SPANDIDOS PUBLICATIONS

Differential expression of peroxisomal proliferator activated receptors α and δ in skeletal muscle in response to changes in diet and exercise

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Abstract. Peroxisome proliferator-activated receptors (PPARs) α, δ and γ are nuclear transcription factors that control key genes involved in fatty acid metabolism and energy homeostasis. Little is known about PPAR activation *in vivo* and the existence of overlapping functions between PPARα, -δ and -γ. As skeletal muscle is an important site for insulin action and acts as a significant sensor for life-style-induced influences in whole-body energy metabolism, we investigated the expression of PPARα, -δ and -γ in rat skeletal muscle in response to exercise after four- and twelve-weeks of high-fat feeding, respectively. PPARα mRNA expression in skeletal muscle increased in parallel with other signs of developing metabolic syndrome such as increased visceral fat pad volymes, plasma free fatty acids and muscle triglyceride concentrations. PPARα mRNA expression was up-regulated 3-fold after four weeks of high-fat feeding ($p<0.01$). Exercise reversed the high-fat induced increase in PPARα expression in young lean rats ($p<0.05$), but did not change the PPARα, -δ and -γ expression in the skeletal muscle in the normal nutritional state. The increase in PPARα expression declined during a longer term of high-fat feeding. In contrast, exercise increased PPARδ mRNA and protein expression 3- to 6-fold in skeletal muscle after longer-term high-fat feeding ($p<0.05$). This effect was accompanied by a reduction in skeletal muscle fat content. These findings suggest that parallel activation of PPARα and -δ expression in skeletal muscle may be an important adaptive mechanism in response to increased fatty acid loads in young, lean animals, protecting them from insulin resistance, whereas exercise might be needed to mediate the same positive effects in older animals.

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Introduction

Skeletal muscle is an important mediator of insulin resistance, since up to 75% of insulin-dependent glucose uptake occurs in this tissue. Infusions of lipid emulsion rapidly raise plasma free fatty acid (FFA) concentrations and induce profound insulin resistance in rat and human skeletal muscle (1-3). Randle *et al* have hypothesized that fatty acids (FAs) compete with glucose for substrate oxidation in the isolated rat heart and rat diaphragm muscle (4). Later studies suggest that increases in plasma FA concentrations induce insulin resistance by inhibiting glucose transport and phosphorylation activity with a subsequent reduction in muscle glycogen synthesis and glucose oxidation (5). An effective mechanism to enhance glucose up-take and insulin sensitivity would therefore entail increased lipid utilization and decreased FA deposition in skeletal muscle.

The three isoforms of peroxisome proliferator activated receptors (PPARs) α, δ and γ are activated by FAs and/or their metabolites and control several genes involved in glucose and lipid metabolism (6). The major insulin sensitizing effect of the different PPARs is their function to clear plasma lipids by stimulating FA catabolism in liver and skeletal muscle and moving fat towards subcutaneous adipose tissue depots.

The different PPARs show distinct features, including different tissue distribution and expression patterns. PPARα is expressed in metabolically active tissue, where it controls genes involved in mitochondrial ß- and ω-oxidation of fatty acids (6,7). Gene-targeting studies indicate that PPARα is essential for the up-regulation of the gene during fasting (8). PPARα knock-out (KO) mice are protected from insulin resistance during high-fat feeding and show decreased tolerance for fasting, therefore indicating a key role for PPARα

Table I. Primer/probe sets used for real-time PCR.

mRNA	Access no.	Sense primer	Antisense primer	Probe
PPARα	M88592	5'-TGGAGTCCACGCATGTGAAG	5'-CGCCAGCTTTAGCCGAATAG	5'-CTGCAAGGGCTTCTTTCGGCGA
PPARδ	U75918	5'-TGTCCCACAACGCTATCCG	5'-CCGCCACCAGCTTCCTCT	5'-TTTGGAAGGATGCCGGAGGCC
PPARγ	AF156666	5'-AGCATCAGGCTTCCACTATGGA	5'-AATCGGATGGTTCTTCGGAAA	5'-TCCATGCTTGTGAAGGATGCAAGGG
PDK4	NM_053551	5'-TCTAACGTCGCCAGAATTAAAGC	5'-GGAACGTACACGATGTGGATTG	5'-ACACAAGTCAATGGAAAATTTCCAGGCCAA
ß2-microglb.	NM_012512	5'-GAAGGAGCCCAAAACCGTC	5'-GTCCAGATGATTCAGAGCTCCAT	5'-CCTGGGACCGAGACATGTAATCAAGCTC
18S rRNA	V01270	5'-TTGCAAAGCTGAAACTTAAAGGAA	5'-AATTAAGCCGCAGGCTCCA	5'-TGACGGAAGGGCACCACCAGG
ß-actin	NM_031144	5'-AGAGGGAAATCGTGCGTGAC	5'-CAATAGTGATGACCTGGCCGT	5'-CACTGCCGCATCCTCTTCCTCCC

in gluconeogenesis (8,9). PPARδ is ubiquitously expressed and implicated in cholesterol metabolism and adiposity (10,11). PPARδ-null mice have reduced adiposity, which is not observed in adipocyte-specific PPARδ deficiency, suggesting that PPARδ controls lipid and glucose metabolism in tissues other than adipose tissue (12). PPARγ is expressed predominantly in adipose tissue and plays a crucial role for adipocyte differentiation and fat deposition (6). PPARγ-null mice show lipodystrophy and have fatty livers (13).

Administration of PPARα and -δ agonists up-regulates genes, such as pyruvate dehydrogenase kinase (PDK)4 and uncoupling protein (UCP)3, and pathways that co-operatively promote FA oxidation in myotubes (14). Further investigation has demonstrated that PPARδ, but not PPARα, is a major regulator of skeletal muscle mitochondrial FA catabolism and energy dissipation (15). Furthermore, PPARδ co-activates CPT1 with PPARγ co-activator (PGC)-1, a mitochondrial biogenesis factor, and stimulates mitochondrial FA oxidation by up-regulating malonyl-CoA decarboxylase in skeletal muscle (15). PPARδ is identified as a sensor for very low-density lipoprotein (VLDL)-signaling in macrophages (16), and its overexpression in adipose tissue leads to a lean phenotype (17). The mechanism by which PPARγ agonists, such as thiazolidinediones (TZDs), exert an insulin-sensitizing effect on skeletal muscle is not completely understood (6).

The relative importance *in vivo* of the three PPARs in modulating skeletal muscle insulin sensitivity in the setting of developing metabolic syndrome has been difficult to establish, as all three isoforms are expressed in muscle and the KO models of at least PPARα and -δ show a redundancy in function (14). Furthermore, little is known about the coordinated regulation of these PPAR isoforms in skeletal muscle and adipose tissue in response to physiological perturbations that either increase energy expenditure, such as exercise training, or lead to an oversupply of nutrients, such as high-fat diets. Against this background, we hypothesized that expression of the different PPARs in rat skeletal muscle and visceral adipose tissue would reflect changes in lifestyle and risk factors involved in developing insulin resistance.

Materials and methods

Animals, diets and exercise protocol. Female Wistar rats (120-130 g) were purchased from B&K Universal, Sollentuna, Sweden, treated as described (18) and housed in the animal facility of the Karolinska Institute for five weeks prior to use. Rats were kept on a 12-h light-dark cycle. Animals received either standard rodent chow or a high-fat diet (55% of calories from fat, TD93075 Harlan Teklad, WI) and had free access to food and water. After four (n=38) or twelve weeks (n=57), chow-fed and fat-fed rats were randomly assigned to two subgroups: exercised-trained or sedentary control. The exercise protocol has been validated (18). Glucose tolerance was determined in all rats as described (19). The anthropometric characteristics of the animals were measured and omental fat pads were removed and weighed after sacrifice of the animals approximately 16 h after the last exercise bout. Hindlimb muscles were removed and immediately frozen for further analysis as described below. mRNA measurements were made on all rats, whereas protein analyses were accomplished in only 40 rats in the twelve-week study due to insufficient material. The Animal Ethics Committee of the Karolinska Institutet approved the study protocol.

Skeletal muscle glycogen and triglyceride content. Glycogen content in gastrocnemius muscles was analyzed fluorometically as described (20). Results are expressed as mmol glucose per kg wet weight. Total triglyceride was extracted from gastrocnemius muscles according to a modification of the chloroform-methanol method (21). The liberated glycerol was thereafter determined spectrophotometrically using a commercially available enzymatic kit (Sigma, MO, USA).

Blood chemistry analysis. The plasma insulin concentration was measured by enzyme immunoassay using a commercial kit (Mercodia, Uppsala, Sweden). FFAs in serum were quantitated using an enzymatic colorimetric method (NEFA C test kit, Wako Chemicals, USA).

Tissue preparation and real-time quantitative PCR. Frozen muscle biopsies from rat gastrocnemius were homogenized with a polytron, and the total RNA was prepared by using Tri Reagent according to the manufacturer's protocol (Sigma-Aldrich AB, Sweden) and quantified by spectrophotometry (Beckman, Germany). mRNA (3 μg) was reverse transcribed using the superscript II kit for real-time PCR (Life technologies, Rockville, MD). Primer/probe sets (Table I) for real-time PCR were designed using the manufacturer's software and sequences available in the Genebank. The primer cDNA specificity was verified in real-time PCR using both the RNA and cDNA as a template. cDNA (3 μl) were amplified with 1X TaqMan Buffer, 5 mM $MgCl_2$, 200 μM of each dNTP, 200 μM of

ıer, 1.25 pM of probe, 0.25 U Amp-Erase Uracil ;ylase, 1.25 U AmpliTaq Gold (PE Applied Biosystems, Foster City, CA) in real-time quantitative PCR (RTQ-PCR) using an ABI PRISM 7000 Sequence detection system instrument and software (PE Applied Biosystems). Expression levels were quantified (in arbitrary units) by generating a 6-point serial standard curve. ß2-microglobulin, ß-actin and 18S rRNA were tested to determine which of the genes were suitable as endogenous control for RNA loading. Both ß2-microglobulin and ß-actin were unaffected by feeding and exercise protocols, while 18S rRNA was inappropriate. ß2-microglobulin was used as reference gene because its mRNA expression is known to be unaffected by exercise in skeletal muscle (22).

Protein extraction and Western blot analysis. Muscle specimens (15-30 mg wet weight) were homogenised using a polytron, and the total protein was prepared in 400 μl ice-cold lysis buffer containing 50 mM Tris (pH 7.5), 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, 5 mM $Na_4P_2O_7$, 50 mM NaF, 1 mM Na_3VO_4, 0.1% Triton X-100, 10 mM ß-glycerolphosphate, 500 μM phenylmethylsulfonyl fluoride, 400 μM DTT, 1 μM Microcystin and 10 μg/ml each of aprotinin and leupeptin. Insoluble material was removed by centrifugation (12000 x g for 10 min at 4˚C). Proteins were quantitated using the Bradford reagent (Bio-Rad Laboratories, Inc.) and measured spectrophotometrically at 590 nm.

To examine PPARα, -δ and -γ protein expression in muscle in the twelve-week study, 100 μg of protein were loaded to measure PPARα and -γ, and 50 μg of protein to measure PPARδ expression, and then subjected to SDS-polyacrylamide gel electrophoresis, performed under reducing conditions on 10% polyacrylamide as described by Laemmli (23). The resolved proteins were transferred to a nitrocellulose sheet as detailed by Towbin *et al* (24). The nitrocellulose membrane was then incubated with rabbit polyclonal antibodies against PPARα and -δ at 1:5000 dilution, transcription factor IIB (TFIIB) at 1:500 diluition and mouse polyclonal antibodies against PPARγ at 1:5000 dilution (Santa Cruz Biotechnology, USA). The blots incubated with primary antibody were probed with the corresponding secondary antibodies to IgG (Dakopatts, Sweden, 1:50000 dilution) conjugated to horseradish peroxidase. The ECL Advance Western blot detection system (Amersham, UK) was used according to the manufacturer's instructions and light emitted was detected on X-ray film. In addition, the light emitted from the membrane was quantified using FUJI LAS 1000. The resulting bands were confirmed by comparing the size of the protein in the cell extract with known molecular markers (Bio-Rad Laboratories, Germany). A standard consisting of 50 μg control protein was used to compare the light intensity between different membranes. For normalization of protein loading, TFIIB was used as a control.

Histology. Tissue samples from the animals treated for twelve weeks were collected from the gastrocnemius muscle and immediately stored in phosphor-buffered saline (PBS) solution (Medicago AB, Uppsala, Sweden). After collection, the samples were snap-frozen in Tissue-Tek (Sakura Finetek Europe BV) and stored at -80˚C for cryosections. Lipid deposits were visualized by staining with hematoxylin and oil-red O on 10-μm cryosections as described (25). Muscle lipid infiltration was observed and photographed using a Leica DMRB microscope operating at x1000 magnification and linked to the Leica Q500IW image analysis system.

Statistics. Results are expressed as fold change and presented as means ± SE. Statistical analyses were performed using StatView software (SAS, Cary, NC). Differences between groups of rats were analyzed by ANOVA. The compared residuals were controlled for normal distribution and, when necessary, subjected to logarithmic transformation. Categorical variables were compared using the Bonferroni/Dunn procedure. Correlation coefficients were calculated using simple regression or logarithmic regression after comparison with the Spearman correlation.

Results

Wistar rats treated for twelve weeks with high-fat feeding showed significantly lower insulin-stimulated muscle glucose uptake and significantly higher insulin tolerance but normal blood glucose levels and only slightly more pronounced glucose intolerance when compared with animals treated for four weeks (data not shown). In addition, rats treated for twelve weeks with high-fat feeding were eight weeks older and heavier than the rats participating in the four-week study (Table II). The exercise protocol was added to study the insulin-sensitizing actions of exercise on gene expression. Female rats were chosen for the study since they cope better with the exercise protocol than male rats.

Characteristics of animals in the four-week study. Physical and biochemical characteristics of the rats in the four-week feeding and exercise study are shown in Table II. Neither exercise nor high-fat feeding resulted in significant changes in body weight. The blood glucose, plasma insulin and muscle triglyceride concentrations did not differ significantly between the different treatment groups. High fat-fed sedentary rats showed increased fat pad weights compared with all the other animals. Furthermore, high fat-fed sedentary animals had higher serum FFA concentrations compared with both sedentary and exercised animals on chow diet. The high fat-fed exercised rats did not show significantly lower FFA concentrations than high fat-fed sedentary animals. High-fat feeding in sedentary rats decreased muscle glycogen concentrations whereas chow-diet and exercise together stimulated glycogen accumulation.

PPAR mRNA expression levels in rat skeletal muscle and their relations to metabolic parameters in the four-week study. The expression of the PPARα gene in skeletal muscle was significantly higher in sedentary fat-fed rats compared with sedentary chow-fed rats. In fat-fed rats, exercise restored PPARα gene expression to similar levels to those observed in the chow-fed sedentary rats (Fig. 1A). In the chow-fed rats, exercise itself did not change the PPARα expression level. Skeletal muscle PPARα mRNA expression correlated positively with skeletal muscle triglyceride concentration ($r=0.35$, $p<0.05$) and amount of omental fat ($r=0.43$, $p<0.01$) (Table III). PPARδ gene expression was not significantly increased after

Table II. Physical and biochemical characteristics of the rats in the four- and twelve-week studies.

Anthropometrical parameters (four-week study)	CS (n=9)	CT (n=9)	FS (n=10)	FT (n=10)
Weight (g)	155±8	149±5	160±5	151±6
Blood glucose (mmol/l)	4.40±0.14	4.46±0.12	4.34±0.09	4.27±0.18
Plasma insulin (mU/l)	0.34±0.09	0.26±0.06	0.39±0.04	0.34±0.06
Serum FFA (mmol/l)	**0.19±0.01**[c]	**0.18±0.01**[c]	0.25±0.01	0.21±0.01
Muscle Tg (mmol x kg wet weight^{-1})	5.18±0.48	5.41±0.67	6.84±0.59	6.69±0.60
Fat pad weight (g)	**1.44±0.22**[f]	**0.72±0.08**[f,a,d]	5.14±0.56	2.48±0.18[f]
Muscle glycogen (mmol x kg wet weight^{-1})	**35.6±1.6**[f,b,d]	57.5±8.1	30.1±1.9	**34.7±1.8**[b,d]
Anthropometrical parameters (twelve-week study)	CS (n=7)	CT (n=6)	FS (n=7)	FT (n=7)
Weight (g)	**256±4**[c]	**242±10**[d]	295±8	265±9
Blood glucose (mmol/l)	3.41±0.21	3.33±0.17	3.34±0.10	3.47±0.19
Fat pad weight (g)	**9.61±1.56**[f]	**8.27±1.70**[f,a,c]	24.16±1.36	16.42±2.33

FFA, free fatty acids; Tg, triglyceride. Groups are compared with the FS-group, [a]compared with the FT-group and [b]compared with the CT-group. [c]p<0.05; [d]p<0.01; [e]p<0.001; [f]p<0.0001. All data are given as mean ± SE.

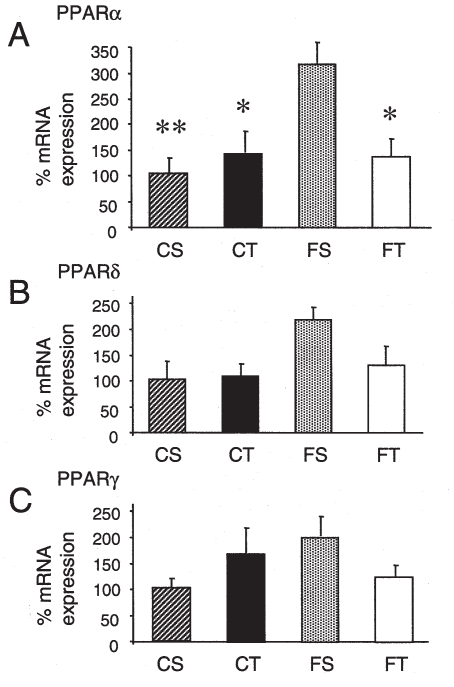

Figure 1. PPAR mRNA expression in the gastrocnemius muscle of differently treated rats in the four-week study. Expression of different PPARs is related to ß2-microglobulin expression. (A) PPARα, (B) PPARδ, (C) PPARγ. The CS group was set to 100%. Bars indicate means ± SE for each treatment group. CS, control chow-fed sedentary (n=9); FS, high fat-fed sedentary (n=10); CT, chow-fed exercised (n=9); and FT, high fat-fed exercised (n=10) animals. All values are compared with FS. *p<0.05; **p<0.01.

four weeks of high-fat feeding compared with all other groups of rats, even if exercise tended to decrease the PPARδ gene expression during high-fat feeding (Fig. 1B). Skeletal muscle PPARδ gene expression was positively correlated with serum FFA concentration (r=40, p<0.05) and amount of omental fat (r=0.44, p<0.01) (Table III). PPARγ gene expression did not differ significantly between the separate groups of rats (Fig. 1C). However, PPARγ mRNA expression was positively correlated with triglyceride concentration in skeletal muscle (r=0.37, p<0.05) (Table III).

Table III. Relationships between rat PPAR mRNA expression and metabolic parameters after four weeks of high-fat feeding.

	PPARα		PPARδ		PPARγ	
Muscle	r	p	r	p	r	p
Blood glucose	0.17	0.30	0.08	0.65	0.06	0.73
Plasma insulin (mU/l)	0.01	0.97	0.03	0.88	0.02	0.93
Serum FFA (mmol/l)	0.28	0.11	**0.40**	**0.02**	0.10	0.57
Muscle Tg	**0.35**	**0.03**	0.23	0.16	**0.37**	**0.03**
Muscle glycogen	0.27	0.14	0.24	0.20	0.10	0.60
Omental fat	**0.43**	**0.01**	**0.44**	**0.01**	0.08	0.64

FFA, free fatty acids; Tg, triglyceride. R-values are correlation coefficients.

PPAR mRNA expression in relation to expression of the PPAR target gene, PDK4, in the four-week study. To further explore whether the observed differences in PPAR mRNA expression levels resulted in changes in PPAR activity, we studied the expression of the PPAR target gene, PDK4. Muscle PPARα, -δ and -γ gene expression correlated positively with PDK4 gene expression (r=0.81, p<0.0001; r=0.75, p<0.0001; r=0.64, p<0.01, respectively). In addition, PPARα and -δ as well as PPARα and -γ gene expression correlated positively with each other (r=0.78 and r=0.60, respectively, p<0.0001).

PPAR mRNA expression levels in rat visceral adipose tissue and their relations to metabolic parameters in the four-week

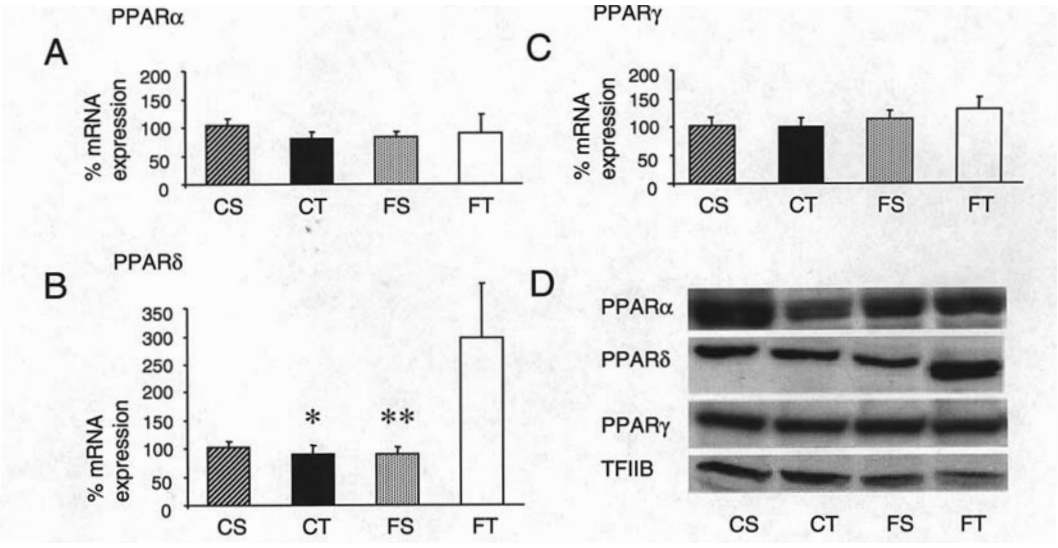

Figure 2. PPARα, -δ and -γ mRNA expression (A-C) and protein expression (D) in the gastrocnemius muscle of differently treated rats in the twelve-week study. The mRNA expression of different PPARs is related to ß2-microglobulin expression. Representative Western blot analyses showing (D) PPARα, PPARδ, PPARγ and TFIIB protein expression in rat gastrocnemius muscle of differently treated rats in the twelve-week study. The CS group was set to 100%. Bars indicates means ± SE for each treatment group. CS, control chow-fed sedentary for mRNA (n=21), for protein (n=13); FS, high fat-fed sedentary for mRNA (n=23), for protein (n=13); CT, chow-fed exercised for mRNA (n=6), for protein (n=7); and FT, high fat-fed exercised for mRNA (n=7) and for protein (n=7) animals. All values are compared with FT. $^{*}p<0.05$; $^{**}p<0.01$.

study. No significant differences were seen between the various treatments with respect to PPAR gene expression in omental adipose tissue. PPARγ gene expression in omental adipose tissue was negatively correlated with the plasma insulin concentration (r=0.60, p<0.001). No correlations were seen between PPAR expression in omental adipose tissue and blood glucose, serum FFA, muscle triglyceride concentration or amount of omental fat.

PPAR mRNA expression levels and their relations to metabolic parameters in the twelve-week study. The physical and biochemical characteristics of the rats after twelve weeks of feeding and exercise protocol are shown in Table II. Unlike the animals in the four-week study, high fat-fed sedentary animals were significantly heavier and showed increased fat pad weights compared with chow-fed animals (Table II). PPARδ gene expression was only induced in the high fat-fed and exercised rats (Fig. 2B). Also PPARδ protein expression was 6-fold increased in these rats compared with exercised chow-fed rats (Fig. 2D). PPARδ mRNA and protein expression did not differ between sedentary animals on different diets. Exercise in chow-fed animals did not change the PPARδ expression. Neither twelve weeks of high-fat feeding nor five days of exercise training led to any statistically significant effect on PPARα or -γ mRNA or protein expression in skeletal muscle (Fig. 2A, C and D). In contrast to the four-week study, skeletal muscle PPAR expression did not correlate with fat pad weights in the twelve-week study. Furthermore, there were no changes in PPAR expression in omental adipose tissue depending on different training or feeding protocols.

PPAR mRNA expression in relation to expression of the PPAR target gene, PDK4, in the twelve-week study. Similarly to the four-week study, PDK4 mRNA expression was positively correlated with PPARα and -γ mRNA expression and tended to correlate with PPARδ mRNA expression in rat skeletal muscle (r=0.53, p<0.0001; r=0.29, p<0.05; r=0.26, p<0.06, respectively). PPARδ mRNA expression was positively correlated with PPARα and -γ mRNA expression (r=0.90, p<0.0001; r=0.82, p<0.0001, respectively) and PPARα mRNA correlated positively with PPARγ expression (r=0.62, p<0.001).

Distribution of fat in rat skeletal muscle in the twelve-week study. Histological analyses of fat deposits in skeletal muscle from differently treated animals were performed in the twelve-week study (Fig. 3). Sedentary high fat-fed rats had considerable fat deposits in the muscle, which were not seen to the same extent in the other three groups of rats. The fat deposits in muscle tissue had almost disappeared in the high fat-fed and exercised rats.

Discussion

FAs are natural ligands for the different PPARs, which can regulate FA oxidation. Activation of either PPARα or -δ by synthetic specific ligands results in decreased serum triglyceride concentrations and improvement of blood lipid profiles (11,26,27). Skeletal muscle is an important site of action for both PPARα and -δ, and a target tissue for FA clearance by oxidation. Thus, we hypothesized that the endogenous expression of different PPARs in skeletal muscle would be modulated during circumstances when FA oxidation is required. These include situations with an increased FA load, such as high-fat feeding, since increased clearance of FAs is essential to prevent their intracellular accumulation, and exercise, since increased ß-oxidation is needed to generate cellular energy (28).

Endogenous PPAR expression in rat skeletal muscle was closely associated with features of obesity and insulin resistance after four weeks of high-fat feeding. PPARα gene expression

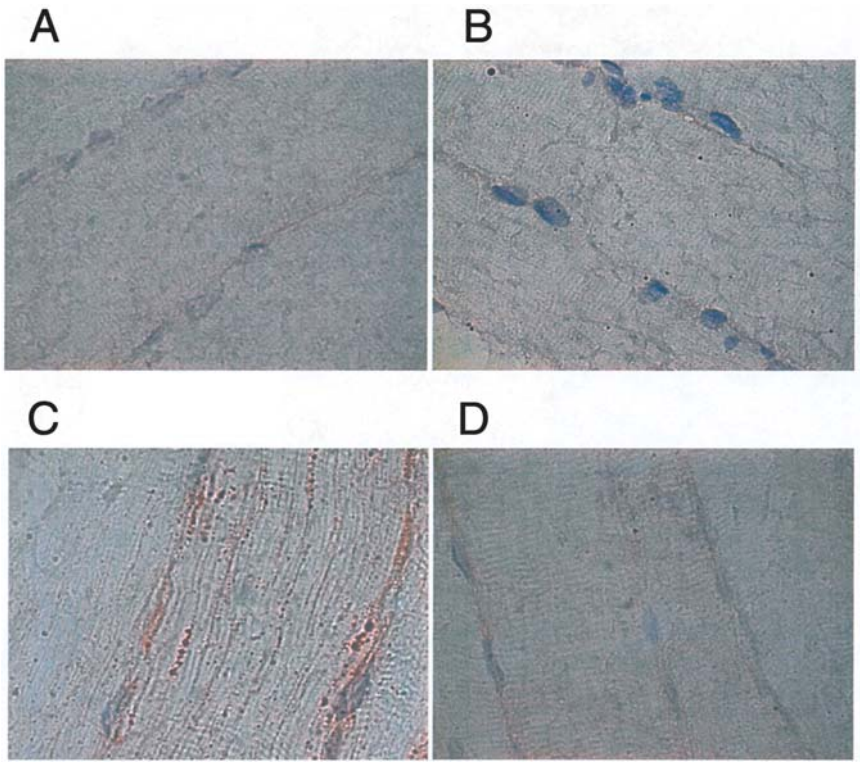

Figure 3. Oil-red O and hematoxylin staining in x1000 magnification of rat gastrocnemius muscle in differently treated animals in the twelve-week study. (A) CS and (B) CT groups show small accumulations of oil-red O coloring in the myocytes, (C) the FS group shows large accumulations of fat widely dispersed in the myocytes and (D) the FT group shows approximately the same muscle oil-red O coloring as the CS-group. CS, control chow-fed sedentary; FS, high fat-fed sedentary; CT, chow-fed exercised; and FT, high fat-fed exercised animals.

was distinctly up-regulated and correlated with intramuscular triglyceride accumulation. Also, PPARδ expression was increased, although non-significantly, and correlated with serum FFA levels. Moreover, a close association between increased PPARα and -δ expression and accumulation of visceral fat pads was observed. Interestingly, the high-fat diet-induced increase in muscle PPARα and -δ gene expression was almost completely reversed by exercise, with a simultaneous decrease in fat pad weight, increase in muscle glycogen and unchanged muscle triglyceride content.

It is well established that high-fat diets increase FA uptake in visceral adipose tissue and skeletal muscle (29,30). PPAR expression may be induced in response to an increased amount of intramuscular FAs, leading to increased plasma FA clearance. During exercise, muscle contractions are shown to stimulate the expression of PGC-1, initiating mitochondrial biogenesis and formation of oxidative type I muscle fibers (31). This results in improved muscle oxidative capacity and enables more effective usage of the FAs as fuel. Further, FAs are also increasingly deposited as triglycerides in oxidative type I muscle fibers (31).

Based on these considerations, FA but not triglyceride content might be decreased in high fat-fed muscle after five days of extensive long-lasting exercise. Depletion of intramuscular FAs may in turn result in decreased PPAR expression. Also, decreased muscle FA content could contribute to exercise-associated improvement in insulin sensitivity by releasing the inhibition of insulin signalling. Muscle contractions are also known to stimulate AMPK by directing muscle GLUT4 protein to the cell surface in an insulin-independent manner (18). In the present study, exercise resulted in increased glycogen synthesis, which might be due to an increase in glucose uptake (5).

An increased muscle PPARα and -δ expression was not observed after twelve weeks of high-fat feeding. Instead, the same levels were encountered as seen after chow feeding. The FA accumulation was simultaneously increased as demonstrated in the microscopic view of skeletal muscle from high fat-fed versus chow-sedentary rats. Consistent with our results, it has been shown that prolonged high-fat feeding leads to increased intramuscular accumulation of FAs (29). There were no consistent correlations between PPARα and -δ expression and fat pad mass, which were greatly increased (Table II). The induced muscle PPARα and -δ expression disappeared in parallel with increased insulin resistance as shown by the significantly lower insulin-stimulated glucose uptake into muscle observed when comparing the four and twelve weeks of high-fat feeding.

In contrast, muscle PPARδ expression was increased in exercised high fat-fed rats in the twelve-week study in contrast to the four-week study where PPARα and -δ expression were reversed to similar expression levels as observed for the chow-fed animals. The increase in PPARδ protein expression was associated with markedly decreased amounts of FAs in muscle, as observed by microscopic examination and ORO-staining, suggestive of an increase in FA oxidation. Exercise induces oxidative type I muscle fibers that have been shown to express higher levels of PPARδ mRNA (32). Previous reports have suggested that it is the activating signal or ligand and not the receptor, per se, that limits the PPARδ effect

Thus, skeletal muscle FA content serving as a PPARδ ligand could be markedly higher after twelve weeks of high-fat feeding compared with the four-week study, and FAs could still be present in sufficient amounts to activate muscle PPARδ after five days of long-term exercise.

Measurable levels of both PPARγ mRNA and protein were detected in rat skeletal muscle. PPARγ is known to up-regulate lipogenic enzymes, and PPARγ expression in skeletal muscle and other oxidative tissue has been suggested to be involved in lipogenesis, non-oxidative FA esterification and lipotoxicity (34). On the other hand, the PPARγ agonist, TZD, exerts insulin-sensitizing effects on skeletal muscle through unknown mechanisms. In the present study, skeletal muscle PPARγ mRNA expression levels correlated with muscle triglyceride content after four weeks of high-fat feeding. As in adipose tissue, skeletal muscle PPARγ activation might contribute to increased muscle triglyceride storage. Since triglyceride accumulation is generally considered as a safe way to store lipids in muscle (35), this function of PPARγ could act to protect the muscle against lipotoxicity. However, it is unclear how PPARγ expression is regulated in skeletal muscle since neither exercise nor a high-fat diet influenced its expression in the present study. This finding is in agreement with other studies that show no change in PPARγ expression after exercise (31,36,37).

The mechanism by which FAs would regulate PPAR expression is not fully known. During starvation, a condition with elevated plasma FAs, skeletal muscle PPARα and -δ expression and various PPAR-responsive genes are dramatically up-regulated (7,38). It has been speculated that elevated levels of circulating FFAs are sensed by PPAR target tissue and are directly responsible for alterations in gene expression (38). In the present study, PPARα expression correlated positively with PPARδ expression, which is in agreement with recent reports showing that PPARδ have overlapping functions with PPARα (39). Also, it has been shown that PPARδ acts as a potential repressor of PPARα-mediated transcription in 3T3 pre-adipocytes (12). Consistent with a potential role of PPARδ as an 'FA sensor', intracellular FAs could activate PPARδ by ligand-binding, leading to autoregulation of PPAR expression and potentiation of the transcriptional effect of FAs.

Since increased gene expression is not necessarily accompanied by increased protein activity, we also measured the expression of the PPAR target gene, PDK4. Skeletal muscle PDK4 expression was closely correlated with the skeletal muscle expression of all PPARs in the four-week study, and with PPARα and PPARγ expression in the twelve-week study. Also, a borderline significant correlation between PPARδ and PDK4 mRNA expression was seen in the twelve-week study. This suggests that the lower PPAR expression seen in high fat-fed and exercise-trained animals in the four-week study, compared with the sedentary high fat-fed rats, leads to lower PDK4 activation, contributing to the increased glycolysis in skeletal muscle, also consistent with the increased muscle glycogen contents seen in these animals. However, it is not clear whether PDK4 is a direct target of PPARγ in rat skeletal muscles. It has been reported that PDK4 mRNA levels increase in skeletal muscles after 6-h treatment with the PPARγ-specific agonist, GW1929, but decrease after 24 hours and 7 days, respectively (40).

In the present study, we investigated PPAR expression *in vivo* during different states of insulin resistance. Exercise training reversed the four-week high-fat diet-induced PPARα expression in skeletal muscle, which is associated with mild insulin resistance and early signs of the metabolic syndrome, such as higher serum FFA and muscle triglyceride concentrations and visceral fat accumulation. In contrast, exercise training after a twelve-week period of high-fat feeding was not associated with changes in PPARα expression, but rather with increases in skeletal muscle PPARδ mRNA and protein expression. These results indicate that endogenous expression of PPARα and PPARδ is induced in response to over-feeding. This might be an adaptive mechanism sufficient to protect against the insulin resistance that prevails after a short period of high-fat feeding in young lean rats. In older animals, the availability of endogenous PPAR ligands such as FAs, along with heavy exercise that increases PPARδ expression, leads to decreased FA content in muscle, which in turn may increase insulin sensitivity.

Together, these data suggest that PPARα, -δ and probably also PPARγ, may play important analogous roles in mediating the metabolic responses in skeletal muscle to different lifestyle factors, such as dietary fat and exercise.

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