Association of Increased Intramyocellular Lipid Content With Insulin Resistance in Lean Nondiabetic Offspring of Type 2 Diabetic Subjects

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Insulin resistance plays an important role in the pathogenesis of type 2 diabetes; however, the multiple mechanisms causing insulin resistance are not yet fully understood. The aim of this study was to explore the possible contribution of intramyocellular lipid content in the pathogenesis of skeletal muscle insulin resistance. We compared insulin-resistant and insulin-sensitive subjects. To meet stringent matching criteria for other known confounders of insulin resistance, these individuals were selected from an extensively metabolically characterized group of 280 first-degree relatives of type 2 diabetic subjects. Some 13 lean insulin-resistant and 13 lean insulin-sensitive subjects were matched for sex, age, BMI, percent body fat, physical fitness, and waist-to-hip ratio. Insulin sensitivity was determined by the hyperinsulinemic-euglycemic clamp method (for insulin-resistant subjects, glucose metabolic clearance rate [MCR] was 5.77 ± 0.28 ml · kg^{-1} · min^{-1} [mean ± SE]; for insulin-sensitive subjects, MCR was 10.15 ± 0.7 ml · kg^{-1} · min^{-1}; $P < 0.002$). Proton magnetic resonance spectroscopy (MRS) was used to measure intramyocellular lipid content (IMCL) in both groups. MRS studies demonstrated that in soleus muscle, IMCL was increased by 84% (11.8 ± 1.6 vs. 6.4 ± 0.59 arbitrary units; $P = 0.008$), and in tibialis anterior muscle, IMCL was increased by 57% (3.26 ± 0.36 vs. 2.08 ± 0.3 arbitrary units; $P = 0.017$) in the insulin-resistant offspring, whereas the extramyocellular lipid content and total muscle lipid content were not statistically different between the two groups. These data demonstrate that in these well-matched groups of lean subjects, IMCL is increased in insulin-resistant offspring of type 2 diabetic subjects when compared with an insulin-sensitive group matched for age, BMI, body fat distribution, percent body fat, and degree of physical fitness. These results indicate that increased IMCL represents an early abnormality in the pathogenesis of insulin resistance and suggest that increased IMCL may contribute to the defective glucose uptake in skeletal muscle in insulin-resistant subjects. *Diabetes* 48:1113–1119, 1999

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EMCL, extramyocellular lipid content; FFA, free fatty acid; IMCL, intramyocellular lipid content; MCR, metabolic clearance rate; MRS, magnetic resonance spectroscopy; WHR, waist-to-hip ratio.

Type 2 diabetes is characterized by impaired β-cell function and a decrease in insulin-stimulated glucose uptake, predominantly of skeletal muscle (1). Insulin resistance is thought to play an important role in the development of type 2 diabetes (1,2). This alteration can already be demonstrated in a large proportion of asymptomatic offspring of patients with type 2 diabetes, a group of normoglycemic subjects known to be at high risk to develop type 2 diabetes (1,3–5).

The exact molecular mechanism causing the defective insulin-stimulated glucose uptake in skeletal muscle of type 2 diabetic subjects remains to be elucidated (1,2). It has been demonstrated that the number of GLUT4 glucose transporters is not significantly different in skeletal muscle membranes derived from insulin-resistant diabetic patients when compared with an insulin-sensitive control group. Whether the translocation mechanism or the docking and fusion process of GLUT4 with the plasma membrane is defective in skeletal muscle of type 2 diabetic patients is currently not known. Furthermore, increased fatty acid concentrations reduce insulin-stimulated glucose uptake by various mechanisms such as substrate competition (Randle mechanism) (6); however, the significance of increased intracellular fatty acids is still under discussion (1,7,8).

To minimize the influence of other confounding variables, such as hyperglycemia or vascular alterations (7–12), that may contribute to the pathogenesis of insulin resistance in later stages of the development toward clinically overt type 2 diabetes, it seems appropriate to focus on the group of asymptomatic and normoglycemic but insulin-resistant subjects (3,4) to study the role of intramyocellular lipid content on the pathogenesis of skeletal muscle insulin resistance.

Besides genetic traits, insulin resistance is affected by acquired factors such as obesity (13), body fat distribution (5,14–16), smoking (17), physical activity level (18,19), and dietary habits (20,21). In this regard, not only the amount of calories but also the composition of the diet seems to be important. Epidemiologic studies indicate that a high intake of saturated fat is associated with insulin resistance (22,23) and a higher risk to develop type 2 diabetes (21). Moreover, in animal studies, insulin resistance can be induced by high-fat feeding (24–26). This manipulation also results in an augmented content of lipids in the muscle, and the latter was found to be closely correlated to the degree of insulin resis-

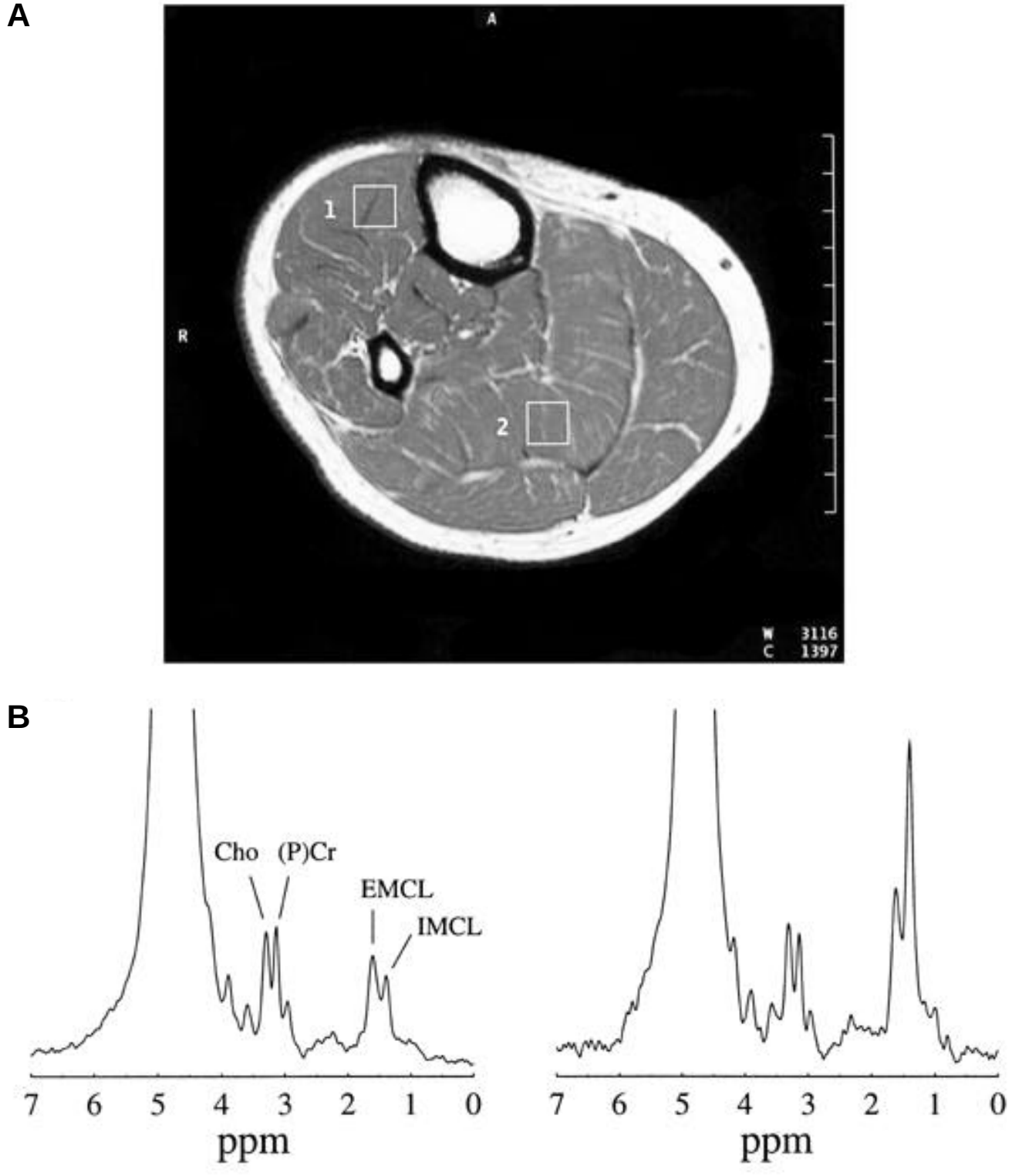

FIG. 1. ***A*****: Magnetic resonance image of the calf, indicating the two volumes of interest, the tibialis anterior muscle (*1*) and the soleus muscle (*2*); the voxel was selected to be free of visible fat.** ***B*****: Proton spectra of the tibialis muscle representing the IMCL and EMCL of a subject with normal (*left panel*) and reduced (*right panel*) insulin sensitivity.**

tance (24,25,27). These observations suggest that an oversupply of dietary fat resulting in an increased lipid accumulation in the muscle could play an important role in the pathogenesis of type 2 diabetes (28,29). And indeed, recent clinical studies indicate that fat content is also increased in muscle biopsies of patients with type 2 diabetes when compared with healthy control subjects (30). It is conceivable that if the muscular fat accumulates directly within the cytosol, that is, in the intramyocellular compartment, this lipid and its metabolism could contribute to the development of insulin resistance and hence to the pathogenesis of type 2 diabetes.

Because of methodologic limitations, a muscle biopsy cannot distinguish between intramyocellular and extramyocellular lipid content. Recently, however, a magnetic resonance spectroscopy (MRS) method was developed that clearly distinguishes between extra- and intramyocellular lipids (31–33).

If an increased lipid content plays a role in the pathogenesis of insulin resistance and type 2 diabetes, an augmented muscular fat accumulation, specifically within the cytosol, should be found in normoglycemic but insulin-resistant offspring.

In the present study, we wanted to test this hypothesis by applying MRS to assess the muscular lipid content in calf muscles and quantify specifically the intramyocellular lipid accumulation in two different fiber types in a metabolically well-characterized group of lean insulin-resistant offspring of type 2 diabetic subjects.

In a pilot study, we found a marked increase in intramyocellular lipid content in insulin-resistant subjects (S.J., F.S., K.R., J.M., A.V., W.R., C.-D.C., H.-U.H., unpublished observations); however, in that unselected group of offspring, the insulin-resistant subjects were significantly more obese and were characterized by a markedly higher body fat content. To focus on the relationship between intramyocellular lipids and insulin sensitivity, it is essential to minimize confounding variables such as obesity and body fat distribution. Therefore, we selected 13 lean insulin-resistant subjects from a large pool of more than 280 healthy offspring of type 2 diabetic subjects who have been extensively metabolically characterized by oral glucose tolerance test, hyperinsulinemic glucose clamp, determination of body composition by body impedance analysis, measurement of physical fitness (Vo_{2max}), and endothelial function (using high resolution ultrasound), as well as by extensive laboratory parameters, including free fatty acids (FFAs) and diverse hormonal parameters, and screening for genetic polymorphisms (34–36). These lean insulin-resistant subjects were compared with a group of 13 insulin-sensitive offspring carefully matched for sex, age, BMI, percent body fat, physical fitness, and waist-to-hip ratio (WHR). The aim of the present study was to evaluate *1*) whether the intramyocellular muscle lipid content is related to insulin sensitivity of glucose uptake, *2*) whether these lipid stores are increased in insulin-resistant offspring, and *3*) whether this association is independent of other factors known to alter insulin sensitivity, such as sex, BMI, body composition, body fat distribution, and physical fitness. The results indicate that intramyocellular lipid content is increased in insulin-resistant offspring of patients with type 2 diabetes, and this increase may contribute to the pathogenesis of insulin resistance.

RESEARCH DESIGN AND METHODS

Subject characteristics. In the Tübingen Family Screening Study for prediabetic patients (TÜFF), >280 healthy offspring of type 2 diabetic subjects have been extensively metabolically characterized (34–36).

According to glucose clamp–derived insulin sensitivity, the whole TÜFF study population was arbitrarily subdivided into two groups, one classified as insulin resistant (glucose metabolic clearance rate [MCR] <7.0 ml · kg^{-1} · min^{-1}) and the other as insulin sensitive (MCR >7.0 ml · kg^{-1} · min^{-1}). To minimize confounding variables, we selected 13 lean, normoglycemic but insulin-resistant offspring from the whole study group. These study participants were matched to a group of insulin-sensitive offspring as described above.

Metabolic analyses. All subjects were instructed to maintain a standard diet and to refrain from heavy exercise for at least 3 days before each metabolic test. All volunteers had an oral glucose tolerance test (75 g dextrose; Boehringer Mannheim) with simultaneous assessment of glucose and insulin. A euglycemic-hyperinsulinemic glucose clamp, with minor modifications of the protocol of DeFronzo et al. (37), was performed on an additional day to quantify insulin sensitivity of glucose uptake as described previously (38). Glucose MCR was used as an index of insulin sensitivity.

Anthropometry. Each subject's weight, height, and waist and hip circumferences were measured, BMI and WHR were calculated, and body composition was assessed using bioimpedance analysis. Physical fitness was quantified by a computerized standardized ramp test and is given as milliliters per kilogram. Laboratory analyses were done using commercially available assays as reported previously (38).

Magnetic resonance spectroscopy. The intramyocellular lipid content (IMCL) in the tibialis anterior and the soleus muscle was quantified by MRS on a 1.5-tesla whole-body system (Magnetom Vision; Siemens, Erlangen, Germany). A stimulated echo acquisition mode (STEAM) single voxel technique was applied, with a repetition time of 2 s and an echo time of 10 ms. The water signal was suppressed using a frequency-selective prepulse. The volumes of interest with a size of 2.5 ml were positioned in areas with low content (tibialis anterior) or representative content (soleus) of intermuscular fat septa visible on standard T1-weighted imaging (Fig. 1). IMCL was quantified by the integral of methylene signals in a range between 1.3 and 1.5 ppm. Extramyocellular lipids (EMCL), with their methylene signal centered at 1.6 ppm (integration range from 1.5 to 1.8 ppm), were assessed in the soleus muscle, since the examined voxel covered a representative area with several fatty septa. In contrast, the distribution of intermuscular fat in the tibialis anterior is rather inhomogeneous. Thus, representative data of EMCL in the latter muscle could not be achieved using a single voxel. The creatine signal at 3.1 ppm (integration range from 3.0 to 3.2 ppm) served as internal reference for IMCL and EMCL quantification (31,32). Since the relaxation times of signals from lipids and creatine are intra- and interindividually constant (31) and the applied echo time was short, results were not corrected for relaxation effects.

Ethics. The study was approved by the ethics committee of the University of Tübingen; each subject was informed about the purpose and the scope of the study and gave written consent.

Statistics. Values are reported as means ± SE. Simple linear regression analyses were performed to evaluate the associations between IMCL and anthropometric and metabolic data. The two study groups were compared with Student's *t* test.

RESULTS

The characteristics of the study population are shown in Table 1. There were no differences concerning sex, age, BMI, WHR, physical fitness, or percent body fat.

All participants had a normal oral glucose tolerance test according to the American Diabetes Association criteria. In the insulin-resistant subjects, insulin sensitivity was only 57% of the sensitive group (Table 2); also, fasting insulin and FFAs

TABLE 1
Characteristic of the study population

	Insulin-sensitive (MCR >7.0 ml · kg^{-1} · min^{-1})	Insulin-resistant (MCR ≤7.0 ml · kg^{-1} · min^{-1})	*P* value
Sex (M/F)	7/6	7/6	NS
Age (years)	31.8 ± 2.4	29.4 ± 1.5	NS
BMI (kg/m^2)	22.9 ± 0.6	23.4 ± 0.35	NS
Vo_{2max} (ml/kg)	35.3 ± 2.7	33.5 ± 2.4	NS
Percent body fat	19.1 ± 2.0	22.1 ± 1.5	NS
WHR	0.83 ± 0.02	0.8 ± 0.02	NS

Data are means ± SE.

TABLE 2
Glucose and lipid measures of the study population

	Insulin-sensitive (MCR >7.0 ml · kg^{-1} · min^{-1})	Insulin-resistant (MCR ≤7.0 ml · kg^{-1} · min^{-1})	*P* value
MCR (ml · kg^{-1} · min^{-1})	10.15 ± 0.7	5.77 ± 0.22	0.0001
Fasting glucose (mmol/l)	4.82 ± 0.15	4.75 ± 0.12	NS
Fasting insulin (μU/ml)	5.45 ± 0.79	7.83 ± 0.75	0.04
Triglycerides (mmol/l)	0.8 ± 0.14	1.25 ± 0.28	NS
HDL cholesterol (mmol/l)	1.54 ± 0.09	1.69 ± 0.12	NS
FFAs (mmol/l)	0.384 ± 0.03	0.581 ± 0.06	0.009

Data are means ± SE.

were significantly higher, whereas fasting glucose, triglycerides, and HDL cholesterol were comparable to the insulin-sensitive group.

As shown in Table 3, IMCL varied over a wide range in the two muscles examined. IMCL was more than three times higher in soleus than in tibialis anterior ($P < 0.01$).

The intramyocellular muscle lipid content showed an inverse relation to insulin sensitivity (MCR), i.e., the higher the muscular fat content, the lower the MCR (Fig. 2). Simple regression analysis revealed a negative relation between insulin sensitivity and the IMCL of both soleus ($r = –0.35$; $P = 0.1$) and tibialis anterior ($r = –0.53$; $P < 0.01$).

IMCL was markedly higher in the insulin-resistant subjects than in the insulin-sensitive group (Fig. 3). In tibialis anterior, IMCL was about 57% higher ($P < 0.017$), and IMCL in soleus showed an even more pronounced difference (84%; $P < 0.008$) between offspring with normal and low insulin sensitivity (Table 3).

In contrast, EMLC and total muscle lipid content in both muscles analyzed revealed no differences between groups (Table 3).

DISCUSSION

To gain further insight into the mechanisms involved in defective insulin-stimulated glucose uptake in skeletal muscle of insulin-resistant subjects, we used MRS to determine IMCL in insulin-resistant offspring of type 2 diabetic patients in comparison with matched insulin-sensitive offspring of type 2 diabetic patients. The results demonstrate that insulin resistance is correlated with IMCL. In both soleus and tibialis anterior muscles, the increased IMCL was found to be associated with a decreased insulin sensitivity of glucose uptake.

Factors such as obesity, body fat distribution, and level of physical activity have a profound effect on insulin sensitivity, and hence possibly also on IMCL (5,14–16,19,39). Because the groups were matched for those particular parameters, the difference in IMCL cannot be attributed to them.

The hyperinsulinemic glucose clamp technique is considered to be the gold standard to quantify insulin-stimulated glucose disposal. During this procedure, more than 70% of the glucose will be taken up by the skeletal muscle. Thus, a glucose clamp–derived index of insulin sensitivity will predominantly mirror the insulin sensitivity of skeletal muscle. Because the results of the present study show a close association between the amounts of IMCL and the glucose clamp–derived measures of insulin sensitivity, the data strongly support a role of cytosolic lipid accumulation in affecting skeletal muscle insulin-stimulated glucose uptake.

Previous investigations have described an increased muscular lipid content in patients with type 2 diabetes (30) or in nondiabetic subjects with insulin resistance (40–42). The association with insulin sensitivity was independent of measures of obesity such as BMI, percent body fat, or WHR (40,41). Fat accumulation was assessed by either muscle biopsies (30,40,41) or computerized tomography (CT) (42) of different muscles, such as the rectus abdominis, gastrocnemius (41), or vastus lateralis (40,42). Due to methodologic limitations, neither procedure can distinguish the lipids according to their anatomic location, namely, the lipid depot between the muscle fibers (extramyocellular lipid) or within the myocyte itself (intramyocellular lipid). It seems reasonable that due to their specific location within the cytosol, intramyocellular lipid stores could influence insulin's action to a higher degree than the extramyocellular lipid depots. Therefore, we have used MRS to distinguish between intra- and extramyocellular lipid content (31,32). This technique has been shown to be capable of differentiating between intra- and

TABLE 3
Intramyocellular, extramyocellular, and total myocellular lipid content of soleus and tibialis anterior muscles

	Insulin-sensitive (MCR >7.0 ml · kg^{-1} · min^{-1})	Insulin-resistant (MCR ≤7.0 ml · kg^{-1} · min^{-1})	*P* value
IMCL			
Soleus	6.4 ± 0.59	11.8 ± 1.6	0.008
Tibialis	2.08 ± 0.28	3.26 ± 0.36	0.017
EMCL			
Soleus	10.66 ± 1.6	8.87 ± 1.02	NS
Tibialis anterior	5.22 ± 1.1	8.5 ± 2.9	NS
TML			
Soleus	20.7 ± 2.4	23.7 ± 2.15	NS
Tibialis anterior	8.8 ± 1.4	13.7 ± 3.2	NS

Data are means ± SE.

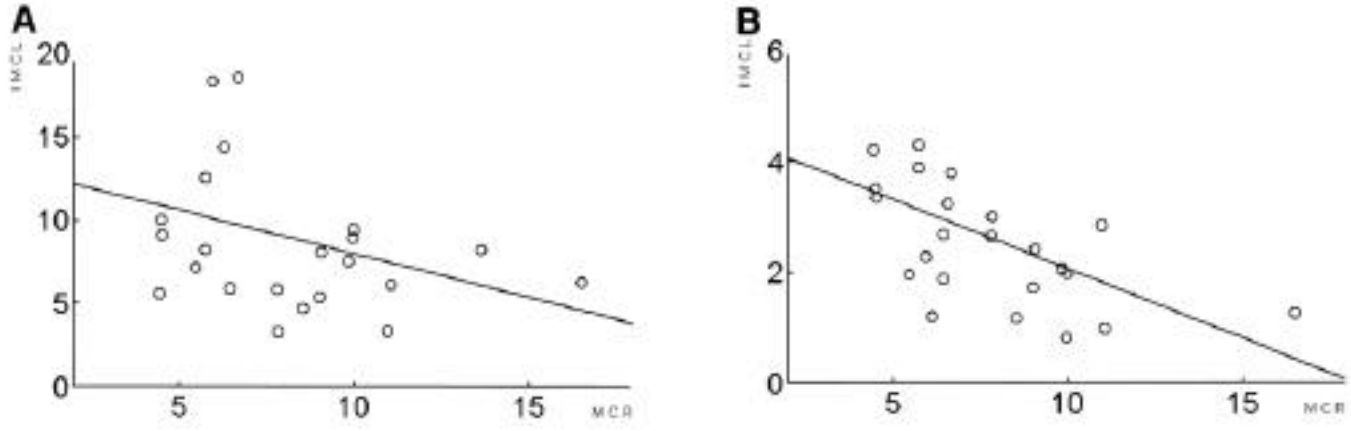

FIG. 2. Correlation between the intramyocellular lipid content and insulin sensitivity in the soleus (*A*) and the tibialis anterior (*B*).

extramyocellular lipid content, as recently demonstrated in patients with congenital lipodystrophy who do not have any extramyocellular lipid content (33).

The novel finding of the current MRS study is that this lipid accumulation is located specifically within the myocyte and the cytosolic fat content of the soleus and tibialis anterior muscles is increased in insulin-resistant asymptomatic offspring of type 2 diabetic subjects when compared with the well-matched insulin-sensitive group. Recently, two other groups using a similar MRS approach have also reported an inverse relationship between insulin sensitivity and IMCL. Perseghin et al. (43) reported in abstract form a close association between insulin sensitivity and the intramuscular lipids in soleus muscle in a similar group of first-degree relatives of type 2 diabetic patients, while Stein et al. (44) describe, also in abstract form, an association between insulin sensitivity and IMCL of tibialis anterior muscle as assessed in a general population without a specific genetic predisposition.

Animal studies indicate an important effect of muscular fat on insulin sensitivity, since high fat feeding was shown to increase lipid content in the muscle and induce insulin resistance (24,25). A close association between tissue triglyceride levels and insulin sensitivity was demonstrated in different animal models (27,45). In subsequent studies, it was shown that a reduction of muscular lipids resulted in an improvement of insulin sensitivity (46–50).

Epidemiologic data are in support of this notion, since a diet rich in saturated fat was found to be a risk factor for the development of type 2 diabetes (21). Furthermore, normoglycemic subjects with a high fat intake are characterized by insulin resistance (22,23). Additionally, it was demonstrated that an increased fat intake resulted in an elevation of intramuscular lipids (51).

The mechanism by which increased IMCL causes insulin resistance remains speculative. However, recent clinical studies using the microdialysis technique indicate that skeletal muscle lipid stores are under hormonal control similar to that of the adipose tissue (52). Thus, it is conceivable that the hydrolysis of triglycerides located within the muscle cell will readily supply acyl-CoAs into the cytosol. These intramyocellular FFAs could compete with glucose at the level of the mitochondria, resulting in a decrease of glucose uptake through various mechanisms (7,8,53–57). Furthermore, it is tempting to speculate that these cytosolic fatty acids could also directly interfere with elements of the insulin signal transduction cascade (46,58,59).

In insulin-resistant subjects, insulin-mediated suppression of FFAs is reduced (8,55,60). Therefore, it seems intriguing to speculate that a dysregulation of lipolysis together with an increased IMCL, as found in the present study, could result in an increased availability of cytosolic FFAs and thus contribute significantly to the development of insulin resistance. However, currently it is unknown whether an increase in IMCL induces insulin resistance (as a primary event) or whether it is the result of diminished insulin sensitivity (secondary event). To answer this question, a long-term prospec-

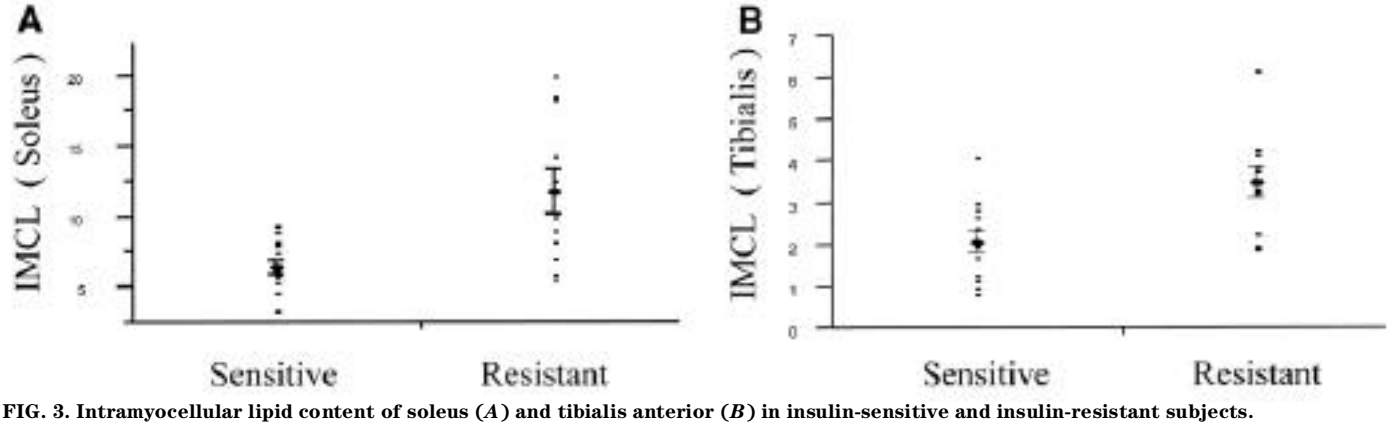

FIG. 3. Intramyocellular lipid content of soleus (*A*) and tibialis anterior (*B*) in insulin-sensitive and insulin-resistant subjects.

tive study with young subjects is needed.

In summary, the present study in offspring of type 2 diabetic patients indicates that insulin-resistant subjects have markedly increased IMCL when compared with insulin-sensitive control subjects. The findings of this study suggest that intramuscular lipid stores could play an unique role in the pathogenesis of skeletal muscle insulin resistance. The evaluation of the underlying molecular mechanism might shed new light on the pathogenesis of insulin resistance and type 2 diabetes.

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