0021-972X/05/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 90(4):2233–2239

doi: 10.1210/jc.2004-1830

A Microarray Search for Genes Predominantly Expressed in Human Omental Adipocytes: Adipose Tissue as a Major Production Site of Serum Amyloid A

Kajsa Sjöholm, Jenny Palming, Louise E. Olofsson, Anders Gummesson, Per-Arne Svensson, Theodore C. Lystig, Eva Jennische, John Brandberg, Jarl S. Torgerson, Björn Carlsson, and Lena M. S. Carlsson

Research Centre for Endocrinology and Metabolism (K.S., J.P., L.E.O., P.-A.S., B.C., L.M.S.C.), Division of Body Composition and Metabolism (A.G., T.C.L., J.B., J.S.T., B.C.), Department of Internal Medicine, Department of Radiology (J.B.), Sahlgrenska University Hospital, and Institute of Anatomy and Cell Biology (E.J.), The Sahlgrenska Academy at Göteborg University, SE-413 45 Göteborg, Sweden

To identify genes predominantly expressed in omental adipocytes, microarray expression profiles from 33 human tissues or cell types were analyzed, using an algorithm developed for identification of transcripts predominantly expressed in a certain tissue. Both known adipocyte-specific and more unexpected genes were among the 28 genes identified. To validate the approach, adipocyte expression of three of these genes, acute-phase serum amyloid A (A-SAA), aquaporin 7, and transport secretion protein-2.2, was compared with 17 other human tissues by real-time PCR. The unexpectedly high expression of A-SAA in adipocytes was further verified by Northern blot and immunohistochemistry. The liver, reported to be the main production site for A-SAA, displayed the second highest expression using microarray and real-time PCR. In obese subjects, adipose tissue mRNA and serum A-SAA levels were down-regulated during an 18-wk diet regime ($P < 0.05$ and $P < 0.0001$, respectively). A-SAA serum levels were highly correlated to adipose tissue mRNA levels ($P < 0.001$) and to the total ($P < 0.0001$) and sc ($P < 0.0001$) adipose tissue areas, as analyzed by computed tomography.

We show that adipose tissue is a major expression site of A-SAA during the nonacute-phase reaction condition. This provides a direct link between adipose tissue mass and a marker for low-grade inflammation and cardiovascular risk. (*J Clin Endocrinol Metab* 90: 2233–2239, 2005)

IN ADDITION TO its role in energy storage, it has been established that the adipose tissue is an endocrine organ that produces and secretes adipose tissue-specific or enriched hormones, known as adipokines. Such proteins have a variety of local, peripheral, and central effects and may be of importance for the development of obesity and obesity-associated diseases. Many adipokines have been described, and their potential role in obesity is under investigation (reviewed in Ref. 1). However, some key genes, not known to have high expression in adipocytes, may remain to be discovered.

Expression profiling by microarray has emerged as a powerful tool when screening for regulated genes (2–5). Another application for expression profiling is the identification of tissue- or cell-specific genes (6). This approach demands a high number of expression profiles from different tissues and cells to obtain a reliable analysis. Due to the specialized function of adipose tissue, it can be assumed that adipocytes express a specific set of genes that other types of cells do not express or only express at low levels. It is possible that the identification of genes predominantly expressed in adipocytes can give new insights into adipocyte function and may thereby provide important information about genes involved in the development of obesity and obesity-associated metabolic disease. Several studies have shown that central obesity, in particular an increase in visceral fat mass, is tightly linked to components of the metabolic syndrome (7). The aim of this study was to search for genes that are predominantly expressed in omental adipocytes. The search was performed in gene expression profiles from human omental adipocytes and 32 other human tissues and cells (2–4, 6, 8).

We here report that adipose tissue is a major production site of acute-phase serum amyloid A (A-SAA), a known risk factor for coronary artery disease (9, 10). Therefore, our results provide additional support for an important role of adipose tissue and obesity in the development of cardiovascular disease.

First Published Online December 28, 2004

Abbreviations: ACTB, β-Actin; AD, average difference; AQP7, aquaporin 7; A-SAA, acute-phase serum amyloid A; BMI, body mass index; CRP, C-reactive protein; CT, computed tomography; hs-CRP, high sensitive CRP; PPIA, peptidyl-prolyl isomerase A; RPLP0, large ribosomal protein P0; TTS-2.2, transport secretion protein-2.2; VLCD, very-low-calorie diet.

Subjects and Methods

Subjects and samples

Adipose tissue biopsies from the abdominal sc depot and the major omentum were obtained from 10 obese men (2) and four obese women undergoing laparoscopic bariatric surgery. Adipocytes were prepared from sc and omental adipose tissue from three of the obese men (2) using the procedure outlined by Smith *et al.* (11). A healthy liver sample was used for immunohistochemistry. Abdominal sc adipose tissue biopsies

and fasting blood samples were obtained from obese women and men before (wk 0), during (wk 8 and 16), and after (wk 18) diet-induced weight loss using a very-low-calorie diet (VLCD, 450 kcal/d). Subjects were treated with VLCD for 16 wk and then a normal diet was gradually reintroduced. The adipose tissue RNA content was analyzed using microarray and/or real-time PCR. Adipose tissue from one female subject undergoing abdominal surgery was used for Northern blot analysis of A-SAA expression in adipose tissue and adipocytes. Monocytes from healthy blood donors were prepared and differentiated into macrophages as previously described (12). Pooled macrophage RNA was used for microarray analysis as well as for verification with real-time PCR. RNA pools from nasal mucosa biopsies (3), as well as T cells (4), were analyzed by microarray. The Medical Ethics Committee at Göteborg University approved all studies, and all participants gave written informed consent. RNA from the Human Total RNA Master Panel II (Clontech, Palo Alto, CA) was used for verification of the gene expression in different human tissues by real-time PCR. Liver RNA from the RNA panel was used for Northern blot analysis of A-SAA expression.

Measurements

Body composition was determined with computed tomography (CT) before (wk 0) and during (wk 16) the VLCD treatment, using the same parameters as previously described (2). Total, sc, and visceral adipose tissue areas, from the CT scan at the lumbar 4 vertebrae, were used for correlations. The serum A-SAA concentration was measured in duplicate using an ELISA (Biosource International, Camarillo, CA). Other blood chemistry analyses [high sensitive C-reactive protein (hs-CRP), high-density lipoprotein (HDL), and insulin] were performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital. Patient characteristics used for correlations are stated in Table 1 (adipose tissue samples from these patients were analyzed using U133A microarrays; Affymetrix, Santa Clara, CA).

Microarray expression profiles

Hu95A microarray (Affymetrix) hybridization to RNA from omental adipocytes, sc and omental adipose tissue, nasal mucosa, T cells, and macrophages was performed as previously described (2–4). RNA from sc adipose tissue biopsies before, during, and after VLCD treatment was hybridized to U133A microarrays (Affymetrix). Hu95A expression profiles from fetal brain, whole brain, cortex, caudate nucleus, amygdala, thalamus, corpus callosum, pituitary gland, cerebellum, spinal cord, dorsal root ganglia, whole blood, testis, prostate, ovary, uterus, salivary gland, trachea, lung, thymus, spleen, adrenal gland, pancreas, thyroid, kidney, fetal liver, liver, and heart were obtained from Su *et al.* (http://expression.gnf.org; Ref. 6). The expression profile from normal muscle generated by Bakay *et al.* (8) was obtained from http://microarray.cnmcresearch.org. The Hu95A microarrays of sc and omental adipose tissue as well as omental adipocytes, nasal mucosa, T cells, and macrophages were scaled to an average intensity of 200, the same intensity as the public expression profiles. All VLCD samples were analyzed with wk 0 as baseline. An average difference (AD) was calculated for the replicates of each tissue or cell type and used for all analysis.

Data analysis for Hu95A microarrays

Genes having an AD of less than 20 were given an AD of 20 (13). To identify genes predominantly expressed in adipocytes, two criteria were used: 1) the genes should have an AD of 10 SD higher than the mean AD of all the other tissues, and 2) the genes should have at least a 3-fold higher expression level than the tissue with the second highest AD. These two criteria allow us to identify genes that have an expression level that is clearly separated from both the average expression level of all the tissues, but also from any other highly expressing tissue or cell type (14).

Real-time PCR analysis

Reagents for real-time PCR analysis of aquaporin 7 (AQP7), transport secretion protein-2.2 (TTS-2.2), peptidyl-prolyl isomerase A (PPIA), and large ribosomal protein P0 (RPLP0) (Assays-on-Demand, TaqMan Reverse Transcriptase reagents, and TaqMan Universal PCR Master mix) were purchased from Applied Biosystems (Foster City, CA) and the conditions used were according to the manufacturer's protocol. The high sequence similarity between the SAA1 and SAA2 isoforms made it impossible to study these isoforms separately. Consequently, probe and primer sequences for A-SAA (Cybergene, Huddinge, Sweden) were designed to span an exon-exon boundary and detect both the SAA1 and SAA2 isoforms, denoted A-SAA (sequences are available upon request). cDNA synthesis was performed in a total reaction volume of 50 μl using 500 ng total RNA. cDNA corresponding to 10 ng RNA per reaction was used for real-time PCR amplification. Amplification and detection of specific products was performed with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) using default cycle parameters. A standard curve was plotted for each primer-probe set with a serial dilution of pooled adipose tissue cDNA. Human PPIA or RPLP0 was used as reference to normalize the expression levels between samples. All standards and samples were analyzed in triplicate.

RT-PCR and Northern blot

RT-PCR amplification of A-SAA (primer sequences are available upon request) and SAA3 (primers as previously described in Ref. 15) was performed. The PCRs were purified using the Qiaquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced using the BigDye Terminator v3.1 cycle sequencing kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). The A-SAA fragment was subcloned into the pCRII vector (Invitrogen, San Diego, CA). β-Actin (ACTB) (Ambion, Austin, TX) was used as an internal control. Northern blot analysis was performed using total RNA (5 μg) from liver, adipocytes, and adipose tissue. Membranes were probed with ^{32}P-labeled antisense A-SAA and ACTB RNA.

TABLE 1. Characteristics of the patients included in the VLCD study

	wk 0	wk 8	wk 16	wk 18	*P* value
N (women/men)	6/34	5/26	5/24	5/24	
BMI (kg/m^2)	37.0 ± 4.3	31.0 ± 3.8	27.9 ± 3.8	28.3 ± 3.6	<0.0001
WHR	1.02 ± 0.07	0.97 ± 0.07	0.94 ± 0.08	0.94 ± 0.08	<0.0001
Waist (cm)	121.0 ± 11.3	107.8 ± 12.7	98.0 ± 12.8	98.2 ± 12.3	<0.0001
TAT (cm^2)	725 ± 176		392 ± 158		<0.0001
VAT (cm^2)	227 ± 78		90 ± 45		<0.0001
SAT (cm^2)	490 ± 159		296 ± 124		<0.0001
A-SAA (μg/ml)	31.1 ± 47.1	15.2 ± 23.0	10.6 ± 7.5	13.5 ± 10.5	<0.0001
Hs-CRP (mg/liter)	4.5 ± 4.9	3.3 ± 3.4	2.4 ± 1.4	2.2 ± 2.2	<0.001
HDL (mmol/liter)	1.3 ± 0.3	1.2 ± 0.3	1.4 ± 0.3	1.4 ± 0.2	n.s.
Insulin (mU/liter)	17.3 ± 8.1	6.6 ± 3.9	4.3 ± 2.2	6.7 ± 3.9	<0.0001

Serum samples were obtained before, during, and after VLCD. CT scans performed at wk 0 and wk 16 were used for adipose tissue area calculations. Serum A-SAA, hs-CRP, HDL, and insulin were measured during VLCD. A *P* value for down- or up- (HDL) regulation during the diet was calculated for each parameter. TAT, Total adipose tissue; VAT, visceral adipose tissue; SAT, sc adipose tissue; n.s., not significant. Mean ± SD.

Immunohistochemistry

Omental and sc adipocytes were fixed in buffered paraformaldehyde (4%), embedded in agar (3%), dehydrated in a graded series of ethanol, and embedded in paraffin using standard procedures. Biopsy sections were prepared as previously described (2). SAA1 was localized by incubation with a monoclonal antibody to human SAA1 (HyCult Biotechnology, Uden, The Netherlands) as previously described (2).

Statistical analysis

All analyses were initially performed with standard linear regression. Correlation of within-person longitudinal measurements was addressed with the use of generalized estimating equations (16). Tests of parameters within these models were performed with generalized Wald tests (17), which yielded χ^2 statistics. Due to the skewed nature of the measurements, logarithmic transformations were applied to the various A-SAA variables and predictors. For each gene analyzed in Fig. 1, the full model considered had main effects for both depot and gender, as well as an interaction term for potential gender-specific depot effects. In the tests for depot effect, the null hypothesis was that the measurements differed only by gender. In the tests for gender effect, the null hypothesis was that the measurements differed only by depot type. Under each null hypothesis, the test statistic was distributed as χ^2 with two degrees of freedom. An exact binomial test was used to assess down- (or up-) regulation. In the absence of down-regulation, maximum levels are equally likely to occur at any time point. In the presence of down-regulation, maximum levels will have a disproportionately higher frequency at baseline.

Results

Identification of genes predominantly expressed in omental adipocytes

Using microarray datasets from 33 human tissues and cells, 28 genes were identified as predominantly expressed in omental adipocytes (Table 2). Some of these genes were expected (*e.g.* adiponectin, adipose specific 2, hormone-sensitive lipase, AQP7), whereas others were more unexpected (*e.g.* A-SAA) or not well studied (*e.g.* TTS-2.2). Literature and database searches were performed to select genes for further validation. To validate the approach used in this study, we confirmed the predominant expression in omental

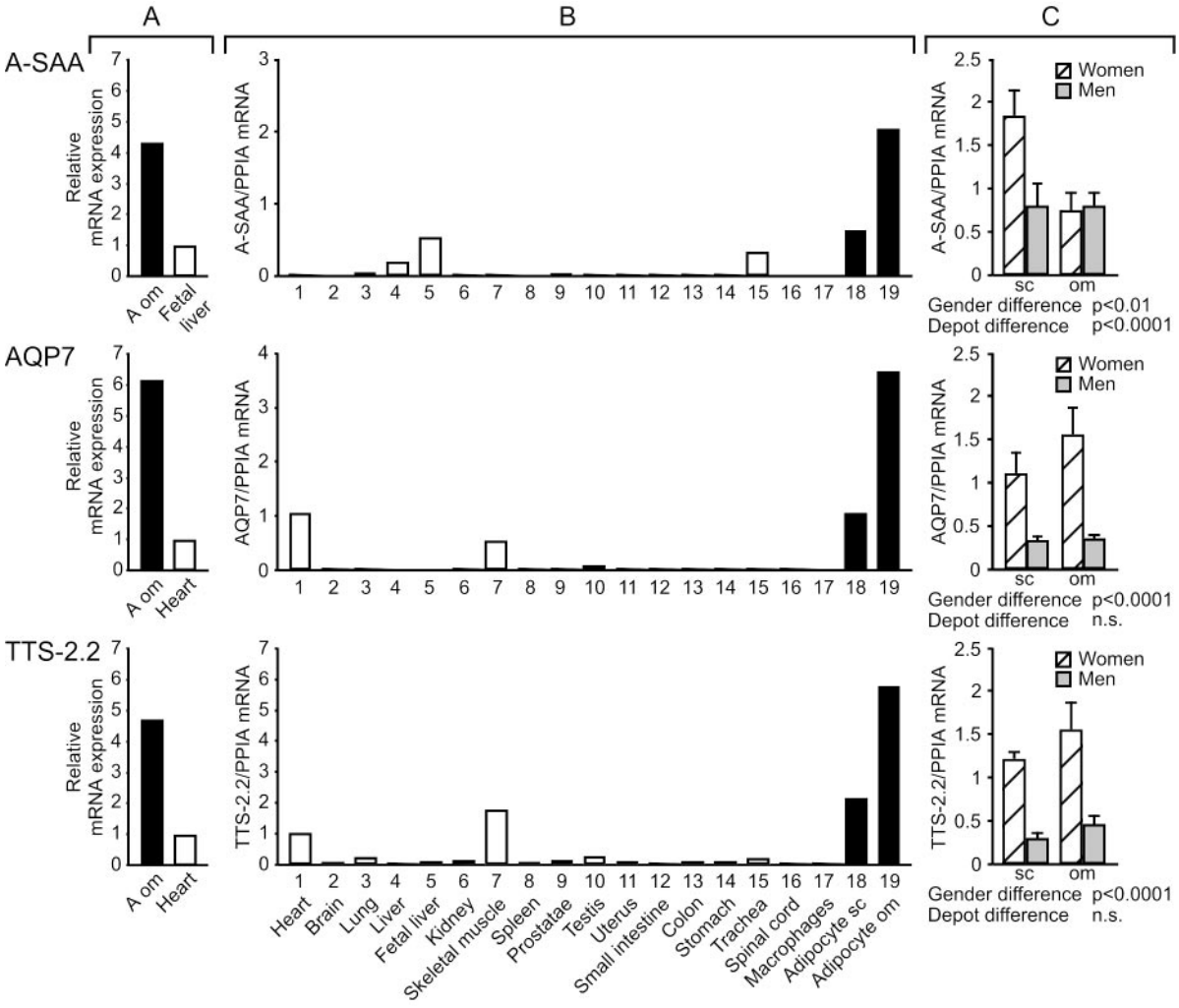

FIG. 1. Gene expression of A-SAA, AQP7, and TTS-2.2 as analyzed by microarray and real-time PCR. The relative expression for A-SAA, AQP7, and TTS-2.2 is shown for omental adipocytes (A om; *black bars*) and the tissue with second highest expression (*white bar*) (A). Expression of A-SAA, AQP7, and TTS-2.2 in 17 different tissues/cell types as well as sc (sc, n = 2) and omental adipocytes (*black bars*, n = 2) (B). Expression of A-SAA, AQP7, and TTS-2.2 in sc and omental adipose tissue in women (n = 4) and men (n = 7) (mean ± SEM) (C). *P* values for gender differences after depot adjustment and depot differences after gender adjustment for each gene are shown *below the graphs* (n.s., not significant). Expression of A-SAA, AQP7, and TTS-2.2 was normalized to PPIA in all samples when analyzed by real-time PCR.

TABLE 2. Genes predominantly expressed in human omental (om) adipocytes (expression profiles generated using U95A microarrays)

Gene symbol	Gene description	AD om adipocytes	Second tissue	AD in second tissue
ACACB	Acetyl-coenzyme A carboxylase β	1480	Liver	491
	Acetyl-coenzyme A carboxylase β	998	Kidney	110
AGPAT2	1-Acylglycerol-3-phosphate *O*-acyltransferase 2	9129	Liver	1750
AOC3	Amine oxidase, copper containing 3	6012	DRG	995
ACDC	Adipocyte, C1Q and collagen domain containing (adiponectin)	6438	DRG	2005
	Adipocyte, C1Q and collagen domain containing (adiponectin)	4163	DRG	1160
APM2	Adipose specific 2	13326	Prostate	2139
AQP7	Aquaporin 7	6239	Heart	619
	Aquaporin 7	7582	Heart	1625
CD36	CD36 antigen	535	Macrophages	84
C10orf10	Chromosome 10 open reading frame 10	4903	DRG	1618
DF	Complement factor D (adipsin)	20504	Heart	4050
DPT	Dermatopontin	4964	Uterus	1518
	Dermatopontin	2737	Uterus	596
FABP4	Fatty acid binding protein 4	23207	DRG	5407
FLJ32389	Hypothetical protein FLJ32389	1848	Heart	520
GPD1	Glycerol-3-phosphate dehydrogenase 1	7622	Muscle	1114
GYG2	Glycogenin 2	2039	Spinal cord	254
HRASLS3	HRAS-like suppressor 3	3501	Corpus callosum	1125
ITGB1BP1	Integrin β_1 binding protein 1	1637	Thalamus	247
	Integrin β_1 binding protein 1	3017	Fetal brain	524
ITSN1	Intersectin 1	2300	Ovary	666
LIPE	Hormone-sensitive lipase	12901	Corpus callosum	1070
	Hormone-sensitive lipase	2838	Not detectable	
NPR1	Natriuretic peptide receptor A	830	Spleen	173
NQO1	NAD(P)H dehydrogenase, quinone 1	4072	Trachea	676
PFKFB3	6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	1216	Salivary gland	346
PLIN	Perilipin	18271	DRG	3102
PPARG	Peroxisome proliferative activated receptor, γ	1492	Macrophages	328
PRKAR2B	Protein kinase, cAMP-dependent, regulatory, type II, β	2188	Fetal brain	701
PEX19	Peroxisomal biogenesis factor 19	1215	Macrophages	349
RDH5	Retinol dehydrogenase 5	4009	DRG	60
SAA1	Serum amyloid A1	19650	Fetal liver	4531
TTS-2.2	Transport secretion protein-2.2	11896	Heart	2519

The tissue with the second highest expression is indicated along with the expression value for that tissue. Some genes are represented by two probe sets. Values represent Affymetrix AD. DGR, Dorsal root ganglia.

adipocytes for AQP7, TTS-2.2, and A-SAA. In line with previous results (18), AQP7 was shown to be predominantly expressed in adipocytes as determined by real-time PCR analysis. Heart displayed the second highest expression in both the microarray and real-time PCR analysis (Fig. 1, A and B). The AQP7 expression levels in women were significantly higher than in men after adjusting for depot differences ($P < 0.0001$; Fig. 1C). However, no difference in AQP7 expression between sc and omental adipose tissue depots could be detected (Fig. 1C). TTS-2.2, which has recently been shown to catalyze the initial step in triglyceride hydrolysis (19), displayed very high expression levels in omental adipocytes, when analyzed by microarray. According to the microarray analysis, the second highest expression was found in heart (Fig. 1A). The TTS-2.2 expression level was three times higher in omental adipocytes as compared with the second highest expression level, detected in skeletal muscle as analyzed with real-time PCR, whereas heart showed the third highest expression (Fig. 1B). The TTS-2.2 expression levels were significantly higher in women compared with men after adjusting for depot differences ($P < 0.0001$; Fig. 1C). No difference in TTS-2.2 expression between the adipose tissue depots or during diet-induced weight loss (data not shown) could be detected.

A-SAA

Tissue panel and adipose tissue depot expression of A-SAA. Liver has previously been reported to be the main production site for A-SAA (reviewed in Refs. 20 and 21). However, analysis of the microarray datasets showed that A-SAA expression was even higher in human adipocytes, whereas fetal and adult liver displayed the second and third highest expression level (Fig. 1A). This finding was confirmed in two subjects using real-time PCR (Fig. 1B), showing that the omental adipocyte A-SAA expression was four times higher than the expression level in fetal liver and adult liver. There was a significant difference between expression levels both between the adipose tissue depots, where sc adipose tissue showed the highest expression, and gender, where women displayed the highest expression ($P < 0.0001$ and $P < 0.01$, respectively; Fig. 1C).

Northern blot, RT-PCR, and immunohistochemistry. A-SAA expression was also analyzed using Northern blot and RT-PCR. Northern blot confirmed a high expression of A-SAA in adipocytes and adipose tissue, but unexpectedly low signal was detected in human liver (Fig. 2A). Using RT-PCR, A-SAA expression was detected in adipocytes and adipose tissue, as well as in liver (data not shown). To confirm that

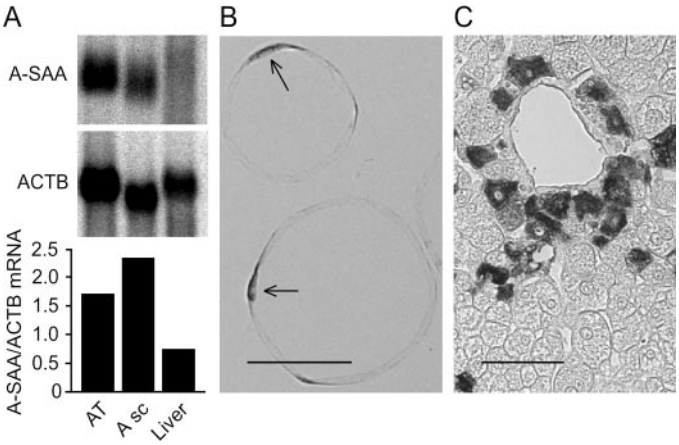

FIG. 2. Expression of A-SAA mRNA and protein in human adipose tissue and liver. Northern blot analysis of A-SAA expression in human adipose tissue (AT), sc adipocytes (A sc), and liver. The graph illustrates the A-SAA levels relative to ACTB expression as analyzed by densiometry (A). Paraffin sections of human omental adipocytes (B) and human liver (C) demonstrating SAA1 immunoreactivity. Positive signal appears *dark gray* (indicated by *arrows* in the adipocytes). *Bar*, 50 μm.

the amplified A-SAA fragment corresponds to that reported in GenBank, it was verified by sequencing. Immunohistochemistry showed that SAA1 is expressed also at the protein level in both human omental and sc adipocytes as well as in liver (Fig. 2, B and C). The SAA1 staining in adipocytes is focused around the nucleus, where most of the cytoplasm is gathered. In the liver, only some of the hepatocytes stained positive for SAA1, and the most intense staining was seen in the hepatocytes around the central venules.

The SAA family is comprised of four different isoforms (21). The SAA1 and SAA2 genes display a very high homology at both the RNA and protein levels, which makes it is difficult to distinguish between these two isoforms. SAA1 and SAA2 are refered to as "acute phase SAA," and these isoforms increase drastically in the circulation in response to infection. The third member, SAA3, has previously been detected in mouse adipose tissue (22, 23) and, therefore, we wanted to make sure that SAA3 expression did not affect the results of this study. Most studies refer to human SAA3 as a pseudogene (24), but in one study SAA3 has been detected in human mammary gland epithelial cells after lipopolysacharide or prolactin stimulation (15). We were not able to detect SAA3 in human adipose tissue, adipocytes, or liver using RT-PCR or real-time PCR, suggesting that this gene is not expressed in these tissues. SAA4 is constitutively expressed and referred to as a "housekeeping" gene (21). The mRNA levels of SAA4 were not regulated during VLCD, and the expression levels were much lower than the ones detected for A-SAA (data not shown).

mRNA and serum levels of A-SAA during diet-induced weight loss. Together with C-reactive protein (CRP), A-SAA has recently been recognized as a cardiovascular risk factor (9, 10, 25). Short-term studies on intentional weight loss have shown drastic improvement in all aspects of the metabolic syndrome. This, together with our observation that A-SAA is highly expressed in adipocytes, prompted us to examine the possible regulation of A-SAA during diet-induced weight loss. During VLCD, A-SAA mRNA levels were down-regulated, according to the microarray ($P < 0.05$; Fig. 3A). The A-SAA serum levels were also regulated in response to diet-induced weight loss ($P < 0.0001$; Fig. 3B), and correlated with A-SAA mRNA expression in sc adipose tissue ($P < 0.001$) as measured by microarray. The A-SAA mRNA expression was significantly higher in women compared with men as analyzed by microarray ($P < 0.0001$). The down-regulation of mRNA levels between baseline and wk 8 during VLCD was confirmed in a different population, using real-time PCR (n = 30, $P < 0.0001$; Fig. 3C).

Correlations to body composition and blood parameters during diet-induced weight loss. The A-SAA serum levels were highly correlated to total ($P < 0.0001$) and sc ($P < 0.0001$) abdominal adipose tissue area as well as waist/hip ratio ($P < 0.05$), waist ($P < 0.0001$), and body mass index (BMI; $P = 0.0001$) during

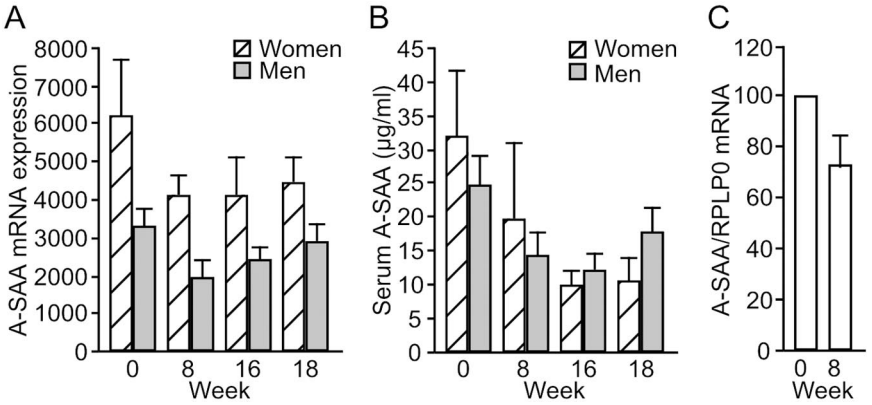

FIG. 3. Effect of diet-induced weight loss on A-SAA mRNA and serum levels. A-SAA gene expression in sc adipose tissue during VLCD according to the U133A microarray (A). Values correspond to the 214456_x_at probeset. The A-SAA expression was significantly decreased between wk 0 and wk 8; $P < 0.01$. The A-SAA expression levels in women were significantly higher compared with men; $P < 0.0001$ (n = 5 women, n = 16 men). Serum levels of SAA during VLCD (B) (n = 5–6 women, n = 24–34 men, see Table 2). A-SAA serum levels were down-regulated during VLCD ($P < 0.0001$; n = 5 women, n = 23 men). mRNA levels of A-SAA during VLCD as analyzed by real-time PCR (C). A-SAA mRNA levels were down-regulated between baseline and wk 8 ($P < 0.0001$; n = 30). Expression of A-SAA was normalized to RPLP0 when analyzed by real-time PCR.

diet-induced weight loss. The A-SAA serum levels were also correlated to hs-CRP ($P < 0.0001$) and fasting insulin levels ($P < 0.05$). No correlation was found between A-SAA serum levels and visceral adipose tissue or HDL levels.

Discussion

The metabolic syndrome, affecting one fourth of the adult U.S. population, is defined by a cluster of risk factors, predisposing for cardiovascular disease (26). Components of the metabolic syndrome, such as obesity and type 2 diabetes, are associated with a systemic increase in inflammatory markers (27, 28). Modestly increased levels of acute-phase proteins, including A-SAA and hs-CRP, are independent risk factors for coronary artery disease in both men and women (9, 10, 25). We demonstrate in this study that adipose tissue is a major site of A-SAA production. Because adipocytes can make a large contribution to whole body weight, particularly in obese individuals, it is possible that the contribution of adipose tissue to the amount of A-SAA present in the blood can be significant. This is supported by the strong correlation between A-SAA serum levels and BMI (10). In addition, we show that A-SAA serum levels are correlated to both adipose tissue mRNA levels and to total and sc abdominal adipose tissue in obese subjects during weight loss. Obesity is recognized as an important component of the metabolic syndrome, and research during the last decade has demonstrated that adipose tissue produces several hormones, including leptin and adiponectin that regulate key aspects of metabolism (1). It has also been suggested that A-SAA may exert metabolic functions (29). Interestingly, in addition to being expressed at very high levels in adipocytes we found that A-SAA expression was regulated in response to diet-induced weight loss.

Serum levels of CRP and A-SAA are highly correlated (28), and this correlation was confirmed also in our patient material. In contrast to A-SAA, we failed to detect CRP mRNA expression in adipose tissue. In a recent publication, obesity was shown to be the major determinant of elevated CRP in subjects with the metabolic syndrome (30). However, it has been proposed that the correlation between BMI and hs-CRP may be driven by adipose-derived cytokines, such as IL-6 (31). It should be emphasized that our data demonstrate that adipose tissue is a major site of A-SAA production under conditions that do not evoke a strong systemic acute-phase response. We show SAA1 immunoreactivity in the cytoplasm around the adipocyte nucleus. In the liver, SAA1 staining is concentrated to hepatocytes surrounding central venules. It may be speculated that the more than 1000-fold increase in serum A-SAA, seen during an infection, could be due to instant release of the A-SAA protein that has been stored in hepatocytes close to the central venules, whereas the more moderate increased A-SAA serum levels, seen in obese patients (10, 28), may be due to a constant secretion of A-SAA from the adipocytes.

The finding that adipose tissue expression of A-SAA was regulated in response to diet-induced weight loss raises the question of the biological role of A-SAA. Although most studies have focused on A-SAA as a marker of inflammation, there are indications that it may influence HDL function. During the acute-phase reaction, A-SAA rapidly associates with HDL, thereby displacing apolipoprotein A1 (20), which normally plays a central role in reverse cholesterol transport from peripheral cells to the liver. Altered activities of HDL-associated enzymes are probably responsible for the impaired antioxidative properties of HDL during the acute phase, suggesting that SAA-HDL is a proinflammatory molecule (32). It has also been speculated that increased levels of A-SAA are responsible for the lowered HDL levels seen during the acute-phase response (33). Therefore, A-SAA production in adipose tissue may provide at least a partial explanation for the low HDL-cholesterol levels frequently seen in obesity. Furthermore, it has been proposed that, in the mouse, A-SAA is a ligand of the cell surface receptor Tanis (29), which is expressed in rat liver and 3T3-L1 cells (29). Tanis displays homology with the human protein AD-015 (29), which we detected in human adipose tissue using real-time PCR (data not shown). Tanis has been suggested as a link between diabetes, inflammatory response, and cardiovascular disease, because it is regulated by glucose and the expression is altered in type 2 diabetes in an animal model (29). Furthermore, we found a significant correlation between SAA serum levels and fasting insulin levels in our patient material.

This report describes an approach for identification of genes predominantly expressed by human omental adipocytes, using microarray expression profiles from a large number of tissues and cells. Efforts have previously been made to identify genes specifically expressed in human adipose tissue. Maeda *et al.* (34) constructed an adipose tissue cDNA library and compared the expressed genes with those detected in cDNA libraries of 10 other tissues. The approach used in that study is limited by the number of clones that are analyzed and how well they represent the genes expressed in the specific tissue. However, microarray data, in combination with bioinformatic tools, can be used to more extensively and rapidly explore gene expression. In the future, the strategy used here can be applied to datasets from the new generation of microarrays containing all known genes present in the human genome. Hence, it is likely that the list of adipocyte-specific genes will grow. The simplicity of this approach makes it useful for detection of specific gene expression in any particular tissue or cell type of interest.

In the current study, several genes previously known to be expressed predominantly in human adipose tissue were identified, including PPAR-γ, adiponectin, adipose specific 2, adipsin, and AQP7 (1, 18, 34). To validate the approach used in this study, the expression of AQP7, was verified. The predominant expression of TTS-2.2, which has recently been shown to catalyze the initial step in triglyceride hydrolysis (19), was also verified. TTS-2.2 was first identified in a cDNA screening project (35), and the protein sequence displays similarities with adiponutrin, an adipose-specific gene that was recently discovered in mice (36, 37) and gene sequence 2 (37). Both the adiponutrin and TTS-2.2 genes are induced early in the differentiation of 3T3-L1 cells (19, 36, 37).

In conclusion, we show in this study that adipose tissue is a major site of production for A-SAA during conditions of the nonacute phase. Previous studies indicate that adipose tissue is an important source of proinflammatory cytokines. Taken together, this suggests that adipose tissue may contribute to the low-grade systemic inflammation seen in obesity. Be-

cause SAA-HDL has been proposed to be proatherogenic, the production of A-SAA in adipose tissue may be a link between obesity and atherosclerosis.

Acknowledgments

We thank members of Research Centre for Endocrinology and Metabolism II, Dr. Lars Lönn, and Dr. Lars Sjöström for fruitful discussions, and Margareta Jernås for running the microarrays. VLCD was provided by Cambridgekuren Sverige AB.

Received September 15, 2004. Accepted December 20, 2004.

Address all correspondence and requests for reprints to: Kajsa Sjöholm, Research Centre for Endocrinology and Metabolism, Vita Stråket 12, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden. E-mail: kajsa.sjoholm@medic.gu.se.

This work was supported by grants from The Swedish Research Council (11285, 529-2002-6671, and 521-2002-6536), IngaBritt och Arne Lundbergs forskningsstiftelse, Swegene, Fredrik och Ingrid Thurings foundation, Magn. Bergvalls foundation, the Tore Nilssons foundation, and the foundations of the National Board of Health and Welfare.

References

1. **Rajala MW, Scherer PE** 2003 Minireview: the adipocyte—at the crossroads of energy homeostasis, inflammation, and atherosclerosis. Endocrinology 144: 3765–3773
2. **Gabrielsson BG, Johansson JM, Jennische E, Jernås M, Itoh Y, Peltonen M, Olbers T, Lönn L, Lönroth H, Sjöström L, Carlsson B, Carlsson LM, Lönn M** 2002 Depot-specific expression of fibroblast growth factors in human adipose tissue. Obes Res 10:608–616
3. **Benson M, Svensson PA, Carlsson B, Jernås M, Reinholdt J, Cardell LO, Carlsson L** 2002 DNA microarrays to study gene expression in allergic airways. Clin Exp Allergy 32:301–308
4. **Olsson B, Andersson PO, Jernås M, Jacobsson S, Carlsson B, Carlsson LM, Wadenvik H** 2003 T-cell-mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. Nat Med 9:1123–1124
5. **Svensson PA, Englund MC, Markström E, Ohlsson BG, Jernås M, Billig H, Torgerson JS, Wiklund O, Carlsson LM, Carlsson B** 2003 Copper induces the expression of cholesterogenic genes in human macrophages. Atherosclerosis 169:71–76
6. **Su AI, Cooke MP, Ching KA, Hakak Y, Walker JR, Wiltshire T, Orth AP, Vega RG, Sapinoso LM, Moqrich A, Patapoutian A, Hampton GM, Schultz PG, Hogenesch JB** 2002 Large-scale analysis of the human and mouse transcriptomes. Proc Natl Acad Sci USA 99:4465–4470
7. **Montague CT, O'Rahilly S** 2000 The perils of portliness: causes and consequences of visceral adiposity. Diabetes 49:883–888
8. **Bakay M, Zhao P, Chen J, Hoffman EP** 2002 A web-accessible complete transcriptome of normal human and DMD muscle. Neuromuscul Disord 12(Suppl 1):S125–S141
9. **Fyfe AI, Rothenberg LS, DeBeer FC, Cantor RM, Rotter JI, Lusis AJ** 1997 Association between serum amyloid A proteins and coronary artery disease: evidence from two distinct arteriosclerotic processes. Circulation 96:2914–2919
10. **Johnson BD, Kip KE, Marroquin OC, Ridker PM, Kelsey SF, Shaw LJ, Pepine CJ, Sharaf B, Bairey Merz CN, Sopko G, Olson MB, Reis SE** 2004 Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). Circulation 109:726–732
11. **Smith U, Sjöström L, Björntorp P** 1972 Comparison of two methods for determining human adipose cell size. J Lipid Res 13:822–824
12. **Ohlsson BG, Englund MC, Karlsson AL, Knutsen E, Erixon C, Skribeck H, Liu Y, Bondjers G, Wiklund O** 1996 Oxidized low density lipoprotein inhibits lipopolysaccharide-induced binding of nuclear factor-κB to DNA and the subsequent expression of tumor necrosis factor-α and interleukin-1β in macrophages. J Clin Invest 98:78–89
13. **Kaminski N, Allard JD, Pittet JF, Zuo F, Griffiths MJ, Morris D, Huang X, Sheppard D, Heller RA** 2000 Global analysis of gene expression in pulmonary fibrosis reveals distinct programs regulating lung inflammation and fibrosis. Proc Natl Acad Sci USA 97:1778–1783
14. **Svensson PA, Hägg DA, Jernås M, Englund MC, Hulten LM, Ohlsson BG, Hulthe J, Wiklund O, Carlsson B, Fagerberg B, Carlsson LM** 2004 Identification of genes predominantly expressed in human macrophages. Atherosclerosis 177:287–290
15. **Larson MA, Wei SH, Weber A, Weber AT, McDonald TL** 2003 Induction of human mammary-associated serum amyloid A3 expression by prolactin or lipopolysaccharide. Biochem Biophys Res Commun 301:1030–1037
16. **Zeger SL, Liang KY** 1986 Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42:121–130
17. **Rotnitzky A, Jewell NP** 1990 Hypothesis testing of regression parameters in semiparametric generalized linear models for cluster correlated data. Biometrika 77:485–497
18. **Kuriyama H, Kawamoto S, Ishida N, Ohno I, Mita S, Matsuzawa Y, Matsubara K, Okubo K** 1997 Molecular cloning and expression of a novel human aquaporin from adipose tissue with glycerol permeability. Biochem Biophys Res Commun 241:53–58
19. **Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R** 2004 Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 306:1383–1386
20. **Yamada T** 1999 Serum amyloid A (SAA): a concise review of biology, assay methods and clinical usefulness. Clin Chem Lab Med 37:381–388
21. **Urieli-Shoval S, Linke RP, Matzner Y** 2000 Expression and function of serum amyloid A, a major acute-phase protein, in normal and disease states. Curr Opin Hematol 7:64–69
22. **Benditt EP, Meek RL** 1989 Expression of the third member of the serum amyloid A gene family in mouse adipocytes. J Exp Med 169:1841–1846
23. **Lin Y, Rajala MW, Berger JP, Moller DE, Barzilai N, Scherer PE** 2001 Hyperglycemia-induced production of acute phase reactants in adipose tissue. J Biol Chem 276:42077–42083
24. **Kluve-Beckerman B, Drumm ML, Benson MD** 1991 Nonexpression of the human serum amyloid A three (SAA3) gene. DNA Cell Biol 10:651–661
25. **Arroyo-Espliguero R, Avanzas P, Cosin-Sales J, Aldama G, Pizzi C, Kaski JC** 2004 C-reactive protein elevation and disease activity in patients with coronary artery disease. Eur Heart J 25:401–408
26. **Ford ES, Giles WH, Dietz WH** 2002 Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA 287:356–359
27. **Pickup JC, Mattock MB, Chusney GD, Burt D** 1997 NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 40:1286–1292
28. **van Dielen FM, van't Veer C, Schols AM, Soeters PB, Buurman WA, Greve JW** 2001 Increased leptin concentrations correlate with increased concentrations of inflammatory markers in morbidly obese individuals. Int J Obes Relat Metab Disord 25:1759–1766
29. **Walder K, Kantham L, McMillan JS, Trevaskis J, Kerr L, De Silva A, Sunderland T, Godde N, Gao Y, Bishara N, Windmill K, Tenne-Brown J, Augert G, Zimmet PZ, Collier GR** 2002 Tanis: a link between type 2 diabetes and inflammation? Diabetes 51:1859–1866
30. **Aronson D, Bartha P, Zinder O, Kerner A, Markiewicz W, Avizohar O, Brook GJ, Levy Y** 2004 Obesity is the major determinant of elevated C-reactive protein in subjects with the metabolic syndrome. Int J Obes Relat Metab Disord 28: 674–679
31. **Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM** 2001 C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 286:327–334
32. **Van Lenten BJ, Hama SY, de Beer FC, Stafforini DM, McIntyre TM, Prescott SM, La Du BN, Fogelman AM, Navab M** 1995 Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. J Clin Invest 96:2758–2767
33. **Salazar A, Pinto X, Mana J** 2001 Serum amyloid A and high-density lipoprotein cholesterol: serum markers of inflammation in sarcoidosis and other systemic disorders. Eur J Clin Invest 31:1070–1077
34. **Maeda K, Okubo K, Shimomura I, Mizuno K, Matsuzawa Y, Matsubara K** 1997 Analysis of an expression profile of genes in the human adipose tissue. Gene 190:227–235
35. **Strausberg RL, Feingold EA, Grouse LH, Derge JG, Klausner RD, Collins FS, Wagner L, Shenmen CM, Schuler GD, Altschul SF, Zeeberg B, Buetow KH, Schaefer CF, Bhat NK, Hopkins RF, Jordan H, Moore T, Max SI, Wang J, Hsieh F, Diatchenko L, Marusina K, Farmer AA, Rubin GM, Hong L,** *et al.* 2002 Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. Proc Natl Acad Sci USA 99:16899–16903
36. **Baulande S, Lasnier F, Lucas M, Pairault J** 2001 Adiponutrin, a transmembrane protein corresponding to a novel dietary- and obesity-linked mRNA specifically expressed in the adipose lineage. J Biol Chem 276:33336–33344
37. **Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW** 2004 Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A_2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. J Biol Chem 279:48968–48975