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Acute in vivo effects of insulin on gene expression in adipose tissue in insulin-resistant and insulin-sensitive subjects

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Abstract *Aims/hypothesis:* We determined the response of selected genes to in vivo insulin in adipose tissue in 21 non-diabetic women. *Materials and methods:* The women were divided into insulin-sensitive and -resistant groups based on their median whole-body insulin sensitivity (8.7±0.4 vs 4.2±0.3 mg kg^{-1} min^{-1} for insulin-sensitive vs -resistant group). Subcutaneous adipose tissue biopsies were obtained before and after 3 and 6 h of i.v. maintained euglycaemic hyperinsulinaemia. Adipose tissue mRNA concentrations of facilitated glucose transporter, member 1 (*SLC2A1*, previously known as *GLUT1*), facilitated glucose transporter, member 4 (*SLC2A4*, previously known as GLUT4), peroxisome proliferator-activated receptor γ (*PPARG*), peroxisome proliferator-activated receptor γ co-activator 1α (*PPARGC1A*), 11β-hydroxysteroid dehydrogenase-1 (*HSD11B1*), *TNF*, adiponectin (*ADIPOQ*), *IL6* and the macrophage marker *CD68* were measured using real-time PCR. *Results:* Basal expression of 'insulin-sensitivity genes' *SLC2A4* and *ADIPOQ* was lower while that of 'insulin-resistance genes', *HSD11B1* and *IL6* was significantly higher in the insulin-resistant than in the insulin-sensitive group. Insulin significantly increased expression of 'insulin-sensitivity genes' *SLC2A4*, *PPARG*, *PPARGC1A* and *ADIPOQ* in the insulin-sensitive group, while only expression of *PPARG* and *PPARGC1A* was increased in the insulin-resistant group. The expression of 'insulin-resistance genes' *HSD11B1* and *IL6* was increased by insulin in the insulin-resistant group, but insulin failed to increase *HSD11B1* expression in the insulin-sensitive group. At 6 h, expression of *HSD11B1*, *TNF* and *IL6* was significantly higher in the insulin-resistant than in the insulin-sensitive group. *IL6* expression increased significantly more in response to insulin in the insulin-resistant than in the insulin-sensitive group. *CD68* was overexpressed in the insulin-resistant as compared with the insulin-sensitive group at both 0 and 6 h. *Conclusions/interpretation:* These data suggest that genes adversely affecting insulin sensitivity hyperrespond to insulin, while genes enhancing insulin sensitivity hyporespond to insulin in insulin-resistant human adipose tissue in vivo.

Keywords Adipocytokines · Adiponectin · Cortisol · Interleukin · Macrophages · PGC-1 · TNF

Abbreviations *ACTB*: gene encoding β-actin · *ADIPOQ*: gene encoding adiponectin · *HSD11B1*: gene encoding 11β-hydroxysteroid dehydrogenase-1 · PPARG: peroxisome proliferator-activated receptor γ · PPARGC1A: peroxisome proliferator-activated receptor γ co-activator α · SLC2A1: facilitated glucose transporter, member 1 · SLC2A4: facilitated glucose transporter, member 4 · *TBP*: gene encoding TATA-box binding protein

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Introduction

Low-grade systemic inflammation and insulin resistance frequently coexist. Serum concentrations of cytokines, such as IL6 [1–6] and in some studies TNF [6, 7] correlate with markers of insulin resistance. Adipose tissue is one site of inflammation in insulin-resistant conditions. The number of macrophages is increased in both obese and lipoatrophic insulin-resistant subjects [8–10], and the expression of genes encoding IL6 [2, 6] and TNF [2, 6, 7] is increased in human

adipose tissue. IL6 impairs insulin signalling in 3T3-L1 adipocytes in vitro [11], and adipose tissue IL6 content correlates inversely with insulin action in vivo in humans [12]. The increased *TNF* expression in adipose tissue was recently suggested to originate exclusively from macrophages [8].

Insulin-resistant adipose tissue also overexpresses other genes that possibly contribute to insulin resistance, e.g. the gene encoding the enzyme that converts cortisone to cortisol, 11β-hydroxysteroid dehydrogenase-1 (*HSD11B1*), which is expressed in both adipocytes and macrophages [13, 14]. Overexpression of this enzyme in mice results in insulin resistance and visceral obesity [15]. In addition to these 'insulin-resistance' genes, another set of genes ('insulin-sensitivity genes') are underexpressed in insulin-resistant adipose tissue. These include the gene encoding adiponectin (*ADIPOQ*), which is deficient in both serum and adipose tissue of obese [16–18] and lipoatrophic [19] subjects, as well as the genes encoding the insulin-sensitive facilitated glucose transporter, member 4 (*SLC2A4*, previously known as GLUT4) [20], the adipogenic transcription factor peroxisome proliferator-activated receptor-γ (*PPARG*) [21, 22] and its coactivator 1α (*PPARGC1A*) [23, 24].

Regarding acute regulation of 'insulin-resistance' genes, insulin has been found to increase *IL6* expression in vitro in human adipocytes [25] and in 3T3-L1 cells [26], and transiently in a study in vivo in human adipose tissue [27]. Data on *TNF* expression are inconsistent, with one study reporting a transient increase in *TNF* mRNA by insulin in vivo with no change in serum TNF [27], while another in vivo study [28] and one in vitro study using human adipose tissue [29] found no effect of insulin on *TNF* expression. Insulin has been reported to decrease *HSD11B1* expression in vitro [13], and not to change expression during a 3-h insulin infusion in human adipose tissue [28]. Insulin acutely increases *SLC2A4* [30, 31] and *PPARG* [32] expression, but there are no in vivo data on acute regulation of *PPARGC1A* or *ADIPOQ* in vivo in human adipose tissue.

No studies to date have compared the acute effects of insulin on 'insulin-resistance' and '-sensitivity' genes in insulin-resistant and -sensitive subjects. In the present study, we compared expression of selected 'insulin-resistance' genes (*IL6*, *TNF*, *HSD11B1*) and 'insulin-sensitivity' genes (*SLC2A4*, *PPARG*, *PPARGC1A*, *ADIPOQ*) in subcutaneous adipose tissue biopsies of insulin-sensitive and insulin-resistant subjects, and compared responses of these genes to acute hyperinsulinaemia by repeating the biopsies after 3 and 6 h of insulin infusion. We also determined whether expression of the macrophage marker *CD68* is increased in the insulin-resistant vs -sensitive subjects, and whether its expression is regulated by insulin.

Subjects, materials and methods

Subjects and study designs A total of 21 non-diabetic apparently healthy Caucasian women were recruited on the basis of the following inclusion criteria: (1) age 18–60 years, and (2) no known acute or chronic disease other than obesity based on history and physical examination and standard laboratory tests (blood counts, serum creatinine, thyroid-stimulating hormone, electrolyte concentrations and electrocardiogram). Other exclusion criteria included pregnancy and treatment with drugs that may alter glucose tolerance. In each subject, whole-body insulin sensitivity was measured using the euglycaemic insulin clamp technique (insulin infusion rate 1 mU kg^{-1} min^{-1} for 6 h) and needle biopsies of adipose tissue were taken before and after 3 and 6 h of hyperinsulinaemia. The women were divided into insulin-sensitive (n=11) and insulin-resistant (n=10) groups, on the basis of their median rate of whole-body insulin sensitivity.

The nature and potential risks of the study were explained to all subjects prior to obtaining their written informed consent. The study was carried out in accordance with the principles of the Declaration of Helsinki. The protocol was approved by the ethics committee of Helsinki University Central Hospital.

Whole-body insulin sensitivity Whole-body insulin sensitivity was measured using the insulin clamp technique [33]. The study was begun at 07:30 hours after an overnight fast. Two 18-gauge catheters (Venflon; Viggo-Spectramed, Helsingborg, Sweden) were inserted, one in an antecubital vein for infusion of insulin and glucose, and another retrogradely in a heated hand vein to obtain arterialised venous blood for measurement of glucose concentrations every 5 min and serum free insulin concentration every 30 min. Regular human insulin (Insulin Actrapid; Novo Nordisk, Denmark) was infused in a primed-continuous fashion. The rate of the continuous insulin infusion was 1 mU kg^{-1} min^{-1} for 6 h. Normoglycaemia was maintained by adjusting the rate of a 20% glucose infusion based on plasma glucose measurements from arterialised venous blood every 5 min. Whole-body insulin sensitivity was determined from the glucose infusion rate required to maintain normoglycaemia between 30 and 360 min [33].

Adipose tissue biopsy and total RNA cDNA preparation A needle aspiration biopsy of abdominal subcutaneous fat was taken under local intracutaneous anaesthesia at baseline and after 3 and 6 h of hyperinsulinaemia [34]. Each biopsy sample was taken from different locations in the left, middle and right lower abdominal region. The sample was immediately frozen and stored in liquid nitrogen until analysis. Frozen tissue samples (50–150 mg) were homogenised in 2 ml RNA STAT-60 (Tel-Test, Friendswood, TX, USA) and total RNA isolated according to the manufacturer's instructions. After DNase treatment (RNase-free DNase Set; Qiagen, Hilden, Germany) RNA was purified using the RNeasy Minikit (Qiagen). RNA concentrations were measured using the RiboGreen fluorescent nucleic acid stain (RNA Quantification Kit; Molecular Probes, Eugene, OR, USA). The quality of RNA was checked by agarose gel electrophoresis. Isolated RNA was stored at −80°C until quantification of target mRNAs. A total of 0.1 μg RNA was transcribed into cDNA using Moloney murine leukaemia virus reverse transcriptase (Life Technologies, Paisley, UK) and oligo $(dT)_{12–18}$ primers.

Quantification of mRNA concentrations Quantification of the gene encoding β-actin (*ACTB*), *PPARG* and *ADIPOQ* mRNA was performed in Helsinki (E.K.) by real-time PCR using LightCycler technology (Roche Diagnostics, Mannheim, Germany). An aliquot of 2 μl 1:10 diluted cDNA was brought to a final volume of 20 μl, which contained 3 mmol/l magnesium chloride, 2 μl LightCycler-FastStart DNA SYBR Green I Mix (Roche Diagnostics) and 0.5 μmol/l primers. After the initial activation of the DNA polymerase at 95°C for 10 min, the amplification conditions were as follows: 40 cycles consisting of denaturation at 95°C for 15 s, annealing for 5 s at 57°C (*ACTB*), 56°C (*PPARG*) and 58°C (*ADIPOQ*) and extension at 72°C. The extension times (s) were calculated from the amplicon size (bp/25). Fluorescent data were acquired at the end of each extension phase. After amplification, a melting curve analysis from 65°C to 95°C with a heating rate of 0.1°C/s with a continuous fluorescence acquisition was made. The primers for *PPARG*, *ADIPOQ* and *ACTB* have been described [35]. A standard curve for *PPARG* was created using purified cloned plasmid cDNA (QIAquick PCR Purification Kit; Qiagen). For human *ACTB* and *ADIPOQ* expression, standard curves were created from a specific PCR product. To account for differences in RNA loading, *PPARG* and *ADIPOQ* were expressed relative to *ACTB*.

The mRNA expression levels of facilitated glucose transporter, member 1 (*SLC2A1*, previously known as *GLUT1*), *SLC2A4, PPARGC1A, TNF,HSD11B1* and the gene encoding TATA-box binding protein (*TBP*) were measured in Stockholm (K.K., M.K., R.M.F.) using TaqMan real-time PCR according to the manufacturer's protocol using an ABI PRISM 7000 Sequence Detection System instrument and software (PE Applied Biosystems, Foster City, CA, USA). cDNA synthesised from 15 ng total RNA was mixed with TaqMan Universal PCR Master Mix (Applied Biosystems). Primer and probe sets for *SLC2A1, SLC2A4, PPARGC1A* and *HSD11B1* were designed using the manufacturer's software and sequences available in GeneBank, and the sequences have been published previously [35, 36]. Differences in the loading of cDNA were adjusted for by expressing results relative to *TBP*. Expression levels were quantified in arbitrary units by generating a six-point serial standard curve. After these analyses were performed, obese subjects were reported to have an excess of macrophages in adipose tissue [8, 9], and IL6 was shown to cause insulin resistance in adipocytes [11]. Therefore *CD68* and *IL6* were quantified later in remaining 0- and 6-h samples and expressed relative to TBP. *TNF,CD68, IL6* and *TBP* were measured using Pre-Developed TaqMan Assay Reagents (assay numbers Hs00174128_m1, Hs00154355_m1, Hs0 0174131_m1 and Hs99999910_m1, respectively; Applied Biosystems). The specificity of each primer and probe set was confirmed by visualisation of a single PCR product by agarose gel electrophoresis.

Other measurements Blood samples were taken after an overnight fast for measurement of plasma glucose, serum insulin and C-peptide, serum triglyceride and total and HDL cholesterol concentrations. The percentage (%) of body fat was determined by using bioelectrical impedance analysis (BioElectrical Impedance Analyzer System Model #BIA-101A; RJL Systems, Detroit, MI, USA) [37]. Waist circumference was measured midway between spina iliaca superior and the lower rib margin, and hip circumference at the level of the greater trochanters [38].

Analytical procedures Plasma glucose concentrations were measured in duplicate with the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Fullerton, CA, USA) [39]. Serum free insulin concentrations were measured using the Auto-DELFIA kit from Wallac (Turku, Finland) and C-peptide concentrations by RIA [40]. HbA_1c was measured by HPLC using the fully automated Glycosylated Hemoglobin Analyzer System (BioRad, Richmond, CA, USA) [41]. Serum total cholesterol, HDL cholesterol and triglyceride concentrations were measured with enzymatic kits from Roche Diagnostics using an autoanalyser (Roche Diagnostics Hitachi 917; Hitachi, Tokyo, Japan). LDL cholesterol concentration was calculated using the formula of Friedewald [42]. Serum adiponectin concentrations were measured using an ELISA kit from B-Bridge International (San Jose, CA, USA).

Table 1 Physical and biochemical characteristics of the study subjects divided into insulin-sensitive and -resistant groups on the basis of their median whole-body insulin sensitivity

	Insulin-sensitive	Insulin-resistant	*p* value
Number	11	10	–
Age (years)	32±3	40±3	NS
Body weight (kg)	69±4	90±4	<0.01
BMI (kg/m^2)	24.7±1.1	32.7±1.8	<0.001
Whole-body fat (%)	28±2	36±1	<0.001
Fat mass (kg)	20±2	35±4	<0.01
Waist-to-hip-ratio	0.86±0.01	0.91±0.01	<0.01
Fasting plasma glucose (mmol/l)	5.1±0.1	5.6±0.2	<0.01
Fasting serum insulin (mU/l)	3±1	10±1	<0.001
Fasting serum C-peptide (nmol/l)	0.4±0.1	0.8±0.1	<0.001
Fasting serum LDL cholesterol (mmol/l)	2.2±0.1	3.1±0.1	<0.01
Fasting serum triglycerides (mmol/l)	0.8±0.1	1.4±0.2	<0.01
Fasting serum HDL cholesterol (mmol/l)	1.4±0.1	1.3±0.1	<0.001
Fasting serum adiponectin (mg/l)	18±2	12±1	<0.01

Data are means±SEM

Statistical analyses All parameters were analysed using non-parametric methods. Insulin-sensitive and -resistant groups were compared using the Mann–Whitney test. Effects of insulin were analysed using Friedman's test followed by Dunn's post hoc test to compare single measurements. Correlations were calculated using Spearman's rank correlation coefficient. $p>0.05$ was considered statistically significant. The calculations were performed using SPSS 11.0 for Windows (SPSS, Chicago, IL, USA). All data are shown as means±SEM.

Results

Clinical characteristics Characteristics of the groups are given in Table 1. The insulin-resistant group was more obese than the insulin-sensitive group. Markers of insulin resistance, including serum fasting insulin, C-peptide and triglyceride concentrations were higher and HDL cholesterol and adiponectin concentrations were lower in the insulin-resistant than in the insulin-sensitive group.

Table 2 The relationship (Spearman's *r*) between gene expression in adipose tissue and whole-body insulin sensitivity

Gene	0 h	3 h	6 h
SLC2A1/TBP	−0.08	−0.29	0.04
Insulin-sensitivity genes			
SLC2A4/TBP	0.44#	0.66**	0.73***
PPARG/ACTB	0.14	−0.37	−0.14
PPARGC1A/TBP	0.19	0.55*	0.34
ADIPOQ/ACTB	0.48*	−0.16	−0.24
Insulin-resistance genes			
IL6/TBP	−0.48*	ND	−0.71***
TNF/TBP	−0.44*	−0.53*	−0.46*
HSD11B1/TBP	−0.55*	−0.74***	−0.82***
Macrophage marker			
CD 68/TBP	−0.66**	ND	−0.68***

ND not determined
*$p<0.05$
**$p<0.01$
***$p<0.001$
#$p=0.06$

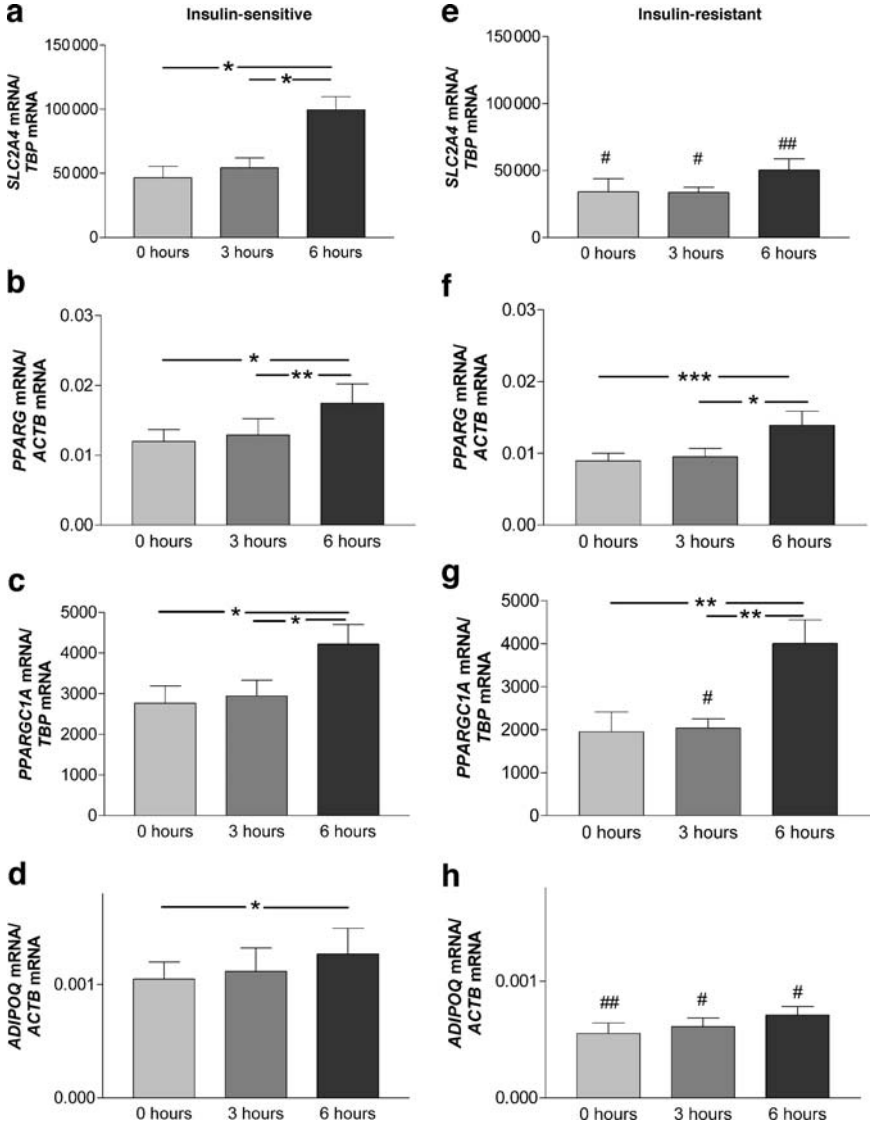

Fig. 1 The expression of 'insulin-sensitivity genes' *SLC2A4*, *PPARG*, *PPARGC1A* and *ADIPOQ* in (**a**–**d**) insulin-sensitive ($n=11$) and (**e**–**h**) insulin-resistant ($n=10$) subjects at 0, 3 and 6 h during euglycaemic hyperinsulinaemia (rate of continuous insulin infusion 1 mU kg^{-1} min^{-1}). *$p<0.05$, **$p<0.01$, ***$p<0.001$ for change between time points; #$p<0.05$, ##$p<0.01$ for difference between groups

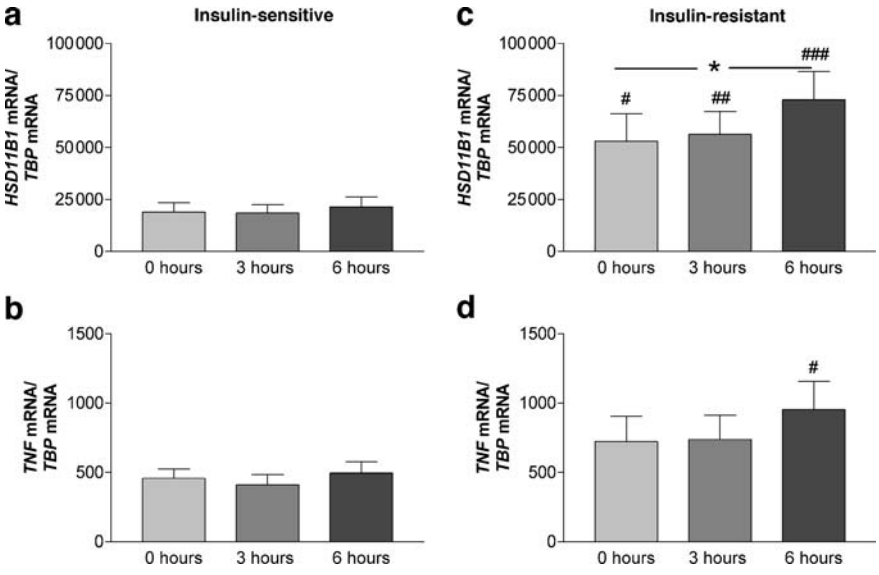

Fig. 2 The expression of 'insulin-resistance genes' *HSD11B1* and *TNF* in (**a, b**) insulin-sensitive (n=11) and (**c, d**) insulin-resistant (n=10) subjects at 0, 3 and 6 h during euglycaemic hyperinsulinaemia (rate of continuous insulin infusion 1 mU kg^{-1} min^{-1}). *p<0.05 for change between time points; #p<0.05, ##p<0.01, ###p<0.001 for difference between groups

During the insulin infusion, serum insulin concentrations were similar in insulin-sensitive and -resistant groups (69±4 vs 76±4 mU/l, respectively; NS). By definition, whole-body insulin sensitivity was 107% higher in the insulin-sensitive than the insulin-resistant group (8.7±0.4 vs 4.2±0.3 mg kg^{-1} min^{-1}, p<0.0001).

Expression of genes encoding SLC2A1, SLC2A4, PPARG, PPARGC1A and ADIPOQ in adipose tissue Before the start of the insulin infusion, the mRNA concentrations of *SLC2A1* (1033±543 vs 722±251, insulin-sensitive vs -resistant; NS), *PPARG* and *PPARGC1A* (Fig. 1) were comparable between the two groups. *SLC2A4* gene expression was lower in the insulin-resistant than the insulin-sensitive group at baseline (p<0.05). The mRNA concentrations of *SLC2A1* and the housekeeping genes (*ACTB* and *TBP*) were comparable between the groups and remained unchanged during insulin infusion in both groups (data not shown). During the insulin infusion, *SLC2A4* mRNA concentrations increased 2.2-fold in the insulin-sensitive group, whereas expression of *SLC2A4* remained unchanged in the insulin-resistant group (Fig. 1). At 6 h, *SLC2A4* expression was significantly higher in the insulin-sensitive than the insulin-resistant group. In univariate correlation analysis (all subjects analysed as one group), *SLC2A4* mRNA correlated with whole-body insulin sensitivity at 3 h (Spearman's r=0.66, p<0.01) and 6 h (r=0.73, p<0.001) and almost significantly at 0 h (Table 2). The expression of *PPARG* and *PPARGC1A* increased significantly during insulin infusion in both groups with no differences between the groups (Fig. 1). *PPARGC1A* and *SLC2A4* mRNA concentrations correlated with each other at 0 h (r=0.66, p<0.01) and 6 h (r=0.38, p<0.05). The changes in gene expression between 0 and 6 h of insulin infusion were confirmed in independent assays for the quantification of *SLC2A4*, *PPARG* and *PPARGC1A* (data not shown).

ADIPOQ gene expression at all time points was significantly higher in the insulin-sensitive than the insulin-resistant group (Fig. 1). *ADIPOQ* mRNA concentrations increased significantly in the former but not in the latter group (Fig. 1). Serum adiponectin concentrations were significantly higher in the insulin-sensitive than the insulin-resistant group before (Table 1) and during insulin infusion at 3 h (16±2 vs 11±1 mg/l, respectively; p<0.01) and 6 h (16±2 vs 11±1 mg/l, p<0.01).

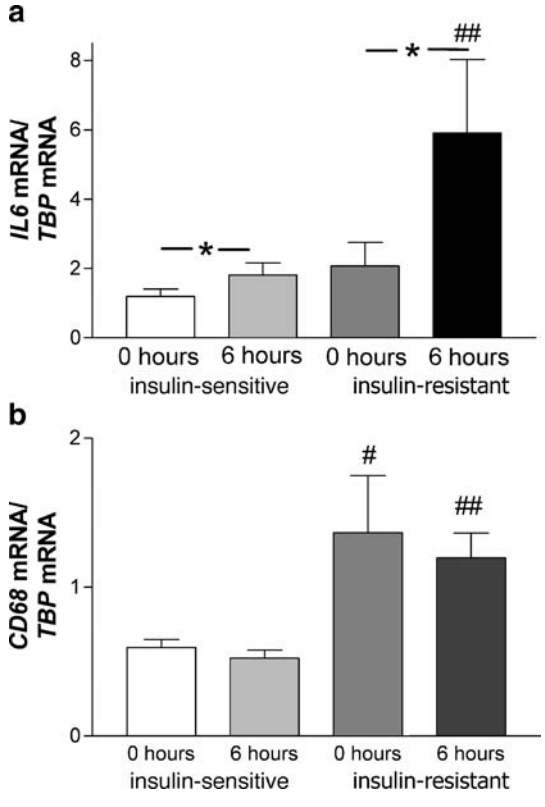

Fig. 3 The expression of (**a**) *IL6* and (**b**) the macrophage marker *CD68* in insulin-sensitive and insulin-resistant subjects at 0 and 6 h during euglycaemic hyperinsulinaemia (rate of continuous insulin infusion 1 mU kg^{-1} min^{-1}). *p<0.05 for change between time points; #p<0.05, ##p<0.01 for difference between groups

HSD11B1, TNF and *IL6* Before insulin infusion, *HSD11B1* mRNA concentrations were 2.4-fold higher in the insulin-resistant than the insulin-sensitive group (Fig. 2). Insulin further increased *HSD11B1* mRNA concentrations in the former group, while there were no changes in the insulin-sensitive group. *TNF* mRNA concentrations tended to be higher in the insulin-resistant than the insulin-sensitive group at all time points with a significant difference at 6 h (Fig. 2). Insulin did not change *TNF* concentrations significantly. *IL6* gene expression increased by insulin in both groups and expression was significantly higher in the insulin-resistant than in the insulin-sensitive group at 6 h (Fig. 3). Also, the increase in *IL6* gene expression by insulin was significantly higher in the insulin-resistant than the insulin-sensitive group ($p<0.02$).

Expression of macrophage marker CD68 Before and during the insulin infusion, *CD68* mRNA concentrations were significantly higher in the insulin-resistant than the insulin-sensitive group (Fig. 3).

Discussion

The present data are the first to compare the responses of genes thought to counteract insulin action ('insulin-resistance genes') and enhance insulin action ('insulin-sensitivity genes') to insulin in vivo in adipose tissue. The study was performed in apparently healthy subjects (except for obesity) who were arbitrarily divided into less insulin-sensitive (insulin-resistant group) and more insulin-sensitive (insulin-sensitive group) based on their median whole-body insulin sensitivity. The results show that insulin resistance is not only characterised by failure of insulin to normally increase expression of 'insulin-sensitivity genes' (*SLC2A4*, *ADIPOQ*) but also by hyperresponsiveness of 'insulin-resistance genes' (*IL6*, *HSD11B1*, *TNF*) to insulin.

The multitude of genes involved in regulating insulin action and the discovery that adipose tissue of obese subjects contains an excess of macrophages [8], which also express insulin-action genes, makes interpretation of the present data complex. Because of the need for repeated biopsies, it was unfeasible to take enough adipose tissue to allow separation of macrophages and adipose cells, and therefore we were unable to allocate the observed changes in gene expression to a given cell type. Theoretically, this implies that any of the following scenarios might have taken place. Firstly, genes hyperresponding to insulin could reside in a different cell type than those hyporesponding to insulin. For example, the hyperresponse to insulin of *IL6*, which is expressed both in macrophages and in adipocytes, could have occurred in macrophages and the hyporesponse of adiponectin in adipocytes, which is the only cell type expressing adiponectin [43]. Secondly, both the hyper- and hyporesponse could have occurred in the same cell type but the increased basal or insulin-stimulated expression of 'insulin-resistance genes' in the insulin-resistant group could have modulated the response to insulin. Thirdly, a combination of both mechanisms might have been involved. These possibilities will be further discussed below in conjunction with the individual genes.

We divided the present study subjects on the basis of their whole-body insulin sensitivity. As expected the insulin-resistant subjects were more obese and had more whole-body fat than the insulin-sensitive subjects. We cannot therefore distinguish between effects of obesity as compared with insulin sensitivity on gene expression in adipose tissue. Adipocyte cell size is increased in insulin-resistant obese subjects and may influence adipose gene expression [44]. There are abundant, albeit controversial, data on differences in gene expression between different adipose tissue depots [45]. Repeated sampling of intra-abdominal fat was not ethically justifiable in healthy subjects.

Several previous studies have compared expression of one or several of the genes in the basal state, i.e. after an overnight fast, between non-obese insulin-sensitive and obese insulin-resistant subjects. These include reports of decreased expression of the 'insulin-sensitivity genes' *SLC2A4* [20, 46, 47], *PPARGC1A* [23] and *ADIPOQ* [16, 48]. These findings were confirmed in the present study. Deletion or overexpression of each of these genes has been shown to modulate insulin action in mouse models [21, 49–52]. Data are inconsistent regarding *PPARG*, with reports of decreased [53, 54] or unchanged [55, 56] expression of the gene encoding PPARG1, and decreased [57], unchanged [53, 55] or increased [54, 56] expression of the gene encoding PPARG2 in obese as compared with lean subjects. The relative abundance of the mRNAs of *PPARG1* and *PPARG2* is also controversial [32, 55–57]. In the present study, *PPARG* expression was comparable between the insulin-resistant and the insulin-sensitive group (Fig. 1). Regarding expression of the 'insulin-resistance genes', we confirm reports of increased basal expression of *HSD11B1* [58, 59]. In addition, at 6 h of insulin infusion gene expression of *TNF* and *IL6* was greater in the insulin-resistant than in the insulin-sensitive group (Figs. 2 and 3).

Regarding acute regulation of gene expression by insulin, the present data are, to the best of our knowledge, novel in comparing acute changes in gene expression in insulin-sensitive and -resistant subjects and in demonstrating that insulin increases mRNA concentrations of *HSD11B1* and *PPARGC1A* in human adipose tissue in vivo. Previous studies have shown that insulin increases *SLC2A4* expression in skeletal muscle [30, 60], and that this effect is blunted in skeletal muscle in insulin-resistant as compared with insulin-sensitive subjects [30, 60]. This was confirmed in adipose tissue in the present study. PPARGC1A is a transcriptional coactivator, which induces gene expression of *SLC2A4* in skeletal muscle [61]. It is absent from white adipose tissue in mice but is expressed in human adipose tissue [23]. We confirm the presence of *PPARGC1A* in human adipose tissue [23] and extend previous findings by demonstrating that insulin acutely increases *PPARGC1A* expression in vivo. *PPARGC1A* and *SLC2A4* expression was significantly correlated, which is in keeping with data showing that overexpression of *PPARGC1A* in skeletal muscle increases *SLC2A4* content and insulin sensitivity

[24]. Overexpression of *PPARGC1A* increases energy expenditure by stimulating thermogenesis in brown fat in mice [62]. This increases the need for fuels such as glucose. Increases in gluconeogenesis in the liver and glucose utilisation in peripheral tissues by PPARGC1A could help to maintain energy supply, even if regulated in a tissue-specific fashion by insulin. Insulin has been shown to acutely (i.e. within 3 h) increase expression of *PPARG*, but the response was similar in lean and obese subjects and in type 2 diabetic patients [32]. In the present study, we also found that *PPARG* gene expression responded similarly to insulin in both groups studied (Fig. 1).

Regarding 'insulin-resistance genes', *HSD11B1* was higher at all time points in the insulin-resistant as compared with the insulin-sensitive group, and increased in response to insulin in the former but not in the latter group (Fig. 2). Data regarding insulin regulation of *HSD11B1* gene expression and activity are not unequivocal. In preadipocytes, insulin attenuates HSD11B1 activity but synergises with glucocorticoids to stimulate adipocyte differentiation that is associated with induction of HSD11B1 activity [63]. In vivo, consistent with the present data at the 3 h time point, 3 h of hyperinsulinaemia has been reported not to change *HSD11B1* expression in type 2 diabetic or healthy men [28]. *HSD11B1* is a gene that is abundantly expressed in macrophages in addition to adipocytes [14]. Therefore the increase in *HSD11B1* in the insulin-resistant group could also be due to insulin action in macrophages, although this possibility remains hypothetical. Like *HSD11B1*, *IL6* was increased significantly more by insulin in the insulin-resistant than the insulin-sensitive subjects (Fig. 3). This most probably occurred in non-adipose cells, since isolated adipocytes account for only 10% of total IL6 release from human adipose tissue [4]. *IL6* may antagonise insulin action [1] by several mechanisms. It has been shown to down-regulate *SLC2A4*, *PPARG* and *ADIPOQ* expression in 3T3-L1 adipocytes [11, 64, 65], and increase HSD11B1 activity in primary cultures of adipose stromal cells [66]. The interstitial IL6 concentration in human adipose tissue is ~100 times higher than that in plasma [67] and IL6, in contrast to TNF, is released from adipose tissue systemically [3]. The high local concentration of IL6 and the existence of all components important for IL6 signalling in human fat cells suggest that IL6 originating from non-fat cells in part induces insulin resistance in a paracrine fashion [11].

TNF expression after 6 h of insulin infusion was higher in insulin-resistant than in insulin-sensitive subjects. Two recent studies have found virtually all TNF to be derived from macrophages [9] or non-fat cells [68] in human adipose tissue. In a study examining the direct effect of insulin on macrophage gene expression using an array technique, insulin stimulated *TNF* the most among all genes in the analysis [69]. In an earlier study, lipopolysaccharide was found to increase TNF production more robustly from human whole-adipose tissue than from isolated adipocytes [29]. In the latter study, insulin had no effect on TNFproduction. In a study that also used the insulin clamp technique and measured *TNF* expression in a group of normal subjects, insulin was found to increase *TNF* expression in adipose tissue transiently after 2 h [27]. In another study, 4 h of in vivo hyperinsulinaemia had no effect on subcutaneous adipose tissue *TNF* mRNA concentration in lean or obese subjects [28]. Our data suggest that the response of *TNF* to insulin is exaggerated in insulin-resistant subjects, although the increase by insulin failed to reach statistical significance. Whether this is because an increased number of macrophages (vide infra) hyperrespond to insulin remains to be determined.

In the study reporting macrophage accumulation in adipose tissue in obesity, immunohistochemical detection and quantification of *CD68*-expressing cells in subcutaneous adipose tissue showed that average adipocyte size and BMI were strong predictors of *CD68*-expressing cells [9]. The increase in the present study of *CD68* expression in insulin-resistant obese subjects therefore most probably reflects an increase in the number of macrophages in adipose tissue.

ADIPOQ mRNA concentration increased significantly by 6 h in the insulin-sensitive but not the insulin-resistant group (Fig. 1). *ADIPOQ* expression has been studied in two previous in vivo studies, which found no change after 2.5 h [48] and 3 h [28] of insulin infusion, consistent with the present data after 3 h. In vitro, in 3T3-L1 adipocytes, insulin has been reported both to increase [70] and decrease [71] adiponectin expression. In human adipocytes, insulin has been reported to increase adiponectin secretion from omental but not subcutaneous adipocytes [72].

We conclude that acute insulin regulation of gene expression in insulin-resistant adipose tissue is characterised not only by hyporesponsiveness of insulin-sensitivity genes such as *SLC2A4* to insulin but also by hyperresponsiveness of insulin-resistance genes (*IL6*, *TNF*, *HSD11B1*). Although attempts made to date in humans using anti-inflammatory approaches such as TNF antagonists to ameliorate insulin resistance have been unsuccessful, the present study supports continued development of such therapies.

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