Insulin Resistance Directly Correlates With Increased Saturated Fatty Acids in Skeletal Muscle Triglycerides

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A close relationship between elevated plasma free fatty acid (FFA) levels and insulin resistance is commonly reported in obese subjects. The aim of the present study was to evaluate the role of intramuscular triglyceride (mTG) and FFA levels in insulin sensitivity in 30 nondiabetic normal-weight or obese subjects (18 with body mass index [BMI] = 21.8 ± 3.3 kg/m² and 12 with BMI = 34.6 ± 2.7 kg/m²) who underwent minor abdominal surgery. Body composition was estimated by isotopic dilution, substrate oxidation by indirect calorimetry, and whole-body glucose uptake by euglycemic-hyperinsulinemic clamp (EHC). Glucose uptake (M) value negatively correlated with the MTG level ($R^2 = -.56$, $P < .0001$), which was increased in obese patients (11.6 ± 2.2 *v* 6.2 ± 1.4 μmol/g wet weight muscle tissue, $P < .0001$). The TG fatty acid profile was significantly different in the 2 groups: an increased concentration of saturated fat was present in obese patients (unsaturated to saturated ratio, 1.89 ± 0.40 *v* 2.19 ± 0.07, $P < .0001$). Stepwise linear regression analysis of total mTGs and palmitic and oleic fractions on the M value showed that only TGs and palmitic acid were significantly related to glucose uptake ($R^2 = .66$, $P < .0001$). Furthermore, among the other anthropometric variables, only the BMI was significantly correlated with MTGs ($R^2 = .71$, $P < .0001$). In conclusion, not only the MTG concentration but also the FFA pattern seems to affect insulin-mediated glucose uptake. A pivotal role might be played by a high saturated fatty acid content in the TGs.

INSULIN RESISTANCE is defined as an impaired biological response to either exogenous or endogenous insulin.[1] It is often present when circulating levels of either free fatty acids (FFAs) or triglycerides (TGs) are abnormally high.[2-5] The negative effect of increased tissue lipid content, which likely reflects the plasma lipid concentration, on insulin sensitivity is commonly ascribed to the competition between acetyl-coenzyme As deriving from FFA β-oxidation and those deriving from glucose oxidation for entrance into the Krebs into cycle, according to Randle's hypothesis.[2] Obese subjects have a large potential to release FFA from intra-abdominal reserves, since fat cell size and, consequently, the lipid content are increased in both portal and nonportal tissues.[5] Major fractional primary FFA reesterification reduces FFA turnover per fat mass in obese subjects and seems to favor subcutaneous fat accumulation.[6]

The mechanisms underlying the association between visceral obesity and the cluster of metabolic disturbances characterizing the insulin-resistant state have been extensively investigated. Different regional fat distribution, subcutaneous or visceral, is associated with different values for important metabolic variables.[7] Both circulating TGs and skeletal muscle triglycerides (mTGs) influence insulin sensitivity. Few data[8-15] are available in the literature concerning the amount of TGs and their fatty acid pattern in skeletal muscle. Recently, direct evidence of a significant correlation between the intramyocellular lipid concentration and insulin sensitivity was found by Krssak et al[8] in healthy subjects by ^{1}H-nuclear magnetic resonance spectroscopy.

Pan et al[9] found a significant association between the degree of insulin resistance and the mTG content in vastus lateralis muscle biopsies in nondiabetic Pima Indians. High levels of mTG also have been observed in rectus abdominis muscle biopsies in patients with type 2 diabetes.[10] The fatty acid profile also has been shown to be closely linked to insulin action; in fact, a higher proportion of saturated fats is associated with hormone resistance.[11]

This study aimed to evaluate in skeletal muscle biopsies whether the absolute content of TGs and their fatty acid profile are different in lean compared with obese subjects, and whether these parameters are related to insulin action as evaluated by the euglycemic-hyperinsulinemic clamp (EHC).

SUBJECTS AND METHODS

Subjects

The subjects were 30 nondiabetic patients, 15 males and 15 females, who underwent abdominal surgery for cholecystectomy due to the presence of cholesterol gallstones (n = 20, 7 obese and 13 lean subjects) or hernia of the abdominal wall (n = 10, 5 obese and 5 lean subjects). They had a mean age of 32.4 ± 9.5 years (range, 22 to 52), height 169.3 ± 9.3 cm (range, 154 to 187), weight 77.7 ± 22.5 kg (range, 41 to 119), and body mass index (BMI) 27.0 ± 7.0 kg/m² (range, 13.25 to 38.13). The plasma lipid pattern was not statistically different in the 2 groups as determined by the Fisher exact test. The subjects were clinically euthyroid, had no evidence of renal, cardiac, or hepatic dysfunction, and were not treated with medication that could affect carbohydrate or insulin metabolism. None of the subjects smoked cigarettes. They were divided into 2 groups. The control group consisted of 18 subjects, 8 men and 10 women aged 33.7 ± 9.8 years with a BMI of 21.8 ± 3.3 kg/m². The obese group consisted of 12 patients, 5 men and 7 women with a mean age of 30.4 ± 9.0 years and a BMI of 34.6 ± 2.7 kg/m². All subjects consumed a weight-maintaining diet consisting of at least 250 g carbohydrate per day for 1 week before the study.

Body Composition

Body composition was estimated on the basis of total body water (TBW)[7] measured by isotopic dilution. On the day preceding the clamp study, each subject received 80 μCi tritiated water (specific activity, 100 mCi/mL) in 5 mL saline solution as an intravenous bolus injection. Blood samples were obtained every 30 minutes for 180 minutes, and plasma radioactivity was counted with a β-scintillation counter (Canberra-Packard, model 1600 TR; Meriden, CT). Each determination was

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obtained in duplicate with 0.5 mL plasma. The resulting values (dpm) were plotted against time (minutes), thus obtaining the steady-state concentration (dpm per milliliter) of the tracer and, consequently, its apparent volume of distribution (TBW in liters). The fat-free mass (kilograms) was approximated by dividing TBW by 0.73.[7]

EHC

Subjects underwent a EHC 2 weeks before surgery. All subjects were admitted to the Department of Metabolic Diseases of the Catholic University School of Medicine in Rome at 7 PM on the day before the study. At 7 AM the following morning in the postabsorptive state after a 12- to 14-hour overnight fast, indirect calorimetry monitoring was started. The infusion catheter was inserted into an antecubital vein. The sampling catheter was placed in the contralateral dorsal hand vein, and this hand was kept in a heated box (60°C) to obtain arterialized blood. A basal blood sample was obtained for insulin and glucose determination. At 8 AM after a 12- to 14-hour overnight fast, the EHC was performed according to the method of De Fronzo et al.[16] A priming dose of short-acting human insulin was given during the initial 10 minutes in a logarithmically decreasing manner to acutely increase plasma insulin to the desired level. Insulinemia was then maintained with a continuous infusion of insulin at a rate of 40 $mU \cdot m^{-2} \cdot min^{-1}$ for 110 minutes. During the clamp, the glucose level was monitored every 5 minutes and the infusion rate of a 20% glucose solution was adjusted following the algorithm detailed by DeFronzo et al.[16] Because serum potassium tends to decrease during this procedure, KCl was administered at a rate of 15 to 20 mEq/h to maintain serum potassium between 3.5 and 4.5 mEq/L. The rates of glucose uptake for the last 40 minutes (between 80 and 120 minutes of the clamp) were averaged to calculate the overall glucose disposal rate (M value). Arterialized blood samples were collected every 20 minutes during the clamp study to measure the insulin concentration. The subjects emptied their bladder before starting the study; during the clamp, urine was collected to measure urinary nitrogen loss for each subject, which was used for the calorimetry computations. Respiratory gas exchange was measured by an open-circuit ventilated-hood system (monitor MBM-100, Deltatrac; Datex Instrumentarium, Helsinki, Finland). The energy expenditure, respiratory quotient, and substrate oxidation rates were calculated from oxygen consumption, carbon dioxide production, and urinary nitrogen excretion according to the method of Ferrannini.[17] Respiratory gas exchange measurements were started 45 minutes before the clamp to measure resting energy expenditure, and continued during the 120 minutes of the clamp.

The nature and purpose of the investigations were explained to each subject before they agreed to participate in the study. The protocol followed the guidelines of the hospital ethics committee.

Analytical Methods

The serum glucose level was measured by the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin was determined by microparticle enzyme immunoassay (Abbott Imx, Pasadena, CA).

Skeletal Muscle Lipid Analysis

During surgery, a rectus abdominis muscle biopsy was obtained (1 g). Each sample was free of visible contamination from subcutaneous fat. A specimen of 500 mg was taken and immediately placed in calcium-free Hanks solution with EDTA and bubbled with O_2 95% and CO_2 5%. The sample was washed and then immersed in fresh Hanks solution with collagenase type IV 50 mg and calcium ions and agitated in a Dubnoff waterbath (Asal 720/D, Milan, Italy) maintained at 37°C until the tissue appeared soft. At this point, the specimen was gently removed, and the cells were brushed with a blunted spatula, filtered, suspended in phosphate-buffered saline, and centrifuged twice at 50 × *g* for 2 minutes. The supernatant was discarded, and the muscle cells were dried under a nitrogen stream. After protein precipitation with 5 to 10 mg trichloroacetic acid, lipids were extracted twice with 8 vol chloroform:methanol (2:1 vol/vol) by stirring the solutions at 60°C for 15 minutes. The combined extracts were dried in a GyroVap apparatus (GV1; Gio. DeVita, Rome, Italy) operating at 60°C, coupled to a vacuum pump and a gas trap (FTS System, Stone Ridge, NY). The dry weight of lipid extracts was obtained by weighing the sample tube before and after drying the extracts. The extracts were redissolved in chloroform:methanol (2:1 vol/vol) and fractionated into their various components by thin-layer chromatography (TLC) on standard thin-layer plates (Stratocrom SI AP; Carlo Erba, Milan, Italy) coated with a 0.25-mm layer of silica gel and activated by heating at 120°C for 20 minutes. The plates were developed in successive solvent systems as described by Passi et al.[18] The area of silica gel corresponding to the ratio of fraction time (R_f) of a triolein and tripalmitin standard mixture was scraped off and extracted with peroxide-free diethyl ether. The TG fraction eluted from the TLC plates was saponified by treatment with 2N KOH in methanol and successive acidification to pH 2 to 3 with 2N HCl. FFAs were thus obtained and finally separated and measured according to a previously described method.[18] The same procedure was applied to a 200-mg skeletal muscle specimen to extract total lipids and then to measure the FFA content of the TG fraction.

Statistical Analysis

Results are presented as the mean ± SD unless otherwise specified. Each value represents the average of triplicate determinations for high-performance liquid chromatography (HPLC) analyses. Intergroup comparisons were made by a Mann-Whitney *U* test. Correlations between anthropometric and metabolic data were assessed by the Spearman rank nonparametric correlation coefficient. Linear regression was used to evaluate relationships between continuous variables.

The Fisher exact test was used to assess differences between groups of categorical variables like sex.

RESULTS

The control and obese groups did not differ significantly by height, age, and sex (Table 1). During the insulin clamp session, the steady-state plasma glucose was maintained close to the initial values, with an average coefficient of variation less than 10%. Table 2 lists the values of clamp-derived metabolic variables for the 2 groups under study. In particular, fasting insulin was up to 2 times higher in obese versus control subjects.

Hepatic glucose output (HGO) was not estimated in this study, but insulin levels definitely higher than those usually considered necessary to inhibit liver glucose production were attained.[19-21] However, in case HGO was not completely blocked, the M value would be underestimated and the difference in the M value between normal and obese subjects should

Table 1. Anthropometric Characteristics of the Subjects (mean ± SD)

Characteristic	Control Subjects (n = 18)	Obese Patients (n = 12)
Age (yr)	33.7 ± 9.8	30.4 ± 9.0
Sex ratio (male/female)	8/10	7/5
Height (cm)	168 ± 10	171 ± 7
Weight (kg)	62.1 ± 12.6	101.2 ± 9.0
BMI (kg/m^2)	21.8 ± 3.3	34.6 ± 2.7
FFM (kg)	50.6 ± 12.0	66.8 ± 6.1
Fat mass (kg)	11.4 ± 5.8	34.4 ± 7.3

Table 2. Mean Values for Metabolic Variable (mean ± SD)

Variable	Control Subjects (n = 18)	Obese Patients (n = 12)
Fasting plasma insulin (pmol/L)	26.9 ± 13.3	62.0 ± 12.3*
TG (μmol/g wet weight)	6.2 ± 1.4	11.6 ± 2.2*
M value (μmol · kg FFM^{-1} · min^{-1})	38.5 ± 6.4	17.0 ± 4.1*
Glucose storage (μmol · kg FFM^{-1} · min^{-1})	20.5 ± 3.7	8.7 ± 2.6*
Glucose oxidation (μmol · kg FFM^{-1} · min^{-1})	17.9 ± 3.2	8.4 ± 2.4*
End-clamp plasma insulin (pmol/L)	509.14 ± 46.27	615.15 ± 51.7

*$P < .0001$.

be even larger, since it is ascertained that HGO is inhibited to a lesser extent in obese individuals. The M value was significantly greater in control compared with obese subjects, and it was negatively related ($R^2 = -.56$, $P < .0001$) with mTG content (Fig 1). The glucose oxidation rate as evaluated by indirect calorimetry was higher in controls versus obese subjects.

The difference between the rate of whole-body glucose uptake and the rate of glucose oxidation, which is an indirect measure of glucose storage, was also greater in control subjects.

The analysis of mTGs showed 2-fold higher levels in obese subjects compared with controls. The FFA pattern—both the individual absolute level and the percent value—was statistically different in the 2 groups (Table 3), with a lower unsaturated to saturated ratio in obese subjects. The only exception was the percent myristic acid, which did not reach statistical significance.

Stepwise linear regression analysis of mTG and palmitic and oleic fractions on the M value showed that only mTG and palmitic acid were significantly related to the M value ($R^2 = .66$, $P < .0001$). Stepwise linear regression analysis of total mTG on anthropometric variables (like BMI, age, FFM, and fat mass) showed that the BMI was significantly related to this parameter ($R^2 = .71$, $P < .0001$).

DISCUSSION

A great deal of attention has been focused on the role of FFAs and TGs as major determinants of the insulin resistance

Table 3. HPLC Fatty Acid Profile of mTGs (mean ± SD)

Fatty Acid	Control Subjects (n = 18) μmol/g Wet Weight	Control Subjects (n = 18) %	Obese Patients (n = 12) μmol/g Wet Weight	Obese Patients (n = 12) %
Myristic acid (C14:0)	0.03 ± 0.00	0.45 ± 0.14	0.06 ± 0.010†	0.47 ± 0.00
Palmitoleic acid (C16:1)	0.40 ± 0.09	6.46 ± 0.30	0.72 ± 0.13†	6.23 ± 4.76†
Oleic acid (C18:1)	3.00 ± 0.69	48.74 ± 1.27	5.45 ± 0.99†	46.94 ± 0.36†
Palmitic acid (C16:0)	1.54 ± 0.37	24.96 ± 1.10	3.23 ± 0.69†	27.67 ± 0.78†
Linoleic acid (C18:2)	0.85 ± 0.22	13.71 ± 1.12	1.42 ± 0.26†	12.25 ± 0.09†
Stearic acid (C18:0)	0.34 ± 0.09	5.61 ± 0.8	0.74 ± 0.10†	6.41 ± 0.49*
Unsaturated/ saturated ratio	2.19 ± 0.07		1.89 ± 0.04†	

*$P < .05$.
†$P < .0001$.

syndrome in humans.[22-52] The muscle TG content represents an important source of energy for cellular metabolic processes. Intracellular storage of TGs has been demonstrated in the skeletal muscle of a variety of subjects, including healthy volunteers, trained athletes, type 1 and type 2 diabetic patients, and obese Pima Indians.[9,10,13,22,36] However, while athletes and healthy controls show a good ability to mobilize these depots together with good insulin sensitivity,[13,36] type 1 diabetics demonstrate slower resting FFA turnover.[37]

Lipids seem not only to directly influence glucose metabolism but also to enhance glucose-stimulated insulin secretion in intact animals and humans.[38-45] However, the mechanism by which lipids act on insulin-mediated glucose disposal is still unknown. In fact, it is not clear whether the accumulation of intracellular TGs is secondary to the metabolic derangement due to insulin resistance or whether increased mTG stores might be responsible for a reduced insulin sensitivity. It is possible that, similar to the results of decreasing plasma TG levels, the reduction of mTG content may improve insulin sensitivity. The

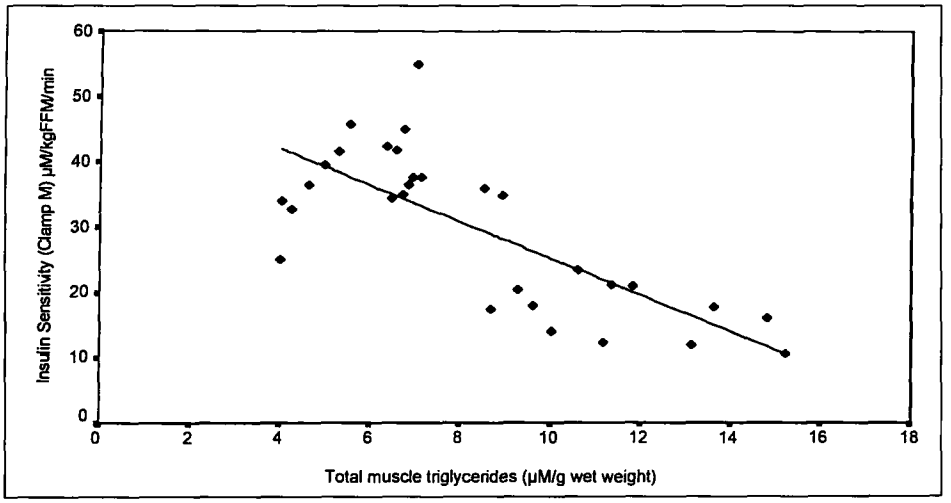

Fig 1. Linear regression ($R^2 = .56$; $P < .0001$) between whole-body glucose uptake (M value) and TG content in skeletal muscle specimens (mTG). M = −2.8 + 53.30 · mTG.

metabolic competition between lipid and glucose is well known.[2,28,35] Elevated fasting FFA levels or hypertriglyceridemia are known to be associated with an increased risk for both impaired glucose tolerance and type 2 diabetes mellitus.[31,32] Also, we have shown that insulin resistance is reversible in morbidly obese individuals independently of weight loss but correlating with a significant decrease of both TG and FFA plasma concentrations.[25] A surgically induced lipid malabsorption with subsequent drastic reduction of plasma TGs was also responsible for the disappearance of frank diabetes mellitus in patients with familial chylomicronemia.[26,27]

A recent review on the role of fatty acids in the pathogenesis of insulin resistance,[28] highlights two possible mechanisms: a fat-related inhibition of glucose transport or phosphorylation that appears after a few hours of fat infusion, and a decrease in muscle glycogen synthase activity that is apparent after a longer period of lipid infusion. However, the hypothesis advanced by Boden concerns only short-term experiments in both humans and experimental animals.

In the present study, total mTGs and their respective FFA patterns were investigated in specimens of rectus abdominis in both control and obese caucasian subjects. These parameters also have been correlated with insulin sensitivity as measured by the EHC. mTG content and whole-body glucose uptake were statistically different in the 2 groups and showed a highly significant negative correlation throughout the entire study sample (Fig 1). Although this observation suggests that mTG might play an important role in the determination of insulin resistance, there is not a clear cause-effect relationship between them, at least in our series.

High concentrations of mTG in a group of obese nondiabetic, Pima Indians with insulin resistance were observed by Pan et al.[9] No significant relationship was demonstrated between skeletal mTG content and insulin sensitivity indices in both healthy controls[13,22,32] and type 1 diabetics.[10] A possible explanation for these discordant findings could be either the different method used in the measurement of insulin sensitivity (a short insulin resistance test instead of EHC)[13] or the small quantity of muscle biopsy specimens. Nevertheless, in control subjects, increased mTG stores were definitely associated with decreased insulin-stimulated muscle glycogen synthase activity, suggesting that mTGs may represent one of the causes of the impairment of glycogen synthesis.[13] Concerning type 1 diabetic patients, the different origin of muscle and blood FFAs, as well as their different profile, might be responsible for the different results.[22]

In our series, a relevant finding is the different muscle FFA pattern found in control subjects compared with obese subjects. In fact, not only the total saturated fat but also the absolute amount of palmitic acid were significantly higher in obese versus control subjects. This result supports the data[46-52] showing that dietary fats containing a higher proportion of saturated fatty acids correlate with the prevalence of insulin resistance. In this study, it was also hypothesized that the dietary fatty acid profile affects the fatty acid profile of skeletal muscle lipids.

In our investigation, a positive correlation between the mTG and BMI was also found. It is likely that the wide range of BMI values, from 13.3 to 38.1 kg/m^2, a large muscle specimen, and a different origin of the muscle biopsy (rectus abdominis instead of vastus lateralis) may account for the different results obtained with respect to those reported by Pan et al.[9] In fact, these investigators did not find any relationship between different measures of adiposity and mTG muscle content in obese Pima Indians.[9]

In conclusion, these data lend more support to the hypothesis that both mTG concentrations and FFA patterns can affect insulin-mediated whole-body glucose uptake. A pivotal role may be played by a high saturated fatty acid content in the TGs. The pattern of intracellular lipids, in fact, seems to affect glucose uptake much more than the degree of body fat accumulation. The mechanism responsible for this relationship is still unclear and needs further investigation.

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