agreement with a number of researches stating that s25(OH)D is at a low level in obesity and inversely correlated to the amount of body fat. Also The correlation between s25(OH)D and sPTH was inverse, supportive the commonly supposed concept that secondary hyperparathyroidism is a consequence of vitamin D insufficiency occurred as s25(OH)D decreases less than 30 ng/ml[16].

Increased serum PTH concentrations were found in this study and also are well described in

Table III. Multiple regression models.

	Coefficient	Partial R^2	*p*-value	Model
PTH (pg/ml)				
Intercept	-4.66			
Leptin (ng/ml)	0.527	0.747	0.0001	$R^2 = 0.55$
β-CTX (ng/ml)	8.79	0.336	0.043	$p = 0.0001$
25D (ng/ml)				
Intercept	32.18			
PTH (pg/ml)	-0.181	-0.452	0.034	$R^2 = 0.21$
Calcium (mmol/L)	-0.292	-0.328	0.147	$p = 0.0001$
β-CTx (ng/ml)				
Intercept	0.403			
Osteocalcin (pg/ml)	0.008	0.646	0.0001	$R^2 = 0.498$
Leptin (pg/ml)	-0.011	-0.261	0.043	$p = 0.0001$
25(OH)D (ng/ml)	-0.003	0.057	0.0647	
Osteocalcin (ng/ml)				
Intercept	27.85			
PTH (pg/ml)	0.38	0.28	0.01	$R^2 = 0.47$
β-CTx (ng/ml)	40.21	0.53	0.0001	$p = 0.0001$

Results are shown as mean ± SD. [a]Student's *t*-test.

obesity in adults[16]. Several investigators accredited these elevations to secondary hyperparathyroidism in reply to decreased s25(OH)D,which in obesity seems to be a noticeable observation. Others reported no correlation between s25(OH)D and PTH[17]. The s25(OH)D levels in this study ranged from 10-56.9 ng/ml in both groups the obese children and the control group joined, but a negative significant correlation existed between PTH and s25(OH)D in obese children. In contrast, a significant positive relationship existed between PTH and leptin when leptin ranged from 6.1 to 44.83 ng/ml in obese children. Because children with primary or secondary hyperparathyroidism were heavyweight than others in the control group, it may be proposed that raised PTH levels participate in obesity. Conversely, PTH levels declined after reducing weight in one research, in accord with most investigations done on obese adults indicating that the raised serum PTH is not a cause of obesity but an outcome[16].

Multiple regression analysis showed that serum leptin and β-CTx were the most important predictive variables for PTH while s25(OH)D offering no contribution to PTH prediction. Thus, low s25(OH)D did not participate to the increased sPTH. Consequently, the results favor the theory that in obesity the inverse relationship between s25-(OH)D and sPTH is not causal, but that the change in their levels are a straight effect of obesity per se. The existence of an inverse correlation between s25(OH)D and sPTH must not be used to denote the existence of vitamin D deficiency in obesity. In obesity the independence of sPTH from s25(OH)D levels was reported by other studies[17]. The reason of low s25(OH)D in obesity is uncertain. Several proposals have been mentioned, involving removal of 25(OH)D by adipose tissue, causing it to become less accessible for transformation to s1,25$(OH)_2$D as well as decreased the duration of exposure to sunlight[18]. The inverse relationship between 25(OH)D and PTH obtained in this study, proposing that inhibition of 25(OH)D production in obesity, overruled any stimulatory influence of PTH. Significantly, intervention study giving vitamin D supplementation for an 8-wk in obese subjects who were suffering from insufficient levels of vitamin D displayed no result on intact sPTH and an insignificant reduction in sPTH (1-84) levels in spite of a significant rise of s25(OH)D[18]. In addition to weight in some studies, age was another factor added to the decrease in s25(OH)D with a well recorded association[19]. We have reported a significant correlation between s25(OH)D and weight status in term of inverse correlation, which does not essentially indicate a cause-and-effect link. Therefore, these changes may just be connected to other influences affecting both weight status and the vitamin D endocrine system.

A possible mechanism of increase PTH could be through influence of leptin on PTH production. As reported in this study and other studies, a confirmed association between serum leptin and sPTH was demonstrated in obesity[15]. Moreover, the implication that leptin is a PTH secretagogue brought about by injections of leptin in the leptin-deficient (*ob*/*ob*) mouse significantly raise sPTH levels[20]. If leptin influences PTH production directly either through paracrine or endocrine mechanisms, it implied that the relationship between leptin and PTH is causative. Unfortunately, no direct studies were done to confirm or disprove the paracrine or the endocrine mechanisms. Nevertheless, primary hyperparathyroidism in patients with obesity proven to have elevated level of PTH and greater parathyroid tumor weight than non-obese, proposing that increase parathyroid cell mass is mediated by leptin. Some studies suggest that leptin could be functioning as a mitogen working through paracrine mechanisms to control parathyroid cell mass[21].

The direct association between bone mass and body fat mass could be linked to the direct correlations between serum leptin and sPTH[22] as PTH controls sclerostin production, which is the main regulator of bone mass[23]. Hyperparathyroidism pathogenesis in obesity stays unclear. Some researches prove that hyperparathyroidism in morbid obesity fall back with weight loss supporting the strong role played by fat by itself and some investigations had revealed that it changes and decreases with weight alterations in healthy women[25], proposing a causal association. However, the analysis in our work was a case control study cross sectional and not longitudinal, rendering it not easy to assume causality.

This study showed the association between obesity and the changed serum levels of vitamin D and PTH, the calciotropic hormones controlling calcium and phosphate homeostasis. There was no change in Ca levels denoting no noticeable influence of PTH on Ca, proposing that PTH changes is not big enough to give a response on this objective variable or that additional controlling circumstances stand out. In our re-

port, Pi level was found to be higher in obese children but investigating renal tubular reabsorption of Pi directly was not determined, the finding is similar to the results of Lenders et al[25]. Other study found that no significant alteration in Pi although the hormone FGF23 regulating phosphate homeostasis was increased. FGF23 is produced by bone cells and cause down-regulation of phosphate reabsorption and suppressing renal 1,25-$(OH)_2$D formation[26]. Marked 25(OH)D deficiency happens at s25(OH)D concentration constantly less than 10 ng/ml and lead to significant deficiency in calcium absorption, decrease calcium levels, secondary increase in PTH, stimulated bone turnover, and rickets in children and osteomalacia in adults. Alternatively, vitamin D insufficiency arises when s25(OH)D is persistently lower than 30 ng/ml but goes above deficiency concentration of 20 ng/ml which was the case for some of the overweight and obese children in our research. While there is common concept that mild secondary hyperparathyroidism occurs as a biochemical manifestation resulting from vitamin D insufficiency. The consequences of vitamin D insufficiency remain controversial regarding mineral homeostasis. Cardiovascular disease, hypertension, insulin resistance, type 2 diabetes, and osteoarthritis all are comorbid condition associated with obesity in which vitamin D insufficiency may have a role to play in their pathogenesis.

In our study no significant changes in circulating OCN and β-CTx were found between obese and lean children but the effect of leptin on bone turn over markers was found by multiple regression analysis as β-CTx was predicted by leptin and OCN but not s25(OH)D suggesting a direct or indirect effect of leptin on osteoclasts. Also PTH predicted OCN, be indicative of its influence on osteoblasts. Bone is an active organ that constantly undertakes considerable turnover, an activity called modeling/remodeling including bone production and growth by osteoblasts and bone resorption by osteoclasts[27]. The process of bone remodeling preserves the quality of the skeleton by constantly substituting old bone with new one. Therefore, the balance between bone production and destruction at any specific time has an effect on bone mass. As others have demonstrated earlier, in obesity bone turnover markers were increased[16]. Serum β-CTx is a preferable marker than other resorption markers as urine desoxypyridinoline/creatinine (DPD/cre) because it is creatinine-independent. A positive correlation existed between a oesteogenesis marker, OCN and a osteolysis marker, β-CTx and OCN levels demonstrated positive correlation with age ($r = 0.347$ $p < 0.01$). In the human life cycle, OCN concentrations are at their peak during adolescence[28]. Others reported decrease levels of OCN in obese children[29] but elevated OCN values are described in patients suffering from increased bone formation as primary hyperparathyroidism. These findings in children were emphasized through an investigation showing that substantial decrease in weight was also correlated with a rise in OCN[29]. As OCN concentrations may vary with age, gender, height, growth velocity, puberty, and time at blood draw[30] The majority of these investigations showed a connection between OCN and obesity. Individuals at Tanner stage II-III were more likely to have higher OCN levels and those with 25(OH)D deficiency were more likely to have lower OCN using a sample of obese adolescents of mixed race/ethnicity[25].

In some researches in the morbid obese patients, markers of both osteogenesis and osteolysis were raised. Weight was responsible for the rise in the bone formation marker, bone alkaline phosphatase which may imply that adipokines have an effect on bone formation directly. Furthermore, low bone mineral density and content with reduced femora length was observed in the leptin-deficient (*ob*/*ob*) mouse. Also *in vitro* studies found leptin to be responsible for the reduction of bone fragility and the boosting of osteoblast differentiation and proliferation[21]. Therapeutically, providing leptin can inhibit osteogenesis working via the central nervous system or increase bone formation acting peripherally[32]. The role for leptin in bone formation can be endorsed by the presence of profuse functional leptin receptors in osteoblasts. In consequence, many evidences support that bone turnover is directly affected by leptin. Leptin effects on bone may be mediated through harmoniously interaction with calciotropic hormones.

The mechanism by which PTH affects bone formation and resorbtion is by upregulation of the expression of and to elevate levels of RANKL, an important cytokine for osteoclastogenesis[33]. PTH is also known to negatively regulate the production of sclerostin by osteocytes. Sclerostin inhibit both Wnts and several members of the bone morphogenetic protein (BMPs) actions which are critical for osteoblastogenesis and synchronize the activity of mature osteoblasts. PTH may in-

crease bone formation through direct influences on osteocytes by decreasing the expression of the osteocyte specific gene Sost (producing sclerostin)[34]. Rises osteoblast number and bone construction occurs also as a result of chronic PTH elevation. Increase osteoblast formation happens as a consequence of increase release of growth factors embedded in the bone matrix as consequent of stimulation of bone resorption. Also strong anti-apoptotic effects on osteoblasts have been related to PTH[35].

Many processes have been suggested to clarify the interaction between obesity and bone metabolism, involving raised proinflammatory cytokines and increased leptin production. There are many new evidences suggesting that at both young and old age, the infiltration of bone marrow by fat has harmful effect on bone remodeling unit affecting its function and also decreasing bone strength and density. Significantly, adipocytes in bone marrow inhibit osteoblastogenesis affecting bone formation as well as secrete inflammatory cytokines which have the capability to recruit osteoclasts. Adipocytes through the amplified production of leptin and/or reduced formation of adiponectin, influence bone formation directly or influence bone resorption indirectly. Additionally through the increased production of the proinflammatory cytokine as IL-6, IL-7, IL-1 α, IL-1 β, and TNF-α which are in particular raised in obesity and recognized to stimulate osteoclastogenesis, through altering the receptor activator of NF-κB (RANK)/RANK ligand/osteoprotegerin pathway[36].

The strength of the research is being a cross sectional case control study in overweight and obese children that cover a wide range of BMI. It verifies our assumption that serum leptin is one of the factors which have a direct effect on the processes leading to alterations in the mineral-regulating hormones. Thought, there was no major correlation between OCN and markers of body weight. However, we cannot eliminate a small influence of OCN on the very obese children.

This study has a small number of hypothetical shortcomings. BMI percentiles for age and weight were applied to categorize overweight and obese children, but BMI is considered to be only one of many markers of fat mass. Second, a cross sectional study cannot detect causal relationship, longitudinal researches are desirable for a clear understanding and recognition of assumed connections between these markers and fat tissue throughout the change from normal weight to obesity, to determine the reasons that create the alteration in the mineral-regulating hormones, and how these change with decrease in fat mass. Finally, we have no data on gender-specific hormone measurements which could of help in understanding the gender differences we noticed with β-CTx. Another possible weakness is the sample size which may have limited the ability to identify a meaningful connection between leptin and osteocalcin.

In summary, this cross sectional case control study of obese children reveals that they have elevated PTH levels and that these alteration clearly associate with changes in leptin and not due to s25(OH)D levels considered to be in the insufficiency and deficiency ranges. Our finding proposes that leptin has an endocrine or paracrine influence on PTH secretion. Serum 25(OH)D was decreased and seemed to be the consequence of boosted regulatory mechanisms. The associated bone turn over markers with PTH, shown in obesity could be a result of direct effect of leptin which support our hypothesis that that leptin can participate in the processes causing alteration in calcitropic hormones and bone turnover.

Conclusions

The obese children could be at a bigger risk of producing increased level of leptin and PTH with strong associated with bone turn over marker OCN and β-CTx and also deficiency of 25(OH)D which could have an important role to play in the pathogenesis of obesity and linked bone metabolic risk diseases as osteoporosis and fractures. While calciotropic hormones are finely tuned to the control of mineral metabolism, they also impact and are influenced by hormonal factors directly involved in the modulation of energy metabolism.

These studies provide clear evidence of the interrelationship among leptin, calciotropic hormones and bone turn over markers showing the integrative role of fat on skeleton and bone metabolic homeostasis.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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