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Inflammatory markers, tbars and vitamin E in class II and III obese patients before undergoing bariatric surgery

ORIGINAL

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Abstract

Background: Obesity and its comorbidities such as insulin resistance, endothelial dysfunction, hypertension, type 2 diabetes and atherosclerosis have been associated with chronic inflammation and oxidative stress. Weight loss with maintenance is not always possible. Bariatric surgery has been proved to be the only efficient method for weight reduction in patients with clinically severe obesity. It leads to significant weight loss and improvement and/or remission of comorbidities. On the other hand, considering that surgical intervention per se induces oxidative stress and inflammatory response, the objective of the present study was to verify if some inflammatory marker, TBARS and/or Vitamin E could be evaluated before gastric surgery and the relationship with clinical characteristics of the obesity.

Methods: A quantitative cross-sectional study was carried out at Clinica Ana Rosa (Santo André, SP, Brazil). 100 obese patients (BMI ≥ 35-40 kg/m^2) awaiting bariatric surgery were selected for this study. The control group included 29 individuals, BMI < 35 kg/m^2, with or without associated comorbidities. The patients completed a structured interview. Weight, height, waist and hip circumferences were also collected. Blood was collected. Glucose (G), total cholesterol (TC), HDL cholesterol (HDLc), LDL cholesterol (LDLc), VLDL cholesterol (VLDLc), triglycerides (TG), high-sensitivity C-reactive protein (hs-CRP), vitamin E (VIT E), fibrinogen (FIB), IL-6 (human interleukin-6), TNF-alpha and thiobarbituric acid reactive substances (TBARS) were measured.

Results: Patients have shown increased serum levels of hs-CRP and FIB. In this study, class II and III obese patients awaiting bariatric surgery and the control group had lower than normal VIT E levels and no significant changes in TBARS levels were observed. Conclusions: Of all markers evaluated hs-PCR and fibrinogen were found altered in obesity group patients. Therefore, our results reinforce the importance of evaluating inflammatory markers in obese patients before bariatric surgery.

Keywords
Bariatric surgery, inflammatory markers, obesity, TBARS, vitamin E

Introduction

Obesity and its comorbidities such as insulin resistance, endothelial dysfunction, hypertension, type 2 diabetes and atherosclerosis have been associated with chronic inflammation and oxidative stress [1].

Obesity is characterized by an inflammatory state reflected by chronic increases of inflammatory markers, including pro-inflammatory cytokines (e.g., IL-6, TNF-α) and C-reactive protein (CRP). Higher expression of inflammatory markers has been reported in the adipose tissue of obese patient compared with lean subjects, and this expression was reduced after weight loss [2].

The presence of oxidant stress can be measured by using indirect indicators of lipid peroxidation such as TBARS (thiobarbituric acid-reactive substances), which is a good indicator of cell damage [3].

Obesity has been associated with low levels of antioxidant vitamins such as vitamin E but the reasons are not clear. Vitamin E is one of the most important elements of the non-enzymatic antioxidant defense in biologic system [4].

Weight loss with maintenance is not always possible. Bariatric surgery has been proved to be the only efficient method for weight reduction in patients with clinically severe obesity [body mass index (BMI) ≥ 40 kg/m^2]. It leads to significant weight loss and improvement and/or remission of comorbidities (1).

On the other hand, considering that surgical intervention per se induces oxidative stress (5) and inflammatory response (2), the objective of the present study was to verify if some inflammatory marker, TBARS and/or Vitamin E could be evaluated before gastric surgery and if them were different between obesity classes.

Patients and methods

A quantitative cross-sectional study was carried out at Clinica Ana Rosa (Santo André, SP, Brazil). The study protocol was approved by the Committee for Ethics in Research in humans of the Faculdade de Medicina do ABC, and it was in line with the World Medical Association Declaration of Helsinki.

The patients completed a structured interview to obtain the following data: gender, age, medical history, drug consumption and diseases. Weight, height, waist and hip circumferences were also collected. Body mass index (BMI) was calculated according to the formula: actual body weight (kg)/height (m2). The cutoff points used were those recommended by the WHO for classifying normal weight and excess weight, with the study participants qualifying as class II obesity, with BMI ≥ 35 kg/m^2 < 40 kg/m^2 or class III obesity, with BMI ≥ 40 kg/m^2. Bioelectrical Impedance Analysis (BIA) and skinfold measurements were also performed in order to obtain the muscle mass (MM).

100 obese patients (BMI ≥ 35-40 kg/m^2) awaiting bariatric surgery were selected for this study.

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The control group included 29 individuals, BMI < 35 kg/m^2, with or without associated comorbidities. Inclusion criteria were: 20 to 59 years-old adult patients, with no gender restrictions. Patients excluded from the study were younger than 20 or older than 60 years-old, with positive serological testing for hepatitis B or C or the human immunodeficiency virus, and/or patients suffering from endogenous obesity.

Blood samples were collected after a 12-h fast. The plasma and serum samples were separated by centrifugation and immediately aliquoted and stored at -80°C until analysis.

Biochemical parameters were measured in duplicate. Glucose (G), total cholesterol (TC), HDL cholesterol (HDLc), LDL cholesterol (LDLc), VLDL cholesterol (VLDLc) and triglycerides (TG) were determined by standard enzymatic methods. High-sensitivity C-reactive protein (hs-CRP) levels were measured by ELISA kit. Serum vitamin E (VIT E) concentration was determined by high-performance liquid chromatography (HPLC) with UV detection and cholesterol concentration, using a Roche analyser and reagents. Fibrinogen (FIB) levels in plasma were measured by the Clauss method. IL-6 (human interleukin-6) levels were measured with Thermo Scientific Human IL-6 ELISA Kits. TNF-alpha assay kits were used to measure levels of tumor necrosis factor alpha (TNF-α). For quantitative determination of thiobarbituric acid reactive substances (TBARS) was used QuantiChrom™ TBARS Assay Kit.

The statistical analysis was carried out using SigmaStat 3.1 Software (Sigma Stat Software Inc., Richmond, CA, USA). The rejection level for a null hypothesis was 0.05. Normality was evaluated by the Kolmogorov-Smirnov test. Differences between groups were tested using the t-test or the Mann-Whitney test. Correlations between levels of TBARS, VIT E, FIB, IL-6, TNF-α and hs-CRP were tested using the Spearman correlation test.

Two models of multiple linear regressions with stepwise backward elimination were used to verify the correlation between MM and BMI (as dependent variables) and all other studied variables (known as independent variables). Independent variables were the following ones: age, gender, TBARS, TC, HDLc, LDLc, VLDLc, TG, G, FIB, IL-6, TNF-α, VIT E, and hs-CRP. Variables with deviation from the normal distribution were submitted to logarithmic transformations before the logistic regression analysis.

Results

Table 1 shows clinical and laboratory parameters from the obese patients (BMI ≥ 35-40 kg/m^2) awaiting bariatric surgery and the control group. Differences between age (38.70 versus 44.32) and LDLc

Table 1. Gender, age, BMI, TBARS, TC, HDLc, LDLc, VLDLc, TG and G in class II and III obese patients before undergoing bariatric surgery and control group.

Group	N	Gender	Age (years)	BMI (kg/m2)	TBARS (mg/dL)	TC (mg/dL)	HDLc (mg/dL)	LDLc (mg/dL)	VLDLc (mg/dL)	TG (mg/dL)	G (mg/dL)
Class II and III obese patients	100	86 W 14 M	38.70* (13.29)	41.38 (4.68)	3.42 (1.46)	196.62 (39.78)	44.14 (12.50)	122.88* (36.28)	23.88 (11.03)	129.22 (67.30)	104.13 (52.96)
Control	29	22 W 7 M	44.32* (10.90)	32.42 (1.39)	3.49 (1.32)	208.04 (41.20)	46.51 (12.87)	142.25* (37.79)	23.66 (15.31)	119.41 (74.16)	96.85 (21.15)

Results as mean and SD. W: women. M: men. BMI: body mass index. TBARS: thiobarbituric acid-reactive substances. TC: total cholesterol. HDLc: HDL cholesterol. LDLc: LDL cholesterol. VLDLc: VLDL cholesterol. TG: triglycerides. G: glucose. * $P < 0.05$ (t-test).

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(122.88 versus 142.25) were evident between the groups.

Using Mann-Whitney test, it was possible to detect that hs-CRP (11.31 versus 7.89) was significant and statistically different between groups **(Table 2)**. Moreover, it was possible to verify a direct correlation between VIT E (17.36) and hs-CRP (11.31) (P = 0.047) in class II and III obese patients and between VIT E (19.11) and FIB (117.04) (P = 0.001) in control group.

The multiple linear regression analysis with stepwise backward elimination was used to determine the correlation between muscular mass and the independent variables (BMI, TBARS, HDLc, LDLc, VLDLc, TG, G, FIB, IL-6, TNF-α, VIT E and hs-CRP) **(Table 3)**. Statistic program eliminated those independent variables that have not shown significant correlation. Data have shown that higher values of MM were associated to greater TNF-α levels. On the other hand, lower values of MM showed significantly greater VIT E and IL-6 levels.

Table 4 shows the relationship between BMI independent variables that have shown significant correlation. BMI was associated to a negative correlation with age and a positive correlation with glucose. This model explains 33.7% of the BMI variation between the individuals with a test power of 88.8% considering an alpha level of 0.05.

Table 2. VIT E, IL-6, TNF-α, hs-CRP and FIB in class II and III obese patients before undergoing bariatric surgery and control group.

Group	N	VIT E (mg/dL)	IL-6 (mg/dL)	TNF-α (mg/dL)	hs-CRP (mg/dL)	FIB (mg/dL)
Class II and III obese patients	100	17.36 (5.89)	2.51 (1.49)	10.89 (4.86)	11.31* † (7.99)	109.73 (50.51)
Control	29	19.11 (6.85)	2.74 (2.78)	8.96 (3.36)	7.89* (9.13)	117.04 ‡ (44.30)

Results as mean and SD. VIT E: vitamin E. IL-6: Interleukin 6. TNF-α: tumor necrosis factor alpha. Hs-CRP: high-sensitivity C reactive protein. FIB: fibrinogen. † P = 0.047; r = 0.213 (Spearman's correlation). ‡ P = 0.001; r = 0.613 (Spearman's correlation). * P < 0.05 (Mann-Whitney test).

Table 3. Multiple linear regression analysis to test the relation between muscular mass and independent variables.

Dependent variable	Independent variables	Coefficient	P-value	Meaning of the Model		
				R^2	P-value	Test power (α = 0.05)
ln (MM %)	Constant	3.759	-	0.591	< 0.001	1.000
	Gender	-0.156	< 0.001			
	Age	0.003	0.016			
	ln (VLDLc)	0.089	0.013			
	ln (IL-6)	-0.095	0.027			
	ln (TNF-α)	0.073	0.037			
	VIT E	-0.007	0.029			

MM: muscular mass. VLDLc: VLDL cholesterol. IL-6: Interleukin 6. TNF-α: tumor necrosis factor alpha. VIT E: vitamin E. ln: logarithmic transformation. Normality test: *P* = 0.653. Constant variance assumption: *P* = 0.250

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Table 4. Multiple linear regression analysis to test the relation between body mass index and independent variables.

Dependent variable	Independent variables	Coefficient	P-value	Meaning of the Model		
				R^2	P-value	Test power (α = 0.05)
BMI	Constant	2.97	-	0.337	0.007	0.888
	Age	-0.003	0.010			
	ln (G)	0.183	0.006			

BMI: body mass index. G: glucose. ln: logarithmic transformation. Normality test: P = 0.654. Constant variance assumption: P = 0.620.

Discussion

In this study, class II and III obese patients awaiting bariatric surgery and the control group had lower than normal reference of VIT E levels. Many authors have shown that obesity contributes to the development of chronic diseases and systemic oxidative stress, mainly because of the reduction in antioxidant defenses [5, 6]. Interestingly, in this study were not observed differences because all patients evaluated were obese (Control group and Class II and III obese patients).

The two studied groups showed increased hs-CRP levels. It has now been firmly established that adipose tissue is associated with increased inflammatory markers, proinflammatory adipokines, such as high-sensitivity C-reactive protein (hs-CRP), IL-6 and TNF-α [7-10]. It is already known that the evident rise of the inflammatory status in obesity may be associated to the development of other disorders, such as insulin resistance (IR) and metabolic syndrome (MS) [11]. Furthermore, of the inflammatory markers studied the hs-PCR seems to be elected to show differences between the obesity classes.

Some authors have shown that the expression of TNF-α mRNA in the adipose tissue is positively correlated to the body fat, insulin and TG levels [12]. High TNF-α levels in obese patients may reduce the insulin sensibility by influencing the phosphorylation status at the insulin receptor [13]. In this present study no significant change has been observed at TNF-α level.

At the adipose tissue, IL-6 and its receptor are expressed by both adipocytes and matrix of the adipose tissue. IL-6 expression at the adipose tissue and its plasma concentrations are positively related to obesity, to glucose intolerance, to insulin resistance and to hs-CRP production associated to cardiovascular complications [14]. Despite the relation between hs-CRP and IL-6 has been observed in the literature, no correlation between them was observed in the studied groups.

Cabrera et al. [15] have shown that before the surgery obese patients usually present higher levels of inflammatory markers and lower levels of antioxidant substances compared to control group. One year after Roux-en-Y bypass gastroplasty the antioxidant protection improved and the inflammatory markers had decreased.

Class II and III obese patients awaiting bariatric surgery and the control group have shown increased serum FIB levels. Fibrinogen promotes clot formation by increasing the fibrin formation, the platelet aggregation and the plasma viscosity. It also promotes atherosclerosis by the proliferation of endothelial cells in the smooth vascular muscular layer. High FIB levels increase the prevalence and the incidence of cardiovascular diseases and are correlated to obesity [16].

The excessive nutrient intake by obese patients seems to contribute to overproduction of reactive oxygen species (ROS). In this study, we did not observe TBARS changes between the groups. However, bariatric surgery seems to promote a protective effect in the protein oxidative damage and to improve inflammation and obesity biomarkers. A probable mechanism involved in the reduction of the oxidative stress is the decrease of fat mass, blood lipids, and the partial normalization of endogenous antioxidant defense. As food intake after bariatric surgery is limited, lower amount of ROS is generated at the mitochondria and, consequently, less oxidative damage is produced [17].

Excess weight and accumulated fat in subjects with severe obesity seem to be related to increased inflammatory response. We suggest the inclusion of some biomarkers to verify inflammation in obese patients. It is important investigating VIT E, hs-CRP, and FIB serum levels in patients candidates to bariatric surgery. Our results suggest differences in hs-PCR and fibrinogen levels between obesity classes and them can be predictive to bariatric surgery.

Conflict of Interest

The authors declare there is no conflict of interest.

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