

# Peroxisome proliferator activated receptor gamma activity is low in mature primary human visceral adipocytes

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## Abstract

**Aims/hypothesis** The amount of visceral fat mass strongly relates to insulin resistance in humans. The transcription factor peroxisome proliferator activated receptor gamma (PPARG) is abundant in adipocytes and regulates genes of importance for insulin sensitivity. Our objective was to study PPARG activity in human visceral and subcutaneous adipocytes and to compare this with the most common model for human disease, the mouse.

**Materials and methods** We transfected primary human adipocytes with a plasmid encoding firefly luciferase controlled by PPARG response element (PPRE) from the

acyl-CoA-oxidase gene and measured PPRE activity by emission of light.

**Results** We found that PPRE activity was 6.6-fold higher (median) in adipocytes from subcutaneous than from omental fat from the same subjects ( $n=23$ ). The activity was also 6.2-fold higher in subcutaneous than in intra-abdominal fat cells when we used a PPARG ligand-binding domain-GAL4 fusion protein as reporter, demonstrating that the difference in PPRE activity was due to different levels of activity of the PPARG receptor in the two fat depots. Stimulation with 5  $\mu\text{mol/l}$  rosiglitazone did not induce a PPRE activity in visceral adipocytes that was as high as basal levels in subcutaneous adipocytes. Interestingly, in mice of two different strains the PPRE activity was similar in visceral and subcutaneous fat cells.

**Conclusions/interpretation** We found considerably lower PPARG activity in visceral than in subcutaneous primary human adipocytes. Further studies of the molecular mechanisms behind this difference could lead to development of drugs that target the adverse effects of visceral obesity.

**Electronic supplementary material** Supplementary material is available in the online version of this article at <http://dx.doi.org/10.1007/s00125-006-0515-x> and is accessible to authorised users.

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## Abbreviations

GAL4	DNA-binding transcription factor required for the activation of the GAL4 genes
Luc	Firefly luciferase
N-CoR	Nuclear receptor co-repressor
PPAR	Peroxisome proliferator activated receptor
PPARG	Peroxisome proliferator activated receptor gamma
PPRE	PPARG response element
Rluc	Renilla luciferase
RXR	Retinoid X receptor

SMRT	Silencing mediator of retinoic acid and thyroid hormone receptor
SRC-1	Steroid receptor co-activator-1
TIF2	Transcription intermediary factor 2

## Introduction

The regional distribution of fat mass affects both the sensitivity to insulin and prognosis of cardiovascular disease in humans [1]. The male abdominal fat accumulation pattern is associated with high levels of NEFA in plasma, development of type 2 diabetes, hypertension and dyslipidaemia, i.e. components of the metabolic syndrome [2]. It is generally considered that the unfavourable prognosis in subjects with abdominal obesity is due to large amounts of intra-abdominal fat [3–6]. Human omental fat cells display a high number of beta-adrenergic receptors and reduced antilipolytic effect of insulin, compared with subcutaneous fat cells, resulting in a high release of fatty acids from this fat cell depot [7, 8]. Fatty acids from fat tissue within the abdominal cavity are drained to the hepatic vein resulting in delivery of venous blood with high concentrations of fatty acids directly to the liver, which can affect liver metabolism to cause dyslipidaemia and increased production of glucose. Prospective clinical studies have demonstrated the importance of intra-abdominal fat depots for the metabolic syndrome. Surgical removal of 18 to 19% of total fat mass taken from subcutaneous depots does not affect insulin sensitivity in humans [9], while removal of the omentum in severely obese subjects decreased insulin resistance despite the fact that the omental fat mass corresponded to only 0.8% of total body fat [10].

The transcription factor peroxisome proliferator activated receptor (PPAR) gamma (PPARG) is highly expressed in adipocytes and has been shown to affect genes of importance for differentiation of fat cells and for insulin sensitivity [11, 12]. PPARG heterodimerises with the retinoid X receptor (RXR) following ligand binding and this complex can activate the PPARG response element (PPRE) that controls several genes of great importance for insulin sensitivity. Dominant negative mutations of PPARG have been found in subjects that are severely insulin-resistant and who also develop type 2 diabetes at an early age [13, 14], demonstrating the importance of PPARG signalling for normal insulin sensitivity in humans. Conversely, treatment with synthetic PPARG activators of the thiazolidinediones class, such as rosiglitazone and pioglitazone, is currently a treatment option in patients with type 2 diabetes. These PPARG ligands lower plasma glucose levels as well as improving other components of the

metabolic syndrome such as high levels of NEFA, dyslipidaemia and high blood pressure [11]. PPARG receptor activity is also regulated by binding of co-activators and co-repressors. Known co-activators to PPARG are: steroid receptor co-activator-1 (SRC-1), co-integrator-associated protein, transcription intermediary factor 2 (TIF2), PPARG co-activator 1 and PPARG-binding protein. The nuclear receptor co-repressor (N-CoR) or the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT), on the other hand, reduce PPARG activity when recruited [15].

We have developed a technique to transfect primary human adipocytes by electroporation [16]. In this study we used this technique to transfect cells with a plasmid (labelled pAOX) encoding firefly luciferase (Luc) cDNA under control of a PPRE from the acyl-CoA-oxidase gene. In this assay, an endogenous activator of PPRE, i.e. PPARG, activates PPRE to produce luciferase. Luciferase in turn produces light when the substrate luciferin is added to cell lysates and the amount of light emission is quantified using a luminometer. The fat cells in this paper were co-transfected with a small amount of another plasmid containing cDNA for a related light-emitting protein, Renilla luciferase (Rluc). Transcription from this plasmid is controlled by a constitutively active promoter, making the amount of light emission in lysates proportional to the efficiency of the transfection procedure. By relating the PPRE-inducible firefly luciferase light emission to that of Rluc in the same sample we are able to overcome differences in the amount of transfected cells that inevitably occur in fat cells from humans. In some experiments a further construct instead of pAOX was used in combination with the Rluc standard. This construct uses cDNA encoding a fusion protein of the ligand-binding domain of PPARG with a yeast-cell derived DNA-binding transcription factor required for the activation of the GAL4 genes' (GAL4) DNA-binding domain. To measure activation of this recombinant protein a third plasmid was added that contained firefly luciferase under control of the yeast GAL4 response element. This allows quantification of PPARG receptor activity in the transfected cells without causing confounding effects resulting from overexpressing of a fully functioning PPARG receptor, as the yeast GAL4 binding domain is not expected to affect mammalian cell mRNA expression. Our aim was to compare PPRE activity in visceral and subcutaneous fat cells and also to compare human fat cells with those from mice in this regard.

## Subjects and methods

*Subjects* Abdominal subcutaneous and visceral (omental and from appendices epiploicae) human adipose tissue was

removed during surgery [16]. The patients were women undergoing surgery for various gynaecological diseases. PPRE activity was measured in subcutaneous and intra-abdominal adipose tissue from 23 subjects with a mean age of  $68 \pm 18$  (range 28–95) years and a mean BMI of  $27 \pm 7$  (range 16–44)  $\text{kg}/\text{m}^2$ .

**Mice** Standard lean male NMRI mice ( $n=10$ ) were used as indicated (B and K Universal, Södertälje, Sweden). We also used obese male mice ( $n=10$ ) expressing the gene for human islet amyloid polypeptide, but deficient for endogenous murine islet amyloid polypeptide expression; these mice were derived from our own laboratory [17]. Mice were caged individually with a 12-h light–dark cycle and had free access to a high-energy diet consisting of water, standard chow R 70 (Lactamin, Kimstad, Sweden) and lard (Ellico Food, Kävlinge, Sweden) for 11 months before being killed. Principles of laboratory animal care according to National Institutes of Health (NIH publications no. 85–23, revised 1985; <http://grants1.nih.gov/grants/olaw/references/phspol.htm>) and Swedish animal care regulations were followed.

**Tissue sampling** The adipose tissue was cleared from vascular and fibrous structures and rinsed in 0.9% (w/v) NaCl. Using scissors, 5 to 10 g of fat tissue was cut into millimetre-sized pieces and digested in equal volume (1 g/ml) of Krebs–Ringer solution (0.12 mol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l  $\text{CaCl}_2$ , 1.2 mmol/l  $\text{MgSO}_4$ , 1.2 mmol/l  $\text{KH}_2\text{PO}_4$ ) containing 20 mmol/l HEPES, pH 7.4, 3.5% (w/v) fatty-acid-free bovine serum albumin (Roche, Mannheim, Germany), 200 nmol/l adenosine, 2 mmol/l glucose and 260 U/ml collagenase for 1.5 h at  $37^\circ\text{C}$  in a water-bath with agitation. After collagenase (Worthington, Lakewood, NJ, USA) digestion, the adipocytes were separated from connective tissue debris by filtering. The adipocytes were then washed (at 40% cells by volume) in the Krebs–Ringer solution containing 20 mmol/l HEPES, pH 7.4, 1% (w/v) fatty-acid-free bovine serum albumin, 200 nmol/l adenosine and 2 mmol/l glucose and kept in a water-bath with agitation at  $37^\circ\text{C}$  for a maximum of 30 min until electroporation or RNA extraction.

**Electroporation and luciferase assay** Each 0.4-cm gap electroporation cuvette was filled with 200  $\mu\text{l}$  of the adipocyte solution. An additional 200  $\mu\text{l}$  of PBS buffer (137 mmol/l NaCl, 2.7 mmol/l KCl, 10 mmol/l  $\text{Na}_2\text{HPO}_4$ , 1.8 mmol/l  $\text{KH}_2\text{PO}_4$ , pH 7.5) containing 2  $\mu\text{g}$  pAOX-Luc [18] and 0.1  $\mu\text{g}$  pRluc plasmid (BioSignal, Packard, CT, USA) DNA was then mixed with the cells to allow determination of PPRE activity. The activity of a transfected human PPARG receptor was measured in the corresponding manner by using a construct of human PPARG ligand-binding domain fused to the yeast GAL4

DNA-binding domain [19], kindly provided by B. Staels (UR 545 INSERM, Institut Pasteur de Lille, Lille, France), and a plasmid encoding firefly luciferase under control of five copies of a GAL4 response element ( $5 \times \text{GAL4-TK-LUC}$ ) (2  $\mu\text{g}$  of each construct/cuvette) [20].

Electroporation was carried out with 400 V 4 ms square-wave pulse using a pulser (GenePulser II; Bio-Rad Laboratories, Hercules, CA, USA) as described in detail earlier [20].

Luciferase activity was analysed after incubation for 18 h at  $37^\circ\text{C}$  in 10%  $\text{CO}_2$ , following electroporation and application of a technique described in detail earlier [20]. In brief, cells were lysed and assayed for firefly and Rluc using a reporter assay system (Dual-Luciferase Reporter Assay Systems; Promega, Madison, WI, USA). Firefly and Rluc activities were measured using a multilabel counter (Victor 1420; Wallac, Turku, Finland). The induced amount of firefly luciferase was normalised according to the constitutively expressed Rluc, thus correcting for any differences in the amount of transfected cells.

**RNA extraction and amplification** Total RNA was extracted from isolated subcutaneous and omental adipocytes from eight women with no history of diabetes. Next 1 ml of floated layer of adipocytes was decanted into a sterile tube, and total RNA was immediately extracted by homogenisation in 2 ml Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was quantified by spectrophotometry and the samples were then stored at  $-80^\circ\text{C}$  until further use. First-stranded cDNA was synthesised from 1  $\mu\text{g}$  of total RNA using a kit (Enhanced Avian RT First Strand Synthesis Kit; Sigma, Saint Louis, MO, USA).

PCR amplification of the cDNA was done using ThermoWhite Taq DNA polymerase (Saveen Werner, Malmö, Sweden). The primers of the studied genes and the cycling parameters are available in the Electronic supplementary material (ESM) Table 1. The amplified products were quantified by fluorescence imaging (LAS 1000; Fuji, Tokyo, Japan) after separation in 1.5% agarose gel, and normalised to mRNA levels of beta-actin.

**Immunoblotting** Whole cell lysates were subjected to SDS-PAGE, and transferred to nitrocellulose membranes for immunoblotting with antibodies against human PPARG (detecting both isoform 1 and 2), RXR alpha, beta-actin or N-CoR (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Bound antibodies were detected with horseradish peroxidase-conjugated anti-IgG according to the enhanced chemoluminescence protocol (Amersham Biosciences, Uppsala, Sweden).

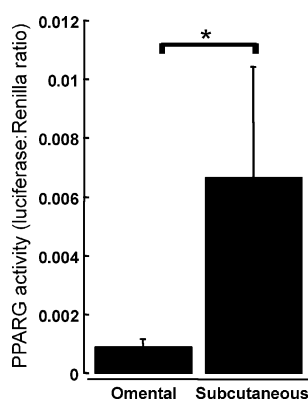
**Ethics** The study was approved by the human and animal Ethics Committees of Linköping University and performed

in accordance with the Declaration of Helsinki. Informed consent was obtained from all participating patients.

**Statistics** Statistical calculations, except ANOVA, were done using StatView 4.5 (Abacus Concepts, Berkeley, CA, USA) software. Comparisons within and between groups were made with Student's paired and unpaired two-tailed *t* test and linear correlations with Pearson's test or as stated in the text. Mean±SD is given unless otherwise stated. Statistical significance was considered to be given at the 5% level ( $p \leq 0.05$ ). The ANOVA calculations were carried out using SPSS 11.5 (SPSS, Chicago, IL, USA).

## Results

Basal PPRE activity differed substantially in subcutaneous and omental adipocytes from the same subjects (median 6.6, range 1.9–59, for subcutaneous:intra-abdominal PPRE activity ratio, average of  $13 \pm 17$ -fold higher in subcutaneous adipocytes). This was due to different activity levels of the PPARG receptor since, by using a GAL4-PPARG fusion protein (amino acids 174–475 of PPARG corresponding to the ligand-binding domain) and a second plasmid encoding luciferase under control of five copies of a GAL4 response element ( $5 \times \text{GAL4-TK-LUC}$ ) as reporters of PPARG receptor activity, we found a median of 6.2-fold higher activity in subcutaneous than in intra-abdominal fat cells (mean value  $5.8 \pm 3$ ,  $p = 0.04$  in paired *t* test,  $n = 5$ ; Fig. 1). There was no correlation between BMI and the basal subcutaneous:intra-abdominal PPRE activity ratio in the total material nor in that from patients classified as

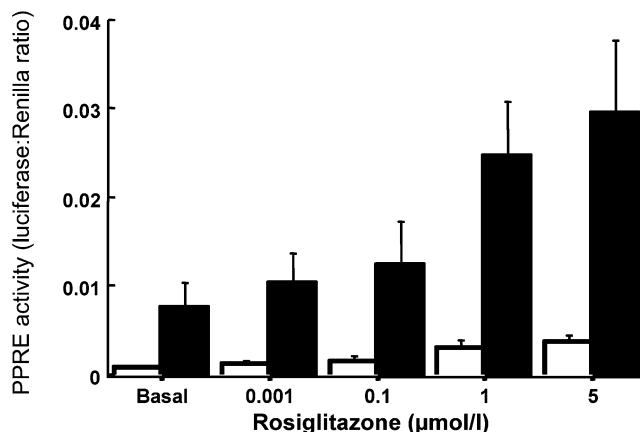


**Fig. 1** PPRE activity in omental and subcutaneous adipocytes. Primary human fat cells were transfected with plasmids encoding a GAL4-PPARG fusion protein and firefly luciferase under control of the GAL4 response element and also the constitutively expressed Rluc. After 18 h of incubation luciferase activities were measured by luminometry. Light emission from firefly luciferase was normalised to that of Rluc. In paired comparison, activity in subcutaneous fat cells was on average 6.2-fold higher than in intra-abdominal fat cells (mean value  $5.8 \pm 3$ ,  $*p < 0.05$  in paired *t* test,  $n = 5$ ). Error bars: mean±SEM

having a low ( $< 18.5 \text{ kg/m}^2$ ), normal ( $18.5\text{--}24.99 \text{ kg/m}^2$ ) or high ( $\geq 25 \text{ kg/m}^2$ ) BMI according to WHO criteria. Stimulation with 0.01 to 5  $\mu\text{mol/l}$  rosiglitazone increased the PPRE activity in a dose-dependent manner in both tissues. However, not even the highest dose of rosiglitazone induced a PPRE activity in intra-abdominal adipocytes that was as high as basal levels in subcutaneous adipocytes ( $p < 0.05$  paired *t* test; Fig. 2).

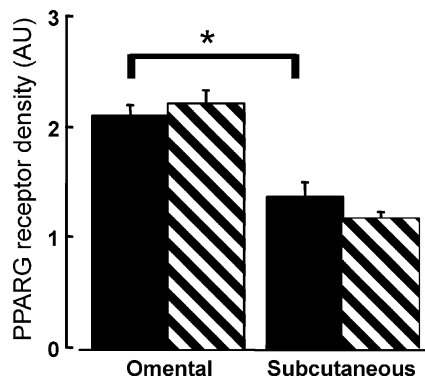
No statistically significant correlation was observed between basal PPRE activity and subject age or BMI, either in subcutaneous or in intra-abdominal adipocytes in the total material, or after separate analysis in the three BMI groups. There was a positive correlation between BMI and the increase in PPRE activity induced by 5  $\mu\text{mol/l}$  rosiglitazone in subcutaneous adipocytes in the total material ( $r = 0.66$ ,  $p = 0.006$ ,  $n = 17$ ), but this was not detectable when separately analysed in the three BMI categories. No such correlation in the omental adipocytes from the same subjects was seen ( $p = 0.3$ ), although after analysis in the three BMI groups, there was a positive correlation between BMI and the response to 5  $\mu\text{mol/l}$  rosiglitazone in the overweight group ( $r = 0.62$ ,  $p = 0.01$ ,  $n = 16$ ).

Levels of PPARG receptor were 55% higher in the omental fat cells, and this was unaffected by incubation with rosiglitazone (Fig. 3). To test whether the low PPRE activity in human omental fat was caused by low activity of the RXR receptor (i.e. the other half of the heterodimerised receptor), we added 100 nmol/l of the highly specific RXR agonist LG1069 to omental fat cells. This did not affect



**Fig. 2** Effect of rosiglitazone on PPRE activity in primary human adipocytes from subcutaneous and omental fat cells. Primary human adipocytes isolated from subcutaneous and omental fat tissue were transfected with pAOX-Luc (plasmid encoding PPARG response element controlling transcription of cDNA for firefly luciferase protein) and pRluc (a plasmid encoding Rluc) and incubated with indicated doses of rosiglitazone. After 18 h of incubation luciferase activities were measured in omental ( $n = 3$ , open bars) and subcutaneous ( $n = 4$  filled bars) fat cells. Error bars are mean±SEM. The increase in PPRE activity was statistically significant compared with 5  $\mu\text{mol/l}$  rosiglitazone except for the comparison with 1  $\mu\text{mol/l}$ ;  $p < 0.05$ , ANOVA with post hoc Tukey test



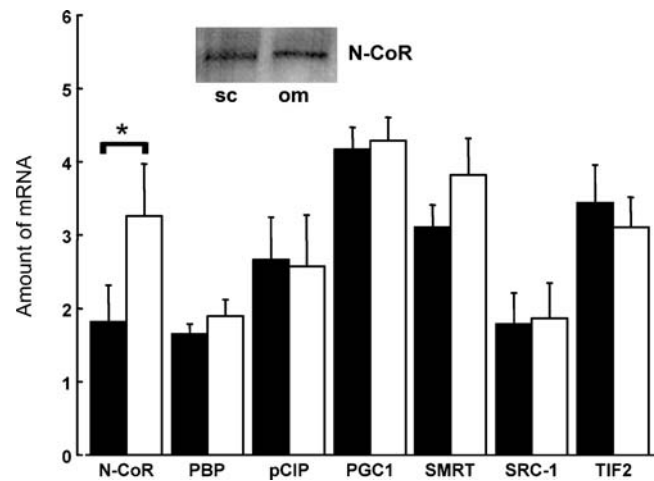


**Fig. 3** Effect of rosiglitazone on the amount of PPARG receptor in human adipocytes from subcutaneous and omental fat cells. The subcutaneous and omental cells were incubated with (hatched bars) or without (filled bars) 5  $\mu\text{mol/l}$  rosiglitazone for 18 h and whole cell lysates were prepared and separated by SDS-PAGE, electrotransferred to nitrocellulose membranes and immunoblotted with anti-PPARG and beta-actin antibodies. The amount of PPARG was normalised to beta-actin in each lane. The level of PPARG receptor was 55% higher in omental than in subcutaneous fat cells and was unaffected by incubation with rosiglitazone;  $n=4$ ;  $*p<0.05$  in paired  $t$  test. AU, arbitrary units

PPRE activity ( $n=3$ ,  $p=0.3$  in paired  $t$  test), indicating that the RXR receptor is not rate-limiting. There was no difference with regard to RLuc activity, a measure of survival and transfection efficiency, in omental compared with subcutaneous adipocytes in the human fat cells (paired  $t$  test,  $n=8$ ,  $p=0.4$ ). Nor could we detect any difference in transfection efficiency by microscopy after transfection with green fluorescent protein (15% in fat cells from both depots, not shown).

To further study the potential mechanisms behind the difference in PPRE activity, we examined the levels of mRNA of known co-activators and co-repressors in subcutaneous and intra-abdominal fat cells. We found about a twofold higher level of the co-repressor N-CoR mRNA in intra-abdominal than in subcutaneous fat cells (Fig. 4). However, quantification of the N-CoR protein by immunoblot did not reveal any difference between the two fat depots (Fig. 4 insert). Our measurement of PPRE activity relies on luciferase production during incubation for 18 h. Thus the amount of co-activators and/or co-repressors could have changed during incubation. To examine this possibility, we analysed the levels of mRNA for the co-activators/repressors PPARG co-activator 1 ( $n=3$ ), PPARG-binding protein ( $n=5$ ), co-integrator-associated protein ( $n=3$ ), SRC-1 ( $n=3$ ), TIF2 ( $n=3$ ), SMRT ( $n=3$ ), and N-CoR ( $n=5$ ) when the cells were incubated for 9 or 18 h (not shown). None of these co-activators/repressors changed in concentration during this incubation period compared with baseline values (paired  $t$  test, after normalisation to amount of beta-actin).

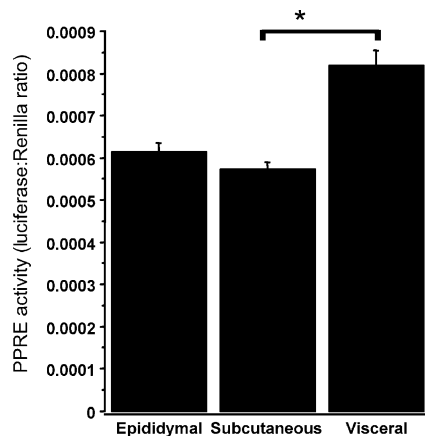
Rodents do not normally acquire much subcutaneous fat, but male mice exhibit considerable amounts of epididymal



**Fig. 4** Levels of mRNA for PPARG co-activators and co-repressors in mature subcutaneous (filled bars) compared with omental (open bars) primary human adipocytes. Total RNA was extracted from isolated adipocytes from eight subjects as described above. Amplified gene products were quantified after separation in 1.5% agarose gel and normalised to levels of beta-actin from the same cell lysates. Error bars are mean $\pm$ SEM. The insert shows levels of N-CoR protein in adipocytes isolated from subcutaneous (sc) and omental (om) fat, from which whole cell lysates were prepared and separated by SDS-PAGE, electrotransferred to nitrocellulose membranes, and immunoblotted with anti-N-CoR antibodies (a representative experiment of three in which the same amount of protein of each lysate was loaded on the gel).  $*p<0.05$ . N-CoR, nuclear receptor co-repressor; PBP, PPARG gamma-binding protein; pCIP, co-integrator-associated protein; PGC1, peroxisome proliferative activated receptor gamma, coactivator 1; SMRT, silencing mediator of retinoic acid and thyroid hormone receptor; SRC-1, steroid receptor co-activator-1; TIF2, transcription intermediary factor 2

fat tissue under regular feeding conditions, which subsequently is the most common source of primary fat cells in rodents. To study subcutaneous fat cells from mice, we first examined a strain of obese mice that had been on a high-energy diet for 11 months. The level of PPRE activity in murine fat cells taken from subcutaneous or epididymal fat tissue was identical in these mice (Fig. 5). As we could not find omental fat tissue in the animals, we examined intra-abdominal fat from appendices epiploicae, which are fatty structures along the anterior part of the intestines. Interestingly, fat cells from this intra-abdominal fat depot displayed a slightly higher, 43%, basal PPRE activity compared with subcutaneous fat cells ( $p<0.05$  in paired  $t$  test, Fig. 5).

We confirmed that the PPRE activity was similar in subcutaneous and visceral fat cells in another strain of mice, NMRI mice. In two experiments on fat pooled from five lean NMRI mice, the PPRE activity was 12% and 23% higher, respectively in visceral than in subcutaneous fat cells. We verified in humans that basal PPRE activity in fat cells from both appendices epiploicae and the omentum majus were lower than in subcutaneous fat cells (omental adipocytes  $7.0\pm 2.2$  and epiploic adipocytes  $11\pm 3.7$  times lower basal



**Fig. 5** Basal PPARE activity in fat cells from mice. Adipocytes from epididymal and subcutaneous fat tissues were compared with those from appendices epiploicae (visceral) in mice. The cells were isolated and transfected in the same way as human adipocytes and the PPARE activity normalised to Rluc. The PPARE activity was 43% higher in the visceral than in subcutaneous fat cells (ten mice were used and the fat cells from each depot divided into three groups). Error bars are mean  $\pm$  SEM. \* $p < 0.05$

PPRE activity than subcutaneous adipocytes, paired *t* test between omental and epiploic fat cells,  $p = 0.13$ ,  $n = 4$ ).

## Discussion

We found considerably lower PPARG and PPARE activity in intra-abdominal than in subcutaneous primary human adipocytes. Although it could be assumed that the difference between the fat depots could be caused by lower levels of an endogenous ligand to PPARG in the omental fat cells, such a difference should disappear upon addition of a strong synthetic ligand of PPARG such as rosiglitazone or upon treatment of the RXR part of the dimeric receptor by LG1069. However, this was not the case, indeed, not even at the highest concentration of rosiglitazone did the PPARE activity in omental fat cells reach basal levels in subcutaneous fat cells. Since increased PPARG activity through treatment with synthetic PPARG activators leads to reduced adipose tissue lipolytic activity and lowered levels of NEFA in plasma, this novel finding of low PPARG activity is in line with the high lipolytic activity in intra-abdominal fat tissue [8].

The low PPARG receptor and PPARE activities were not a consequence of particularly poor transfection rate of intra-abdominal adipocytes, since we found no difference in transfection efficiency by microscopy after transfection with green fluorescent protein, nor was any difference with regard to absolute Rluc activity seen in the two fat depots. Moreover, any difference in transfection efficiency in individual experiments was corrected for by using Rluc as an internal standard. Using cDNAs encoding a GAL4-PPARG fusion protein and luciferase under control of the

GAL4 response element as reporters of PPARG receptor activity, we demonstrated that the large difference in PPARG activity in subcutaneous compared with intra-abdominal fat cells was indeed a consequence of differences in PPARG receptor activity, and not caused by other potential transcription factors affecting the PPARE. Since this measurement of PPARG activity used a transfected recombinant fusion protein, it also shows that the difference between the two fat depots was not caused by a lower amount of the PPARG receptor in the intra-abdominal fat cells. This was confirmed by quantification of PPARG receptor in the two tissues, showing 55% higher levels of the receptor in omental fat cells. Furthermore, Montague et al. have shown that the levels of mRNA for PPARG are not different in subcutaneous and omental human fat cells [21].

It has earlier been demonstrated that the insulin sensitive GLUT4 (now known as solute carrier family 2 [facilitated glucose transporter], member 4 [SLC2A4]) promoter is repressed by PPARG in primary adipocytes [22]. Consequently, the low PPARG activity in the intra-abdominal fat cells is in line with the high levels of GLUT4 in intra-abdominal as compared with subcutaneous adipocytes that were reported by us earlier [23].

Since insulin sensitivity is reduced in older versus younger subjects, we analysed PPARE activity in relation to age. Despite a very wide age range in our study material, we found no significant correlation between age and PPARE activity in the subcutaneous or intra-abdominal fat cells. It has earlier been shown that BMI and mRNA for PPARG are inversely related in subcutaneous fat cells [21]. However, we found no correlation between PPARE activity and BMI in either subcutaneous or intra-abdominal fat cells. The levels of mRNA for co-activators of PPARG were similar in visceral and subcutaneous fat cells. When examining co-repressors of PPARG, we found twofold higher levels of N-CoR mRNA in the intra-abdominal than in subcutaneous fat cells. This did not translate into a corresponding difference in N-CoR protein levels as assayed by immunoblotting. However, in an individual cell, co-activators and co-repressors can be recruited by different transcription factors and since they are generally not abundant, a measurement of the absolute amount does not exclude the possibility that varying amounts are available to the particular transcription factor PPARG [18].

Mice normally have very little subcutaneous fat. Therefore primary fat cells are usually taken from male mice that quite readily acquire fat in the epididymal region. Since many studies related to insulin signalling in primary fat cells are performed on cells obtained from male rodents, we tested PPARE activity in fat cells from three different depots in male mice. Interestingly, in contrast to our findings in human adipocytes, PPARE activity was slightly higher in the visceral than in subcutaneous fat cells in these animals,

while epididymal fat cell activity was similar to that in subcutaneous adipocytes. Although we found no difference in PPRE activity in human epiploic and omental fat cells, this does not rule out other functional differences in these two fat depots. Our findings highlight important species differences between mice and humans with regard to PPAR $\gamma$  activity in fat cells and emphasise the importance of using intra-abdominal fat cells from humans when studying mechanisms behind the insulin resistance that is linked to visceral obesity in man. It is, however, conceivable that some other animals display a similar PPRE difference in omental and subcutaneous fat, and would therefore be more suitable than mice in this regard.

The finding of considerably lower PPRE activity in human omental than in subcutaneous primary adipocytes sheds new light on the mechanisms behind the increased cardiovascular risk related to an enlarged intra-abdominal fat mass. Our study also shows that the difference cannot be compensated even by the potent synthetic PPAR $\gamma$  agonist rosiglitazone. Further studies into the molecular mechanisms behind the large difference in PPAR $\gamma$  signalling in the two adipose tissue depots could lead to the development of drugs that reduce the adverse metabolic effect of abdominal obesity and the poor cardiovascular prognosis linked to this condition [2, 8, 24].

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**Duality of interest** There were no dualities of interest with regard to this paper.

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