Distribution of peroxisome proliferator-activated receptors (PPARs) in human skeletal muscle and adipose tissue: relation to insulin action

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Abstract

Aims/hypothesis. To evaluate the tissue distribution and possible role of the peroxisome proliferator-activated receptors (PPARs) in insulin action in fat and muscle biopsy specimens from lean, obese and subjects with Type II (non-insulin-dependent) diabetes mellitus.
Methods. We measured PPARα, PPARβ(δ) and PPARγ protein expression by western blot analysis. The PPARγ protein was also measured in muscle before and after 3-h hyperinsulinaemic (300 mU · m^{-2} · min^{-1}) euglycaemic clamps.
Results. The PPARα protein was expressed preferentially in muscle relative to fat (more than sevenfold). The PPARβ protein was similar in fat and muscle. The amount of PPARγ protein found in muscle was, on average, two-thirds of that present in fat. There was no statistically significant difference between non-diabetic and diabetic subjects in baseline (pre-clamp) muscle PPAR (α, β or γ) protein expression. Subgroup analysis showed, however, significantly higher PPARγ protein in the most insulin resistant diabetic subjects with glucose disposal rates of 3–6 mg · kg^{-1} · min^{-1} compared with their age and weight matched counterparts with glucose disposal rates of 6–9 (147 ± 23 vs 88 ± 10 AU/μg protein, $p \leq 0.01$ in diabetic and vs 94 ± 15, $p \leq 0.04$ in non-diabetic subjects). Muscle PPARγ protein and glucose disposal rates were inversely correlated in diabetic subjects ($r = -0.47$, $p \leq 0.05$).
Conclusion/interpretation. All PPARs (α, β or γ) are present in skeletal muscle and adipose tissue with different relative distributions. The PPARγ protein is abundant in skeletal muscle as well as adipose tissue. The altered expression of skeletal muscle PPARγ is consistent with a role for this nuclear protein in the impaired insulin action of Type II diabetes. [Diabetologia (2000) 43: 304–311]

Keywords Type II diabetes, insulin resistance, PPARα, PPARβ, PPARγ, protein, obesity, skeletal muscle, adipose tissue, human.

The peroxisome proliferator activated receptors (PPARs) are members of the nuclear receptor superfamily, which are involved in gene regulation [1, 2].

The PPARα protein is expressed in several human tissues, including skeletal muscle, liver, kidney and vascular endothelial cells [3]. It has been suggested that it is involved in the control of lipoprotein metabolism [4, 5], fatty acid oxidation [6, 7] and the cellular uptake of fatty acids [8]. The transcriptional activity of PPARα is stimulated by insulin [9], fibrates and the selective agonist 4-chloro-6(2,3-xylidino)-2-pyrimidinyl-thioacetic acid (WY14643). The PPARβ (also designated PPARδ and NUC1) protein has been found in a number of different tissues in the adult rat [10] but its distribution, regulation and function in human tissue is still to be determined. The last

of these nuclear receptors, PPARγ, is known to play a critical part in adipocyte differentiation and fat deposition and is highly expressed in this tissue [1, 11]. The PPARγ receptor exists as two protein isoforms, γ1 and γ2, the products of alternative splicing. The PPARγ2 isoform contains an N-terminal 30 amino-acid extension and has a lower mobility on SDS-PAGE gel [12]. The PPARγ receptor is bound to and activated by the natural prostaglandin derivative 15-deoxy-^{12,14}-prostaglandin J$_2$ [13], as well as the synthetic insulin sensitizing thiazolidinediones such as troglitazone, pioglitazone and rosiglitazone [14–19]. A close relation between the ability of different thiazolidinediones to activate PPARγ and their potency as antidiabetic agents supports the importance of PPARγ in the regulation of glucose and lipid metabolism and possibly insulin action [17].

In addition PPARγ mRNA has been identified in skeletal muscle [20–22]; the major site of impaired insulin action in Type II (non-insulin-dependent) diabetes mellitus and obesity. Furthermore, PPARγ mRNA expression has been found to be increased in adipose and muscle tissue of some insulin resistant obese rodents and humans [21–24], further suggesting this receptor is involved in regulation of metabolism and insulin action. Despite its presence in human skeletal muscle, PPARγ mRNA abundance is generally 5% or less of that in adipose tissue on the basis of total RNA. This discrepancy in mRNA abundance between tissues has led some investigators to minimise or exclude a role for muscle PPARγ in the control of glucose/lipid metabolism in this tissue. Recognition that PPARγ protein, not message, is responsible for its gene effects, has, however, received little attention until recently when specific immunoblotting antibodies for PPARγ protein became available. Recently, immunohistochemistry was used to show that PPARγ protein is present within nuclei of myocytes [25].

In previous studies from our laboratory, *PPARγ* gene expression (mRNA) in skeletal muscle was found to be rapidly up-regulated in response to insulin treatment in both non-diabetic control and Type II diabetic subjects [22]. In the current report, other than comparing tissue distribution of PPARs in human muscle and fat, we sought to determine PPARα, PPARβ and PPARγ protein expression in the tissues of subjects with and without Type II diabetes. Further, we evaluated whether metabolic variables such as glucose, insulin or triglycerides, characteristically increased in Type II diabetes, might contribute to any differences in PPAR expression between these groups.

Subjects and methods

Materials. All radioisotopes were obtained from DuPont-NEN (Boston, Mass., USA). Bovine serum albumin (BSA, fraction V) was purchased from Boehringer Mannheim (Indianapolis, Ind., USA). Reagents for electrophoresis were purchased from Bio-Rad (Richmond, Calif., USA). Polyclonal antibodies for PPARγ were obtained from BioMol (Plymouth Meeting, Pa., USA), for PPARβ from Santa Cruz Biotechnology (Santa Cruz, Calif., USA) and for PPARγ2 from Affinity BioReagents (Golden, Colo., USA). A monoclonal antibody generated against the amino terminal region of human PPARα (residues 2–101) was a gift from Dr. D. A. Winegar at Glaxo Wellcome (Research Triangle Park, N.C., USA). Anti-rabbit and anti-mouse IgG conjugated with horseradish peroxidase were purchased from Amersham (Arlington Heights, Ill., USA). SuperSubstrate chemiluminescence substrate was from Pierce (Rockford, Ill., USA). All other reagents were purchased from Sigma (St. Louis, Mo., USA), as was a monoclonal antibody against α-sarcomeric actin.

Subjects. Adipose tissue was obtained from the abdominal region, under lidocaine anaesthesia, using a side-cutting needle [26]. Muscle biopsy specimens were obtained from the vastus lateralis of 22 non-diabetic control subjects without a family history of diabetes and 14 subjects with Type II diabetes before and after a 3-h hyperinsulinaemic (300 mU · m^{-2} · min^{-1}) euglycaemic (5.0–5.5 mmol/l) clamp as described in detail previously [27]. Of the subjects with diabetes, seven were treated with oral antidiabetic agents (sulphonylurea, biguanide), three with insulin and four with diet only. None of the subjects was treated with thiazolidinedione agents. Glucose tolerance was determined in all subjects after a 75-g oral glucose tolerance test [28]. Subjects with diabetes had their medication withheld on the morning of biopsy. None of the control subjects were receiving any medications known to influence glucose metabolism. The Committee on Human Investigation of the University of California, San Diego approved the experimental protocol. Informed written consent was obtained from all subjects after explanation of the protocol.

Western blotting of PPAR proteins. Tissue protein lysates were prepared as described previously [29]. Western blot analysis was done by the method of Burnette [30] as detailed previously [29]. Equal amounts of total protein were loaded. Total protein content was determined by a dye-binding method [31]. All blots contained an extract of human adipocytes as an internal standard. The results were normalized to this standard to correct for blot to blot variation. The validity of the PPARγ antibody was established by several criteria. The antibody recognised a doublet of 62000–65000 M$_r$ in 3T3-L1 adipocytes, the intensity of this band increased considerably upon differentiation from the fibroblast phenotype (Fig. 1). A band of similar size was found with another commercially available polyclonal antibody generated in rabbits against human PPARγ (Santa Cruz Biotechnology, Santa Cruz, Calif., USA). Blocking with a peptide specific for this antibody abolished the detection of this band. The antibody did not recognize overexpressed retinoid X receptor (RXR)α, β or γ (kind gift of Dr. R. Mukherjee, Ligand Pharmaceuticals, San Diego, Calif., USA), nor PPARα or β. The PPARγ2 protein content was determined with a commercially available polyclonal antibody generated in rabbits against a peptide sequence described to be specific for human PPARγ2 [32]. The secondary antibody for PPARγ, PPARγ2, and PPARβ was anti-rabbit IgG conjugated with horseradish peroxidase and a similarly conjugated anti-mouse IgG was used for PPARα. Muscle biopsy specimen proteins were visualised with SuperSignal, exposed to high performance chemiluminescence film (Hyperfilm ECL, Amersham Life Science, Little Chatfont, England) and quantified with the NIH image software (Bethesda, Md., USA). To rule out possible inaccuracies due to the limited range of linearity of chemiluminescence

film, all muscle to fat comparisons were also quantified with a low light imaging system (ChemiImager 4000, Alpha Innotech, San Leandro, Calif., USA). Results are presented as 'arbitrary units' of the intensity of the western blot bands as detected by the indicated imaging software. These results are normalised to the value for an internal control sample included on all blots to account for blot-to-blot variation.

For the analysis of other proteins, adipocyte-specific binding protein (ALBP) detection was used with a polyclonal antibody generously provided by Dr. D. Bernlohr (University of Minnesota, USA). Sarcomeric-specific α-actin was detected with a monoclonal antibody using a horseradish peroxidase conjugated anti-mouse IgG as the secondary antibody. Muscle tissue content of muscle-specific fatty-acid binding protein (mFABP) was measured by ELISA according to a previously reported method [33].

Northern blot analysis of tissue specific genes. To evaluate the potential adipose tissue content of muscle biopsy specimens, samples were probed for the mRNA of muscle (myogenin and mFABP) and adipose ALBP tissue specific genes. From the total RNA 10 µg was size-separated by electrophoresis through denaturing formaldehyde 1–1.5 % agarose gel and transferred to a nitrocellulose membrane (Nytran, Schleicher & Schuell, Keene, N. H., USA). To control for gel loading, the membranes were stained with methylene blue and relative intensities of the ribosomal bands were compared quantitatively using computer imaging (NIH Image). Myogenin and mFABP DNA probes for northern analysis were labelled by the hexamer priming method in the presence of α-^{32}P dCTP using the Ambion DECAprime II Random Priming Kit (Ambion, Austin, Tex., USA). The myogenin probe consisted of a 1.5 kb *EcoR1* fragment from pBS. For the mFABP probe, a 0.5 kb *EcoRI* fragment of pSP6.5 containing the cDNA for mFABP was used. Specific antisense RNA probes (riboprobes) for human-ALBP were synthesised from vector pAP2, (gift of Dr. D. Bernlohr, University of Minnesota). Hybridization of DNA and RNA probes was carried out at 68 °C in 10 ml of QuickHyb (Stratagene, San Diego, Calif., USA) according to the manufacturer's instructions. Relative intensities of transcript signals in the linear range were compared quantitatively using computer imaging (NIH Image). The mRNA for PPARγ1,2 was measured by a quantitative reverse transcriptase-PCR assay, as described previously [22].

Statistical analysis. Statistical significance was evaluated using Student's *t* test for dependent and for independent samples and regression analysis. Sample sizes were pre-calculated on the basis of the first three western blot results for PPARγ in each group to achieve a statistical power of 95 % or more. Statistical power in the subgroup analysis of severely diabetic subjects compared with moderately diabetic subjects, as classified by glucose disposal rate (GDR) groups, was 80 % or more (StatSoft, STATISTICA, Tulsa, Okla., USA and GraphPad PRISM, San Diego, Calif., USA). Significance was accepted at *p* 0.05 or less.

Results

Subjects. Clinical characteristics (Table 1) showed fasting concentrations of blood glucose, serum insulin and HbA$_{1c}$ values all significantly higher in the subjects with Type II diabetes than in the non-diabetic subjects without a family history of diabetes. The glu-

Table 1. Clinical characteristics of the study groups

	Type II diabetic subjects (n = 14)	Non-diabetic subjects	
		Obese (n = 8)	Lean (n = 14)
Age (years)	47 ± 1.7	47 ± 2.3	41 ± 2.6[a]
BMI (kg/m²)	35 ± 2.4	32 ± 1.8[b]	23 ± 0.4[a]
HbA$_{1c}$ (%)	8.1 ± 0.8	5.2 ± 0.2[a]	5.2 ± 0.1[a]
Fasting			
– blood glucose (mmol/l)	8.9 ± 0.9	4.9 ± 0.1[a]	4.7 ± 0.1[a]
– serum insulin (pmol/l)	179 ± 37	68 ± 18[a]	31 ± 7[a]
– triglycerides (mmol/l)	2.35 ± 0.40	2.50 ± 0.70[b]	0.83 ± 0.09[a]
– NEFA (g/l)	0.85 ± 0.09	0.64 ± 0.13	0.51 ± 0.09[a]
OGTT (2-hr glucose) (mmol/l)	15.3 ± 1.29	6.5 ± 0.32[a]	5.7 ± 0.33[a]
GDR (mg · kg⁻¹ · min⁻¹)	6.1 ± 0.5	8.2 ± 0.8[a,b]	13.3 ± 0.7[a]

Results are means ± SEM, [a] signifies $p \leq 0.05$ vs Type II diabetic subjects and [b] $p \leq 0.05$ vs lean, non-diabetic subjects

Table 2. Baseline protein expression

	Type II diabetic subjects	Non-diabetic subjects
PPARα (AU/µg protein)	100 ± 13 (n = 14)	104 ± 13 (n = 22)
PPARβ (AU/µg protein)	412 ± 114 (n = 8)	427 ± 58 (n = 14)
PPARγ (AU/µg protein)	113 ± 13 (n = 14)	108 ± 11 (n = 22)
mFABP (pmol/mg protein)	270 ± 31 (n = 13)	285 ± 24 (n = 20)
α-Actin (AU/µg protein)	233 ± 46 (n = 14)	295 ± 30 (n = 21)

Results are given as arbitrary units (AU), number of subjects are given in brackets (*n*)

cose and insulin responses to an oral glucose load were also higher in the diabetes group. Insulin resistance was confirmed in the diabetes group by a significantly reduced maximum insulin-stimulated glucose disposal rate.

Baseline expression of PPARs. All three known PPAR forms are present in appreciable amounts in skeletal muscle (Fig. 2). The PPARα protein expression was consistently greater in muscle than in adipose tissue from the same subject (five to ninefold of the value in paired fat tissue, on average 7.6-fold). Although displaying considerable intersubject variability, PPARβ protein was detected at approximately the same abundance in both tissues. The PPARγ protein was readily detected in muscle tissue, although the relative expression between muscle and fat (31–100 % of the value in paired fat, average = 69 %, n = 8) varied over a broad range between subjects. The relative expression of PPARγ protein in fat and muscle was similar in non-diabetic and diabetic subjects. The PPARγ2 protein was detectable in fat but not in muscle tissue (Fig. 1). Thus PPARγ1 seems to be the predominant isoform expressed in skeletal
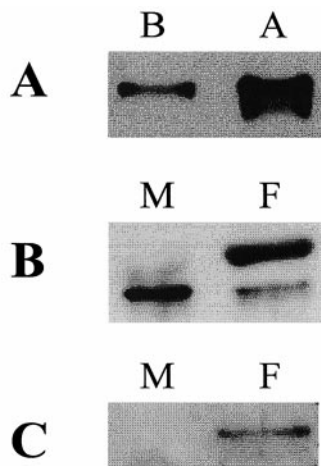
Fig. 1 A–C. Representative western blots for **A** PPARγ in NIH3T3 cells before (B) and after differentiation into adipocytes (A), **B** total PPARγ and **C** PPARγ2 in muscle (M) and fat (F)

muscle. This could also explain why the PPARγ band detected in muscle consistently displays a greater mobility than that in fat, which seems to contain both PPARγ1 and the larger γ2 isoform. (Fig. 2). Electrophoresis conditions were selected to permit analysis of multiple proteins on the same membrane. Under other conditions a doublet for PPARγ was observed in adipose tissue samples (Fig. 1).

No difference in muscle PPARα, β or γ protein expression was observed between non-diabetic and Type II diabetic subjects (Table 2). The relative expression of PPARγ mRNA and protein was similar in muscle tissue with significant correlations between PPARγ protein and mRNA in both non-diabetic ($r = 0.61$, $p \leq 0.05$) and Type II diabetic ($r = 0.80$, $p \leq 0.05$) groups.

Protein expression of muscle-specific fatty acid-binding protein (mFABP), which is known to be critical for utilisation of cellular fatty acids, and of α-sarcomeric actin, a marker for the muscle phenotype [34] was also similar in both groups (Table 2). Human adipose lipid binding protein (hALBP) mRNA and protein were detectable in adipose but not muscle tissue, consistent with minimal adipose content of muscle samples.

Regulation of baseline PPAR expression. To determine whether the in vivo metabolic environment might influence the expression of the PPARs in muscle, the relations between protein expression of these nuclear receptors and clinical characteristics were investigated. Regression analysis showed no influence of age, BMI, fasting serum lipids (non-esterified fatty acids and triglycerides), blood glucose, glycated haemoglobin and fasting serum insulin on protein expression of any PPAR in the whole study group or the diabetic and non-diabetic groups individually. Be-
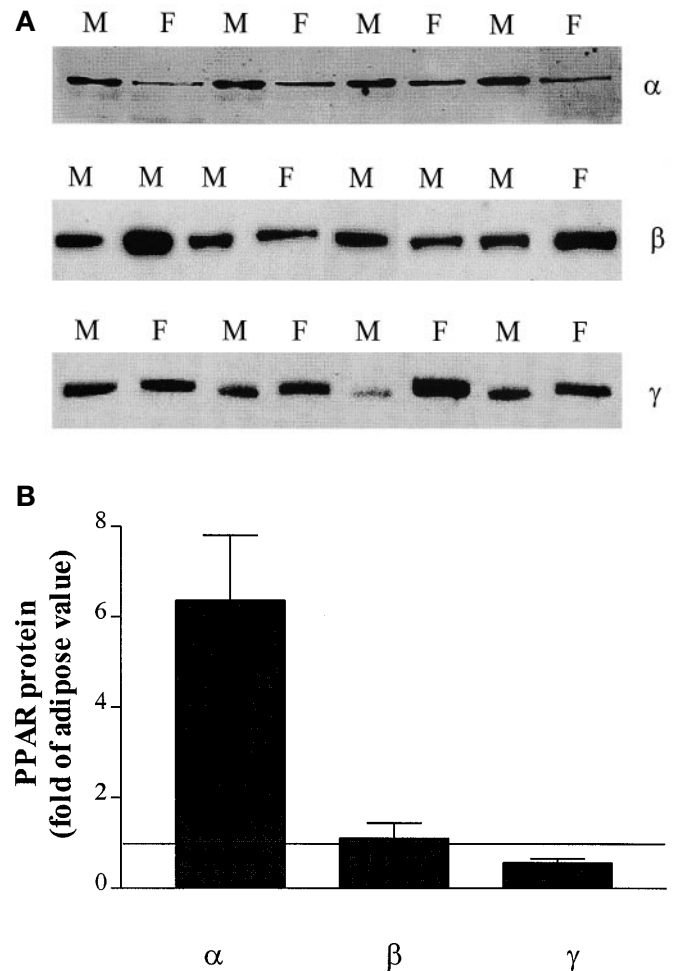

Fig. 2. **A** Expression of PPARα (top panel), PPARβ (middle panel) and PPARγ (bottom panel) proteins in human skeletal muscle and isolated adipocytes. Tissue was obtained and protein extracts prepared as described in the Methods section. Equal amounts (10 μg) of muscle (M) and adipocyte (F) protein were separated by SDS-PAGE and western blotted by antisera against PPARα, PPARβ and PPARγ. For PPARα and PPARγ, tissues from the same subject were run in adjacent lanes. **B** Relative expression of PPAR protein in skeletal muscle. Results presented as a fold of the expression in adipocytes from the same subjects. Average ± SEM for PPARα ($n = 5$), PPARβ ($n = 6$), PPARγ ($n = 8$)

cause PPARγ protein has been suggested as being involved in the regulation of skeletal muscle insulin action, the relation between these two variables was explored further. The insulin-stimulated GDR, determined in each subject during the last 30 min of a hyperinsulinaemic (300 mU · m⁻² · min⁻¹), euglycaemic clamp, was used as a measure of maximum insulin action in muscle. A statistically significant inverse relation was detected between muscle PPARγ protein and maximum GDR in the diabetic subjects ($r = -0.47$, $p \leq 0.05$). Conversely, in the non-diabetic subjects, there was a trend for PPARγ protein to increase with increasing GDR (Fig. 3A), the opposite of the behaviour observed in diabetic subjects,
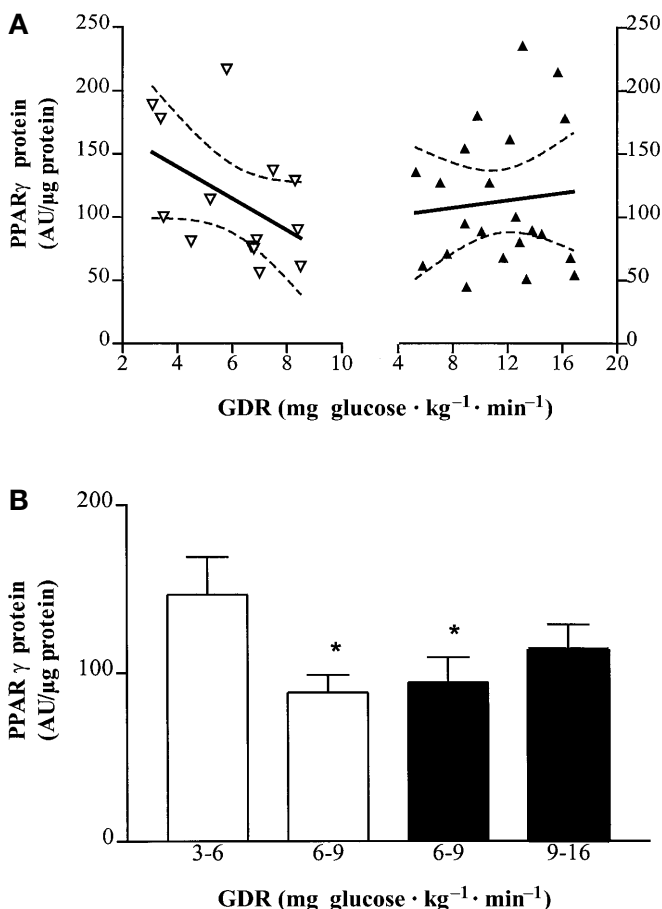
Fig. 3 A, B. Expression of PPARγ protein as a function of maximum insulin action (insulin-stimulated GDR). **A** Correlation of PPARγ protein and GDR in diabetic ($r = 0.47, p \leq 0.05,$ Δ) and non-diabetic (not significant, ▲) subjects. **B** Non-diabetic (■) and Type II diabetic (□) subjects grouped by GDR. Muscle biopsy specimens obtained before insulin infusion. Average ± SEM. *$p \leq 0.05$ vs GDR = 3–6

though this correlation did not attain statistical significance. Diabetic subjects with severe insulin resistance (GDR = 3–6 mg · kg^{-1} · min, mean = 4.3, $n = 6$) were compared to diabetic subjects with moderate to mild resistance (GDR = 6–9, mean = 7.5, $n = 8$) and to non-diabetic subjects with low (GDR = 6–9, mean = 7.5, $n = 7$) and high (GDR = 9–16, mean = 13.3, $n = 15$) insulin responsiveness. This subgroup analysis showed significantly higher muscle PPARγ protein in diabetic subjects with severe insulin resistance (GDR = 3–6) compared with their diabetic counterparts matched with them for age and weight with moderate to mild resistance (147 ± 23 vs 88 ± 10 AU/μg protein, $p \leq 0.01$) and non-diabetic subjects (147 ± 23 vs 94 ± 15, $p \leq 0.04$) with GDR = 6–9 (Fig. 3B). Thus, the most insulin resistant diabetic subjects have the highest muscle PPARγ protein expression. Except for the lean non-diabetic subjects, the subgroups were matched for BMI, eliminating obesity as a variable. Age, BMI, non-esterified fatty

acids, fasting glucose, glycated haemoglobin and fasting insulin did not differ between the two diabetic subgroups with GDR = 3–6 and GDR = 6–9. The PPARα, β, mFABP and α-sarcomeric actin protein also did not differ between the four GDR subgroups (not shown).

Short-term effect on insulin on protein expression. The expression of muscle PPARγ protein was not significantly changed by 3 h of insulin infusion (300 mU · m^{-2} · min^{-1}) from baseline values. They remained similar in the two groups (diabetic: 144 ± 20, non-diabetic: 146 ± 14 AU/μg protein, post-clamp, see Table 2 for baseline values). The PPARγ protein was also unaltered after insulin infusion, (diabetic, 107 ± 7, non-diabetic: 107 ± 13 AU/μg protein, post-clamp).

Protein expression of mFABP was also not significantly different after the clamp in both subjects with diabetes (206 ± 30 vs 224 ± 11 pmol/mg protein, baseline vs clamp, respectively) and non-diabetic subjects (195 ± 17 vs 207 ± 16). The protein expression of the muscle marker, α-sarcomeric actin, was not influenced by short-term insulin exposure in either diabetic or non-diabetic subjects (not shown).

Discussion

A number of recent reports support roles for PPARα and PPARγ in the regulation of glucose and lipid metabolism [2, 4–8, 14–19]. Although PPARβ is detectable in a wide range of tissues [11], including skeletal muscle and fat, it has not yet been assigned a specific role, partly due to its ubiquitous expression. For PPARα, investigators have focused their attention primarily on the liver, the tissue where PPARα was described to be predominantly expressed in rat [11]. There is, however, also information indicating that the relative expression of PPARα mRNA in human skeletal muscle is much higher than in rodent skeletal muscle [3]. Although the focus of research is widening to other cell types such as adipocytes, where PPARα is up-regulated in hyperleptinaemia-induced adipocyte dedifferentiation [35], the role of PPARα in skeletal muscle has not yet been addressed. The high expression of PPARα protein in muscle relative to fat reported here (7.6 : 1), suggests that this nuclear protein could have an important role in muscle fatty acid metabolism.

The PPARγ protein is expressed early during adipocyte differentiation [36]. Several lines of evidence suggest an involvement of PPARγ in insulin action. There is a close relation between the antidiabetic, insulin-sensitising actions of the thiazolidinedione agents and their ability to bind to [18] and activate PPARγ [1, 14–17, 36, 37]. Adipose tissue PPARγ mRNA is positively correlated with obesity (BMI)

and insulin concentrations [21–24]. Weight loss leads to reductions in PPARγ expression in adipocytes, together with improvement in glycaemia and lipidaemia [21]. The majority of these observations were made in adipose tissue, yet PPARγ action in skeletal muscle could differ from that in the adipocyte. We [22] and others [12, 20, 21] have identified PPARγ mRNA in skeletal muscle, although in lower abundance than in adipose tissue and investigated its regulation and possible involvement in insulin action.

In this study we focused our attention on protein expression because it is the protein that is responsible for the gene effects of PPARγ. Given the abundance of PPARγ in adipose tissue [20–22], a key concern is that the current results obtained in skeletal muscle could be influenced by the presence of adipose tissue in the biopsy samples. The following factors indicate that the contribution of adipose tissue to our results is limited. Neither mRNA, nor protein of the adipocyte specific gene *ALBP* was detectable in muscle biopsy specimens, indicating that the adipose content was below the detection sensitivity of these methods. If a higher adipose tissue content did contribute to differences in the PPARγ protein abundance detected, then muscle biopsy samples from obese subjects could be expected to have a higher PPARγ protein content. In the analysis done on the subgroups, lean non-diabetic subjects have more muscle PPARγ protein than obese non-diabetic subjects who would be expected to have more fat in their muscle. Another point to note is the different mobilities of PPARγ protein detected in the two tissues. The results of our PPARγ2 blots suggest that the larger form seen in adipose tissue is most likely the γ2 isoform. This band is not apparent in the muscle samples but it should be there if appreciable contamination by adipose tissue were present.

Although PPARγ protein expression was similar in the total diabetic and non-diabetic groups, diabetic subjects with severe insulin resistance (GDR = 3–6) had a significantly higher PPARγ protein compared to their more insulin sensitive counterparts matched with them for age and weight with and without diabetes (GDR = 6–9). Diabetic and non-diabetic subjects of intermediate insulin responsiveness (GDR = 6–9) were matched for BMI and have equal amounts of PPARγ protein. The most insulin resistant diabetic subjects (GDR = 3–6) were matched for BMI with both intermediate groups yet have the highest PPARγ protein, indicating that the key characteristic is the degree of insulin resistance and not obesity.

The increased expression of skeletal muscle PPARγ protein in Type II diabetic subjects with the most severe insulin resistance could reflect either an acquired or inherited abnormality. We did not find any direct relation between metabolic variables that are characteristically increased in Type II diabetes such as glucose, insulin or lipid concentrations,

and PPARγ protein expression. Whether other factors unique to the metabolic environment of the diabetic state are involved in the regulation of expression or function of PPARγ protein in diabetes is still to be resolved. Functionally active mutations in the *PPARγ* gene, as possibly present in these severely insulin resistant subjects with Type II diabetes, could influence the function of the protein and lead to the abundance of PPARγ as observed in these patients. It should be remembered that the final action of PPARγ is dependent on a number of factors. These include, but are not limited to, the expression and function of PPARγ, the abundance of the relevant endogenous ligands/activators, numerous co-activators or co-repressors and the expression and function of RXRs, the companion nuclear receptors essential for formation of the active heterodimeric complex [1].

We have previously shown that insulin infusion rapidly increases PPARγ mRNA expression in muscle [22]. That protein expression of any PPAR was not up-regulated after 3 h of insulin exposure could be due to translational kinetics which do not permit complete synthesis and processing of detectable additional quantities of the proteins in that time. In agreement with our previous finding in muscle, a recent report also found PPARγ mRNA in adipose tissue to be increased after a 3-h hyperinsulinaemic, euglycaemic clamp [38]. Other investigators found no change in skeletal muscle PPARγ1 mRNA after 5 h of hyperinsulinaemia [39]. This discrepancy could also represent a potential transient nature of the insulin effect to increase gene expression, it could have returned to baseline by 5 h. There are other examples of such transient responses in gene expression to insulin exposure [40].

Several conclusions can be drawn from the current work. First, the PPAR proteins are all expressed in skeletal muscle and adipose tissue but with distinct patterns. Since in vivo activation of PPARγ seems to influence both glucose and lipid concentrations whereas PPARα agonists influence triglyceride metabolism [41], these receptors could be regulating different but complementary events in skeletal muscle. As ligands that activate PPARβ have no effect on glucose or lipid concentrations [41], the role for PPARβ in skeletal muscle is still uncertain. Of these three nuclear receptors, only skeletal muscle PPARγ shows differences between groups, with high protein expression in the most severely insulin resistant subjects. Furthermore, the relation between PPARγ protein expression in skeletal muscle and peripheral insulin action/glucose disposal, either as a cause or a consequence, is altered in Type II diabetic compared with non-diabetic subjects. This suggests diabetes-related differences in the function or regulation of the PPARγ system in skeletal muscle. No such relation appears to exist for PPARα protein. Thus, PPARγ

content in skeletal muscle could be an important factor through which insulin controls glucose and lipid metabolism.

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