

Bone Health in Relation to Vitamin-D Status and Serum Adipokines in Obese Egyptian Children

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Childhood obesity has been linked to an increase in fracture risk, so the impact of obesity on bone metabolism is becoming a focus of attention to identify factors that may affect bone health in obese children. Therefore, this study aimed to examine the association between serum 25-Hydroxy vitamin D [25(OH) D], adipokines and bone status in obese children. This case control study was executed in the Child Health Clinic in Medical and Scientific Centre of Excellence, National Research Centre (NRC), 100 obese and 80 non-obese age- and sex-matched children were enrolled in our study with mean age of (10.12±2.34 & 9.62±1.67 years) respectively. Anthropometric measurements, femoral neck bone mineral density (BMD) and its Z-score, bone mineral content (BMC) were measured using dual-energy X-ray absorptiometry (DXA) in relation to body weight (kg), we also determined serum 25(OH) D, adiponectin, leptin and lipid profile. HOMA-IR was calculated to assess insulin resistance. It was found that BMC and BMD Z-score adjusted for weight were significantly lower in obese children as compared to controls (all p<0.05). Obese children had lower levels of 25(OH) D and adiponectin (P<0.01), while higher levels of leptin, total cholesterol (TC) and triglycerides (TG) compared to controls (P<0.01). Both BMC and BMD Z-score showed positive association with 25(OH) D and adiponectin (P<0.01) and negative association with HOMA-IR, TG and TC (P<0.05). Linear regression analysis showed that 25(OH) D was the most effective factor predicting BMD Z-score and BMC in obese children. It is concluded that, obesity is negatively related to bone health in childhood. Those obese children are at increased risk for vitamin D insufficiency, which shows an obvious relationship to lower bone mass, raising the question of supplementation to prevent the deleterious effect of its deficiency on bones and reducing future risk of fracture and osteoporosis.

Keywords: Childhood Obesity; Bone Health; Serum 25(OH) D; Adipokines.

Increasing recrudescence of childhood obesity is a crucial public health concern.^{1,2}The vast predominance of childhood overweight or obesity is in developing countries, with above 30% increased rate higher than that of developed countries.³Some studies have demonstrated a

counter association of obesity with bone mineral density and bone health in children and adolescents.^{4,5,6} Along with the higher risk of sustaining fractures which are more complex in obese children as compared to normal weight peers.^{4,7}

Vitamin D has a significant role in bone growth, obese children have been found to possess lower concentration of vitamin D^{8,9}, that may contribute to lower BMD, as suggested by some studies.^{6,10}

Adipokines are adipose tissue secreted cytokines, playing a major role in regulating metabolic processes, from which leptin and adiponectin have a major role in obesity-related comorbidities and complications.¹¹ Both of them have been found to perform both a direct and centrally mediated influence on skeletal metabolism and development.¹²

Leptin, an adipocyte-derived hormone known for its role in energy homeostasis, has also been suggested to play a major role in bone metabolism, via its direct influence on skeletal tissue and concomitantly through modulating the bone-regulating hormones.^{13,14} The fat amount and thence leptin concentration, possess a dose-mediated influence on skeletal metabolism; little concentrations of leptin represent an osteoprotective effect, but at higher concentrations, bone wastage is augmented by bone resorption and slashed bone formation.¹²

Adiponectin, the most prevailing adipocytokine in plasma, which is implicated in glucose regulation and fatty acid breakdown and has a protective effect in various processes such as energy metabolism, inflammation and cell proliferation.¹⁵ An effective role of adiponectin in bone metabolism with a counter relation between adiponectin and bone has been brightened up.¹⁶

There is a highlighted interaction between insulin resistance and bone health, which may be interposed by visceral adiposity and the levels of circulating insulin.¹⁷

The aim of this study was to assess the impact of obesity on bone metabolism and to clarify the role of vitamin D status, adipokines and other factors that may affect bone health in Egyptian obese children.

MATERIALS AND METHODS

This was a case-control study, enrolling one hundred obese children compared to a control group of age and sex matched eighty apparently healthy non-obese children, recruited from the

Child Health Clinic in Medical and Scientific Centre of Excellence, National Research Centre (NRC). The study was approved by the Medical Ethical Committee of the National Research Centre, Cairo, Egypt. A written informed consent was obtained from parents of all participants after explanation of the objectives of the study.

Involved children fulfilled the inclusion criteria of simple obesity with age ranged between 5-15 years from both sexes who accepted to participate in the study, excluding children with endocrinal or genetic causes of obesity, chronic debilitating diseases that may interfere with bone health such as: e.g. (renal or hepatic disease), or mal-absorptive disorders (Crohn's disease, celiac disease and cystic fibrosis) and cancer. Children taking medications such as systemic glucocorticoids or anticonvulsants or those taking vitamin D, calcium or multivitamin supplements.

All enrolled children in the study were subjected to careful history taking, thorough clinical examination and anthropometric measurements; weight and height were measured in light clothing with no shoes. Body mass index (BMI) was calculated as weight (kg)/height (m²), Weight for age, height for age and BMI Z-score were calculated with the help of Anthro-plus Program for personal computers based on the WHO growth standards¹⁸, obesity was considered with BMI-Z-score >2. Triceps skinfold (SFT) was obtained by measuring the mid-point between the tip of the shoulder and the tip of the elbow (olecranon process and the acromium), parallel to the long axis of a slightly flexed arm, using the Holtain skinfold calipers, the circumference of the left upper arm, at the same mid-point giving the mid-arm circumference (MAC). Waist circumference at the smallest point between the rib cage and the iliac crest, Hip circumference at the largest width over the greater trochanters (the widest diameter around the buttocks), all of them have been measured with a non-elastic flexible tape and recorded to the nearest 0.1 cm, waist / Hip ratio (W/H R) was then calculated.

Laboratory Determinations: after fasting for 10-12 hours, venous blood sample was taken and left to clot, centrifuged, Sera were separated and stored at -20 ° C until assay.

• Fasting serum glucose, Cholesterol, Triglycerides

(TG), low density lipoprotein (LDL) and high-density lipoprotein (HDL) were measured by enzymatic calorimetric method using Bio-diagnostic kit (Egypt)

- The homeostasis model (HOMA-IR) was calculated according to the known formula [fasting insulin (mIU/ml) \times fasting glucose (mg/dl)/405]. Insulin resistance has been considered if HOMA-IR index > 3.16 , the greater the HOMA-IR value, the greater insulin resistance degree.
- Serum levels of insulin, adiponectin, Leptin and vitamin D were measured by a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle, using Enzyme immunoassay kit of insulin (Chemux Bioscience, Inc, USA), adiponectin (Assay pro, USA), leptin (Diagnostic Automation/Cortez Diagnostics, Inc. USA) and vitamin D following instructions of the kits purchased from (Epitepe Diagnostic, Inc. San Diego, CA 92121, USA).
- Measurement of bone mineral content (BMC) in grams, bone mineral density (BMD) in (g/cm²), bone area (cm²) was done by trained radiology technologist using dual energy X-ray absorptiometry (DXA) (Norland Capital- XR46; Norland Medical systems Inc., Fort Atkinson, WI) according to the procedures recommended

by the manufacturer. Measurements were taken in separate regions of interest: femur neck, and lumbar spine (L1–L4).

Statistical analysis

All data were collected, verified, coded, entered and analyzed using IBM SPSS Statistics v. 22. Comparison between two groups regarding quantitative data was done using Independent t-test. DXA, bone parameters values were compared after adjustment for total body weight using a 1-way analysis of covariance. The relation between two quantitative parameters in the same group was assessed by Pearson correlation analysis. Linear regression analysis was performed to identify possible determinants associated with the Z-scores for BMD and BMC after adjusting for effects of weight. P-value < 0.05 was considered significant.

RESULTS

The present study enrolled 100 obese and 80 non-obese age- and sex-matched Egyptian children with mean age (10.12 \pm 2.34 & 9.62 \pm 1.67 years), respectively. Obese children had statistically significant higher anthropometric indices (weight Z-score, BMI Z-score, Triceps skinfold, mid-arm circumference and waist / Hip ratio, $P < 0.05$ for

Table 1. Demographic data, anthropometric and bone health indices of obese children versus controls

| Variables | Obese group n=100 Mean \pm SD | Control group n=80 Mean \pm SD | p-value |
|-----------------------------|------------------------------------|-------------------------------------|-------------|
| Age (years) | 10.12 \pm 2.34 | 9.62 \pm 1.67 | 0.175 |
| Weight Z-score | 2.64 \pm 0.93 | 0.9684 \pm 0.22 | $< 0.001^*$ |
| Height Z-score | -0.31 \pm 0.69 | -0.70 \pm 0.72 | 0.656 |
| BMI Z-score | 2.67 \pm 0.82 | 1.10 \pm 0.47 | $< 0.001^*$ |
| Skin fold (mm) | 20.60 \pm 4.78 | 10.1 \pm 2.58 | $< 0.001^*$ |
| waist / Hip ratio | 0.90 \pm 0.1 | 0.76 \pm 0.2 | 0.04* |
| Mid-arm (cm) | 36.40 \pm 8.71 | 20.3 \pm 4.1 | $< 0.001^*$ |
| BMD.FN (g/cm ²) | 0.76 \pm 0.16 | 0.80 \pm 0.17 | 0.33 |
| BMC.FN (g) | 3.61 \pm 0.98 | 4.22 \pm 0.74 | 0.004* |
| BMD.FN. Z-score | -0.18 \pm 0.61 | 0.35 \pm 0.85 | 0.002* |
| BMD.LV (g/cm ²) | 0.65 \pm 0.17 | 0.58 \pm 0.13 | 0.067 |
| BMC.LV (g) | 35.07 \pm 7.11 | 38.72 \pm 4.29 | 0.013* |
| BMD.LV. Z-score | -0.40 \pm 0.71 | 0.02 \pm 0.8 | 0.016* |

*($P < 0.05$): obese significantly different from controls

BMC: bone mineral content; BMD: bone mineral density; BMI: Body mass index; FN: femoral neck; LV: lumbar vertebra; SD: Standard deviation.

all) than non-obese group. After adjustment for body weight, BMC and BMD Z-score at femoral neck (FN) and lumbar vertebra (LV2-LV4) were significantly lower in obese children (all $p < 0.05$), while no difference in BMD at any site of analysis as compared with normal-weight children (Table 1).

Table (2) shows that after adjustment for body weight, obese children had lower levels of vitamin D (25(OH) D), adiponectin and high density lipoprotein (HDL) ($P < 0.01$), while higher levels of leptin, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) & HOMA-IR compared to controls ($P < 0.01$).

BMC.FN showed positive association with 25(OH) D (Figure 1) and negative association with Wt. Z-score ($r = -.306$, $p = .031$), BMI. Z-score ($r = -0.291$, $p = 0.04$), Cholesterol ($r = -0.373$, $p = 0.008$) (Figure 2), HDL ($r = -0.513$, $p = 0.000$) & HOMA-IR, ($r = -0.286$, $p = 0.044$).

BMD.FN. Z-score showed positive association with 25(OH) D ($r = 0.376$, $p = 0.007$) & Adiponectin ($r = 0.367$, $p = 0.009$) (figure 3) and negative association with TG ($r = -0.327$, $p = 0.020$), Cholesterol ($r = -0.295$, $p = 0.037$) HDL ($r = -0.474$, $p = 0.001$) & HOMA-IR ($r = -.350$, $p = .013$).

BMD.LV. Z-score showed positive association with 25(OH) D ($r = 0.298$, $p = 0.036$) & Adiponectin ($r = 0.337$, $p = 0.017$) and negative association with HDL ($r = -0.283$, $p = 0.046$) & HOMA-IR ($r = -0.312$, $p = 0.027$). Leptin was insignificantly correlated to bone variables in obese cases.

Linear regression analysis showed that 25(OH) D was the most effective factor predicting BMC.FN ($P < 0.001$) and BMD.FN. Z-score ($P = 0.05$) in obese children (Table 3 & 4).

DISCUSSION

This study was conducted to test the hypothesis that obesity is a risk factor for poor bone health in children and adolescents. After adjustment for body weight, BMC and BMD. Z-score were significantly lower in obese children ($p < 0.05$), compared to control group. While the correlation analysis revealed an inverse association between BMC.FN and BMI Z-score. Conflicting results have been reported in several studies, regarding the relation between bone health and obesity. In close agreement, with our findings, a large scale national survey of U.S. young population from the NHANES found that both total body BMD and lumbar spine BMD had an inverse relation with degree of obesity, the authors suggested regional differences in the relation between adiposity and BMD.¹⁹ Another cross sectional study conducted on Indian children and adolescents found that total body BMC, bone area and BMD adjusted for Tanner stage and weight were significantly lower in obese children relative to overweight and normal weight children.⁴

However in contrast to these findings, other studies confirmed a positive association between BMD and fat mass.²⁰ Similarly, Kim *et al.*²¹ evidenced a positive impact of fat mass and

Table 2. Laboratory features of obese children versus controls

| Variables | Obese group n=100 Mean±SD | Control group n=80 Mean±SD | p-value |
|---------------------|------------------------------|-------------------------------|---------|
| 25(OH) D (ng/mL) | 10.13±3.22 | 17.68±11.64 | <0.001* |
| Cholesterol | 189.74±47.98 | 89.52±17.1 | <0.001* |
| Triglycerides | 131.63±34.36 | 81.68±24.76 | <0.001* |
| HDL | 49.28±11.6 | 58.26±11.21 | <0.001* |
| LDL | 129.40±28.42 | 47.94±10.62 | <0.001* |
| Adiponectin (ng/mL) | 37.4±8.5 | 54.7±11.4 | 0.03* |
| Leptin (ng/mL) | 41.34±11.3 | 4.7±2.4 | <0.001* |
| HOMA.IR | 5.12±1.34 | 1.85±0.6 | 0.001* |

* $p < 0.05$ the relation is statistically significant

25(OH) D: 25-Hydroxy vitamin D; HOMA-IR: homeostasis model assessment of insulin resistance, LDL: low-density lipoprotein; HDL: high-density lipoprotein; SD: Standard deviation.

lean mass on bone density in Korean adolescents. This could match the findings of a recent meta-analysis about the comparison of BMD between obese or overweight children and controls, which found a significant higher BMD in the obese group.²²

In the same context, the increased adiposity has traditionally been accounted to have a beneficial effect on bone status, as the mechanical loading was found to be a potent enhancer of

the proliferation of osteoblasts and increased bone strength.²³ Thus, this can partly explain the positive relation between adiposity and bone status. However, after adjustment for body weight, this positive association is no longer significant,²⁴ or is completely absent.²⁵

Hence, it was indicated that the positive influence of body weight on BMD could not counteract the deleterious effects of obesity on bone health. However, the precise mechanism

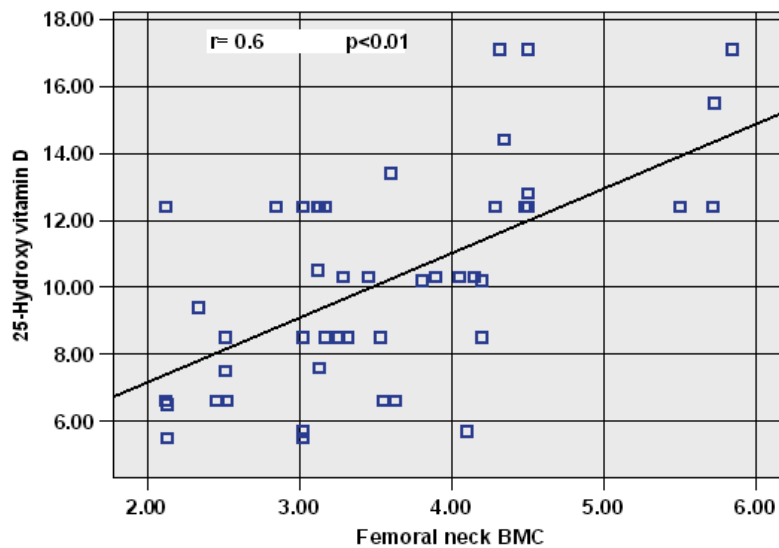


Fig. 1. Correlation between BMC.FN and 25(OH) vitamin D in obese cases

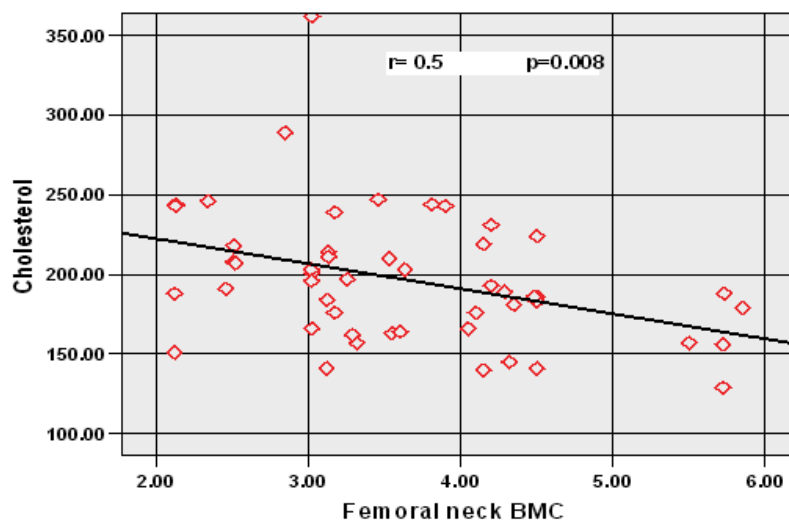


Fig. 2. Correlation between BMC.FN and cholesterol in obese cases

implying for deteriorated bone status in the obese is not completely elucidated yet and further studies are required.²⁶

The influence of vitamin D as one of the most important bone-regulators is well-known, furthermore, accumulating evidences indicated the contribution of vitamin D to lower

BMD.²⁷ Simultaneously, serum concentrations of vitamin D had evidenced to be decreased in obese individuals²⁸, and inversely associated with BMI.⁷ These findings match our results as we observed a significant lower levels of vitamin D in obese

Table 3. Linear regression analysis for independent predictors of BMC.FN among the studied obese children

| Predictors | BMC.FN | |
|---------------|--------------------------------|---------|
| | Standardized Coefficients Beta | Sig. |
| (Constant) | | 0.006 |
| 25(OH) D | 0.542 | <0.001* |
| Cholesterol | -0.147 | 0.283 |
| Triglycerides | -0.062 | 0.652 |
| Adiponectin | 0.002 | 0.990 |
| HOMA.IR | -0.054 | 0.690 |

* p<0.05 the relation is statistically significant
25(OH) D: 25-Hydroxy vitamin D;HOMA-IR: homeostasis model assessment of insulin resistance, BMC: bone mineral content; FN: femoral neck.

Table 4. Linear regression analysis for independent predictors of BMD.FN. Zscore among the studied obese children

| Predictors | BMD.FN. Z-score | |
|---------------|--------------------------------|-------|
| | Standardized Coefficients Beta | Sig. |
| (Constant) | | 0.260 |
| BMI z-score | 0.155 | 0.327 |
| 25(OH) D | 0.299 | 0.05* |
| Cholesterol | -0.097 | 0.526 |
| Triglycerides | -0.114 | 0.467 |
| Adiponectin | 0.246 | 0.094 |
| HOMA.IR | -0.159 | 0.317 |

* p<0.05 the relation is statistically significant
BMD: bone mineral density; BMI: Body mass index;FN: femoral neck,25(OH) D: 25-Hydroxy vitamin D;HOMA-IR: homeostasis model assessment of insulin resistance

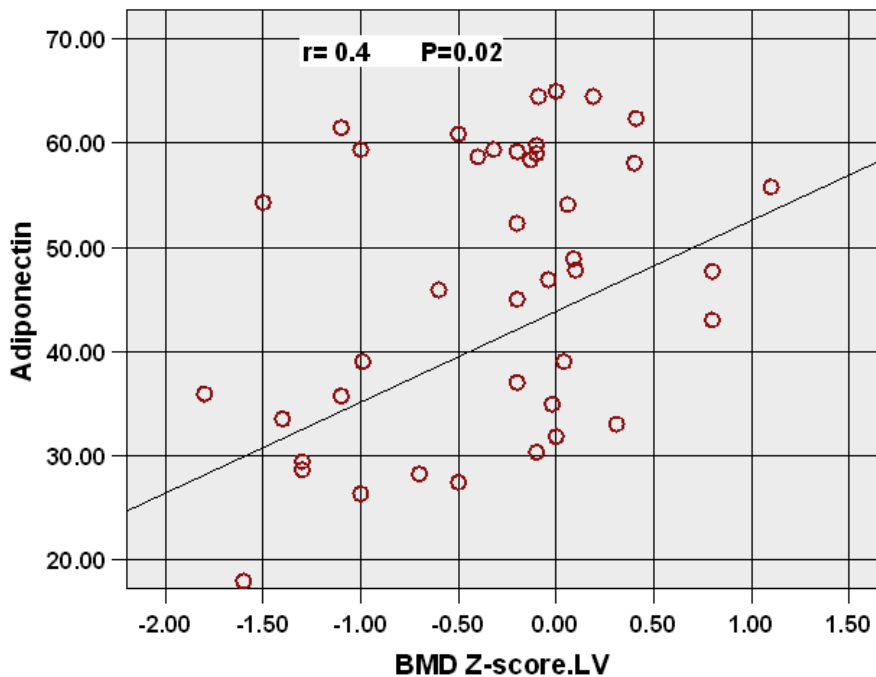


Fig. 3. Correlation between BMD.LV. Z-score and Adiponectin in obese cases

subjects compared to control group. In addition, a negative association was found between vitamin D and BMI Z-score, BMC.FN and BMD.FN Z-score. Vitamin D also was a strong predictor of BMC.FN and for BMD.FN Z-score by linear regression in this study.

Recently it was suggested that decreased serum vitamin D is an outcome of obesity and the findings from the clinical trials that explored the association between vitamin D and obesity, are still infrequent and inconclusive. On the other hand, it was evidenced that vitamin D prevents fat deposition, promotes insulin synthesis, and reduces insulin resistance and hunger, that favor controlling obesity and T2DM.²⁹

The contribution of some adipokines in bone remodeling had been considered as another probable mechanism. These molecules are released from fat cells and some of them interfere with both bone formation and resorption.³⁰

Existed data regarding the role of leptin was inconsistent, Rhee and his colleagues³¹, suggested that it plays a beneficial role on bone mass and density in prepubertal girls.

However, higher concentrations of leptin, had been proposed to stimulate inflammatory cascades in osteoblasts that may contribute to poor bone health.³²

Although obese subjects in this study showed significantly higher levels of leptin, there was no significant correlation with any bone variables.

In accordance, in a previous review study, leptin was not associated with BMD after adjustment for BMI and fat mass in variable studies.³³ The finding of another recent review may explain this controversy, as they reported that leptin can improve bone health in cases with deficient leptin blood levels, otherwise it is probably had no significant effect when it is not deficient.¹³

On the other hand, the role of adiponectin as a mediator of the relation between bone health and obesity was investigated, there was a direct association between BMD FN.Z-score and adiponectin, while its correlations with other bone health indices were insignificant, which match the finding of a previous longitudinal study, conducted on 96 adolescent healthy males.³⁴

In contrast with the current study, the majority of the clinical studies, which had been

discussed in a recent review, reported a negative association between adiponectin and bone health in populations with different age groups. However, most of *in vitro* studies that searched its signaling and mechanisms of actions partially supported our findings and revealed that the activities of adiponectin predicted a positive effect on bone, as it enhanced the proliferation and differentiation of osteoblasts. Moreover, the indirect effect of adiponectin was considered, through modulation of the sympathetic tone and the regulation of insulin sensitivity and energy homeostasis.³⁵

Similarly, China *et al.*³⁶ reported that adiponectin positively influenced skeletal health, and they attributed the discrepancy found in literatures to the use of various structural forms of adiponectin in preclinical research.

In another respect, this study suggested the potential contribution of the metabolic disturbances associated with obesity to poor bone health as there were inverse associations between BMD.FN. Z-score with TG, Cholesterol, HDL & HOMA-IR. Furthermore, BMD.LV. Z-score showed negative association with HOMA-IR.

In accordance, the interrelation between dyslipidemia and low mineral density of bone was evidenced in several studies; elevated cholesterol had been proved a negative regulator of bone.³⁷

In addition, HOMA-IR was used to evaluate insulin resistance in a study conducted by Pollock *et al.*³⁸; they suggested the contribution of insulin resistance in the link between obesity and low bone mass. Moreover, vitamin D concentrations were inversely associated with insulin resistance in the present study, that match other findings.^{39,40}

Furthermore, in agreement with our findings it was indicated that disturbed glucose regulation negatively affected the growing skeleton. However, the exact mechanism needs more elucidation.⁴¹

The major limitation of this study is being a cross-sectional one, thus studying the causal relations could not be inferred. Further longitudinal studies are needed, on larger sample size for better understanding of the complex situation.

CONCLUSION

In conclusion, rising concerns regarding the relation between childhood obesity and bone

health have emerged nowadays. The current study demonstrated a significantly lower value of most of the studied bone health indices in obese children relative to controls, and a negative association was found between bone mineral density at femoral neck and BMI. Vitamin D represented the most effective predictor of bone health, indicating its important role in the mechanism implicated in mal-affecting bone status in obese. These findings may contribute in enhancing the preventive and therapeutic strategies to counteract the linked risks of obesity and poor bone health.

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