

Research article

REGULATION OF HUMAN ALDOKETOREDUCTASE 1C3 (AKR1C3) GENE EXPRESSION IN THE ADIPOSE TISSUE

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Abstract: Aldoketoreductase 1C3 (AKR1C3) is a functional prostaglandin F synthase and a negative modulator of the availability of ligands for the nuclear receptor peroxisome proliferator-activated receptor-gamma (PPAR γ). AKR1C3 expression is known to be associated with adiposity, one of the components of the metabolic syndrome. The aim of this study was to characterize the expression of AKR1C3 in the adipose tissue and adipocytes and to investigate its potential role in the metabolic syndrome. Using microarray analysis and real-time PCR, we studied the expression of AKR1C3 in adipose tissue samples from obese subjects with or without metabolic complications, during very low calorie diet-induced weight loss, and its expression in isolated human adipocytes of different sizes. The adipose tissue AKR1C3 expression levels were marginally lower in obese subjects with the metabolic syndrome compared with the levels in healthy obese subjects when analyzed using microarray ($p = 0.078$) and real-time PCR ($p < 0.05$), suggesting a secondary or compensatory effect. The adipose tissue mRNA levels of AKR1C3 were reduced during and after diet-induced weight-loss compared to the levels before the start of the diet

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Abbreviations used: 15d-PGJ₂ – 15-deoxy-^{12,14}-prostaglandin J₂; AKR1C3 – aldoketoreductase 1C3; BMI – body mass index; HDL – high density lipoprotein; hs-CRP – high sensitivity C-reactive protein; LDL – low density lipoprotein; om – omental; PPAR γ – peroxisome proliferator-activated receptor-gamma; sc – subcutaneous; VLCD – very low calorie diet; WHR – waist-to-hip ratio

($p < 0.001$ at all time-points). The gene expression of AKR1C3 correlated with both adipose tissue mRNA levels and serum levels of leptin before the start of the diet ($p < 0.05$ and $p < 0.01$, respectively). Furthermore, large adipocytes displayed a higher expression of AKR1C3 than small adipocytes (1.5-fold, $p < 0.01$). In conclusion, adipose tissue AKR1C3 expression may be affected by metabolic disease, and its levels are significantly reduced in response to diet-induced weight loss and correlate with leptin levels.

Key words: Metabolic syndrome, Adipose tissue, Adipocytes, Diet-induced weight loss, Aldoketoreductase 1C3, 15-deoxy-^{12,14}-prostaglandin J₂

INTRODUCTION

The metabolic syndrome is a cluster of risk factors that together increase the risk of the development of cardiovascular diseases. Obesity is recognized as a major driver of the metabolic syndrome, and voluntary weight-loss improves metabolic function and reduces overall mortality [1, 2]. However, some obese subjects remain healthy for relatively long periods of time. The reason for this apparent resistance is unknown.

Adipose tissue was once considered to be a passive storage depot, releasing energy as fatty acids and glycerol in times of fasting or starvation. During the last decade, it became apparent that adipocytes are active endocrine cells that secrete important hormones, cytokines, vasoactive substances and other peptides with potential roles in the development of metabolic disease [3]. It is well known that the accumulation of visceral fat is strongly associated with metabolic disease, independent of the total adipose tissue mass [4-7]. This suggests functional differences between the adipose tissue depots. It has been shown that visceral adipocytes are less responsive to the antilipolytic effects of insulin and more sensitive to adrenergic lipolysis [8]. Researchers including ourselves have previously shown that a number of genes display different expression levels between the subcutaneous (sc) and the visceral depots [9-11]. One gene which has been shown in several studies to be expressed at higher levels in sc adipose tissue is aldoketoreductase 1C3 (AKR1C3; also known as type 5 17 β -hydroxysteroid dehydrogenase; 17 β -HSD-5) [12-15].

AKR1C3 is a multifunctional enzyme that is able to metabolize a diverse array of substrates, including various types of hydroxysteroids [16, 17]. However, AKR1C3 also participates in prostaglandin metabolism, and plays a suppressive role in the formation of endogenous ligands (15-deoxy-^{12,14}-prostaglandin J₂; 15d-PGJ₂) for the nuclear receptor peroxisome proliferator-activated receptor-gamma (PPAR γ) [18]. PPAR γ plays a pivotal role in adipocyte differentiation and is also a target for insulin-sensitizing drugs [19]. It has been shown that all the enzymes required for the synthesis of 15d-PGJ₂ are expressed in human adipose tissue. These enzymes also have distinct expression patterns with higher expression levels in omental compared to sc adipose tissue [14].

The aim of this study was to characterize the expression of AKR1C3 in human adipose tissue and adipocytes, and to investigate its potential role in the metabolic syndrome.

MATERIALS AND METHODS

Subjects and samples

During a diet-induced weight loss study, abdominal sc adipose tissue biopsies and fasting blood samples were obtained from 6 obese women and 18 men before (week 0), during (weeks 8 and 16) and after (week 18) treatment with a very low calorie diet (VLCD; 450 kcal as 8 g fat, 51 g protein, and 50 g carbohydrate per day). The subjects were on the VLCD for 16 weeks, then a normal diet was gradually re-introduced [20-22]. For the baseline analysis, the subjects were also divided into two groups: those who were healthy obese and those who were obese with the metabolic syndrome (Tab. 1). The metabolic

Tab. 1. The patient characteristics of the subjects included in the microarray analysis (means \pm SD).

		Wk 0	Wk 8	Wk 16	Wk 18
Gender (male/female)	MetS	9/3			
	Non-MetS	9/3			
Age (years; min-max)	MetS	51 (28-61)			
	Non-MetS	45 (29-59)			
BMI (kg/m ²)	MetS	37.7 \pm 5.2	31.6 \pm 4.5	28.4 \pm 4.6	28.7 \pm 4.2
	Non-MetS	37.6 \pm 4.8	32.0 \pm 3.9	28.9 \pm 3.7	29.0 \pm 3.7
Waist (cm)	MetS	122 \pm 12	108 \pm 12	98 \pm 12	99 \pm 11
	Non-MetS	124 \pm 13	112 \pm 13	103 \pm 14	103 \pm 14
WHR	MetS	1.0 \pm 0.08	0.98 \pm 0.08	0.95 \pm 0.07	0.95 \pm 0.06
	Non-MetS	1.0 \pm 0.08	0.99 \pm 0.08	0.95 \pm 0.09	0.96 \pm 0.10
Systolic BP (mmHg)	MetS	147 \pm 17	123 \pm 12	120 \pm 12	131 \pm 14
	Non-MetS	129 \pm 12	118 \pm 12	114 \pm 16	117 \pm 15
Diastolic BP (mmHg)	MetS	91 \pm 16	74 \pm 9.4	71 \pm 9.9	77 \pm 13
	Non-MetS	86 \pm 10	77 \pm 12	73 \pm 12	75 \pm 11
Fasting Glucose (mmol/l)	MetS	6.9 \pm 1.8	4.6 \pm 0.74	4.6 \pm 0.92	5.4 \pm 1.2
	Non-MetS	5.1 \pm 0.58	4.5 \pm 0.61	4.4 \pm 0.35	4.5 \pm 0.32
OGTT 2h Glucose (mmol/l)	MetS	11 \pm 3.2	8.1 \pm 2.0	7.7 \pm 2.9	7.0 \pm 2.7
	Non-MetS	5.4 \pm 1.5	6.0 \pm 1.2	6.2 \pm 2.0	4.9 \pm 0.88
LDL Cholesterol (mmol/l)	MetS	3.6 \pm 1.0	2.1 \pm 0.83	2.5 \pm 0.79	3.0 \pm 0.71
	Non-MetS	3.7 \pm 1.0	2.4 \pm 0.76	2.6 \pm 0.49	2.8 \pm 0.65
HDL Cholesterol (mmol/l)	MetS	1.4 \pm 0.41	1.2 \pm 0.32	1.4 \pm 0.39	1.4 \pm 0.29
	Non-MetS	1.3 \pm 0.36	1.2 \pm 0.26	1.3 \pm 0.35	1.4 \pm 0.29
Triglycerides (mmol/l)	MetS	2.2 \pm 1.3	0.97 \pm 0.21	0.83 \pm 0.20	1.3 \pm 0.46
	Non-MetS	1.4 \pm 0.29	1.1 \pm 0.26	0.99 \pm 0.23	1.1 \pm 0.53
Insulin (mU/l)	MetS	15 \pm 6.5	5.3 \pm 1.9	3.7 \pm 1.4	6.2 \pm 3.7
	Non-MetS	17 \pm 8.4	8.8 \pm 5.0	5.0 \pm 2.7	6.4 \pm 3.8
hs-CRP (mg/l)	MetS	5.0 \pm 4.2	5.3 \pm 6.6	2.4 \pm 1.7	2.5 \pm 2.6
	Non-MetS	5.5 \pm 7.3	3.9 \pm 3.9	2.5 \pm 1.4	2.4 \pm 2.0

syndrome was diagnosed according to the World Health Organization (WHO) definition [23], in which there is a requirement for type 2 diabetes, insulin resistance or impaired glucose tolerance, and two of the following conditions: hypertension, dyslipidaemia, central obesity or microalbuminuria.

For the real-time PCR study, abdominal sc adipose tissue biopsies were obtained from 4 obese men and 8 obese women without the metabolic syndrome and 5 obese men and 7 obese women with the metabolic syndrome. For the details of the study, see the previous report by Torgerson *et al.* [24]. The patient characteristics for the subjects included in this study are shown in Tab. 2. The ethical review board at the University of Gothenburg approved all of the studies, and all participants gave their written informed consent.

Tab. 2. Subjects included in the real-time PCR analysis of AKR1C3 (mean \pm SD), n = 12 except for HDL in the Metabolic syndrome group where n = 11.

	Healthy	Metabolic syndrome
Gender (male/female)	4/8	5/7
Age	47.4 \pm 9.7	49.5 \pm 7.6
BMI (kg/m ²)	41.0 \pm 5.4	42.5 \pm 7.3
Waist	119 \pm 15	127 \pm 17.
WHR	0.96 \pm 0.12	1.04 \pm 0.06
Fasting glucose (mmol/l)	3.9 \pm 0.6	8.4 \pm 2.7
Fasting insulin (mU/l)	23.7 \pm 14.9	30.0 \pm 13.5
Systolic BP (mmHg)	130 \pm 16	134 \pm 18
Diastolic BP (mmHg)	82 \pm 11	83 \pm 14
Cholesterol (mmol/l)	5.7 \pm 1.5	5.6 \pm 0.6
HDL Cholesterol (mmol/l)	1.3 \pm 0.4	1.1 \pm 0.2
Triglycerides (mmol/l)	1.9 \pm 0.7	2.4 \pm 1.4

Tissue distribution analysis

AKR1C3 gene expression in different human tissues was assessed using DNA microarray expression profiles. Affymetrix U133 Plus 2.0 DNA microarray expression profiles were downloaded from the Gene Expression Omnibus database (GEO accession No. GSE3526; <http://www.ncbi.nlm.nih.gov/geo/>). This dataset contains 353 expression profiles from 65 human tissues (3-9 replicates from different individuals per tissue). AKR1C3 expression was investigated using the 209160_at probe-set.

RNA isolation

Total RNA from the adipose tissue for the DNA microarray diet study and from isolated adipocytes was isolated using the RNeasy Lipid Tissue kit (Qiagen, Hilden, Germany). Total RNA from the adipose tissue for the real-time PCR study was prepared with the phenol-chloroform extraction method of Chomczynski and Sacchi [25] and the RNeasy Lipid Tissue kit (Qiagen).

DNA microarray analysis

For the diet and adipocyte size studies, cRNA was prepared and hybridized as recommended in the Affymetrix Gene Chip Expression Analysis manual. Briefly, biotin-labeled target cRNA was prepared by *in vitro* transcription (Enzo Diagnostics, Farmingdale, NY, USA) and hybridized to Human Genome U133A DNA microarrays (Affymetrix, Santa Clara, CA, USA) according to the Minimum Information About a Microarray Experiment (MIAME) guideline [26]. DNA microarrays were scanned with a confocal laser scanner (GeneArray scanner36 G2500A Hewlett Packard, Palo Alto, CA, USA). In the diet study, the scanned output files were analyzed with Affymetrix Microarray Suite Version 5.0 (MAS 5.0) software as previously described [27]. The raw data was globally scaled to an arbitrary target intensity value of 100.

For the adipocyte size study, DNA microarray data was analyzed using Weighted Analysis of Microarray Experiments (WAME) as previously described [28]. AKR1C3 expression was analyzed using the 209160_at probe-set.

RT-PCR analysis

Reagents for the real-time PCR analysis of AKR1C3 (Hs00366267_m1) and LRP10 (Assays-on-Demand, TaqMan Reverse Transcriptase reagents, and TaqMan Universal PCR Master mix) were purchased from Applied Biosystems (Foster City, CA, USA). The conditions used were as per the manufacturer's protocol. cDNA synthesis was performed in a total reaction volume of 50 μ l using 1000 ng total RNA. cDNA corresponding to 10 ng RNA per reaction was used for real-time PCR amplification. The amplification and detection of specific products was performed with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) using the default cycle parameters. A standard curve was plotted for each primer-probe set with a serial dilution of pooled adipose tissue cDNA. Human LRP10 [29] was used as a reference to normalize the expression levels between the samples. All the standards and samples were analyzed in triplicate.

Statistical analysis

The values are expressed as means \pm SEM, unless stated otherwise. The statistical analyses were performed using the SPSS package (version 13.0; SPSS, Chicago, IL, USA). Correlations between AKR1C3 expression and anthropometric and biochemical markers were performed using the Spearman rank correlation test. Differences in the AKR1C3 expression levels between the healthy subjects and subjects with the metabolic syndrome were analyzed using the Mann-Whitney U test. For the diet-induced weight loss study, differences in the AKR1C3 expression levels between different weeks were analyzed using the Friedman test followed by Wilcoxon's signed ranks test.

RESULTS

AKR1C3 expression is reduced during diet-induced weight loss

Weight-loss is associated with an improvement in the metabolic parameters [1]. Obese subjects were on a 16-week VLCD (450 kcal/day) followed by two weeks of gradual reintroduction of normal food. The average weight loss was 27 kg. This diet-induced weight loss resulted in a reduced expression of AKR1C3 in the sc adipose tissue after 8 weeks and 16 weeks of VLCD compared to the baseline levels ($p < 0.001$ and $p < 0.001$, respectively; Fig. 1). The AKR1C3 expression levels also remained significantly reduced after the two weeks (week 18) of gradual normal food reintroduction ($p < 0.001$; Fig. 1), as determined by DNA microarray analysis.

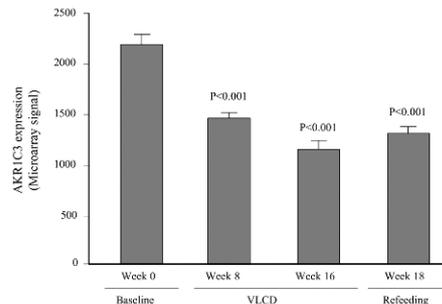


Fig. 1. The expression of AKR1C3 in sc adipose tissue during diet-induced weight-loss analyzed using DNA microarrays. Obese subjects ($n = 24$) were put on a VLCD for 16 weeks followed by a two-week gradual re-introduction of a normal diet. The data is presented as means \pm SEM.

Subjects with the metabolic syndrome have lower expression levels of AKR1C3

We examined whether AKR1C3 expression in sc adipose tissue is altered in obese subjects with the metabolic syndrome compared with healthy obese subjects without the metabolic syndrome. The metabolic syndrome subjects in the diet-induced weight-loss study displayed a tendency towards a lower AKR1C3 expression compared to healthy obese subjects at baseline when analyzed by DNA microarray ($p = 0.078$, Fig. 2A). Significantly lower levels of AKR1C3 expression in the adipose tissue from obese subjects with the metabolic syndrome were observed when a second group of subjects was analyzed using real-time PCR ($p = 0.039$, Fig. 2B).

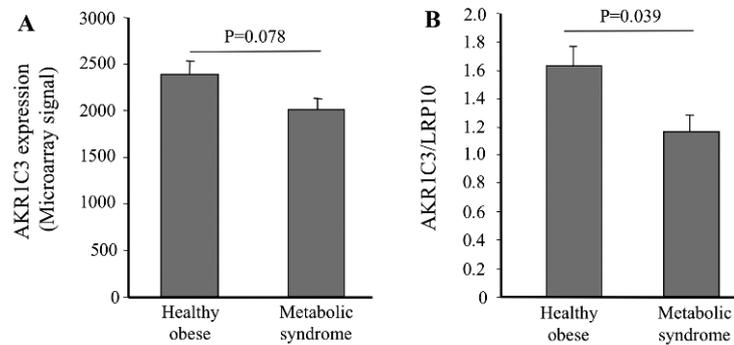


Fig. 2. AKR1C3 expression in sc adipose tissue in obese subjects with or without the metabolic syndrome. AKR1C3 expression was analyzed in two groups of subjects using DNA microarray (A) and RT-PCR (B). The data is presented as means \pm SEM.

Correlations between AKR1C3 expression and metabolic parameters

To further investigate the potential link between adipose AKR1C3 and metabolic disease, baseline AKR1C3 expression levels in the subjects participating in the dietary study were correlated to anthropometric and biochemical parameters relevant to the metabolic syndrome (Tab. 1). There was no correlation of baseline AKR1C3 gene expression with fasting levels of insulin or glucose, the homeostasis model assessment of insulin resistance (HOMA), triglyceride levels, high density lipoprotein (HDL) cholesterol levels and total cholesterol levels, body mass index (BMI) or waist-to-hip ratio (WHR).

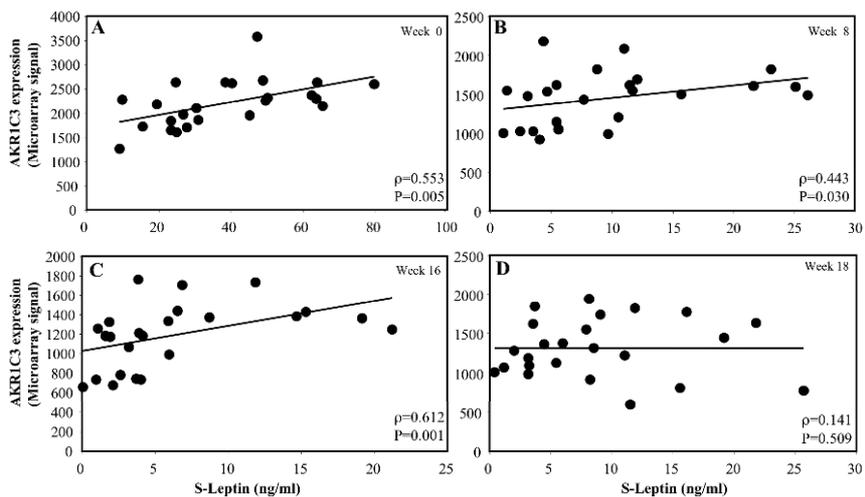


Fig. 3. Correlations between serum leptin levels and AKR1C3 expression in sc adipose tissue in 24 obese subjects who were on a VLCD. Scatter plots of serum leptin levels and AKR1C3 expression before (A – week 0), during (B – week 8, C – week 16) and after (D – week 18) the VLCD.

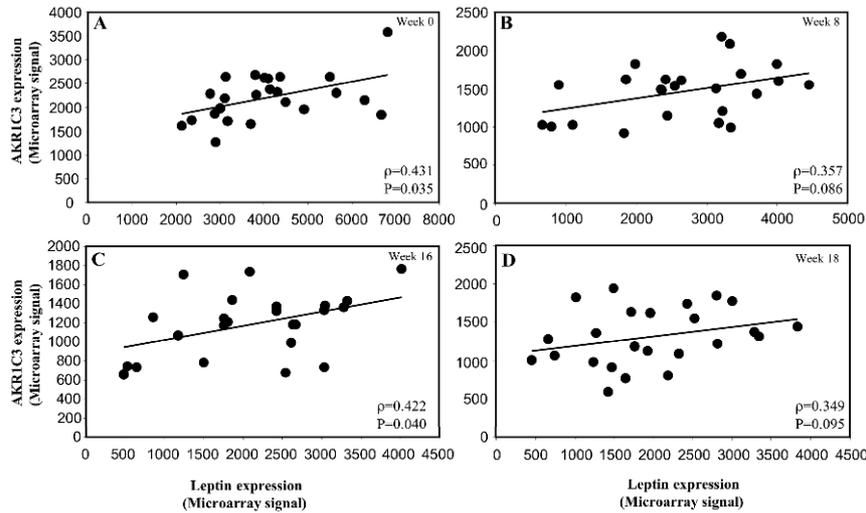


Fig. 4. Correlations between leptin expression and AKR1C3 expression levels in sc adipose tissue in 24 obese subjects who were on a VLCD. Scatter plots of leptin expression and AKR1C3 expression levels before (A – week 0), during (B – week 8, C – week 16) and after (D – week 18) the VLCD.

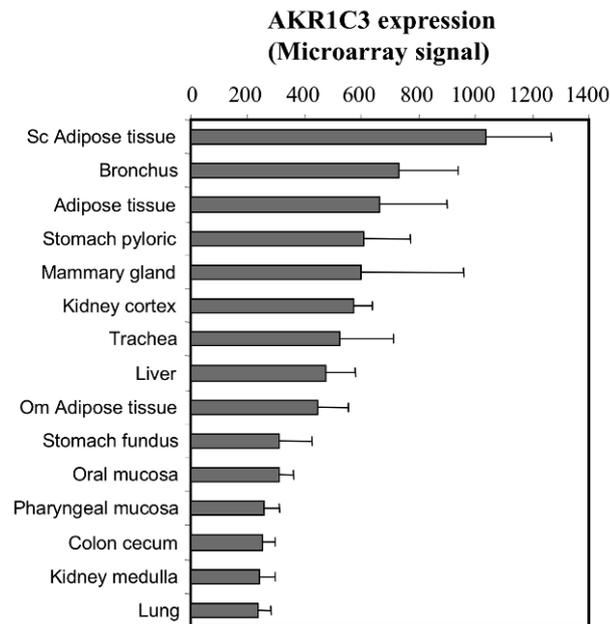


Fig. 5. The tissue distribution of human AKR1C3 expression. Public expression profiles from 65 human tissues were downloaded from the Gene Expression omnibus database (GEO accession No. GSE3526; <http://www.ncbi.nlm.nih.gov/geo/>). The figure illustrates the 15 tissues with the highest AKR1C3 expression as determined by DNA microarray analysis. The data is presented as means \pm SEM.

However, there was a positive correlation between AKR1C3 expression and the serum levels of leptin at baseline and at all time points during diet-induced weight loss ($p = 0.005$, $p = 0.030$ and $p = 0.001$, respectively, Fig. 3A-C). However, no correlation was observed after the reintroduction of a normal diet (week 18, Fig. 3D). There was also a correlation between adipose tissue AKR1C3 expression and leptin expression levels at baseline and at week 16 ($p = 0.035$ and $p = 0.04$, respectively, Fig. 4A and C), but only a borderline significant correlation at weeks 8 and 18 (Fig. 4B and D).

AKR1C3 is highly expressed in the sc adipose tissue depot

Using publicly available DNA microarray profiles from 65 human tissues (GEO accession No. GSE3526; <http://www.ncbi.nlm.nih.gov/geo/>), we analyzed the tissue distribution of AKR1C3 expression. Sc adipose tissue displayed the highest expression level of AKR1C3 for all the tissues included in the analysis. The tissues with the top 15 expression levels are shown in Fig. 5. As in previous studies [12-15], omental adipose tissue displayed a lower expression level than sc adipose tissue.

AKR1C3 expression in isolated human adipocytes

Increased adipose tissue mass and fat cell size are associated with the development of metabolic disease [4, 30]. We used microarray expression profiles to investigate AKR1C3 expression in size-separated human adipocytes derived from the same adipose tissue biopsies [28]. The analysis showed that large adipocytes have significantly higher expression levels of AKR1C3 than small adipocytes from the same subject (1.5-fold, $p = 0.009$, data not shown).

Type 2 diabetes, impaired glucose tolerance or insulin resistance is a requirement of the WHO definition for the metabolic syndrome [23]. To further investigate a possible link between glucose intolerance and AKR1C3, we analyzed AKR1C3 expression in isolated adipocytes treated with 1 mU/ml of insulin for 6 h. However, no significant alteration of AKR1C3 expression was observed (data not shown).

DISCUSSION

This study showed that the AKR1C3 mRNA expression levels in sc adipose tissue are lower in obese subjects suffering from metabolic complications, and that AKR1C3 gene expression was reduced during diet-induced weight loss. Furthermore, AKR1C3 expression correlated with leptin levels and expression was higher in enlarged adipocytes.

AKR1C3 expression in adipose tissue has previously mainly been investigated in relation to its role in steroid hormone conversion [12, 13, 15, 31]. However, to the best of our knowledge, a direct link between adipose tissue AKR1C3 expression and the metabolic syndrome has not been investigated. The putative role of adipose tissue AKR1C3 in the metabolic syndrome may be related to its suppressive effects on PPAR γ activation [14, 18]. The key function of AKR1C3

in the reduction of PPAR γ agonist (15d-PGJ₂) availability was elegantly demonstrated in a macrophage-like cell line (HL60), where the overexpression of AKR1C3 promoted phenotypic changes similar to those observed in HL60 cells exposed to PPAR γ antagonists [18]. In addition, AKR1C3 can affect PPAR γ activation, not only by reducing agonist formation, but also by directly increasing the level of PGF_{2 α} , which is a PPAR γ antagonist [32]. Quinkler *et al.* recently showed that all the enzymes required for the synthesis of the endogenous ligands (15d-PGJ₂) for PPAR γ are expressed in human adipose tissue. They suggested that the higher expression in omental adipose tissue may be involved in some of the observed depot-specific differences in adipocyte differentiation [14].

The interpretation of the reduced AKR1C3 expression in adipose tissue from subjects with the metabolic syndrome is unclear, but it may be viewed as a secondary compensatory mechanism to increase PPAR γ ligand availability thereby reducing insulin resistance. However, reduced AKR1C3 expression may also lead to reduced androgen generation in sc adipose tissue in subjects with the metabolic syndrome [15]. This is in line with studies showing that low androgen levels are associated with components of the metabolic syndrome, such as type 2 diabetes [33] and visceral obesity [34]. However, the alternate definitions for the metabolic syndrome contain different criteria for the assessment of glucose intolerance, adiposity, dyslipidaemia and hypertension [35]. That we were unable to detect a correlation between AKR1C3 expression and any of the metabolic parameters analyzed argues against a major role for it in metabolic disease. Larger study cohorts are needed to precisely define the potential impact of glucose levels, dyslipidaemia and hypertension on the regulation of AKR1C3 expression and to assess whether AKR1C3 has a role in metabolic disease or whether marginal changes in expression are due to compensatory or secondary effects of metabolic disease.

Obesity, and central obesity in particular, is one of the key components of the metabolic syndrome. Previous studies have found an association of AKR1C3 expression in adipose tissue with BMI [15] and waist-to-hip ratio [31]. We could not verify an association between the AKR1C3 gene expression in adipose tissue and BMI in our diet-induced weight loss study, but we did find that AKR1C3 expression in sc adipose tissue was positively correlated with serum leptin levels, which supports an association with adiposity. There may also be an indirect effect of high AKR1C3 expression on leptin levels, as a recent study showed that PGJ₂ inhibits leptin expression and secretion in 3T3-L1 adipocytes. Hence, high levels of AKR1C3 would reduce levels of PGJ₂, and this would ultimately lead to a reduced suppression of leptin levels [36], which could be relevant to our observation of a weaker positive correlation between leptin and AKR1C3 mRNA levels in sc adipose tissue. The reason why we do not see a correlation between AKR1C3 expression and BMI in our study is not clear, but may be explained by the fact that the included subjects were leaner (median BMI 28 kg/m² and mean BMI 26 kg/m², respectively) in the other two studies

compared to our subjects (mean BMI ≥ 41 kg/m² in both groups). The narrower BMI range in our study cohort could also explain the lack of an observed correlation. Other potential explanations for this lack of correlation between AKR1C3 expression and the measurements of adiposity could be an insufficient number of subjects in our study or experimental variability in the expression analysis.

In a previous study, Quinkler *et al.*, showed that AKR1C3 expression in gluteal adipose tissue from women was reduced after a ten-week VLCD regime [15]. Our study supports and expands on this finding, using sc abdominal adipose tissue from both men and women. The unaltered AKR1C3 expression observed during the reintroduction of normal food (weeks 16-18) indicates that AKR1C3 expression is related to adiposity *per se* rather than to the reduced caloric intake during the diet-regime. This data in combination with the data showing the association of AKR1C3 expression with measurements of adiposity [15, 31] indicates that altered AKR1C3 expression is a consequence of obesity rather than a cause of obesity. Decreased AKR1C3 expression during weight-loss could result in increased levels of PPAR γ ligands and thus may partially mediate the increased adipose tissue insulin sensitivity observed with weight loss.

Increased mean adipocyte size has been shown to be associated with adiposity and insulin resistance [37] and has been identified as an independent predictor of type 2 diabetes in Pima Indians [30]. One finding of our study is that large adipocytes have a 1.5-fold higher AKR1C3 expression than small adipocytes isolated from sc adipose tissue biopsies from the same subject. This may be interpreted as reduced levels of PPAR γ ligands and possibly reduced insulin sensitivity in enlarged adipocytes. There is an apparent paradox in the finding that enlarged adipocytes are associated with insulin resistance and our findings that subjects with the metabolic syndrome, as well as impaired glucose tolerance, show lower sc adipose tissue AKR1C3 gene expression. However, it was recently shown that insulin-resistant subjects had a higher ratio of small to large sc abdominal adipocytes, and this was associated with a lower expression of adipocyte differentiation markers in the adipose tissue [38]. The authors proposed that insulin resistance is associated with an inability of adipose tissue to store excess energy because small adipocytes cannot fully differentiate into mature fat cells [38]. Hence, if AKR1C3 is primarily expressed in fully matured adipocytes, the lower expression in adipose tissue from obese subjects with the metabolic syndrome could reflect a larger proportion of immature adipocytes in the sc adipose tissue.

Several studies have reported a depot difference in AKR1C3 expression, with sc adipose tissue displaying the highest expression [12-15]. Our study expands on these findings by showing that AKR1C3 has the highest expression levels in sc adipose tissue when compared with 64 other human tissues. Quinkler *et al.* have previously shown that AKR1C3 expression is higher in the human liver compared to sc adipose tissue [15]. The observed differences may be due to

different expression analysis techniques and the target sequence for the analysis (microarray compared to real time PCR).

The multiple enzymatic functions of AKR1C3 are challenging when trying to understand its role in the adipose tissue and adipocytes. Most of the information on AKR1C3 in the adipose tissue is currently limited to data from expression analyses. Further analyses on AKR1C3 protein expression and function in PPAR γ ligand formation are needed to increase our understanding of the role of AKR1C3 in the development of obesity and metabolic disease.

In conclusion, this study shows that AKR1C3 expression in adipose tissue is decreased in subjects with the metabolic syndrome and that the expression in sc abdominal adipose tissue is downregulated during diet-induced weight-loss. Furthermore, AKR1C3 expression levels correlated with leptin levels, and the expression was higher in enlarged adipocytes.

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