Skeletal Muscle Triglyceride Levels Are Inversely Related to Insulin Action

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In animal studies, increased amounts of triglyceride associated with skeletal muscle (mTG) correlate with reduced skeletal muscle and whole body insulin action. The aim of this study was to test this relationship in humans. Subjects were 38 nondiabetic male Pima Indians (mean age 28 ± 1 years). Insulin sensitivity at physiological (*M*) and supraphysiological (*MZ*) insulin levels was assessed by the euglycemic clamp. Lipid and carbohydrate oxidation were determined by indirect calorimetry before and during insulin administration. mTG was determined in vastus lateralis muscles obtained by percutaneous biopsy. Percentage of body fat (mean 29 ± 1%, range 14–44%) was measured by underwater weighing. In simple regressions, negative relationships were found between mTG (mean 5.4 ± 0.3 μmol/g, range 1.3–1.9 μmol/g) and $\log_{10}M$ ($r = -0.53$, $P \leq 0.001$), *MZ* ($r = -0.44$, $P = 0.006$), and nonoxidative glucose disposal ($r = -0.48$ and -0.47 at physiological and supraphysiological insulin levels, respectively, both $P = 0.005$) but not glucose or lipid oxidation. mTG was not related to any measure of adiposity. In multiple regressions, measures of insulin resistance ($\log_{10}M$, *MZ*, $\log_{10}$[fasting insulin]) were significantly related to mTG independent of all measures of obesity (percentage of body fat, BMI, waist-to-thigh ratio). In turn, all measures of obesity were related to the insulin resistance measures independent of mTG. The obesity measures and mTG accounted for similar proportions of the variance in insulin resistance in these relationships. The results suggest that in this human population, as in animal models, skeletal muscle insulin sensitivity is strongly influenced by local supplies of triglycerides, as well as by remote depots and circulating lipids. The mechanism(s) underlying the relationship between mTG and insulin action on skeletal muscle glycogen synthesis may be central to an understanding of insulin resistance. *Diabetes* 46:983–988, 1997

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LFPIns, log fasting plasma insulin; *M* and *MZ*, low-dose (physiological) and high-dose (supraphysiological) in vivo insulin-mediated glucose disposal rates, respectively; mTG, triglyceride associated with skeletal muscle; *S* and *SZ*, nonoxidative components of total glucose disposal at physiological and supraphysiological insulin concentrations, respectively; W/T, waist-to-thigh ratio.

NIDDM, obesity, hypertension, dyslipidemias, and cardiovascular disease are all prevalent diseases that have been linked with impaired insulin action (insulin resistance) (1). Skeletal muscle has been identified as the primary site of insulin-stimulated glucose disposal at euglycemia (2,3) and, as such, is a major locus of insulin resistance. However, the mechanisms responsible for muscle insulin resistance remain unclear.

Increased circulation lipid supply has been shown to impair glucose metabolism through effects on both glucose oxidation and storage (4–9). The corollary that decreasing lipid supply will increase glucose metabolism has also received strong experimental support (10,11). These studies have generally addressed circulating lipids; little information is available about the role of stored muscle lipids.

Animal studies have shown that triglyceride associated with skeletal muscle (mTG) is an important source of lipid for energy within muscle (12,13). In addition, there is evidence to suggest that an intramuscular lipid pool may exist within human skeletal muscle as an immediate source of energy (14). In studies in the high-fat–fed rat, increases in storage triglyceride within single hindlimb muscles have been clearly associated with impairment in insulin-stimulated glucose metabolism in those same muscles (15). No such studies are available in humans. The aim of the present study was to examine the relationship between mTG and measures of insulin action in humans.

RESEARCH DESIGN AND METHODS

The sample cohort was 38 male Pima Indian volunteers of the Gila River Indian Community who were participating in a longitudinal study of the development of NIDDM (16). Subjects had a mean age of 28 ± 1 years, height 171.1 ± 0.8 cm, and weight 95.8 ± 3.1 kg and were in good health as assessed by medical history and physical examination. All subjects gave informed consent, and the studies were approved by the ethics committees of the National Institutes of Health, the Indian Health Service, and the Gila River Indian Community.

All subjects were admitted to the Phoenix Clinical Research Unit of the National Institutes of Health and stabilized for 2 days or more on a weight-maintenance diet (50% carbohydrate, 30% fat, and 20% protein). Glucose tolerance was assessed after administration of a 75-g oral glucose load using World Health Organization criteria (17). Glucose tolerance was normal in all subjects. Body composition (percent body fat) was estimated by hydrodensitometry with simultaneous determination of lung residual volume (18,19).

Euglycemic-hyperinsulinemic clamp. In vivo insulin-mediated glucose disposal rate was measured by a two-step euglycemic-hyperinsulinemic clamp (4). The clamp was performed by primed continuous low- and high-dose insulin infusions (290 and 2,900 pmol · min^{-1} · m^{-2}, respectively), each of which was continued for 100 min while the plasma glucose was maintained at ~5.5 mmol/l. In vivo insulin action was determined during the period from 60 to 100 min. During this time, plasma glucose uptake approached steady state, but it is unlikely that a precise steady state was achieved. Nevertheless, in these studies, because compar-

TABLE 1
Mean values and ranges of anthropometric and metabolic variable and simple correlations with skeletal muscle associated triglyceride concentration (mTG)

	n	Value	*r*	*P**
BMI (kg/m^2)	38	32.7 ± 1.1 (19.0–52)	0.175	0.3
Percentage body fat	38	29 ± 1 (18–44)	0.232	0.2
Waist/thigh	38	1.6 ± 0.1 (1.3–2.2)	0.026	0.9
Fasting plasma glucose (mmol/l)	38	5.0 ± 0.1 (4.1–6.0)	0.100	0.6
Fasting plasma insulin (pmol/l)	38	220 ± 17 (85–677)	0.435	0.006
Total *M* (mg · min^{-1} ·kg FFM^{-1} + 17.7)	38	3.2 ± 0.2 (1.6–7.2)	–0.531	0.001
Total *MZ* (mg · min^{-1} ·kg FFM^{-1} + 17.7)	38	8.8 ± 0.3 (3.9–13.4)	–0.438	0.006
Basal oxidation (mg · min^{-1} ·kg FFM^{-1})	32			
Carbohydrate		1.64 ± 0.09 (0.70–3.10)	0.035	0.8
Lipid		0.58 ± 0.04 (0.14–0.93)	0.225	0.2
M oxidation (mg · min^{-1} ·kg FFM^{-1})	32			
Carbohydrate		2.27 ± 0.09 (1.31–3.50)	0.202	0.3
Lipid		0.32 ± 0.04 (-0.36–0.73)	0.265	0.1
MZ oxidation (mg · min^{-1} ·kg FFM^{-1})	32			
Carbohydrate		3.40 ± 0.10 (2.34–4.69)	0.192	0.3
Lipid		–0.02 ± 0.04 (-0.47–0.38)	0.256	0.2
S (glucose storage component of *M*)	32	0.93 ± 0.22 (-0.68–4.47)	–0.475	0.005
SZ (glucose storage component of *MZ*)	32	5.36 ± 0.31 (0.57–9.57)	–0.474	0.005

Data are *n* or means ± SE (range), unless otherwise indicated. FFM, fat-free mass. *r* values were determined by simple regression against mTG. Regression against mTG was performed with the $\log_{10}$ of fasting plasma insulin and total *M* values. *Significance level associated with *r*.

isons are made between individuals and not the calculation of rates of glucose disposal or hepatic glucose production, this is not critical. Plasma glucose was measured by the glucose oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA) and plasma insulin by radioimmunoassay using a radioassay analyzer (Concept 4, ICN, Horsham, PA).

Indirect calorimetry. At 40 min before the initial insulin infusion and for the last 40 min during each of the insulin infusions, oxygen consumption and carbon dioxide production were determined by open-circuit indirect calorimetry (4). A transparent plastic hood was placed over the subject's head and secured around the neck with a soft collar. The subjects were supine and were asked to remain motionless and awake during the test. The flow rate was measured by using a pneumotachograph attached to a Fleisch flow transducer (Gould, Cleveland, OH). A constant fraction of the expired gases was withdrawn and analyzed for oxygen and carbon dioxide concentrations. Oxygen was measured by a zirconium cell analyzer and carbon dioxide by an infrared analyzer (Applied Electrochemistry, Sunnyvale, CA). The analyzers and flowmeter outputs were connected to a desktop computer (Hewlett Packard, Palo Alto, CA), which recorded continuous integrated calorimetric measurements over 5-min intervals. The basal and insulin-stimulated carbohydrate oxidation and lipid oxidation rates were calculated from indirect calorimetry data (20). Six subjects did not have indirect calorimetry determined.

Muscle biopsy. At 2 days after the euglycemic-hyperinsulinemic clamp, a percutaneous biopsy of the vastus lateralis muscle was obtained. After a 12-h overnight fast, the skin and suprafascial tissue of the thigh were anesthetized with 8–10 ml of 1% lidocaine HCl (Astra Pharmaceutical Products, Westborough, MA), and the biopsy was performed with a Bergström needle (Depuy, Phoenix, AZ). The specimen was immediately frozen and stored in liquid nitrogen for later analysis.

Skeletal muscle triglyceride analysis. Extraction and isolation of the lipid components of skeletal muscle have been described previously (21). In brief, muscle tissue was homogenized in 2:1 (vol/vol) chloroform:methanol and total lipids extracted. Nonpolar lipids were isolated by solid-phase extraction, and triglyceride content then was determined spectrophotometrically at 500 nm by using an enzymatic colorimetric test kit, Triglycerides GPO-PAP (Boehringer Mannheim, Sydney, Australia) and a Cary 3 spectrophotometer (Varian, Mulgrave, Australia). Samples were quantitated against a standard curve of Precimat glycerol (Boehringer Mannheim).

Calculations. Total glucose disposal (at physiological [*M*] and supraphysiological [*MZ*] levels) was expressed as milligrams per minute per kilogram fat-free mass plus 17.7 based on the regression relationship observed in a large sample from the same population as the study group (20). Measures of glucose storage (at physiological [*S*] and supraphysiological [*SZ*] insulin concentrations) were calculated from the difference between the raw glucose disposal measures and carbohydrate oxidation measures and then expressed per kilogram of fat-free mass.

Statistics. All statistical analyses were performed using the Statview SE+ graphics package (Abacus Concepts, Berkeley, CA). Relationships between variables were analyzed by simple correlation and by multiple and stepwise linear regressions. The significance of simple correlation coefficients was assessed using Fisher's *Z* transformation. The significance of independent relationships in the multiple and stepwise regressions were assessed by a *t* test on the regression coefficients. Variables showing evidence of skewed distributions in larger published data sets in Pima Indians were log-transformed before analysis (*M* and fasting insulin). Stepwise regressions were performed in the forward direction with *F* for entry set to 4. Data are presented as means ± SE.

RESULTS

Members of the study group were young but overweight (Table 1), with mean body fat of 29 ± 1% (range 18 to 44%). Other subject characteristics, including measures of insulin action at *M* and *MZ* and basal and clamp carbohydrate/lipid oxidation values, are also listed in Table 1.

The mean value obtained for mTG in the skeletal muscles of the study group was 5.4 ± 0.3 μmol/g wet wt, with a range of 1.3–9.8 μmol/g wet wt. A direct relationship was observed between mTG and the log fasting plasma insulin (LFPIns) values ($r = 0.44$, $P < 0.006$). A negative relationship was found between the insulin action at the low dose of the euglycemic-hyperinsulinemic clamp and the triglyceride content ($r = -0.53$, $P < 0.0006$; Fig. 1). A similar relationship was found between skeletal mTG and insulin action at the supraphysiological insulin level ($r = -0.44$, $P < 0.006$).

Measures of adiposity as determined by percent body fat, BMI, and waist-to-thigh ratio (W/T; as a measure of central adiposity) were significantly negatively related to insulin action (percent body fat and *M*: $r = -0.50$, $P < 0.001$; BMI and *M*: $r = -0.49$, $P < 0.002$; W/T and *M*: $r = -0.49$, $P < 0.002$). However, in contrast to the relationships observed

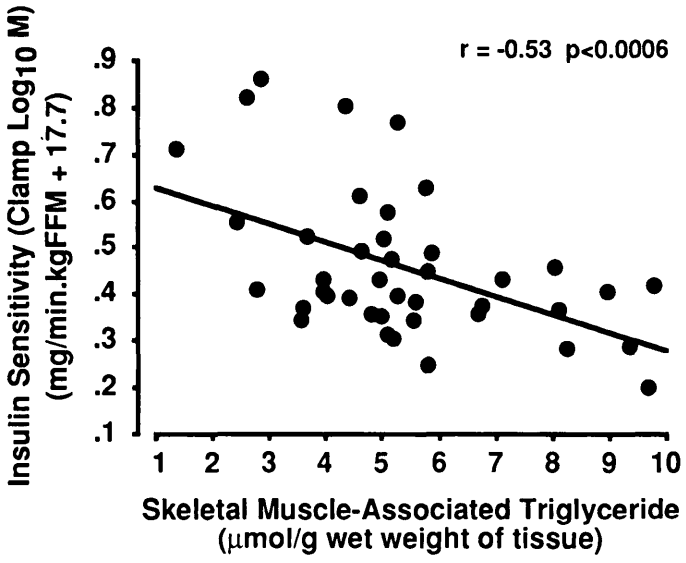

FIG. 1. Relationship between the logarithm of insulin sensitivity and skeletal muscle-associated triglyceride concentration. The index of insulin sensitivity at physiological insulin level (*M*; 290 pmol · min^{-1} · m^{-2}) was derived from the euglycemic-hyperinsulinemic clamp technique and is expressed derived from resting metabolic measurements with hydrodensitometry. FFM, fat-free mass.

TABLE 2
Multiple regression results for the equation $y = a_0 + a_1 x_1 + a_2 x_2$

	mTG = *y*	
Independent variables	*t*	*P*
% fat	0.037	<0.97
LFPIns	2.421	<0.02
BMI	0.254	<0.80
LFPIns	2.636	<0.01
W/T	1.318	<0.20
LFPIns	3.210	<0.003
% fat	0.242	<0.81
Log*M*	3.340	<0.002
BMI	0.671	<0.51
Log*M*	3.582	<0.001
W/T	1.546	<0.13
Log*M*	4.123	<0.0002
% fat	0.669	<0.51
MZ	2.544	<0.02
BMI	0.305	<0.76
MZ	2.660	<0.01
W/T	0.844	<0.40
MZ	3.023	<0.005

t and *P*, significance of coefficients for x_1 and x_2 (i.e., independent effects as predictors); % fat, percent body fat.

between mTG and the three measures of insulin action, no significant relationships were found between mTG and any measure of adiposity (Table 1).

Tables 2 and 3 show all possible permutations of the independent relationships between mTG, insulin action, and adiposity. mTG content was related to LFPIns, log *M*, and *MZ* independent of percent body fat, BMI, and W/T. In turn, all three measures of adiposity were related to all three measures of insulin action independent of mTG. However, adiposity and muscle triglyceride were not significantly related to each other in any manner.

In a stepwise regression analysis of the various predictors of $\log_{10}M$, only mTG ($t = 4.12$, $P = 0.0002$) and W/T ($t = 3.19$, $P = 0.003$) were identified as independent correlates, with percent body fat and BMI not entering the regression. Together, mTG and W/T accounted for 44% of the variance in $\log_{10}M$ (29% of *MZ*).

Both *M* ($r = -0.51$, $P < 0.002$) and *MZ* ($r = -0.53$, $P < 0.001$) were significantly negatively correlated with the respective clamp lipid oxidation rates. mTG was not related to any measure of lipid or carbohydrate oxidation either basally or during the low- or high-dose insulin infusions.

mTG was significantly ($P < 0.005$) negatively related to *S* ($r = -0.48$) and *SZ* ($r = -0.47$), as shown in Table 1. Also, significant relationships were found between percent body fat, BMI, and W/T with *S* ($r = -0.37$, -0.39, and -0.36, respectively; all $P < 0.05$) but not with *SZ* (all $P > 0.05$). However, the relationship between mTG and glucose storage was independent of all measures of adiposity.

DISCUSSION

An oversupply of lipid has been shown to lead to insulin resistance, whereas a reduction in lipid or reduced lipid oxidation and availability increases insulin action (11,23–25). Much of the experimental work has focused on circulating lipid supply. Infusions of triglyceride previously have been shown to reduce insulin-stimulated nonoxidative glucose

TABLE 3
Multiple regression results for the equation $y = a_0 + a_1 x_1 + a_2 x_2$

	LFPIns = *y*		Log*M* = *y*		*MZ* = *y*	
Independent variables	*t*	*P*	*t*	*P*	*t*	*P*
% fat	3.246	<0.003	2.985	<0.005	1.462	<0.15
mTG	2.421	<0.02	3.340	<0.002	2.544	<0.016
BMI	3.022	<0.005	3.167	<0.003	1.533	<0.13
mTG	2.636	<0.01	3.582	<0.001	2.660	<0.01
W/T	3.394	<0.002	3.194	<0.003	2.269	<0.03
mTG	3.210	<0.003	4.123	<0.0002	3.023	<0.005

t and *P*, significance of coefficients for x_1 and x_2 (i.e., independent effects as predictors); % fat, percent body fat.

disposal (9,26), whereas increased supply of free fatty acids is associated with reduced muscle glycogen accumulation (7,27). However, the present findings suggest that lipids other than those immediately available from the circulation may have major effects on insulin action. Even more surprising is that these postulated lipid effects are not only independent of adiposity but equally as powerful as the well-known effects of obesity on insulin action. Skeletal muscle contains energetically significant quantities of triglyceride (28), and studies in animals have shown that its content is negatively related to insulin action (15). The present study has provided strong support for a significant role of mTG in humans as a source of lipid to impair insulin-mediated glucose metabolism in muscle, in particular the nonoxidative component.

Earlier work in animals (15) showed modulation of mTG by high-fat diets (59% of total calories as fat) with varying fatty acid profiles. Insulin action was directly assessed within individual tissues and triglyceride content determined within that same tissue. This current study assessed insulin action, via three different measures, at the *whole* body level, whereas triglyceride content was determined in a single muscle. Despite these methodological differences, a significant negative relationship between insulin action and skeletal muscle triglyceride content was again observed. This highlights the importance of skeletal muscle triglyceride content as a major variable in insulin-mediated glucose disposal in the body. These results alone cannot demonstrate either the existence or direction of causality. Equally, the Pima Indians are a particularly diabetes-prone group, and the present results require confirmation in other more insulin-sensitive populations. However, in conjunction with the animal studies where dietary (15) or pharmaceutical (25) manipulation of muscle triglyceride levels consistently alters insulin action in the predicted direction, they suggest that mTG may be a contributing cause of insulin resistance.

Until now, circulating lipid has been considered the major lipid influence on glucose metabolism. However, clearly skeletal mTG represents another important factor. This point is emphasized by the lack of relationship between measures of whole body adiposity and mTG. In keeping with this, Groop et al. (30) have demonstrated that increasing insulin levels in obese individuals resulted in a similar decline in plasma free fatty acid oxidation to that in lean individuals. However, inhibition of total lipid oxidation (circulating and endogenous) was less sensitive to insulin in the obese, apparently due to a relative failure to suppress endogenous muscle lipolysis. Furthermore, insulin resistance has been linked with an indirect index of elevated lipid in muscle (reduced Hounsfield attenuation on computed tomography scans [31]). This latter study showed a strong negative correlation between glucose storage and this measure of increased muscle fat, findings consistent with the present results. These workers also found—again in line with the findings of this study—that measures of muscle fat and visceral obesity were independent predictors of insulin resistance. Moreover, stepwise regression revealed that an increased measure of muscle fat had the strongest predictive value for insulin resistance and together with visceral fat content accounted for 57% of the variance in glucose storage in the leg muscle that they analyzed. The parallels between these two sets of findings emphasize the dual but independent importance of mTG and visceral adiposity, possibly contributing through the influence on circulating lipids, in insulin-mediated glucose uptake.

The amount of triglyceride in muscle found here is energetically significant. If fully oxidized for energy, it would provide ~50% of the energy potentially available from the glycogen levels reported in a similar group of Pima Indians (32). The only other study of insulin-resistant subjects found that triglyceride associated with rectus abdominis muscle biopsies obtained during surgery ranged from ~40 to 600 μmol/g wet wt (5). These extraordinarily high values were most likely due to contamination from adipose depots. In keeping with the findings in this paper, other studies that have measured mTG yielded values of <50 μmol/g tissue (33–36). The skeletal muscle biopsies obtained in this study were from deep within the vastus lateralis and free of any visible signs of contamination from subcutaneous or other adipose depot stores. Thus, while the triglyceride was not localized to within the muscle fibers, early evidence suggests that it is (28); that is consistent with the excellent relation with insulin action independent of total body adiposity, which should relate more closely to intermuscle fat. However, it must be emphasized that we have not localized the lipid, and this is a matter of importance for further work.

With regard to this latter point, trained athletes and animals show the same or higher levels of muscle triglycerides as sedentary controls (33–35,37) but have improved insulin action (38,39). This is unexpected in light of the relationship exposed in the current investigation, and the question is why do higher levels of triglyceride in trained individuals not lead to insulin resistance? The answer may lie in the distribution of that triglyceride. Endurance exercise increases both the mitochondrial volume and distribution in skeletal muscle (40). In addition, recent electron microscopy studies have revealed that in trained dogs, mitochondria appear virtually in direct contact with triglyceride droplets (37), in contrast with the lack of association with mitochondria in untrained animals. That may account both for the improved ability of trained individuals to mobilize fat for energy during moderate-intensity exercise and for the lack of a detrimental effect of such storage lipid on glucose metabolism. In contrast, in sedentary individuals the lipid may be located within neutral lipid domains of the plasma membrane itself (41,42), which may then affect second-messenger systems (43). Localization of lipid in one or more membranes may affect translocation of glucose transporters (44) or insulin receptor tyrosine kinase activity (45,46). Electron microscopy localization studies in sedentary and trained individuals would be instructive in this regard.

Recently, we have shown in Pima Indians that the fatty acid profile of the major muscle membrane lipid (phospholipid) was closely linked to insulin action such that a higher proportion of saturated fats was associated with insulin resistance (47). In this subset of that study group, mTG was negatively related to the phospholipid unsaturation index (r = –0.323, $P < 0.05$; data not shown), such that the greater the proportion of unsaturated phospholipid fatty acids, the lower the stored skeletal muscle triglyceride. The mechanisms involved in this relationship are not proven, but it is consistent with the notion that saturated fats reduce the energy requirements of the cell due to reduced ion permeability (48,49), thereby tipping the balance away from lipid utilization for energy and toward storage.

In summary, this study finds a significant negative relationship between muscle triglyceride content and nonoxidative glucose disposal in humans, concordant with and extending previous findings in rats. This relationship is independent of measures of total and central adiposity. Although the mechanisms responsible for this relationship are unclear, they may be central to an understanding of the link between lipid metabolism and insulin resistance.

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