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Abstract

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Objective: Free fatty acid (FFA) oxidation is reduced in subjects with type 2 diabetes mellitus and impaired glucose tolerance (IGT). Weight reduction does not improve these impairments. Because exercise training is known to increase fatty acid (FA) oxidation, we investigated whether a combined diet and physical activity intervention program can improve FA oxidation in subjects with IGT.

Research Methods and Procedures: Sixteen subjects with IGT were studied before and after 1 year of a lifestyle intervention program [nine intervention (INT) subjects, seven controls (CON)]. INT subjects received regular (i.e., every 3 months) dietary advice and were stimulated to increase their level of physical activity. Glucose tolerance, anthropometric characteristics, and substrate use at rest and during exercise were evaluated before and after 1 year. Substrate oxidation was measured at rest and during moderate intensity exercise using indirect calorimetry in combination with stable isotope infusion ([U-^{13}C]palmitate and [6,6-$^{2}H_2$-]glucose).

Results: After 1 year, no differences were seen in substrate use at rest. During exercise, total fat and plasma FFA oxidation were slightly increased in the INT group and decreased in the CON group, with the change being significantly different (change after 1 year: INT, $+2.0 \pm 1.4$ and $+1.9 \pm 0.9$ μmol/kg per minute; CON, -3.5 ± 1.6 and -1.8 ± 0.5 μmol/kg per minute for total and plasma FFA, respectively; $p < 0.05$).

Discussion: A combined diet and physical activity intervention program can prevent further deterioration of impaired FA oxidation during exercise in subjects with IGT.

Key words: impaired glucose tolerance, fatty acid metabolism, lifestyle intervention, stable isotopes, type 2 diabetes

Introduction

Disturbances in skeletal muscle fatty acid (FA)[1] metabolism may play an important role in the development of insulin resistance and type 2 diabetes (1,2). Several studies have shown that the uptake and oxidation of free FA (FFA) are impaired in insulin resistant (viscerally) obese subjects (3) and type 2 diabetic subjects (4–6). An imbalance between uptake and oxidation of plasma FFA could lead to accumulation of lipids within the muscle, which, in turn, is strongly associated with insulin resistance (7,8).

It is difficult to assess the pathophysiological abnormalities leading to type 2 diabetes when the type 2 diabetic state has already developed because it is impossible to differentiate between primary factors and adaptational responses. Considerable weight reduction has not been shown to improve the abnormalities in FA metabolism in type 2 diabetic and obese subjects, suggesting that the abnormal FA metabolism is primary rather than acquired (9). Recent findings that impairments in FA uptake and oxidation are already present in the prediabetic state of impaired glucose tolerance (IGT) (10,11) suggest that these disturbances could play a role in the progression from IGT to type 2 diabetes.

It is well-known that endurance exercise training can alter the capacity to oxidize FAs (12–14). Sial et al. (13) dem-

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[1] Nonstandard abbreviations: FA, fatty acid; FFA, free FA; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; HOMA-IR, index for insulin resistance with the homeostasis model assessment; FFM, fat-free mass; BW, body weight; ARF, acetate recovery factor; GC, gas chromatograph; TTR, tracer-to-tracee ratio; TG, triglyceride; Ra, rate of appearance; Rd, rate of disappearance; ACC, acetyl CoA-carboxylase; HAD, 3-hydroxyacyl-CoA dehydrogenase.

Table 1. Subject characteristics at baseline

	Intervention	Control
Number (men/women)	9 (5/4)	7 (5/2)
Age (years)	54.3 ± 1.8	62.4 ± 1.7*
Body weight (kg)	84.6 ± 3.9	80.1 ± 3.1
Body fat (%)	34.0 ± 2.0	32.9 ± 2.9
BMI (kg/m^2)	29.3 ± 0.6	27.9 ± 1.2
Waist-to-hip ratio	0.97 ± 0.02	0.97 ± 0.03
$V\text{O}_{2\text{max}}$ (L/min)	2.22 ± 0.17	2.22 ± 0.15
Fasting glucose (mM)	6.1 ± 0.5	5.7 ± 0.2
2-Hour glucose (mM)	8.4 ± 0.4	8.4 ± 0.2
Fasting insulin (mU/L)	13.8 ± 2.4	13.5 ± 2.7
HOMA-IR index	3.9 ± 1.0	3.4 ± 0.6

Data are mean ± SE.
* $p < 0.01$ between groups.

onstrated that in elderly subjects, exercise training increased total fat oxidation without a change in lipolysis or FFA availability. Comparable data were found in obese and healthy lean subjects using a low-intensity exercise program for only a few hours weekly (12,14), a regimen much more applicable for (obese) insulin-resistant subjects. Recently, several well-controlled studies clearly showed that lifestyle changes (i.e., changes in dietary and physical activity) substantially reduced the incidence of type 2 diabetes in high-risk subjects (15–17). So far, little to no information is available about the effect of such intervention programs on the impaired FA metabolism found in IGT and type 2 diabetes. In the present study, a group of glucose-intolerant subjects was studied before and after 1 year of a lifestyle intervention program. Our aim was to investigate whether a combined diet and physical activity intervention program altered the capacity for (plasma-free) FA oxidation in subjects with IGT, at rest as well as during exercise.

Research Methods and Procedures

The study on lifestyle intervention and IGT, Maastricht, was designed to study whether a diet/physical activity intervention program can improve glucose tolerance in subjects with a high risk of developing type 2 diabetes. A detailed description of the study can be found elsewhere (18). In the present study, 16 subjects with IGT [nine intervention (INT) subjects, seven controls (CON)] participating in the larger intervention trial underwent additional measurements to evaluate the effect of this lifestyle intervention program on substrate use and oxidation (for characteristics, see Table 1). Subjects had no other reported health problems and did not use any medication that could interfere with substrate metabolism. The Medical Ethical Review Committee of Maastricht University approved the study protocol, and all subjects gave their written informed consent before the start of the study.

Lifestyle Intervention

For a detailed description, see reference 18. Briefly, the intervention program consisted of a dietary and a physical activity component. Dietary recommendations were based on the Dutch guidelines for a healthy diet (±55 energy% carbohydrates; <30 to 35 energy% fat intake, with <10 energy% intake of saturated FAs; a cholesterol intake of <33 mg/MJ; protein intake of 10 to 15 energy%; and an intake of dietary fiber of at least 3 g/MJ). Dietary advice was given by a skilled dietitian on an individual basis every 3 months. Subjects were stimulated to increase their physical activity to at least 30 minutes of moderate physical activity a day for at least 5 d/wk. Individual advice was given on how to increase their daily physical activity (walking, cycling, swimming). Furthermore, subjects were encouraged to participate in an exercise program consisting of components of aerobic exercise training and components of resistance training.

Subjects in the CON group were informed about the beneficial effects of a healthy diet, weight loss, and increased physical activity, but no individual advice or programs were provided. No additional appointments were scheduled.

Methods

Before and after 1 year of the lifestyle intervention program, several measurements were performed.

Glucose Tolerance Test. A standard oral glucose tolerance test (OGTT), with blood sampling at t = 0, 30, 60, and 120 minutes, was performed to measure glucose tolerance. Fasting plasma glucose (millimolar) and insulin concentration (milliunits per liter) were used to calculate an index for insulin resistance with the homeostasis model assessment (HOMA-IR) described by Matthews et al. (19).

Aerobic Capacity. An incremental exhaustive exercise test was performed on an electronically braked bicycle ergometer (Lode, Groningen, The Netherlands) to determine the maximal aerobic power output and oxygen consumption ($V\text{O}_{2\text{max}}$). The test started at a workload of 0.75 W/kg fat-free mass (FFM) for 3 minutes, followed by 3 minutes at 1.5 W/kg FFM. Thereafter, the workload was increased every 3 minutes by 0.5 W/kg FFM until exhaustion (respiratory quotient above 1.1 and no further increase in oxygen uptake). During the experimental trials, subjects exercised before and after 1 year at the same absolute workload, i.e., 55% baseline $V\text{O}_{2\text{max}}$.

Body Composition. Body weight (BW) was determined on an electronic scale; body composition was determined by hydrostatic weighing with simultaneous lung volume mea-

surement (Volugraph 2000; Mijnhardt, Bunnik, The Netherlands), and calculated according to Siri (20). Waist and hip circumference measurements were made to the nearest 1 cm with subjects standing in an upright position, one-half way between the iliac spine and the last rib and at the level of the trochanter major, respectively.

Substrate Use. Subjects participated before and after 1 year in two stable-isotope trials, separated by at least 1 week. Trials were performed in random order. Subjects were asked not to participate in any (exhausting) physical activity the last 3 days before the trials and not to consume any products of high natural ^{13}C-abundance during the last week before both tests because this may disturb the $^{13}C/^{12}C$ measurement in blood and expired air (21).

Experimental Trial. Subjects came to the laboratory at 8 AM after an overnight fast. Two cannulae were inserted, one into an antecubital vein for the infusion of tracers and one in retrograde direction into a contralateral dorsal hand vein for blood sampling. The cannulated hand was placed in a hot box to obtain arterialized venous blood. Background blood and breath samples were taken 30 minutes after placement of the cannulae. At t = 0, an intravenous dose of 0.085 mg/kg BW $NaH^{13}CO_3$ was given to prime the bicarbonate pool, followed by a constant rate continuous infusion of [U-^{13}C]palmitate (0.0067 μmol/kg BW per minute). After 60 minutes, a continuous infusion of [6,6-$^{2}H_2$]glucose (0.3 μmol/kg BW per minute) was started after a priming dose had been given (18 μmol/kg BW per minute). Tracers were administered through a calibrated infusion pump (IVAC560 pump; IVAC, San Diego, CA). During the last 20 minutes of the resting period (t = 100, 110, and 120 minutes), breath and blood samples were taken, and V_{O_2} consumption and V_{CO_2} production were determined. Thereafter, exercise was started for 1 hour (120 to 180 minutes). [U-^{13}C]palmitate infusion was doubled at the start of the exercise. During the last 20 minutes, blood and breath samples were taken, and V_{O_2} consumption and V_{CO_2} production were determined (t = 160, 170, and 180 minutes).

In a second trial, the acetate recovery factor (ARF), necessary for correction of palmitate oxidation rates (22), was determined as described before (11)

Tracers. The [U-^{13}C]palmitate tracer (99% enriched; Cambridge Isotope laboratories, Andover, MA) was dissolved in heated sterile water and passed through a 0.2-μm filter into 5% warm human serum albumin (Central Blood Bank, Leiden, The Netherlands) to make a 0.65 mM solution. The [6,6-$^{2}H_2$]glucose and [1,2-^{13}C]acetate tracer (99% enriched, Cambridge Isotope Laboratories) were dissolved in 0.9% saline to make an 18.7 and 3.0 mM solution, respectively. The exact infusion rates of [U-^{13}C]palmitate, [6,6-$^{2}H_2$]glucose, and [1,2-^{13}C]acetate were determined for each experiment by measuring the concentration of the infusate (see "Biochemical Methods").

Breath, Blood, and Urine Sampling. Breath samples were obtained by having the subjects breathe normally for at least 3 minutes into a mouthpiece connected to a 6.75-liter mixing chamber. Breath samples were collected into a 20-mL Vacutainer tube (Becton Dickinson, Meyland Cedex, France) to determine the enrichment of CO_2 ($^{13}C/^{12}C$ ratio). V_{O_2} and V_{CO_2} were determined by means of open-circuit spirometry (Oxycon Beta; Mijnhardt). Arterialized blood samples were collected in EDTA-containing tubes and were immediately centrifuged at 3000 rpm at 4 °C; the plasma was frozen in liquid nitrogen and stored at −80 °C until analysis. Urine was collected overnight to determine nitrogen excretion for calculating the non-protein respiratory exchange ratio.

Biochemical Methods. Breath samples were analyzed for $^{13}C/^{12}C$ ratio by injecting 20 μL of the gaseous headspace into a gas chromatograph (GC)-isotope ratio mass spectrometer (Finnigan MAT 252; Finnigan, Bremen, Germany). Total plasma FFA, glucose, and infusate acetate concentrations were measured using standard enzymatic techniques (for FFA, FFA-C test kit, Wako Chemicals, Neuss, Germany; for glucose, Roche Unikit III, Hoffman-La Roche, Basel, Switzerland; for acetate, kit no. 148261, Boehringer Mannheim, Mannheim, Germany). Insulin concentration during the experimental trial was measured using a double-antibody radioimmunoassay (Insulin RIA-100; Kabi Pharmacia, Uppsala, Sweden); plasma insulin levels during the OGTT were measured with an enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). For the determination of plasma palmitate concentration and enrichment, FFAs were extracted from plasma, isolated by thin-layer chromatography, and derivatized to their methyl esters. Palmitate concentrations were determined on an analytical GC with ion-flame detection using heptadecanoic acid as an internal standard; on average, palmitate concentration was 27 ± 1% of total FFA concentration. Isotopic enrichment of palmitate was determined by GC-isotope ratio mass spectrometer after on-line combustion of FAs to CO_2 (Finnigan MAT 252), with correction for the extra methyl group in the derivate. The concentration of infusate palmitate was determined as described above for plasma samples. For determination of glucose enrichment in plasma, aliquots of EDTA plasma were extracted with methanol:chloroform and chloroform:water. The clear water layer was dried, and a butylboronic acid-acetyl derivate was made. Subsequently, the enrichment of the glucose derivate was determined by electron ionization/gas chromatography mass spectrometry (Finnigan INCOS XL; Finnigan, San Jose, CA).

Calculations. Metabolic rate was calculated from V_{O_2} and V_{CO_2} according to the equation of Weir (23). Carbohydrate and fat oxidation rates were calculated from V_{O_2} and V_{CO_2} and urinary nitrogen excretion (24). Protein oxidation (as calculated from nitrogen excretion) was assumed to be

similar during the overnight fasted state and during exercise. Total FA oxidation was calculated by converting the rate of fat oxidation [triglyceride (TG) oxidation] to its molecular equivalent, with the assumption of the average molecular weight of TG to be 860 g/mol. Subsequently, the molar rate of TG oxidation was multiplied by three to obtain FA oxidation.

Enrichment of breath CO_2 and plasma palmitate, acetate, and glucose is given as the tracer-to-tracee ratio [TTR; $=(^{13}C/^{12}C)$sample $-$ $(^{13}C/^{12}C)$background]. Fractional recovery of label in breath CO_2, derived from the infusion of labeled acetate, was calculated as follows: acetate recovery = $(TTRCO_2 \times Vco_2)/2F$, where F is the infusion rate of acetate, and the number 2 in the denominator is to correct for the number of ^{13}C molecules in acetate.

Rate of appearance (Ra) and rate of disappearance (Rd) were calculated according to Steele's equation for steady state (palmitate at rest) and Steele's single-pool non-steady state equations adapted for use with stable isotopes (palmitate during exercise and glucose at rest and during exercise). Volume of distribution was assumed to be 0.040 L/kg for palmitate and 0.160 L/kg for glucose. Ra and Rd of FFA were calculated by dividing palmitate Ra and Rd by the fractional contribution of palmitate to the total FFA concentration. Percentage of infused [U-^{13}C]palmitate oxidized was calculated with the formula: percentage infused tracer oxidized = $((TTR\ CO_2 \times Vco_2)/(16 \times F \times$ acetate recovery$)) \times 100\%$, where F is the infusion rate of palmitate, and the number 16 in the denominator is to correct for the number of ^{13}C molecules in palmitate. Plasma FFA oxidation was calculated as: Rd FFA $\times$ percentage of infused palmitate tracer oxidized, and TG-derived FA oxidation was calculated as: total FA oxidation $-$ plasma FFA oxidation. During exercise, the Rd of glucose is identical to the measured oxidation rate (25); therefore, muscle glycogen oxidation rates during exercise are calculated as: total glucose oxidation $-$ plasma glucose oxidation.

Statistical Analysis

Data are presented as means $\pm$ SE. Oxidation rates and Ra and Rd are expressed as micromoles per kilogram of FFM per minute. Differences between groups were analyzed with a two-tailed Student's *t* test for unpaired data, and changes within groups were analyzed with a two-tailed Student's *t* test for paired data. Changes in concentration of metabolites over time between groups were analyzed with a two-way repeated-measures ANOVA. Statistical significance was set at $p < 0.05$.

Results

Subject Characteristics

No differences were found in baseline BW, BMI, body composition, and maximal aerobic capacity between groups (see Table 1). Subjects in the CON group were of older age (INT, 54.3 $\pm$ 1.8 years; CON, 62.4 $\pm$ 1.7 years; $p < 0.01$). Baseline glucose tolerance and insulin resistance were comparable (see Table 1).

Table 2. Change in subject characteristics

	Intervention ($n = 9$)	Control ($n = 7$)
Body weight (kg)	-0.8 ± 1.1	$+1.0 \pm 0.9$
Body fat (%)	-0.3 ± 0.9	$+0.2 \pm 1.0$
BMI (kg/m^2)	-0.3 ± 0.4	$+0.3 \pm 0.4$
Waist-to-hip ratio	-0.02 ± 0.01	$+0.02 \pm 0.01$*
$V_{O_{2max}}$ (L/min)	$+0.03 \pm 0.06$	-0.03 ± 0.07
Fasting glucose (mM)	$+0.1 \pm 0.1$	$+0.1 \pm 0.1$
2-Hour glucose (mM)	$+0.3 \pm 0.4$	$+0.5 \pm 0.8$
Fasting insulin (mU/L)	-0.4 ± 1.5	$+2.9 \pm 2.4$
HOMA-IR index	-0.1 ± 0.5	$+0.8 \pm 0.6$

Data are mean $\pm$ SE.
* $p < 0.01$ between groups.

After 1 year, BW, body composition, aerobic capacity, and insulin resistance showed a tendency to improve in the INT group; however, differences between groups were not statistically significant (Table 2). Waist-to-hip ratio decreased more in the INT group compared with the CON group (INT, -0.02 ± 0.01; CON, $+0.02 \pm 0.01$; $p < 0.05$).

Relative workload at baseline and after 1 year of intervention was comparable between groups (INT, before 51.5 $\pm$ 2.1%, after 52.6 $\pm$ 3.0% $V_{O_{2max}}$; CON, before 56.5 $\pm$ 1.3%, after 57.4 $\pm$ 3.0% $V_{O_{2max}}$). During the last 20 minutes of exercise, oxygen consumption had reached a plateau.

Arterialized Concentrations of Metabolites

Table 3 depicts the concentration of circulating glucose, FFA, and insulin at rest and during the last 20 minutes of exercise before and after 1 year, for both groups. No differences were observed between groups. FFA levels increased and insulin levels decreased throughout the exercise period to the same extent in both groups (ANOVA time effect, $p < 0.05$). Plasma palmitate, expressed as a fraction of total FFA, was not different between groups either before or after 1 year (mean 27 $\pm$ 1% of total FFA).

Tracer Kinetics

Plasma glucose TTR at rest and during exercise and plasma palmitate TTR during exercise slightly decreased over time (data not shown); therefore, Steele's non-steady state equations were used to calculate substrate kinetics. No differences were observed between groups in plasma palmi-

Table 3. Circulating metabolites

		Exercise			ANOVA		
	Rest	160	170	180	Group	Time	Inter.
Glucose (mM)							
Before							
INT	5.6 ± 0.3	5.4 ± 0.2	5.4 ± 0.2	5.3 ± 0.2	—	—	—
CON	5.3 ± 0.1	5.4 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	—	—	—
After							
INT	5.8 ± 0.5	5.6 ± 0.3	5.5 ± 0.3	5.5 ± 0.3	—	—	—
CON	5.5 ± 0.1	5.4 ± 0.1	5.4 ± 0.2	5.5 ± 0.2	—	—	—
FFA (μM)							
Before							
INT	718 ± 105	733 ± 103	818 ± 110	874 ± 121			
CON	641 ± 69	742 ± 84	856 ± 90	859 ± 72	—	<0.01	—
After							
INT	708 ± 80	736 ± 96	813 ± 98	852 ± 95			
CON	681 ± 93	700 ± 70	806 ± 85	885 ± 99	—	<0.01	—
Insulin (mU/L)							
Before							
INT	9.4 ± 2.0	8.2 ± 1.2	8.2 ± 1.4	6.5 ± 0.6			
CON	9.5 ± 1.2	7.0 ± 0.8	6.8 ± 0.9	6.7 ± 0.8	—	<0.05	—
After							
INT	11.2 ± 2.1	9.2 ± 1.4	8.8 ± 0.4	9.0 ± 1.2			
CON	11.7 ± 1.4	9.5 ± 1.0	9.6 ± 0.9	8.5 ± 0.9	—	<0.01	—

Date are mean ± SE. inter., group × time interaction.
160, 170, and 180 are mintues into the trial and represent the last 20 minutes of the 60-minute exercise period of the trial.

tate and glucose TTR. Furthermore, no differences were found between baseline and year 1 in plasma palmitate and glucose TTR (data not shown). No significant differences in ARF were observed between groups at rest (before, 23.8 ± 1.0% vs. 21.8 ± 1.2%; after, 22.9 ± 1.0% vs. 21.3 ± 0.9% for INT and CON, respectively) or during exercise (before, 60.7 ± 3.3% vs. 61.0 ± 1.9%; after, 59.4 ± 3.9% vs. 68.3 ± 2.4% for INT and CON, respectively).

Energy Expenditure and Substrate Oxidation

Energy expenditure was comparable between groups and did not significantly differ before and after 1 year, at rest and during moderate intensity exercise (data not shown). Ra and Rd of glucose and FAs were comparable between groups, before and after 1 year of the lifestyle intervention program (see Table 4).

Oxidation rates are given in Table 5 (expressed as micromoles per kilogram of FFM per minute) and Figure 1 (expressed as relative contribution to energy expenditure during exercise). Total FA oxidation during exercise was slightly elevated in the CON group at baseline ($p < 0.05$). However, when corrected for total energy expenditure, no differences were observed between groups in substrate oxidation at baseline (Figure 1). After 1 year of intervention, resting carbohydrate oxidation was lower in the INT group, without a significant change in FA oxidation. During exercise, total carbohydrate and glycogen oxidation were increased in the CON group, whereas these variables slightly decreased in the INT group, with the change after 1 year being significantly different between groups ($p < 0.05$; Table 4 and Figure 1). Total FA and plasma FFA oxidation during exercise were increased in the INT group and decreased in the CON group (p for difference in change $<$ 0.05). After 1 year, subjects in the INT group tended to rely more on FA oxidation during exercise compared with CON (INT, 53.0 ± 4.2% vs. CON, 43.1 ± 1.8% of total energy expenditure; $p = 0.07$; see Figure 1).

Discussion

Several studies have shown that the uptake and oxidation of FFA are impaired in type 2 diabetic patients (4,5) and in

Table 4. Substrate kinetics for intervention ($n = 9$) and control ($n = 7$) groups

	Baseline		1 Year	
	Intervention	**Control**	**Intervention**	**Control**
Rest				
Plasma Glucose Ra	16.5 ± 1.5	17.5 ± 0.7	15.8 ± 1.5	16.6 ± 1.3
Plasma Glucose Rd	17.4 ± 1.3	17.7 ± 0.5	17.2 ± 1.7	17.1 ± 1.3
Plasma FFA flux	14.6 ± 2.1	12.1 ± 1.3	15.7 ± 1.8	12.2 ± 1.4
Exercise				
Plasma glucose Ra	27.4 ± 1.6	29.5 ± 2.9	26.0 ± 2.3	29.3 ± 2.7
Plasma glucose Rd	27.7 ± 1.8	29.3 ± 3.0	26.7 ± 2.5	28.4 ± 2.9
Plasma FFA Ra	22.5 ± 2.6	21.5 ± 1.8	24.3 ± 2.3	21.8 ± 1.5
Plasma FFA Rd	22.5 ± 2.6	21.5 ± 1.8	24.3 ± 2.3	21.8 ± 1.5

Data are mean ± SE and are expressed as micromoles per kilogram of FFM per minute.

the prediabetic condition of IGT (11). In the present study, subjects with IGT were restudied after 1 year of a lifestyle intervention program. The most important finding was that subjects in the INT group maintained their capacity to oxidize (plasma free) FAs, whereas in the CON group, (plasma free) FA oxidation was decreased. Thus, a combined diet and physical activity intervention program may be able to prevent a further impairment in FA use in subjects with IGT.

Methodological Considerations

FA kinetics were determined with use of the stable isotope [U-^{13}C]palmitate in combination with indirect calorimetry. To correct for the loss of ^{13}C label due to fixation in products of the tricarboxylic acid cycle and the bicarbonate pool, Sidossis introduced the ARF (26). Failure to use the ARF may lead to a substantial underestimation of plasma FFA oxidation. The ARF needs to be determined in every subject under the same circumstances and at the same

Table 5. Fuel oxidation rates for intervention ($n = 9$) and control ($n = 7$) groups

	Baseline		1 Year		Change	
	Intervention	**Control**	**Intervention**	**Control**	**Intervention**	**Control**
Rest						
Carbohydrate	10.4 ± 1.1	11.9 ± 0.8	8.2 ± 1.1	11.4 ± 0.8†	−2.2 ± 1.4	−0.5 ± 1.2
Total FA	3.8 ± 0.5	3.2 ± 0.1	3.7 ± 0.3	3.1 ± 0.3	−0.2 ± 0.6	−0.1 ± 0.4
Plasma FFA	5.5 ± 0.8	4.4 ± 0.4	5.5 ± 0.5	4.7 ± 0.8	+0.0 ± 0.6	+0.3 ± 0.7
TG-derived FA	−1.6 ± 0.5	−1.2 ± 0.5	−1.8 ± 0.5	−1.7 ± 0.7	−0.2 ± 0.5	−0.4 ± 0.6
Exercise						
Carbohydrate	71.5 ± 8.0	74.8 ± 9.2	67.7 ± 9.5	90.4 ± 6.5*	−3.9 ± 5.7	+15.6 ± 4.5†
Plasma glucose	27.7 ± 1.8	29.3 ± 3.0	26.7 ± 2.5	28.4 ± 2.9	−0.9 ± 1.4	−0.8 ± 2.0
Glycogen	43.9 ± 6.7	45.5 ± 8.2	40.9 ± 8.1	62.0 ± 7.1*	−2.2 ± 5.6	+16.5 ± 3.4†
Total FA	16.7 ± 1.6	21.5 ± 1.2	18.7 ± 1.2	18.0 ± 1.3	+2.0 ± 1.4	−3.5 ± 1.6†
Plasma FFA	15.7 ± 1.7	17.9 ± 1.7	17.6 ± 1.5	16.0 ± 1.7*	+1.9 ± 0.9	−1.8 ± 0.5‡
TG-derived FA	1.0 ± 1.5	3.6 ± 1.3	1.2 ± 1.6	2.0 ± 1.6	+0.1 ± 1.8	−1.6 ± 1.2

Data are mean ± SE and are expressed as micromoles per kilogram of FFM per minute.
* $p < 0.05$ for difference between baseline and 1 year within groups.
† $p < 0.05$ for difference between groups.
‡ $p < 0.01$ for difference between groups.

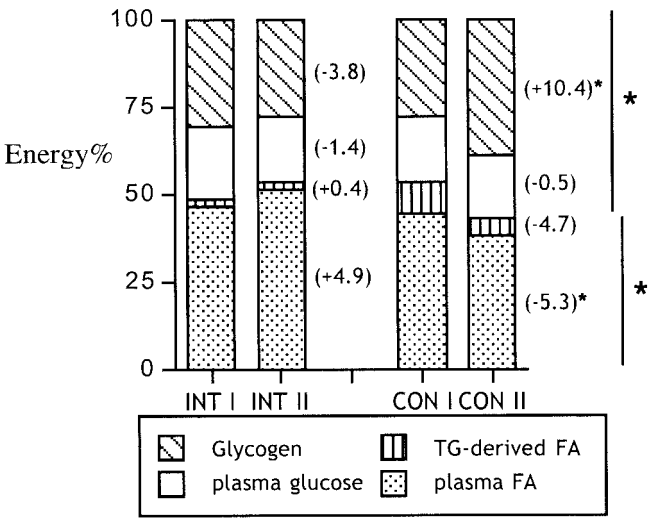

Figure 1: Fuel oxidation during exercise for INT ($n = 9$) and CON ($n = 7$) groups. Data are expressed as relative contribution to total energy expenditure (Energy%). Change over time is depicted between parentheses. * $p < 0.05$ for difference in change over time between groups.

time-points as the palmitate infusion test because of a large inter-individual variation in the ARF, especially during exercise (22).

Part of the (labeled) palmitate taken up by muscle at rest can be incorporated into intramyocelluar TGs. These labeled FAs can be oxidized during exercise and appear as $^{13}CO_2$ in breath. Although intramyocelluar TG is used as an energy substrate during exercise, its relative contribution is not completely clear. Thus, a small proportion of the $^{13}CO_2$ appearing in breath during exercise can be due to TG-derived FA oxidation and not plasma FFA oxidation. This overestimation of plasma FFA oxidation made during exercise introduces only a small error, which has little impact on the interpretation of our results.

The FFA kinetic data presented in this manuscript are quite comparable with results obtained, and published, in earlier studies in our laboratory, with comparable protocols and patients (11,14,27). However, compared with findings in other studies, FFA flux at rest is relatively high (28–30). Several factors can underlie this difference, i.e., metabolic status (glucose-intolerant vs. normoglycemic subjects), period of measurement, protocol (stable isotopes vs. radioactive isotopes; arterialized venous blood sampling vs. arterial sampling), and subject characteristics (sex; aerobic capacity). In the present study, substrate kinetics were measured before and after intervention in experimental and CON groups. Conclusions are based on observed changes within groups and between groups.

Lifestyle Intervention

The intervention strategy we used in this study was based on general public health recommendations for dietary intake and physical activity (18). Such a regimen is much more suitable for preventing diabetes because it is less time consuming and much better tolerated than very intensive tightly controlled intervention programs. Dietary intake was monitored with a 3-day food record. However, this method does not give valid results for small groups (as was the case in this study). In the larger group from which the subjects were selected, evaluation of the nutrient intake data clearly showed beneficial changes: a decrease in total and saturated fat intake and an increase in fiber intake [see Mensink et al. (17)].

As a consequence of our intervention strategy, changes in subject characteristics were less profound as compared with more intense diet and exercise programs. Changes in body composition and glucose homeostasis after 1 year of intervention found in the present study, however, reflected the results seen after 1 year in the larger intervention trial [INT group ($n = 47$), BW, -2.7 ± 0.5 kg; $V_{O_{2max}}$, $+0.10 \pm 0.03$ L/min; 2-hour glucose, -0.8 ± 0.3 mM; CON group ($n = 55$), BW -0.2 ± 0.5 kg; $V_{O_{2max}}$, -0.0 ± 0.03 L/min; 2-hour glucose, $+0.2 \pm 0.3$ mM; $p < 0.05$ for difference in change; see Mensink et al. (17)], indicating that to detect statistically significant differences in such parameters as glucose tolerance (measured by an OGTT) and body composition, larger groups are required. Nevertheless, for the purpose of this study, substrate use and oxidation measured by stable isotopes, group size is sufficient.

Substrate Oxidation

The main novel finding of the present study was that, after 1 year of a lifestyle intervention program, subjects in the INT group maintained their capacity to oxidize (plasma-free) FAs during exercise, whereas in the CON group, (plasma-free) FA oxidation was decreased. Due to the slightly higher total FA oxidation in the CON vs. INT groups at baseline (Table 5), it cannot be excluded that some regression to the mean underlies the observed changes. However, the altered fat oxidative capacity is supported by the observation in a comparable group of subjects on the same intervention program that lifestyle changes resulted in changes in expression of genes and proteins, reflecting a better capacity to oxidize FAs (26). In that study, a down-regulation of acetyl CoA-carboxylase (ACC)-2 and an increase in 3-hydroxyacyl-CoA dehydrogenase (HAD) protein content in skeletal muscle were observed in the INT group, compared with an increase of ACC2 and no change in HAD in the CON group (31).

It has been shown that substantial weight loss does not improve the impaired capacity to use plasma FFA in type 2 diabetic subjects (9). Our data indicate that a combined program of aerobic exercise, changes in diet composition,

and weight loss may be able to compensate for the impaired oxidation of (plasma-free) FAs, compared with the lack of improvement found after weight loss per se. It is possible that the positive effects on fat metabolism can be ascribed to the inclusion of physical activity in the program. Indeed, it has been shown that aerobic exercise training can increase fat oxidation in different populations (12–14). A 16-week training program increased fat oxidation during exercise in elderly subjects (mean age 74 years), which was likely related to alterations in skeletal muscle FA metabolism (13). Comparable data were found in healthy lean (12) and obese (14) men after low-intensity training sessions for only a few hours weekly, a regimen more comparable with the present intervention program. The observed maintenance or even slight improvement of the capacity to oxidize (plasma-free) FAs in the INT group suggests that the positive effects of exercise can also be translated to the prediabetic condition of IGT. Secondly, besides weight reduction, the diet intervention program was directed toward a reduction of the amount of fat, particularly saturated fat, in the diet. Changes in dietary composition can also have an effect on substrate use and oxidation. For example, a change in the dietary polyunsaturated-to-saturated fat ratio can modulate the oxidation of fat and carbohydrate not only acutely after a meal but also after chronic feeding (32).

Thus, the present study shows, for the first time, to our knowledge, that the combination of an increased level of physical activity, weight loss, and a change in dietary intake can slow down the worsening of disturbances in FA metabolism in the prediabetic condition of IGT. This could be important in relation to the development of insulin resistance and the progression from IGT to type 2 diabetes.

Underlying Mechanisms

Several mechanisms could be responsible for the diminished capacity of the (pre)diabetic muscle to oxidize FAs. An increased concentration of malonyl-CoA, possibly due to dysregulation by ACC and malonyl CoA decarboxylase or hyperglycemia, leading to inhibition of carnitine-palmitoyl transferase-1, has been suggested to explain the diminished ability to oxidize FAs (2,33). As stated earlier, we observed that in a comparable group of subjects, lifestyle changes resulted in a down-regulation of ACC-2 in skeletal muscle (31). In line with this, Schrauwen et al. (12) showed that a minimal amount of physical activity significantly decreased ACC-2 mRNA expression in human skeletal muscle and increased fat oxidation. Furthermore, endurance training increases skeletal muscle capillarization and mitochondrial density (34) and increases the activity of several enzymes involved in FA transport, transfer of FAs into mitochondria (carnitine-palmitoyl transferase), and β-oxidation (HAD) (35,36). Muscle biopsy data indicate that the insulin-resistant skeletal muscle is characterized by a diminished content of oxidative enzymes (5,37). Thus, an increase in (mitochondrial) enzyme activity could underlie the differences in FA oxidation between groups found in this study after 1 year of intervention.

In conclusion, after 1 year of a combined diet and physical activity intervention program, the INT group showed a maintenance of the capacity to oxidize (plasma-free) FAs during exercise as compared with a reduced capacity in the CON group. This indicates that a lifestyle intervention program can prevent a further deterioration of disturbances in FA metabolism found in subjects with IGT.

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