Skeletal Muscle Peroxisome Proliferator–activated Receptor-γ Expression in Obesity and Non–insulin-dependent Diabetes Mellitus

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Abstract

The two isoforms of peroxisome proliferator–activated receptor-γ (PPARγ1 and PPARγ2), are ligand-activated transcription factors that are the intracellular targets of a new class of insulin sensitizing agents, the thiazolidinediones. The observation that thiazolidinediones enhance skeletal muscle insulin sensitivity in obesity and in patients with non–insulin-dependent diabetes mellitus (NIDDM), by activating PPARγ, and possibly by inducing its expression, suggests that PPARγ expression in skeletal muscle plays a key role in determining tissue sensitivity to insulin, and that PPARγ expression may be decreased in insulin resistant subjects. We used a sensitive ribonuclease protection assay, that permits simultaneous measurement of the two isoforms, to examine the effects of obesity and NIDDM, and the effects of insulin, on skeletal muscle levels of PPARγ1 and PPARγ2 mRNA. We studied seven patients with NIDDM (body mass index, 32±1 kg/m^2), seven lean (24±1 kg/m^2), and six obese (36±1 kg/m^2) normal subjects. Biopsies from the vastus lateralis muscle were taken before and after a 5-h hyperinsulinemic (80 mU/m^2 per minute) euglycemic clamp. The obese controls and NIDDM patients were insulin resistant with glucose disposal rates during the last 30 min of the clamp that were 67 and 31%, respectively, of those found in the lean controls. PPARγ1, but not PPARγ2 mRNA was detected in skeletal muscle at 10–15% of the level found in adipose tissue. No difference was found in PPARγ1 levels between the three groups, and there was no change in PPARγ1 levels after 5 h of hyperinsulinemia. In obese subjects, PPARγ1 correlated with clamp glucose disposal rates ($r = 0.92$, $P < 0.01$). In the lean and NIDDM patients, muscle PPARγ1 levels correlated with percentage body fat ($r = 0.76$ and $r = 0.82$, respectively, both $P < 0.05$) but not with body mass index. In conclusion: (*a*) skeletal muscle PPARγ1 expression does not differ between normal and diabetic subjects, and is not induced by short-term hyperinsulinemia; (*b*) skeletal muscle PPARγ1 expression was higher in subjects whose percent body fat exceeded 25%, and this may be a compensatory phenomenon in an attempt to maintain normal insulin sensitivity. (*J. Clin. Invest.* 1998. 101:543–548.) Key words: insulin resistance • glucose kinetics • euglycemic clamp • body fat • mRNA

Introduction

Peroxisome proliferator–activated receptors (PPARs)[1] are members of the steroid/thyroid hormone receptor superfamily of transcription factors (1–3). Members of this family of transcription factors are encoded by three PPAR genes, PPARα, PPARδ (also known as NUC 1), and PPARγ (2, 4–7). The two isoforms, PPARγ1 and PPARγ2, are derived from the same gene by alternative promoter usage and differential mRNA splicing, so that PPARγ2 has an additional 30 amino acids at the NH_2 terminus (8, 9). Endogenous ligands for PPARγ include fatty acids, especially the polyunsaturated fatty acids (linoleic, linolenic, and arachidonic acids) and the prostanoid derivative prostaglandin J2 (10–13). A new class of insulin-sensitizing agents, the thiazolidinediones, have also been shown to be high affinity ligands for PPARγ (14). Binding occurs within the same concentration range as that needed for transactivation of a heterologous promoter (14), and preliminary evidence suggests that the in vivo activity of the thiazolidinediones, correlates with their affinity for PPARγ receptors (15). These data suggest that PPARγ receptors and their endogenous ligands may play an important role in determining tissue sensitivity to insulin.

Skeletal muscle is the major site of insulin-stimulated glucose disposal in humans, accounting for ~ 80% of the enhanced rate of whole body glucose disposal under hyperinsulinemic glucose clamp conditions (16). Skeletal muscle is the predominant site of peripheral insulin resistance in obese and NIDDM subjects (16), and improvement of insulin action by thiazolidinediones in these subjects is associated with enhanced peripheral (primarily muscle) glucose uptake (4, 17, 18). In vitro studies suggest important direct effects of these agents on insulin signaling and glucose uptake in muscle and adipocyte cell lines (19–21), and in human skeletal muscle cultures (22), implying skeletal muscle expression of PPARγ receptors. Indeed, we and others have shown recently that skeletal muscle from humans and rodents expresses PPARγ at 10–25% the level found in adipocytes (9, 23, 24). While we found only PPARγ1 in skeletal muscle, Vidal-Puig and colleagues (23, 24) found a small amount of PPARγ2 in addition to PPARγ1.

Expression of PPARγ receptors in adipose tissue of rodents has been shown to be regulated by insulin and nutri-

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1. *Abbreviations used in this paper:* aP2, adipocyte protein 2; BMI, body mass index; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NIDDM, non–insulin-dependent diabetes mellitus; PPARγ, peroxisome proliferator–activated receptor-γ; Ra, appearance rate; Rd, disappearance rate; RNase, ribonuclease.

tional state (24). Adipocyte PPARγ expression was decreased by streptozotocin induced insulinopenic diabetes and partly restored by insulin therapy. Expression of both isoforms in adipose tissue was also reduced 60–80% after 48 h starvation. Regulation of muscle PPARγ receptors probably differs from that in adipocytes: muscle PPARγ2 fell by 25% in response to 48 h starvation, but PPARγ1, the predominant isoform in skeletal muscle was not affected (24).

Given the potential role of PPARγ receptors as mediators of insulin sensitivity, and the central role of skeletal muscle in determining whole body insulin sensitivity, we sought to determine first, whether the mRNA for these receptors is reduced in skeletal muscle of insulin resistant obese subjects and patients with NIDDM, and, second, whether muscle PPARγ expression is enhanced by in vivo hyperinsulinemia during a 5 h glucose clamp.

Methods

Seven patients with NIDDM, and 13 normal control subjects (7 lean, body mass index [BMI] < 27 kg/m^2; 6 obese, BMI > 27 kg/m^2) were studied. Normal glucose tolerance was confirmed in all the control subjects by a 75-g oral glucose tolerance test. None of the control subjects took any medication known to alter glucose tolerance. The clinical characteristics of the subjects are given in Table I. None of the diabetic patients had clinical evidence of long-term diabetic complications. All subjects had normal renal and liver function tests. Percent body fat was determined from under-water weighing. One diabetic patient refused to be weighed under water, and in her, percentage body fat was determined from triplicate skinfold thickness measurements at four sites (biceps, triceps, subscapular, and suprailiac) using Holtain skinfold calipers (CMS Weighing Equipment Ltd., London, United Kingdom). Five of the NIDDM patients were on treatment with sulfonylureas for their diabetes, and two were on diet alone. The diabetic patients were asked to continue with their usual diet but to stop their oral hypoglycemic agents for at least 2 wk before study. The study was approved by the Human Subjects Internal Review Board of the University of California, San Diego; written informed consent was obtained from each subject.

Hyperinsulinemic euglycemic clamps and muscle biopsies. Studies were performed in the morning after a 10–12-h overnight fast. At 0300 h, an 18-gauge cannula was inserted into an antecubital vein and a constant infusion of 3-^{3}H-glucose (0.25 μCi/min) (New England Nuclear, Boston, MA) started. For blood sampling, a venous cannula was inserted retrograde into a distal forearm vein, the hand being maintained in a hand warmer at 70°C. After each blood sample was taken this cannula was flushed with 0.15 mol/liter NaCl in water. After four basal blood samples were taken between 0800 and 0830 h for estimation of plasma glucose concentration and specific activity and serum insulin concentration, an IV infusion of insulin (Humulin S; Eli Lilly and Co., Indianapolis, IN) diluted in 0.15 mol/liter saline containing 1% wt/vol human albumin was begun at 80 mU/m^2 per minute from a Harvard syringe pump. Potassium and phosphate were given intravenously to compensate for the intracellular movement of these ions and to maintain normal serum levels. Plasma glucose was measured at 5 min intervals by a glucose oxidase method (YSI 2700 analyzer; Yellow Springs Instrument Co., Yellow Springs, OH) and the blood glucose level clamped at 4.4 mmol/liter for 5.5 h by adjustment of the rate of infusion of a solution of 20% (wt/vol) glucose in water (25). The 20% glucose solution was labeled with 3-^{3}H-glucose so as to maintain plasma glucose specific activities during the clamp close to basal levels (26). Blood samples for glucose concentration and specific activity were taken every 15 min, and for insulin concentrations every 30 min, until +270 min, and then every 10 min until +300 min.

A muscle biopsy (200–300 mg) was obtained from the vastus lateralis (27), under local lignocaine anaesthesia, before the basal samples and again at +300 min of the glucose clamp. Tissue was blotted free of blood and immediately frozen in liquid nitrogen, under which it was stored until used for analysis of RNA.

RNA analysis. Total RNA was prepared from muscle samples using the method of Chomczynski and Sacchi (28). PPARγ1 and PPARγ2 expression were determined by a ribonuclease (RNase) protection assay using a probe that distinguishes these two isoforms (9). A partial cDNA corresponding to nucleotides 1-252 of hPPARγ2 was subcloned into the pCRII vector (Invitrogen Corp., San Diego, CA). This was linearized with Cel II and labeled antisense riboprobe made with the T7 RNA polymerase and Maxiscript in vitro transcription kit (Ambion, Inc., Austin, TX). RNase protection assay was done with an Ambion, Inc. direct protect lysate assay kit. Band intensities were quantitated by a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

To determine the extent of contamination of the muscle samples by adipose tissue, the abundance of the adipocyte protein 2 (aP2) mRNA was determined, since aP2 gene expression is fat specific (29). RNase protection assays for aP2 were performed as previously described (9).

To verify the integrity of the RNA preparations, and to normalize for RNA content in the samples, 5 μg of RNA from each sample was analyzed by Northern blotting and a probe specific to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). To normalize the relative expression levels, the intensity of the PPARγ1 band from each sample in the RNase protection assay was divided by the intensity of the band from the sample in the Northern blot.

Analytical procedures. For determination of [^{3}H]glucose-specific activity 1 ml of plasma was deproteinized with $Ba(OH)_2/ZnSO_4$ (30). After centrifugation the neutral supernatant was evaporated and the residue dissolved in 1 ml water. After adding 10 ml of scintillation fluid (Ecoscint, Manville, NJ), ^{3}H disintegrations per minute were determined in an ICN 36014 liquid scintillation counter (Titertek Instruments, Inc., Huntsville, AL) using an external standard to correct for quenching. Quadruplicate aliquots of the labeled glucose infusate were added to non-radioactive plasma and processed in parallel with the plasma samples to allow calculation of the 3-^{3}H-glucose infusion rate.

Serum insulin was measured by a double antibody technique (31). The intra- and interassay coefficients of variation were 6.8 and 7.9%, respectively.

Calculation of glucose appearance and disappearance rates. In the basal state rates of glucose appearance (Ra) and disappearance rate (Rd) were calculated by dividing the 3-^{3}H-glucose infusion rate by the plasma glucose specific activity using the mean of the four basal plasma samples. During the clamp total glucose Ra and Rd were calculated from the 3-^{3}H-glucose data using the nonsteady state equations of Steele (32). A distribution volume of 0.19 liters/kg and a pool fraction of 0.5 were used in the calculation (33). In the NIDDM patients basal glucose Rd was corrected for urinary glucose excretion.

Table I. Clinical Characteristics of the Subjects Studied

	Normal subjects		NIDDM patients
	Lean	Obese	
n	7	6	7
Age (yr)	38±3	43±3	48±5
Weight (kg)	78±5	102±4	92±5
BMI (kg/m^2)	24.3±1.0	35.5±1.3	31.8±1.3
Percent body fat*	18.2±3.2	31.7±1.9	29.1±1.4
Waist/hip ratio	0.89±0.01	0.97±0.02	0.97±0.02
HbA1C (percent)	5.1±0.3	5.1±0.3	9.2±1.0

Mean±SEM. *Determined from underwater weighing.

Hepatic glucose output was calculated by subtracting the exogenous glucose infusion rate from the total glucose Ra.

Statistical analysis. Results are expressed as mean±SEM unless otherwise indicated. The significance of differences within groups was tested by Student's paired *t* test, and between groups by analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Correlations were sought by Pearson's least squares method. A P value of < 0.05 was considered statistically significant.

Results

Plasma glucose and insulin levels. Plasma glucose and insulin levels in the basal state and during the last 30 min of the glucose clamp are shown in Table II. Fasting plasma glucose levels were higher in the diabetic patients ($P < 0.001$) but similar in the obese and lean control subjects. Fasting serum insulin levels were higher in both the NIDDM patients, and obese control subjects, by comparison with the lean normal subjects (Table II). Plasma glucose levels during the last half hour of the clamp were similar in the three groups as was the coefficient of variation of plasma glucose calculated for each subject (lean controls, 4.6±1.9 (SD) percent, obese controls, 3.6±2.3%, NIDDM, 2.0±1.4%). Steady state insulin levels during the clamp were similar in the three groups (Table II).

Glucose appearance and disappearance rates. In the basal state, total glucose Ra was higher in the NIDDM patients (3.50±0.20 mg/kg per minute) than in the lean (2.51±0.18) or obese control subjects (1.95±0.22 mg/kg per minute). Glucose Rd, corrected for urinary glucose excretion was also higher in the NIDDM patients (Fig. 1). Although, glucose Ra and Rd expressed per kilogram body weight tended to be lower in the obese subjects than in the lean normal subjects, these differences were not statistically significant.

During the glucose clamp, the glucose requirement to maintain the desired plasma glucose level was lower in the NIDDM patients (2.17±0.45 mg/kg per minute) than in the lean (9.85±0.69 mg/kg per minute) or obese control subjects (6.53±1.04 mg/kg per minute) ($P < 0.001$ for both). 3-^{3}H-glucose turnover (Ra) was also lower in the NIDDM patients (3.15±0.36 mg/kg per minute) than in the two control groups (lean, 10.01±0.63; obese, 6.81±1.09 mg/kg per minute, $P < 0.001$ and $P < 0.05$, respectively). The difference between glucose Ra and the glucose infusion rate represents endogenous production of glucose by the liver, and hepatic glucose output during the last 30 min of the clamp was completely suppressed in the lean (0.16±0.17 mg/kg per minute) and obese control subjects (0.28±0.18 mg/kg per minute) but not in the NIDDM patients (0.97±0.16 mg/kg per minute, $P < 0.01$ and $P < 0.05$ versus lean and obese controls, respectively).

In the lean control subjects, glucose Rd during the last 30 min of the clamp was increased fourfold over that in the basal state. In the NIDDM patients glucose Rd during the clamp showed little change from basal and was markedly reduced (3.14±0.34 mg/kg per minute) by comparison with the lean (10.06±0.62) and obese control groups (6.78±1.09) (Fig. 1, $P < 0.001$ for both).

Skeletal muscle PPARγ expression. PPARγ mRNA was present in the muscle samples at 10–15% of the level found in adipose tissue, when expressed per microgram RNA. In contrast to adipose tissue, which expresses similar amounts of PPARγ1 and PPARγ2 isoforms, only PPARγ1 was found in skeletal muscle (Fig. 2). Control experiments with a fat-specific aP2 riboprobe indicate that there was very little fat contaminating the muscle samples (< 3%), and thus the presence

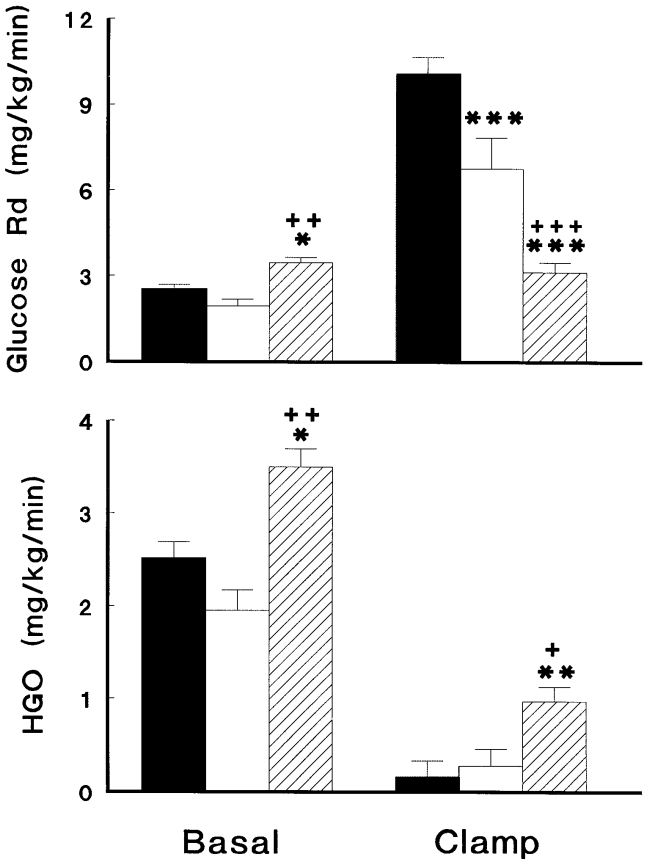

Figure 1. Rates of glucose Rd and hepatic glucose output in the basal state and during the last half hour of a 5-h 80 mU/m^2 per minute hyperinsulinemic euglycemic clamp. Seven lean normal subjects (*black bars*); six obese normal subjects (*white bars*); seven NIDDM patients (*hatched bars*). Mean±SEM. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus lean control subjects. +$P < 0.05$, ++$P < 0.01$, and +++$P < 0.001$ versus obese controls.

Table II. Plasma Glucose and Insulin Levels in the Basal State and at the End of the Glucose Clamp

	Normal subjects		NIDDM patients
	Lean	Obese	
n	7	6	7
Basal			
Plasma glucose (mmol/liter)	5.1±0.1	5.1±0.1	9.1±0.7***+++
Serum insulin (mU/liter)	3.5±0.6	12.1±3.7	23.5±5.7**
Clamp			
Plasma glucose (mmol/liter)	4.4±0.1	4.3±0.1	4.4±0.1
Serum insulin (mU/liter)	154±14	156±14	159±7

Mean±SEM. **$P < 0.01$; ***$P < 0.001$ versus normal controls. +++$P < 0.001$ versus obese controls.

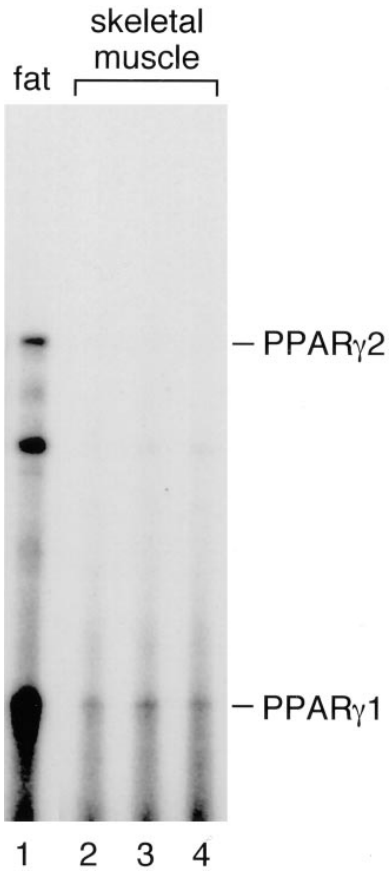

Figure 2. PPARγ1 but not PPARγ2 is expressed in human skeletal muscle. 10 μg of RNA from each muscle sample was analyzed by a RNase protection assay as described in Methods. As control, 10 μg of human fat RNA was also analyzed. A set of basal samples from three normal subjects are shown. Protected bands corresponding to PPARγ1 and PPARγ2 are indicated.

of PPARγ1 mRNA in skeletal muscle is not explained by fat cells contaminating the muscle sample. No significant differences were found in the basal levels of PPARγ1 mRNA in muscle from patients with NIDDM, obese normal subjects, or lean control subjects (F = 0.68, NS) (Fig. 3), and no relationship was found between skeletal muscle PPARγ mRNA levels and either BMI or the waist/hip ratio in any of the groups. Although BMI is the most commonly used index of obesity, it provides only an indirect estimate of excess body fat. Two of our normal subjects with a BMI below 27 kg/m^2 had a percentage body fat in the range found in the obese and NIDDM patients, while one of the NIDDM patients with a BMI of 31.8 kg/m^2 had a relatively low percentage body fat (Fig. 4). A strong relationship was found between skeletal muscle PPARγ1 mRNA expression and percent body fat, as determined from underwater weighing, in both the lean control subjects ($r = 0.76$, $P < 0.05$) and the NIDDM patients ($r = 0.82$, $P < 0.05$) (Fig. 4). When the study subjects were arbitrarily subdivided into two groups on the basis of whether fat accounted for more or less than 25% of body weight, then basal skeletal muscle PPARγ1 mRNA expression was significantly higher in those with the higher fat content (0.604±0.074 versus 0.347±0.058, $P < 0.02$).

PPARγ1 mRNA levels were unchanged, in all three groups, by the 5-h insulin infusion during the glucose clamp studies. No relationship was found between muscle PPARγ1 mRNA levels and fasting serum insulin levels. Muscle PPARγ1 mRNA expression correlated with the glucose disposal rate during the clamp in the obese subjects ($r = 0.92$, $P < 0.01$) but not in the lean ($r = 0.41$) or NIDDM subjects ($r = 0.21$).

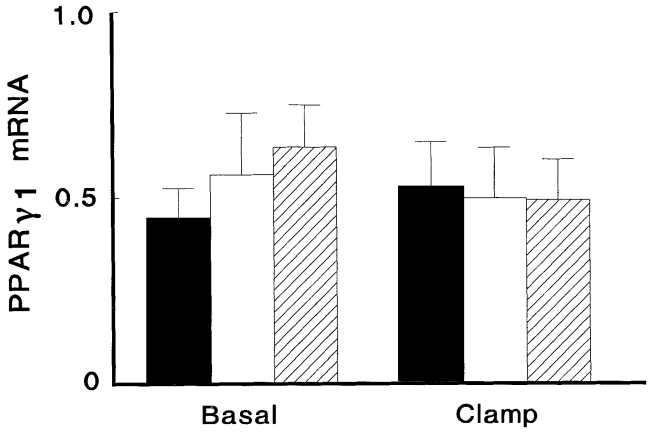

Figure 3. Skeletal muscle PPARγ1 mRNA levels in arbitrary units normalized in relation to GAPDH expression, in the basal state and at the end of a 5-h 80 mU/m^2 per minute hyperinsulinemic euglycemic clamp in seven lean normal subjects (*black bars*), six obese normal subjects (*white bars*), and seven NIDDM patients (*hatched bars*). Mean±SEM.

Discussion

The identification of PPARγ as the receptor for the thiazolidinediones, a new class of insulin-sensitizing agents, with direct effects on skeletal muscle insulin action (22) raises the possibility that PPARγ receptors play a key role in determining tissue insulin sensitivity. Indeed insulin has been shown to cause the phosphorylation and activation of PPARγ in 3T3-L1 cells (34). If PPARγ receptors play a role in insulin signaling, then reduced muscle PPARγ levels in obesity and NIDDM could contribute to insulin resistance. In this study, we used a sensitive RNase protection assay (9) that distinguishes between the two isoforms, PPARγ1 and PPARγ2, to examine the effects of obesity and NIDDM on skeletal muscle levels of these receptor mRNAs and their regulation by insulin.

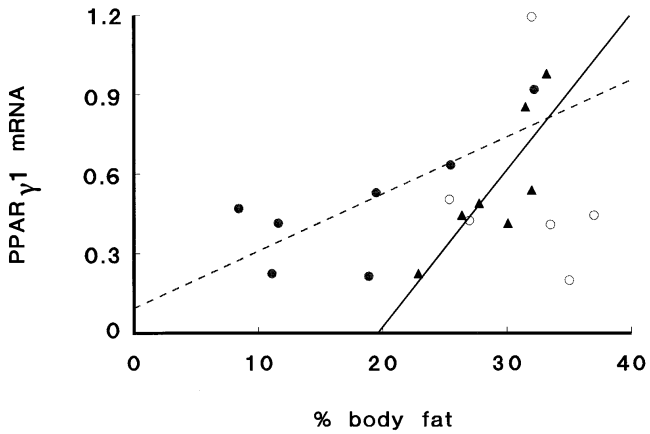

Figure 4. Relationship between skeletal muscle PPARγ1 mRNA levels (arbitrary units) and percent body fat determined from underwater weighing. Seven lean controls (*black circles*), six obese controls (*white circles*), seven NIDDM patients (*black triangles*). PPARγ1 mRNA levels are the mean of the basal and clamp biopsy values for each subject. For lean controls (----), $r = 0.76$, $P < 0.05$ ($y = 0.022x + 0.095$); for NIDDM patients (—), $r = 0.82$, $P < 0.05$ ($y = 0.059x - 1.162$).

In keeping with our previous studies (9) we found that PPARγ is expressed in human skeletal muscle at ~ 10–15% of the level found in adipose tissue, and that this is entirely due to expression of PPARγ1 (Fig. 2). We do not find any PPARγ2 in human skeletal muscle. This contrasts with findings of Vidal-Puig et al. who found expression of both PPARγ isoforms in human skeletal muscle, with the γ1 isoform predominating (23). A possible explanation for their finding of PPARγ2 is the presence of adipocytes within the muscle biopsy sample. We are confident that expression of PPARγ1 mRNA in our muscle samples is not due to the presence of adipocytes. First, RNase protection assays for the fat cell–specific aP2 gene showed < 3% of the aP2 mRNA levels found in fat cells. Second, since both PPARγ1 and PPARγ2 are found in fat cells (9, 23, 24), contamination should result in expression of both PPARγ isoforms and not selective expression of PPARγ1 as found in our study. Both PPARγ1 and PPARγ2 are biologically active (35). The observation that a truncated form of PPARγ2 lacking the NH_2-terminal 127 amino acids was more effective in mediating adipogenic differentiation of fibroblasts suggests that the PPARγ1 isoform may be more active (35).

We hypothesized that the expression of PPARγ receptors is reduced in skeletal muscle of insulin resistant obese subjects and patients with NIDDM. Both the NIDDM patients and the obese control subjects were markedly insulin resistant, with glucose disposal rates during the last 30 min of the glucose clamp that were only 31 and 67%, respectively, of those found in the lean control subjects (Fig. 1). Despite these marked differences in insulin sensitivity no differences in skeletal muscle PPARγ1 mRNA levels were found between the three groups (Fig. 3). However, within the obese group skeletal muscle PPARγ1 expression correlated positively with the clamp glucose disposal rate ($r = 0.92$, $P < 0.01$). The physiological significance of this finding is unclear given the small number of subjects studied. One might speculate that a high level of PPARγ1 expression in skeletal muscle might confer some protection against the development of insulin resistance associated with obesity. This would fit with the idea that the thiazolidinediones may in part improve insulin action in skeletal muscle by upregulating the expression of PPARγ (22). Although we did not measure PPARγ protein levels in skeletal muscle, preliminary evidence suggests that PPARγ protein levels parallel changes in the mRNA (24).

In rodents, PPARγ receptor expression in adipose tissue is regulated by insulin and nutritional state (23). 6 d after the induction of insulinopenic diabetes in mice, adipose tissue PPARγ expression is markedly reduced (23). Therefore, we examined the effects of a hyperinsulinemic euglycemic clamp on muscle PPARγ levels. Despite the supraphysiological insulin levels (150–160 mU/liter), and relatively long duration of the clamp, skeletal muscle PPARγ1 levels were unchanged after 5 h of insulin infusion (Fig. 3). This agrees with the lack of effect of nutritional state on PPARγ1 expression in rodent skeletal muscle (23), and the lack of effect of insulin on PPARγ expression in isolated human adipocytes even after exposure to 10^{-7} M insulin for 72 h (24). Our data do not preclude a role for insulin in the long-term regulation of skeletal muscle PPARγ expression. However, given the lack of correlation between muscle PPARγ1 levels and fasting insulin levels, it would appear that variations in muscle insulin exposure within the physiological range are not a major determinant of PPARγ1 levels.

A strong correlation between adipocyte PPARγ expression and BMI has been reported (24). This is consistent with the key role that PPARγ plays in adipocyte differentiation and maintenance of the adipocyte phenotype (1, 2, 35, 36), and suggests that PPARγ is important in the increase in adipose tissue mass in obesity. Although BMI is the most commonly used index of obesity, it provides only an indirect estimate of excess body fat. In a study of 173 normal subjects with a BMI below 30 kg/m^2, BMI explained only 32 and 34% of the variance in relative fat mass determined by dual-energy x-ray absorptiometry, in males and females, respectively (37). Two of our normal subjects with a BMI below 27 kg/m^2 had a percentage body fat in the range found in the obese and NIDDM patients, while one of the NIDDM patients with a BMI of 31.8 kg/m^2 had a relatively low percentage body fat (Fig. 4). Although we found no correlation between skeletal muscle PPARγ expression and BMI, we did find a significant correlation between skeletal muscle PPARγ1 expression and percentage body fat in both the lean normal subjects ($r = 0.76$, $P < 0.05$) and the NIDDM patients ($r = 0.82$, $P < 0.05$). However, we did not observe such a relationship in the obese non-diabetic subjects. Because the number of subjects in each group was relatively small, one must be cautious about the interpretation of these data. However, if we take all of the groups together, then, in subjects in whom fat comprised > 25% of body weight, muscle PPARγ1 mRNA levels were higher than in those with a percentage body fat below this level ($P < 0.02$). Although still speculative, this raises the possibility that as body fat content increases, skeletal muscle PPARγ1 expression also increases. Insulin does not appear to be responsible for this upregulation, given the lack of any correlation with fasting insulin levels and unchanged muscle PPARγ1 mRNA levels after 5 h of insulin infusion.

One hypothesis put forward to explain the development of skeletal muscle insulin resistance in obesity and NIDDM is increased expression of tumor necrosis factor-α (TNF-α) (16, 38). Increased adipose tissue TNF-α mRNA levels are found in obese and diabetic humans (39, 40) and obese rodent models of insulin resistance (38, 40). Although circulating levels of TNF-α are normal in obesity and diabetes, it has been suggested that adipose tissue depots in proximity to skeletal muscle, or even skeletal muscle itself (41), elaborate increased amounts of TNF-α which causes insulin resistance in the local environment. A prominent action of TNF-α in adipocytes is increased lipolysis and a dose-dependent loss of the adipocyte phenotype, associated with a marked reduction in the expression of several adipocyte-specific genes (42). PPARγ is one of the earliest genes to be downregulated. If PPARγ in skeletal muscle is similarly regulated by TNF-α, then our findings that PPARγ mRNA is not decreased in obese or NIDDM subjects, and that muscle PPARγ mRNA levels tend to be higher as body fat increases, do not support the idea of increased TNF-α stimulation of skeletal muscle in NIDDM or obesity.

Acknowledgments

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