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Lifestyle changes and lipid metabolism gene expression and protein content in skeletal muscle of subjects with impaired glucose tolerance

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Abstract

Aims/hypothesis. Skeletal muscle of pre-diabetic patients is characterised by a diminished capacity to handle fatty acids. A diminished content of several enzymes involved in fatty-acid transport and oxidation have been suggested to underlie these defects. The aim of this study was to investigate whether the combination of dietary advice, increased physical activity and weight loss improves lipid metabolic gene and protein expression in skeletal muscle of subjects with impaired glucose tolerance.

Methods. Before and after 1 year of a lifestyle-intervention programme, expression of several genes and proteins involved in lipid metabolism were measured in *vastus lateralis* muscle biopsies from subjects in the intervention ($n=7$) and control group ($n=6$).

Results. After 1 year the intervention group had an improved glycaemic control and reduced body fat compared to the control group. Significant differences

were observed for acetyl CoA-carboxylase 2 and uncoupling protein 2 expression (ACC2: $-16.8\pm 12.4\%$ vs $+51.5\pm 32.3\%$ for the intervention and control group respectively; $p<0.05$) (UCP2: $-26.9\pm 10.3\%$ vs $+10.5\pm 6.2\%$ for the intervention and control group respectively; $p<0.05$). Change in 3-hydroxyacyl-CoA dehydrogenase protein content tended to be different between groups ($+3.2\pm 1.1$ vs -0.9 ± 1.9 U/mg.ww for the intervention and control group, $p=0.07$).

Conclusions/interpretation. Lifestyle changes leading to an improved glycaemic control and reduced adiposity, resulted in a down-regulation of ACC-2 and UCP2 expression and in an increase in HAD protein content, reflecting a better capacity to utilise fatty acids. [Diabetologia (2003) 46:1082–1089]

Keywords Impaired glucose tolerance, acetyl CoA carboxylase, muscle biopsy, lipid metabolism, uncoupling protein, lifestyle-intervention, fatty acids, fat oxidation, insulin resistance.

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Abbreviations: FFM, Fat free mass; CPT-2, carnitine palmitoyl transferase-2; CPT-1m, carnitine palmitoyl transferase-1 muscle form; ACC-2, acetyl Co-enzyme A carboxylase-2; LPL, lipoprotein lipase; PPAR α , peroxisome proliferator-activated receptor- α ; UCP-2, uncoupling protein 2; UCP-3, uncoupling protein 3; FAT/CD36, fatty acid translocase/CD36; FABPc, cytosolic fatty acid binding protein; CS, citrate synthase; HAD, 3-hydroxyacyl-coA dehydrogenase; MJ, megajoule.

Skeletal muscle has an important role in the overall utilisation and oxidation of fatty acids because of its large mass and because lipids are the principal oxidative substrate after an overnight fast and during moderate exercise. Type 2 diabetes mellitus is characterised by abnormalities in skeletal muscle glucose and lipid metabolism [1]. Uptake and oxidation of NEFA are diminished under postabsorptive conditions in Type 2 diabetes [2, 3] and IGT [4], whereas during postprandial conditions there is impaired suppression of muscle NEFA uptake in Type 2 diabetes [2], favouring increased storage of lipids. Indeed, the diabetic muscle is characterised by increased levels of triacylglycerols (intramuscular triacylglycerols, IMTG) [5, 6], which is strongly associated with the degree of in-

sulin resistance, even in non-diabetic subjects [7, 8]. Thus, alterations in fatty-acid metabolism can play an important role in the development of insulin resistance and Type 2 diabetes mellitus [1, 9].

Several mechanisms have been suggested to underlie the diminished capacity of the pre-diabetic muscle to oxidise fatty acids. Several studies have shown that the insulin-resistant skeletal muscle is characterised by a diminished content of proteins or enzymes involved in fatty-acid transport (cytosolic fatty acid binding protein, FABPc), β -oxidation (3-hydroxy fatty-acyl CoA dehydrogenase, HAD) and the Krebs cycle (citrate synthase, CS) [3, 10]. The metabolic capacity of the insulin-resistant skeletal muscle seems to be organised towards fat esterification rather than oxidation [10]. Reduced activity of the rate-limiting step in fatty acid oxidation, carnitine-palmitoyl transferase 1 (CPT-1), either to decreased expression or more pronounced inhibition by malonyl-CoA, has been suggested to explain the diminished ability to oxidise fatty acids [11]. Finally, a role is suggested for uncoupling proteins (UCP) in the disturbed handling of fatty acids in Type 2 diabetes [12, 13].

Weight reduction is known to improve insulin sensitivity and reduce the degree of glucose intolerance. However substantial (dietary-induced) weight reduction did not improve the disturbed plasma-derived fatty acid oxidation in Type 2 diabetic subjects [14]. Neither did weight reduction correct the metabolic derangement's of skeletal muscle partitioning fat towards esterification rather than oxidation, in obese non-diabetic subjects [10]. In contrast, physical exercise is a potent enhancer of insulin sensitivity and also increases the capacity of the skeletal muscle to oxidise fatty acids. The enhanced fat oxidation after exercise training is associated with upregulation of genes involved in fatty-acid uptake such as membrane-associated fatty-acid binding protein (*FABPpm*) and fatty-acid translocase (*FAT/CD36*) [15, 16], and genes involved in fatty-acid oxidation, as *CPT-1*, acetyl-CoA carboxylase (*ACC*) and *HAD* [16, 17, 18]. Therefore, combining exercise, dietary advice and weight-loss could be an effective strategy because this would have the potential to compensate for the impaired capacity to oxidise fatty acids and stimulate expression of genes involved in lipid metabolism in skeletal muscle of insulin resistant subjects, in comparison to the lack of improvement after weight-loss alone. In addition, the intake of nutrients is another important environmental factor regulating gene expression. Especially the fatty-acid composition of dietary lipids has a marked effect on gene expression, and influences fuel homeostasis in liver and skeletal muscle [19].

We addressed whether the combination of dietary advice, increased physical activity and moderate weight loss, a so-called lifestyle-intervention, improves the lipid metabolic mRNA and protein profile of skeletal muscle in subjects with IGT. Epidemiologi-

cal studies have shown that such a lifestyle intervention program, based on general public health recommendations reduces the progression from IGT to diabetes with almost 60% over a 3-year period [20, 21]. Before and after 1 year of a lifestyle-intervention program, gene and protein expression related to skeletal muscle lipid metabolism was measured in biopsies from the *m. vastus lateralis*.

Subjects and methods

Subjects and design. The Study on Lifestyle-intervention and IGT, Maastricht (SLIM) was designed to study whether a diet and physical activity intervention program can improve glucose tolerance in subjects with a high risk of developing Type 2 diabetes mellitus. A detailed description of the study can be found elsewhere [22]. In the present study, thirteen subjects with IGT participating in the larger intervention trial underwent additional measurements to evaluate the effect of this lifestyle-intervention program on skeletal-muscle gene expression and protein content, seven from the intervention group (four male, three female) and six subjects from the control group (six male). Subjects had no health problems and did not use any medication which could interfere with substrate metabolism. Before and after 1 year of a lifestyle-intervention program, glucose tolerance, body composition and maximal aerobic capacity was measured and muscle biopsies were taken. Due to inadequate biopsy material we could not measure mRNA and protein concentration in one subject from the control group. The Medical Ethics 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. The intervention program involved changes in diet and physical activity. Dietary recommendations were based on the Dutch guidelines for a healthy diet (± 55 energy % carbohydrates; <30 – 35 energy % fat intake, with <10 energy % intake of saturated fatty acids; a cholesterol intake of less than 33 mg per megajoule (MJ); protein intake of 10–15 energy% and an intake of dietary fibre of at least 3 g per MJ). The goal for body weight was a BMI of less than 25 kg/m², but for practical reasons a body weight loss of 5 to 10 kg was aimed at depending on the degree of obesity. Dietary advice was given by a skilled dietician on an individual basis every 3 months. Subjects were stimulated to increase their physical activity to at least 30 min of moderate physical activity per day for at least 5 days a week. Individual advice was given on how to increase their daily physical activity (walking, cycling, swimming) and goals were set. Furthermore, subjects were encouraged to participate in a supervised exercise program, consisting of components of aerobic exercise training and components of resistance training. Participation in both, the dietary visits and the exercise sessions was recorded.

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

Measurements. A standard 75-g OGTT, with blood-sampling at $t=0$, $t=30$, $t=60$ and $t=120$ min, was done to measure glucose tolerance and insulin concentrations. Body weight was determined on an electronic scale; body composition was determined by hydrostatic weighing with simultaneous lung volume

Table 1. List of primers used for the different RT-PCR assays

Names	Forward primers	Reverse primers
PPAR α	5'-GCT-ATC-ATT-ACG-GAG-TCC-ACG-3'	5'-AGC-TGC-GGT-CGC-ACT-TGT-CA-3'
UCP2	5'-GAC-CTA-TGA-CCT-CAT-CAA-GG-3'	5'-ATA-GGT-GAC-GAA-CAT-CAC-CAC-G-3'
UCP3	5'-ATG-GAC-GCC-TAC-AGA-ACC-AT-3'	5'-CTG-GGC-CAC-CAT-CTT-TAT-CA-3'
m-CPT1	5'-TAC-AAC-AGG-TGG-TTT-GAC-A-3'	5'-CAG-AGG-TGC-CCA-ATG-ATG-3'
CPT2	5'-TTA-AAG-ACA-GCA-CTC-AGA-CC-3'	5'-GTT-TTC-ATG-GTG-GCA-TCA-AAC-3'
ACC2	5'-GG-TGG-AGA-TGA-ACC-GAC-TTC-3'	5'-ACT-CTC-CTC-CTT-CCT-CGA-TG-3'
LPL	5'-GGT-CGA-AGC-ATT-GGA-ATC-CAG-3'	5'-TAG-GGC-ATC-TGA-GAA-CGA-GTC-3'

measurement (Volugraph 2000, Mijnhardt, Bunnik, the Netherlands), and calculated according to [23]. We measured the waist circumference of the subjects, while they were standing, to the nearest 1 cm half way between the spina iliaca and the last rib.

The subjects underwent an incremental exhaustive exercise test on an electronically braked bicycle ergometer to determine maximal aerobic capacity ($VO_{2\max}$). The test started at a workload of 0.75 W per kg fat free mass (FFM) for 3 min, followed by 3 min at 1.5 W per kg FFM. Thereafter, the workload was increased every 3 min by 0.5 W per kg FFM until the subjects became exhausted (RQ above 1.1 and no further increase in oxygen uptake).

Needle muscle biopsies were taken before and after 1 year, percutaneously from the *vastus lateralis* muscle after an overnight fast, using the Bergstrom method with suction [24]. Biopsies were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Biochemical methods. Blood samples were collected in sodium fluoride (glucose) or heparin (insulin) containing tubes on ice and were immediately centrifuged at 4°C ; plasma was stored at -80°C until analysis. Plasma glucose concentration was measured with a standard enzymatic technique automated on the Cobas Fara centrifugal analyser (Glucose HK 125, ABX diagnostics, Montpellier, France). Plasma insulin concentration was measured with an ELISA assay (Mercodia, Uppsala, Sweden), which shows no cross-reactivity with pro-insulin.

Quantification of mRNA levels. For determination of the expression of carnitine palmitoyl transferase-2 (CPT-2), carnitine palmitoyl transferase-1 muscle form (CPT-1m), acetyl Co-enzyme A carboxylase-2 (ACC-2), lipoprotein lipase (LPL), peroxisome proliferator-activated receptor- α (PPAR α), uncoupling protein 2 (UCP-2), uncoupling protein 3 (UCP-3), total RNA was isolated using the acid phenol method of Chomczynski and Sacchi [25] with an additional DNase digestion step with concomitant acid phenol extraction and ethanol precipitation. The level of specific mRNA was determined using either RT-competitive PCR assays (CPT2 and ACC2) or real-time RT-PCR assays (CPT1muscle, LPL, PPAR α , UCP2 and total UCP3). For both competitive and real-time PCR, the first strand cDNA was synthesised with the specific reverse primers and from 0.1 μg of total RNA in experimental conditions that warranted optimal efficiency of the reaction [26]. During the competitive PCR, Cy-5 5'-end labelled sense primers were used to generate fluorescent PCR products that were analysed with an automated laser fluorescence DNA sequencer (ALFexpress, Pharmacia, Uppsala, Sweden) in 4% denaturing polyacrylamide gels. The initial concentration of target mRNA was determined at the competition equivalence point as described previously [26]. The real-time PCR was done by using a LightCycler (Roche Diagnostics, Meylan, France) with the FastStart

DNA Master SYBR Green kit (Roche Diagnostics). After amplification, a melting curve analysis was done to verify the specificity of the reaction. For quantification, a standard curve was systematically generated with six different amounts (150 to 30 000 molecules/tube) of purified target cDNA cloned in the pGEM plasmid (Promega). The LightCycler software (Roche Diagnostics) was used for the analysis. The list of the primers used for the different RT-PCR assays is available in Table 1.

Protein expression. To determine the protein content, muscle biopsies were homogenised in ice-cold tris-EDTA buffer at pH 7.4. Homogenates were subsequently sonicated for 15 s, four times. Membrane protein fatty acid translocase/CD36 (FAT/CD36) was measured by an sandwich-type ELISA [27]. To determine the cytosolic proteins, the homogenates were centrifuged at 10 000 g for 2 min to remove cell debris (of membranes). Tissue content of muscle-type cytosolic fatty acid binding protein (FABPc) in skeletal muscle was measured by means of an ELISA (Hycult Biotechnology, Uden, Netherlands), using recombinant human FABPc as standard [28]. Citrate synthase (CS) was determined according to the method of Sherperd and Garland [29], whereas 3-hydroxyacyl-coA dehydrogenase (HAD) was assayed according to Bergmeyer [30].

Statistical analysis. Data are presented as means \pm SEM. Differences between groups were analysed with a two-tailed Student's *t* test for unpaired data. Pearson correlation coefficients were calculated to assess the relation between changes in body weight, waist circumference, $VO_{2\max}$ and 2-h glucose on one side and changes in expression of genes and proteins on the other side. Statistical significance was set at a *p* value of less than 0.05.

Results

Subject characteristics. All 13 subjects participating in this study were participants from an ongoing intervention trial, studying the effect of lifestyle changes on glucose tolerance [22]; seven from the intervention group (four men, three women) and six from the control group (six men). Baseline characteristics are depicted in Table 2. There were no statistically significant differences in baseline characteristics between groups. All seven subjects in the intervention group were compliant to the intervention program, they attended all the scheduled dietary visits and participated at least 1 h a week in the supervised exercise sessions throughout the year.

Table 2. Baseline characteristics

		Intervention group	Control group	<i>p</i> value
<i>n</i>	(male/female)	7 (4/3)	6 (6/0)	
Age	(year)	54.4±2.2	57.2±3.6	0.52
Body weight	(kg)	93.7±6.7	84.0±3.7	0.26
Waist	(cm)	107.0±4.2	100.4±2.3	0.22
VO _{2max}	(ml/kg/min)	26.3±1.6	29.9±1.4	0.12
2-h glucose	(mmol/l)	8.4±0.7	9.4±0.8	0.34
NEFA	(µmol/l)	545±34	582±53	0.55

Data are means ± SEM

Table 3. Changes after 1 year in subjects characteristics

		Intervention group	Control group	<i>p</i> value
Body weight	(kg)	-1.5±1.2	+0.8±0.8	0.15
Waist	(cm)	-3.5±1.0	+0.3±1.1	0.03
VO _{2max}	(ml/kg/min)	+1.6±1.0	-0.9±1.0	0.12
2-h glucose	(mmol/l)	-0.6±0.8	+1.0±0.8	0.19
Fast insulin	(mU/l)	-1.7±2.0	+3.3±1.7	0.09
2-h insulin	(mU/l)	-15.8±5.3	+41.8±22.1	0.02
Fast NEFA	(µmol/l)	-125±46	-79±28	0.46
Triglycerides	(mmol/l)	-0.31±0.22	+0.40±0.21	0.03

Data are mean ± SEM

The intervention group included male and female subjects, while the control group included only male subjects. Analysing the results in male subjects only, however, resulted in comparable differences in gene expression and protein content between control and intervention group. Because of the small numbers in both groups we included the results obtained in the female subjects in our final analysis.

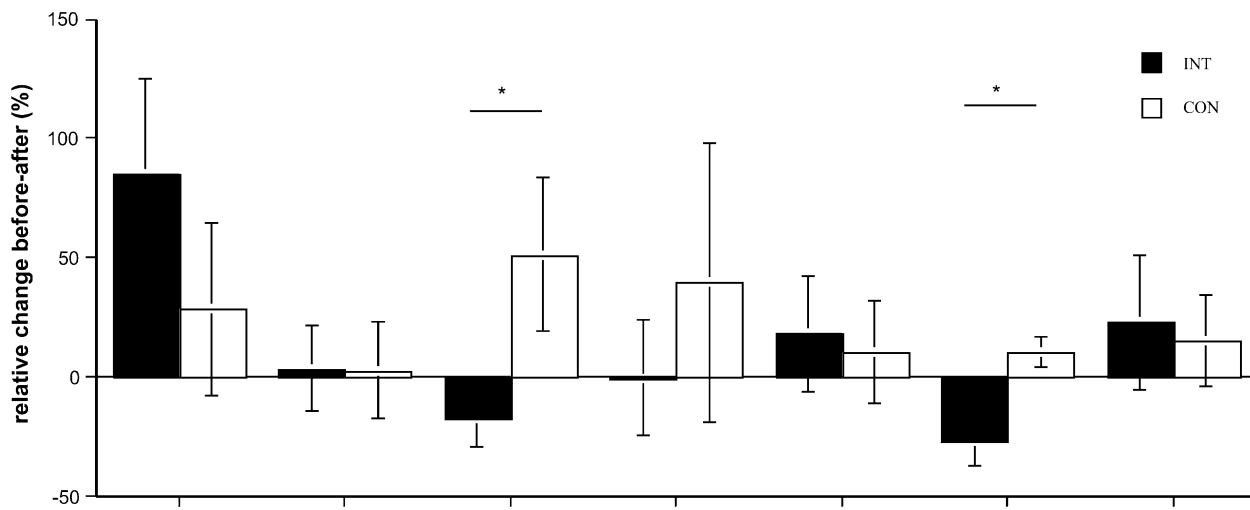
Changes after 1 year are depicted in Table 3. Body weight and waist circumference had decreased in the intervention group and in the control group. The change in waist circumference after 1 year was significantly different between groups (-3.5±1.0 cm vs +0.3±1.1 cm for intervention and control respectively; $p=0.04$). After 1 year, 2-h plasma glucose and fasting insulin tended to decrease in the intervention group, compared to a slight increase in the control group (NS). Additionally, 2-h insulin decreased in the intervention group and increased in the control group (-15.8±5.3 mU/L vs +41.8±22.1 mU/L for intervention and control respectively; $p=0.03$).

Gene expression. Relative changes after 1 year in genes involved in lipid metabolism in skeletal muscle, are shown in Fig. 1. CPT-2 was upregulated in the intervention group, but this increase was not different from the increase observed in the control group after 1 year ($p=0.33$). Differences in change in gene expression between groups were observed for ACC-2 and UCP-2. Both were down-regulated in the intervention group and up-regulated in the control group after

1 year (relative change: ACC2: -16.8±12.4% vs +51.5±32.3% for intervention and control respectively; $p<0.05$) (relative change: UCP2: -26.9±10.3% vs +10.5±6.2% for intervention and control respectively; $p<0.05$). No differences were observed in change in expression of CPT-1 muscle, LPL, PPAR α and UCP3 total between groups (Fig. 1).

Protein content. Changes in content of proteins involved in fatty acid transport, β -oxidation and the Krebs-cycle are shown in Fig. 2. HAD tended to increase after 1 year of intervention in the active group, and slightly decreased in the control group (+3.2±1.1 U/mg-ww vs -0.9±1.9 U/mg-ww respectively; $p=0.07$ for difference in change). No significant differences were seen in FABPc, CS and FAT/CD36 between groups after 1 year.

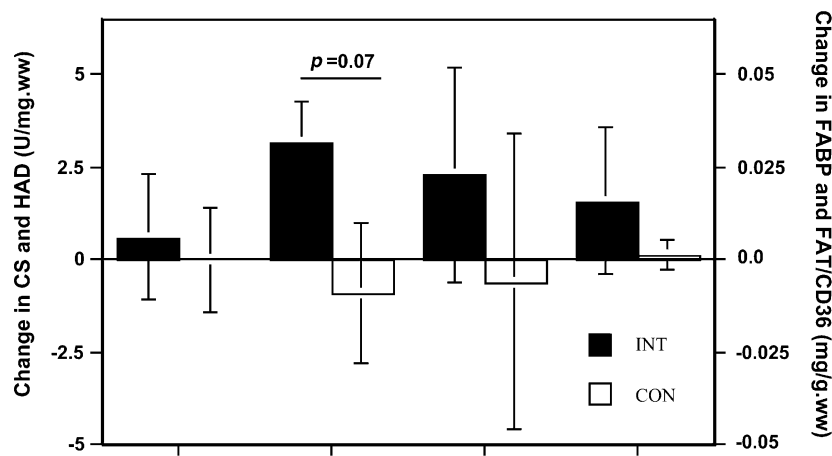
Correlation between changes in subjects characteristics and mRNA and protein concentrations. Correlation between changes in body-weight, waist circumference, VO_{2max} and 2-h glucose and changes in expression of genes and proteins were calculated. A correlation was observed between change in UCP2 and waist, change in HAD and 2-h glucose and between change in FABPc and 2-h glucose (Fig. 3). Inclusion of 'group' as dummy variable yielded similar results. A decrease in UCP2 expression was associated with a decreased waist circumference after 1 year ($R=0.71$; $p<0.01$). Furthermore, an increase in FABPc and HAD protein content both tended to be correlated with a de-



	CPT 2 (atmol/ μ gRNA)	CPT 1m (atmol/ μ gRNA)	ACC 2 (atmol/ μ gRNA)	LPL (atmol/ μ gRNA)	PPARalpha (atmol/ μ gRNA)	UCP 2 (atmol/ μ gRNA)	UCP 3 (atmol/ μ gRNA)	
INT	0.23 \pm 0.01	4.71 \pm 0.71	43.7 \pm 3.8	1.96 \pm 0.68	6.18 \pm 1.18	2.61 \pm 0.34	16.2 \pm 1.64	before
	0.44 \pm 0.10	4.32 \pm 0.59	35.9 \pm 5.3	1.24 \pm 0.22	6.28 \pm 0.77	1.81 \pm 0.25	17.6 \pm 1.70	after
CON	0.28 \pm 0.05	4.73 \pm 0.29	49.3 \pm 6.2	1.54 \pm 0.49	6.21 \pm 0.83	1.29 \pm 0.19*	13.6 \pm 3.89	before
	0.30 \pm 0.04	4.69 \pm 0.71	73.3 \pm 16.0*	1.37 \pm 0.26	6.28 \pm 0.48	1.50 \pm 0.18	12.8 \pm 0.29*	after

Fig. 1. Relative (%) and absolute changes (atmol/ μ gRNA) in expression of several genes involved in lipid metabolism in skeletal muscle of glucose-intolerant subjects before and after

1 year of a lifestyle intervention in the intervention (■ INT) and control group (□ CON). Data are means \pm SEM * p <0.05 between groups



	CS (U/mg.ww)	HAD (U/mg.ww)	FABP (mg/g.ww)	FAT/CD36 (mg/g.ww)	
INT	3.09 \pm 1.20	5.05 \pm 1.03	0.07 \pm 0.02	0.02 \pm 0.01	before
	3.67 \pm 1.11	8.29 \pm 0.68	0.09 \pm 0.10	0.03 \pm 0.02	after
CON	6.88 \pm 2.65	9.95 \pm 2.38	0.13 \pm 0.02	0.02 \pm 0.01	before
	6.88 \pm 2.12	9.05 \pm 0.98	0.12 \pm 0.02	0.02 \pm 0.01	after

Fig. 2. Changes in protein content involved in lipid metabolism in skeletal muscle of glucose intolerant subjects before

and after 1 year of a lifestyle intervention in the intervention (■ INT) and control group (□ CON). Data are means \pm SEM

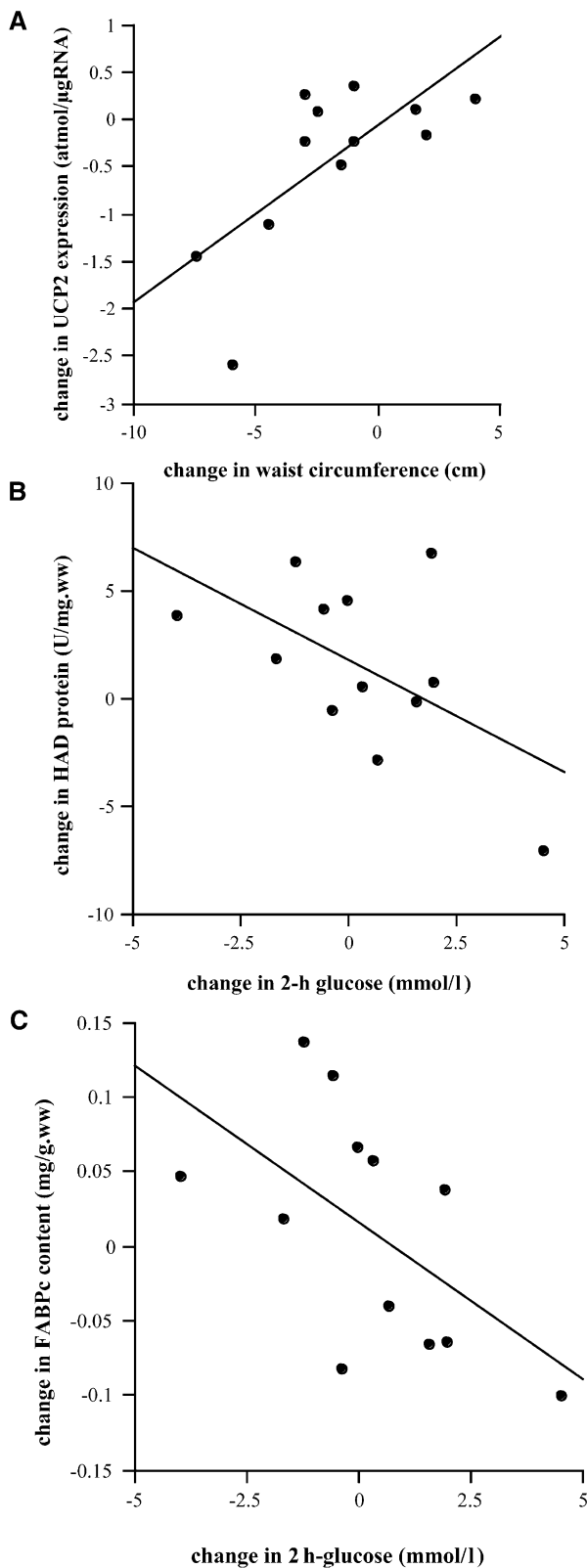


Fig. 3 A–C Simple correlation between change in waist and change in UCP2 expression (A) ($r=0.71$, $p<0.01$); change in 2-h glucose and change in HAD protein (B) ($r=-0.56$, $p=0.06$); change in 2-h glucose and change in FABPc protein content (C) ($r=-0.57$, $p=0.06$)

crease in postload glucose concentration ($R=-0.57$ and $R=-0.56$ respectively; $p=0.06$). Other changes in subject characteristics were not correlated to any changes in gene and protein expression.

Discussion

Skeletal muscle of pre-diabetic patients is characterised by a diminished capacity to handle fatty acids [2, 3, 4]. A diminished muscle content of several enzymes involved in fatty acid transport and oxidation has been suggested to underlie these defects [3, 10]. Body weight reduction per se did not correct the metabolic derangements in skeletal muscle in Type 2 diabetic and obese non-diabetic subjects [10, 14]. The effect on the expression of genes and proteins involved in lipid metabolism was examined in skeletal muscle biopsies of subjects with IGT, before and after 1 year of a combined diet and exercise intervention program, knowing to improve glucose tolerance and decrease the degree of insulin resistance [22].

Expression of several genes involved in (skeletal muscle) lipid metabolism was altered after 1 year of intervention. ACC-2 mRNA and UCP-2 mRNA had decreased in the intervention group but increased in the control group, while HAD protein content tended to increase in the intervention group compared to the control group. Therefore, a combined diet and exercise intervention program that leads to an improved metabolic profile, can induce alterations in expression of selected genes and proteins involved in lipid metabolism in muscle of glucose intolerant subjects, generally reflecting a better capacity to handle fatty acids.

Our lifestyle-intervention program resulted in several marked changes in subjects characteristics after 1 year. Despite the small number of subjects, differences were observed in changes in waist circumference and in 2-h insulin concentrations already. Both variables decreased in the intervention group and (slightly) increased in the control group. Changes in body weight, glucose tolerance, and insulin concentrations in this study mimicked the results seen after 1 year in the larger intervention trial, but were less pronounced (large trial: intervention group ($n=47$): body weight -2.7 ± 0.5 kg, 2-h glucose -0.8 ± 0.3 mmol/l, fasting insulin -2.5 ± 0.9 mU/l; control group ($n=55$) body weight -0.2 ± 0.5 kg, 2-h glucose $+0.2\pm 0.3$ mmol/l, fasting insulin $+0.4\pm 0.6$ mU/l; $p<0.05$ for difference in change; see [22]). These results confirm the known beneficial effects of changing dietary habits and improving physical activity on glucose tolerance and subsequent progression to Type 2 diabetes mellitus [20, 21].

Skeletal muscle of subjects with Type 2 diabetes or the pre-diabetic condition of IGT is characterised by a reduced capacity to oxidise (plasma) fatty acids [2, 3, 4]. This impaired fatty acid oxidation can be an im-

portant mechanism underlying the development of the insulin-resistant state [1, 9]. Reduced activities of several key enzymes involved in fatty acid oxidation (CS, HAD and CPT) was observed in muscle biopsies of insulin-resistant obese subjects, and it was concluded that the insulin-resistant muscle is organised toward fat esterification rather than oxidation [10]. Weight-loss, induced by a dietary intervention, did not improve this situation [10]. We combined a dietary intervention with an exercise protocol, resulting in several (small) changes in the lipid metabolic profile of skeletal muscle of subjects with IGT compared to the control subjects with IGT. Acetyl CoA carboxylase-2 (ACC-2) slightly decreased in the intervention group and increased in the control group ($p < 0.05$ for difference in change). Our data match the observation that a 3-month low-intensity endurance training program decreased ACC-2 mRNA expression and improved fat oxidation in healthy non-obese men [18]. ACC-2 plays an important role in the control of fatty acid oxidation; it catalyses the formation of malonyl-CoA, an intermediate that inhibits the activity of CPT-1. CPT-1 is responsible for the transport of fatty acyl-CoA into mitochondria. Although changes in CPT-1 mRNA levels were not different between groups in our study, we can not rule out a difference in protein concentration or activity of CPT-1. It is tempting to speculate that the observed differences in ACC-2 expression underlie (via malonyl-CoA and CPT-1) the corresponding changes in insulin resistance and glucose tolerance.

The combination of a decreased ACC-2 expression and an increased HAD protein content ($p = 0.07$) after 1 year suggests an improved capacity to oxidise fatty acids in the intervention group. This matches our observation, in a comparable group of subjects, that total fat oxidation and plasma NEFA oxidation during exercise were slightly improved in the intervention group and decreased in the control group after 1 year of this lifestyle-intervention programme [31]. Furthermore, we also found an association between increased HAD protein content and improved glucose tolerance (i.e. decreased 2-h glucose). The inclusion of exercise in the protocol is most probably responsible for the changes observed in our study, as weight loss per se has been reported not to change the expression of several important genes involved in skeletal muscle lipid metabolism [10]. Moreover, exercise training is a powerful stimulator of lipid oxidation by increasing the expression of genes which encode for key enzymes in lipid metabolism [16].

Another interesting observation is the difference found in UCP-2 expression between groups after 1 year: a decrease in the intervention group and an increase in the control group. The decrease in UCP-2 mRNA expression after 1 year was associated with a reduced abdominal adiposity, i.e. reduced waist circumference. These results are in agreement with earlier observations that UCP2mRNA expression is

increased in skeletal muscle of obese [12] and Type 2 diabetic subjects [32]. Moreover it has been shown that UCP2 mRNA expression is correlated with obesity [12], and decreases after (diet-induced) weight loss [13]. Although the physiological function of UCP2 is still not known, a role in reactive oxygen species (ROS) production, and lipid metabolism in skeletal muscle has been suggested [33]. Of interest, [12] an increased UCP-2 content in obese muscle was in parallel with a reduced lipid utilisation. In our study UCP-2 mRNA expression decreased after a combined diet and exercise intervention program, in combination with an improvement in genes and proteins involved in lipid utilisation (i.e. ACC-2 mRNA, HAD and to a lesser extent CPT-1 mRNA), suggesting a role for UCP-2 in lipid metabolism. However, it has also been shown that UCP2 is up-regulated by fatty acids [34]. In addition, lower NEFA concentrations in the intervention group were responsible for the decreased UCP2 expression. Further studies are needed to clarify the exact role of UCP2.

Apart from changes in the capacity to oxidise fat, an impaired capacity to transport fatty acids has been suggested to play a role in the impaired capacity to utilise fat in skeletal muscle of insulin-resistant subjects [3]. Although FAT/CD36 and FABPc were increased in the intervention group, the changes after 1 year were not statistically different from the control group. However, the change in FABPc protein content was correlated to improved glucose tolerance (i.e. decreased 2-h glucose), suggesting that fatty-acid transport is related to an improvement in glucose tolerance.

In summary, earlier studies have shown that skeletal muscle of pre-diabetic patients is characterised by a diminished capacity to utilise fatty acids [2, 3, 4]. Body weight reduction per se did not correct these metabolic derangements [10, 14]. In this study, a one-year combined diet and physical activity intervention program improved the metabolic profile of glucose-intolerant subjects compared to the control subjects. This improvement was paralleled by a decreased expression of ACC-2 mRNA and UCP-2 mRNA and an increased HAD protein content. These changes indicate a better capacity to utilise fatty acids in skeletal muscle. This improved handling of lipids by muscle could be one of the mechanisms underlying the beneficial effects of lifestyle-changes on glucose tolerance.

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References

1. Kelley DE, Mandarino LJ (2000) Fuel selection in human skeletal muscle in insulin resistance: a reexamination. *Diabetes* 49:677–683
2. Kelley DE, Simoneau JA (1994) Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *J Clin Invest* 94:2349–2356
3. Blaak EE, Wagenmakers AJ, Glatz JF et al. (2000) Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle. *Am J Physiol Endocrinol Metab* 279:E146–E154
4. Mensink M, Blaak EE, Baak MA van, Wagenmakers AJ, Saris WH (2001) Plasma free fatty acid uptake and oxidation are already diminished in subjects at high risk for developing type 2 diabetes. *Diabetes* 50:2548–2554
5. Falholt K, Jensen I, Lindkaer Jensen S et al. (1988) Carbohydrate and lipid metabolism of skeletal muscle in type 2 diabetic patients. *Diabet Med* 5:27–31
6. Goodpaster BH, He J, Watkins S, Kelley DE (2001) Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 86:5755–5761
7. Pan DA, Lillioja S, Kriketos AD et al. (1997) Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes* 46:983–988
8. Perseghin G, Scifo P, De Cobelli F et al. (1999) Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a ^1H - ^{13}C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48:1600–1606
9. McGarry JD (2002) Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 51:7–18
10. Simoneau JA, Veerkamp JH, Turcotte LP and Kelley DE (1999) Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. *FASEB J* 13:2051–2060
11. Ruderman NB, Saha AK, Vavvas D, Witters LA (1999) Malonyl-CoA, fuel sensing, and insulin resistance. *Am J Physiol* 276:E1–E18
12. Simoneau JA, Kelley DE, Neverova M and Warden CH (1998) Overexpression of muscle uncoupling protein 2 content in human obesity associates with reduced skeletal muscle lipid utilization. *FASEB J* 12:1739–1745
13. Schrauwen P, Schaart G, Saris WH et al. (2000) The effect of weight reduction on skeletal muscle UCP2 and UCP3 mRNA expression and UCP3 protein content in Type II diabetic subjects. *Diabetologia* 43:1408–1416
14. Blaak EE, Wolffenbuttel BH, Saris WH, Pelsers MM, Wagenmakers AJ (2001) Weight reduction and the impaired plasma-derived free fatty acid oxidation in type 2 diabetic subjects. *J Clin Endocrinol Metab* 86:1638–1644
15. Kiens B (1997) Effect of endurance training on fatty acid metabolism: local adaptations. *Med Sci Sports Exerc* 29:640–645
16. Tunstall RJ, Mehan KA, Wadley GD et al. (2002) Exercise training increases lipid metabolism gene expression in human skeletal muscle. *Am J Physiol Endocrinol Metab* 283:E66–E72
17. Spina RJ, Chi MM, Hopkins MG, Nemeth PM, Lowry OH, Holloszy JO (1996) Mitochondrial enzymes increase in muscle in response to 7–10 days of cycle exercise. *J Appl Physiol* 80:2250–2254
18. Schrauwen P, Van Aggel-Leijssen DP, Hul G et al. (2002) The effect of a 3-month low-intensity endurance training program on fat oxidation and acetyl-coA carboxylase-2 expression. *Diabetes* 51:2220–2226
19. Clarke SD, Gasperikova D, Nelson C, Lapillonne A, Heird WC (2002) Fatty acid regulation of gene expression: a genomic explanation for the benefits of the mediterranean diet. *Ann NY Acad Sci* 967:283–298
20. Tuomilehto J, Lindstrom J, Eriksson JG et al. (2001) Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 344:1343–1350
21. Knowler WC, Barrett-Connor E, Fowler SE, et al. (2002) Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 346:393–403
22. Mensink M, Feskens EJ, Saris WH, De Bruin TW, Blaak EE (2003) Study on Lifestyle Intervention and Impaired Glucose Tolerance Maastricht (SLIM): preliminary results after one year. *Int J Obes Relat Metab Disord* 27:377–384
23. Siri W (1956) The gross composition of the body. *Adv Bio Med Physiol* 4:239–280
24. Bergstrom J, Hermansen L, Hultman E, Saltin B (1967) Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71:140–150
25. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159
26. Auboeuf D, Vidal H (1997) The use of the reverse transcription-competitive polymerase chain reaction to investigate the in vivo regulation of gene expression in small tissue samples. *Anal Biochem* 245:141–148
27. Pelsers MM, Lutgerink JT, Nieuwenhoven FA et al. (1999) A sensitive immunoassay for rat fatty acid translocase (CD36) using phage antibodies selected on cell transfectants: abundant presence of fatty acid translocase/CD36 in cardiac and red skeletal muscle and up-regulation in diabetes. *Biochem J* 337:407–414
28. Wodzig KW, Pelsers MM, van der Vusse GJ, Roos W, Glatz JF (1997) One-step enzyme-linked immunosorbent assay (ELISA) for plasma fatty acid-binding protein. *Ann Clin Biochem* 34:263–268
29. Shepherd D, Garland PB (1969) The kinetic properties of citrate synthase from rat liver mitochondria. *Biochem J* 114:597–610
30. Bergmeyer H (1974) 3-Hydroxyacyl CoA dehydrogenase. Lactate dehydrogenase. In: Bergmeyer H (ed.) *Methods of enzymatic analysis*, vol 1. Academic Press, New York
31. Mensink M, Blaak E, Wagenmakers A, Saris W (2002) Fatty acid metabolism and impaired glucose tolerance: effect of a one-year lifestyle-intervention program (SLIM). *Diabetes* 51:A336 (Abstract)
32. Vidal H, Langin D, Andreelli F, Millet L, Larrouy D, Laville M (1999) Lack of skeletal muscle uncoupling protein 2 and 3 mRNA induction during fasting in type-2 diabetic subjects. *Am J Physiol* 277:E830–E837
33. Saleh MC, Wheeler MB, Chan CB (2002) Uncoupling protein-2: evidence for its function as a metabolic regulator. *Diabetologia* 45:174–187
34. Khalfallah Y, Fages S, Laville M, Langin D, Vidal H (2000) Regulation of uncoupling protein-2 and uncoupling protein-3 mRNA expression during lipid infusion in human skeletal muscle and subcutaneous adipose tissue. *Diabetes* 49:25–31