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Effects of Diet and Exercise on Muscle and Liver Intracellular Lipid Contents and Insulin Sensitivity in Type 2 Diabetic Patients

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Insulin resistance is associated with the circulating free fatty acid (FFA) level and intracellular lipid content in muscle and liver. We investigated the effect of 2-wk diet and exercise therapy on total adiposity, circulating FFA, intracellular lipid content in muscle and liver, and peripheral insulin sensitivity. Type 2 diabetic patients were divided into a diet group (n = 7) and a diet plus exercise group (n = 7). We performed a hyperinsulinemic-euglycemic clamp study before and after treatment. Intramyocellular lipid (IMCL) in the tibialis anterior muscle and intrahepatic lipid (IHL) were evaluated by ^{1}H-magnetic resonance spectroscopy. Fasting FFA were not altered, and total body fat showed a slight, but significant, decrease in both groups after treatment. IMCL was decreased by 19%, and the glucose infusion rate was increased by 57% in the diet plus exercise group, whereas neither IMCL nor glucose infusion rate was significantly altered in the diet group. However, IHL showed a significant decrease in both groups. In summary, we found that 2 wk of diet and exercise decreased IMCL and increased muscle insulin-mediated glucose uptake, whereas diet with or without exercise decreased IHL. These effects were evident despite a small decrease in body fat and were observed independently of fasting FFA levels. (***J Clin Endocrinol Metab* 90: 3191–3196, 2005**)

TYPE 2 DIABETES IS characterized by insulin resistance in skeletal muscle and liver. Many previous studies have suggested that an elevated plasma level of free fatty acid (FFA) is a key factor linking obesity with insulin resistance (1–5). In contrast, recent cross-sectional studies using ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) or biopsy have demonstrated that the intramyocellular lipid (IMCL) level is negatively associated with insulin sensitivity in both obese and nonobese subjects (3, 6–10). In addition, a higher IMCL level is associated with impairment of early insulin signal transduction in muscle biopsies from healthy subjects (10). These reports suggest that IMCL itself or related intracellular substances, such as diacylglycerol or protein kinase C (PKC), are important regulators of insulin sensitivity in skeletal muscle (1, 3, 4, 10). Similarly, intrahepatic lipid (IHL) accumulation was reported to be associated with impaired hepatic glucose metabolism. A suppressive effect of insulin on hepatic glucose production was negatively correlated with the IHL content in both healthy subjects (11) and type 2 diabetics (12). From all these reports, intracellular lipid accumulation in the insulin target organs as well as increased circulating FFA levels may be some of the mechanisms of insulin resistance.

Exercise improves insulin resistance in skeletal muscle, but the exact mechanism of this effect is not fully understood (13). IMCL is oxidized during moderate intensity exercise (14), and recent reports suggest that a 1-h cycling at 65% maximum oxygen uptake reduces IMCL by 11.5–28.5% in healthy men (15). Therefore, a decrease in IMCL may be involved in the mechanisms of exercise-induced amelioration of insulin resistance in skeletal muscle. However, there have been no studies assessing the effect of exercise on IMCL and insulin sensitivity in type 2 diabetes. In addition, it is still unclear whether diet or exercise decreases IHL in diabetic patients.

Accordingly, we investigated whether 2 wk of diet and exercise could reduce the intracellular lipid content in skeletal muscle and liver and improve insulin resistance in type 2 diabetic patients. To evaluate the effects of diet therapy and diet plus exercise therapy, we treated type 2 diabetic patients with diet therapy alone (25–30 kcal/kg ideal body weight and no increase in physical activity) or diet therapy plus exercise therapy (25–30 kcal/kg ideal body weight and a mean exercise increment of ~170 kcal/d). To monitor each treatment strictly, all patients were admitted to the hospital for 14 d and received a controlled diet and exercise regimen.

Subjects and Methods

We studied 14 type 2 diabetic patients. None of the subjects had cardiovascular disease, liver diseases, or diabetic vascular complications. Before starting the study, the subjects were divided randomly into a diet group (D group; n = 7) and a diet plus exercise group (D+E group; n = 7). In the D group, three subjects were taking sulfonylureas (SU),

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Abbreviations: AMPK, AMP-activated protein kinase; BMI, body mass index; Cre, creatine; D group, diet group; D+E group, diet plus exercise group; FFA, free fatty acid; α-GI, α-glucosidase inhibitor; GIR, glucose infusion rate; ^{1}H-MRS, ^{1}H-magnetic resonance spectroscopy; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; MET, metformin; NS, not significant; PKC, protein kinase C; ppm, parts per million; S-fat, methylene signal intensity; SGU, splanchnic glucose uptake; SU, sulfonylurea; TA, tibialis anterior muscle.

two subjects were taking SU and metformin (MET), and the other two subjects were using an α-glucosidase inhibitor (α-GI). In the D+E group, three subjects were being treated with SU, three patients were taking SU and MET, and one subject was using α-GI. During this study, the MET or α-GI dose was not changed, but the SU dose was reduced if the fasting plasma glucose level decreased to less than 90 mg/dl. All subjects gave written informed consent to the study, which was approved by the ethics committee of Juntendo University.

Study design

The baseline total body fat content was examined by the gas dilution method using a specific analyzer (Bod Pod, Life Measurement, Concord, CA), and the IMCL in right tibialis anterior muscle and the IHL of segment 6 of the liver were measured by ^{1}H-MRS (VISART EX V4.40, Toshiba, Tokyo, Japan). Peripheral insulin sensitivity was evaluated by the euglycemic hyperinsulinemic clamp method using an artificial pancreas (STG 22, Nikkiso, Shizuoka, Japan). These parameters were examined on separate days within 1 wk before admission, because the intervention protocols were started from the first day of admission. A well trained dietician calculated total energy intake and composition of daily fat intake from food diaries. After baseline evaluation, all subjects were admitted to Juntendo University Hospital and placed on a carefully strictly calculated diet (60% carbohydrate, 25% fat, and 15% protein; mean total energy intake of 27.9 kcal/kg ideal body weight) for 2 wk in both groups. In the D+E group, the subjects were also instructed to perform two or three sessions of exercise (30 min each) by walking on 5–6 d per wk. Exercise intensity was targeted at 50–60% of maximum oxygen uptake, which was checked by a pulse rate monitor (PL6000, Cateye, Dallas, TX), and mean physical activity level in 2 wk was estimated with an ambulatory accelerometer (Lifecorder, Suzuken, Nagoya, Japan). In the D group, patients were directed to maintain their physical activity at the same intensity as before admission, which was also monitored by the same methods as in the D+E group. We reevaluated IMCL and IHL on d 11 and reassessed peripheral insulin sensitivity on d 13. Both examinations were performed 24 h after the last exercise on the previous day.

Proton magnetic resonance spectroscopy

Before and after treatment, IMCL and IHL were measured at 1400 h as described previously (12, 16). Briefly, IMCL of the left tibialis anterior muscle (TA) and IHL of segment 6 in the liver were measured by ^{1}H-MRS using a knee coil and a whole body coil, respectively. Voxels ($1.2 \times 1.2 \times 1.2$ cm^3 for TA and $2 \times 2 \times 2$ cm^3 for liver) were positioned in the TA muscle or liver avoiding visible interfascial fat and blood vessels, and the voxel sites were carefully matched at each examination. Imaging parameters were set as follows; repetition time of 1500 msec, echo time of 136 msec (TA) or 10 msec (liver), acquisition numbers of 192 (TA) or 8 (liver), and 1024 data points over a 1000-kHz spectral width. After examination, resonances were quantified by reference to the methylene signal intensity (S-fat), with peaks being observed at approximately 1.25 parts/million (ppm) in TA and at approximately 1.3 ppm in liver. IMCL was quantified by S-fat and the creatine signal at 3.0 ppm (Cre) as the reference and was calculated as a ratio relative to Cre (S-fat/Cre). IHL was quantified by S-fat and H_2O at approximately 4.7 ppm as the internal reference and calculated as a percentage of H_2O and S-fat (S-fat $\times$ 100/(H_2O + S-fat)) as described previously (12).

Euglycemic hyperinsulinemic clamp study

Patients were fasted overnight from 2100–0800 h on the day for the clamp study. Intravenous cannulas were placed in both forearms (one was for insulin and glucose infusion and the other for continuous blood glucose monitoring). The dorsal vein of the foot was also cannulated for blood sampling. Using an artificial pancreas, a euglycemic hyperinsulinemic clamp study (target plasma glucose level of 95 mg/dl and insulin infusion rate of 100 mU/m^2·min) was performed as reported previously (17). The steady-state glucose infusion rate (GIR) was observed from 90–120 min, and the mean GIR during that period was used as a marker of peripheral insulin sensitivity.

Statistical analysis

All data are expressed as the mean ± SE. Comparison of results between before and after treatment was performed using the paired *t* test. Differences between the two groups were compared by Student's *t* test. Simple linear regression analysis was performed to evaluate the associations between IMCL or IHL and other parameters. Statistical significance was set at $P < 0.05$.

Results

The baseline clinical characteristics are shown in Table 1. No significant differences between the two groups were observed. At baseline, neither IHL nor IMCL was correlated with body mass index [BMI; IHL: r = 0.008; P = not significant (NS); IMCL: r = −0.389; P = NS), body fat content (IHL: r = 0.233; P = NS; IMCL: r = −0.447; P = NS), or the fasting serum FFA level (IHL: r = 0.072; P = NS; IMCL: r = −0.125; P = NS). Body fat content was not correlated with the fasting serum FFA level (r = 0.256; P = NS).

Table 2 shows the changes in parameters between baseline and after 2 wk of treatment. BMI and body fat were slightly, but significantly, decreased in both groups (BMI: −1.5 ± 0.003% in the D group, −2.3 ± 0.004% in the D+E group; body fat: −8.2 ± 0.03% in the D group, −9.6 ± 0.02% in the D+E group). Glycated albumin, fasting plasma glucose, and serum triglycerides also decreased in both groups, whereas fasting insulin levels were not significantly changed. Serum high density lipoprotein cholesterol was only significantly decreased in the D group, and the other parameters were not significantly changed in either group. Although physical activity did not change in the D group, it was significantly increased in the D+E group. The reductions of total energy intake and saturated fatty acid intake were not different between the two groups.

As shown in Fig. 1A (*left*), IMCL decreased by 19% (3.80 ± 0.44 to 3.05 ± 0.38; $P < 0.03$) in the D+E group, but no significant change was observed in the D group. The mean GIR, mainly indicating skeletal muscle insulin sensitivity, was significantly increased by 57% in the D+E group (from 5.26 ± 0.33 to 8.22 ± 0.47 mg/kg·min; $P < 0.001$), whereas no significant change was observed in the D group (from 6.12 ± 0.93 to 6.49 ± 0.33 mg/kg·min; P = NS; Fig. 1A, *right*). The percent change in IMCL in all subjects was not correlated with the percent change in body fat (r = 0.339; P = NS) or with fasting FFA (r = 0.042; P = NS). However, it was

TABLE 1. Clinical characteristics of the subjects

	D group	D+E group
n	7	7
Sex (M/F)	4/3	4/3
Age (yr)	55.0 ± 4.8	46.3 ± 2.8
BMI (kg/m^2)	27.4 ± 3.2	27.1 ± 2.9
Body fat (%)	30.7 ± 4.9	30.8 ± 3.7
Glycated albumin (%)	22.4 ± 2.1	23.6 ± 1.6
IMCL (IMCL/Cr)	4.04 ± 0.67	3.80 ± 0.44
IHL (%)	10.26 ± 2.89	7.31 ± 1.93
GIR (mg/kg·min)	6.12 ± 0.93	5.26 ± 0.33
Physical activity (kcal/day)	324.4 ± 36.5	239.0 ± 36.8
Energy intake (kcal/ideal body weight)	35.4 ± 3.4	35.6 ± 1.8
Energy intake (kcal/day)	2052.9 ± 156.9	2115.0 ± 153.9

Data are the mean ± SE. Conversion factors are as follows: physical activity and energy intake, kJ = kcal × 0.239. M, Male; F, female.

TABLE 2. Clinical parameters before and after 2 wk of treatment

	D group		D+E group	
	Before	After	Before	After
BMI (kg/m^2)	27.4 ± 3.2	27.0 ± 3.2[a]	27.1 ± 2.9	26.4 ± 2.8[b]
Body fat (%)	30.7 ± 4.9	28.3 ± 4.7[c]	30.8 ± 3.7	28.0 ± 3.7[b]
Glycated albumin (%)	22.4 ± 2.1	20.5 ± 2.1[d]	23.6 ± 1.6	19.9 ± 2.0[d]
Fasting plasma glucose (mg/dl)	198.1 ± 23.2	135.7 ± 6.63[c]	177.6 ± 19.6	132.6 ± 14.0[d]
Fasting plasma insulin (μU/ml)	16.8 ± 7.3	14.8 ± 6.8	13.3 ± 2.8	10.3 ± 3.0
Triglyceride (mg/dl)	177.1 ± 37.3	119.7 ± 16.7[c]	216.3 ± 65.4	103.1 ± 21.8[c]
Free fatty acid (mmol/liter)	0.53 ± 0.07	0.65 ± 0.05	0.73 ± 0.10	0.63 ± 0.07
Total cholesterol (mg/dl)	186.6 ± 8.8	179.3 ± 8.5	197.6 ± 8.5	186.0 ± 9.5
HDL cholesterol (mg/dl)	43.9 ± 6.5	39.7 ± 6.5[b]	49.1 ± 6.9	44.3 ± 4.9
Hypersensitive CRP (μg/ml)	4.01 ± 2.23	1.57 ± 0.48	1.92 ± 1.15	1.58 ± 1.05
AST (IU/liter)	25.9 ± 2.4	26.9 ± 3.2	22.3 ± 6.2	24.1 ± 7.3
ALT (IU/liter)	31.3 ± 6.6	24.1 ± 7.3	27.9 ± 8.5	33.0 ± 13.3
γ-Glutamyl transpeptidase (IU/liter)	59.3 ± 19.6	40.3 ± 10.3	46.3 ± 13.6	36.0 ± 10.3
Leptin (ng/ml)	11.84 ± 4.81	9.24 ± 4.13	7.50 ± 1.84	6.59 ± 2.72
Physical activity (kcal/day)	324.4 ± 36.5	294.9 ± 25.5	239.0 ± 36.8	410.8 ± 68.2[d]
Energy intake (kcal/ideal body weight)	35.4 ± 3.4	27.7 ± 0.4[d]	35.6 ± 1.8	28.1 ± 1.0[b]
Saturated fatty acid (kcal/kg body weight)	2.87 ± 0.30	1.91 ± 0.17[c]	3.21 ± 0.33	1.96 ± 0.17[c]
Energy intake (kcal/day)	2052.9 ± 156.9	1628.6 ± 80.8[d]	2115.0 ± 153.9	1657.1 ± 71.9[b]

Data are the mean ± SE. Conversion factors are as follows: glucose, mmol/liter = mg/dl × 0.05551; insulin, pmol/liter = μU/ml × 60; triglyceride, mmol/liter = mg/dl × 0.0112; total cholesterol and HDL cholesterol, mmol/liter = mg/dl × 0.02586; physical activity, energy intake and saturated fatty acid, kJ = kcal × 0.239. ALT, Alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; HDL, high density lipoprotein.

[a] $P < 0.001$ *vs.* baseline.
[b] $P < 0.01$ *vs.* baseline.
[c] $P < 0.05$ *vs.* baseline.
[d] $P < 0.03$ *vs.* baseline.

significantly correlated with the percent change in physical activity (r = −0.623; $P = 0.0154$; Fig. 2). The change in mean GIR in all subjects was not correlated with the percent change in body fat (r = −0.326; P = NS), but was significantly correlated with the change in physical activity (r = 0.563; $P = 0.0345$) and tended to show correlation with the percent change in fasting serum FFA (r = −0.470; $P = 0.092$) and with IMCL (r = −0.461; $P = 0.097$).

IHL was significantly decreased in both groups, as shown in Fig. 1B (D group: from 10.26 ± 2.89 to 8.08 ± 2.34, $P < 0.03$; D+E group: from 7.31 ± 1.93 to 5.83 ± 1.97, $P < 0.03$). The percent change in IHL in all subjects was not correlated with either the percent change in body fat (r = 0.276; P = NS) or fasting serum FFA (r = 0.131; P = NS). The percent change in glycated albumin was not correlated with the percent change in IHL (r = −0.182; P = NS), IMCL (r = −0.019; P = NS), or body fat content (r = −0.12; P = NS).

Discussion

The present study demonstrated the effects of 2 wk of diet with or without exercise on IMCL, IHL, serum FFA, and insulin sensitivity in type 2 diabetic patients. There were three interesting results with regard to the relationships among lifestyle modification and these metabolic parameters. First, we found that diet plus exercise, but not diet alone, decreased IMCL by 19% and increased mean GIR by 55%. Second, both diet and diet plus exercise significantly reduced IHL by 27%. Third, both regimens had less effect on body fat (1.9% reduction) and fasting FFA levels compared with the marked effects on IMCL and IHL.

The percent decrease in IMCL and the percent increase in GIR (which mainly reflects muscle insulin sensitivity) were higher in D+E group compared with D group. In addition, the percent changes of IMCL and GIR in all subjects were significantly correlated with the percent change of physical activity, respectively. These results suggest that exercise therapy may be required for IMCL reduction and improvement of muscle insulin resistance in the short-term intervention. Regarding the effect of exercise on muscle glucose metabolism, many reports have shown that exercise improves both insulin-dependent and -independent glucose uptake by skeletal muscle (13, 18, 19). Several studies have demonstrated that exercise increases the activity of AMP-activated protein kinase (AMPK) in muscle, which, in turn, promotes translocation of glucose transporter-4 from the cytosol to the plasma membrane and increases insulin-independent glucose uptake by muscle (13, 19). Interestingly, activation of muscle AMPK also promotes intracellular β-oxidation of fat (20). Recently, an important influence of fat oxidation on IMCL and insulin sensitivity has been reported. It has been suggested that a higher level of fasting lipid oxidation is associated with a normal IMCL content and insulin sensitivity in obese subjects (21). In addition, another study demonstrated that enhanced oxidation of fat through physical activity was associated with improvement of insulin sensitivity in obese subjects (22). Recent studies have suggested that intramyocellular nonoxidized fat may partly be metabolized to diacylglycerol, which activates PKC that, in turn, phosphorylates the serine residues of insulin receptor substrate-1. Serine-phosphorylated insulin receptor substrate-1 is insensitive to insulin, leading to impairment of early insulin signaling, which may be a mechanism of muscle insulin resistance (1). Taken together, exercise-induced AMPK activation and enhanced oxidation of fat may be at least partly associated with IMCL reduction and improvement of muscle insulin sensitivity in the D+E subjects, although we did not

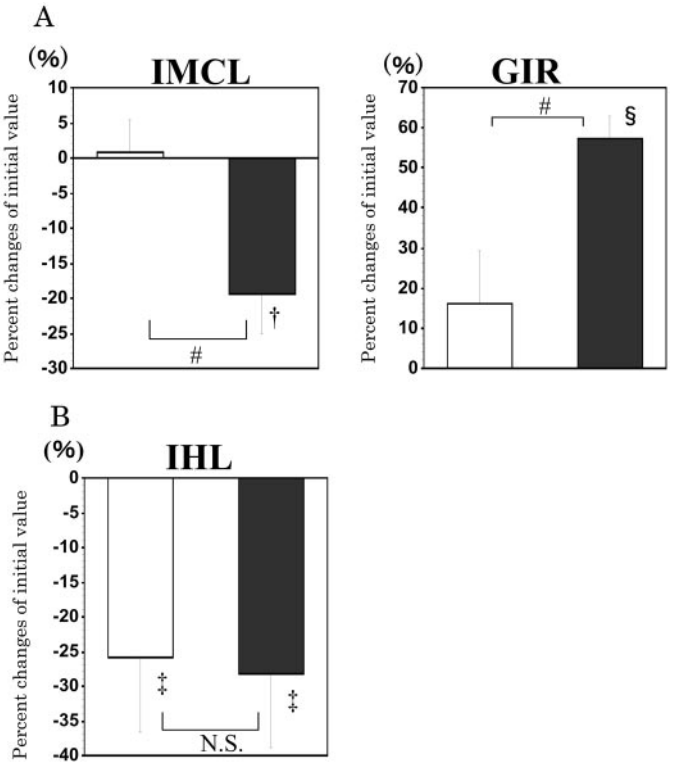

FIG. 1. A, Changes in IMCL (*left*) and GIR (*right*) after 2 wk of treatment. B, Changes in IHL after 2 wk of treatment. □, D group; ■, D+E group. Data are the mean ± SE.§, $P < 0.0001$; †, $P < 0.03$; ‡, $P < 0.05$ (*vs.* baseline). #, $P < 0.03$ (diet *vs.* diet plus exercise).

directly evaluate fat oxidation or the activities of AMPK and PKC.

It is still unclear whether the amelioration of IMCL and GIR observed in the D+E group reflects the last bout of exercise. It has been demonstrated that IMCL is decreased by one bout of exercise and recovered to basal levels 24 h after exercise in female runners (23). However, the effect of one bout of exercise on IMCL in type 2 diabetes has not been investigated. In terms of GIR, the effect of one bout of exercise on insulin sensitivity in skeletal muscle is still controversial in type 2 diabetes (24, 25). Thus, we need to examine the extent of the effect of one bout of exercise on muscle glucose metabolism and IMCL in type 2 diabetic patients.

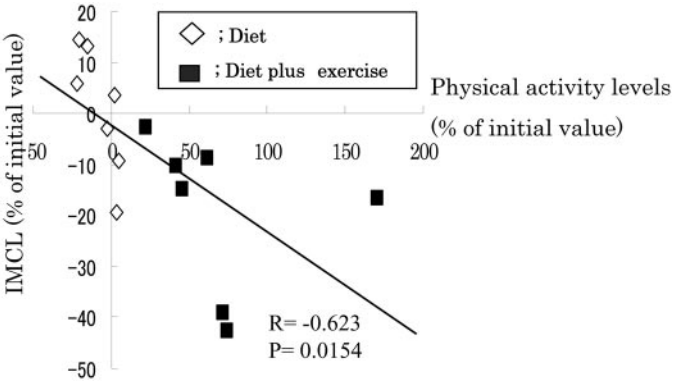

FIG. 2. Relationship between relative changes in IMCL and physical activity. ◇, D group; ■, D+E group. The change in IMCL was significantly correlated with the percent change in physical activity ($r = -0.623$; $P = 0.0154$).

In the present study we did not observe significant changes in circulating FFA, IMCL, and muscle insulin sensitivity in a short-term diet with calorie restriction. However, we should keep in mind that the present results are based on a small number of the subjects, and thus we cannot conclude that 2-wk diet therapy is not effective on those parameters. It is also possible that chronic calorie restriction may have a positive effect on the muscles. Previous reports have indicated that IMCL levels were decreased by remarkable weight reduction after gastric surgery in nondiabetic obese subjects, and that insulin sensitivity was improved (26). In patients with type 2 diabetes, there is only one report that IMCL was decreased after weight reduction by chronic calorie restriction for 4 months, but the association between IMCL and insulin sensitivity was not evaluated in that study (27). Thus, additional investigations are needed to evaluate the effects of both short-term and chronic diet therapies on IMCL and muscle glucose metabolism in type 2 diabetic patients.

Calorie restriction for 2 wk with or without exercise caused a 27% decrease in IHL despite a minimal change in body fat (−1.9%) in the present study, suggesting that diet therapy was necessary for IHL reduction. A major mechanism of fatty liver is thought to be excess FFA flux into the liver, with sources being exogenous dietary FFA and endogenous FFA derived from fat cells (28, 29). We observed that total saturated fatty acid intake was significantly decreased in both groups. Thus, restriction of saturated fat intake may at least partly contribute to a reduction of IHL. Consistent with our study, Tiikkainen *et al.* (29) reported that restriction of total calorie and saturated fat intake in obese women for 18–19 wk decreased IHL by 39% and showed a positive relationship between restriction of saturated fat intake and IHL reduction. Compared with their chronic study, it is interesting that even a short period of diet therapy could decrease IHL despite a very slight effect on body fat. In contrast, it is still unclear whether 2-wk lifestyle modification can alter endogenous FFA flux into the liver. Future studies using a tracer method will directly prove the importance of short-term lifestyle modification on endogenous FFA flux.

Previous studies have demonstrated a negative correlation between the hepatic triglyceride content and insulin-induced suppression of hepatic glucose production in healthy subjects (11) and type 2 diabetics (12). Thus, excessive accumulation of fat in the liver may induce hepatic insulin resistance, although the exact mechanism of such insulin resistance is not fully understood. We did not examine insulin-induced suppression of hepatic glucose production in the present study, but we preliminarily measured splanchnic glucose uptake (SGU) mainly reflecting hepatic glucose uptake by euglycemic hyperinsulinemic clamp with an oral glucose-loading method as described in our previous reports (17, 30). As shown in Fig. 3, SGU in six subjects (three from each group) showed a significant increase from 38.3 ± 6.9% to 49.6 ± 6.2% ($P < 0.05$). Thus, diet with or without exercise therapy may cause a decrease in IHL and an increase in SGU under hyperinsulinemic euglycemic conditions. Consistent with this observation, pioglitazone treatment also decreases IHL and increases SGU (31). IHL depletion may, therefore,

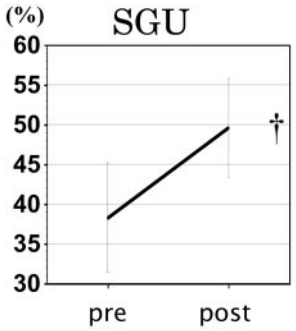

FIG. 3. Changes in SGU after 2 wk of treatment. SGU was measured in six subjects (three from each group). Data are the mean ± SE. †, $P < 0.05$ (*vs.* baseline).

be used as a marker of improved hepatic glucose metabolism after treatments. Interestingly, in the present study diet treatment decreased levels of fasting plasma glucose, glycated albumin, and IHL, whereas few changes in IMCL and GIR were observed, suggesting that reduction of IHL and improvement of hepatic glucose metabolism may be important factors for short-term amelioration of glycemic control.

Many previous studies have suggested that an increase in circulating FFA is a key factor mediating the link between obesity and insulin resistance (1–5). We observed that total body fat was slightly, but significantly, decreased, although fasting serum FFA showed little change after 2 wk of diet with or without exercise. It is possible that a slight decrease in body fat without a decline in leptin, a marker of total adiposity (32), might not be sufficient to decrease FFA. These results suggest that short-term lifestyle modification may improve lipid accumulation and insulin sensitivity in muscles and liver independently of an effect on FFA levels, and they also reinforce the idea that IMCL and IHL are important for insulin resistance as well as plasma FFA levels.

In conclusion, 2 wk of diet plus exercise therapy decreased IMCL and increased muscle insulin-mediated glucose uptake, whereas both diet alone and diet plus exercise significantly decreased IHL. These metabolic changes occurred despite a small decrease in body fat and were observed independently of fasting FFA levels.

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