Diabetologia (2005) 48: 96–104
DOI 10.1007/s00125-004-1612-3

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A. Hammarstedt · V. Rotter Sopasakis · S. Gogg · P.-A. Jansson · U. Smith

Improved insulin sensitivity and adipose tissue dysregulation after short-term treatment with pioglitazone in non-diabetic, insulin-resistant subjects

Received: 16 July 2004 / Accepted: 14 September 2004 / Published online: 29 December 2004

Abstract *Aims/hypothesis:* We examined whether short-term treatment with a thiazolidinedione improves insulin sensitivity in non-obese but insulin-resistant subjects and whether this is associated with an improvement in dysregulated adipose tissue (reduced expression of IRS-1, GLUT4, PPARγ co-activator 1 and markers of terminal differentiation) that we have previously documented to be associated with insulin resistance. *Methods:* Ten non-diabetic subjects, identified as having low IRS-1 and GLUT-4 protein in adipose cells as markers of insulin resistance, underwent 3 weeks of treatment with pioglitazone. The euglycaemic–hyperinsulinaemic clamp technique was used to measure insulin sensitivity before and after treatment. Serum samples were analysed for glucose, insulin, lipids, total and high-molecular-weight (HMW) adiponectin levels. Biopsies from abdominal subcutaneous adipose tissue were taken, cell size measured, mRNA and protein extracted and quantified using real-time RT-PCR and Western blot. *Results:* Insulin sensitivity was improved after 3 weeks treatment and circulating total as well as HMW adiponectin increased in all subjects, while no effect was seen on serum lipids. In the adipose cells, gene and protein expression of IRS-1 and PPARγ co-activator 1 remained unchanged, while adiponectin, adipocyte P 2, uncoupling protein 2, GLUT4 and liver X receptor-α increased. Insulin-stimulated tyrosine phosphorylation and p-ser-PKB/Akt increased, while no significant effect of thiazolidinedione treatment was seen on the inflammatory status of the adipose tissue in these non-obese subjects. *Conclusions/interpretation:* Short-term treatment with pioglitazone improved insulin sensitivity in the absence of any changes in circulating NEFA or lipid levels. Several markers of adipose cell differentiation, previously shown to be reduced in insulin resistance, were augmented, supporting the concept that insulin resistance in these individuals is associated with impaired terminal differentiation of the adipose cells.

Keywords Adiponectin · Adipose tissue · GLUT4 · Insulin resistance · IRS-1 · LXR · Thiazolidinediones

Abbreviations AdipoR: Adiponectin receptor · aP2: Adipocyte P 2 · C/EBP: CCAAT/Enhancer binding protein · GIR: Glucose infusion rate · HMW: High molecular weight · IR: Insulin receptor · LBM: Lean body mass · LXR: Liver X receptor · MCP1: Monocyte chemoattractant protein-1 · PGC-1: PPARγ Co-activator 1 · PI3-kinase: Phosphoinositide-3-kinase · PKB: 3-Phosphoinositide-dependent protein kinase B · PPAR: Peroxisome proliferator-activated receptor · PTP: Phosphotyrosine phosphatase · SOCS: Suppressors of cytokine signalling · PTP-1C: Phosphotyrosine phosphatase 1C · RXR: Retinoic X receptor · UCP-2: Uncoupling protein 2

Introduction

Thiazolidinediones like pioglitazone and rosiglitazone are powerful insulin sensitisers used in the treatment of type 2 diabetes. The nuclear receptor for thiazolidinedione, peroxisome proliferator-activated receptor (PPAR) γ, is highly expressed in adipocytes suggesting that thiazolidinediones mainly exert their insulin-sensitising effect through the adipose tissue although controversial results have been published [1, 2]. Thiazolidinediones improve insulin sensitivity both in man and in different animal models of insulin resistance [3, 4]. The improvement in insulin resistance is, at least in rodent models, accompanied

A. Hammarstedt · V. Rotter Sopasakis · S. Gogg · P.-A. Jansson · U. Smith
The Lundberg Laboratory for Diabetes Research, Department of Internal Medicine, The Sahlgrenska Academy at Göteborg University,
Göteborg, Sweden

U. Smith (✉)
The Lundberg Laboratory for Diabetes Research, Department of Internal Medicine, Sahlgrenska University Hospital,
413 45 Göteborg, Sweden
e-mail: ulf.smith@medic.gu.se
Tel.: +46-31-3421104
Fax: +46-31-829138

by a remodelling of the adipose tissue, where large adipocytes are replaced by an increased recruitment of small and more insulin-sensitive cells [5, 6]. However, to what extent this occurs in human adipose tissue is still not clear [7].

The adipose tissue is an endocrine organ, secreting several factors (adipokines) that can affect whole-body insulin sensitivity. One such molecule of great current interest is adiponectin, which is only secreted by the adipose cells [8–10]. Thiazolidinediones not only increase the concentration of total circulating adiponectin but also alter the relative abundance of the different molecular weight complexes [11].

Growing evidence has pointed to a relationship between low-grade chronic systemic inflammation and insulin resistance. Studies in man have shown an increase in inflammatory markers and cytokines in different states of insulin resistance [12]. Recent studies have also shown that the adipose tissue becomes "inflamed" in obesity as a consequence of an increased infiltration of inflammatory cells [13–15]. Furthermore, the adipose tissue expression of macrophage-related genes is positively correlated with BMI and adipocyte cell size [13]. Similarly, we have recently shown that there is a correlation between fat cell size and IL-6 secretion in vitro as well as the interstitial concentrations in the adipose tissue in vivo [16].

We have also recently shown that there is a clear association between insulin resistance, type 2 diabetes and adipose tissue dysfunction. For instance, several key molecules for insulin signalling and action, such as IRS-1 and GLUT4, are reduced in adipose cells from insulin-resistant subjects with or without type 2 diabetes [17, 18].

The insulin-stimulated activation of phosphoinositide-3-kinase (PI3-kinase), phosphoinositide-dependent protein kinase B (PKB)/Akt and glucose transport in the adipose cells are also impaired in insulin-resistant states [17, 18]. In addition to these perturbations, we have identified other markers for dysregulated adipose tissue in insulin resistance suggesting impaired adipose cell differentiation, i.e. reduced expression of adiponectin and adipocyte P 2 (aP2), low circulating adiponectin levels, increased fat cell size [19, 20] and a reduced expression of the PPARγ co-activator 1 (PGC-1) [21].

In the present study, we examined whether a 3-week treatment with the thiazolidinedione, pioglitazone, improves insulin sensitivity in insulin-resistant but non-obese and non-diabetic subjects exhibiting the markers of adipose tissue dysfunction and, if so, whether this is related to an improvement in these markers. In addition, we examined if the thiazolidinedione in this group changed the expression of inflammatory markers related to monocyte/macrophage infiltration in the adipose tissue.

Materials and methods

Subjects This study was approved by the Ethical Committee of Göteborg University and informed consent was obtained from each subject. Ten non-diabetic subjects, previously identified as having low expression of IRS-1 and GLUT4 protein in the adipose cells as markers of insulin resistance [17, 18], volunteered to undergo 3 weeks of treatment with pioglitazone (Actos, 30 mg/day). Body weight and height were recorded with standard techniques. Waist and hip circumferences were measured as described [22] and WHR calculated. Lean body mass (LBM) was calculated from bioimpedance analysis (BIA-101, Akern, Florence, Italy) [23].

Euglycaemic–hyperinsulinaemic clamp Insulin sensitivity was measured with the euglycaemic–hyperinsulinaemic clamp technique [24], previously described in detail [25]. In brief, insulin was infused at a constant rate of 40 $mU \cdot m^{2} \cdot min^{-1}$ into an intravenous cannula placed in the antecubital vein. By infusing glucose at a variable rate, blood glucose was maintained at 5 mmol/l. Steady state was reached after 60–90 min, and the average rate of glucose infusion required to maintain euglycaemia was calculated over the final 30 min (90–120 min) and expressed per kilogram LBM. Glucose was analysed in venous blood using an automatic glucose analyser (Yellow Springs Instruments, Yellow Springs, OH, USA) and insulin with a standard radioimmunoassay (Amersham, Uppsala, Sweden).

Biochemical analyses Non-esterified fatty acids in serum were measured by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany) while other plasma lipid concentrations were determined with an automated Cobra Mira analyser (Hoffman-LaRoche, Basel, Switzerland) [26]. Circulating adiponectin levels were measured in plasma by an ELISA (B-Bridge International, Sunnyvale, CA, USA).

Velocity sedimentation of adiponectin Five to 20% sucrose gradients were poured stepwise in thin-walled ultracentrifuge tubes (Beckman, Palo Alto, CA, USA) and allowed to equilibrate overnight at 4°C. Following layering of the sample on top, gradients were centrifuged at 55,000×*g* for 4 h at 4°C in a TLS55 rotor in a Beckman TL-100 ultracentrifuge. 150-µl gradient fractions were sequentially retrieved and analysed by quantitative Western blot analysis as described below.

Fat cell isolation Human abdominal subcutaneous adipose tissue was obtained in the fasting state by a surgical incision. Isolation of human adipose cells was performed essentially as described previously [27]. Briefly, biopsies were washed to remove traces of blood and treated with 0.8 mg/ml collagenase (Sigma, St. Louis, MO, USA) for ~60 min at 37°C. Isolated adipose cells were filtered through a 250-µm nylon mesh, washed four times with fresh medium to remove collagenase and the cell size was then measured [27].

Cell lysate and immunoblotting Isolated human adipocytes were separated from medium by centrifugation through dinonyl phthalate. Lysis buffer was added, samples briefly

vortexed and rocked for 2 h at 4°C. Detergent-insoluble material was sedimented through centrifugation at 12,000×*g*; this was done for 10 min at 4°C. Supernatants were collected and stored at −80°C prior to use [17]. Protein concentration was measured using the bicinchonic acid method (Pierce, Rockford, IL, USA).

Lysate proteins were separated on SDS-PAGE as described [18] and immunoblotted with anti-adiponectin (Alexis, Lausen, Switzerland), anti-IRS-1, anti-IRS-2 (Upstate Biotechnology, Lake Placid, NY, USA), anti-PGC-1, anti-phosphotyrosine (Santa Cruz, Santa Cruz, CA, USA), anti-PKB/Akt, anti-phospho-serine-PKB/Akt (p-ser-PKB/Akt) (Cell Signaling Technology, Beverly, MA, USA), anti-GLUT4 (Chemicon, Temecula, CA, USA) and anti-perilipin (PROGEN Biotech, Heidelberg, Germany) antibodies according to the manufacturer's recommendations.

Enhanced chemiluminescence was used to detect the proteins (Amersham, Buckinghamshire, UK). Band intensities were quantified using a Personal Densitometer (Molecular Dynamics, Sunnyvale, CA, USA) and analysed using ImageQuant software provided by the manufacturer.

mRNA isolation and quantification Total cellular RNA was extracted with the guanidinium thiocyanate method as described [28] and TaqMan Real Time RT-PCR was used to quantify mRNA expression.

The RNA samples were treated with DNAse and single-stranded random-hexamer-primed cDNA was synthesised. Gene-specific probes and primer pairs were designed using Primer Express software (Applied Biosystems, Foster City, CA, USA). The quantification was performed using the standard protocol of ABI PRISM 7700 (Applied Biosystems). For each primer/probe set (available upon request), a standard curve was generated. Each sample was run in duplicate and the mean value was used to calculate mRNA levels. The quantity of a particular gene in each sample was normalised to that of 18s.

Statistical analysis Conventional statistical methods were used (Stat View; SAS Institute, Cary, NC, USA). Differences were tested with two-tailed Student's *t*-test for paired comparison, with a *p* value of less than 0.05 considered to be significant.

Results

Clinical characteristics and effect of thiazolidinedione The clinical characteristics of the subjects before and after treatment are shown in Table 1. The subjects were non-obese and neither body weight nor WHR changed during the treatment period. There were also no significant changes in fasting blood glucose or lipid levels after the treatment. Insulin sensitivity, measured with the hyperinsulinaemic–euglycaemic clamp technique, was improved by 20% after 3 weeks treatment ($p<0.01$). This improvement was seen in all but one subject. Surprisingly, both fasting and NEFA levels during the clamp (180 min) were similar before and after thiazolidinedione treatment. Average fat cell size decreased in most subjects (Table 1). However, a decreased mean fat cell size was not a prerequisite for improved insulin sensitivity.

Circulating adiponectin levels increased by approximately 100% after thiazolidinedione treatment ($p<0.001$, Table 1, Fig. 1a) and, in contrast to the glucose infusion rate (GIR), all individuals increased their adiponectin levels. No significant correlation was seen between circulating adiponectin levels and the GIR before or after treatment with thiazolidinedione (Fig. 1c, e). Furthermore, there was no correlation between the increase in circulating adiponectin concentrations and the improvement in insulin sensitivity (data not shown).

We also measured the ratio between the high-molecular weight (HMW) adiponectin and the total amount of adiponectin. The relative amount of HMW adiponectin increased in all subjects after treatment (average increase 11%; $p<0.01$) (Fig. 1b). However, neither the levels before or after thiazolidinedione correlated significantly with whole-body insulin sensitivity, measured as GIR (Fig. 1d, f), nor did the absolute change following pioglitazone treatment (data not shown). Interestingly, there was no relationship between HMW adiponectin, or the increase

Table 1 Clinical characteristics and metabolic variables

	Before treatment	After treatment	*p* Value
Age (years)	43.3±1.9		
BMI (kg/m^2)	26.9±1.1	26.9±1.0	NS
WHR	0.95±0.01	0.95±0.01	NS
f-Glucose (mmol/l)	4.94±0.20	4.65±0.16	0.06
f-Insulin (mU/l)	9.39±1.32	8.34±1.05	NS
f-HDL (mmol/l)	1.39±0.10	1.45±0.14	NS
f-Cholesterol (mmol/l)	5.65±0.31	5.57±0.25	NS
f-TG (mmol/l)	1.78±0.29	1.66±0.28	NS
NEFA baseline (mmol/l)	0.53±0.03	0.45±0.06	NS
NEFA 180 min (mmol/l)	0.11±0.02	0.13±0.02	NS
GIR (mg·kg LBM^{-1} min^{-1})	7.70±0.76	9.17±0.92	<0.01
Cell size (μg)	0.46±0.03	0.42±0.04	NS
Adiponectin (μg/ml)	7.16±1.36	13.51±1.87	<0.001

Values are expressed as mean ± SEM.
TG Triglycerides, *GIR* glucose infusion rate

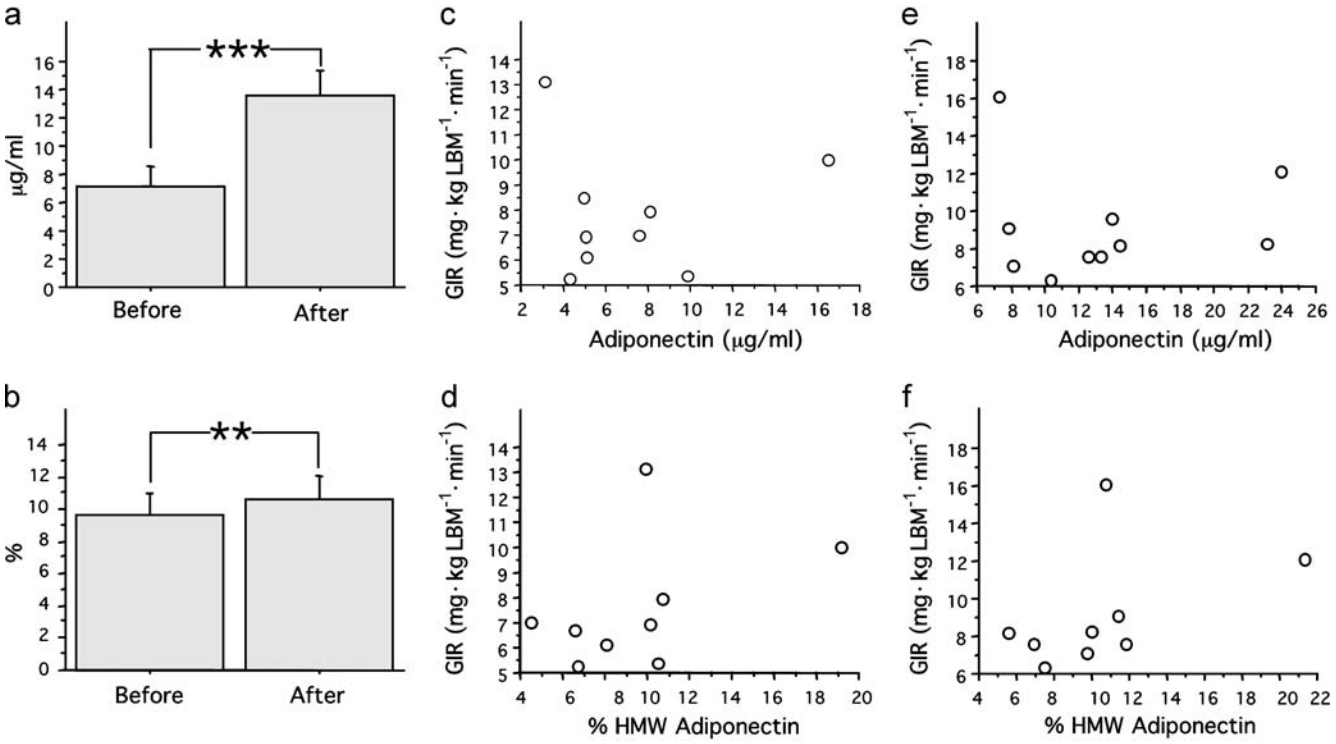

Fig. 1 Circulating total (**a**) and relative amounts of HMW (**b**) adiponectin before and after thiazolidinedione treatment. Correlation analyses between circulating and relative levels and insulin sensitivity measured as the glucose infusion rate (GIR) during a euglycaemic–hyperinsulinaemic clamp is shown before (**c**, **d**) and after (**e**, **f**) thiazolidinedione treatment, respectively. $^{**}p<0.01$, $^{***}p<0.001$. HMW, high-molecular weight

following pioglitazone, and the total circulating levels of adiponectin.

Adipose cell differentiation The expression of several genes before and after pioglitazone treatment was examined in isolated adipose cells (Table 2) as well as in intact biopsies. In general, similar effects were found. In all subjects, mRNA levels of adiponectin, like circulating levels, increased after treatment, but the magnitude was much smaller (30%, $p<0.05$) (Table 2) and did not correlate with total circulating or HMW adiponectin levels or with insulin sensitivity (data not shown).

The mRNA for another marker of adipocyte differentiation, aP2, was also increased after pioglitazone treatment ($p=0.06$). However, neither adipose differentiation-related protein nor lipin nor perilipin expression was changed (Table 2, Fig. 2a).

No significant changes were seen in the mRNA levels of the putative adiponectin receptors 1 or 2 (Table 2), and the expression of either receptor did not correlate with total circulating or HMW adiponectin levels or with insulin sensitivity (data not shown). In contrast to adiponectin mRNA, there was no change in the expression level of either AMP-activated kinase alpha subunit 1 or 2 in the isolated adipocytes after 3 weeks of treatment with pioglitazone (Table 2).

GLUT4, PGC-1 and liver X receptor-α The mRNA expression of GLUT4 increased ($p<0.01$, Table 2) and this

Table 2 Gene expression in isolated adipocytes

	Before treatment	After treatment	*p* Value
Insulin receptor	1.08±0.16	1.02±0.13	NS
IRS1	1.12±0.19	1.14±0.11	NS
IRS2	1.53±0.21	1.49±0.12	NS
GLUT4	1.16±0.21	1.67±0.23	<0.005
PGC1	1.31±0.31	1.23±0.21	NS
LXRα	0.74±0.06	0.86±0.07	0.06
PPARγ2	3.12±0.92	2.88±0.62	NS
Adiponectin	2.08±0.48	2.51±0.43	<0.05
aP2	0.87±0.04	1.00±0.07	0.06
ADRP	1.66±0.19	1.69±0.18	NS
Perilipin	1.82±0.17	1.81±0.11	NS
Lipin	1.18±0.18	1.36±0.29	NS
AdipoR1	2.10±0.74	2.00±0.77	NS
AdipoR2	1.61±0.33	1.70±0.42	NS
AMPKα1	0.79±0.27	0.64±0.15	NS
AMPKα2	0.60±0.17	0.54±0.12	NS
FOXC2	2.39±0.50	2.40±0.64	NS
UCP2	1.46±0.23	2.22±0.31	<0.005

Values are expressed as mean ± SEM.
PGC1 PPARγ co-activator 1, *LXRα* liver X receptor, *PPARγ2* peroxisome proliferator-activated receptor γ2, *ap2* adipocyte P 2, *ADRP* adipose differentiation-related protein, *AdipoR1/2* adiponectin receptor 1/2, *AMPK* AMP-activated kinase, *FOXC2* forkheadox C2, *UCP2* uncoupling protein 2

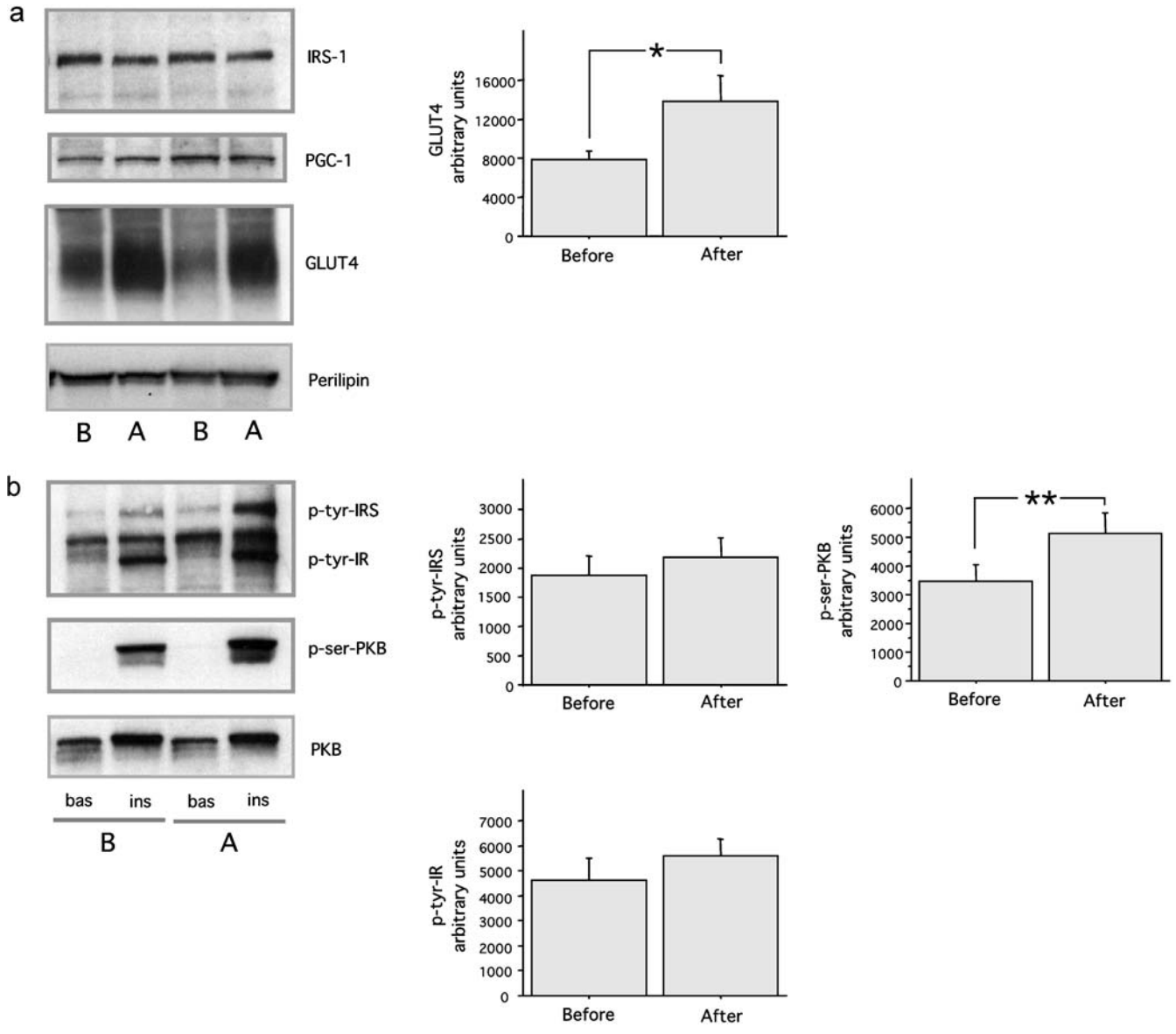

Fig. 2 **a** Representative Western blots of IRS-1, PGC-1, GLUT4 and perilipin performed on isolated cells from two individuals, before (B) and after (A) treatment with thiazolidinedione. *Bars* show the densitometry scanning of GLUT4 protein expression (n=8). **b** Representative Western blots of insulin-stimulated phosphorylation of the insulin receptor (IR), IRS and PKB/Akt protein and serine phosphorylation performed on cells from one individual, before (B) and after (A) treatment with thiazolidinedione (n=4). Bars show the densitometry scanning of the respective bands. Values are expressed as means ± SEM. *p<0.05, **p<0.01

was accompanied by an identical increase in GLUT4 protein expression (72%, p<0.05) (Fig. 2a). The mRNA expression of GLUT4 correlated positively with that of adiponectin, both in isolated adipose cells (r^2=0.43, p=0.07; Fig. 3a) and in adipose tissue (r^2=0.67, p<0.01; Fig. 3b), but there was no significant correlation between either plasma total or HMW adiponectin and the expression of GLUT4 mRNA or protein (data not shown). PGC-1 is expressed in human white adipose cells and the expression level is reduced in insulin resistance [21]. PGC-1 has also been found to increase GLUT4 expression in skeletal muscle [29]. However, there was no change in *PGC-1* gene or protein expression in the adipose cells when comparing before and after pioglitazone treatment (Table 2, Fig. 2a). Another transcription factor which has been shown to increase GLUT4 expression in adipose cells is liver X receptor (LXRα) [30]. Interestingly, LXRα expression was increased after pioglitazone in isolated adipose cells (Table 2), as well as in intact tissue (p<0.01).

Forkheadox C2 and uncoupling protein 2 We also examined mRNA levels of these proteins, which are considered to be important for energy expenditure. Forkheadox C2 remained unchanged while uncoupling protein 2 (UCP2) was markedly increased in isolated adipose cells (Table 2) and in adipose tissue (p<0.05).

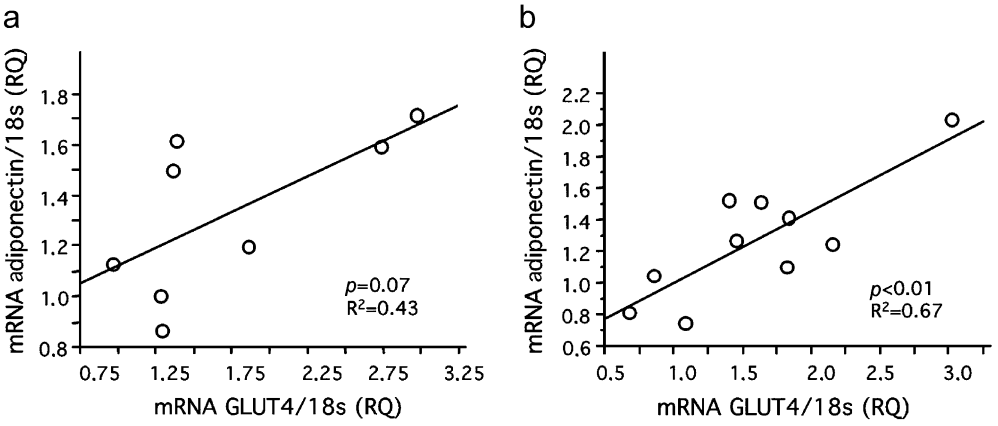

Fig. 3 Correlation analysis of GLUT4 and adiponectin mRNA measured by real-time RT-PCR in isolated adipose cells (**a**) and adipose tissue (**b**)

Insulin signalling in the adipose cells The effect of insulin, when added for 15 min to the cells in vitro, on the downstream signalling, which was measured as serine phosphorylation of PKB/Akt, as well as the upstream tyrosine phosphorylation of the insulin receptor (IR) and the IRS, was examined in four individuals from whom enough tissue was obtained both before and after treatment with pioglitazone. As shown in Fig. 2, p-ser-PKB/Akt was significantly increased after thiazolidinedione and this was associated with increased tyrosine phosphorylation of IR and IRS (Fig. 2b). The increased p-ser-PKB/Akt was not due to increased protein expression, nor was IR or *IRS-1* gene expression (Table 2) or IRS-1 protein increased (Fig. 2a). We also examined whether IRS-2 was increased, but no such consistent effect was found after the short-term pioglitazone treatment (data not shown).

Thus, the increased downstream activation of PKB/Akt following pioglitazone could not be attributed to effects on protein expression of PKB/Akt or the expression of upstream signalling molecules. We then examined whether pioglitazone changed the expression of the phosphotyrosine phosphatase (PTP)-1B and PTP-1D. However, the mRNA levels of both these phosphatases remained unchanged (data not shown).

Table 3 Gene expression of inflammatory markers in adipose tissue

	Before treatment	After treatment	*p* Value
IL6	0.29±0.06	0.23±0.05	NS
Gp130	0.87±0.19	0.96±0.25	NS
SOCS1	0.58±0.08	0.60±0.07	NS
SOCS3	0.11±0.03	0.10±0.03	NS
TNFα	1.09±0.15	1.20±0.14	NS
IL8	0.20±0.04	0.16±0.03	0.06
CD68	1.06±0.11	1.32±0.13	0.06
MAC1	0.93±0.11	1.00±0.11	NS
MCP1	0.50±0.12	0.46±0.04	NS
EMR1	0.76±0.11	0.88±0.08	NS

Values are expressed as mean ± SEM.
SOCS1/3 Suppressors of cytokine signalling 1/3, *CD68* macrophage antigen CD68, *MAC1* macrophage antigen alpha polypeptide, *MCP1* monocyte chemoattractant protein-1, *EMR1* egf-like module containing, mucin-like, hormone receptor-like 1

Inflammatory markers in the adipose tissue The gene expression of several pro-inflammatory cytokines known to be increased in states of insulin resistance, such as IL-6, TNFα and IL-8, was measured in intact adipose tissue (Table 3) and in isolated adipose cells. We also measured the mRNA levels of proteins involved in cytokine signalling (Table 3).

None of these genes changed their expression levels significantly after pioglitazone either in the isolated adipose cells (not shown) or in the adipose tissue, although there was a trend for expression of IL-8 to be decreased in the adipose tissue (p=0.06; Table 3).

The inflammatory process that occurs in adipose tissue in obesity is also reflected by an elevation of macrophage attractants and markers [14] suggesting that adipose tissue becomes inflamed in obesity [15]. We, therefore, also measured specific markers for macrophage infiltration in the adipose tissue before and after treatment. However, no change was seen in either the macrophage surface markers (macrophage antigen alpha polypeptide, egf-like module containing, mucin-like, hormone receptor-like 1 or macrophage antigen CD68) or in monocyte chemoattractant protein-1 (MCP1), a member of the small inducible cytokines family, which, like IL-8, can play an important role in the recruitment of macrophages (Table 3).

Discussion

Previous studies from this laboratory have shown an early association between adipose tissue dysfunction and insulin resistance [17–21, 31].

In the present study, we examined whether thiazolidinedione treatment improved some, or all, of the identified markers of adipose tissue dysfunction in this group of individuals. Since the subjects were non-diabetic, confounding effects of lowering the degree of glycaemia would not be expected.

The salient results of the present study are: (1) insulin sensitivity was improved in the non-diabetic subjects after only 3 weeks of treatment with pioglitazone; (2) this improvement was seen in the absence of any reduction in the circulating levels of NEFA or other lipids; (3) circulating adiponectin levels, including HMW adiponectin,

were consistently increased; (4) markers of terminal differentiation of the adipose cells, like aP2 and adiponectin, were increased; (5) GLUT4, LXRα and UCP2 were increased while no difference was seen in IRS-1, PGC-1 or the markers of adipose tissue infiltration of inflammatory cells; and (6) insulin-induced intracellular signalling was improved.

Taken together, these findings are consistent with an improved terminal differentiation of the adipose cells and/or an increased recruitment of pre-adipocytes along the adipose lineage. The small difference in mean adipose cell size seen after 3 weeks tends to favour an important role for the former possibility. Surprisingly, IRS-1 expression, like PGC-1, was not increased by the thiazolidinedione, suggesting the involvement of additional mechanisms than merely impaired differentiation of the adipose cells.

In contrast to IRS-1 and PGC-1, thiazolidinedione treatment was associated with a substantial and consistent increase in the expression of GLUT4 mRNA and protein. Interestingly, a significant correlation was found between the expression of GLUT4 and adiponectin, both in isolated adipocytes and in adipose tissue biopsies, supporting an important role for enhanced terminal differentiation of the adipose cells.

Although the insulin-stimulated glucose uptake in adipose tissue only accounts for ~10% of total glucose disposal, GLUT4 expression levels in adipose cells appear to play a prominent role for whole-body insulin sensitivity, since adipose-selective ablation of the *GLUT4* gene impairs insulin action in the liver and skeletal muscle in vivo [32]. This finding clearly indicates that factors secreted from the adipose tissue influence liver and skeletal muscle insulin sensitivity.

Previous studies of the effect of PPARγ agonists on the expression of GLUT4 in the adipose cells have been contradictory [33–36]. However, a recent study has shown that the PPAR/retinoic X receptor heterodimer exerts an inhibitory effect on GLUT4 promoter activity, and that thiazolidinedione binding to the receptor alleviates this repression [37]. However, other mechanisms could also be involved. GLUT4 expression is induced by the PPARγ co-activator PGC-1, at least in skeletal muscles [29]. In addition, a recent study showed that agonists for the LXRα receptor increased GLUT4 expression in 3T3-L1 adipocytes [30]. LXRα, in contrast to PGC-1, was increased by thiazolidinedione. Thus, the present data would support a role of LXRα in increasing GLUT4 expression in response to thiazolidinedione. It has previously been shown that *LXRα* is a gene responsive to PPAR response element [38]. It should be emphasised that activation of LXRα, in contrast to PPARγ, does not enhance the terminal differentiation of adipose cells, but it does augment lipid accretion [39]. However, such cross talk between these nuclear receptors could contribute to the multitude of effects induced by thiazolidinedione.

We found no significant correlation between circulating adiponectin levels and degree of insulin sensitivity in the present study. This somewhat unexpected finding is probably due to the small number of individuals studied and/or their fairly narrow range of insulin sensitivity. Most previous studies have included larger groups with greater variation in insulin sensitivity.

However, circulating adiponectin levels increased in all subjects after treatment with pioglitazone. This finding is in line with previous publications that thiazolidinedione increases plasma adiponectin levels in obese and in type 2 diabetic patients, as well as in normal subjects to a similar extent. This is accompanied by improvements in different metabolic variables in obese and diabetic, but not in lean insulin-sensitive subjects [40]. This discrepancy has been recently elucidated by the finding that it is not the absolute amount, but rather the distribution of low- and high-molecular weight adiponectin that is important for improved insulin sensitivity in the liver [11]. We also found HMW adiponectin to be increased, but no correlation was seen with the improvement in whole-body insulin sensitivity measured as glucose disposal rate. Since the liver seems to be the primary site of action for HMW adiponectin, this finding was not unexpected and is in agreement with the recent study [11].

Since perilipin is induced by PPARγ agonists [41], such an effect of thiazolidinedione could account for the reduced NEFA levels that have been frequently reported [42, 43]. However, perilipin mRNA and protein levels were not changed by thiazolidinedione in the present study nor were the NEFA levels changed. Thus, it is possible that this effect of thiazolidinedione depends on the presence of obesity, since perilipin expression seems to be reduced in the obese state [44]. Irrespective of mechanisms, the present study clearly shows that insulin sensitivity is improved by thiazolidinedione before any changes can be seen in circulating NEFA levels.

Growing evidence has indicated an association between insulin resistance and inflammation both systemically and in the adipose tissue. Pro-inflammatory markers, such as TNFα and IL-6 are overexpressed in adipose tissue in obesity [45, 46], and in non-obese states of insulin resistance [47] IL-6 has also been shown to reduce the expression of aP2, adiponectin and PPARγ2 [16, 48], markers of terminal adipocyte differentiation. There were no significant changes in the expression of either cytokine in the present study, which makes it unlikely that the increase in adiponectin and aP2 following thiazolidinedione treatment was due to an amelioration of local inflammation. However, it should be emphasised that the subjects in the present study were non-obese.

The inflammatory process in the adipose tissue of obese and insulin-resistant individuals is not only characterised by elevation of inflammatory cytokines in the adipose cells, but also by the infiltration of inflammatory cells that may play a role in the insulin resistance [13, 14].

The adipocyte-secreted MCP-1 was recently demonstrated to be induced by IL-6 and TNFα and to decrease insulin-stimulated glucose uptake as well as the expression of GLUT4, aP2 and PPARγ [49]. Furthermore, MCP-1 and other macrophage-related genes were reduced in *ob/ob* mice following thiazolidinedione treatment [14]. However, we did not find any significant effect of thiazolidinedione

on several macrophage markers in our non-obese individuals. This is consistent with the concept that the insulin-sensitising effect of thiazolidinedione is not necessarily dependent on the anti-inflammatory effect of these agents, but that this may be of additional importance in the obese state.

Thiazolidinedione increased the expression of UCP-2, which is in agreement with some [50] but not all [51] previously published findings. However, the physiological effect of the increased UCP-2 mRNA levels following thiazolidinedione is currently unclear and requires further examination.

Finally, the present study also shows that the insulin intracellular signalling cascade was improved by short-term treatment with thiazolidinedione. This was evident both from the increased tyrosine phosphorylation of the IR and IRS, and from the serine phosphorylation of PKB/Akt.

However, the mechanism for this remains obscure since there was no up-regulation of these proteins nor could we document a change in the gene expression of PTP-1C or PTP-1D. Insufficient availability of tissue prevented us from examining directly whether the activity of these or other tyrosine phosphatases was changed, which, in fact, would appear likely.

In conclusion, the present study shows for the first time that short-term treatment with pioglitazone leads to improved insulin sensitivity in non-obese and insulin-resistant individuals in the absence of any changes in circulating NEFA and other lipid levels. Several markers of adipose cell differentiation were augmented, supporting the concept that insulin resistance in these non-obese and non-diabetic subjects was associated with an impaired terminal differentiation of the adipose cells. In addition, the upstream intracellular signalling cascade for insulin was improved but the precise mechanism for this remains unclear. The increased expression of LXRα adds another dimension whereby thiazolidinedione can improve insulin action.

Acknowledgements This study was supported by grants from the Swedish Research Council (K2001-72X-03506-30B), the Swedish Diabetes Association, the Sonya Hedenbratt Memorial Fund, the IngaBritt and Arne Lundberg Foundation, the Novo-Nordisk Foundation and the Torsten and Ragnar Söderberg Foundation.

Duality of interest: none.

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