Associations Between the Fatty Acid Content of Triglyceride, Visceral Adipose Tissue Accumulation, and Components of the Insulin Resistance Syndrome

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Many factors are involved in the development of the insulin resistance syndrome, such as visceral obesity and the type of dietary fat. The main purpose of this study was to investigate the relationships between fatty acid content of triglyceride (TG), visceral adipose tissue (AT) accumulation, and metabolic components of the insulin resistance syndrome in a group of 97 Caucasian men with a mean age of 45.1 ± 7.2 years (29 to 63 years). To reach these objectives, Spearman correlations, group comparisons, and stepwise multiple regression analyses were performed. The proportion of palmitic acid (16:0) in the TG fraction was positively associated with plasma fasting insulin ($r = .25$, $P = .03$), diastolic ($r = .45$, $P < .001$), and systolic ($r = .29$, $P = .003$) blood pressure. On the other hand, the proportion of α-linolenic acid (18:3n-3) was associated negatively with apolipoprotein (apo) B ($r = -.29$, $P = .005$) and positively with low-density lipoprotein (LDL) diameter ($r = .29$, $P = .007$), while the proportion of γ-linolenic acid (18:3n-6) was associated negatively with plasma TG ($r = -.33$, $P = .003$), diastolic ($r = -.29$, $P = .01$), and systolic ($r = -.35$, $P = .002$) blood pressure and plasma fasting insulin ($r = -.37$, $P = .0005$) and positively with high-density lipoprotein ($HDL)_2$-cholesterol ($r = .27$, $P = .01$) and LDL diameter ($r = .25$, $P = .02$). Stepwise multiple regression analyses were conducted to determine the contribution of visceral AT, body fat mass, and the fatty acid content of TG to the variance of metabolic variables studied. It was found that visceral AT contributed significantly to the variance in plasma TG ($R^2 = 20.7\%$, $P < .0001$), apo B ($R^2 = 9.0\%$, $P = .007$), HDL_2-cholesterol ($R^2 = 17.9\%$, $P < .0001$), LDL diameter ($R^2 = 4.9\%$, $P = .02$), and area under the glucose curve (AUC-glucose) ($R^2 = 8.2\%$, $P = .006$). On the other hand, body fat mass contributed significantly to the variance in fasting insulin ($R^2 = 19.7\%$, $P < .0001$) and diastolic ($R^2 = 6.8\%$, $P = .007$) and systolic ($R^2 = 10.5\%$, $P = .01$) blood pressure. At least one fatty acid made a significant contribution to the variance of each metabolic variable studied. In fact, the proportion of 18:3n-6 contributed significantly to the variance in both TG ($R^2 = 8.9\%$, $P = 0.007$) and HDL_2-cholesterol ($R^2 = 6.0\%$, $P = .01$). Moreover, 18:3n-3 contributed to the variance of apo B ($R^2 = 7.0\%$, $P = .02$), while 18:3n-6 made the largest contribution to the variance of LDL diameter ($R^2 = 7.6\%$, $P = .02$). Finally, 16:0 significantly contributed to the variance of AUC-glucose ($R^2 = 11.4\%$, $P = .0003$), diastolic ($R^2 = 25.2\%$, $P < .0001$), and systolic ($R^2 = 6.8\%$, $P = .002$) blood pressure. In summary, results of this study suggest that the fatty acid content of TG is associated with many metabolic variables of the insulin resistance syndrome independently of body fat mass or visceral AT accumulation.

THERE IS MUCH scientific evidence suggesting that a high accumulation of visceral adipose tissue (AT) is associated with a cluster of metabolic abnormalities that increases the risk of cardiovascular disease (CVD) and type 2 diabetes.[1,2] This cluster has been referred to as the insulin resistance syndrome and includes high triglycerides (TG) and low high-density lipoprotein-cholesterol (HDL-chol), hyperapolipoprotein B, small dense low-density lipoprotein (LDL), glucose intolerance, insulin resistance, hypertension, impaired fibrinolytic activity, increased concentrations of prothrombotic factors, elevated cytokines and inflammation markers, altered postprandial lipemia, and hyperuricemia.[3,4] It has been suggested that visceral obesity is a central component of the insulin resistance syndrome and could be causally involved in the etiology of many components of the syndrome.[2,5] The potential mechanisms responsible for the adverse metabolic effects of abdominal fat are, however, not fully understood. In addition, although the associations between visceral obesity and metabolic parameters are well recognized, it is also well established that there is significant heterogeneity in the relationships between visceral fat and metabolic features of the insulin resistance syndrome.[6]

Besides visceral obesity, other factors can modulate the development of the insulin resistance syndrome, such as the type of dietary lipids. In many studies, it has been shown that saturated fatty acids are associated with an increase in total cholesterol and TG levels, blood pressure, and insulin resistance.[7-9] Saturated fatty acids are also related to deterioration in glucose tolerance and fibrinolytic activity.[10,11] Monounsaturated fatty acids are generally associated with a decrease in total cholesterol concentration and an improved glucose tolerance and insulin sensitivity, as well as with an improved fibrinolysis.[7,11,12-15] Polyunsaturated fatty acids are known to decrease LDL-chol levels.[16] Furthermore, a high proportion of n-3 and n-6 fatty acids can improve glucose tolerance, fibrinolytic activity, and reduce the risk of developing peripheral insulin resistance by increasing insulin sensitivity.[15,17-19]

A positive association has also been reported between the contribution of total dietary fat to energy intake and the level of obesity.[20] However, few studies have examined the relationship between the type of dietary fat, the corresponding fatty acid content of TG, and the level of obesity. To our knowledge, no study has yet examined how the fatty acid content of TG could

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modulate the relationships between visceral AT and the features of the metabolic syndrome. According to some studies,[21,22] increased TG levels may be an independent risk factor for CVD, and higher TG concentrations are associated with a higher level of visceral AT accumulation.[23] However, the difficulties in establishing TG as an independent risk factor for CVD may be due, in part, to the heterogeneity in TG fatty acid composition.

Therefore, in the present study, we wanted to examine the associations between the fatty acid content of TG, which reflects the dietary fatty acid intake of the last few days[24] and components of the insulin resistance syndrome. We also wanted to examine whether the contribution of the fatty acid content of TG to the establishment of metabolic parameters of the insulin resistance syndrome was independent of visceral AT accumulation and could therefore explain some of the heterogeneity of the relationships between visceral obesity and variables of the metabolic profile.

SUBJECTS AND METHODS

Subjects

A total of 97 Caucasian men were recruited by solicitation through the media, with the preoccupation to cover a wide range of body mass index (BMI) values. This study was approved by the Medical Ethics Committee of Laval University. Before entering the study, participants were subjected to a complete medical examination and were asked to sign an informed consent document. The mean age of the sample was 45.3 $\pm$ 7.3 years and ranged from 29 to 63 years. All subjects were apparently healthy and were not undergoing treatment for diabetes, hypercholesterolemia, coronary heart disease, or endocrine disorders.

Body Composition and Anthropometry

Body density was measured by the hydrostatic weighing technique,[25] and the mean of 6 measurements was used in the calculation of body density. Pulmonary residual volume was measured before immersion in the hydrostatic tank by using the helium dilution method of Meneely and Kaltreider.[26] Percent body fat mass was derived from body density using the equation of Siri.[27] Height, body weight, BMI, and waist and hip circumferences were measured following the procedures recommended by the Airlie Conference,[28] and the waist-to-hip ratio was calculated.

Computed Tomography

Measurements of visceral AT areas were performed by computed tomography (CT) with a Siemens Somatom DHR scanner (Erlanger, Germany) by using the procedure of Sjöström et al[29] as previously described.[30] Briefly, subjects were examined in the supine position with both arms stretched above their head. The CT scan was performed at the abdominal level between L4 and L5 vertebrae using a radiograph of the skeleton as a reference to establish the position of the scan to the nearest millimeter. Total visceral fat areas were calculated by delineating these areas with a graph pen and then computing the AT surfaces using an attenuation range of -190 to -30 Hounsfield units (HU).[29,31] The visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the amount of visceral fat from the total abdominal AT area.

Oral Glucose Tolerance Test

A 75-g oral glucose tolerance test (OGTT) was performed in the morning after an overnight fast. Blood samples were collected through a venous catheter from an antecubital vein into vacutainer tubes containing EDTA (ethylenediamine tetraacetic acid) at -15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes for determination of plasma glucose and insulin concentrations. Plasma insulin was measured by radioimmunoassay with polyethylene glycol separation,[32] whereas plasma glucose was measured enzymatically.[33] The total glucose and insulin areas under the curve (AUC) during the OGTT were determined with the trapezoid method.

Lipid and Lipoprotein Measurements

Blood samples were collected after a 12-hour overnight fast from an antecubital vein into tubes containing EDTA for measurement of plasma lipid and lipoprotein levels. TG[34] and cholesterol[35] concentrations in the plasma and in the lipoprotein subfractions were measured using a Technicon RA-500 (Bayer, Tarrytown, NY) and enzymatic reagents obtained from Randox (Randox Laboratories, Crumlin, UK). Plasma very–low-density lipoprotein (VLDL) ($d < 1.006$ g/mL) were isolated by ultracentrifugation,[36] and the HDL fraction was obtained after precipitation of LDL in the infranatant ($d > 1.006$ g/mL) with heparin and $MnCl_2$.[37] The cholesterol content of the infranatant was measured before and after precipitation, and the concentration of LDL-chol was obtained by difference. Apo B concentrations were measured by the rocket immunoelectrophoretic method of Laurell, as previously described[38], in the fasting plasma and in the LDL fraction ($d > 1.006$ g/ml).[39] Lyophilized serum standards for apo B measurements were prepared in our Laboratory and calibrated with reference standards obtained from Center for Disease Control and Prevention, (Atlanta, GA). LDL peak particle diameter was assessed with nondenaturing 2% to 16% polyacrylamide gel electrophoresis of whole plasma as previously described.[40]

Measurements of Blood Pressure

The measurement of seating blood pressure was recorded using a sphygmomanometer and a stethoscope after a 15-minute rest period. Systolic blood pressure was considered as the first detectable sound (phase 1) and diastolic blood pressure was measured at the disappearance of Korotkoff's sounds (phase 5) in accordance with the recommendations of the American Heart Association.[41]

Sample Preparation for the Determination pf Plasma Fatty Acid Profile

A total lipid extract was obtained from 500 μL plasma according to the procedure of Folch et al.[42] To obtain the different lipid subfractions, the total lipid extract was processed on a thin-layer chromatography on 20×20 cm silica gel plates (Fisher, Ontario, Canada #06-600 A gel G TLC plates) using heptane-ethyl ether-acetic acid (80:20:2), isolated and methylated with a 6% methanol-H_2SO_4 solution.[43] Hexane was the solvent used to dissolve fatty acid methyl esters before injecting in the gas chromatography system. Only results of the TG fraction are reported in the present study.

Determination of the Fatty Acid Content of TG by Gas Chromatography

Relative concentrations of individual fatty acid in the TG fraction were determined using gas chromatography (Hewlett Packard [HP], CA, 5890A) equipped with a 25 m-by-0.20 mm capillary column (DB-225, SN 9682721; J & W Scientific, CA) to separate the fatty acid methyl ester (FAME). The instrument temperature was programmed from 100°C to 220°C at 20°C/min. The detector temperature was 240°C and the injector temperature 235°C. Helium was used as carrier gas and the split ratio was 1:13. The FAMEs were identified by comparison with authentic FAME standards, and peak areas were integrated as relative weight (wt%) by using a microprocessor (Hewlett

Table 1. Characteristics of the 97 Subjects

Variables	Mean ± SD	Range
Age (yr)	45.1 ± 7.2	29-63
Waist circumference (cm)	96.2 ± 10.1	68.7-119.0
BMI (kg/m^2)	28.1 ± 4.3	18.7-41.0
Body fat mass (kg)	23.9 ± 8.4	8.1-44.9
Visceral AT (cm^2)	144.5 ± 60.7	50.8-357.1
Total chol (mmol/L)	5.1 ± 0.8	3.3-7.2
LDL-chol (mmol/L)	3.4 ± 0.7	1.5-5.0
HDL-chol (mmol/L)	1.0 ± 0.2	0.6-1.6
HDL_2-chol (mmol/L)	0.4 ± 0.2	0.01-0.9
Triglycerides (mmol/L)	1.9 ± 1.0	0.5-6.3
Apo B (g/L)	1.1 ± 0.2	0.6-1.5
LDL diameter (Å)	252.0 ± 5.4	240.6-264.4
Systolic blood pressure (mm Hg)	120.6 ± 11.0	100-155
Diastolic blood pressure (mm Hg)	84.3 ± 9.2	69-113
Fasting glucose (mmol/L)	5.5 ± 0.6	4.3-6.9
Fasting insulin (pmol/L)	73.8 ± 51.4	1.0-389.0
AUC-glucose (mmol/L/min)	1,219.7 ± 254.9	680.6-2,018.3
AUC-insulin (pmol/L/min)	87,481.1 ± 64,774.5	22,358-498,420

Abbreviations: AT, adipose tissue; Chol, cholesterol; apo, apolipoprotein; AUC, area under the curve; BMI, body mass index.

Packard, Chem Station). The coefficient of variation was 5.11% for the largest peaks, 5.69% for medium peaks, and 7.38% for the smallest peaks.

Statistical Analyses

Spearman correlation coefficients were used to quantify the univariate associations among variables. Fatty acid content of TG of men matched one by one for their visceral AT levels, but presenting a notable difference in the value of the metabolic variable studied were compared. More specifically, each pair showed a maximal difference of 10 cm^2 for their visceral AT level, and there was at least 1 standard deviation (SD) between the 2 values of the metabolic variable studied. This matching procedure also resulted in similar values for age and body fat mass for the 2 groups compared. The number of pairs that it was possible to form varied depending upon the metabolic variable studied (n = 31 pairs for TG and HDL_2-chol; n = 33 pairs for apo B concentrations; n = 38 pairs for LDL diameter, diastolic, and systolic blood pressure; n = 23 pairs for plasma insulin concentrations, and n = 30 pairs for the AUC-glucose). The Student's unpaired *t* test was used to compare the 2 groups of subjects formed following the matching procedure. For each variable studied, it was determined whether variances were equal or unequal. In the presence of unequal variances, the approximate *t* statistic was computed.[44] A stepwise multiple regression analysis was performed to quantify the independent contribution of abdominal and total adiposity and variables of the fatty acid content of TG to the determination of metabolic variables of the insulin resistance syndrome. In fact, for each metabolic parameter, visceral AT accumulation, fat mass, and the 2 fatty acids displaying the highest univariate correlation coefficient with the metabolic variable studied were included in the model. For variables not normally distributed (18:3n-3; 18:3n-6) their log-transformed values were used for analyses. All statistical analyses were performed with the SAS statistical package (SAS, Cary, NC).

RESULTS

Table 1 shows characteristics of the sample of 97 men. Values covered a wide range for BMI, waist circumference, fat mass,and visceral AT. Table 2 shows the proportion of each fatty acid in the TG fraction. Oleic acid (18:1) was the main fatty acid found in the TG fraction with a mean proportion of 44.3% ± 4.0% followed by palmitic acid (16:0) (31.2% ± 4.6%) and linoleic acid (18:2) (12.1% ± 3.7%).

Table 3 shows Spearman correlation coefficients between the proportion of the different fatty acids in the TG fraction and anthropometric variables. BMI was positively correlated with the proportion of 16:1 ($r = .27$) and negatively with the proportion of 18:3n-6 ($r = -.27$). Moreover, body fat mass was negatively associated with 18:3n-6 proportion in the TG fraction ($r = -.23$). No significant associations were observed between visceral AT and the different fatty acids studied.

Spearman correlation coefficients between the proportion of the different fatty acids in the TG fraction and metabolic variables of the insulin resistance syndrome are shown in Table 4. Apo B levels were negatively associated with 18:3n-3 proportion ($r = -.29$). Plasma TG concentration was positively correlated with 16:1 proportion ($r = .24$) and negatively with 18:3n-6 ($r = -.33$). Plasma concentration of HDL_2-chol was positively associated with the proportion of stearic (18:0) and 18:3n-6 ($r = .27$ and $r = .27$) and negatively with palmitoleic acid (16:1) ($r = -.30$). In addition, Table 4 shows that there was a positive association between LDL diameter and both 18:3n-3 and 18:3n-6 proportions ($r = .29$ and $r = .25$). Diastolic and systolic blood pressure values were positively associated with 16:0 ($r = .45$ and $r = .29$) and negatively with 18:2 ($r = -.37$ and $r = -.21$) and 18:3n-6 proportions ($r = -.29$ and $r = -.35$). Fasting insulin concentrations were positively correlated with 16:0 ($r = .25$) and negatively with the 18:3n-6 proportion ($r = -.37$). The AUC-glucose measured during the OGTT was positively associated with the proportion of 16:0 and negatively with the proportion of 18:1. Finally, significant inverse association between LDL-chol concentration and 18:3n-3 proportion was also observed ($r = -.29$, $P = .004$) (data not shown).

Significant relationships were observed between visceral AT accumulation and metabolic variables of the insulin resistance syndrome (TG, HDL_2-chol, insulin, apo B, LDL diameter, glucose and blood pressure). However, an important heterogeneity was noticed for these associations as visceral fat explained no more than 20% of the variance for the different metabolic variables studied (results not shown). Therefore, we evaluated whether the fatty acid content of TG could explain some of this heterogeneity by comparing subjects with similar visceral AT levels, but with very different values of the meta-

Table 2. Proportions (%) of Various Fatty Acids in the TG Fraction

Fatty Acids	Proportion (% ± SD)	Range (%)
14:0 (myristic acid)	2.8 ± 1.4	1.3-11.9
16:0 (palmitic acid)	31.2 ± 4.6	20.2-42.2
16:1 (palmitoleic acid)	3.3 ± 1.2	1.0-7.2
18:0 (stearic acid)	5.2 ± 1.6	2.3-15.7
18:1 (oleic acid)	44.3 ± 4.0	33.9-56.0
18:2 (linoleic acid)	12.1 ± 3.7	1.5-23.9
18:3n-3 (α-linolenic acid)	0.9 ± 0.4	0.3-2.4
18:3n-6 (γ-linolenic acid)	0.4 ± 0.3	0.09-2.0

NOTE. n = 97.

Table 3. Spearman Correlation Coefficients for the Associations Between Various Triglyceride Fatty Acids and Anthropometric Variables

Variable	14:0	16:0	16:1	18:0	18:1	18:2	18:3n-3	18:3n-6
Age	0.02	0.05	0.05	0.11	−0.23*	0.06	0.21*	0.14
BMI	0.05	0.18	0.27†	−0.01	−0.11	−0.16	−0.20	−0.27*
Fat mass	0.04	0.12	0.20	−0.06	−0.12	−0.10	−0.10	−0.23*
VAT	−0.17	0.09	0.12	−0.07	−0.02	−0.09	−0.09	−0.17

Abbreviations: BMI, body mass index; VAT, visceral adipose tissue.
*$P < .05$, †$P < .01$, n = 97.

bolic variables studied (see the Subjects and Methods section for the details on matching procedure used).

In Fig 1, it is shown that for a similar visceral AT accumulation, men with higher TG as well as men with lower HDL_2-chol had a significantly lower 18:3n-6 proportion ($P = .04$). For a similar visceral fat accumulation, the group of men with high levels of apo B displayed a lower 18:3n-3 proportion ($P = .02$). Moreover, men with smaller LDL had lower proportions of 18:3n-3 ($P = .006$) and 18:3n-6 ($P = .04$).

Figure 2 shows that for a given visceral AT accumulation, the group with higher fasting insulin levels and higher AUC-glucose had higher 16:0 proportion ($P = .03$). In addition, the group with a higher systolic blood pressure had higher 16:0 ($P = .02$) and lower 18:3n-6 ($P = .04$) proportions in comparison with the group characterized by a lower systolic blood pressure. Furthermore, the group with a higher diastolic blood pressure had higher 16:0 ($P = .003$) and lower 18:2 ($P = .007$) proportions in the TG fraction than the group of men with lower diastolic blood pressure, but similar visceral AT accumulation.

Stepwise multiple regression analyses were performed to better sort out the contribution of visceral AT, body fat mass, and the different fatty acids to the variance of metabolic variables of the insulin resistance syndrome. As shown in Table 5, visceral AT contributed between 4.9% to 20.7% of the variance for TG, apo B, HDL_2-chol, LDL diameter, and AUC-glucose. On the other hand, body fat mass significantly contributed to fasting insulin ($R^2 = 19.7\%$) and blood pressure (systolic: $R^2 = 10.6\%$; diastolic: $R^2 = 6.8\%$). For each metabolic variable, at least 1 fatty acid made a significant contribution to the variance. In fact, both TG and HDL_2-chol were explained by the proportion of 18:3n-6 in the TG fraction ($R^2 = 8.9\%$ and 6.0%). Moreover, 18:3n-3 contributed signifcantly to the variance of apo B ($R^2 = 7.0\%$) while 18:3n-6 was the fatty acid making the largest contribution to the variance of the LDL diameter ($R^2 = 7.6\%$). Finally, 16:0 contributed significantly to the variance of the AUC-glucose and that of systolic and diastolic blood pressure ($6.8\% \leq R^2 \leq 25.2\%$).

DISCUSSION

A high accumulation of AT at the abdominal level has been suggested to play an important role in the etiology of the insulin resistance syndrome.[2] More specifically, a high accumulation of visceral AT has been related to insulin resistance, hyperinsulinemia, glucose intolerance, high levels of apo B, dense and small LDL particles, altered postprandial lipemia, hypertension, inflammatory profile, a decrease of the fibrinolytic activity, as well as hyperuricemia, which are parameters that have been proposed as components of the insulin resistance syndrome.[3,5,45] The type of dietary fat and the corresponding plasma fatty acid profile have been suggested as important relevant factors involved in the etiology of the insulin resistance syndrome. In fact, many studies have related the plasma fatty acid profile to plasma glucose-insulin homeostasis,[46] to the plasma lipid-lipoprotein profile,[47-49] as well as to blood pressure.[9] In this study, the main objective was to determine the respective contributions of visceral AT accumulation and the fatty acid content of TG, reflecting the average dietary fatty acid composition during the last days[24] to the variance of metabolic parameters of the insulin resistance syndrome.

As it is commonly the case in studies, which have measured the fatty acid profile of the plasma TG fraction, we did not report value for the longer chain fatty acids in our study because their concentrations in the TG fraction were too low or even not detectable in some subjects.[48] We also reported that there are not much significant associations between the fatty acid content of TG and morphologic variables. In fact, no association was found between visceral AT and the different

Table 4. Spearman Correlation Coefficients for the Associations Between Various Triglyceride Fatty Acids and Metabolic Variables of the Insulin Resistance Syndrome

Variables	14:0	16:0	16:1	18:0	18:1	18:2	18:3n-3	18:3n-6
TG	0.00	0.05	0.24*	−0.12	−0.08	0.06	−0.13	−0.33†
Apo B	−0.07	−0.03	0.07	0.02	0.01	0.03	−0.29†	−0.13
HDL_2-chol	−0.06	0.14	−0.30‡	0.27†	−0.02	−0.17	0.18	0.27*
LDL diam	−0.10	−0.09	0.10	−0.04	0.06	0.03	0.29†	0.25*
Diastolic BP	0.09	0.45‡	0.00	0.12	−0.13	−0.37‡	−0.20	−0.29†
Systolic BP	−0.11	0.29†	−0.07	0.10	0.02	−0.21*	−0.20	−0.35†
Insulin	0.11	0.25*	0.04	0.15	−0.11	−0.19	−0.10	−0.37‡
Glucose area	0.08	0.22*	0.10	0.05	−0.22*	−0.10	−0.17	0.00

Abbreviations: TG, triglycerides; apo, apolipoprotein; chol, cholesterol; diam, diameter; BP, blood pressure.
*$P < .05$, †$P < .01$, ‡$P < .001$, n = 97.

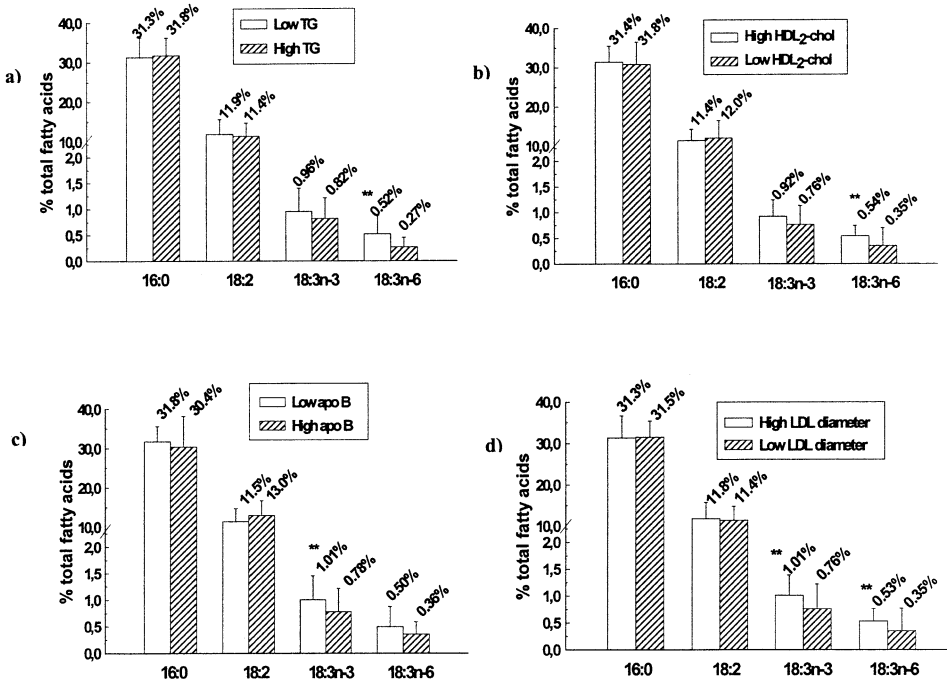

Fig 1. Fatty acid proportion in TG fraction for 2 groups of men paired for visceral AT level, but presenting different values for the metabolic variable studied. (A) TG (n = 31 in each group): subjects with either low (1.07 ± 0.32 mmol/L) or high (2.84 ± 0.93 mmol/L) TG concentrations.(B) HDL_2-chol (n = 31 in each group): subjects with either high (0.55 ± 0.16 mmol/L) or low (0.23 ± 0.12 mmol/L) HDL_2-chol concentrations. (C) Apo B (n = 33 in each group): subjects with either low (0.85 ± 0.13 g/L) or high (1.23 ± 0.14 g/L) apo B concentrations. (D) LDL diameter (n = 38 in each group): subjects with either high (256.86 ± 3.66 Å) or low (248.01 ± 3.39 Å) LDL diameter.

fatty acids studied. Moreover, only a few variables of the fatty acid content of TG were correlated with age, BMI and fat mass. To our knowledge, we are the first to report correlations between the fatty acid content of TG and visceral AT measured by CT. A study by Rössner et al[24] revealed that non-obese subjects had a different plasma fatty acid profile in the TG fraction when compared with obese patients. In fact, the 18:2 and 18:3 proportions were lower in obese patients, while the proportions of 16:0 and 16:1 were higher. Another study showed weak correlations between the plasma fatty acid profile of the cholesterol ester fraction and both BMI and the waist-to-hip ratio, a finding concordant with our results.[50] These

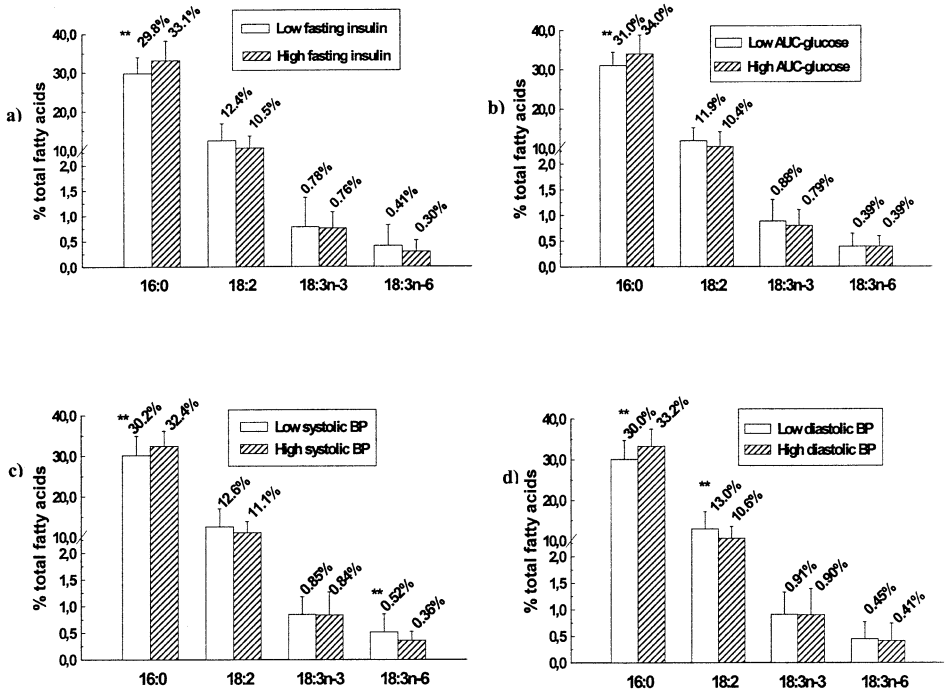

Fig 2. Fatty acid proportion in TG fraction for 2 groups of men paired for visceral AT level, but presenting different values for the metabolic variable studied. (A) Insulin levels (n = 23 in each group): subjects with either low (37.4 ± 16.4 pmol/L) or high (131.0 ± 71.0 pmol/L) insulin levels. (B) AUC-glucose (n = 30 in each group): subjects with either low (1033.26 ± 153.74 mmol/L/min) or high (1,468.19 ± 220.14 mmol/L/min) AUC-glucose. (C) Systolic blood pressure (n = 38 in each group): subjects with either low (111.1 ± 6.1 mm Hg) or high (129.6 ± 9.0 mm Hg) systolic blood pressure. (D) Diastolic blood pressure (n = 38 in each group): subjects with either low (77.0 ± 4.5 mm Hg) or high (92.6 ± 7.2 mm Hg) diastolic blood pressure.

Table 5. Stepwise Multiple Regression Analysis Showing Independent Contributions of Visceral AT, Fat Mass, and Fatty Acids to the Variance of Metabolic Variables of the Insulin Resistance Syndrome

Dependent Variable	Independent Variables	Partial ($R^2 \times 100$)	*P* Value
Triglycerides	Visceral AT	20.7	<.0001
	18:3n-6	8.9	.0007
Apolipoprotein B	Visceral AT	9.0	.007
	18:3n-3	7.0	.002
HDL_2-cholesterol	Visceral AT	17.9	<.0001
	18:3n-6	6.0	.01
LDL diameter	18:3n-6	7.6	.002
	Visceral AT	4.9	.02
Glucose area	16:0	11.4	.0003
	Visceral AT	8.2	.006
Insulin	Fat mass	19.7	<.0001
Systolic blood pressure	Fat mass	10.5	.007
	16:0	6.8	.002
Diastolic blood pressure	16:0	25.2	<.0001
	Fat mass	6.8	.01

NOTE. The stepwise multiple regression model included fat mass, visceral fat, and the 2 fatty acids displaying the highest univariate correlation coefficient with the metabolic variable studied.

results suggest that body composition and body fat distribution variables are weak correlates of the fatty acid content of TG.

It has been suggested that the fatty acid profile in circulating lipids seems to reflect the day-to-day variation of the diet rather than the dietary intake over a long period of time.[24] For that reason, we made sure that blood samples for the different analyses (OGTT, lipid profile, and fatty acid profile) were collected at the same time for each subject to avoid a bias from day-to-day variation in the type of dietary fatty acids ingested. Associations were examined between the fatty acid content of TG and metabolic variables of the insulin resistance syndrome. Our results provide further support to the notion that saturated fatty acids increase the risk of hypertension and CVD.[50] In fact, 16:0 was strongly and positively correlated with blood pressure. It is possible that the association attributed to saturated fatty acids is a result of competing effects of nutrients on blood pressure regulation, because plasma fatty acid composition also reflects a certain dietary pattern. For example, individuals eating more saturated fat may also have a higher total fat intake and a lower dietary intake of complex carbohydrates, fiber, polyunsaturated fatty acids, and antioxidants, which may all have an effect on blood pressure regulation.[50] Moreover, the proportion of 16:0 was positively associated with insulin levels and the AUC-glucose. This could be explained by the fact that saturated fatty acids decrease membrane fluidity, which may affect insulin sensitivity of different tissues.[51,52] It must also be underlined that a wide variation in plasma insulin concentrations was found among our sample, a finding concordant with previous observations.[53,54] The large variation in adiposity of our sample can contribute to explain this finding. For 16:1, our results suggest that this fatty acid behaves more like a saturated than a monounsaturated fatty acid, because its proportion was positively associated with TG and negatively with HDL_2-chol, a finding concordant with results reported by Siguel et al.[55]

The proportions of 18:1,18:2, 18:3n-3, and 18:3n-6 in the TG fraction seem to be associated with a more favorable metabolic profile. Indeed, the proportion of 18:1 was negatively associated with the AUC-glucose. Furthermore, our results showed a negative correlation between 18:2 and blood pressure. We also found negative associations between 18:3n-3 and both total cholesterol and apo B concentrations, while a positive association was found with LDL diameter. Finally, 18:3n-6 showed a negative association with TG, blood pressure, and fasting insulin levels and positive associations with HDL_2-chol and LDL diameter. Many potential mechanisms are proposed to explain the "protective" effect of n-3 and n-6 fatty acids against CVD risk factors. The first mechanism proposed is the increase of the membrane fluidity that improves insulin action. Accordingly, fasting insulin, which is a fair indicator of insulin resistance in nondiabetic subjects, was negatively associated with 18:3n-6.[18] Furthermore, it has been suggested that n-6 fatty acids decrease total cholesterol concentration mainly in the LDL fraction when these fatty acids replace saturated fatty acids in the diet.[7] Some mechanisms have been proposed to explain this finding, such as an increase in LDL-receptor activity, inhibition of hepatic synthesis of apolipoprotein B-containing lipoproteins, or enhanced excretion of fecal steroids.[7] In our study, the TG levels were also negatively associated with 18:3n-6, and the mechanisms of this reduction remain unclear. In fact, a TG-lowering action for n-6 fatty acids is not a consistent phenomenon; many patients with hypertriglyceridemia do not respond with a lowering of serum TG when fed a diet high in linoleic acid.[56] The ingestion of n-3 fatty acids has usually a better impact on chylomicron metabolism than n-6 fatty acids.[57]

It is well known that certain heterogeneity exists between visceral AT accumulation and metabolic parameters of the insulin resistance syndrome, and our results also reflected this heterogeneity. Epidemiologic studies have reported a lower incidence of CVD among Inuits from Greenland compared with Caucasians from North American populations, probably due to their high dietary intake of polyunsaturated fatty acids, mainly n-3 fatty acids, despite the fact that Inuits are more obese than Caucasians from North America.[17,58,59] In our study, we examined whether the fatty acid content of TG could explain some of the heterogeneity of the relationships involving visceral AT with metabolic parameters. We found that for a given visceral fat accumulation, subjects with a deteriorated metabolic profile were characterized by significant differences in their fatty acid content of TG compared with subjects with a favorable metabolic profile. These results suggest that a fatty acid content of TG characterized by low 16:0 and high 18:3 (both n-3 and n-6) proportions may protect visceral obese individuals against the development of metabolic alterations of the insulin resistance syndrome. Our stepwise multivariate analyses were also supportive of this hypothesis, as they showed that the fatty acid content of TG made a significant contribution to the variance of the metabolic variables studied, independently of total adiposity and visceral fat accumulation. These results may have clinical implications for the treatment of individuals with high visceral fat accumulation. Because body weight loss is often difficult to achieve and maintain, changes in the type of dietary fat, which would result in

changes in the fatty acid content of TG, could be an alternate therapeutic strategy to achieve a better metabolic profile in visceral obese men.

In conclusion, we reported many significant associations between the fatty acid content of TG and metabolic variables of the insulin resistance syndrome, but weak or absent relationships between the fatty acid profile and either body composition or body fat distribution. It is suggested that part of the heterogeneity found in the relationships between abdominal AT accumulation and metabolic variables of the insulin resistance syndrome could be explained by the fatty acid content of TG.

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