



Human adipose tissue gene expression of solute carrier family 19 member 3 (*SLC19A3*); relation to obesity and weight-loss

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Abstract

Objective: Adipose tissue is a specialized endocrine organ that is involved in modulating whole-body energy homeostasis and expresses a specific subset of genes, which may play a role in adipose tissue metabolism. The aim of this study was to search for novel adipose tissue-specific genes using a tissue panel of RNAseq expression profiles.

Methods: RNAseq expression profiles from 53 human tissues were downloaded from the GTex database. *SLC19A3* expression was analyzed by microarray or real-time PCR in two sets of paired subcutaneous and omental adipose tissue samples, in two studies with adipose tissue from persons with high or low body mass index (BMI), in adipose tissue from patients who underwent weight loss with a very-low caloric diet and during preadipocyte-adipocyte differentiation.

Results: The RNAseq-based tissue distribution expression screen identified *SLC19A3* (encoding the thiamine transporter 2) as adipose tissue-specific. *SLC19A3* expression was higher in subcutaneous compared with omental adipose tissue in both sample sets ($p = 0.043$ and $p < 0.001$). Preadipocyte differentiation towards adipocytes resulted in increased *SLC19A3* gene expression ($p = 0.018$ or less at all-time points). Subcutaneous adipose tissue expression of *SLC19A3* was lower in persons with high BMI in both cohorts ($p = 0.008$, and $p < 0.001$) and increased during a weight-loss intervention ($p = 0.006$).

Conclusion: The specific adipose tissue expression pattern of *SLC19A3*, together with its regulation in obesity and during weight loss, indicate that it plays a key role in adipocyte metabolism.

KEYWORDS

adipose tissue, obesity, *SLC19A3*, thiamine transporter, weight-loss

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1 | INTRODUCTION

The central function of adipose tissue and adipocytes is to control lipid metabolism.¹ In conditions of excess nutrients, the adipocyte store energy in the form of lipids, whereas in the post-prandial period or during starvation, it supplies lipids to other tissues through lipolysis.² In response to energy overconsumption, the adipose tissue is expanded through an increase in the number and/or size of adipocytes.³ Adipose tissue expansion also affects the cellular composition and function of the stromal vascular fraction cells of the adipose tissue. This includes macrophage infiltration and low-grade adipose tissue inflammation,⁴ which has been shown to contribute to obesity-related metabolic comorbidities.⁴

Research during the last 3 decades has described many additional specific functions of adipose tissue. Adipose tissue is currently defined as an endocrine organ with secretion of signaling molecules, so-called adipokines, from adipocytes.^{5,6} Many of these adipokines play key roles in the regulation of energy homeostasis and contribute to obesity-related metabolic disorders.⁷

In addition to the regular white adipose tissue, humans also have smaller amounts of brown adipose tissue.⁸ The key feature of brown adipose tissue is the generation of heat via the uncoupling of the respiratory chain.⁸ Clearly, also brown adipose tissue expresses a set of unique genes which are related to its unique function, and uncoupling protein 1 (*UCP1*) is the clearest example of this.⁸

The specific functions of the adipocytes and adipose tissue are mediated by proteins that, in many cases, are specifically expressed in the adipocytes. Hence, adipose tissue and adipocytes have distinct expression profiles, and some genes are uniquely expressed by adipocytes. Adipose tissue dysfunction has been linked to several obesity-related comorbidities, and adipocyte-specific genes have been shown to play a part also in these comorbidities.^{5,6}

Analysis of mRNA expression levels has been used to gain insights into the function of genes, and the development of techniques such as microarrays has enabled screening-based approaches to find genes of relevance for many different functions in the human body and different pathological conditions. Microarray-based methods have previously been used to search for genes with a tissue/cell type-specific expression pattern.⁹ This methodology is based on the comparison of expression profiles from multiple human tissues and cell types. Our group has used this methodology for the identification of several novel adipocyte/adipose tissue-specific genes, including *CIDEA*, *CIDEA*, *NQO1*, *TNMD*, *SAA1* and *ITIH5*.¹⁰⁻¹⁵ The rationale behind this approach is that genes that are exclusively expressed in a specific tissue most likely have important functions in that tissue. Dysregulation of genes with key functions in adipose tissue may be linked to obesity-related metabolic dysfunction. RNA sequencing (RNAseq) has now emerged as an advantageous tool for expression profiling, compared to microarrays, since it is not limited by the probe composition of the microarray.¹⁶ RNAseq data from multiple human tissues and cell types can potentially also be used in searches for novel adipose tissue-specific genes.

This study hypothesized that adipose tissue express several unique genes that play key roles in adipose tissue function and that dysregulation of such genes may be linked to obesity-related metabolic dysfunction. This study aimed to use an RNAseq dataset with multiple human tissues to search for novel adipose tissue-specific genes and to characterize the expression pattern of such genes.

2 | MATERIALS AND METHOD

2.1 | Gene expression dataset and search for adipose tissue-specific genes

Tissue distribution of gene expression was analyzed in a publicly available RNAseq dataset, downloaded from the GTEx portal (Version 7, <https://www.gtexportal.org/home/datasets>). The RNAseq dataset contains 53 tissues and cell types, and data for each tissue/cell type originate from analysis of 5 to 564 samples, but the replicates of each sample were combined into a mean expression profile for each tissue or cell type. The dataset contains expression profiles from both subcutaneous and omental adipose tissue. Gene expression levels were calculated as transcripts per kilobase million (TPM). To identify genes specifically expressed in subcutaneous adipose tissue, genes that had an expression level (TPM) that was at least 10 standard deviations (SD) higher than the mean TPM of all the other tissues and at least 5-fold higher TPM than the tissue with the second-highest TPM (second tissue) were selected. In these analyses, expression profiles for omental adipose tissue and mammary tissue were excluded due to the high degree of similarities to subcutaneous adipose.

A second publicly available RNAseq dataset (HPA RNA-seq normal tissues, <https://www.ncbi.nlm.nih.gov/gene/80704>) was also analysed since this dataset contained tissues not included in the first dataset.¹⁷ This dataset contains samples from 95 human individuals representing 27 different tissues (2-7 samples analyses per tissue), and gene expression levels were calculated as reads per kilobase million (RPKM).

2.2 | Paired subcutaneous and omental adipose tissue biopsies

Two groups of patients were used to investigate adipose tissue depot differences in gene expression. In the first group, paired subcutaneous and omental adipose tissue biopsies were obtained from 5 healthy lean women (Body mass index [BMI] 23.0 ± 1.2 Kg/m²) undergoing elective surgery (liposuction and elective gynecological procedures) at the University Hospital Coventry and Warwickshire.¹³ Smokers and subjects with recent weight change, hormone replacement, and malignant diseases were excluded. In the second group, paired subcutaneous and omental adipose tissue was obtained from 27 subjects without diabetes (17 females, 10 males, BMI 37.8 ± 13.3 Kg/m²) undergoing kidney donation at the Sahlgrenska University Hospital, or bariatric surgery at the Uppsala University Hospital, as previously reported.¹⁸

2.3 | The SibPair parents (SPP) study

The SibPair study was designed to study the genetics of obesity and consists of 154 nuclear families with BMI discordant sib-pairs (BMI difference > 10 Kg/m²).¹⁹ In total, 732 subjects were recruited from all over Sweden and examined at the Obesity Unit, the Sahlgrenska University Hospital in Gothenburg. In the current study, subcutaneous adipose tissue needle biopsies were obtained from the parents in the SibPair study ($n = 88$)²⁰ and were used for the analysis of adipose tissue gene expression. This group was also stratified by BMI into individuals being either of normal weight (BMI 18.5–25 Kg/m²; $n = 17$) or with obesity (BMI > 30 Kg/m²; $n = 32$).

2.4 | Subcutaneous adipose tissue study

Subcutaneous adipose tissue needle biopsies were obtained by needle aspiration of the lower part of the abdomen from a cohort of subjects without diabetes (52 female, 20 male; BMI 26.1 ± 2.9 Kg/m²) at the Uppsala University Hospital. The adipose tissue was used for mRNA extraction ($n = 68$), and isolation of adipocytes and stromal vascular fraction ($n = 4$) and expansion of preadipocytes into adipocytes ($n = 8$). Subjects with type 1 diabetes, endocrine disorders, cancer or other major illnesses were excluded, as so were those having ongoing medication with systemic glucocorticoids, beta-blockers and immune-modulating therapies.

2.5 | The very low-calorie diet (VLCD) study

As previously described,¹⁰ 24 persons with obesity (BMI > 30 kg/m²) were treated with a very low-calorie diet (VLCD; 450 kcal/day, Cambridge diet or Modifast) for 16 weeks (wk) at the Sahlgrenska University Hospital in Gothenburg. The VLCD period was followed by a 2 weeks of gradual re-introduction to a normal diet. Abdominal subcutaneous adipose tissue biopsies were obtained before (wk 0), during (wk 8 and 16), and after (wk 18) diet-induced weight loss from 24 subjects (6 women and 18 men), and used for gene expression analysis by DNA microarray. The participants displayed a mean weight loss of 27.7 ± 1.8 kg during the VLCD period (week 16). During wk 17 and 18, the body weight was stabilized.

2.6 | Perirenal adipose tissue study

Biopsies of perirenal adipose tissue were obtained from 55 healthy kidney donors at the Sahlgrenska University Hospital in Gothenburg. The samples were screened for *UCP1* gene expression, and samples with high *UCP1* expression were classified as containing brown adipose tissue (BAT, $n = 10$). A matched control group ($n = 10$) was created from the samples displaying no/low *UCP1* expression.²¹

2.7 | Adipose tissue sampling and RNA preparation

Adipose tissue, obtained by needle aspirations or by surgical biopsies, was snap-frozen and stored at -80°C until analysis. Total RNA was prepared using the RNeasy lipid tissue kit (QIAGEN, Chatsworth, CA), or using the phenol-chloroform extraction method of Chomczynski and Sacchi with further purification with RNeasy clean-up columns.

2.8 | Microarray analysis

Microarray analysis of paired subcutaneous and omental adipose tissue samples and adipose tissue samples from the SibPair parents study was performed using U133 plus 2.0 microarray (Affymetrix, Santa Clara, CA). Microarray analysis of adipose tissue from the VLCD study was performed using U133A microarray (Affymetrix), and perirenal adipose tissue was analyzed using Gene 1.0 ST microarrays (Affymetrix). All microarray analysis was performed according to the manufacturer's instructions (Affymetrix), and expression data were analyzed using the Robust Multi-array Average algorithm. *SLC19A3* expression was assessed using probe sets 220736_at and 8059538 for U133 microarrays and Gene 1.0 ST microarrays, respectively.

2.9 | Real-time PCR

The quantity and quality of the isolated RNA were determined by NanoDrop ND-1000 (NanoDrop Technologies, Inc. Wilmington, New Jersey). cDNA synthesis was performed with High Capacity cDNA Reverse Transcriptase kit (Applied Biosystems, Foster City, CA), according to the manufacturer guidelines. The mRNA expression analyses were performed using the QuantStudio 3 sequence detection system (Applied Biosystems) using a specific Taqman gene expression assay (Applied Biosystems): *SLC19A3* (Hs00228858), *ADIPOQ* (Hs00605917), *PLIN1* (Hs00160173), *CEBPA* (Hs00269972) and *PPARG* (Hs1115513). All samples were run in duplicate, and the data were analysed using $2^{-\Delta\Delta\text{Ct}}$ using *18S* or *GUSB* as a reference gene.

2.10 | Isolation of human adipocytes and stromal vascular cells

Adipocytes and stromal vascular cells were isolated from subcutaneous adipose tissue for preadipocyte differentiation into adipocytes and analyses of *SLC19A3* gene and protein expression. The isolation of preadipocytes was performed as previously reported.²² In brief, adipose tissue was digested with collagenase (1.2 mg/ml, from *Clostridium histolyticum*, Roche, Mannheim, Germany) in Hank's medium (5.6 mM glucose, 4% BSA, 150 nM adenosine) for 60 min in a shaking water-bath. Isolated adipocytes were filtered through a 250 μm nylon mesh, and the media under the adipocytes was collected and centrifuged for 3 min at 1200 rpm for isolation of the

stromal vascular fraction. The mature adipocytes were washed in Hank's media 6 mmol/L glucose, 4% BSA, 150 nM adenosine, pH 7.4 and separated from the medium through centrifugation in dinonyl phthalate (Tokyo Chemical Industry).

2.11 | Differentiation of preadipocytes into adipocytes

2.11.1 | Two human adipocyte differentiation models were used:

Human isolated preadipocytes: The stromal vascular fraction containing preadipocytes was cultured with preadipocyte medium (Dulbecco's modified Eagle's medium (DMEM)/Ham's F12, Gibco) containing 10% foetal bovine serum (FBS, Thermo Fisher), 100 units/ml penicillin and 100 g/ml streptomycin (Life Technology), 0.04mg/ml gentamycin (Gibco), and 4.125 ng/ml basic fibroblast growth factor (Sigma) at 37°C until 70–80% confluence. Preadipocytes were trypsinized and seeded at a density of 15,000 cells/cm² with preadipocyte media. After cells reached 100% confluence (day 0), differentiation was induced by adding differentiation cocktail (DMEM/Ham's F12, 1% PEST, 100 nM (16,666 µU/ml) human insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark), 17 µM pantothenate, 33 µM biotin, 1 µM cortisol, 1 µM rosiglitazone, 250 µM 3-isobutyl-1-methylxanthine (IBMX), 10 µg/ml transferrin human, 2 nM 3, 3', 5'-triiodo L-thyronine [all from Sigma]) for 5 days. The differentiation continued with maintenance medium (composition is similar to that of differentiation cocktail except for IBMX) until day 14. The medium was replenished every 3 days.

SGBS cells: Human Simpson-Golabi-Behmel syndrome (SGBS) adipocytes were kindly provided by Professor Martin Wabitsch (Ulm University Medical Centre, Germany). SGBS were expanded in DMEM-F12 containing 33 µM biotin, 17 µM pantothenate, 1% PEST, and 10% non-heat-inactivated FBS until 80% confluence. Adipocyte differentiation was induced for 4 days with differentiation medium: DMEM-F12 with 1% PEST, 100 nM insulin, 17 µM pantothenate, 33 µM biotin, 1 µM dexamethasone, 1 µM rosiglitazone, 250 µM IBMX, 10 µg/ml transferrin, and 2 nM tri-iodothyronine (T3). The differentiation continued with the maintenance medium (the same composition as the differentiation medium except for IBMX, dexamethasone, and rosiglitazone) until day 14.

The cells were used to determine the SLC19A3 gene and protein expression, and the differentiation rate was confirmed by measuring the expression of the adipogenic markers *PPARG* and *CEBPA*.

2.12 | Immunoblotting

Separated adipocytes or stromal vascular cells were homogenized in lysis buffer (25 mM Tris-HCl; 0.5 mM EGTA; 25 mM NaCl; 1% Nonidet P-40; 1 mM Na₃VO₄; 10 mM NaF (all from Sigma); 100 nM okadaic acid (Alexis Biochemicals, Lausen, Switzerland), 1X Complete protease inhibitor cocktail (Roche, Indianapolis, IN, USA), pH 7.4) for

30 min at 4°C. The samples were centrifuged at 12,000 g for 15 min and 4°C until complete phase separation. The lysate was collected, and the protein concentration was determined using a BCA protein assay kit (Pierce, Thermo Scientific, Rockford, IL, USA). SDS-PAGE and immunoblotting were performed with standard procedures.²² Membranes were incubated overnight with anti-SLC19A3 (1:1000, 13407-1-AP, Proteintech, Manchester, UK) and thereafter with horseradish peroxidase-conjugated anti-rabbit (Cell Signalling Technologies) secondary antibody. Anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1:3000, 5174, Cell Signalling) was used as a loading control protein. Protein bands were then visualized using enhanced chemiluminescence with a high-resolution field and quantified with ChemiDoc™ MP System (BioRad).

2.13 | Data analysis and statistics

Enrichment analysis was performed using the online David functional annotation tool (version 6.8) using default settings.^{23,24} The top 500 genes that positively correlated with *SLC19A3* expression in human subcutaneous adipose tissue (SibPair parents, $n = 88$) were selected, and these 500 genes correspond to 358 unique David IDs. Enrichment analysis was performed with Human Genome U133 plus set as background.

The difference in gene expression in paired subcutaneous and omental adipose tissue biopsies was analyzed using related-samples Wilcoxon signed-rank test. The difference in gene expression in brown adipose tissue and between subjects with differences in BMI was performed using independent sample Mann-Whitney *U* test or Spearman's rank correlation. Gene and protein expression during adipocyte differentiation and expression during caloric restriction was analyzed by related-samples Friedman's Two-Way Analysis of Variance by rank followed by Wilcoxon signed rank test as post-hoc test. Two-sided *p* values less than 0.05 were considered statistically significant. Statistical analysis was performed using IBM SPSS statistics 25.

2.14 | Ethics approval

The Regional Ethics Committee in Gothenburg, Uppsala and the South Birmingham Ethics Committee approved these studies. All study subjects received written and oral information about the respective study before giving informed consent.

3 | RESULTS

3.1 | RNAseq-based search for genes specifically expressed in subcutaneous adipose tissue

The RNAseq based search for adipose tissue specific-genes yielded 14 genes that were classified as specifically expressed in

TABLE 1 Genes specifically expressed in human subcutaneous adipose tissue

Gene ID	Gene symbol	SAT TPM	Fold ^a	Second tissue
ENSG00000174697.4	<i>LEP</i>	229	30,7	Cells-transformed fibroblasts
ENSG00000181092.5	<i>ADIPOQ</i>	621	24,9	Artery-coronary
ENSG00000166819.7	<i>PLIN1</i>	1029	23,0	Nerve-tibial
ENSG00000170323.4	<i>FABP4</i>	5946	15,7	Artery-coronary
ENSG00000187288.6	<i>CIDEA</i>	1029	14,3	Small intestine-terminal ileum
ENSG00000176194.13	<i>CIDEA</i>	108	8,4	Skin-not sun exposed (suprapubic)
ENSG00000133317.10	<i>LGALS12</i>	52	7,9	Whole blood
ENSG00000135917.9	<i>SLC19A3</i>	62	7,5	Liver
ENSG00000000005.5	<i>TNMD</i>	27	6,9	Nerve-tibial
ENSG00000126545.9	<i>CSN1S1</i>	4	6,1	Brain-anterior cingulate cortex (BA24)
ENSG00000184811.3	<i>TUSC5</i>	154	5,9	Artery-tibial
ENSG00000167676.3	<i>PLIN4</i>	1069	5,8	Muscle-skeletal
ENSG00000172901.15	<i>AQPEP</i>	15	5,6	Testis
ENSG00000079435.5	<i>LIPE</i>	369	5,0	Testis

Abbreviation: TPM, transcripts per kilobase million.

^aFold change calculated as the ratio between SAT TPM and TPM of the second tissue. Omental adipose tissue and mammary glands were not included in this analysis.

subcutaneous adipose tissue using the predefined criteria (Table 1). Classical adipocyte-specific genes such as leptin (*LEP*), adiponectin (*ADIPOQ*), Perilipin 1 (*PLIN1*) and fatty acid binding protein 4 (*FABP4*) were classified as the top four on the list of genes specifically expressed in subcutaneous adipose tissue (Table 1). Genes previously identified as adipose/adipocyte-specific genes in microarray-based scans, such as *CIDEA*,¹⁰ *CIDEA*¹¹ and *TNMD*,¹³ were also included on the list. A novel finding from this analysis was that the thiamine transporter 2 (*THTR2*, encoded by the solute carrier family 19 member 3 [*SLC19A3*] gene)²⁵ was specifically expressed in adipose tissue and was therefore selected as a focus for further investigation.

3.2 | Tissue distribution of thiamine transporters in human tissues

The complete tissue distribution of *SLC19A3* expression in the GTEx dataset is shown in Figure 1A. In this dataset, *SLC19A3* was highly expressed in visceral and subcutaneous adipose tissue and in mammary tissue. Similar tissue distribution of *SLC19A3* mRNA expression was observed also in an additional RNAseq dataset (Supplemental Figure 1). In this dataset, adipose tissue displayed the highest *SLC19A3* expression levels, followed by placenta and duodenum. Higher expression of *SLC19A3* in subcutaneous adipose tissue compared with omental adipose tissue was observed in the microarray dataset with paired biopsies from 5 lean women (Figure 1B, $p = 0.043$). This depot difference in *SLC19A3* mRNA expression was verified in an independent dataset with paired biopsies from 27 subjects which were analyzed by real-time PCR (Figure 1C, $p < 0.001$).

3.3 | *SLC19A3* expression in adipocytes and during adipocyte differentiation

SLC19A3 mRNA levels were very low in human preadipocytes but increased during the adipocyte differentiation process ($p = 0.0014$ for differences among repeated differentiation days), and higher *SLC19A3* mRNA levels were observed during all timepoints of differentiation compared with the preadipocytes at day 0 (Figure 2A, $p = 0.018$ or less for all time points). *SLC19A3* protein expression followed the same expression pattern during adipocyte differentiation, but the changes in *SLC19A3* protein expression were not statistically significant (Figure 2B). *SLC19A3* mRNA levels were also investigated in another model of human adipocyte differentiation (SGBS cells). In line with the human isolated preadipocyte data, *SLC19A3* expression was significantly increased after 14 days of adipocyte differentiation (Figure 2D, $p = 0.013$). Adipocyte differentiation was confirmed in both these models by analysis of *PPARG* and *CEBPA* expression (supplement Figure 3 A-D).

SLC19A3 protein levels were also investigated in isolated adipocytes and stromal vascular fraction cells. *SLC19A3* protein was detected in both the adipocytes and the stromal vascular fraction cells, but no significant difference in protein levels were observed (Figure 2 E-F).

3.4 | Adipose tissue expression of *SLC19A3* in obesity and during weight-loss

SLC19A3 expression was lower in subcutaneous adipose tissue from subjects with obesity compared with subjects of normal weight

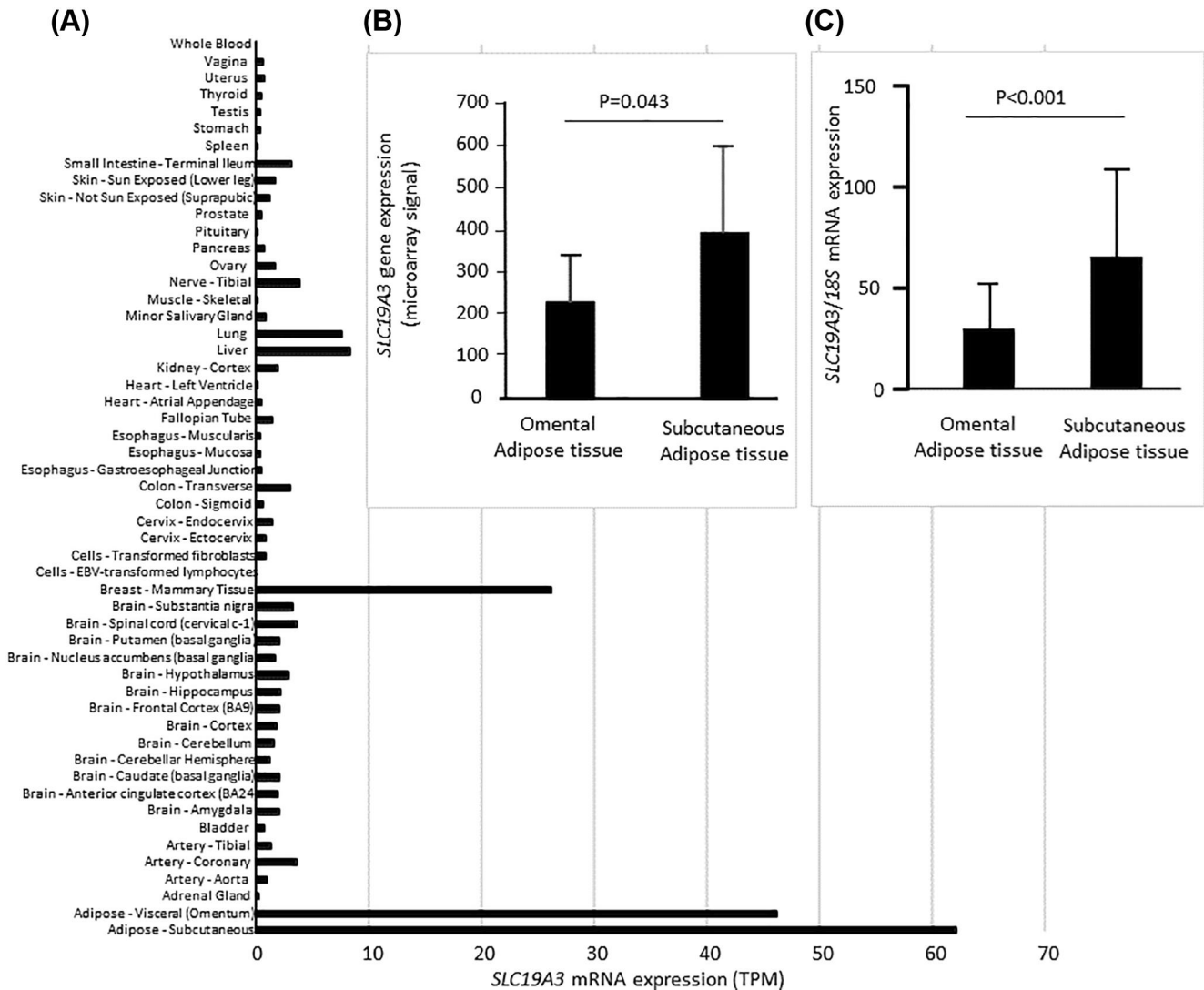


FIGURE 1 Tissue distribution of *SLC19A3* expression. The expression analysis was performed in a panel of human tissues from GTex (A) and in paired subcutaneous and omental adipose tissue biopsies (B, C). Expression analysis was performed by RNA sequencing (A), microarray (B) and real-time PCR (C). Data are presented as mean expression (with SD in B and C). Analysis was performed in 5 and 27 patients, respectively in B and C

($p = 0.008$, Figure 3A). To verify this finding, real-time PCR analysis of *SLC19A3* mRNA expression was performed in subcutaneous adipose tissue from 68 subjects with BMI ranging from 20.2 to 34.5 Kg/m². *SLC19A3* expression showed a negative correlation with BMI (Figure 3B, $\rho = -0.571$, $p < 0.001$). A similar analysis was performed with omental adipose tissue from 27 subjects with BMI ranging from 20.9 to 58.2 Kg/m², but no significant association was found (Supplementary Figure 2 A-B). In addition, mRNA expression analysis of *ADIPOQ* and *PLIN1*, two genes known to be down-regulated in obesity, were performed. Subcutaneous *SLC19A3* mRNA expression was highly correlated to both *ADIPOQ* and *PLIN1* mRNA expression (Supplementary Figure 2 A-B, $p < 0.001$ for both). However, the correlation pattern was less clear for *SLC19A3* mRNA expression in omental adipose tissue (Supplementary Figure 2 A-B).

In a weight-loss study (VLCD study) with 24 participants, subcutaneous adipose tissue *SLC19A3* mRNA expression was increased

during 16 weeks of energy restriction ($p = 0.006$ after 16 weeks, Figure 4). However, when regular food was gradually re-introduced during the last two weeks of the intervention (between week 16 and 18), the mRNA expression of *SLC19A3* was reduced ($p = 0.002$) almost to the level as before the intervention.

3.5 | Function of *SLC19A3* in adipose tissue

To gain insights of the potential function of *SLC19A3* in adipose tissue, a gene set enrichment analysis was performed. The top 500 genes with a positive correlation to *SLC19A3* expression in the subcutaneous adipose tissue in the SibPair parents cohort ($n = 88$) were used for enrichment analysis. Terms that were enriched in this analysis were dominated by links to the mitochondrion (Table 2). In addition, significantly enriched terms included lipid metabolism and branched-chain amino acid catabolism (Table 2).

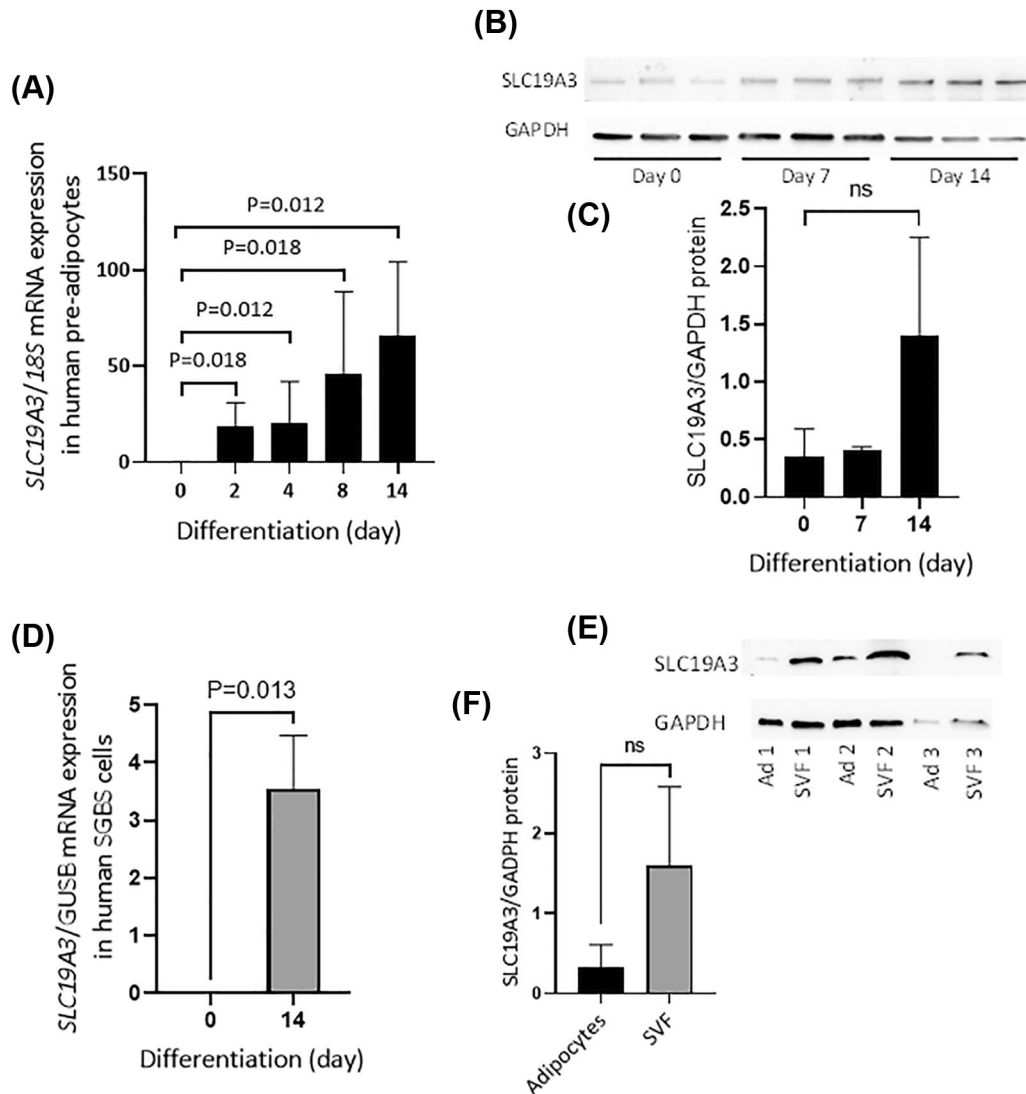


FIGURE 2 Expression of *SLC19A3* during adipocyte differentiation and in isolated adipocytes. Human preadipocytes were differentiated towards adipocytes for 14 days, and *SLC19A3* expression was analyzed by real time PCR (A) and western blot (B and C). Data are presented as mean expression and SD, and the experiment was performed with preadipocytes from 8 and 3 donors, respectively. Differentiation of SGBC cells towards adipocytes and analysis of *SLC19A3* expression before and after 14 days of differentiation (D). Western blot analysis of *SLC19A3* protein expression in isolated adipocytes and stromal vascular fraction cells (SVC) (E and F)

Brown adipocytes have a high abundance of mitochondria, and *SLC19A3* expression may therefore be linked presence of brown adipocytes and energy metabolism in adipose tissue. However, analysis of *SLC19A3* mRNA expression in brown adipocyte containing human perirenal adipose tissue shows that this tissue does not express *SLC19A3* mRNA at a higher level compared with perirenal adipose tissue that does not contain brown adipocytes (Figure 5).

4 | DISCUSSION

This study showed that the thiamine transporter *SLC19A3* was specifically expressed in human adipose tissue and that adipose tissue *SLC19A3* expression was regulated by obesity, weight-loss and

possibly energy intake. Furthermore, an enrichment of genes with mitochondrial function was observed among the genes that correlated with the *SLC19A3* adipose tissue expression. Taken together, these results indicate that *SLC19A3* plays a role in adipose tissue energy metabolism.

Duodenum has been describes as the main site of thiamin uptake in the body.²⁶ The gene product of *SLC19A3* has been suggested to mediate this uptake due to its high expression in duodenum.²⁶ Tissue distribution datasets that contain both adipose tissue and duodenum indicated that the expression levels of *SLC19A3* were several-fold higher in adipose tissue compared with the duodenum. This tissue distribution pattern does not speak against a key role for *SLC19A3* in intestinal thiamine uptake, but it highlights the very high expression which was observed in adipose tissue.

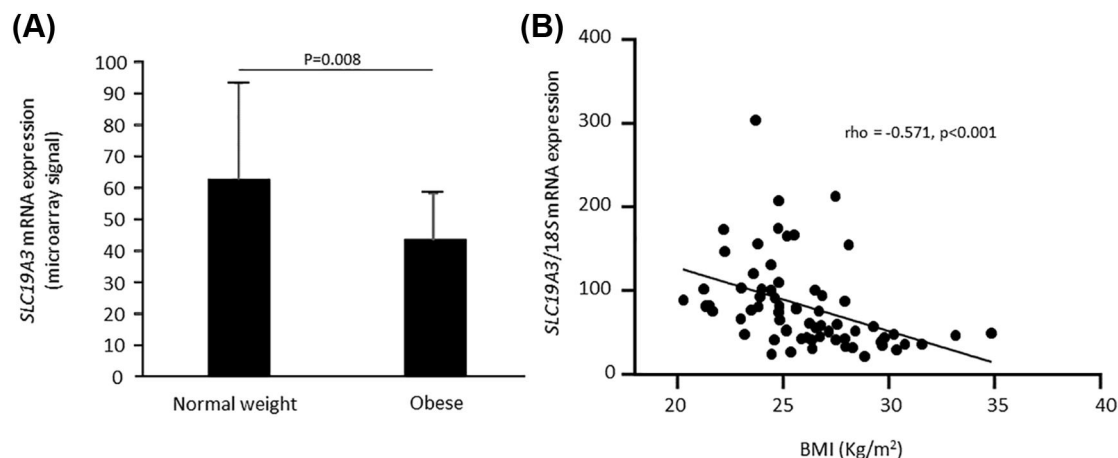


FIGURE 3 Subcutaneous adipose tissue expression of *SLC19A3* and its relation to BMI. Expression analysis of patients with obesity compared with normal weight controls (A). Correlation analysis of *SLC19A3* expression and BMI (B). Expression analysis was performed by microarray (A) and real-time PCR (B). Data are presented as mean expression with SD or as a scatter plot. ns; not significant

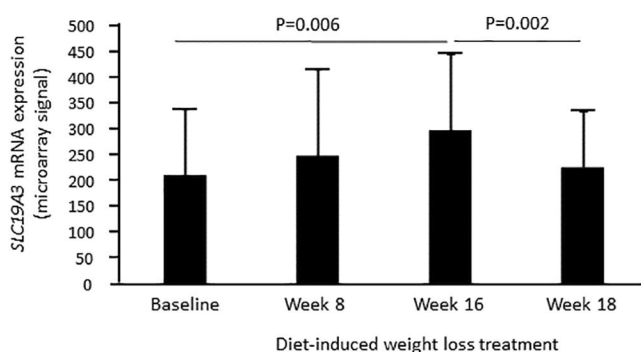


FIGURE 4 Subcutaneous adipose tissue expression of *SLC19A3* during weight-loss intervention. Adipose tissue gene expression of *SLC19A3* from patients with obesity undergoing a 16 weeks diet-induced weight loss ($n = 24$) treatment using VLCD. After week 16 of VLCD, normal food was gradually introduced for 2 weeks. Data are presented as mean and SD. VLCD, very low caloric diet

Thiamine has its main function as a cofactor of the enzyme pyruvate dehydrogenase, an enzyme active in the citric acid cycle.²⁷ Hence, decreased thiamin levels can result in reduced mitochondrial activity, impaired oxidative metabolism, and decreased energy production.²⁷ Skeletal muscle and brown adipose tissue are tissues with high energy demand and an abundance of mitochondria, whereas white adipose tissue has relatively few mitochondria.²⁸ Functional studies²⁷ and the tissue distribution of *SLC19A2* indicate that this transporter was responsible for thiamine delivery to the high energy demanding tissues. This was in line with the findings in the current study regarding human brown adipocytes and *SLC19A3* expression. Cellular studies have confirmed the thiamine transport capacity of *SLC19A3*,^{29,30} however, the exact function of thiamine and *SLC19A3* in adipose tissue needs to be explored in mechanistic studies.

Using a gene identification strategy similar to ours, Ahn et al.³¹ also identified *SLC19A3* as an adipose-specific/enhanced protein-coding gene. Using publicly available expression datasets, they identified increased expression of *SLC19A3* during adipocyte differentiation and 8-fold higher expression in adipocytes compared with the stromal vascular fraction. The adipocyte differentiation findings are well in line with the findings in the current study. However, the current study indicated that *SLC19A3* protein was also expressed in the stromal vascular fraction of adipose tissue. Ahn et al.³¹ also present data from an expression dataset (GSE9624) with omental adipose tissue from prepubertal children, which indicate higher *SLC19A3* expression in children with obesity compared with lean children. In addition, they also present data from subcutaneous adipose tissue (expression data set GSE12050), which indicate no difference in *SLC19A3* expression between persons with obesity and lean persons. These findings were not in line with the findings in the current study, where lower subcutaneous adipose tissue *SLC19A3* expression in persons with obesity was found in two independent populations. The findings from the current study are further strengthened by the results from the weight-loss intervention, where weight-loss and/or reduced energy intake was linked to higher subcutaneous *SLC19A3* expression. Despite these findings, the function of *SLC19A3* in adipose tissue remains unclear. The link between *SLC19A3* expression and the enrichment of genes with mitochondrial functions may be an important guide in future functional experiments aimed at addressing this knowledge gap.

The main limitations of this study are that the majority of the findings are based on analysis of *SLC19A3* mRNA and protein expression, and the lack of functional experiments, which could have pinpointed the role of *SLC19A3* in adipose tissue. Strengths of this study include that findings have been replicated in independent populations and with different mRNA analyzing technologies.

TABLE 2 Top 40 enriched terms among top 500 genes positively correlated with *SLC19A3* expression in subcutaneous adipose tissue (functional annotation chart)

Category	Term	Count	Adj <i>p</i> -value*
GOTERM_CC_DIRECT	Mitochondrion	108	6.70E-37
UP_KEYWORDS	Mitochondrion	97	1.50E-36
UP_SEQ_FEATURE	Transit peptide:Mitochondrion	63	8.40E-32
UP_KEYWORDS	Transit peptide	66	2.00E-32
GOTERM_CC_DIRECT	Mitochondrial matrix	46	2.20E-23
KEGG_PATHWAY	Valine, leucine and isoleucine degradation	18	3.50E-13
UP_KEYWORDS	Acetylation	123	1.90E-12
GOTERM_CC_DIRECT	Mitochondrial inner membrane	38	5.90E-12
COG_ONTOLOGY	Lipid metabolism	16	1.70E-10
GOTERM_BP_DIRECT	Branched-chain amino acid catabolic process	10	7.20E-08
KEGG_PATHWAY	Metabolic pathways	67	3.60E-08
KEGG_PATHWAY	Propanoate metabolism	11	1.10E-07
GOTERM_CC_DIRECT	Mitochondrial outer membrane	18	4.60E-07
KEGG_PATHWAY	Parkinson's disease	19	5.60E-07
GOTERM_BP_DIRECT	Fatty acid beta-oxidation	11	9.80E-06
UP_KEYWORDS	Mitochondrion outer membrane	15	1.40E-06
UP_KEYWORDS	Oxidoreductase	32	4.80E-06
GOTERM_BP_DIRECT	Generation of precursor metabolites and energy	11	4.60E-05
KEGG_PATHWAY	Biosynthesis of antibiotics	22	5.70E-06
UP_KEYWORDS	Mitochondrion inner membrane	21	7.50E-06
KEGG_PATHWAY	NAFLD	18	1.40E-05
KEGG_PATHWAY	Fatty acid degradation	10	4.20E-05
UP_SEQ_FEATURE	Domain:ATP-grasp	6	8.30E-04
INTERPRO	Rudiment single hybrid motif	5	1.70E-03
UP_KEYWORDS	Electron transport	12	1.20E-04
UP_KEYWORDS	Lipid metabolism	24	1.50E-04
UP_SEQ_FEATURE	Binding site:Substrate; via amide nitrogen	7	1.10E-03
INTERPRO	Crotonase superfamily	6	2.00E-03
KEGG_PATHWAY	Oxidative phosphorylation	15	1.40E-04
GOTERM_BP_DIRECT	Biotin metabolic process	6	2.50E-03
UP_KEYWORDS	Disease mutation	78	2.30E-04
INTERPRO	ATP-grasp fold, subdomain 1	6	2.00E-03
KEGG_PATHWAY	Carbon metabolism	14	2.00E-04
UP_KEYWORDS	Multifunctional enzyme	10	2.80E-04
UP_KEYWORDS	Respiratory chain	9	2.60E-04
KEGG_PATHWAY	Huntington's disease	18	2.00E-04
UP_KEYWORDS	Peroxisome	11	3.20E-04
UP_KEYWORDS	Fatty acid metabolism	12	3.90E-04
GOTERM_BP_DIRECT	Metabolic process	14	6.00E-03
GOTERM_CC_DIRECT	Mitochondrial respiratory chain complex I	8	1.40E-03

Abbreviation: NAFLD, non-alcoholic fatty liver disease.

**p*-value adjusted according to Benjamini.

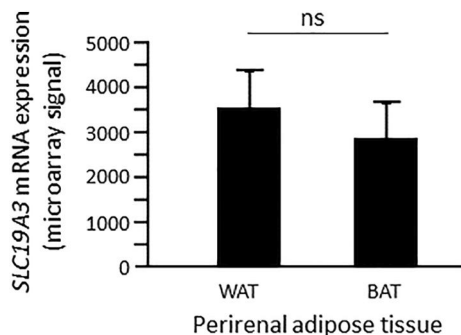


FIGURE 5 *SLC19A3* expression in human brown adipose tissue. Human perirenal adipose tissue was analysed for *UCP1* expression and stratified into two groups, samples containing (BAT, $n = 10$) or not containing (WAT, $n = 10$) brown adipocytes. Data are presented as a mean expression with SD. ns; not significant

5 | CONCLUSION

This study showed that *SLC19A3* was highly expressed in adipose tissue and expression characterization of *SLC19A3* expression in human adipose tissue and adipocytes was performed. The regulation of *SLC19A3* indicate that this gene may play a key metabolic role in adipose tissue but its role in thiamine uptake in adipocytes or other functions in adipocytes needs to be further investigated.

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CONFLICT OF INTEREST

Prof. Lena M.S. Carlsson reports receiving consulting fees from Johnson & Johnson Healthcare Systems and grant support, paid to her institution. Dr Taube has a patent and holds stocks in Umecrine AB. Dr K Sjöholm and Dr M Pereira reports grant support, paid to the institutions. The other authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

Maria J. Pereira, Lena M. S. Carlsson and Per-Arne Svensson planned the study. Maria J. Pereira, Johanna C. Andersson-Assarsson, Peter Jacobson, Magdalena Taube, Prasad Kamble, Kajsa Sjöholm and Per-Arne Svensson carried out experiments, performed clinical studies and microarray analysis. All authors were involved in data analysis and writing the paper and had final approval of the submitted and published versions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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