Intramyocellular Triglyceride Content Is a Determinant of in Vivo Insulin Resistance in Humans

A ^{1}H-^{13}C Nuclear Magnetic Resonance Spectroscopy Assessment in Offspring of Type 2 Diabetic Parents

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Insulin resistance is the best prediction factor for the clinical onset of type 2 diabetes. It was suggested that intramuscular triglyceride store may be a primary pathogenic factor for its development. To test this hypothesis, 14 young lean offspring of type 2 diabetic parents, a model of in vivo insulin resistance with increased risk to develop diabetes, and 14 healthy subjects matched for anthropomorphic parameters and life habits were studied with *1*) euglycemic-hyperinsulinemic clamp to assess whole body insulin sensitivity, *2*) localized ^{1}H nuclear magnetic resonance (NMR) spectroscopy of the soleus (higher content of fiber type I, insulin sensitive) and tibialis anterior (higher content of fiber type IIb, less insulin sensitive) muscles to assess intramyocellular triglyceride content, *3*) ^{13}C NMR of the calf subcutaneous adipose tissue to assess composition in saturated/unsaturated carbons of triglyceride fatty acid chains, and *4*) dual X-ray energy absorption to assess body composition. Offspring of diabetic parents, notwithstanding normal fat content and distribution, were characterized by insulin resistance and increased intramyocellular triglyceride content in the soleus ($P < 0.01$) but not in the tibialis anterior ($P = 0.19$), but showed a normal content of saturated/unsaturated carbons in the fatty acid chain of subcutaneous adipocytes. Stepwise regression analysis selected intramyocellular triglyceride soleus content and plasma free fatty acid levels as the main predictors of whole body insulin sensitivity. In conclusion, ^{1}H and ^{13}C NMR spectroscopy revealed intramyocellular abnormalities of lipid metabolism associated with whole body insulin resistance in subjects at high risk of developing diabetes, and might be useful tools for noninvasively monitoring these alterations in diabetes and prediabetic states. *Diabetes* 48:1600–1606, 1999

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DEXA, dual-energy X-ray absorption; NMR, nuclear magnetic resonance; TE, echo time; TR, repetition time.

Insulin resistance plays a primary role in the pathogenesis of type 2 diabetes (1,2), being the most relevant abnormality of glucose homeostasis 1–2 decades before the onset of frank hyperglycemia (3,4). In type 2 diabetic patients, the major site of impairment of insulin action resides in the skeletal muscle, where glucose metabolism is abnormal because of an impairment of insulin-stimulated glycogen synthesis (5) due to a defect in glucose transport/phosphorylation (6,7). The same features characterize young nonobese and nondiabetic first-degree relatives of type 2 diabetic patients (8,9), who have been shown to have a high risk of developing diabetes, suggesting that these alterations are fully expressed before the development of diabetes and, therefore, would be primary for its pathogenesis. A competition between plasma glucose and free fatty acids as fuel for energy production was suggested several years ago (10); it was also recently demonstrated that in healthy humans, an increased plasma free fatty acid availability was acutely able to induce insulin resistance by the same mechanisms responsible for insulin resistance in type 2 diabetic patients and in their nondiabetic first-degree relatives (11). It is very well known that type 2 diabetic patients (12) and their first-degree relatives (13) are characterized by increased plasma free fatty acid concentrations and plasma levels correlated with the severity of insulin resistance (13). We do not know whether a causal link between free fatty acid–induced insulin resistance and insulin resistance in type 2 diabetes exists, nevertheless, the link between increased availability of fatty acids and muscle insulin resistance has been well established. More recently, it was also hypothesized that the increment of plasma free fatty acids may be paralleled by an increased storage of intramyocellular triglycerides (14).

Comparison of signals from human skeletal muscle and fat tissue obtained in vivo by means of ^{1}H nuclear magnetic resonance (NMR) spectroscopy showed that muscle tissue contains two compartments of triglycerides: one of these compartments represents the lipids within the fat cells, and the other compartment, the lipids located as droplets in contact with mitochondria within the cytoplasm of muscle cells (15–18). In addition, ^{13}C NMR spectroscopy allows measurements of the major classes of unsaturated and saturated fatty acids constituting tissue triglycerides (19,20).

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The aim of this work was to test the hypothesis that intramuscular triglyceride content is related to whole body insulin sensitivity by studying young nonobese and nondiabetic offspring of type 2 diabetic parents, who are at high risk of developing diabetes because of the presence of insulin resistance. To pursue this goal, we used ^{1}H NMR spectroscopy of the skeletal muscle in combination with the euglycemic clamp technique for the assessment of whole body insulin sensitivity and with dual-energy X-ray absorption (DEXA) for the assessment of body composition. In addition, we also tested, using ^{13}C NMR spectroscopy, whether this model of insulin resistance was characterized by an abnormal pattern of saturation/unsaturation of the fatty acids constituting tissue triglycerides.

RESEARCH DESIGN AND METHODS

Subjects. First-degree relatives of type 2 diabetic patients were recruited at Istituto Scientifico H San Raffaele. The main criteria for their inclusion in the study were as follows: *1*) both parents with type 2 diabetes or one parent and a first- or second-degree relative with type 2 diabetes (diagnosis of diabetes was based on fasting glucose concentration >7.8 mmol/l and/or history of oral hypoglycemic agent assumption); *2*) age (24–45 years); *3*) white race; *4*) body weight within 12% of their ideal body weight according to the 1983 Metropolitan Life Insurance tables; *5*) sedentary life style; *6*) no history of hypertension, endocrine/metabolic disease, or cigarette smoking. Habitual physical activity was assessed using a questionnaire (21). Fourteen (7 male and 7 female) first-degree relatives of type 2 diabetic parents who were not participating in heavy physical activity were selected. Each single offspring of type 2 diabetic parents was matched with a single control subject for anthropomorphic characteristics, with the difference that the 14 healthy normal subjects had no family history of diabetes, obesity, or hypertension traced through their grandparents. The clinical and laboratory characteristics of the two groups of subjects are summarized in Tables 1 and 2. All subjects were in good health as assessed by medical history, physical examination, hematological testing, and urinalysis. Informed consent was obtained from all subjects after explanation of the purpose, nature, and potential risks of the study. The protocol was approved by the ethical committee of the Istituto Scientifico H San Raffaele.

Experimental protocol. Subjects were instructed to consume an isocaloric diet (~250 g carbohydrate/day) for 3 weeks before the studies and to abstain from exercise activity for at least 2 weeks before the studies. Women were studied between days 3 and 10 of the menstrual cycle. Subjects were studied by means of the euglycemic-hyperinsulinemic clamp to assess whole body insulin sensitivity after a 10-h overnight fast. Within 2–3 days, patients were studied by means of ^{1}H and ^{13}C NMR spectroscopy to assess muscle triglyceride content and degree of saturation/unsaturation of the fatty acids constituting tissue triglycerides, respectively. NMR sessions were performed in the Division of Diagnostic Radiology of the Istituto Scientifico H San Raffaele between 7:00 and 9:00 A.M. after a 10-h overnight fast. For 15 of the 28 patients, ^{1}H and ^{13}C NMR spectroscopy sessions were performed on two separate consecutive days because of technical problems. Within 2–3 days, the patients were also studied by DEXA to assess body composition. DEXA was performed in the Department of Science, Nutrition and Microbiology, Nutrition Section, Università degli Studi di Milano.

Euglycemic-hyperinsulinemic clamp. Subjects were admitted to the Metabolic Unit of the Division of Internal Medicine I of the Istituto Scientifico H San Raffaele at 7:00 a.m. after a 10-h overnight fast. A Teflon catheter was inserted into an antecubital vein for tracers, glucose, and insulin infusions, and an additional catheter was inserted retrogradely into a wrist vein for blood sampling. The hand was kept in a heated box (70°C) throughout the experiment to allow sampling of arterialized venous blood. Blood samples for fasting plasma glucose, HbA_{1c}, total cholesterol, HDL cholesterol, triglycerides, free fatty acids, insulin, proinsulin, glucagon, cortisol, and leptin were performed to measure them in the fasting condition. Thereafter, a bolus (5 mg/kg body wt) was administered, followed by a continuous infusion (0.05 mg · kg^{-1} body wt · min^{-1}) of [6,6-$^2H_2$]glucose obtained from massTrace (Woburn, MA). Blood for determination of plasma glucose and insulin concentrations and for plasma tracer enrichment was drawn every 15 min during the last 30 min of the 2.5-h equilibration period. After the basal equilibration period, a euglycemic-hyperinsulinemic clamp was performed as previously described (22). Insulin was infused at 1 mU · kg^{-1} · min^{-1} to reach a plasma insulin concentration of ~550 pmol/l, and plasma glucose concentration was kept at ~5 mmol/l for an additional 150 min by a variable infusion of 20% dextrose infusion. Blood samples for plasma insulin concentration and plasma [6,6-$^2H_2$]glucose enrichment were drawn every 15 min throughout the study.

^{1}H NMR spectroscopy. ^{1}H NMR spectroscopy was performed on a Signa 1.5 Tesla scanner (General Electric Medical Systems, Milwaukee, WI) using a conventional linear extremity coil. High resolution T_1 weighted images of the right calf were obtained before spectroscopic acquisitions to localize the voxel of interest for the ^{1}H spectroscopy study. The voxel shimming was executed to optimize the homogeneity of the magnetic field within the specific volume of interest. Two ^{1}H spectra were collected from a 15 × 15 × 15 mm^3 volume within the soleus and tibialis anterior muscles. A PRESS pulse sequence (repetition time [TR] = 2,000 ms and echo time [TE] = 60 ms) was used, and 128 averages were accumulated for each spectrum, with a final acquisition time of 4.5 min. The water signal was suppressed during the acquisition, since it would dominate the other metabolite's peak signals of interest. A third ^{1}H spectrum of a triglyceride solution inside a glass sphere, positioned within the extremity coil next to the calf, was also obtained during the same session to have an external standard acquired in the same conditions as the subject's spectra. Postprocessing of the data, executed with the

TABLE 1
Anthropomorphic characteristics of study groups

	Offspring of type 2 diabetic parents		Normal subjects		
	Men	Women	Men	Women	*P* value
n	7	7	7	7	—
Age (years)	31 ± 2	29 ± 2	30 ± 2	30 ± 2	0.78
Body weight (kg)	74 ± 4	58 ± 4	72 ± 3	58 ± 1	0.76
Height (cm)	177 ± 2	166 ± 2	173 ± 1	165 ± 2	0.42
BMI (kg/m^2)	23.4 ± 0.8	21.1 ± 0.7	24.0 ± 0.7	21.2 ± 0.7	0.69
Waist-to-hip ratio	0.91 ± 0.05	0.80 ± 0.02	0.87 ± 0.03	0.74 ± 0.01	0.16
Ideal body weight (%)	107 ± 4	102 ± 4	110 ± 3	104 ± 4	0.66
Total body fat (kg)	16 ± 2	16 ± 2	15 ± 1	16 ± 2	0.88
Body fat (%)	22.4 ± 2.3	28.1 ± 3.1	21.6 ± 1.4	29.7 ± 3.1	0.98
Fat content (%)					
Arms	18.5 ± 1.7	23.7 ± 4.0	17.5 ± 1.8	22.5 ± 3.0	0.56
Trunk	24.7 ± 1.7	23.7 ± 3.6	22.9 ± 1.3	26.3 ± 3.3	0.93
Legs	22.9 ± 1.0	32.8 ± 3.1	22.0 ± 1.9	34.2 ± 2.2	0.86
Physical activity index	8.0 ± 0.4	8.5 ± 0.6	7.7 ± 0.5	8.1 ± 0.2	0.41

Data are means ± SE. The range of possible scores for the physical activity index is 3–15. The lowest value corresponds to the level of physical activity of a clerical worker who plays a light sport (energy expended is <0.76 mJ/h; e.g., bowling) and who participates in sedentary activities during leisure time. The highest value corresponds to the level of physical activity of a person who is very physically active at work (e.g., a construction worker), who plays heavy sports (energy expended is at least 1.76 mJ/h; e.g., boxing, basketball, football, or rugby), and who is very physically active during leisure time (e.g., walking >1 h/day or biking >45 min/day). *P* values were obtained from analyses comparing the offspring of type 2 diabetic patients vs. normal subjects.

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TABLE 2
Laboratory characteristics of study groups

	Offspring of type 2 diabetic parents		Normal subjects		
	Men	Women	Men	Women	*P* value
n	7	7	7	7	—
Plasma glucose (mmol/l)	5.1 ± 0.2	5.0 ± 0.2	4.7 ± 0.2	4.6 ± 0.1	0.01
HbA_{1c} (%)	5.1 ± 0.1	5.0 ± 0.8	4.9 ± 0.2	4.9 ± 0.2	0.39
M value (mg/[kg · min])	4.73 ± 1.39	4.87 ± 0.85	7.01 ± 0.98	5.88 ± 0.69	0.04
Total cholesterol (mmol/l)	4.81 ± 0.26	4.58 ± 0.31	4.68 ± 0.36	5.25 ± 0.36	0.45
HDL cholesterol (mmol/l)	1.61 ± 0.23	2.05 ± 0.13	1.33 ± 0.16	2.00 ± 0.21	0.45
Plasma free fatty acids (mmol/l)	0.636 ± 0.061	0.675 ± 0.049	0.531 ± 0.057	0.563 ± 0.050	0.05
Plasma triglycerides (mmol/l)	1.90 ± 0.55	0.70 ± 0.08	0.94 ± 0.12	0.87 ± 0.16	0.20
Insulin (pmol/l)	67 ± 15	49 ± 10	47 ± 8	45 ± 9	0.08
Total proinsulin (pmol/l)	9.6 ± 2.4	6.0 ± 0.6	6.6 ± 1.5	5.5 ± 1.0	0.23
Intact proinsulin (pmol/l)	3.6 ± 1.0	2.6 ± 0.3	2.6 ± 0.6	2.5 ± 0.5	0.61
Total proinsulin/insulin %	14 ± 3	12 ± 3	14 ± 6	12 ± 2	0.41
Intact proinsulin/insulin %	5 ± 1	5 ± 2	5 ± 1	6 ± 1	0.49
C-peptide (nmol/l)	0.75 ± 0.19	0.57 ± 0.06	0.67 ± 0.05	0.56 ± 0.06	0.19
Leptin (ng/ml)	7.2 ± 2.1	7.8 ± 1.5	4.8 ± 0.7	8.6 ± 0.2	0.81
Glucagon (ng/l)	164 ± 21	67 ± 9	160 ± 15	97 ± 5	0.89
Cortisol (nmol/l)	3,090 ± 552	2,897 ± 552	4,139 ± 497	2,649 ± 303	0.22
Creatinine (μmol/l)	84.0 ± 8.0	68.1 ± 4.4	92.8 ± 3.5	74.3 ± 2.7	0.30

Data are means ± SE. Blood sampling for substrates and hormones was performed after a 10-h overnight fast. *P* values were obtained from analyses comparing the offspring of type 2 diabetic patients vs. normal subjects.

Sage/IDL software, consisted of high pass filtering, spectral apodization, zerofilling, Fourier transformation, and phasing of the spectra. The integral of the area under the peak was calculated using a Marquardt fitting with Lorentzian functions of the peaks of interest. The integral of the methylene signal ($-CH_2$) at 1.35 ppm was used to calculate intramyocellular triglyceride content expressed in arbitrary units (AU) as ratio to the integral of the peak of the external standard × 1,000.

^{13}C NMR spectroscopy. ^{13}C NMR spectroscopy was performed in the same system after the ^{1}H spectroscopy session or on the following morning. The dual-frequency flexible spectroscopy coil (Medical Advances, Milwaukee, WI), consisting of a ^{13}C square surface coil of 11.3 × 11.3 cm and two ^{1}H 14 × 15.5 cm Helmholtz-type coils, was positioned around the right calf. ^{1}H coils were used for the scout image, localized shimming, and the ^{1}H decoupling. Carbon spectra were acquired from a 20-mm slice using a 90° pulse with TR = 3 s. ^{1}H decoupling was performed using WALTZ-4, and an average power of 18 W was applied using a prototype decoupler (General Electric Medical Systems) during the acquisition. The spectra were then analyzed using a correction factor to overcome partial saturation effects. This factor was obtained from three volunteers by measuring the peak areas of spectra acquired at different repetition times (TR = 3 and 10 s) and calculating the change in the spectra. The calculated factor was then applied for the analysis of all spectra at TR = 3 s (19,20). A typical spectrum is shown in Fig. 1. The proportion of saturated or unsaturated fatty acid carbons was obtained as previously described without making any assumption about the composition of tissue under study (23).

Body composition. DEXA was performed with a Lunar-DPX-IQ scanner (Lunar, Madison, WI). A different scan mode was chosen with respect to each subject's body size, as suggested by the manufacturer's operator manual. For regional analysis, three-compartment processing was performed in the arms, trunk, and legs (24). Fat content is expressed as kilograms of fat mass and as percent of soft tissues.

Analytical procedures. Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA) (25). Plasma free fatty acids were measured by microfluorometric assay (26). Fasting serum triglycerides, total cholesterol, HDL cholesterol, and creatinine were measured as previously described (27). Plasma insulin, total proinsulin, and intact proinsulin were measured by a highly specific two-site monoclonal antibody-based immunosorbent assay (ELISA; Dako Diagnostics, Cambridgeshire, U.K.) (28). C-peptide was measured by a radioimmunoassay using a double antibody (25); glucagon and cortisol were measured as previously described (25). Plasma concentration of leptin was determined by radioimmunoassay with a human kit (Linco Research, St. Charles, MO) by using the manufacturer's assay protocol (29). The [6,6-$^2H_2$]glucose enrichment was measured by gas chromatography–mass spectrometry as previously described (30). Glucose kinetics were calculated using Steele's equations for the non–steady state (31). M value was calculated by adding the rate of residual endogenous glucose production to the glucose infusion rate.

Statistical analysis. All data are presented as means ± SE. Comparison between different groups was performed using analysis of variance (ANOVA) with Scheffè's post hoc testing when appropriate. Forward and backward stepwise regression analysis were performed using F ratio-to-remove of 4 and F ratio-to-enter of 3.996 to assess which variable was most useful in predicting the M value.

RESULTS

Clinical and laboratory characteristics. Healthy volunteers and offspring of type 2 diabetic parents were selected to be comparable for age, body weight, height, BMI, waist-to-hip ratio, and ideal body weight (Table 1). Total body fat, assessed by DEXA, was similar in the two groups, and the fat distribution in different body compartments (arms, trunk, and legs) was also comparable between normal healthy subjects and offspring of type 2 diabetic parents, demonstrating identical total fat content and a similar pattern of fat distribution. Nonetheless, women were characterized by an increased fat storage when compared with men (28.9 ± 2.1 vs. 22.0 ± 1.3%, $P < 0.01$), and this was particularly evident at the level of the legs (33.5 ± 1.8 vs. 22.5 ± 1.1%, $P < 0.01$, respectively, in women and men). Laboratory characteristics are summarized in Table 2. Fasting plasma glucose concentration, even if within the normal range, was increased in the offspring of type 2 diabetic parents ($P < 0.01$). Glycosylated hemoglobin was, nevertheless, comparable between the two groups. Fasting plasma free fatty acids were also slightly higher in the offspring of type 2 diabetic parents compared with healthy control subjects ($P = 0.05$). On the contrary, total cholesterol, HDL cholesterol, and triglycerides were comparable between the two groups studied. The hormone profile was also comparable: fasting insulin showed a trend for higher concentrations in the offspring of type 2 diabetic parents compared with normal subjects ($P = 0.08$); total proinsulin (intact proinsulin and fragments) and intact proinsulin concentrations were also comparable. Finally, fasting C-peptide, leptin, glucagon, and cortisol concentrations were comparable between the two study groups.

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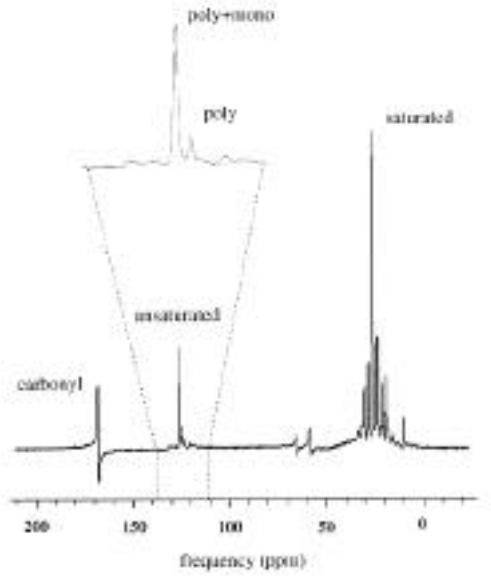

FIG. 1. Decoupled ^{13}C spectrum of calf tissues. Signals from saturated fatty acid carbons are in the region from 10 to 45 ppm; peaks from unsaturated fatty acid carbons are in the region from 110 to 140 ppm; and peak from carbonyl is in the region from 165 to 175 ppm. The window represents an enlargement of the unsaturated region at 110–140 ppm, which contains one pair of resonances: the smaller peak of carbons on the inside of consecutive double bonds (–C=C–C–C=C–, "inner" olefinic carbons) is characteristic of polyunsaturated fatty acids (poly), and the larger peak of carbons external to the double bonds (–C=C–C–C=C–) resonates around the same chemical shift as the olefinic carbons from monounsaturated fatty acids (mono) (–C=C–) and are called "outer" olefinic carbons.

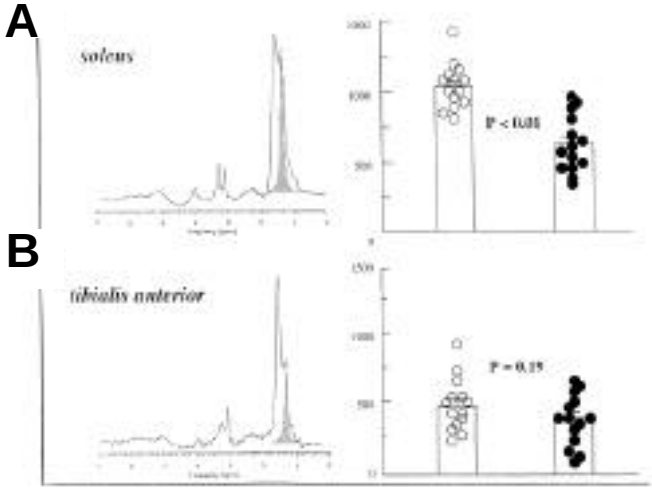

FIG. 2. ^{1}H spectra of the soleus and tibialis anterior muscles. *A, left panel*: a spectrum typical of the soleus muscle; *B, left panel*: a spectrum typical of the tibialis anterior muscle. The shaded peaks in the spectra represent the best fit of the CH_2– residue peaks of intramyocellular triglycerides (1.35 ppm). At 1.55 ppm, the CH_2– residue peak of extramyocellular triglycerides is represented. In the right panels, bar graphs representing the intramyocellular content in the soleus (*A*) and tibialis anterior (*B*) for each offspring of type 2 diabetic parents (○) and normal subjects (●) and means ± SE for the series of values are depicted.

Euglycemic-hyperinsulinemic clamp. During the insulin clamp, plasma glucose concentration was kept at baseline levels and was comparable between the two groups (4.99 ± 0.75 vs. 4.81 ± 0.74 mmol/l in the offspring of type 2 diabetic parents and normal subjects, respectively). Plasma insulin concentration was also comparable (542 ± 22 vs. 527 ± 14 pmol/l during the last 30 min of the clamp in the offspring of type 2 diabetic parents and normal subjects, respectively). Rate of glucose appearance (R_a) was comparable in both postabsorptive (2.32 ± 0.21 vs. 2.17 ± 0.15 mg · kg^{-1} · min^{-1} in the offspring of type 2 diabetic parents and normal subjects, respectively, $P = 0.759$) and clamp (0.18 ± 0.05 vs. 0.11 ± 0.09 mg · kg^{-1} · min^{-1} in the offspring of type 2 diabetic parents and normal subjects, respectively, $P = 0.745$) conditions. Insulin sensitivity, measured as the M value, was impaired in the offspring of type 2 diabetic parents (4.80 ± 0.91 vs. 6.45 ± 0.72 mg · kg^{-1} body wt · min^{-1}, $P = 0.04$) compared with that in the normal subjects, and the difference was more pronounced in men ($P = 0.01$) than in women ($P = 0.07$).

^{1}H NMR spectroscopy

Effect of muscle type. Intramyocellular triglyceride content in the soleus muscle (Fig. 2*A*, *left panel*, shaded peak) was higher than in the tibialis anterior muscle (Fig. 2*B*, *left panel*, shaded peak) in both normal subjects (636 ± 52 vs. 395 ± 51 AU, $P < 0.01$) and offspring of type 2 diabetic parents (1,047 ± 40 vs. 493 ± 52 AU, $P < 0.01$). This is summarized in the right panels of Fig. 2*A* (soleus) and *B* (tibialis anterior), taking into account that the scale on the *y*-axes was kept identical in the two panels. A linear relationship between the soleus and tibialis anterior muscle lipid content was found in normal subjects ($r = 0.52$, $P = 0.04$), but not in the offspring of type 2 diabetic parents ($r = –0.08$, $P = 0.79$).

Effect of sex. There was a trend for higher intramyocellular triglyceride content in women than in men in the tibialis anterior in both normal subjects (489 ± 43 vs. 302 ± 82 AU, $P = 0.07$) and offspring of type 2 diabetic parents (614 ± 70 vs. 371 ± 45 AU, $P = 0.02$). On the contrary, there was no sex effect with regard to the soleus muscle: the content was similar in both normal subjects (684 ± 84 vs. 589 ± 64 AU, respectively, in women and men, $P = 0.39$) and offspring of type 2 diabetic parents (999 ± 34 vs. 1094 ± 70 AU, respectively, in women and men, $P = 0.24$).

Effect of family history of type 2 diabetes. Intramyocellular triglyceride content in the soleus muscle (Fig. 2*A*, *right panel*) was higher in offspring of type 2 diabetic parents (1,047 ± 40 AU) than in normal subjects (636 ± 52 AU, $P < 0.01$). On the contrary, the tibialis anterior muscle (Fig. 2*B*, *left panel*) showed just a trend for higher content in the offspring of type 2 diabetic parents (493 ± 52 vs. 395 ± 51 AU, $P = 0.19$).

^{13}C NMR spectroscopy. Offspring of type 2 diabetic parents had similar content of saturated (89.74 ± 0.41 vs. 90.19 ± 0.44%, $P = 0.44$) and unsaturated (10.25 ± 0.41 vs. 9.81 ± 0.38%, $P = 0.44$) carbons when compared with normal subjects, and the content of polyunsaturated (2.03 ± 0.14 vs. 1.96 ± 0.11%, $P = 0.72$) and monounsaturated (8.23 ± 0.33 vs. 7.85 ± 0.32%, $P = 0.43$) carbons was also comparable between the two groups of study.

Multivariate analysis. To establish which variable was most useful in predicting insulin sensitivity, both forward and backward stepwise regression analyses were performed including the following independent variables: age, BMI, total body fat content, total body percent fat content, percent fat in the trunk, waist-to-hip ratio, physical activity index, fasting plasma free fatty acids, fasting plasma glucose, serum triglyceride, plasma leptin, triglyceride content in the soleus

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and tibialis anterior muscles, percentual composition in monounsaturated carbons, polyunsaturated carbons, and saturated carbons in fatty acids constituting the triglyceride pool of calf tissue. The analysis selected the soleus triglyceride content as the most useful variable in predicting insulin sensitivity (simple regression analysis: $R^2 = 0.29$, $P < 0.01$), and plasma free fatty acids concentration was also useful (simple regression analysis: $R^2 = 0.21$, $P = 0.03$).

DISCUSSION

In this work, ^{1}H and ^{13}C NMR spectroscopy methods were used in offspring of type 2 diabetic parents to look for a relationship between intramyocellular muscle content and/or adipose tissue composition in saturated/unsaturated fatty acids and whole body insulin resistance. This is the first application of the combination of the two techniques to study, noninvasively, intracellular lipid store in physiology and pathophysiology of diabetes. The major findings of this research are that healthy young lean offspring of type 2 diabetic parents, an in vivo model of increased risk to develop diabetes in the future, were characterized by *1*) increased muscle triglyceride content that correlated with the severity of whole body insulin resistance and *2*) no change in the content of unsaturated/saturated carbons of fatty acid chains in adipose tissue when compared with healthy normal subjects without a family history of diabetes.

The assessment of whole body insulin sensitivity measured by the clamp technique showed that offspring of type 2 diabetic parents were characterized by a reduced glucose disposal rate (Table 2). They had normal serum total cholesterol, HDL cholesterol, and triglycerides when compared with normal subjects. On the contrary, plasma free fatty acid levels were increased notwithstanding a trend for higher fasting insulin levels (Table 2). It was crucial to determine whether the storage of this substrate in the skeletal muscle was normal or somehow defective (14).

^{1}H NMR spectroscopy allows the distinction of two intramuscular triglyceride compartments in human skeletal muscle, and one of these represents the intramyocellular fraction (15). The study of the soleus muscle resulted in increased triglyceride content in both the normal subjects and the offspring of type 2 diabetic parents compared with the tibialis anterior. All human muscles are of mixed fiber type (32), but the soleus is prevalently composed of slow-twitch oxidative fibers (fiber type I), and the tibialis anterior has a higher content of fast-twitch glycolytic fibers (fiber type IIb) (33). This is in agreement with previous papers showing that intramuscular triglyceride concentrations are graded according to the following sequence (from highest to lowest concentration): slow-twitch oxidative, fast-twitch oxidative, and fast-twitch glycolytic (34,35). We also found a sex effect with regard to the tibialis anterior, in which the triglyceride content was higher in women than in men in both offspring of type 2 diabetic parents and normal subjects.

The most important finding was the higher triglyceride content in the soleus muscle, but not in the tibialis anterior, of the offspring of type 2 diabetic parents compared with that in the normal subjects. We think that this divergent behavior of the two muscles is due to the fact that different fiber types have different insulin sensitivity and responsiveness (36). The observation in animal models that slow-twitch oxidative (type I) and fast-twitch oxidative (type IIa) fibers have the greatest insulin sensitivity, whereas the fast-twitch glycolytic fibers (type IIb) have the least (37–39), supports this theory. A study in humans (Pima Indians and Caucasians) also showed a positive correlation between insulin action and the percentage of fiber type I and a negative correlation with the percentage of fiber type IIb in biopsies of the vastus lateralis (40). As a consequence, the metabolic abnormality had to be more evident in the more insulin-sensitive fibers (type I), and thus in the soleus muscle, which is supposed to contain more type I fibers than the tibialis anterior muscle. In fact, the result of the multivariate analysis showed that triglyceride content in the soleus muscle was the best predictor of whole body insulin sensitivity; on the contrary, the triglyceride content of the tibialis anterior was not significantly related. The relationship between the triglyceride content in the soleus muscle and whole body insulin sensitivity is in agreement with a previous study in which the same relationship was found in male Pima Indians using assessment of triglyceride content in the vastus lateralis muscle by means of a needle biopsy (41). With respect to that article, our study was performed in both male and female Caucasians, which group is not as diabetes-prone as Pima Indians; in addition, we specifically selected lean subjects (BMI 22.2 ± 0.6 in the offspring of type 2 diabetic parents and 22.6 ± 0.6 in normal subjects vs. 32.7 ± 1.1 kg/m^2 in subjects studied by Pan et al. [41]) to avoid any confounding effect of adiposity, which is also well known to affect insulin action. All study subjects performed a DEXA exam to properly assess whether total and regional body fat (in particular the trunk) were comparable between offspring of type 2 diabetic parents and normal subjects. The two groups of study were tightly controlled for these variables, allowing us to demonstrate the strong relationship between intramyocellular triglyceride levels and insulin sensitivity independent of total body fat content.

The mechanisms responsible for abnormal storage of triglycerides in the cytoplasm of skeletal muscle cell are unclear. This abnormality may simply reflect a different fiber type composition in the muscle under study: it was recently found in a Danish population of insulin-resistant first-degree relatives of patients with type 2 diabetes that the vastus lateralis muscle had an increased fraction of fiber type IIb compared with healthy subjects without a family history of diabetes (42), but the study did not determine whether this was due to reduced physical fitness. Physical exercise, which may also have a profound effect on intramyocellular lipid storage (33), was comparable, as assessed by a questionnaire (21), in offspring of type 2 diabetic parents and normal subjects because we specifically selected subjects with a sedentary lifestyle. Therefore, we do not think that a genetic or fitness-related change in muscle fiber type composition may explain our findings without a metabolic alteration standing as a background. The main reason for this being that we found that a muscle rich in fiber type IIb (tibialis anterior) had a lower triglyceride content than a muscle rich in fiber type I (soleus), and, as a consequence, if offspring of type 2 diabetic parents had higher type IIb fibers, they should have been characterized by a lower rather than a higher content of intramyocellular lipids, for the reasons previously discussed. Because intramuscular fat content was always assessed on bioptic specimens and different fiber types, and therefore different muscles, have different behavior, much of the data regarding the effects of the hormone milieu is controversial.

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In our study, the hormone profile was similar between the two study groups (Table 2), fasting plasma insulin concentration being the only exception because of a trend for higher concentration ($P = 0.08$) in the offspring of type 2 diabetic parents. Proinsulin fractions, C-peptide, glucagon, and cortisol were comparable. Because leptin directly regulates both adiposity and energy homeostasis (43,44) and its gene expression takes place not only in the adipose tissue, but also in the muscle (45), we also measured plasma leptin concentrations in our study groups to test whether the increased muscle triglyceride store in offspring of type 2 diabetic parents could be associated with abnormal leptin expression, but we did not find any significant difference (Table 2). If fat deposition within muscle is increased, fatty acids uptake has to be increased or fatty acid oxidation has to be reduced. A reduced Hounsfield attenuation on computed tomography scans (which would stand for increased fat depositions) was found in the muscle of nondiabetic obese women and was strongly related to reduced muscle oxidative capacity, suggesting that an impaired capacity of skeletal muscle for fat oxidation would drive fat to intramuscular accumulation (46). On the other hand, an increased muscle lipoprotein lipase activity might lead to increased lipid uptake in muscle; in this regard, insulin-resistant subjects (Pima Indians) showed increased muscle lipoprotein lipase activity during insulin administration with respect to the postabsorptive condition rather than a decrement, as observed in normal subjects (47). This point of discussion on the basis of our data is just a matter of speculation, since this work was not specifically designed to address these issues.

In muscle biopsies of Pima Indians (48) and Caucasians (49), an inverse relationship between insulin sensitivity and saturated fats of muscle membrane phospholipids was found. ^{13}C NMR spectroscopy gives the unique possibility of noninvasively assessing the fatty acid status (19,20,23,50,51) because the main ^{13}C NMR signals visible in in vivo spectra are from fatty acids in subcutaneous fat. Therefore, we also performed ^{13}C NMR spectroscopy of the calf in the study groups but did not find any abnormality of the percentage of saturated/unsaturated (mono- and poly-) carbons of fatty acid chain in the offspring of type 2 diabetic patients. In the multivariate analysis, they did not reveal any significant role in explaining whole body insulin sensitivity. The dichotomy with the results of the plasma membrane of insulin-resistant subjects (48,49) may be explained by two factors. First, we assessed the degree of saturation/unsaturation of the fatty acids of triglycerides mainly stored in adipocytes; therefore, the sampled pools (adipose vs. muscle tissue) and the compartment (cytoplasm vs. plasma membrane) were different. Second, polyunsaturated fatty acids are derived exclusively from the diet because essential fatty acids have a concentration that is proportional to the dietary intake (52). Therefore, the similar fatty acid composition in the study groups was probably due to the similar diet habits. The normal pattern of fatty acid composition found in the adipose tissue of the offspring of type 2 diabetic parents taken together with the alterations found in the plasma membrane phospholipids suggests that the metabolic alterations are differently expressed in body tissues and compartments (membranes and cytoplasm).

In conclusion, the results of this work demonstrate that subjects at high risk of developing type 2 diabetes have abnormal intramuscular triglyceride storage that seems to be selective for muscle (or fiber) type. The strong relationship between this abnormal muscle triglyceride storage and whole body insulin sensitivity suggests that it may play a major role in the pathogenesis of type 2 diabetes. Whether these differences might be fully due to a genetic background or to an environmental factor is not known, but ^{1}H and ^{13}C NMR spectroscopy, noninvasive successful techniques for the study of muscle and liver glucose metabolism, have been shown to be useful tools for the study of intracellular lipids and might eventually be useful for monitoring the effects of preventive interventions in diabetes and prediabetic states.

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