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Syeda Sadia Fatima

*Aga Khan University*, [sadia.fatima@aku.edu](mailto:sadia.fatima@aku.edu)

Saad Farooq

*Aga Khan University*

Maaida Asif Tauni

*Aga Khan University*

Omar Irfan

*Aga Khan University*

Faiza Alam

*Aga Khan University*

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## Effect of raised body fat on vitamin D, leptin and bone mass

Syeda Sadia Fatima,<sup>1</sup> Saad Farooq,<sup>2</sup> Maaida Asif Tauni,<sup>3</sup> Omar Irfan,<sup>4</sup> Faiza Alam<sup>5</sup>

### Abstract

**Objectives:** To estimate leptin, vitamin D and bone mineral density levels in individuals with high fat mass, and to assess any correlation.

**Methods:** The cross-sectional study was conducted at the Basic Medical Sciences Institute, Jinnah Post Graduate Medical Centre, Karachi, and Aga Khan University, Karachi, from August 2012 to July 2014, and comprised healthy male volunteers between the ages of 18-60 years. Body fat percentage was determined using bioelectrical impedance analysis and the participants were classified as: Group A (15-21.9); Group B (22-27.9); and Group C (>28). Bone mineral density was calculated by ultrasound bone densitometer (T-score between +1 and ?1 considered normal). Enzyme-linked immunosorbent assay kits were used to determine the levels of vitamin D and leptin. SPSS 19 was used for statistical analysis.

**Results:** A total of 132 male subjects participated in this study, with each of the 3 groups having 44(33.3%). Despite all groups having low Vitamin D, a marked decrease was observed in group C compared to groups A and B ( $p < 0.018$ ). Bone mineral density T-score was  $< -1$ ; total calcium was within normal range in all three groups. Serum leptin was raised in Group C compared to group A and B ( $p = 0.03$ ). Body fat percentage was negatively associated with vitamin D ( $p = 0.004$ ;  $r = -0.351$ ), while it was positively correlated with leptin ( $p = 0.038$ ;  $r = 0.256$ ).

**Conclusion:** Excess of body fat percentage led to decreased vitamin D and raised leptin. However, bone mineral density and calcium levels were within normal range, suggesting that other factors might have played a role in maintaining bone mass in obese individuals, such as leptin.

**Keywords:** Obesity, Bone weight, Leptin, Vitamin D, Bioelectric impedance analysis, Bone mineral density. (JPMA 65: 1315; 2015)

### Introduction

Obesity either in the form of raised body mass index (BMI) or excess body fat (BF) deposition is exceedingly harmful for the human body. Decreased exercise, sedentary lifestyles and inappropriate dietary habits, combined with a "thrifty genotype" have made obesity a global pandemic. In Pakistan more than 22 per cent individuals over 15 years are obese.<sup>1</sup> Obesity not only has psychosocial issues like low self-esteem, but it predisposes a person to other life-threatening complications such as cardiovascular diseases, type II diabetes, high blood cholesterol levels etc.<sup>2</sup> Of these myriad effects of obesity, the effects on the vitamin D and leptin levels and their effect on maintenance of bone mineral density (BMD) in obese individuals are highly intriguing.

Other than dietary sources like fish, cheese and eggs, skin also contributes towards the vitamin D (cholecalciferol) pool when it is exposed to ultraviolet (UV) radiation from the sun, which is converted to its active form in the

kidney.<sup>3</sup> This vitamin is essential for early development and growth and is known to play an integral role in maintaining BMD.<sup>4</sup> The role of vitamin D is to enhance the absorption of calcium, phosphorus, magnesium and zinc from the gut. Deficiency of vitamin D leads to a number of diseases in children such as rickets and osteomalacia and increases the risk for certain cancers, multiple sclerosis, hypertension and type 1 diabetes in adults.<sup>5</sup> Since vitamin D is fat-soluble, it tends to get sequestered in fat tissues and obese individuals may develop an apparent deficiency.<sup>6</sup> Ironically, these reduced levels do not appear to affect the bone mass in obese individuals. In fact, increased BMI has been associated with increased BMD by previous studies, possibly because increased BMI increases mechanical stress on the bones resulting in an amplified BMD.<sup>7</sup>

On the other hand, leptin, a satiety hormone released by adipocytes is the product of obese (ob) gene.<sup>8</sup> It primarily regulates the fat content of the body. Leptin levels increase after a meal and act on satiety centres in arcuate nucleus of the hypothalamus. Increase in BF also causes an increase in baseline leptin levels. Obese people often have hyperleptinaemia because they usually develop leptin resistance,<sup>9</sup> therefore the satiety function of leptin gets depressed. Other known effects of leptin are its

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<sup>1,5</sup>Department of Biological and Biomedical Sciences, <sup>2-4</sup>Medical College, Aga Khan University, Karachi, Pakistan.

**Correspondence:** Faiza Alam. Email: faiza.alam@aku.edu

effects on the nervous and endocrine systems, especially the hypothalamic-pituitary-gonadal axis and insulin biology.<sup>10</sup> Some studies have also suggested that leptin causes increased osteoblastic differentiation and inhibits osteoclast generation, therefore decreasing bone resorption and increasing bone mass.<sup>11</sup> Nevertheless the role of leptin on bone regulation is still highly controversial with different authors suggesting diverse associations.

The relation of BMD, leptin and vitamin D in obesity is not well understood. A number of previous studies have associated excess body fat mass with decreased vitamin D, increased bone mass and increased levels of leptin.<sup>7,9,12</sup>

The current study was planned to estimate the circulatory levels of vitamin D and leptin along with BMD levels in individuals with high fat mass and to assess their correlation.

## Subjects and Methods

The cross-sectional study was conducted at the Basic Medical Sciences Institute (BMSI), Jinnah Post Graduate Medical Centre (JPMC), Karachi, and Aga Khan University (AKU), Karachi, from August 2012 to July 2014, and comprised healthy male volunteers between the ages of 18-60 years.

Body fat percentage (BF%) was estimated by bioelectrical impedance analysis using Diagnostic Scale BG55 (Beurer, Germany). Subjects were asked to stand on the machine in light clothing and in an erect posture. The participants were classified as: Group A (15-21.9); Group B (22-27.9); and Group C (>28).<sup>13</sup>

In order to achieve minimum 80% power with a 15% estimated prevalence of obesity and a two-sided 5% level of significance, the minimum sample size required, according to Power and Sample Size (PASS) version 11, was 44 for each group.

BF% was measured after the study was approved by the ethics review committee of MBSI And all participants gave written informed consent.

Height was measured using a standard stadiometer (ZT - 120 Health Scale, made in China). BMI was calculated using the following formula: weight in kg / height in m<sup>2</sup>.<sup>14</sup> The following was used as reference values for BMI; normal weight 18.0-22.9 kg/m<sup>2</sup>, overweight 23.0-25.9kg/m<sup>2</sup>, and obese >26.0 kg/m<sup>2</sup> as per the South Asian criteria.<sup>15</sup> Waist and hip circumference along with their ratio was measured as previously described.<sup>16</sup>

The heel bone (calcaneous) BMD was measured using an

ultrasound bone densitometer (OsteosysSonost 3000 Bone Densitometry). A T-score between +1 and -1 was considered normal or healthy.<sup>17</sup>

Three ml of blood was collected in sterile Venoject tubes after an overnight fast of 8-12 hours. Blood was centrifuged at 2000xg for 5 minutes to separate the serum. Serum was further aliquoted in small volumes and stored at -80°C until further use.

Vitamin D and leptin levels were measured by commercially available enzyme-linked immunosorbent assay (ELISA) kits (kit cat#KAP197; kit cat# KAP2281 by DIA source Immunoassays S.A. Belgium respectively). The following reference range for vitamin D levels were considered; deficient <10 ng/ml, insufficient =10-29 ng/ml, sufficient = 30-100 ng/ml and toxic = >100 ng/ml.[18] Serum calcium was estimated by automated clinical analysers (Roche).<sup>19</sup>

SPSS 19 was used for statistical analysis. Data on continuous variables, such as age, height, weight, BMI, waist circumference, hip circumference, waist-hip ratio, BF%, BMD, and biochemical (serum calcium, vitamin D and leptin) parameters were calculated as mean ± standard deviation (SD). Mann-Whitney U test was used to compare groups. Spearman's coefficient of correlation (r) was used to determine the correlation between serum vitamin D, leptin levels, BF parameters and BMD. In all statistical analysis, p<0.05 was considered significant.

## Results

A total of 180 individuals volunteered for the study, but 48(26.6%) were excluded on the basis of history of recent illness in the preceding six weeks, chronic diseases like diabetes, tuberculosis, cancer or hypertension, and supplementation of vitamin D or calcium. A total of 132(73.3%) male subjects participated in the study, with each of the three groups having 44(33.3%) subjects. All subjects were age-matched and therefore no significant differences were observed among the groups. Biochemical and biophysical parameters of the subjects were noted (Table).

In all the study groups, values of BMD T-score was in the lower threshold (i.e. <-1); similarly all groups showed normal total calcium levels (mg/dl) (p>0.05). Despite all groups having low levels of Vitamin D, a marked decrease was observed in Group C compared to groups A and B (p =0.018). The low levels of vitamin D, however, did not have any effect on the calcium levels or the bone mass of these individuals. Serum leptin concentration was significantly increased in Group C compared to groups A and group B (p=0.03).

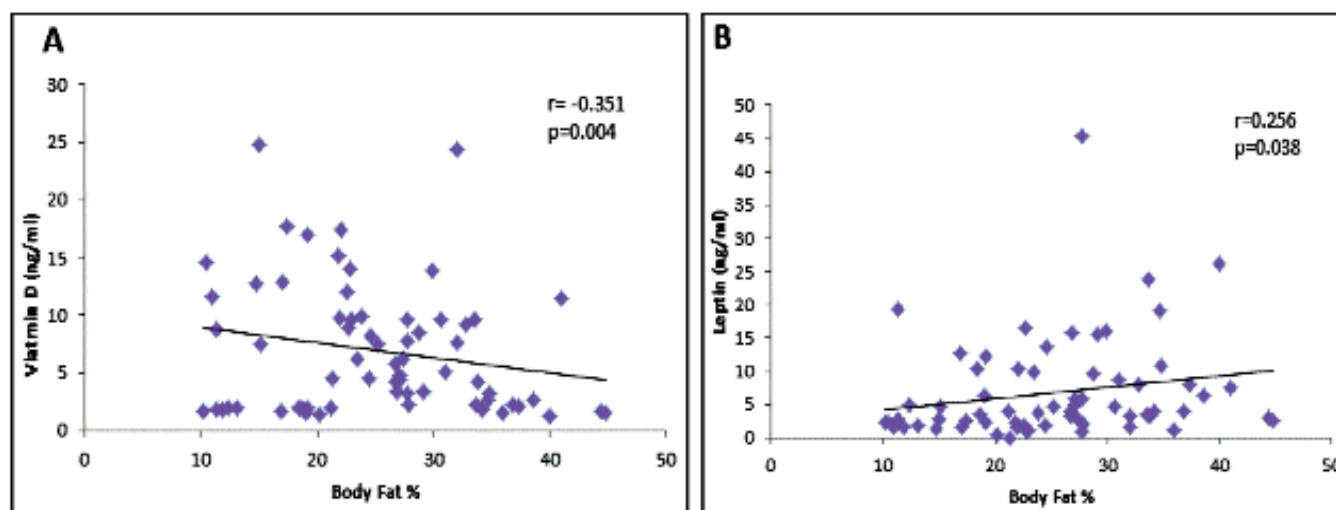
**Table:** Biophysical characteristics.

Parameter	Group A Normal weight (n=44) Mean $\pm$ SD	Group B Overweight (n=44) Mean $\pm$ SD	Group C Obese (n=44) Mean $\pm$ SD	P value
Age (years)	35.7 $\pm$ 11.6	37.2 $\pm$ 12.1	39.3 $\pm$ 11.0	>0.05
Weight (kg)	58.7 $\pm$ 9.3	70.9 $\pm$ 8.00	78.8 $\pm$ 12.5**	<0.001
BMI (kg/m <sup>2</sup> )	19.6 $\pm$ 2.0	24.5 $\pm$ 2.35**	28.9 $\pm$ 7.2**	<0.001
Body fat %	15.7 $\pm$ 3.8	25.4 $\pm$ 2.27**	34.7 $\pm$ 5.2**	<0.001
Waist circumference (cm)	71.8 $\pm$ 7.6	89.4 $\pm$ 9.05	96.0 $\pm$ 14.9**	<0.001
Hip circumference(cm)	85.6 $\pm$ 8.7	102.8 $\pm$ 9.08	102.7 $\pm$ 15.6	<0.001
WHR	0.8 $\pm$ 0.0	0.88 $\pm$ 0.085	0.9 $\pm$ 0.1**	<0.001
BMD value (T-score)	-1.34 $\pm$ 0.6	-1.39 $\pm$ 1.09	-1.2 $\pm$ 0.6	0.264
BMD %	78.8 $\pm$ 9.6	74.6 $\pm$ 17.99	80.6 $\pm$ 11.6	0.227
Bone weight	8.34 $\pm$ 1.7	9.57 $\pm$ 1.42	10.0 $\pm$ 6.8	0.707
Vitamin D (ng/ml)	12.8 $\pm$ 2.1	9.4 $\pm$ 2.10	7.2 $\pm$ 1.4*	0.018
Leptin (ng/ml)	4.7 $\pm$ 0.9	5.51 $\pm$ 1.90	8.3 $\pm$ 1.3*	0.030
Calcium (mg/dl)	8.8 $\pm$ 0.4	8.82 $\pm$ 0.49	9.0 $\pm$ 0.7	0.525

BMI: Body mass index

WHR: Waist-hip ratio)

BMD: Bone mineral density.

\*Statistically significant as compared to control subjects, where  $p < 0.05$ .\*\*Statistically significant as compared to control subjects, where  $p < 0.01$ .**Figure (A-B):** Correlation Graphs.

Total BF% was negatively associated with circulating vitamin D concentrations ( $p = 0.004$ ;  $r = -0.351$ ) while at the same time it was positively correlated with leptin levels ( $p = 0.038$ ;  $r = 0.256$ ) (Figure-1A-B).

## Discussion

Relation of vitamin D and leptin with BF% is scarce, specially in Pakistani population. This population, being overtly vitamin D-deficient without showing any obvious signs of decremental BMD, renders itself as a target for investigating the link between body fat, vitamin D and

leptin with bone mass. To address this, we estimated the levels of vitamin D, leptin and BMD in varying degrees of adiposity and studied their correlation. The present study yielded interesting results, showing that the overall study cohort had low vitamin D levels despite having normal serum calcium. The low vitamin D level is perhaps now considered a common finding among the South Asian population as documented in studies done earlier.<sup>20,21</sup> The current study determined that with increased BF% and vitamin D levels further decreased without affecting the bone mineralisation. This unusual relation of low

vitamin D, normal calcium and a positive correlation with bone mineralisation is in line with a previous study.<sup>22</sup> The lower levels might be due to vitamin D sequestration in adipose tissue, resulting in reduced levels in the blood. The reasons for such a finding to prevail in most studies points towards genetic polymorphisms, leading to hypovitaminosis D. Further research to identify relevant gene polymorphism might be able to shed more light to this intriguing phenomenon.

It is also possible that the reduced vitamin D levels may cause obesity in these individuals as suggested by a previous study.<sup>23</sup> The reduced vitamin D levels may enhance the capacity of the body to store large amounts of fat as a stress response, to ensure better survival. The bone density of the study population revealed no significant difference despite the differences in the vitamin D levels. In fact, the obese population with lower vitamin D levels still had a BMD closer to normal as opposed to those with a relatively higher vitamin D level. An earlier study<sup>12</sup> found that adolescents between 10-16 years of age with obesity had lower vitamin D serum concentration, but had higher age-adjusted BMD than lean subjects.

This was a very thought-provoking finding, suggestive of something additional than vitamin D is playing a part in bone mineralisation. The best candidate for this hypothesis in our study would be increased leptin levels in the obese subjects. As serum leptin concentrations are elevated in obesity, a favourable effect of raised leptin on BMD might play a part in maintaining bone mineralisation,<sup>24</sup> resulting in a better BMD value in our population of interest, although the factual reason was beyond the scope of our study and a large sample size is required to validate this finding. On the other hand, some studies have shown to have a contradictory opinion to this idea.<sup>25</sup> Therefore the increased BMD can possibly be caused by the increased weight loading/stress on the bone which occurs in obesity.

The limitations of the study are owing to its cross-sectional design, due to which a causal relation between these factors could not be implied. Moreover, the role of certain hormones such as of oestrogen, parathyroid hormone (PTH), etc. could not be evaluated. Future longitudinal studies including these factors as well as identifying genetic polymorphisms for vitamin D receptor and collagen synthesis genes are required to elucidate the role of genetic factors maintaining BMD and calcium levels despite hypovitaminosis D in Pakistani population.

## Conclusion

Decreased levels of vitamin D were associated with higher

body fat mass probably due to its sequestration within the adipocytes. Despite low vitamin D, there was no change noticed in BMD which may highlight the bone mineralisation effects of raised leptin. But further longitudinal studies are required to clarify these findings.

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