Effects of Intravenous and Dietary Lipid Challenge on Intramyocellular Lipid Content and the Relation With Insulin Sensitivity in Humans

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An increased intramyocellular lipid (IMCL) content, as quantified by [1]H-magnetic resonance spectroscopy (^{1}H-MRS), is associated with reduced insulin sensitivity. At present, it is unclear which factors determine IMCL formation and how rapidly IMCL accumulation can be induced. We therefore studied the impact of hyperinsulinemia and elevated circulating nonesterified fatty acid (NEFA) levels on IMCL formation and insulin sensitivity. We further evaluated the influence of a high-fat diet on IMCL storage. In the infusion protocol, 12 healthy male subjects underwent a 6-h hyperinsulinemic-euglycemic glucose clamp with concomitant infusion of Intralipid plus heparin. IMCL was quantified by ^{1}H-MRS in soleus (SOL) and tibialis anterior (TA) muscle at baseline and then every hour. IMCL levels started to increase significantly after 2 h, reaching a maximum of 120.8 ± 3.4% (SOL) and 164.2 ± 13.8% (TA) of baseline after 6 h (both $P < 0.05$). In parallel, the glucose infusion rate (GIR) decreased progressively, reaching a minimum of 60.4 ± 5.4% of baseline after 6 h. Over time, the GIR was strongly correlated with IMCL in TA ($r = -0.98$, $P \leq 0.003$) and SOL muscle ($r = -0.97$, $P \leq 0.005$). In the diet protocol, 12 male subjects ingested both a high-fat and low-fat diet for 3 days each. Before and after completion of each diet, IMCL levels and insulin sensitivity were assessed. After the high-fat diet, IMCL levels increased significantly in TA muscle (to 148.0 ± 16.9% of baseline; $P = 0.005$), but not in SOL muscle (to 114.4 ± 8.2% of baseline; NS). Insulin sensitivity decreased to 83.3 ± 5.6% of baseline ($P = 0.033$). There were no significant changes in insulin sensitivity or IMCL levels after the low-fat diet. The effects of the high-fat diet showed greater interindividual variation than those of the infusion protocol. The data from the lipid infusion protocol suggest a functional relationship between IMCL levels and insulin sensitivity. Similar effects could be induced by a high-fat diet, thereby underlining the physiological relevance of these observations. *Diabetes* 50:2579–2584, 2001

Type 2 diabetes is characterized by impaired β-cell function and insulin resistance of skeletal muscle and other tissues (1). Skeletal muscle insulin resistance is also a common feature in obesity, dyslipidemia, and arterial hypertension (1). There is evidence that altered lipid metabolism plays an important role in the pathogenesis of insulin resistance (2–4). In animal studies, high-fat feeding can induce insulin resistance (5). High-fat feeding has also been associated with augmented lipid content in skeletal muscle, and intramyocellular triglyceride levels have been shown to be closely related to insulin sensitivity (5). Moreover, studies in muscle biopsies and computed tomography (CT) scans suggest that an increased lipid content in skeletal muscle is associated with insulin resistance in nondiabetic humans (6).

To understand potential mechanisms linking intramuscular lipids to skeletal muscle insulin resistance, it is important to consider that there are two distinct pools of lipid storage in skeletal muscle: lipids located between muscle fibers (adipocytes)—that is, extramyocellular lipids—and lipids located within the muscle cells (cytosolic triglycerides)—that is, intramyocellular lipids (IMCL). Because of methodological limitations, neither biochemical evaluation of muscle biopsy material nor evaluation by CT can distinguish between these compartments. [1]H-magnetic resonance spectroscopy (^{1}H-MRS) is a well-established method for noninvasive quantification of IMCL content in vivo (7–9). Using ^{1}H-MRS, several investigators recently reported a close negative correlation between IMCL levels of skeletal muscle and insulin sensitivity of glucose uptake (10,11).

Currently, little is known about determinants and the time course of IMCL regulation. A relatively rapid regulation is suggested by clinical studies showing that moderate prolonged exercise decreases IMCL within hours (12). However, which factors control the accumulation of IMCL and how rapidly this occurs is largely unknown. It is also unknown whether an increase in IMCL is directly linked to changes in insulin sensitivity.

In animal studies, hyperinsulinemia has been shown to rapidly increase total skeletal muscle lipids in the presence of elevated nonesterified fatty acids (NEFA) (13). In a recent study, Brechtel et al. (14) showed that magnetic

TABLE 1
Subject characteristics

	Infusion protocol	Diet protocol
n	12	12
Age (years)	28.2 ± 1.0	29.9 ± 1.5
BMI (kg/m^2)	22.1 ± 0.6	23.2 ± 0.6
Waist-to-hip ratio	0.85 ± 0.02	0.85 ± 0.01

Data are means ± SE.

resonance spectroscopy is a sensitive tool for detecting rapid changes in IMCL in humans. In the present study, therefore, we used this technique to investigate the time course of IMCL upregulation during hyperinsulinemia and elevation of circulating NEFA. Simultaneously, we used the hyperinsulinemic-euglycemic glucose clamp to measure changes in insulin sensitivity. We found that an increase in IMCL is accompanied by a decrease in insulin sensitivity. Because the lipid infusion protocol represents an experimental situation with supraphysiologically high plasma NEFA levels, it is unclear whether an extrapolation of the results to the physiological situation is possible. Therefore, in a second protocol we studied the effects of a short-term, high-fat diet on insulin sensitivity and IMCL levels. Similar to the results after lipid infusion, IMCL levels were increased and insulin sensitivity was decreased after the high-fat diet. The results of the diet protocol suggested that, even with high interindividual variation, nutrient fat rapidly modifies IMCL levels and insulin sensitivity.

RESEARCH DESIGN AND METHODS

Subjects. In both protocols, investigations were performed in 12 young, healthy, male, normal-weight, nondiabetic volunteers; 4 subjects underwent both protocols. A precharacterization of the volunteers was obtained in the context of the Tübingen Family Study presently under way (10), including >500 metabolically well-characterized first-degree relatives of type 2 diabetic patients (TÜF study, Tübingen). For subject characteristics, see Table 1. All subjects underwent an oral glucose tolerance test to assess glucose tolerance. Those with a history of hypertension or thyroidal disease were excluded. None of the subjects was taking any medication. Only subjects from the intermediate insulin sensitivity range were selected to eliminate the influence of extreme insulin resistance on the outcome of the experiments (10). Subjects gave informed written consent before participating. Both protocols were approved by the Ethics Committee of the University of Tübingen.
Lipid infusion protocol. In the lipid infusion protocol, IMCLs were quantified at baseline and every 60 min thereafter. The subjects remained in the magnetic resonance tomograph throughout the 6-h study period. In the first step, all 12 volunteers were treated with the combination of hyperinsulinemia and increased circulating NEFA. NEFA levels were raised via infusion of a lipid emulsion plus heparin, as described previously (2,3). After a bolus of 250 IU, heparin was infused at a constant rate of 0.4 IU · kg^{-1} · min^{-1} (Heparin-Sodium Braun Multi 10.000 IU/ml; Braun, Meslungen, Germany) and a 20% lipid emulsion (Intralipid 20; Pharmacia, Erlangen, Germany) with a constant rate of 1.5 ml/min for 6 h.

In the second step, 6 of the 12 subjects underwent an experiment with hyperinsulinemia alone. In this group, a solution of 0.9% saline (Delta-Pharma, Pfullingen, Germany) was infused as a control for the lipid emulsion plus heparin. The details of the method based on these 6 individuals were reported in Brechtel et al. (14). An additional 3 of the 12 subjects received an infusion of lipid emulsion plus heparin without concomitant insulin infusion.
Hyperinsulinemic-euglycemic glucose clamp. After an overnight fast, two Teflon catheters (Vasofix Braunüle; Braun) were inserted for blood drawing and infusions, respectively. In the experiments with hyperinsulinemia, a 6-h glucose-clamp with infusion of 1.0 mU · kg^{-1} · min^{-1} insulin was performed, as previously described (10). The glucose infusion rate (GIR; in mg · kg^{-1} · min^{-1}) necessary to maintain euglycemia was used as a measure of insulin sensitivity. The average GIR of the 60 min preceding the indicated time point was used.

Diet protocol and diet composition. Each subject received a high-fat low-carbohydrate diet and a low-fat high-carbohydrate diet for 3 consecutive days each. Before and after the completion of the diets, IMCL levels were quantified by ^{1}H-MRS and insulin sensitivity was determined by a 2-h hyperinsulinemic-euglycemic glucose clamp. Fasting blood samples were collected for measurement of blood glucose, serum insulin, and lipid concentrations.

The diets were designed by a study dietitian to represent extremes of Western nutritional habits, defined as follows: 1) high-fat diet: high in calories, high in fat (55–60% of energy intake, predominantly saturated fat), and low in carbohydrates (30–35%) and protein (11–16%), with total energy intake of 2,667–2,943 kcal/day; and 2) low-fat diet: normal range of calories, low in saturated fat (18–23%), and high in carbohydrates (62–64% of energy intake) with 16–18% protein and fiber, with total energy intake of 1,901–2,125 kcal/day. On average, the total energy intake was 767 kcal/day lower than with the high-fat diet. The diets consisted of commonly available food items (e.g., pizza, pasta). No alcohol was permitted during the study period. For the 2 days before the metabolic investigations, the subjects were on a prescribed, standardized well-balanced diet. Body weight remained constant with both diets.
In vivo ^{1}H-NMR spectroscopy. Image-guided ^{1}H-MRS was applied in the soleus (SOL) and tibialis anterior (TA) muscles on a 1.5 Tesla whole-body system (Magnetom Vision; Siemens, Erlangen, Germany), as previously described (8,10). A stimulated echo acquisition mode, single voxel technique (repetition time 2 s, echo time 10 ms) with suppression of the predominant water signal was used to determine IMCL levels. Voxel (2.4 ccm) were positioned in areas with low (TA) or representative content (SOL) of intramuscular fat septa visible on standard T1-weighted images. IMCLs were quantified as the area under the curve of the methylene signals of lipids at 1.3–1.5 ppm. The creatine signal at 3.1 ppm served as an internal reference.
Analytical procedures. During the clamp, plasma glucose was measured every 10 min by the glucose-oxidase method (YSI, Yellow Springs, OH). Blood was drawn every 20 min for determination of serum insulin (MEIA Abbott, Wiesbaden, Germany) and NEFA levels (WAKO Chemicals, Neuss, Germany), as previously described (10).
Statistical analysis. Data are reported as means ± SE. Dynamic changes (1–6 h vs. baseline) during the experiments were evaluated by Student's t test and expressed as the percent change from baseline. To examine the relationship between IMCL levels and GIR, least square regression analysis was performed.

RESULTS

Lipid infusion protocol. During the hyperinsulinemic glucose clamp, circulating insulin levels increased comparably in the protocol with (57 ± 4 mmol/l) and without (53 ± 3 mmol/l) concomitant lipid infusion (Fig. 1A). Although glucose levels remained constant during infusion of NEFA only, insulin levels rose slightly (Fig. 1A). The marked increase in circulating NEFA during lipid infusion was similar in the protocol with (3,192 ± 241 µmol/l) and without insulin (3,141 ± 242 µmol/l) (Fig. 1B).

The mean GIR in the insulin-only protocol increased from 7.6 ± 1.4 mg · kg^{-1} · min^{-1} after 2 h to 13.7 ± 0.9 mg · kg^{-1} · min^{-1} after 6 h, whereas in the protocol with concomitant lipid infusion, the GIR declined by ~40%, from 8.8 ± 0.5 mg · kg^{-1} · min^{-1} after 2 h to 5.4 ± 0.7 mg · kg^{-1} · min^{-1} after 6 h (Fig. 2A).

Insulin infusion alone had no effect on IMCL levels in either muscle (TA: 8.8 ± 4.4%; SOL: 1.5 ± 1.6%; both NS), whereas the concomitant infusion of NEFA led to a steady increase in IMCL levels in both muscles. After 2 h, IMCL levels in TA muscle were already significantly higher than at baseline (15.7 ± 5.8%; $P \leq 0.05$). At the end of the intervention, IMCL content was 64.2 ± 13.8% ($P = 0.0003$) higher (Fig. 2B). In comparison, the rise in IMCL levels in SOL muscle achieved significance after only 4 h and reached a maximum increase of 20.7 ± 3.4% ($P = 0.005$) compared to baseline values. (Fig. 2C and D). A strong negative correlation between IMCL content and GIR was observed in both TA ($r = -0.98$; $P \leq 0.003$) and SOL muscle ($r = -0.97$; $P \leq 0.005$). Lipid infusion without
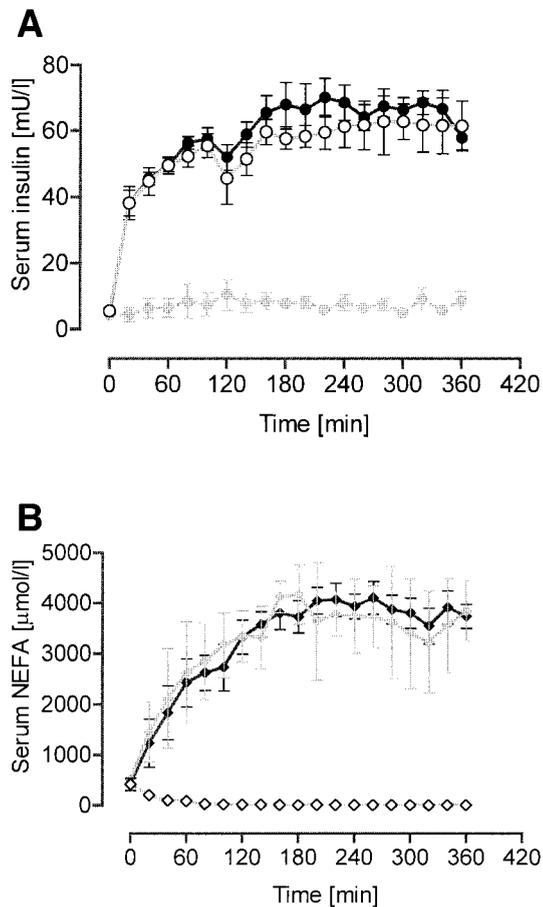
A

B

FIG. 1. *A*: Serum insulin levels during 6-h hyperinsulinemic-euglycemic clamp with (12 subjects; ●) and without (6 subjects; ○) concomitant infusion of Intralipid and heparin-sodium. Insulin levels during infusion of Intralipid and heparin-sodium without clamp are represented by ◐ (3 subjects). *B*: Serum NEFA levels during 6-h hyperinsulinemic-euglycemic clamps with (12 subjects; ●) and without (6 subjects; ○) concomitant infusion of Intralipid and heparin-sodium. NEFA levels during infusion of Intralipid and heparin-sodium without clamp are represented by ◇ (3 subjects).

concomitant insulin infusion did not significantly increase IMCL levels in the 6-h time frame (data not shown).

Diet protocol

Glucose, insulin, and NEFA levels. Fasting plasma glucose remained unchanged during both diet protocols. Fasting insulin levels were not different before and after the high-carbohydrate diet (6 vs. 7 pmol; NS) nor before and after the high-fat diet (8 vs. 6 pmol; NS). Fasting triglyceride and NEFA levels remained nearly unchanged during both protocols (Fig. 3).

Insulin sensitivity. After the high-fat diet, the GIR decreased significantly, from 8.3 ± 0.5 to 6.9 ± 0.6 mg · $kg^{-1} \cdot min^{-1}$, thereby indicating a significant decrease in insulin sensitivity ($P < 0.033$). In contrast, after the low-fat diet, the GIR remained unchanged (7.5 ± 0.5 vs. 7.7 ± 0.6 mg · $kg^{-1} \cdot min^{-1}$; NS) (Table 2, Fig. 3). NEFA levels decreased similarly during the glucose clamp.

IMCL levels. After the high-fat diet, the IMCL content in TA muscle increased by ~50% ($48.0 \pm 16.9\%$; $P < 0.005$), whereas the IMCL content in SOL muscle increased by only 14% and did not reach statistical significance ($14.4 \pm 8.2\%$; NS). However, after the low-fat diet, IMCL content decreased in both TA and SOL muscle, but also did not

reach statistical significance (ΔIMCL TA: -10.1 ± 8.7, NS; ΔIMCL SOL: $7.7 \pm 4.6\%$, NS). Changes in IMCL levels and insulin sensitivity for both dietary periods are presented in Fig. 3.

Figure 4 shows the individual changes in insulin sensitivity and IMCL levels during the intervention. Clearly, in the lipid infusion protocol (Fig. 4*A* and *B*), the interindividual variation was much smaller than in the diet protocol (Fig. 4*C* and *D*). In three subjects, the changes actually went in the opposite direction of the mean during the diet protocol.

DISCUSSION

Lipid infusion protocol. Neither hyperinsulinemia during the clamp alone nor experimental elevation of circulating NEFAs without concomitant infusion of insulin induced a change in IMCL levels. In contrast, the combination of the Intralipid/heparin infusion and clamp, leading to increased circulating NEFA, and insulin resulted in a significant increase in IMCL levels after only 2 h in TA muscle and 4 h in SOL muscle. Although IMCL levels increased significantly in both muscle types, the increase in TA muscle was more pronounced. The increases in IMCL levels and decreases in insulin sensitivity paralleled one another and showed a strong negative correlation over time for both TA ($r = -0.98$; $P \leq 0.003$) and SOL muscle ($r = -0.97$; $P \leq 0.005$). Unfortunately, the methods used did not allow us to discern which event occurred first: the change in IMCL levels or the change in insulin sensitivity.

These findings in humans agree with observations in a rat model reported by Chalkley et al. (13). These authors found an increased triacylglycerol content in the red gastrocnemius muscle under similar conditions (i.e., after a 5-h hyperinsulinemic glucose clamp and infusion of a lipid emulsion plus heparin) (13). In that study, however, no distinction between the extramyocellular lipid and IMCL compartments could be made.

^{1}H-MRS offers a unique noninvasive approach for quantifying the time course of changes in IMCL levels in humans. The close correlation of time course and degree of change in both parameters are suggestive of a potential link between IMCL levels and muscle insulin sensitivity. It is necessary to point out that our use of the hyperinsulinemic clamp measured the net effect on peripheral (mainly muscle) glucose uptake and suppression of endogenous (mainly hepatic) glucose production. Because elevated NEFA not only stimulates basal glucose production (15), but also prevents insulin suppression of glucose production, it is likely that not all of the NEFA effect on insulin

TABLE 2

Change of metabolic parameters during high-fat versus low-fat diet: change from baseline

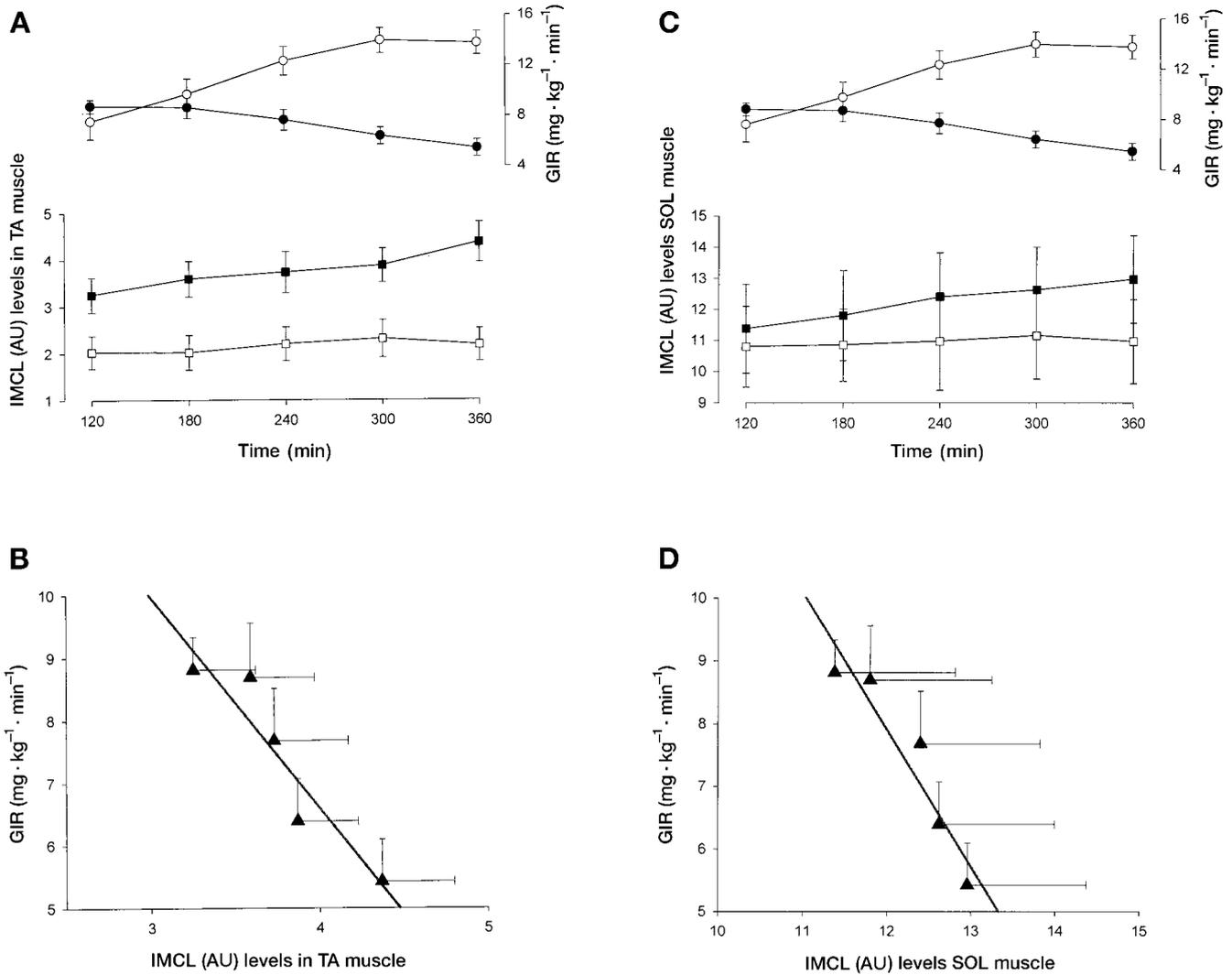|	Δ High-fat diet	P	Δ Low-fat diet	P
Fasting glucose (mmol/l)	0.9 ± 2.2	0.80	-0.4 ± 1.7	0.76
Fasting serum insulin (mmol/l)	1.1 ± 0.9	0.21	-1.8 ± 1.8	0.33
GIR (mg · $kg^{-1} \cdot min^{-1}$)	-1.4 ± 0.6	0.03	0.2 ± 0.4	0.70
NEFA (μmol/l)	-42.4 ± 45.5	0.37	95.8 ± 56.7	0.12
Triglyceride (mg/dl)	-7.7 ± 18.2	0.69	18.3 ± 16.7	0.30

Data are means $\pm$ SE.

FIG. 2. *A*: Upper panel: GIR during 6-h hyperinsulinemic-euglycemic clamps with (12 subjects; ●) and without (6 subjects; ○) concomitant infusion of Intralipid and heparin-sodium. Lower panel: IMCL levels (AU) in TA muscle during 6-h hyperinsulinemic-euglycemic clamps with (12 subjects; ■) and without (6 subjects; □) concomitant infusion of Intralipid and heparin-sodium. *B*: Inverse correlation between increasing IMCL (AU) in TA muscle and decreasing GIR during 6-h hyperinsulinemic-euglycemic clamps with concomitant infusion of Intralipid and heparin-sodium (12 subjects; ▲; $r = -0.98$, $P \leq 0.003$). *C*: Upper panel: GIR during 6-h hyperinsulinemic-euglycemic clamps with (●) and without (○) concomitant infusion of Intralipid and heparin-sodium. *C*: Lower panel: IMCL levels (AU) in SOL muscle during 6-h hyperinsulinemic-euglycemic clamps with (■) and without (□) concomitant infusion of Intralipid and heparin-sodium. *D*: Inverse correlation between increasing IMCL levels (AU) in SOL muscle and decreasing GIR during 6-h hyperinsulinemic-euglycemic clamps with concomitant infusion of Intralipid and heparin-sodium (12 subjects; ▲; $r = -0.97$, $P \leq 0.005$).

sensitivity was attributable to changes in muscle. Nevertheless, it is probably fair to assume that muscle was responsible for ~50% of the effect on insulin sensitivity observed.

The molecular basis of a potential link between increased IMCL levels and decreased insulin sensitivity remains to be determined. Currently, little is known about the mechanisms involved in the regulation of intramyocellular lipogenesis. The increase of IMCL may be induced by several mechanisms, such as the activation of enzymes of lipogenesis (e.g., acetyl-CoA carboxylase and fatty acid synthase) and/or changes in the concentrations of substrates of intracellular lipid metabolism (e.g., malonyl-CoA, long-chain fatty acid-CoA, acetyl-CoA, and citrate) (5).

Diet protocol. To determine the effects of short-term dietary interventions on IMCL levels and insulin sensitivity, we compared two types of diets typical in Western
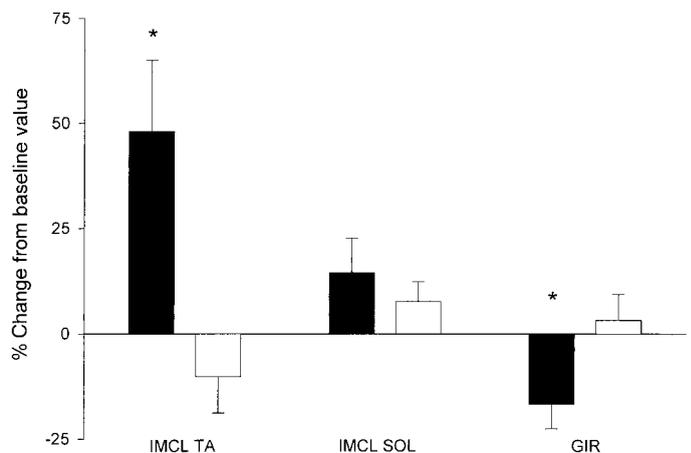

FIG. 3. Relative changes from baseline after high-fat diet (12 subjects; ■) and low-fat diet (12 subjects; □). *$P < 0.05$.
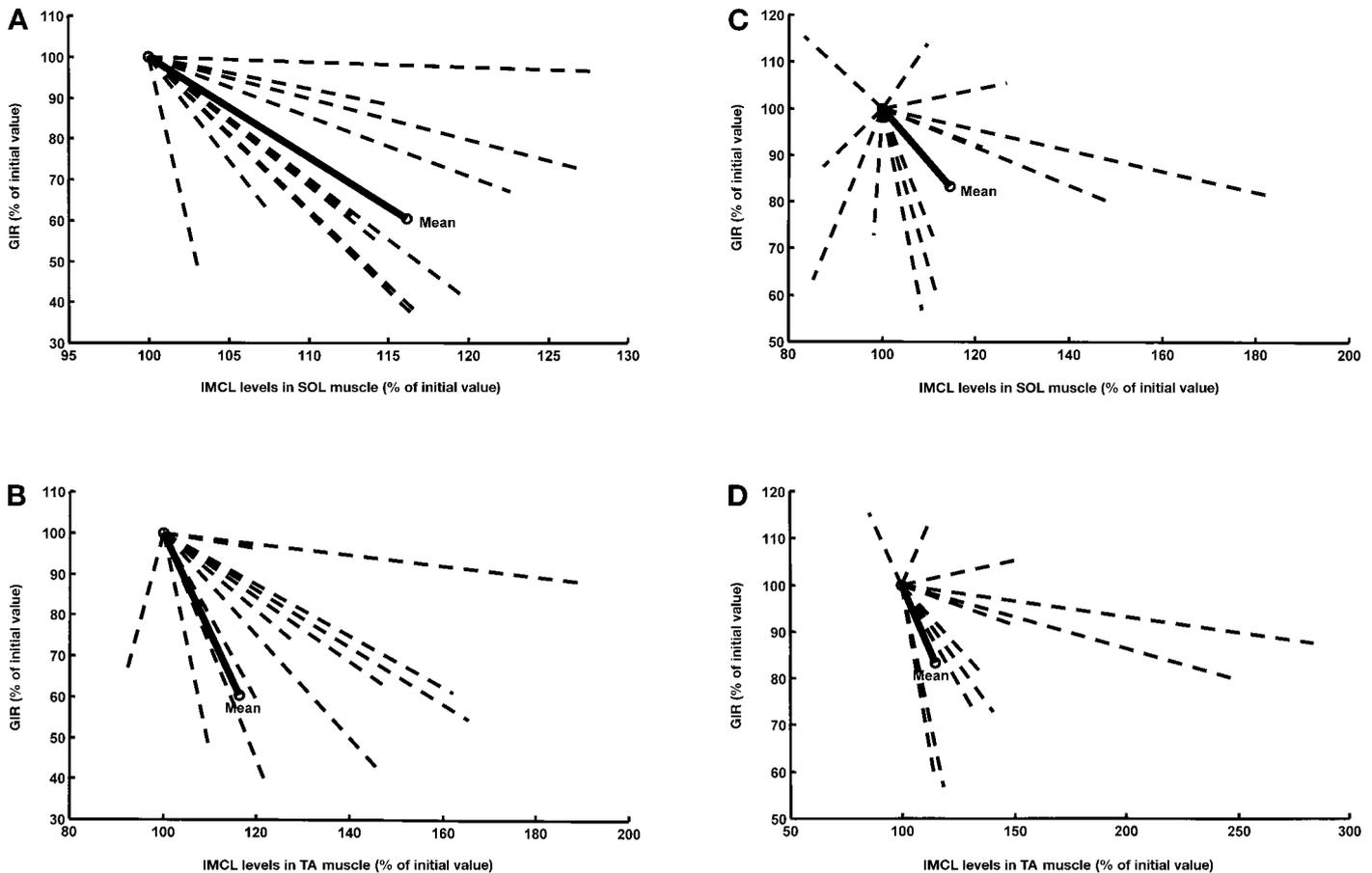
FIG. 4. *A* and *B*: Relative changes from baseline IMCL levels in SOL (*A*) and TA muscle (*B*) and insulin sensitivity from each of the 12 subjects after hyperinsulinemia and lipid infusion. *C* and *D*: Relative changes from baseline IMCL levels in SOL (*C*) and TA muscle (*D*) and insulin sensitivity from each of the 12 subjects after 3 days of high-fat diet.

societies. The high-fat diet contained predominantly saturated fatty acids and was low in carbohydrate. The low-fat diet was high in carbohydrate and low in fat, as is recommended for the general population as well as for diabetic patients (16–20). Total calories were higher in the high-fat diet because fat is an excellent source of energy. We preferred not to match the protocols to avoid conversion of excess carbohydrate-derived calories into fat (21–23). Therefore, we could not separate the influence of caloric imbalance and the contribution of fat intake. However, it is likely that the observed effects were mainly attributable to the increased proportion of fat in the high-fat diet. After ingestion of the high-fat diet for 3 days, both IMCL levels and insulin sensitivity were clearly affected. This observation agrees with the results obtained in the lipid infusion protocol. However, one consideration is whether the result in the diet protocol was attributable to the same mechanism as in the lipid infusion protocol, given that plasma NEFA levels obtained before the clamp remained nearly unchanged. Also, the suppression pattern of NEFAs during the clamp was almost identical for both groups. Therefore, the possibility that the alterations in insulin sensitivity and IMCL levels in this study were caused by factors other than increased postprandial plasma NEFA levels (e.g., molecular mechanisms within the muscle cell itself) cannot be excluded.

This conclusion agrees with findings from several studies that have showed that a fat-rich diet can increase

plasma NEFA levels, and, moreover, that this increase in NEFA levels is accompanied by a decrease in insulin sensitivity (3,10,11,24,25). The same limitations as mentioned above regarding the specificity of the hyperinsulinemic clamp to measure changes in muscle versus liver remain.

Differences in muscle types in the diet protocol. The decrease of insulin sensitivity after a high-fat diet was accompanied by a marked increase in IMCL levels, which was statistically significant only for the TA muscle. In the SOL muscle, neither high fat nor high carbohydrate intake led to significant changes of IMCL content. This result might have been influenced by methodological factors. For example, the reproducibility of ^{1}H-MRS was reported to be 10–15% for the SOL muscle and 5–10% for the TA muscle (9,10,12). Moreover, absolute changes in IMCL content for the SOL muscle seem to be much higher, as this muscle presents a higher baseline value because of its mainly oxidative metabolism (9,10,12,14).

Interindividual variation of the susceptibility to fat loading. The concomitant increase in IMCL levels and decrease in insulin sensitivity is in accordance with earlier studies reporting a negative correlation between IMCL content and insulin sensitivity in healthy subjects as well as in first-degree relatives of patients with type 2 diabetes (3,10–12,24,25). Figure 2 shows the correlation of mean values of the time course of IMCL versus insulin sensitivity change. As shown in Fig. 4, the effect of the infusion

protocol was relatively homogenous compared to the diet protocol. This variation might reflect insufficient control of parameters, such as physical activity during the 3-day protocol. It could also point to a different individual susceptibility to the negative effects of fat loading. However, such susceptibility factors could include family history of diabetes, differences in genotype (e.g., peroxisome proliferator-activated receptor-γ Pro12Ala polymorphism, which affects lipid handling, and other genetic determinants) (26,27). Because the present study was conducted in subjects with intermediate insulin sensitivity, it was not possible to address whether subjects with lower insulin sensitivity are more susceptible to nutrient changes.

In conclusion, this study showed that IMCL formation, induced rapidly through short-term elevation of circulating NEFA and hyperinsulinemia, leads to decreased insulin sensitivity. The results of the diet protocol suggest that this is a physiologically relevant regulatory mechanism.

ACKNOWLEDGMENTS

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