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Higher Content of *Trans* Fatty Acids in Abdominal Visceral Fat of Morbidly Obese Individuals undergoing Bariatric Surgery compared to Non-Obese Subjects

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Background: The purpose of this study was to determine the total content of *trans* fatty acids (TFA) in subcutaneous, retroperitoneal and visceral fat of morbidly obese and non-obese patients submitted to bariatric surgery or plastic and abdominal surgery.

Methods: The adipose tissues were obtained by surgery; lipids were extracted, saponified and esterified. TFA were measured by FTIR-ATR spectroscopy.

Results: The TFA average in obese patients was 6.3% for retroperitoneal and 8.7% for visceral fat. For non-obese patients, the figures were 6.9% (subcutaneous) and 9.3% (visceral). There was no difference between the groups. However, the TFA depot in visceral fat was higher than other fatty tissues for morbidly obese ($P<0.001$) and non-obese ($P<0.05$) patients.

Conclusions: Our values for TFA content in all adipose tissues analyzed are higher than reported in other countries (3-6%). We showed more TFA in visceral adipose tissue than in other abdominal fat (subcutaneous and retroperitoneal) stores. The visceral adipose tissue level is worrisome because the higher rate of lipolysis in this tissue appears to be an important indicator of metabolic alterations and the levels of TFA found in adipose tissue presumably reflect the higher dietary intake of TFA by Brazilians.

Key words: *Trans* fatty acids, subcutaneous adipose tissue, retroperitoneal adipose tissue, visceral adipose tissue, morbid obesity, non-obese

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Introduction

Obesity is a complex metabolic syndrome of multifactorial etiology affecting a progressively increasing number of individuals globally.[1,2] Epidemiological studies have confirmed that this metabolic syndrome is related to abdominal obesity and insulin resistance, diabetes and other metabolic risk factors for coronary heart disease (CHD).[2] Abdominal fat is composed of several distinct anatomic depots: subcutaneous fat and intrabdominal fat, which can be divided into visceral and retroperitoneal.[3]

In obese individuals, there are several abnormalities in free fatty acid (FFA) metabolism. It has been suggested that excess visceral fat is more harmful than excess subcutaneous fat, because lipolysis of visceral adipose tissue triglycerides releases FFAs into the portal vein, which are then delivered directly to the liver.[3] The rate of lipolysis in obese individuals is accelerated in visceral adipose tissue, and the increase in circulating FFAs results in dyslipidemia, hyperinsulinemia and hyperglycemia.[2]

There is a substantial interest in the relationship between dietary intake of specific fatty acids and

chronic diseases.[4] *Trans* fatty acids (TFAs) are mainly present in solid fats produced by partial hydrogenation of oils, and are naturally found in products originating from ruminants.[5] They are well-absorbed by the body, and long-term intake is reflected in the fatty acid composition of adipose tissue.[6]

Several studies have shown that a high intake of TFA raises low density lipoprotein (LDL) cholesterol and lowers high density lipoprotein (HDL) cholesterol, affecting the LDL/HDL cholesterol ratio in an unfavourable manner compared to other fatty acids.[7-11] The dietary *trans* fat can perturb essential fatty acid (EFA) metabolism, leading to changes in the phospholipid fatty acid composition in the aorta, the target tissue of atherogenesis.[12]

Measurements of individual fatty acids and saturated and unsaturated fats suggested that insulin sensitivity might be altered acutely in humans.[13] Lovejoy et al[14] observed that humans fed with 10% elaidic acid had raised insulin levels, resulting in a higher insulin-to-glucose ratio, which suggests that TFA may induce insulin resistance acutely.

Depots of individual fatty acids in adipose tissue reflect their presence in the diet and their differential oxidation in humans. The subtypes of adipose tissue also differ in uptake, storage and mobilization of lipids.[15,16]

The purpose of our study was to describe the total content of TFA in abdominal subcutaneous, retroperitoneal and visceral fat of obese and non-obese patients, as an indicator of dietary exposure in a sample of the Brazilian population.

Materials and Methods

Two groups of patients were studied: Group A consisted of morbidly obese patients (BMI>40) and Group B of non-obese patients (BMI<30). The obese and non-obese patients provided subcutaneous, retroperitoneal and/or visceral adipose tissue. The morbidly obese Group A yielded 31 samples of retroperitoneal fat (eight men, 23 women) and 32 samples of visceral fat (seven men, 25 women). Group B, non-obese, gave 18 samples of subcutaneous fat (two men, 16 women) and nine samples of visceral fat (three men, six women).

Morbidly obese adipose tissue was obtained from patients submitted to bariatric surgery at the São Lucas Hospital - PUCRS Center of Morbid Obesity. Non-obese tissues were obtained by lipoaspiration surgery at the Divina Providancia Hospital and Moinho de Ventos Hospital (Center of Reconstitutive Plastic Surgery) and by abdominal surgery at the Nossa Senhora de Fátima Hospital - RS. Experiments were approved by the ethical committee of the Federal University of Rio Grande do Sul (n° 2003224), and all subjects were informed about the aim of the study and signed the informed consent form.

During surgery, a sample of subcutaneous, retroperitoneal and visceral fat (approximately 15 g) was taken from all patients to enable triplicate analyses.

Lipids were extracted according to Folch et al,[17] and were further saponified and esterified in the presence of diazomethane. Two mL of KOH solution (1:1; v/v methanol/water) were added to the saponification, and placed in a steam bath at 80°C. After the sample had reached the temperature, it was acidified with H_2SO4 1M to pH 3, extracted with diethyl ether, dried with anhydrous sodium sulfate and esterified with diazomethane.[18] Reagents were purchased from Merck Co.

A Fourier transform infrared spectrometer with attenuated total reflection (FTIR-ATR Perkin-Elmer Spectrum One spectrometer) was used to measure total TFA in the samples. ATR quantification was based on the measurement of the integrated area under 966 cm^{-1}. A calibration curve of area vs *trans* fatty acids methyl acids (FAMEs) "percentage" was generated for reference mixtures in the range of 1-60% elaidic acid methyl ester standard in oleic acid methyl ester standard[19] purchased from Sigma Chemical Co. The correlation coefficient was 0.9994. The measurement parameters were 1050-900 cm^{-1} spectral range, 50-scans and 4 cm^{-1} resolution.[20]

Statistical Analyses

All statistical analyses were performed with SPSS version 8, using the non-parametric Exact test. To quantify the sample size (*n*) the MTT0-one test (n Query Advisor 30) was used. The samples were analyzed, one by one, in triplicate, and the results were expressed as means ± SD, and a *P*-value of <0.05 was considered statistically significant.

Results

The method used to quantify the TFA, FTIR-ATR spectroscopy is widely used for the determination of total fatty acids with isolated *trans* double bonds. The FTIR-ATR determination is based on the C-H out-of-plan deformation band at 966 cm^{-1} which is the unique characteristic of isolated double bonds with *trans* configuration.[21,22] Fritsche et al[20] determined the levels of TFAs in human adipose tissue by FTIR-ATR, and concluded that levels found by ATR were usually higher than those determined by Gas Chromatography (GC), partly because the GC method underestimates *trans* C18:1 isomers in favor of *cis* C18:1 isomers.

The total TFA content in the adipose tissue (subcutaneous, retroperitoneal and/or visceral) of both groups is shown in Figure 1 (A, B), and the mean values are found in Table 1. For obese patients, mean TFA values were 6.40% (SD= 0.50%) in retroperitoneal adipose tissue and 8.74% (SD= 0.29%) in visceral adipose tissue (Figure 1B). Non-obese patients had a mean of approximately 6.94% (SD= 0.72%) in subcutaneous adipose tissue and 9.29% (SD= 0.59%) in visceral adipose tissue (Figure 1A).

The total TFA of retroperitoneal and subcutaneous adipose tissue was not significantly different between obese and non-obese patients. Nor was there any significant difference in the total stored TFAs of the visceral adipose tissue in the two experimental groups. In obese patients, there was no difference in concentration of total TFAs in subcutaneous (6.52%, SD=0.69%, n=3) and retroperitoneal adipose tissue (data not shown in the table and figure).

There is a higher concentration of total TFAs in visceral adipose tissues than in subcutaneous fat in non-obese patients ($P<0.05$). This level of significance is lower because of the reduced number of samples; removal of visceral adipose tissue is not always part of the surgical procedure in these patients. A similar situation was seen for retroperitoneal and visceral adipose tissue in obese patients; the total concentration of TFAs was greater in visceral tissue ($P<0.001$).

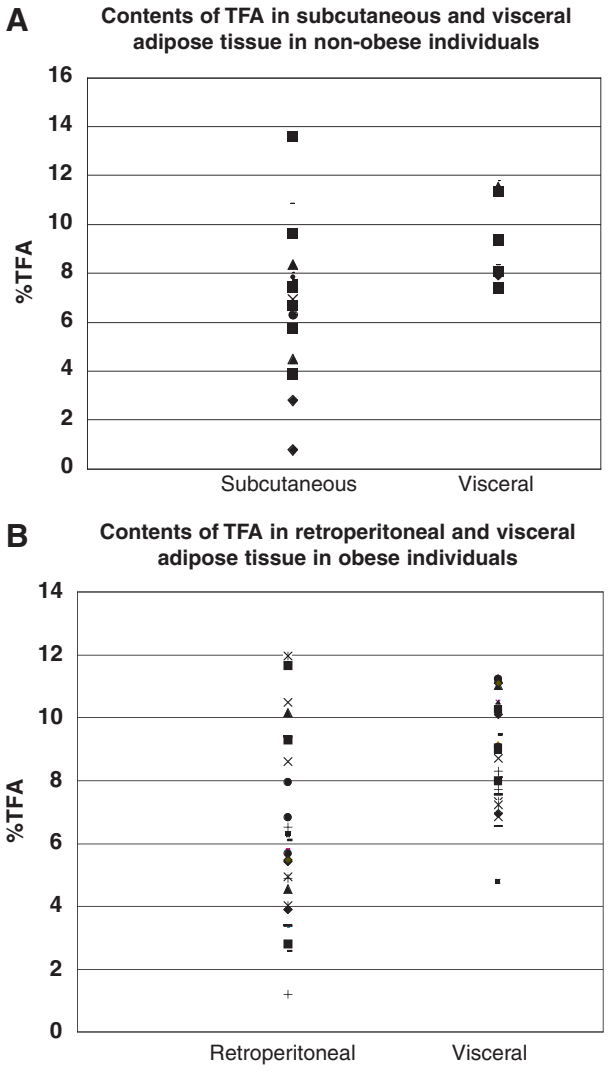

Figure 1. Individual distribution of total TFA in different tissues of obese and non-obese patients. **A**) Individual distribution of total TFA in subcutaneous and visceral adipose tissue of non-obese patients $P<0.05$. **B**) Individual distribution of total TFA in retroperitoneal and visceral adipose tissue of obese patients $P<0.001$.

Discussion

Dietary fats have received much attention from health professionals. Not only the quantity, but also the quality of dietary fat has been studied in relation to the epidemic of obesity, insulin resistance and development of CHD in European countries and USA.[13,23] The fatty acid composition of adipose tissue is an appropriate biomarker for dietary intake for those fatty acids that are not synthesised by

Table 1. Content of *trans* fatty acids in subcutaneous, retroperitoneal and visceral adipose tissue of obese and non-obese individuals

Group	n	Mean %	SD %	*P*
Obese				
Retroperitoneal	31	6.40	0.50	
Visceral	32	8.74	0.29	0.001
Non-obese				
Subcutaneous	18	6.94	0.72	
Visceral	9	9.29	0.59	0.05

P<0.001 when comparing visceral with retroperitoneal adipose tissue of obese group.
P<0.05 when comparing visceral with subcutaneous adipose tissue of non-obese group.

human beings (linoleic acid, n-3 polyunsaturated fatty acids and isomeric *trans* fatty acids). Thus, the fatty acid composition of adipose tissue may provide a particularly useful marker of long-term fatty acid intake over months or years.[4]

We determined the concentration of total TFA in the different adipose tissues of obese patients submitted to bariatric surgery and non-obese patients submitted to different surgery. Our results show that the concentration of total TFAs in subcutaneous or retroperitoneal adipose tissue is greater in both obese and non-obese patients, when compared with other studies. The total TFA content in visceral adipose tissue was statistically significantly higher when compared with subcutaneous and retroperitoneal, in both groups.

The higher values of total TFAs found in all adipose tissue analyzed might be due to the technology of edible fat production in our country and the indiscriminate utilization of hydrogenated vegetable fat.[24,25] Some foods analyzed in Brazil showed higher levels of TFAs, for instance, margarines,[24,26] biscuits,[11] potato chips, pasta, ice-cream, cakes and other foods.[27] Consequently, the consumption of *trans* fatty acids in Brazilian people is higher than in others.[28]

In Brazil there has been recent legislation which will become valid in 2006, compelling manufacturers to include TFA content on the food label.[29] The US FDA intends to proceed with legislation on *trans* fatty acids and concludes that *trans* fat intake increases the risk of coronary heart disease. They recommend that *trans* fat consumption be kept as low as possible.[30] In European countries and the USA, the *trans* fatty acid content has declined to an average 1-2%.[5] The FAO and WHO have also published a guideline "Diet, Nutrition and Prevention of Chronic Diseases", in which the consumption of TFA is recommended to be <1%.[31]

There have been many publications identifying total TFA in subcutaneous fat samples from persons with atherosclerosis,[32] men with acute myocardial infarction,[33,34] cardiac patients,[35-37] French women[38] and US female nurses.[4] Nevertheless, the total TFA content in visceral adipose tissue has not previously been measured.

Visceral obesity in particular is an important risk factor for the metabolic complications that accompany obesity. Only visceral adipose tissue is drained by the portal venous system and has a direct connection with the liver. Mobilization of FFAs is more rapid from visceral than from subcutaneous fat cells, because of the higher lipolytic activity in visceral adipocytes, in both non-obese and obese individuals. It is particularly high in the latter, and this probably contributes significantly to the FFA levels in the systemic circulation. The higher lipolytic activity in visceral fat in comparison to subcutaneous adipose tissue can be attributed to regional variation in the action of the major lipolysis-regulating hormones, catecholamines and insulin, the lipolytic effect of catecholamines being more pronounced and the antilipolytic effect of insulin being weaker in visceral than in subcutaneous adipose tissue.[39]

Our results indicated a higher TFA load than in previous publications. These data show a higher consumption of these fatty acids in the Brazilian diet.

In conclusion, our study has shown higher levels of TFA in visceral adipose tissue than the other abdominal fats (subcutaneous and retroperitoneal). The major concentration of TFA in visceral adipose tissue is worrisome, because the higher rate of lipolysis in this specific tissue appears to be an important indicator for metabolic alterations. Dietary TFA may act as a signaling molecule in lipid metabolism, but further work is necessary to confirm this.

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