

Original Article



OPEN ACCESS

Received: Aug 4, 2020

Revised: Oct 20, 2020

Accepted: Oct 22, 2020

Correspondence to

Sakineh Shab-Bidar

Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, No 44, Hojjat-dost Alley, Naderi St., Keshavarz Blvd, Tehran 14167-53955, Iran.

E-mail: s_shabbidar@tums.ac.ir

Copyright © 2020. The Korean Society of Clinical Nutrition

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID iDs

Parisa Janmohammadi

<https://orcid.org/0000-0001-6315-7310>

Farhang Djafari

<https://orcid.org/0000-0001-6890-8208>

Gholamreza Mohammadi Farsani

<https://orcid.org/0000-0003-2033-9100>

Sakineh Shab-Bidar

<https://orcid.org/0000-0001-8630-6155>

Ali Asghar Haeri Mehrizi

<https://orcid.org/0000-0002-3671-2849>

Conflict of Interest

The authors declare that they have no competing interests.

Parathyroid Hormone and 25-Hydroxyvitamin D Do Not Mediate the Association between Dietary Calcium, Protein and Vitamin D Intake and Adiposity and Lipid Profile in Patients with Type 2 Diabetes: a Structural Equation Modeling Approach

Parisa Janmohammadi ¹, Farhang Djafari ², Gholamreza Mohammadi Farsani ¹, Sakineh Shab-Bidar ², Ali Asghar Haeri Mehrizi ³

¹Department of Clinical Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran 14167-53955, Iran

²Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran 14167-53955, Iran

³Health Metrics Research Center, Iranian Institute for Health Sciences Research, Academic Center for Education, Culture and Research, Tehran 13145-1756, Iran

ABSTRACT

This study aimed to utilize structural equation modeling (SEM) techniques to determine the effects of dietary calcium, protein and vitamin D on adiposity and lipidemia and to assess mediatory effects of parathyroid hormone (PTH) and 25-hydroxyvitamin D (25(OH)D) in patients with type 2 diabetes. In this cross-sectional study, a total of 150 diabetic patients (93 females and 57 males) were randomly selected. Anthropometric measures, biochemical analyses, and fat mass percent were recorded. Nutritional data were collected. SEM was performed. Based on the primary hypothesis, adiposity and lipidemia were fitted in a model. The direct effects of dietary calcium ($\lambda = -0.165$, p value = 0.002) and PTH ($\lambda = -0.143$, p value = 0.011) were significantly associated with lipidemia. There were no significant effects for dietary protein on PTH ($\lambda = -0.270$, p value = 0.057), 25(OH)D ($\lambda = -0.071$, p value = 0.613), lipidemia ($\lambda = -0.044$; p value = 0.638) or adiposity ($\lambda = -0.009$, p value = 0.949) as well as for dietary vitamin D on PTH ($\lambda = -0.119$, p value = 0.194), 25(OH)D ($\lambda = 0.023$, p value = 0.806), lipidemia ($\lambda = 0.034$, p value = 0.587) or adiposity ($\lambda = -0.221$, p value = 0.118). The correlation between calcium intake and lipidemia, and adiposity are not mediated by 25(OH)D and PTH. There were the direct effects of dietary calcium on adiposity in patients with type 2 diabetes. The model can be tested in future longitudinal and intervention studies to identify the predictors of obesity.

Keywords: Adiposity; Dietary calcium; Type 2 diabetes; Vitamin D

INTRODUCTION

Diabetes is considered as a chronic metabolic disorder with about 5.1 million deaths annually and approximately 382 million adult patients [1]. Type 2 diabetes is a preventable disorder; hence the disease has more important than type 1 diabetes in public health [2]. On the other hand, obesity is recognized as a major public health problem and the main risk factor for developing type 2 diabetes [3]. Inappropriate diet promotes excessive fat accumulation in body and causes a metabolic imbalance [4] that can lead to obesity and type 2 diabetes [3].

Shortage or excess in consumption of nutrients has been implied to facilitate or impede fat accumulation in body, of which vitamin D and calcium have recently received much attention [4]. Different studies have shown that calcium reduces fat accumulation in overweight and obese people [5-7] and calcium inadequate diet increases fat accumulation in body [5]. It has been established that there is a significantly higher outbreak of obesity in people with low circulating vitamin D levels compared to that in people with normal vitamin D levels [8]. A recent investigation indicated that the consumption of yogurt fortified with vitamin D and calcium attenuated the elevation of parathyroid hormone (PTH) levels [9]. Contrary to these studies, other investigations reported that no significant relationship was observed between body adiposity and dietary calcium [10-12]. High level of PTH is positively related to high body mass index (BMI) [13]. Also, imbalance in calcium and protein intake may result in increased calcium resorption rate from bones that can influence calcium homeostasis and absorption [9,14]. Increasing dietary protein induces hypercalciuria and a negative calcium balance [15]. Findings on the role of protein in obesity are highly varied in studies among adults [16] and the influence of dietary protein on PTH and 25-hydroxyvitamin D (25[OH]D) is not well understood [17]. The positive link between animal protein intake and adiposity could be related to the enhancement in insulin secretion and adipocyte proliferation and differentiation [18]. Meta-analyses of trials in adults also reported no significant effects of protein intake on high-density lipoprotein (HDL), low-density lipoprotein (LDL) levels [19]. On the other hand, the dietary intake of phosphorus is related to protein intake, which can affect serum PTH levels [20]. Thus, available data are challenging, more studies are necessary to reject or support the association between calcium, protein and vitamin D intake and obesity with the hypothesis that “the dietary intake of calcium, protein, and vitamin D is directly related to blood lipid profiles (triglycerides [TG], LDL cholesterol [LDL-C], and HDL cholesterol [HDL-C]) and the relationship is mediated by calcitropic hormones in patients with type 2 diabetes.” It may be difficult to evaluate the role of dietary vitamin D or calcium in obesity using traditional statistical methods in the field of clinical studies or nutrition epidemiology [4]. Structural equation modeling (SEM) is a valuable technique which considers direct and indirect effects of multiple variables simultaneously versus sequentially. The method can provide important insight into the role of vitamins, minerals and macro-/micro-nutrients in obesity [4]. The aims of the current study were to utilize SEM techniques to determine the effects of dietary calcium, protein and vitamin D on adiposity and lipidemia and test the mediating effects of PTH and 25(OH)D on that association in patients with type 2 diabetes.

MATERIALS AND METHODS

Ethical aspects

This study was approved by the Ethics Committee of Tehran University of Medical Sciences (with code IR.TUMS.VCR.REC.1397.195). Informed consent was obtained from all subjects included in the research prior to data collection.

Participants

The current study was a cross-sectional analysis of dietary intake, body composition and blood parameters in patients with type 2 diabetes in Tehran (center of Iran) during March 2016–October 2017. A total of 150 diabetic patients (93 females and 57 males) were randomly selected. The inclusion criteria were patients with type 2 diabetes without consuming dietary supplements, vitamins and herbal products and/or non-steroidal anti-inflammatory drug, and BMI more than 20. Subjects that consumed the supplements and/or medications which were effective on the endocrine system and/or calcium homeostasis were excluded from the research. In addition, participants with type 1 diabetes, renal disease, cancer, insulin therapy and pregnancy were also excluded.

Anthropometric measurements and nutritional data collection

Height (without shoes) and weight was carefully measured using a stadiometer (Seca, Hamburg, Germany) and a digital scale (Seca 808; Seca), respectively. BMI (kg/m^2) was calculated. Hip (circumference around the buttocks) and waist (the top of the iliac crest around the abdomen) circumferences were determined with a non-flexible, plastic, circumference measuring tape as well as waist to hip ratio was calculated. Fat mass percent (FMP) was determined using bioelectrical impedance analysis (Quadscan 4000 system; Bodystat, Douglas, UK). Usual dietary intake was assessed with the use of a 147-item food frequency questionnaire (FFQ). This questionnaire included 22 groups of food that are frequently consumed by Iranian population. All the questionnaires were administered by trained dietitians. The FFQ consisted of a list of foods with a standard serving size (Willettformat). Participants were asked to report their frequency of consumption of each food item daily (i.e., fruit), weekly (i.e., cheese) and monthly (i.e., noodle) basis during the previous year. Portion sizes of consumed foods were converted from household measures to grams [21]. NUTRITIONIST IV software which was designed for Iranian foods was used to derive nutrient intakes from the respondent-reported information.

Biochemical analyses

In the morning (8–9 a.m.), 10 mL blood specimens were collected after an overnight fasting. Serum PTH and insulin concentrations were measured using an ELISA kit (DRG Diagnostics, Marburg, Germany). Serum intact PTH concentration was determined using enzyme immunoassay (DRG Diagnostics). Serum insulin levels were measured by immune radiometric assay using a commercial kit (Biosource, Dorest, Belgium) and a γ -counter system (Gamma I; Genesys, Maple Park, IL, USA). Serum 25(OH)D was carefully determined by high performance liquid chromatography. Fasting serum glucose, lipid profile including TG, total cholesterol (TC), LDL-C, and HDL-C were determined using enzymatic methods. All these tests were done by commercial kits (all from Pars Azmoon, Tehran, Iran) using an auto-analyzer system (Selectra E; Vitalab, Holliston, the Netherlands). Glycated hemoglobin A1c was determined using a colorimetric method after an initial chromatographic separation (BioSystems, Barcelona, Spain).

Statistical analyses

Descriptive statistics were performed to compare means of vitamin D and calcium intake, and circulating hormone levels and the characteristics of diabetic patients. SEM was performed in 2 parts, the measurement model and the structural model. Relations between manifest or observed variables and latent variables are referred to as the ‘a measurement model’ on a confirmatory factor analysis (CFA) and inter-relations between latent constructs are referred

to as the “a structural model,” known as SEM. Adiposity latent construct was identified using FMP, BMI, hip and waist as multiple indicators and lipidemia latent construct was detected using the levels of HDL, LDL, and TG. CFA was used to evaluate and modify the primary constructs. Dietary vitamin D, calcium and protein were considered as exogenous variables and PTH and 25(OH)D were considered as mediators. Lipidemia and adiposity were our endogenous variables. Indirect/direct effects were evaluated using structural modeling. For SEM analysis, the model was detected and determined according to model fit indicators including: χ^2 test, the ratio of the χ^2 to degrees of freedom (CMIN/DF), incremental fit index (IFI), normed fit index (NFI), comparative fit index (CFI), goodness of fit index (GFI) and root mean square error of approximation (RMSEA). The p value < 0.05 was considered significant. Regression coefficient relating an indicator to a latent variable that is also called a factor loading is presented by λ (lambda). If the factor loading less than 0.3 is considered as a weak relationship, but the factor loading between 0.3 and 0.6 is acceptable, and if it is greater than 0.6 is desirable [22]. The analyses were carried out using IBM SPSS AMOS version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Sample characteristics

Among total number of 150 patients, 93 (62%) were female and 57 (38%) were males. Mean age of participants was 52.43 ± 7.26 , ranged from 29 to 76 years. Participants had an average BMI of 29.6. Mean dietary intake of protein, vitamin D, and calcium and biochemical data of subjects are reported in **Table 1**.

Table 1. The characteristics of diabetic patients (n = 150)

Characteristics	Minimum	Maximum	Value
Age (yr)	29	76	52.43 ± 7.26
Anthropometric measurements			
Height (cm)	141.50	187.00	162.00 ± 7.20
BMI	22.01	41.08	29.32 ± 4.17
Waist (cm)	83.00	127.00	99.85 ± 9.20
Hip (cm)	91.00	132.00	105.00 ± 7.33
Waist to hip ratio	0.84	1.10	0.94 ± 0.05
Fat mass percent	14.20	64.60	37.57 ± 9.70
Biochemical measurements			
Fasting blood sugar (mg/dL)	72	350	183 ± 50.99
HbA1c (%)	5.30	13.80	8.90 ± 1.74
Insulin (IU/mL)	64.30	23.74	22.35 ± 13.71
QUICKI index	24	35	28 ± 0.20
TG (mg/dL)	37	468	177 ± 58.60
HDL (mg/dL)	39	189	46.74 ± 31.16
LDL (mg/dL)	26	167	103 ± 8.64
PTH (pmol/L)	12	130.30	53.85 ± 20.95
25(OH)D (ng/mL)	5.30	103.42	31.16 ± 19.98
Dietary intake data			
Protein (g/day)	30.15	244.69	73.01 ± 35.13
Calcium (mg/day)	334.93	3,957	$1,550 \pm 698.8$
Vitamin D (μ g/day)	0.07	7.92	2.37 ± 1.59

Data are shown as mean \pm standard deviation.

BMI, body mass index; HbA1c, glycated hemoglobin A1c; QUICKI index, quantitative insulin sensitivity check index; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PTH, parathyroid hormone; 25(OH) D, 25-hydroxyvitamin D.

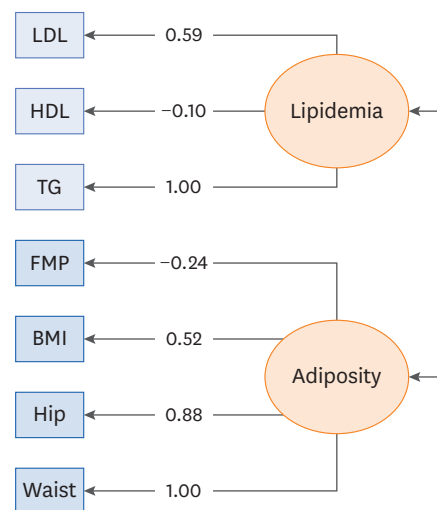


Figure 1. Confirmatory factor analysis model.

Measurement models for unobserved variables. The figure displays each latent construct (adiposity and lipidemia) and the proposed observed variables which define the construct. Factor loadings are illustrated above the pathways.

LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; FMP, fat mass percent; BMI, body mass index.

A confirmatory factor analysis

Figure 1 presents a CFA model and evaluation of Goodness of fit indices for CFA are acceptable (CMIN/DF = 1.570, RMSEA = 0.062, AGFI = 0.896, GFI = 0.944, CFI = 0.972, IFI = 0.967, NFI = 0.913).

Structural equation modeling

A direct model

We assessed the direct effect of exogenous variables on outcomes (adiposity and lipidemia) without evaluating mediating effects of mediators. This model is characterized as a direct model with elimination of mediator variables such as PTH and 25(OH)D. The positive and significant relationship between dietary calcium and adiposity ($p = 0.024$, $\lambda = 0.32$) and negative and significant relationship between dietary calcium and lipidemia ($p = 0.027$, $\lambda = -1.0$) with a good model fit (CMIN/DF = 1.439, RMSEA = 0.054, AGFI = 0.904, GFI = 0.948, CFI = 0.979, IFI = 0.979, NFI = 0.935) (**Figure 2**).

A mediator model

We also assessed the mediating roles of PTH and 25(OH)D on adiposity and lipidemia repeatedly on our structural model, using a mediating test. **Figure 3A** shows the model with reduced dimension of PTH variable. The path between dietary calcium and adiposity was positively ($\lambda = 0.32$) significant ($p = 0.024$) and dietary calcium and lipidemia was negatively ($\lambda = 0.32$) significant ($p = 0.024$). In the model of **Figure 3B**, reduced dimension of 25(OH)D variable was evaluated. The positive significant path between PTH and adiposity ($p = 0.024$, $\lambda = 0.32$) and dietary calcium and adiposity ($p = 0.024$, $\lambda = 0.32$) were seen in this model. All fitting indexes met the criterion of goodness of fit in both models.

A final model

As **Figure 4** demonstrates, all variables were considered in the final model. The direct effects of dietary calcium were significantly observed on adiposity ($p = 0.03$) but there were no

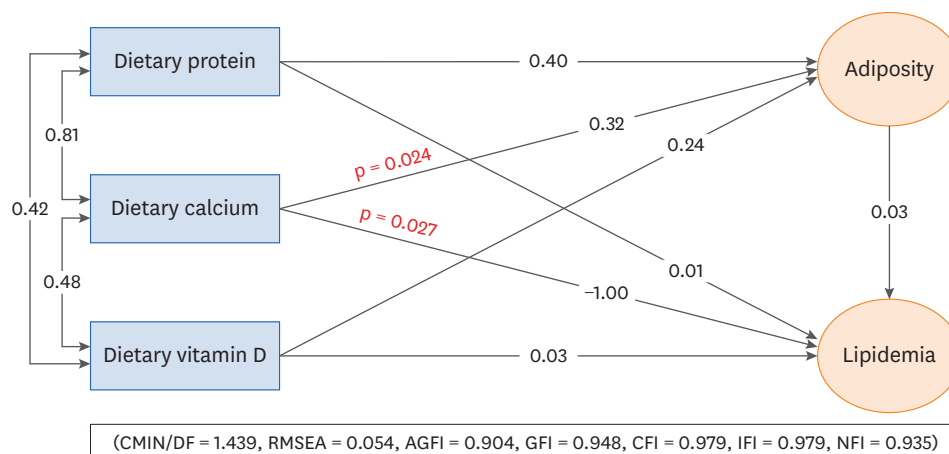


Figure 2. Direct model with standardized parameter estimates.

Model fit indices including, CMIN/DF = 1.439, RMSEA = 0.054, CFI = 0.979, GFI = 0.904, AGFI = 0.904, NFI = 0.935 and IFI = 0.979 display acceptable thresholds and confirm the model appropriateness. Circles represent latent variables; rectangles represent single-item indicators. For readability, only significant p values are shown. Factor loadings are illustrated above the pathways. CMIN/DF, the ratio of the χ^2 to degrees of freedom; RMSEA, root mean square error of approximation; AGFI, adjusted goodness of fit index; GFI, goodness of fit index; CFI, comparative fit index; IFI, incremental fit index; NFI, normed fit index.

significant effects on PTH ($p = 0.115$) and 25(OH)D ($p = 0.831$). Furthermore, there were no significant effects for dietary protein on PTH ($p = 0.137$), 25(OH)D ($p = 0.408$), or adiposity ($p = 0.954$) as well as for dietary vitamin D on PTH ($p = 0.190$), 25(OH)D ($p = 0.690$), or adiposity ($p = 0.088$).

Fit statistics indicated that the model had an adequate fit to data. (CMIN/DF = 1.570, RMSEA = 0.062, AGFI = 0.896, GFI = 0.944, CFI = 0.972, IFI = 0.967, NFI = 0.913).

Thus, the result did not support the hypothesis that calcitropic hormones partially or fully mediate the relationship between diet parameters and adiposity/lipidemia, indicating that PTH and calcitriol did not mediate the relationship between dietary intake variables and adiposity and lipidemia. There was no significant relationship between protein/vitamin D intake and adiposity/lipidemia but there was a significant relationship between dietary calcium and lipidemia, which is evidenced by elevated calcium intake was associated with lower lipidemia.

DISCUSSION

Obesity is the most important risk factor for developing type 2 diabetes [3]. Inappropriate diet can lead to fat accumulation and disturbance of metabolic balance in the body [4]. These metabolic problems lead to obesity and type 2 diabetes [3]. The purpose of this study was to evaluate the effect of dietary vitamin D, calcium and protein and to evaluate direct and mediated (by calcitropic hormones) effects of them on fat accumulation and the lipid profile in people with type 2 diabetes using SEM. The most valuable feature of this model is the simultaneous analysis and processing of relationships among variables of the model of measurement.

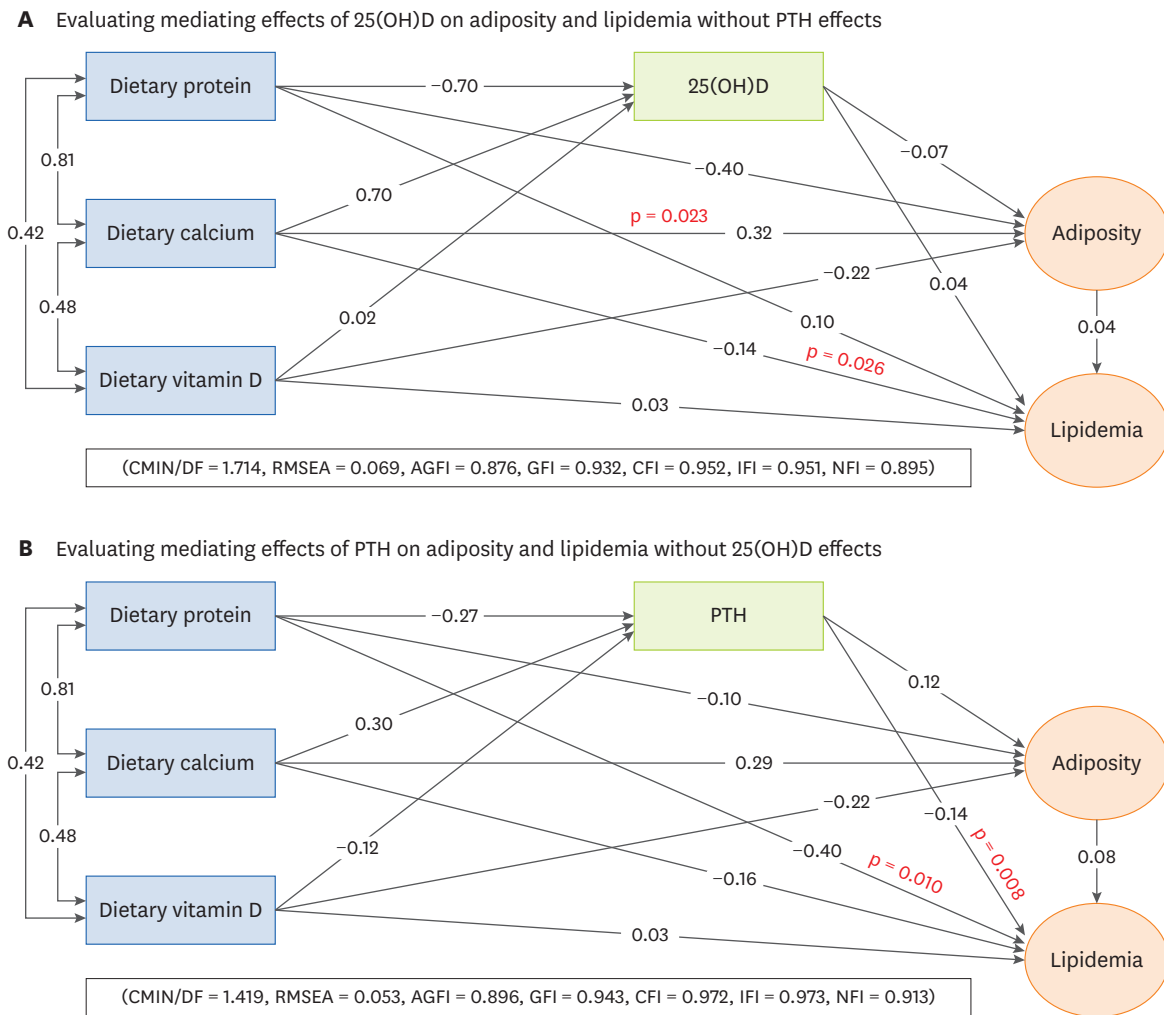


Figure 3. The structural model after testing the mediating effects of (A) 25(OH)D and (B) PTH. (A) Model fit indices including, CMIN/DF = 1.714, RMSEA = 0.069, CFI = 0.952, GFI = 0.932, AGFI = 0.876, NFI = 0.895 and IFI = 0.951 display acceptable thresholds and confirm the model appropriateness. (B) Model fit indices including, CMIN/DF = 1.419, RMSEA = 0.053, CFI = 0.972, GFI = 0.943, AGFI = 0.896, NFI = 0.913 and IFI = 0.973 display acceptable thresholds and confirm the model appropriateness. Circles represent latent variables; rectangles represent single-item indicators. For readability, only significant p values are shown. Factor loadings are illustrated above pathways. 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone; CMIN/DF, the ratio of the χ^2 to degrees of freedom; RMSEA, root mean square error of approximation; AGFI, adjusted goodness of fit index; GFI, goodness of fit index; CFI, comparative fit index; IFI, incremental fit index; NFI, normed fit index.

The results of this cross-sectional study with the hypothesis that “the dietary intake of calcium, protein, and vitamin D is related directly and/or mediated by calcitropic hormones to blood lipid profiles (TG, LDL-C, HDL-C) in patients with type 2 diabetes.” showed that dietary calcium leads to a decrease in lipidemia. Also, there was no significant relationship between dietary protein or vitamin D and lipidemia with/without calcitropic hormones. The results indicate a significant positive relationship between PTH and lipidemia. Calcium has been shown to improve serum lipid parameters that may be contributed to metabolic syndrome and significantly contribute to the improvement of LDL-C, HDL-C and HDL-C/ LDL-C [23]. Clinical trials have also shown that supplementation of calcium [24-26] and the consumption of dairy products [24,25] can be involved in fat oxidation. Nevertheless, there is limited information about the role of calcium supplement to play in weight loss and its effect on lipoproteins. In a study to investigate the effect of calcium on lipid profiles and lipoprotein profiles in obese and overweight women, serum HDL-C, LDL-C, and HDL-C/LDL-C levels of

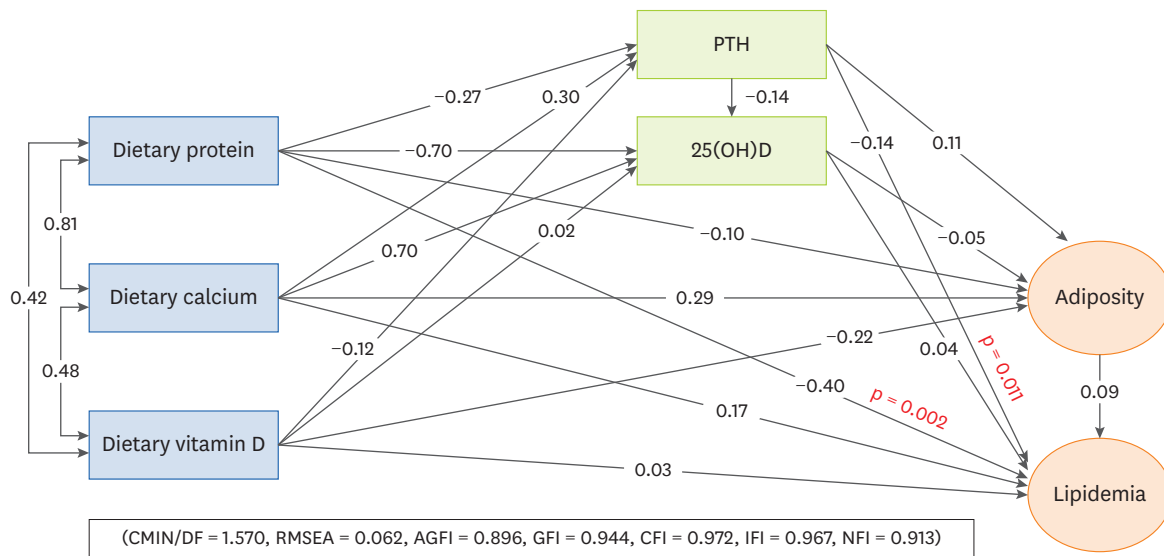


Figure 4. The final structural model with standardized parameter estimates.

Model fit indices including, CMIN/DF = 1.570, RMSEA = 0.062, CFI = 0.972, GFI = 0.944, AGFI = 0.896, NFI = 0.913 and IFI = 0.967 display acceptable thresholds and confirm the model appropriateness. Circles represent latent variables; rectangles represent single-item indicators. For readability, only significant p values are shown. Factor loadings are illustrated above pathways.

PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; CMIN/DF, the ratio of the χ^2 to degrees of freedom; RMSEA, root mean square error of approximation; AGFI, adjusted goodness of fit index; GFI, goodness of fit index; CFI, comparative fit index; IFI, incremental fit index; NFI, normed fit index.

those received calcium supplements were significantly improved. TG and TC also decreased but statistically were not significant [23]. A report was also made on 28 Chinese women, their HDL-C were increased after taking calcium supplements for 26 weeks [27]. In contrast to the findings of this study, Reid et al. [28] showed that calcium supplementation in 323 healthy men had no significant effect on LDL-C, HDL-C, and LDL-C/HDL-C levels. Another study by Bostick et al. [29] reported no significant correlation between calcium supplement consumption with blood lipids. Inadequate calcium intake results in an increase in serum PTH that promotes bone resorption through which calcium homeostasis is influenced.

One of the reasons for the discrepancy in the findings of studies on the effect of dietary calcium on lipidemia can be the difference in dietary protein intake, because it can affect the levels of serum PTH and vitamin D [4]. It was reported that an elevated dietary protein increases calcium excretion in urine [14] and because of this, protein is considered as an important factor in calcium absorption and homeostasis. PTH levels increase along with increasing urinary calcium excretion [30] and studies have shown that increased levels of PTH can lead to body fat accumulation and, consequently increasing the blood lipid levels [31]. Also, in people with metabolic syndrome, PTH may lead to insulin resistance [32,33]. Another reason for the discrepancy in the findings is due to the differences in the amount of calcium intake. The calcium intake in the current population was originated from dairy products (not supplement), which was reflected in the criteria of the study that the participants did not take calcium supplements and vitamin D for three months before the start of the study. In this study, the average calcium intake was about 1,500 mg/day that indicates favorable intake of calcium from diet in this population but this amount was not enough to achieve the desired effects of calcium on the serum lipid profile. In most of the studies mentioned above, the effect of calcium on serum lipids were studied in high doses and in supplement forms.

In current study, most participants were overweight and the mean BMI was 29.32. Studies have shown that leptin in people with diabetes is elevated but adiponectin is decreased. Also, resistance to leptin along with hyperleptinemia is seen in people with diabetes. Obesity and overweight are also associated with insulin resistance and increased serum insulin levels. It has been observed that increased levels of insulin in the blood are associated with an increase in serum LDL-C and TG but decrease in serum HDL-C levels [30,34]. So, in many diabetic patients, lipid and cholesterol-lowering medications such as statins and fibrates are used to reduce blood lipids that are associated with lower levels of leptin in the blood [35]. Dietary calcium also increases the production of adiponectin in overweight and obese individuals [36,37]. These hormones act in an opposite way [38] and in obese people, with an increase in leptin levels, the level of adiponectin is reduced [4]. Therefore, calcium with its role on the levels of adiponectin can affect the blood lipid profile. According to the criteria for entering the study which was not taking any kind of lipid lowering drugs, including fibrates, statins etc., and the role of calcium, mediated by adiponectin, on the level of blood lipids can be justified.

Other dietary factors assessed in this study were protein and vitamin D intake. Kerstetter et al. [14] reported protein intakes are important for regulating the levels of calcitropic hormones. In that clinical trial, a low protein diet (0.7 g/kg), a moderate-protein diet (1 g/kg), and a high protein diet (2 g/kg) plus 800 mg dietary calcium for two weeks indicated that women who received high-protein diet had more urinary calcium excretion and those with low-protein diet had been associated with a significant increase in the levels of calcitropic hormones [14]. In another study, similar results were achieved in the population of postmenopausal women [39]. However, the long-term effects of the amount of protein intake on these hormones are still unknown. In the present study, there was no significant correlation among protein intake and calcitropic hormones with obesity and lipidemia. The mean protein intake of the subjects in present study was 93 g/day, which was much lower than the recommended daily limits.

Calcium can also play a role in lipoproteins by changing the levels of 1, 25(OH)D₃ and PTH [37]. It has been shown that serum vitamin D levels have an inverse relationship with total blood cholesterol and serum vitamin D lower than 15 ng/mL is along with higher LDL [40]. In a study on Iranian people with type 2 diabetes, in people with vitamin D deficiency, TG levels increased, but there was no relationship between serum vitamin D levels and LDL-C or HDL-C levels [41]. The results of a meta-analysis study to determine the relationship between vitamin D and blood lipid profiles including TC, TG, LDL-C, and HDL-C indicated that vitamin D levels have a direct relationship with HDL-C and reverse relationship with LDL-C, TC and TG [42]. In another meta-analysis, the association between vitamin D supplementation and its blood levels with risk factors for cardiovascular diseases including PTH, TG, HDL-C, and LDL-C was studied, and the results indicated that supplementation of vitamin D directly improves blood lipids and helps to reduce the risk of cardiovascular diseases [43]. Receiving vitamin D is also a predictor of serum vitamin D levels and vitamin D deficiency is defined as a serum 25(OH)D level less than 32 ng/mL (< 80 nmol/L). Considering the vitamin D intake in the study population was 80 international units per day, which was much lower than the recommended dietary allowance level of vitamin D (600 international units per day), and 80% of the participants in the study had moderate and severe vitamin D deficiency, it seems factors such as exposure to sunlight, food habits, food enrichment programs, coverage, and the use of sunscreens may be involved in such deficiencies. It is therefore clear that there was no significant relationship between vitamin D and its serum levels with obesity or lipidemia in this study.

The second hypothesis of this study was that “the dietary intake of calcium, protein, and vitamin D is related directly and/or mediated by calcitropic hormones to the obesity indexes (body fat percentage, BMI, waist and hip circumference) in type 2 diabetic patients.” Our results showed that there was not any significant relationship between the dietary intake and the obesity indexes through calcitropic hormones. One of the mechanisms through which calcium is influencing the obesity is an increase in the entry of calcium ions into the cell. Intracellular calcium ions play an important role in the risk of obesity, not only by contributing to lipid storage into the adipocytes, but also by reducing lipolysis and increasing lipogenesis [37,44]. Calcium ions induce the gene expression involved in fatty acid synthesis by stimulating the calcium responsive promoter in this gene [37]. Accordingly, calcium can play a role in the synthesis of fatty acids and the development of obesity. Calcitropic hormones maintain plasma levels of calcium strikingly higher than intracellular calcium by which lead to the balance of extracellular and intracellular calcium at normal physiological levels [5,37]. The reduction of dietary calcium follows by decreased extracellular calcium, in turn, increases the secretion of PTH and 1, 25(OH)2D. Low intakes of dietary protein can also lead to an increase in PTH and the imbalance of intracellular calcium homeostasis causing the accumulation of fat. Elevated levels of intracellular calcium results in increasing the obesity by the mentioned mechanisms [45,46]. The calcitropic hormones modulate this effect by controlling the levels of extracellular and intracellular calcium [5,47,48]. Accordingly, our results showed that there was no significant correlation between the dietary calcium and obesity through PTH and 1, 25(OH)2D [13].

The current study was the first study evaluating the relationship among the intake of calcium, protein and vitamin D with obesity and lipidemia in patients with type 2 diabetes using a structural equation model. Since obesity and lipidemia are often caused by several factors, and therefore it is necessary to consider multiple variables and assess the complex relationships between them. Structural equation models can provide relevant results in this evaluation. According to the statistical logic and outcome, the results could be generalized to all the population in the structural equation model, as the conceptual results of study are fit to the data of the population. Therefore, the difference between theoretical model and real population is very low. However, it should be noted that this is a cross-sectional study, and the causal relationship could not be explored. Based on the limitations of the present study, the first recommendation for future studies is to consider a larger sample size. Second, studies being conducted on different populations with other diseases and the supplements carefully recorded and examined. Third, further researches with considering other parameters that can affect levels of calcitropic hormones such as a physical activity level and the time of sunlight exposure which were not assessed in present study.

CONCLUSION

There was a significant relationship between dietary calcium and lipidemia or obesity, and also PTH was significantly associated with lipidemia. Other variables such as vitamin D or protein did not show significant correlation with lipidemia and obesity in patients with type 2 diabetes. Besides, the mediator variables (PTH and calcitriol) did not have any effect on the mentioned links.

ACKNOWLEDGEMENTS

The current article is obtained from a master thesis (with code 9411468005). Therefore, we would like to acknowledge Tehran University of Medical Sciences for the fund as well as the participants for their assistances.

REFERENCES

1. Toma A, Makonnen E, Mekonnen Y, Debella A, Addisakwattana S. Intestinal α -glucosidase and some pancreatic enzymes inhibitory effect of hydroalcoholic extract of *Moringa stenopetala* leaves. *BMC Complement Altern Med* 2014;14:180.
[PUBMED](#) | [CROSSREF](#)
2. Afrisham R, Aberomand M, Ghaffari MA, Siahpoosh A, Jamalana M. Inhibitory effect of *Heracleum persicum* and *Ziziphus jujuba* on activity of alpha-amylase. *J Bot* 2015;2015:824683.
[CROSSREF](#)
3. Khodabandehloo H, Gorgani-Firuzjaee S, Panahi G, Meshkani R. Molecular and cellular mechanisms linking inflammation to insulin resistance and β -cell dysfunction. *Transl Res* 2016;167:228-56.
[PUBMED](#) | [CROSSREF](#)
4. Lemacks JL, Ilich JZ, Liu PY, Shin H, Ralston PA, Cui M, Wickrama KA. Dietary influence on calcitropic hormones and adiposity in Caucasian and African American postmenopausal women assessed by structural equation modeling (SEM). *J Nutr Health Aging* 2016;20:602-10.
[PUBMED](#) | [CROSSREF](#)
5. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. *FASEB J* 2000;14:1132-8.
[PUBMED](#) | [CROSSREF](#)
6. Shahar DR, Schwarzfuchs D, Fraser D, Vardi H, Thiery J, Fiedler GM, Blüher M, Stumvoll M, Stampfer MJ, Shai I; DIRECT Group. Dairy calcium intake, serum vitamin D, and successful weight loss. *Am J Clin Nutr* 2010;92:1017-22.
[PUBMED](#) | [CROSSREF](#)
7. Lin YC, Lyle RM, McCabe LD, McCabe GP, Weaver CM, Teegarden D. Dairy calcium is related to changes in body composition during a two-year exercise intervention in young women. *J Am Coll Nutr* 2000;19:754-60.
[PUBMED](#) | [CROSSREF](#)
8. Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K. Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States: data from the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 2007;167:1159-65.
[PUBMED](#) | [CROSSREF](#)
9. Bonjour JP, Benoit V, Atkin S, Walrand S. Fortification of yogurts with vitamin D and calcium enhances the inhibition of serum parathyroid hormone and bone resorption markers: a double blind randomized controlled trial in women over 60 living in a community dwelling home. *J Nutr Health Aging* 2015;19:563-9.
[PUBMED](#) | [CROSSREF](#)
10. Bowen J, Noakes M, Clifton PM. Effect of calcium and dairy foods in high protein, energy-restricted diets on weight loss and metabolic parameters in overweight adults. *Int J Obes* 2005;29:957-65.
[PUBMED](#) | [CROSSREF](#)
11. Major GC, Alarie FP, Doré J, Tremblay A. Calcium plus vitamin D supplementation and fat mass loss in female very low-calcium consumers: potential link with a calcium-specific appetite control. *Br J Nutr* 2009;101:659-63.
[PUBMED](#) | [CROSSREF](#)
12. Holecki M, Zahorska-Markiewicz B, Więcek A, Mizia-Stec K, Nieszporek T, Zak-Golab A. Influence of calcium and vitamin D supplementation on weight and fat loss in obese women. *Obes Facts* 2008;1:274-9.
[PUBMED](#) | [CROSSREF](#)
13. Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, Yanovski JA. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab* 2004;89:1196-9.
[PUBMED](#) | [CROSSREF](#)

14. Kerstetter JE, O'Brien KO, Insogna KL. Dietary protein, calcium metabolism, and skeletal homeostasis revisited. *Am J Clin Nutr* 2003;78:584S-592S.
[PUBMED](#) | [CROSSREF](#)
15. Calvez J, Poupin N, Chesneau C, Lassale C, Tomé D. Protein intake, calcium balance and health consequences. *Eur J Clin Nutr* 2012;66:281-95.
[PUBMED](#) | [CROSSREF](#)
16. Astrup A, Raben A, Geiker N. The role of higher protein diets in weight control and obesity-related comorbidities. *Int J Obes* 2015;39:721-6.
[PUBMED](#) | [CROSSREF](#)
17. Kerstetter JE, Caseria DM, Mitnick ME, Ellison AF, Gay LF, Liskov TA, Carpenter TO, Insogna KL. Increased circulating concentrations of parathyroid hormone in healthy, young women consuming a protein-restricted diet. *Am J Clin Nutr* 1997;66:1188-96.
[PUBMED](#) | [CROSSREF](#)
18. Segovia-Siapro G, Khayef G, Pribis P, Oda K, Haddad E, Sabaté J. Animal protein intake is associated with general adiposity in adolescents: the teen food and development study. *Nutrients* 2019;12:110.
[PUBMED](#) | [CROSSREF](#)
19. Santesso N, Akl EA, Bianchi M, Mente A, Mustafa R, Heels-Ansdell D, Schünemann HJ. Effects of higher-versus lower-protein diets on health outcomes: a systematic review and meta-analysis. *Eur J Clin Nutr* 2012;66:780-8.
[PUBMED](#) | [CROSSREF](#)
20. Lorenzo V, Martín M, Rufino M, Jiménez A, Malo AM, Sanchez E, Hernández D, Rodríguez M, Torres A. Protein intake, control of serum phosphorus, and relatively low levels of parathyroid hormone in elderly hemodialysis patients. *Am J Kidney Dis* 2001;37:1260-6.
[PUBMED](#) | [CROSSREF](#)
21. Azadbakht L, Mirmiran P, Esmailzadeh A, Azizi F. Dairy consumption is inversely associated with the prevalence of the metabolic syndrome in Tehranian adults. *Am J Clin Nutr* 2005;82:523-30.
[PUBMED](#) | [CROSSREF](#)
22. Hancock GR, Mueller RO. Structural equation modeling: a second course (quantitative methods in education and the behavioral sciences). Charlotte (NC): Information Age Publishing, Inc; 2006.
23. Major GC, Alarie F, Doré J, Phouttama S, Tremblay A. Supplementation with calcium + vitamin D enhances the beneficial effect of weight loss on plasma lipid and lipoprotein concentrations. *Am J Clin Nutr* 2007;85:54-9.
[PUBMED](#)
24. Melanson EL, Sharp TA, Schneider J, Donahoo WT, Grunwald GK, Hill JO. Relation between calcium intake and fat oxidation in adult humans. *Int J Obes Relat Metab Disord* 2003;27:196-203.
[PUBMED](#) | [CROSSREF](#)
25. Cummings NK, James AP, Soares MJ. The acute effects of different sources of dietary calcium on postprandial energy metabolism. *Br J Nutr* 2006;96:138-44.
[PUBMED](#) | [CROSSREF](#)
26. Teegarden D, White KM, Lyle RM, Zemel MB, Van Loan MD, Matkovic V, Craig BA, Schoeller DA. Calcium and dairy product modulation of lipid utilization and energy expenditure. *Obesity (Silver Spring)* 2008;16:1566-72.
[PUBMED](#) | [CROSSREF](#)
27. Yacowitz H, Fleischman AI, Bierenbaum ML. Effects of oral calcium upon serum lipids in man. *BMJ* 1965;1:1352-4.
[PUBMED](#) | [CROSSREF](#)
28. Reid IR, Ames R, Mason B, Bolland MJ, Bacon CJ, Reid HE, Kyle C, Gamble GD, Grey A, Horne A. Effects of calcium supplementation on lipids, blood pressure, and body composition in healthy older men: a randomized controlled trial. *Am J Clin Nutr* 2010;91:131-9.
[PUBMED](#) | [CROSSREF](#)
29. Bostick RM, Fosdick L, Grandits GA, Grambsch P, Gross M, Louis TA. Effect of calcium supplementation on serum cholesterol and blood pressure. A randomized, double-blind, placebo-controlled, clinical trial. *Arch Fam Med* 2000;9:31-8.
[PUBMED](#) | [CROSSREF](#)
30. Cernea S, Roiban AL, Both E, Huțanu A. Serum leptin and leptin resistance correlations with NAFLD in patients with type 2 diabetes. *Diabetes Metab Res Rev* 2018;34:e3050.
[PUBMED](#) | [CROSSREF](#)
31. Tidwell DK, Valliant MW. Higher amounts of body fat are associated with inadequate intakes of calcium and vitamin D in African American women. *Nutr Res* 2011;31:527-36.
[PUBMED](#) | [CROSSREF](#)

32. Li Y, Wang C, Zhu K, Feng RN, Sun CH. Effects of multivitamin and mineral supplementation on adiposity, energy expenditure and lipid profiles in obese Chinese women. *Int J Obes* 2010;34:1070-7.
[PUBMED](#) | [CROSSREF](#)
33. Baldi S, Natali A, Buzzigoli G, Galvan AQ, Sironi AM, Ferrannini E. In vivo effect of insulin on intracellular calcium concentrations: relation to insulin resistance. *Metabolism* 1996;45:1402-7.
[PUBMED](#) | [CROSSREF](#)
34. Li J, Shen X. Leptin concentration and oxidative stress in diabetic ketoacidosis. *Eur J Clin Invest* 2018;48:e13006.
[PUBMED](#) | [CROSSREF](#)
35. Singh P, Zhang Y, Sharma P, Covassin N, Soucek F, Friedman PA, Somers VK. Statins decrease leptin expression in human white adipocytes. *Physiol Rep* 2018;6:e13566.
[PUBMED](#) | [CROSSREF](#)
36. Zemel MB, Sun X, Sobhani T, Wilson B. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. *Am J Clin Nutr* 2010;91:16-22.
[PUBMED](#) | [CROSSREF](#)
37. Zemel MB. Proposed role of calcium and dairy food components in weight management and metabolic health. *Phys Sportsmed* 2009;37:29-39.
[PUBMED](#) | [CROSSREF](#)
38. Lecke SB, Morsch DM, Spritzer PM. Leptin and adiponectin in the female life course. *Braz J Med Biol Res* 2011;44:381-7.
[PUBMED](#) | [CROSSREF](#)
39. Kerstetter JE, Svastisalee CM, Mitnick ME, Caseria DM, Insogna KL. Dietary protein-induced changes in mineral metabolism are not influenced by age or sex. *J Bone Miner Res* 2000;15:S423.
40. Cutillas-Marco E, Prosper AF, Grant WB, Morales-Suárez-Varela MM. Vitamin D status and hypercholesterolemia in Spanish general population. *Dermatoendocrinol* 2013;5:358-62.
[PUBMED](#) | [CROSSREF](#)
41. Saedisomeolia A, Taheri E, Djalali M, Moghadam AM, Qorbani M. Association between serum level of vitamin D and lipid profiles in type 2 diabetic patients in Iran. *J Diabetes Metab Disord* 2014;13:7.
[PUBMED](#) | [CROSSREF](#)
42. Kelishadi R, Farajzadegan Z, Bahreynian M. Association between vitamin D status and lipid profile in children and adolescents: a systematic review and meta-analysis. *Int J Food Sci Nutr* 2014;65:404-10.
[PUBMED](#) | [CROSSREF](#)
43. Mirhosseini N, Rainsbury J, Kimball SM. Vitamin D supplementation, serum 25(OH)D concentrations and cardiovascular disease risk factors: a systematic review and meta-analysis. *Front Cardiovasc Med* 2018;5:87.
[PUBMED](#) | [CROSSREF](#)
44. Fukuda H, Iritani N, Sugimoto T, Ikeda H. Transcriptional regulation of fatty acid synthase gene by insulin/glucose, polyunsaturated fatty acid and leptin in hepatocytes and adipocytes in normal and genetically obese rats. *Eur J Biochem* 1999;260:505-11.
[PUBMED](#) | [CROSSREF](#)
45. Ahlström T, Hagström E, Larsson A, Rudberg C, Lind L, Hellman P. Correlation between plasma calcium, parathyroid hormone (PTH) and the metabolic syndrome (MetS) in a community-based cohort of men and women. *Clin Endocrinol (Oxf)* 2009;71:673-8.
[PUBMED](#) | [CROSSREF](#)
46. Parikh SJ, Yanovski JA. Calcium intake and adiposity. *Am J Clin Nutr* 2003;77:281-7.
[PUBMED](#) | [CROSSREF](#)
47. Zemel MB. Regulation of adiposity and obesity risk by dietary calcium: mechanisms and implications. *J Am Coll Nutr* 2002;21:146S-151S.
[PUBMED](#) | [CROSSREF](#)
48. Zemel MB. Role of calcium and dairy products in energy partitioning and weight management. *Am J Clin Nutr* 2004;79:907S-912S.
[PUBMED](#) | [CROSSREF](#)