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ORIGINAL ARTICLE

Fatty acid handling protein expression in adipose tissue, fatty acid composition of adipose tissue and serum, and markers of insulin resistance

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Objective: Proteins involved in cellular fatty acid (FA) uptake and metabolism may be of relevance in the context of disturbed FA metabolism associated with insulin resistance. Therefore this study investigated relationships between FA handling protein mRNA expression in adipose tissue, FA composition of adipose tissue and serum, and markers of insulin resistance.
Subjects: 75 subjects with a range of insulin sensitivities recruited from a cohort of 294 healthy 63-year-old Swedish men.
Measurements: Anthropometric and biochemical variables (e.g. waist-hip-ratio (WHR) and homeostasis model assessment (HOMA) index of insulin sensitivity), FA composition of the subcutaneous (s.c.) gluteal adipose tissue, serum nonesterified FA (NEFA) and serum phospholipid compartments (by gas–liquid chromatography; $n=294$), and mRNA levels of FA handling proteins (adipocyte and keratinocyte lipid binding proteins, fatty acid transport protein (FATP) −1 and −4, CD36/fatty acid translocase, plasma membrane fatty acid binding protein, and acyl-CoA synthase-1 (ACS1)) in s.c. gluteal adipose tissue (by quantitative real-time polymerase chain reaction; $n=75$).
Results: ACS1 expression was negatively correlated with measures of insulin resistance and central obesity (ACS1 versus HOMA: $r=-0.28$, $P<0.05$; ACS1 versus WHR: $r=-0.23$, $P<0.05$), with an opposite trend for FATP4. Further analysis of ACS1 expression levels revealed correlations with adipose tissue 16:0 ($r=-0.27$, $P<0.05$) and NEFA 16:1 ($r=0.29$, $P<0.05$), FA composition variables which in turn correlated with HOMA index ($r=0.39$, $P<0.001$ and $r=-0.23$, $P<0.05$, respectively, $n=75$). Moreover, NEFA 16:1 predicted ACS1 expression independently of HOMA, WHR and adipose tissue 16:0 in multiple regression analysis (standardized coefficient = 0.27, $P<0.05$).
Conclusion: Significant associations were found between measures of insulin sensitivity, adipose tissue FA handling protein expression, and specific FA composition variables. Although causal relationships could not be identified these findings suggest a role of FA handling proteins in relation to insulin sensitivity, via their involvement in FA trafficking and metabolism. In particular they indicate links between ACS1 activity, the distribution of 16:0 and 16:1, and insulin sensitivity, which may be of physiological relevance.
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Introduction

The metabolic syndrome is characterized by insulin resistance, central obesity, hypertension, prothrombotic and proinflammatory states, and perturbations of lipid metabolism, including increased triglyceride (TG) concentrations, low high-density lipoprotein (HDL) cholesterol

concentrations, increased formation of small, dense low-density lipoprotein (LDL) particles, and increased nonesterified fatty acid (NEFA) concentrations. According to the recently revised NCEP/ATPIII guidelines, (Grundy *et al*., 2005) diagnosis of the metabolic syndrome is based on the presence of three of the following criteria; elevations of waist circumference, blood pressure, TG or fasting glucose concentrations, or reduced HDL concentrations. Insulin resistance is related to disturbed FA metabolism in adipose tissue and other tissues (reviewed in Frayn, 2002), and serum lipid esters exhibit characteristic FA composition patterns in insulin resistant states (reviewed in Vessby *et al*., 2002). Commonly, the FA composition pattern of serum phospholipids (PLs) and cholesteryl esters (CEs) found to be associated with insulin resistance includes increased proportions of 16:0 (palmitic acid), 16:1 (palmitoleic acid) and 20:3 *n*-6 (dihomo-gamma-linolenic acid), and a decreased proportion of 18:2 *n*-6 (linoleic acid) (Salomaa *et al*., 1990; Vessby *et al*., 1994, 2002; Lovejoy *et al*., 2001; Pelikanova *et al*., 2001; Vessby, 2003). Generally, the proportion of saturated FAs (SFAs) in serum lipid esters is negatively related to insulin sensitivity (Salomaa *et al*., 1990; Pelikanova *et al*., 2001; Vessby, 2003). Tissue FA composition has also been studied in relation to measures of insulin resistance. The proportion of long-chain poly-unsaturated FAs (in particular arachidonic acid, 20:4 *n*-6) and the average degree of FA unsaturation in skeletal muscle membrane PLs has been shown to correlate positively with insulin sensitivity (Borkman *et al*., 1993; Pan *et al*., 1995), whereas the proportion of 16:0 in both skeletal muscle PLs and TGs correlated negatively with insulin sensitivity (Vessby *et al*., 1994). However, studies investigating the relationships between detailed measures of FA composition of adipose tissue and insulin sensitivity in man are warranted.

Several proteins are involved in cellular FA handling and metabolism. The cellular uptake of long-chain NEFAs is facilitated by membrane-associated proteins, including fatty acid translocase (FAT/CD36) (Abumrad *et al*., 1993), plasma membrane fatty acid binding protein (FABP(pm)) (Stremmel *et al*., 1985), and the FA transport proteins (Schaffer and Lodish, 1994; Hirsch *et al*., 1998) (FATPs; FATP1 and FATP4 being the main FATPs in adipose tissue, Stahl *et al*., 2001). Intracellularly, cytosolic fatty acid binding proteins (FABPs; FABP4/adipocyte lipid binding protein (ALBP)/aP2 and FABP5/keratinocyte lipid binding protein (KLBP)/mal1 in adipose tissue) are believed to sequester and direct NEFAs and their derivatives into different metabolic pathways (Coe and Bernlohr, 1998). Acyl-coenzyme-A (CoA) synthases, such as adipose tissue acyl-CoA synthase-1 (ACS1), ligate NEFAs to CoA before further metabolism (e.g. TG, PL and CE synthesis or oxidation; reviewed in Coleman *et al*., 2002). Notably, FATPs also possess acyl-CoA synthase activity (Coe *et al*., 1999).

Previous studies of human adipose tissue FA handling protein expression in relation to insulin resistance and obesity have shown ALBP and KLBP expression to be increased after weight loss in obese subjects, and CD36 expression to be decreased (Fisher *et al*., 2002). Moreover, lipodystrophy (which is strongly related to insulin resistance) in HIV-positive individuals undergoing highly active antiretroviral therapy (HAART) was associated with reduced adipose tissue ACS1 expression (Kannisto *et al*., 2002). Skeletal muscle FATP1 mRNA levels were shown to be negatively correlated with body mass index (BMI) in middle-aged women (but not in men), whereas no such correlation was observed for adipose tissue FATP1 expression, analysed in men only (Binnert *et al*., 2000). In a study of young monozygotic twins, expression levels of FATP4 and CD36 were related to measures of insulin resistance and obesity (Gertow *et al*., 2004). For a recent review on the FATPs in relation to insulin resistance, see Fisher and Gertow (2005).

Hypothesizing that the activities of the above FA handling proteins in adipose tissue are of physiological relevance in determining the distribution of individual FAs within different compartments, and in relation to insulin action, the aim of this study was to investigate relationships between subcutaneous (s.c.) adipose tissue FA handling protein mRNA expression, FA composition of the adipose tissue and serum NEFA compartments, and markers of insulin resistance in 75 Swedish 63-year-old men with a range of insulin sensitivities.

Materials and methods

Subjects

A total of 303 healthy 62–64-year-old men were randomly recruited from a larger population-based cohort of 2039 men living in Stockholm county (Rosell *et al*., 2003). Exclusion criteria were non-Swedish descent, manifest diabetes, cardiovascular disease or cancer, treatment with antihypertensive or lipid-lowering agents, or BMI outside the range 19–35 kg/m^2. Of the original 2039 men, 995 men fulfilled the criteria and were divided into tertiles of fasting plasma insulin concentration in order to ascertain recruitment of subjects with a range of insulin sensitivities. Approximately 100 men, randomly selected from each tertile (participation rate 71%), were included in the current study and subjected to a more detailed investigation including anthropometric measurements, biochemical analyses, and determination of FA composition of both serum and gluteal adipose tissue. Seven subjects with thyroid stimulating hormone >5.0 mmol/l were excluded, leaving 294 subjects. Of these, adipose tissue biopsies for extraction of total mRNA were taken from 87 consecutive subjects (approximately 30 from each insulin tertile), and adequate amounts of RNA for expressional analysis were obtained from 75 individuals (see below). Components of the metabolic syndrome were defined according to the recently revised NCEP/ATPIII guidelines (waist circumference in men $\geqslant 102$ cm, TG $\geqslant 1.7$ mmol/l, HDL cholesterol in men <1.03 mmol/l, systolic blood

pressure $\geqslant$ 130 mm Hg or diastolic blood pressure $\geqslant$ 85 mm Hg, fasting plasma glucose $\geqslant$ 5.6 mmol/l) (Grundy *et al*., 2005), and subjects were divided according to the presence of 0, 1 or 2, or 3 of these components (where the presence of three components is diagnostic of the metabolic syndrome). The Karolinska Hospital Ethics Committee approved of the study and all subjects gave their informed consent.

Clinical procedure

The participants visited the Karolinska Hospital in the morning, having fasted since 2200 hours the night before and refrained from smoking during the morning hours. Sagittal abdominal diameter (SAD) was determined to the nearest 0.1 cm using a ruler and a water level, with the participant lying horizontally. Waist-hip-ratio (WHR) and BMI were measured as described (Rosell *et al*., 2003). Blood samples were drawn into precooled sterile tubes (Vacutainer, Becton Dickinson) containing Na_2 ethylenediamine-*N*, *N*, *N*′, *N*′-tetraacetic acid (final concentration 10 mmol/l) and plasma was recovered by low speed centrifugation (1750 *g*, 10 min, $+4^{\circ}$C) and stored at -70°C within an hour. Needle biopsies of s.c. adipose tissue for FA composition and mRNA analyses were taken from the upper left buttock under local anaesthesia with xylocain.

Laboratory procedures

Blood glucose concentrations were measured by a glucose oxidase method (Kodak Ektachem, Rochester, USA). Insulin concentrations were measured by a commercial enzyme-linked immunosorbant assay (DAKO Diagnostics, Cambridgeshire, UK). Homeostasis model assessment (HOMA) index was calculated as the product of fasting insulin and glucose concentrations (in mU/l and mmol/l, respectively) divided by 22.5. Plasma NEFAs were measured by an enzymatic colorimetric method (WAKO Chemicals, Neuss, Germany). Fasting plasma concentrations of cholesterol and TGs (total, very low-density lipoprotein (VLDL), LDL and HDL) were determined by a combination of preparative ultracentrifugation, precipitation of apolipoprotein B (apoB)-containing lipoproteins, and lipid determinations (ABX Diagnostics and Roche Diagnostics) (Carlson, 1973). The FAs in serum PLs and NEFAs were analysed by as described (Boberg *et al*., 1985). Briefly, serum lipids were extracted in the presence of an antioxidant (butylated hydroxytoluene), separated by thin layer chromatography and analysed by gas–liquid chromatography. The FA composition of adipose tissue (i.e. mainly TG) was determined using the same method except that the thin layer chromatography stage was omitted (i.e. no separation of TG from other minor lipid classes was performed). The relative amount of a given FA was expressed as the percentage of the sum of the FAs that were analysed, by integrating its chromatogram peak area and dividing it by the total area of FAs that were analysed (14:0, 15:0, 16:0, 16:1, 17:0, 18:0, 18:1, 18:2 *n*-6, 18:3 *n*-3, 18:3 *n*-6, 20:3 *n*-6, 20:4 *n*-6, 20:5 *n*-3, 22:5 *n*-3 and 22:6 *n*-3). SFA was defined as the sum of 14:0, 15:0, 16:0, 17:0 and 18:0. The absolute concentration of a given serum NEFA was calculated from its relative amount and the total concentration of plasma NEFAs. Owing to their low prevalence, not all FAs were quantifiable in all samples.

Analysis of mRNA expression

Gluteal s.c. adipose tissue biopsies (mean weight approximately 200 mg) were homogenized in 1 ml phenol-containing TriZol solution (Invitrogen, Carlsbad, CA, USA). After addition of chloroform, the total RNA-containing aqueous phase was treated with DNaseI (Qiagen, Hilden, Germany) and purified on spin columns (RNeasy Mini-Kit, Qiagen). 50–500 ng total RNA was used for cDNA synthesis using SuperScriptII reverse transcriptase (Invitrogen) and oligo-dT(15) primer, in the presence of an RNase inhibitor (RNaseOUT, Invitrogen). Specific FA handling protein (ALBP, KLBP, FATP1, FATP4, CD36, FABP(pm), ACS1) and control TATA-box binding protein (TBP) mRNA expression was quantified by real-time polymerase chain reaction (PCR) using the ABI Prism 7000 Sequence Detection System and software (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol, employing five-point standard curves generated from 10-fold dilutions of purified PCR products. Primer and probe sets for ALBP, KLBP, FATP1, FATP4 and ACS1 were designed using the manufacturer's software and sequences deposited in GenBank (NM001442, NM001444, AX015323, AF055899 and L09229, respectively) (Kannisto *et al*., 2002). CD36, FABP(pm) and TBP were measured with predeveloped Assays-On-Demand (Hs00169627_m1, Hs00751057_s1 (mitochondrial glutamic-oxaloacetic transaminase 2/aspartate aminotransferase 2; proposed to be identical to FABP(pm) (Isola *et al*., 1995)), and Hs00427620_m1, respectively; Applied Biosystems, USA). Owing to inadequate yield of total RNA from 12 biopsies, data on FA handling expression was obtained from 75 individuals. Results were expressed in arbitrary units related to levels of TBP mRNA expression for normalization.

Statistical analyses

Spearman rank correlations were used to analyse relationships between the investigated variables, and multiple regression analysis using the enter method was performed to investigate the independence of associations. Analysis of variance (ANOVA) and Fisher's protected least significant difference (PLSD) tests were used to investigate differences in absolute measurements between groups, for which data are given as mean $\pm$ standard error of the mean (s.e.m.). The analyses were conducted with the StatView software (SAS Institute Inc., Cary, NC, USA). Skewed variables were logarithmically transformed before parametric statistical analysis and for graphical presentation.

Results

Characteristics of the subjects

The median age of the participants was 63.4 years (interquartile range 63.2–63.8 years). Anthropometric and biochemical characteristics of the subjects were (data given as median value with interquartile range in parenthesis): BMI 25.1 (23.3–27.4) kg/m^2, WHR 0.93 (0.89–0.96), waist circumference 95 (88–101) cm, SAD 20.8 (19.1–22.5) cm, systolic blood pressure 131 (122–146) mm Hg, diastolic blood pressure 81 (75–89) mm Hg, insulin 33 (26–44) pmol/l, glucose 4.9 (4.7–5.3) mmol/l, HOMA 1.31 (0.86–1.65), TG 1.18 (0.83–1.60) mmol/l, NEFA 0.47 (0.40–0.57) mmol/l, and HDL cholesterol 1.61 (1.38–1.91) mmol/l.

Correlations of mRNA expression levels with anthropometric and biochemical variables

Correlations of specific gluteal s.c. adipose tissue FA handling protein mRNA expression levels with anthropometric and biochemical variables are shown in Table 1. ACS1 expression correlated negatively with WHR, insulin concentrations and HOMA index (Figure 1). The relationships between ACS1 expression levels and measures of the metabolic syndrome were further analysed by investigating ACS1 expression levels according to prevalence of components of the metabolic syndrome (0, 1 or 2, or 3 or more components according to the recently revised NCEP/ATPIII criteria; (Grundy *et al*., 2005) $n = 26$, 41, and 8, respectively), and according to tertiles of HOMA index. However, ACS1 expression was not significantly different between these groups (data not shown). FABP(pm) expression correlated negatively with NEFA concentrations, and KLBP and CD36 expression correlated negatively with glucose concentrations. Expression levels of control TBP mRNA were not significantly correlated with BMI or any other anthropometric or biochemical variable in Table 1. Of the lipid and lipoprotein variables measured, statistical analyses were focused on those known to be strongly related to insulin resistance and the metabolic syndrome (total TG; i.e. mainly VLDL TG in the fasting state, HDL cholesterol and NEFA).

Correlations of mRNA expression levels with FA composition

Correlations of s.c. adipose tissue FA handling protein expression with relative measures of FAs in the adipose tissue and NEFA compartments were analysed (Table 2). The analysis was focused on ACS1 since its expression levels correlated significantly with measures of insulin resistance and central obesity (Table 1). The FAs chosen for analysis were those whose proportions in serum or skeletal muscle lipid esters have been shown to be related to variations in insulin sensitivity (16:0, 16:1, 18:2 *n*-6, 20:3 *n*-6, 20:4 *n*-6 and SFA) (Salomaa *et al*., 1990; Borkman *et al*., 1993; Vessby *et al*., 1994, 2002; Pan *et al*., 1995; Lovejoy *et al*., 2001; Pelikanova *et al*., 2001; Vessby, 2003). We focused analysis on the adipose tissue and NEFA compartments since the FA

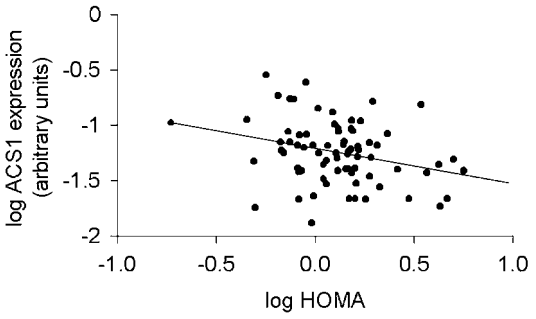

Figure 1 Scatter plot of the relation of s.c. adipose tissue ACS1 mRNA expression with HOMA index. Regression line is shown. For statistics, please refer to Table 1.

Table 1 Correlations between specific s.c. adipose tissue FA handling protein mRNA expression levels and anthropometric and biochemical variables

	ALBP (n = 75)	*KLBP* (n = 75)	*FATP1* (n = 75)	*FATP4* (n = 65)	*CD36* (n = 75)	*FABP(pm)* (n = 71)	*ACS1* (n = 75)
WHR	−0.04	0.09	0.12	0.05	−0.16	0.06	−0.23[b]
SAD	0.03	0.18	0.10	0.23[a]	−0.03	0.06	−0.22[a]
Insulin	0.04	0.0001	0.08	0.21[a]	0.01	0.10	−0.29[c]
Glucose	−0.19	−0.25[b]	−0.11	−0.06	−0.23[b]	−0.13	−0.15
HOMA	−0.03	−0.06	0.07	0.18	−0.06	0.05	−0.28[b]
TG	−0.07	−0.11	−0.03	0.004	−0.12	−0.15	−0.15
NEFA	0.11	0.05	0.02	0.02	0.13	−0.23[b]	0.05
HDL C	0.13	0.04	−0.13	−0.22[a]	0.21[a]	−0.10	0.19

Spearman rank correlation coefficients and significance levels are shown. All anthropometric and biochemical measurements were performed after an over-night fast. For units, see text.

[a] $P = 0.06$–0.09.

[b] $P \leqslant 0.05$.

[c] $P \leqslant 0.01$.

Abbreviations: ACS1, acyl-CoA synthase-1; ALBP, adipocyte lipid binding protein; FA, fatty acids; FABP(pm), plasma membrane fatty acid binding protein; FATP, fatty acid transport protein; HDL C, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; KLBP, keratinocyte lipid binding protein; NEFA, nonesterified fatty acid; s.c., subcutaneous; SAD, sagittal abdominal diameter; TG, triglyceride; WHR, waist-hip ratio.

Table 2 Correlations of the relative content (%) of selected FA in the adipose tissue, serum NEFA and serum phospholipid compartments with expression levels of ACS1 in subcutaneous adipose tissue and HOMA index

	ACS1[a]		*HOMA*[b]		
	Adipose tissue	*Serum*	*Adipose tissue*	*Serum*	*Serum*
	Total	*NEFA*	*Total*	*NEFA*	*PL*
16:0	−0.27[c]	−0.11	0.15[d]	0.30[e]	−0.06
16:1	0.12	0.29[d]	0.01	−0.09	0.06
18:2 *n*-6	0.07	0.02	−0.07	−0.03	−0.14[c]
20:3 *n*-6	−0.03	−0.30[f]	0.33[e]	0.06[g]	0.34[e]
20:4 *n*-6	−0.13	−0.05	0.31[e]	−0.10	−0.04
SFA[h]	−0.23[c]	−0.12	−0.01	0.13[c]	0.29[e]

Spearman rank correlation coefficients and significance levels are shown.
[a]$n=75$.
[b]$n=289$ (AT), $n=292$ (NEFA), $n=291$ (PL).
[c]$P\leqslant 0.05$.
[d]$P\leqslant 0.01$.
[e]$P\leqslant 0.0001$.
[f]$n=15$.
[g]$n=50$.
[h]14:0, 15:0, 16:0, 17:0, 18:0.
For the corresponding correlations with HOMA index in the subset which underwent mRNA expression analyses, please refer to the text.
Abbreviations: ACS1, acyl-CoA synthase-1; FA, fatty acids; HOMA, homeostasis model assessment; NEFA, nonesterified FA; PL, phospholipid; SFA, saturated fatty acid.

composition of these sites (as opposed to the PL compartment) is most likely to be influenced by the investigated FA handling proteins. ACS1 expression correlated negatively with adipose tissue 16:0 and adipose tissue SFA, and positively with NEFA 16:1 (Table 2). Absolute concentrations of individuals FAs in the NEFA fraction could also be calculated. Even though the relative proportion of 16:1 in the NEFA compartment correlated significantly with ACS1 expression levels, the absolute concentrations of NEFA 16:1 did not ($r=0.19$, $P=0.11$). However, NEFA 16:1 concentrations in the lowest quintile of ACS1 expression were 0.017 ± 0.001 mmol/l ($n=15$) compared to 0.028 ± 0.004 mmol/l ($n=16$) in the highest quintile ($P=0.04$, Fisher's PLSD. ANOVA across all quintile groups, $P=0.34$).

To determine independent predictors of ACS1 expression levels, multiple regression analysis was performed with WHR, HOMA, adipose tissue 16:0 and NEFA 16:1 as independent variables. In this analysis, NEFA 16:1 was the only independent predictor of ACS1 expression (data given as the B standardized coefficient and respective *P*-value; intercept; −1.08, $P=0.11$, HOMA; −0.20, $P=0.12$, WHR; −0.06, $P=0.64$, adipose tissue 16:0; −0.04, $P=0.72$, NEFA 16:1; 0.27, $P=0.02$, adjusted $R^2=0.12$, $P=0.01$).

Correlations between FA composition variables and FATP4 expression levels were also investigated (data not shown), since FATP4 mRNA levels correlated with measures of both insulin resistance and central obesity with borderline significance. FATP4 expression correlated positively with adipose tissue 20:4 *n*-6 ($r=0.28$, $P=0.03$).

As expression levels of FABP(pm) were significantly inversely correlated with total plasma NEFA concentrations (Table 1), we also investigated relationships between FABP(pm) expression and individual NEFAs. Expression levels of FABP(pm) correlated significantly with absolute concentrations of some of the investigated individual FAs in the NEFA compartment (16:0; $r=-0.26$, $P=0.03$, 18:2 *n*-6; $r=-0.28$, $P=0.02$), whereas no significant correlations were observed between FABP(pm) expression levels and relative measures of FAs in the NEFA compartment (data not shown).

Correlations of measures of insulin resistance with FA composition

To confirm and complement previous reports linking specific FA composition patterns of serum lipid esters with insulin resistance, we analysed selected FAs (as above) in adipose tissue, NEFAs and PLs in relation to HOMA index in the whole cohort. This revealed positive correlations of HOMA with adipose tissue 16:0, NEFA 16:0, adipose tissue 20:3 *n*-6, PL 20:3 *n*-6, adipose tissue 20:4 *n*-6, NEFA SFA and PL SFA, and a negative correlation of HOMA with PL 18:2 *n*-6 (Table 2). In the subset of subjects who underwent mRNA expression analyses ($n=75$) the majority of these significant correlations were preserved, with the exception of adipose tissue 20:4 *n*-6 ($r=0.20$, $P=0.09$) and PL 18:2 *n*-6 ($r=-0.12$, $P=0.29$). In particular, the correlation between HOMA and adipose tissue 16:0 was preserved and strengthened ($r=0.39$, $P=0.0007$). Moreover, in this subset, NEFA 16:1 was negatively correlated with HOMA ($r=-0.23$, $P=0.048$).

Discussion

This study has investigated the mRNA expression levels in adipose tissue of proteins implicated in different aspects of

FA handling, that is, cellular uptake (CD36, FABP(pm), FATP1, FATP4), acyl-CoA synthesis (which may also drive cellular uptake; ACS1, FATP1, FATP4), and intracellular sequestration and trafficking (ALBP, KLBP). ACS1 expression was negatively correlated with measures of insulin resistance and central obesity (insulin concentrations, HOMA index and WHR). The observation of reduced s.c. adipose tissue ACS1 expression in HAART-associated lipodystrophy (Kannisto *et al*., 2002) is consistent with the current findings. Indeed, obesity-related and lipodystrophy-related insulin resistance are both characterized by impaired adipose tissue FA buffering and ectopic TG accumulation (Frayn, 2002). In this context, ACS1 may play a role in upholding adipose tissue FA buffering and TG storage capacity, since ACS1 is suggested to channel FAs primarily into TG synthetic pathways (Coleman *et al*., 2002). Of the other genes investigated, FATP4 expression showed borderline significant positive correlations with measures of insulin resistance and central obesity (insulin concentrations and SAD). This is in accordance with the finding that FATP4 was found to be upregulated in obesity and insulin resistance in a study of young (mean age 25.7 years) monozygotic twins specifically recruited to exhibit a range of within-pair differences in measures of obesity and insulin resistance (Gertow *et al*., 2004). However, in the twin study no correlations between ACS1 expression and measures of insulin resistance and central obesity were observed, in contrast to the current study of 63-year-old men. This disagreement may be due to differences in study design, namely the fact that the twin study was designed to investigate parameters related to acquired obesity and insulin resistance in young subjects, that is, the development of obesity and insulin resistance due to lifestyle and environmental factors at young age, whereas the current investigation is a population-based cross-sectional study of parameters related to the metabolic syndrome in considerably older individuals. Expression levels of other FA handling proteins were also correlated with markers of the metabolic syndrome, namely KLBP and CD36 expression were negatively correlated with glucose concentrations, and FABP(pm) expression negatively with NEFA concentrations. In this study, with a focus on adipose tissue metabolism, we used HOMA index as measure of insulin sensitivity since a more sensitive measurement of insulin sensitivity, for example, determination of whole-body insulin-stimulated glucose uptake (i.e. mainly into skeletal muscle) by the euglycaemic hyperinsulinaemic clamp procedure, was not available.

In accordance with previous reports on serum PL FA composition in relation to insulin resistance (Salomaa *et al*., 1990; Vessby *et al*., 1994, 2002; Lovejoy *et al*., 2001; Pelikanova *et al*., 2001; Vessby, 2003), the PL relative content of 20:3 *n*-6 and SFA correlated positively, and that of 18:2 *n*-6 negatively, with HOMA in this study. However, no significant correlations were observed between the proportion of PL 16:0, 16:1 or 20:4 *n*-6 and HOMA. In the adipose tissue and/or NEFA compartments, significant correlations of the relative content of all these FAs with HOMA were observed when analysed in the whole cohort, except for 16:1 and 18:2 *n*-6. This is consistent with the serum lipid ester composition pattern commonly observed in insulin resistant states being to some extent reflected in the NEFA and/or adipose tissue compartments.

In order to characterize further the significant associations between ACS1 expression and measures of insulin resistance and central obesity, relationships of ACS1 expression with selected FA composition variables were investigated. ACS1 expression was associated negatively with adipose tissue 16:0 and positively with NEFA 16:1. Both adipose tissue 16:0 and NEFA 16:1 were in turn related to HOMA (positive correlation for 16:0 and negative for 16:1, although the latter association was only significant in the subset of 75 subjects who underwent expression analyses and not in the full cohort). Similarly, FATP4 expression was correlated with adipose tissue 20:4 *n*-6, which in turn was positively correlated with HOMA. These results could indicate that specific FAs link ACS1 and FATP4 expression levels to measures of insulin resistance, although causal relationships cannot be characterized by the current data, as discussed below. However, the fact that NEFA 16:1 was identified as an independent predictor of ACS1 mRNA levels may indicate that 16:1 regulates ACS1 gene transcription, possibly via peroxisome-proliferator activated receptors (PPARs). Both ACS1 and FATP4 are implicated in facilitated FA uptake and acyl-CoA synthesis, but their expression levels show different associations with insulin sensitivity in this study (positively and negatively, respectively). We can only speculate that this could in part be due to differences in their preference towards different substrates (with potentially different biochemical characteristics), and differences in the metabolic fates of these substrates. Indeed, ACS1 has a broad substrate specificity for 12–18 carbon SFAs and 16–20 carbon unsaturated FAs (i.e. medium and long-chain FAs), whereas studies of FATP4 null mice and transfection studies have implicated FATP4 foremost in the esterification of very long-chain fatty acids *in vivo* (Herrmann *et al*., 2001; Hall *et al*., 2005). However, cloned and purified murine FATP4 (i.e. assayed in a cell-free system) showed a preference towards 16:0 compared to 24:0 as substrate for acyl-CoA synthesis (Hall *et al*., 2005). As stated above, adipocyte ACS1 is believed to direct FAs primarily into TG synthetic pathways (Coleman *et al*., 2002). The downstream metabolic pathway(s) preferred by FATP4 in adipose tissue remain to be determined, but observations in FATP4 null mice suggest an important function in membrane lipid synthesis (e.g. PLs and sphingolipids) in other tissues (Herrmann *et al*., 2003). Moreover, in this context, potential differences in intracellular protein localization should be considered. For instance, ACS1 has been shown to associate with glucose transporter-4 (GLUT4) vesicles in primary rat adipocytes (Sleeman *et al*., 1998), indicating an acute upregulation at the plasma membrane in response to insulin, whereas FATP4 did not show any significant upregulation at the plasma membrane

in response to insulin in primary murine adipocytes (although a modest increase was observed in 3T3 L1-adipocytes) (Stahl *et al*., 2002).

The negative correlations of FABP(pm) expression levels with the absolute concentrations of individual NEFAs of 16–18 carbon chain length, and the lack of significant correlations of FABP(pm) expression levels with relative measures of individual NEFAs, may indicate that FABP(pm) facilitates trans-membrane trafficking of 16–18 carbon NEFAs in a concentration-dependent manner, with little or no effect on their relative proportion.

It should be noted that this study only identifies specific s.c. adipose tissue gene expression related to markers of insulin resistance and measures of FA composition, without characterizing causal relationships (e.g. if FA handling protein expression levels influence FA composition and/or vice versa). The FA composition variables investigated are under strong dietary influence, since the FA composition of serum PL and adipose tissue TG reflects the dietary FA composition (albeit with different strengths for different FAs) during the preceding weeks and months to years, respectively (Katan *et al*., 1997; Vessby *et al*., 2002; Vessby, 2003). The FA composition in the (fasting) NEFA compartment reflects the lipolytic release from adipose tissue TG stores and VLDL TG, and the FA uptake into tissues. Of note, the relative lipolytic FA release from adipose tissue has been shown to decrease with FA carbon chain length and increase with degree of unsaturation (Halliwell *et al*., 1996). In this context, the activities and substrate specificities of certain FA handling proteins such as the ones investigated in this study may play a role in determining the FA composition of a given compartment. It should be noted that the mRNA data presented here may not accurately reflect protein levels or protein activity. The current observations in s.c. adipose tissue should not be extrapolated to other tissues, given the tissue-specific expression patterns of several of the investigated FA handling proteins (e.g. FATPs and FABPs), and physiological differences in FA handling between different tissues at a given nutritional state. Thus, further studies are warranted to elucidate the physiological role(s) of FA handling proteins in different tissues.

In summary, we have shown that specific s.c. adipose tissue FA handling protein mRNA expression is related to markers of insulin resistance and central obesity, and to detailed measures of adipose tissue and serum FA composition that are proposed to be of relevance for insulin sensitivity, in 63-year-old men. These findings suggest a physiological and pathological role of FA handling proteins in relation to insulin sensitivity, via their involvement in FA trafficking and metabolism.

Abbreviations

ACS1, acyl-CoA synthase-1; ALBP, adipocyte lipid binding protein; BMI, body mass index; CE, cholesteryl ester; CoA, coenzyme-A; FABP, fatty acid binding protein; FATP, fatty acid transport protein; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; KLBP, keratinocyte lipid binding protein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; PL, phospholipid; SAD, sagittal abdominal diameter; SFA, saturated fatty acid; TG, triglyceride; VLDL, very low-density lipoprotein; WHR, waist-hip-ratio.

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