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# Low vitamin D intake is associated with increase in cardiovascular risk factors in obese adolescents

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**Objectives.** This study aimed to investigate the effect of vitamin D on cardiovascular risk in obese adolescents.

**Methods.** Thirty (16 females/14 males) post-puberty obese adolescents (15-19 years) were involved and measurements of inflammatory biomarkers, body composition, visceral fat, and vitamin D (serum and intake) were performed. The adolescents were submitted to a long-term interdisciplinary therapy with physical exercise, nutritional, psychological, and clinical interventions.

**Results.** Negative correlations between vitamin D intake with plasminogen activator inhibitor-1 (PAI-1) (r=-0.69; p=0.01) and vascular cell adhesion molecule (VCAM-1) (r=-0.82; p=0.001) were found in the population analyzed. Improvement in PAI-1, VCAM-1, body composition, and visceral fat, were observed.

**Conclusions.** We showed that low vitamin D intake is associated with an increase in the cardiovascular risk factors in obese adolescents.

**Key words:** vitamin D, obesity, adolescent, cardiovascular risk

Obesity is a worldwide problem, with a dramatically increasing prevalence in children and adolescents. Recently, a consistent association between increased body mass index (BMI) and lower serum 25-hydroxyvitamin D [25(OH)D] concentrations has been shown in the literature. It has also been reported that body fat content is inversely related to serum 25(OH)D concentration (Arunabh et al. 2003; Vanlint 2013).

The discovery that the vitamin D receptor (VDR) is ubiquitously expressed in almost all body cells, such as immune, vascular or myocardial cells, suggests an involvement of vitamin D-mediated effects in several other systems (Kienreich et al. 2013), which indicates a link between vitamin D deficiency and multiple conditions beyond bone health, including cancer, diabetes, hypertension, infections, and increased risk of cardiovascular disease (Holick 2007; Sun et al. 2011; Eliades et al. 2013).

The effects of vitamin D on hemostasis and its inflammatory mechanisms have been proposed as possible causes of cardiovascular diseases. Epidemiological pooled analysis of prospective observational studies of diverse populations has demonstrated that D hypovitaminosis is associated with a modest risk of cardiovascular events (Galli-Tsinopoulou et al. 2010; Ku et al. 2013).

Reported variations in blood pressure, explained by differences in vitamin D status, have often been relatively very small and therefore, of a questionable clinical relevance. Possible mechanisms for association of vitamin

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D and blood pressure includes the inverse association of vitamin D levels with the renin-angiotensin-aldosterone system (RAAS) activity and association between lower vitamin D and hypertension state (Kienreich et al. 2013).

In addition, vitamin D concentration has been suggested to be associated with the reduction of vascular cell adhesion molecule 1 (VCAM-1), an important endothelial and inflammatory biomarker for a risk of cardiovascular diseases. High level of plasminogen activator inhibitor - type 1 (PAI-1) has been also identified as a risk factor for thrombosis (Mansouritorghabe et al. 2013).

According to several previous data a link between lower vitamin D concentration and cardiovascular risk exists. The purpose of this study was to investigate the effects of vitamin D (serum and intake) on cardiovascular risk in obese adolescents and the effects of interdisciplinary weight loss therapy on biomarkers of cardiovascular diseases in the selected population.

## Subjects and Methods

**Subjects.** Into this study, 30 (16 females/14 males) post-puberty obese adolescents aged 15-19 years were included. Inclusion criteria were Tanner stage five (Tanner and Whithouse 1976), primary obesity and BMI >95th percentile of the CDC reference growth charts. Non-inclusion criteria were the use of birth control pills, cortisone, anti-epileptic drugs, and history of renal disease, alcohol intake, smoking, and secondary obesity due endocrine disorders. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics committee on research at the Universidade Federal de Sao Paulo-UNIFESP (#0135/04 and 152.281), Clinical Trials.gov: NCT01358773. All procedures were clear to those responsible for the participants and an informed consent for research was obtained. The main reasons for dropping out (n=5) in our study were financial and family problems, followed by school and job opportunities.

**Anthropometric measurements.** Body composition and weight were measured by plethysmography scale (BODPOD equipment), where patients wore a minimum clothing as possible and height was measured using a stadiometer (Sanny-model ES 2030). BMI was calculated dividing the weight by height squared (kg/m$^2$).

**Serum analysis.** Blood samples were collected at the outpatient clinic at approximately 8:00 a.m. after an overnight fasting (12 h). The serum concentrations of glucose, insulin, triglycerides, total cholesterol (T-cholesterol), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and very low-density lipoprotein cholesterol (VLDL-c), were determined by enzymatic colorimetric methods (CELM, Barueri, Brazil). To identify individuals with abnormal lipid profiles, we used the reference values from the I Guideline for Preventing Atherosclerosis in Childhood and Adolescence: HDL-C (≥45), LDL-C (<100) and T-cholesterol (<150) (Giuliano et al. 2006). The PAI-1 and VCAM-1 concentrations were measured using a commercially available multiplex assay (EMD Millipore; HMHMAG-34K). Concentration of 25(OH)D was available by chemiluminiscence analysis. The cut-off points used to classify vitamin D deficiency <25 nmol/l (<10 ng/ml) and insufficiency between 25 and 75 nmol/l (10-30 ng/ml) (Bischoff-Ferrari et al. 2006).

**Blood pressure measurements.** Blood pressure was measured on the right arm using a mercury gravity manometer with an appropriately sized cuff. The first appearance of sound (phase 1 Korotkoff sound) was used to define systolic blood pressure and the disappearance of sound (phase 5 Korotkoff sound) was used to define diastolic blood pressure. Two measurements were made after the subjects had been seated for at least 5 min, and the mean value was used for analyses.

**Visceral and subcutaneous adiposity measurements.** Measurements of visceral and subcutaneous adiposities were performed by abdominal ultrasonography. The procedures were performed by the same physician, who evaluated the participants at the baseline time-point and following 1 year therapy (Ribeiro-Filho et al. 2003).

**Interdisciplinary weight loss therapy.** Interdisciplinary therapy occurred three times a week during 2 h per day. The adolescents attended supervised therapy in physical exercise, nutrition, psychological, and clinical interventions.

**Physical exercise intervention.** The physical exercise protocol was performed three times per week for 1 year and included 30 min of aerobic training plus 30 min of resistance training per session. The aerobic training consisted of running on a motor-driven treadmill (Life Fitness-model TR 9700HR) or bicycle at a cardiac frequency intensity representing the ventilatory threshold I (±4 bpm), which was determined by the results of an initial oxygen uptake test for aerobic exercises (ergospirometry). The physical exercise therapy was based on the guidelines from the American College of Sports Medicine (ACSM) (Donnelly et al. 2009). Resistance

training was also designed based on ACSM recommendations (Kraemer et al. 2002).

**Nutritional intervention.** Energy intake was set at the levels recommended by the dietary reference intake for subjects with low levels of physical activity and of the same age and gender following a balanced diet (Dietary Reference Intake 2001). No pharmacotherapies or antioxidants were recommended. Once a week, adolescents had dietetics lessons on the food pyramid, diet record assessment, weight loss diets, and fad diets food labels, dietetics, fat-free and low-calorie foods, and other related topics. At the beginning of the study and 12 months into the program, a 3-day dietary record was collected; this is a validated method to assess dietary consumption (Hill and Davis 2001; Portelinha et al. 2008). These dietary data were transferred into a computer and the nutrient composition was analyzed by a PC program developed at the Universidad Federal de Sao Paulo (Nutwin software, for Windows, 1.5 version, 2002; Sao Paulo - SP, Brazil) that used data from western and local food tables.

**Psychological intervention.** Symptoms of severity of psychological problems such as depression, body image disturbance, anxiety, and binge eating were established according to validated questionnaires that considered some of the psychological problems associated with obesity. All adolescents completed the Portuguese versions of the BES (Freitas et al. 2001) to verify the symptoms of binge eating and BITE to verify Bulimia symptoms, including the purgative subtype (Cordas and Hochgraf 1993). These tests were based on DSM-IV criteria and were validated for obese individuals, including obese adolescents, submitted to weight loss treatment (Gormally et al. 1982; Henderson and Freeman 1987; Isnard et al. 2003). It is relevant to note that the tests were applied only to identify the symptoms and severity of these disorders and not with the purpose of offering a diagnosis, because clinical interviews are

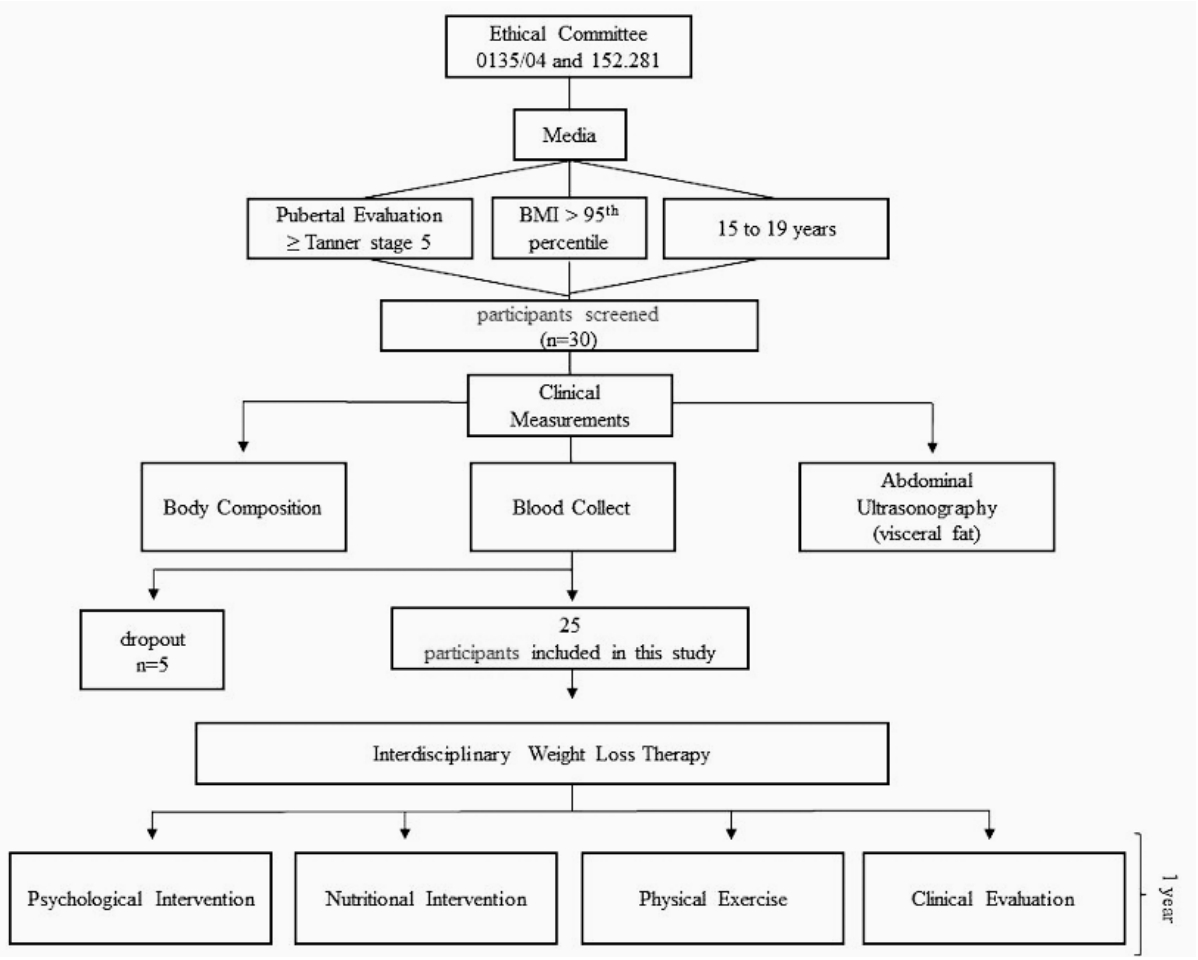


**Fig. 1. Diagram of the interdisciplinary therapy.**

necessary for confirmation (data not showed). Interdisciplinary therapy consisted of a weekly 1 h group and when necessary, individual psychological therapy was recommended when emotional and/or behavioral alterations were found.

**Clinical intervention.** All obese adolescents visited the endocrinologist with their parents once a month. The doctor monitored and evaluated all clinical exams of adolescents including the initial medical history and the physical examination of blood pressure, cardiac frequency, and body weight, parameters associated with the diagnosis of metabolic syndrome, visceral and subcutaneous fat; and treated health problems during therapy. The adolescents were checked for all interdisciplinary therapies compliance (Fig. 1).

**Statistical analysis.** Statistical analysis was performed using the program STATISTICA version 7.0 for Windows. The adopted significant value was $\alpha \leq 5\%$. Data distribution was verified with the Shapiro Wilk test. The parametric data were expressed as mean ± SD; non-parametric data were expressed as median (minimum and maximum values) and analyzed by Z-score correction. To analyze the effects of intervention, Student's t test was applied, dependent on the variables. Correlations were established by Pearson's test.

## Results

**Effects of interdisciplinary weight loss therapy.** One year of interdisciplinary weigh loss therapy promoted a significant statistical reduction in body mass (kg), BMI (kg/m²), body fat mass (kg and %), visceral fat (cm), subcutaneous fat (cm), waist circumference, T-cholesterol (mg/dl), LDL-c (mg/dl), PAI-1 (ng/ml), VCAM-1 (ng/ml), TEI (kcal), and increase in body lean mass (%). No statistical differences were observed on

**Table 1**

**Descriptive data and effects of interdisciplinary therapy in obese adolescents**

| Variables | Baseline | After therapy | p value |
|---|---|---|---|
| Body mass (kg) | 94.39±13.09 | 85.64±11.22 | < 0.0001 |
| BMI (kg/m²) | 33.62±2.98 | 31.20±3.04 | < 0.0001 |
| Body fat mass (kg) | 38.69±7.88 | 33.99±8.19 | < 0.0001 |
| Body fat mass (%) | 41.14±6.64 | 37.41±7.31 | < 0.0001 |
| Body lean mass (kg) | 55.81±10.43 | 54.22±9.49 | 0.38 |
| Body lean mass (%) | 58.86±6.64 | 62.59±7.31 | < 0.0001 |
| Visceral fat (cm) | 4.40±1.09 | 3.64±1.17 | 0.002 |
| Subcutaneous fat (cm) | 3.77±0.68 | 3.54±0.73 | 0.02 |
| Waist circumference (cm) | 95.74±8.37 | 90.41±8.92 | < 0.0001 |
| Glucose (mg/dl) | 90.33±4.55 | 89.94±5.57 | 0.72 |
| SBP (mmHg) | 120 [100/140] | 110 [100/140] | 0.41 |
| DBP (mmHg) | 80 [70/85] | 80 [50/100] | 0.43 |
| Total-cholesterol (mg/dl) | 168.08±37.84 | 151.29±27.98 | 0.002 |
| LDL-cholesterol (mg/dl) | 105.13±36.27 | 87.76±25.10 | 0.002 |
| HDL-cholesterol (mg/dl) | 45.13±8.93 | 46.43±8.54 | 0.15 |
| VLDL-cholesterol (mg/dl) | 16.42±8.53 | 14.19±6.08 | 0.34 |
| PAI-1 (ng/ml) | 17.77±4.03 | 15.01±5.55 | 0.01 |
| VCAM (ng/ml) | 107.15±15.85 | 91.86±27.81 | 0.01 |
| ICAM (ng/ml) | 128.09±37.10 | 109.69±41.89 | 0.18 |
| TEI (kcal) | 1917.48±438.18 | 1402.91±302.60 | < 0.0001 |
| Vitamin D (mcg) | 2.94±1.33 | 2.51±1.70 | 0.55 |
| Vitamin D (ng/ml) | 25.70 [17.6/35.0] | 18 [8.9/29.7] | 0.60 |

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, LDL-cholesterol: low-density lipoprotein cholesterol (<100)*, HDL-cholesterol: high-density lipoprotein cholesterol (≥45)*, Total-cholesterol (<150)*, VLDL-C: very low-density lipoprotein cholesterol, PAI-1: plasminogen activator inhibitor-1, VCAM: vascular cell adhesion molecule, ICAM: intercellular adhesion molecule, TEI: total energy intake, Vitamin D (mcg): measure of vitamin D intake, Vitamin D (ng/ml): serum vitamin D. *Giuliano et al. 2006. Data were expressed as mean ± SD, non-parametric data were expressed as median (minimum and maximum values). Statistical significance: p≤ 0.05.

lean mass (kg), glucose (nmol/l), systolic blood pressure (mmHg), diastolic blood pressure (mmHg), HDL-c, VLDL-c, ICAM (ng/ml), vitamin D intake (mcg), and serum (ng/ml) (Table 1).

**Correlations analysis.** The most important results observed in the present investigation were the negative correlations between vitamin D intake with PAI-1(r=-0.69; p=0.01) and VCAM-1 (r=-0.82; p=0.001) in the population analyzed (Fig. 2).

## Discussion

Recently, an important attention in the scientific community has been acquired to the role of vitamin D in metabolism, especially considering the association between low concentration of D vitamin and the development of cardiovascular risk. Therefore, the most important findings in the present investigation were the negative association between vitamin D intake with PAI-1 and VCAM-1, key biomarkers involved in development of cardiovascular diseases, in obese adolescents. It is important to note that although vitamin D intake was not increased significantly other pro-thrombotic parameters (i.e. lipid profile, body fat ant visceral fat) were improved after 1 year of therapy, which could partly explain the reduction of pro-inflammatory cytokines (VCAM and PAI-1).

PAI-1 is a pro-inflammatory adipokine with pro-thrombotic effects that is also increased in obesity, including children and adolescents. Some studies have reported an association between PAI-1 and the prevalence of metabolic syndrome in obese adolescents (Galli-Tsinopoulou et al. 2010; Corgosinho et al. 2012; Chou et al. 2009).

Adhesion molecules such as VCAM-1 are endothelial and inflammatory markers as well as risk factors for cardiovascular diseases. The mentioned molecule is also associated with enhanced leukocyte adhesion, and enhanced surface thrombogenicity and intravascular coagulation observed in early atherosclerosis (Portelinha et al. 2008).

Vitamin D is a fat-soluble vitamin formed in the skin during exposure to solar ultraviolet B (UVB) radiation. Although, vitamin D can be derived from the diet, few foods naturally contain vitamin D, such as oily fish. First, the vitamin D is metabolized in the liver to 25(OH)D; second, the 25(OH)D is metabolized in the kidneys to the biologically active form 1,25(OH)2D, which exerts its functions through binding to its nuclear receptor (vitamin D receptor, VDR) (Eliades et al. 2013).

In a cross-sectional study, it was verify that the race/ethnicity or gender could influence the dietary, supplemental, and total vitamin D intake in adults aged ≥19 years among USA population. The supplemental vitamin D was significantly higher in females when compared with males, although, the food consumption of vitamin D by females was lower. When examined across gender and race/ethnic groups, it was verified that the total and supplemental vitamin D of non-Hispanic white females was greater than those of non-Hispanic black females and Hispanic females (Moore et al. 2014). In addition, the Fourth Korea National Health and Nutrition Examination Surveys (NHANES IV) research has reported in young people an important influence of gender-dependent skeletal effects of vitamin D, suggesting the necessary of serum levels 25(OH)D up to 20-30 ng/ml or higher in men, especially during the skeletal modeling phase (Lim et al. 2012).

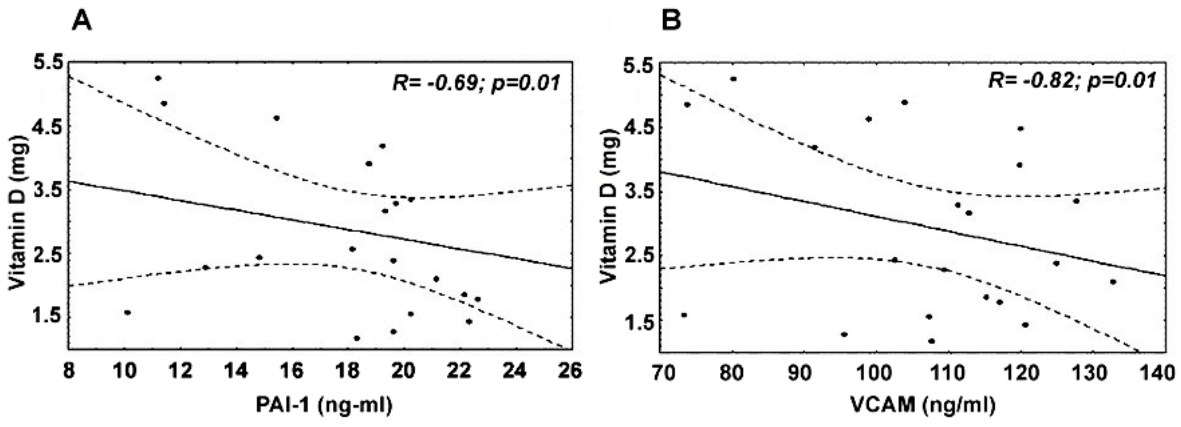


**Fig. 2. Negative correlation between plasminogen activator inhibitor-1 (PAI-1) with vitamin D intake (a) and vascular cell adhesion molecule (VCAM) with vitamin D intake (b) in obese adolescents.;**

As previously described, the vitamin D association with calcium intake could reduce the risk of type 2 diabetes in women (Pittas et al. 2006). In young adults, the dietary plus supplemental vitamin D intake has been shown to be inversely related to the development of metabolic syndrome (Fung et al. 2012) and the higher intake of vitamin D was associated with a lower risk of cardiovascular disease in men (Sun et al. 2011), suggesting that the vitamin D intake is an important biomarker to analyze in view to prevent the development of cardiovascular risk factors.

Evidence suggests that low serum 25(OH)D levels are strongly associated with features of the metabolic syndrome and play an important role in modifying the risk for cardiometabolic outcomes, including diabetes, hypertension, insulin resistance, pancreatic β-cell dysfunction, and cardiovascular disease (Adams and Hewison 2010; Kayaniyil et al. 2010). Accordingly, vitamin D deficiency is associated with increased systematic inflammation. In addition, vitamin D deficiency results in up-regulation of the renin aldosterone system with ensuing cardiac and vascular smooth muscle cell hypertrophy and blood pressure elevation (Li 2003; Mansouritorghabe et al. 2013).

Recent evidence has shown that vitamin D promotes down-regulation in the expression of PAI-1 in human arterial smooth muscle cells, suggesting the anti-inflammatory properties of this vitamin (Zittermann 2006; Deleskog et al. 2012). Corroborating, in the present study we were able to show, as mentioned above, the negative association between deficient vitamin D intake with pro-thrombotic atherogenic biomarkers, suggesting an intrinsic link between quality of diet, obesity, and cardiovascular diseases in precocious life. Together, these results reinforce the importance to consider vitamin D supplementation on the treatment of obese adolescents in view to control the development of cardiovascular diseases associated to obesity and their comorbidities. However, this result is needed to be confirmed in a large cohort of obese adolescents.

In addition, many researches aim to investigate the link between obesity and vitamin D status (Arunabh et al. 2013; Al-Musharaf et al. 2012; Aypak et al. 2014). It was postulated that obese individuals compared with eutrophic individuals are more likely to be vitamin D deficient. Nevertheless, once serum 25(OH)D concentrations in obese individuals are adjusted for body size, there is no longer a difference between obese and non-obese individuals (Drincic et al. 2012). The authors have suggested that in obese individuals, vitamin D dosing for the treatment of deficiency should be based upon body weight (Vanlint 2013).

In the present study, we were not able to compare our findings with a control group (eutrophic adolescents). However, in an important study in Brazilian adolescents, it was observed that serum 25(OH)D levels are negatively associated with non-skeletal outcomes, such as obesity, abdominal obesity, hypercholesterolemia, higher levels of parathyroid hormone, insulin resistance, hyperinsulinemia, and hypertension. Furthermore, lower BMI and waist circumferences were observed in the third tertile of vitamin D intake for all adolescents (25.2±11.1 nmol/l) according to tertile of dietary vitamin D intake among adolescents aged 15 to 17 years (Oliveira et al. 2014). It has been hypothesized that many factors could contribute to this find, including vitamin D storage in adipocytes, decreasing in its activity (Snijder et al. 2005), low dietary vitamin D intake due to poor nutritional habits, and minimal sun exposure due to sedentary indoor lifestyle (Aypak et al. 2014).

Furthermore, another important finding in the present study is that long term interdisciplinary weight loss therapy promoted an improvement in body composition visceral fat and PAI-1 level, which are biomarkers involved in the development of cardiovascular diseases. It is worth noting that visceral fat is associated to secrete a constellation of pro-inflammatory biomarkers, including PAI-1 (Corgosinho et al. 2012).

Contributing to these findings, a positive correlation between visceral fat and PAI-1 concentration was demonstrated in the present study. It is well-established in the literature that fat deposition in visceral compartment is related to production of many pro-inflammatory biomarkers such as PAI-1, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) and consequently involved in the development of many diseases including non-alcoholic fatty liver disease, metabolic syndrome, dyslipidemias, atherosclerosis and cardiovascular complications (Campos et al. 2012a). In accordance with others studies, the interdisciplinary intervention purpose in this research was able to reduce visceral fat and improve the inflammatory profile observed in obese adolescents (Campos et al. 2012b; Damaso et al. 2008; de Piano et al. 2012).

It is important to highlight that in the present investigation non statistical changes were observed for the glucose, systolic, and diastolic blood pressure. However, these variables presented at baseline and after therapy normality values (Zimmet et al. 2007). Moreover, it is possible to note an improvement in lipid profile with

reduction in HDL-c and T-cholesterol, which is an important result, once reduces cardiovascular risk factors (Quijada et al. 2008; Cook and Kavey 2011; Sanches et al. 2014).

Finally, the major result of this study was the negative association between vitamin D intake with PAI-1 and VCAM-1 showing that low vitamin D intake is associated with increase in cardiovascular risk factors in obese adolescents. In addition, interdisciplinary weight loss therapy seems to be an interesting strategy to improve health profile of obese adolescents. However, this study also has some limitations that include a small sample size and the absence of a control group of eutrophic adolescents, suggesting that these findings need to be confirmed by further studies.

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