Increased OCT3 Expression in Adipose Tissue With Aging: Implications for Catecholamine and Lipid Turnover and Insulin Resistance in Women

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Abstract

Background: Catecholamine-stimulated lipolysis is reduced with aging, which may promote adiposity and insulin resistance. Organic cation transporter 3 (OCT3), which is inhibited by estradiol (E2), mediates catecholamine transport into adipocytes for degradation, thus decreasing lipolysis. In this study, we investigated the association of OCT3 mRNA levels in subcutaneous adipose tissue (SAT) with aging and markers of insulin resistance in women.

Methods: SAT biopsies were obtained from 66 women with (19) or without (47) type 2 diabetes (age 22-76 years, 20.0-40.1 kg/m²). OCT3 mRNA and protein levels were measured for group comparisons and correlation analysis. SAT was incubated with E2 and OCT3 mRNA levels were measured. Associations between OCT3 single nucleotide polymorphisms (SNPs) and diabetes-associated traits were assessed.

Results: OCT3 mRNA and protein levels in SAT increased with aging. SAT from postmenopausal women had higher levels of OCT3 than premenopausal women, and there was a dose-dependent reduction in OCT3 mRNA levels in SAT treated with E2. OCT3 mRNA levels were negatively associated with markers of insulin resistance, and ex vivo lipolysis. OCT3 SNPs were associated with BMI, waist to hip ratio, and circulating lipids (eg, triglycerides).

Conclusion: OCT3 mRNA and protein levels in SAT increased with aging, and mRNA levels were negatively associated with markers of insulin resistance. E2 incubation downregulated OCT3 mRNA levels, which may explain lower OCT3 mRNA in premenopausal vs postmenopausal women. High OCT3 protein levels in adipose tissue may result in increased catecholamine degradation, and this can contribute to the reduction in lipolysis observed in women with aging.

Key Words: OCT3, adipose tissue, estradiol, aging, T2D

Abbreviations: AUC, area under the curve; BMI, body mass index; BSA, bovine serum albumin; E2, estradiol; FFA, free fatty acids; FPKM, fragment per kilobase of transcript per million mapped reads; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOME-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; MAO, monoamine oxidase; MRI, magnetic resonance imaging; OCT3, organic cation transporter 3; OGTT, oral glucose tolerance test; PBS, phosphate-buffered saline; RNA-seq, RNA sequencing; SAT, subcutaneous adipose tissue; SEM, standard error of the mean; SNP, single nucleotide polymorphism; SVF, stromal vascular fraction; T2D, type 2 diabetes; VAT, visceral adipose tissue; WHR, waist to hip ratio.

Aging is often characterized by adipose tissue dysfunction, which is implicated in the development of both obesity and type 2 diabetes (T2D) (1). The sympathetic nervous system in part controls adipose tissue function through the release of catecholamines, such as noradrenaline (2). Noradrenaline plays an important role in adipocyte lipid mobilization, as it regulates the breakdown of triglycerides into free fatty acids and glycerol, a process known as lipolysis, and this occurs through binding beta-adrenergic receptors (2). The sympathetic tone is determined by the release, transport, and degradation of catecholamines (2). There are 2 distinct forms of catecholamine transport: neuronal transport and extraneuronal transport. Neuronal transport involves catecholamine uptake to and from nerve endings and this is mainly through the transporters SLC6A2, SLC6A3, and SLC6A4 (3, 4). Extraneuronal transport is mainly mediated by organic cation transporters (OCTs). OCT3 (SLC22A3) was identified in a genome-wide association study as a novel locus with significantly different effects on body mass index (BMI) in younger vs older individuals (5). Moreover, Paquette et al found that an OCT3 single nucleotide polymorphism (SNP) was associated with lipoprotein (a) and cardiovascular disease in individuals with familial hypercholesterolemia, highlighting a possible link between OCT3 expression and dyslipidemia and obesity (6).

OCT3 has been implicated in adipocyte lipolysis and browning (3). Gao et al recently showed that there is an age-induced reduction in catecholamine-stimulated lipolysis in subcutaneous adipose tissue (SAT) from women, which may be due to an upregulation of enzymes related to catecholamine degradation (7). A reduction in noradrenaline-stimulated lipolysis impairs lipid turnover and promotes adipose tissue accumulation, through triglyceride accumulation (hypertrophy) which are features observed with aging and obesity (7). Interestingly,
estradiol, which declines in women after menopause, has been shown to be an inhibitor of OCT3-mediated transport (8). The role of OCT3 in human SAT lipid and glucose metabolism has yet to be well investigated. In this study, we assess the association between OCT3 mRNA and protein levels in SAT with aging, in addition to assessing the association between OCT3 mRNA levels in SAT with markers of obesity and insulin resistance. Understanding whether altered OCT3 expression explains changes in adipose tissue lipolysis may provide insight into a potential target for preventing age-related changes in body fat distribution and the development of insulin resistance.

**Methods and Subjects**

**Subjects**

Abdominal SAT was obtained by needle biopsies from 66 women with \((n = 19)\) or without \((n = 47)\) T2D, in order to obtain subjects with a wide range of insulin sensitivity and resistance. Needle biopsies were obtained by aspiration from the lower part of the abdomen after local dermal anesthesia with lidocaine (Xylocaine, AstraZeneca, Sodertalje, Sweden). The anthropometric characterization (Table 1) of subjects included measurements taken after an overnight fast, with fasting blood samples collected to assess levels of glycated hemoglobin (HbA1c), glucose, lipids, and insulin. Postmenopausal status was defined as a period of 12 months or longer since the last menstruation. One premenopausal woman was taking oral contraceptives (dienogest/etynilestradiol) and one was on a hormone birth control patch (etynilestradiol/norelgestromin). One postmenopausal woman was treated with systemic hormonal replacement therapy (estradiol) and one was treated with local hormonal pessary (estradiol). The subjects with T2D were prescribed a stable dose of metformin for a minimum of 3 months. Additionally, an oral glucose tolerance test was conducted to analyze plasma glucose, insulin, and free fatty acids (FFA). Body composition analysis was performed using whole-body magnetic resonance imaging (MRI), all as previously reported (9). Subjects with type 1 diabetes, other endocrine disorders, cancer, or other major illnesses, as well as ongoing medication with beta-adrenergic blockers, systemic glucocorticoids, or immune-modulating therapies were excluded from the study.

Part of the adipose tissue biopsy was snap-frozen in liquid nitrogen and used for RNA-Seq. In addition, SAT biopsy was used for adipocyte and stromal vascular fraction (SVF) isolation. Isolated mature adipocytes were used for adipocyte size measurements and *ex vivo* analyses of lipolysis and glucose uptake. Not all experiments could be performed in every subject due to the limited amount of aspired adipose tissue. The Regional Ethics Review Board in Uppsala (Dnr 2013/330 and Dnr 2013-183/494) and Gothenburg (Dnr 336-07) approved the studies, and all participants gave their written informed consent.

**Magnetic Resonance Imaging**

MRI was used to assess volumes of abdominal SAT, visceral adipose tissue (VAT), and liver fat percentage as previously described (9).

**Adipose Tissue Incubations**

Adipose tissue \((n = 8)\) was incubated at 37 °C using phenol red–free DMEM media containing 6mM glucose (Invitrogen Corporation, Paisley, OR, USA), 10% charcoal-stripped fetal bovine serum (FBS, Invitrogen), and 1% penicillin-streptomycin. Body composition analysis was performed using whole-body magnetic resonance imaging (MRI), all as previously reported (9). Subjects with type 1 diabetes, other endocrine disorders, cancer, or other major illnesses, as well as ongoing medication with beta-adrenergic blockers, systemic glucocorticoids, or immune-modulating therapies were excluded from the study.

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**Human Preadipocyte Differentiation into Mature Adipocytes**

Preadipocytes were isolated from the SVF of the SAT as previously described (9, 10). Human preadipocytes were differentiated for up to 14 days, as previously reported (10, 11). The cells were seeded at 10 000 to 30 000 cells/cm² into 12 well plates (Thermo Fisher) using preadipocyte media. After cells reached 100% confluence, they were cultured for 1 additional day, after which adipogenesis was induced by adding a differentiation cocktail (phenol red–free DMEM/Ham’s F12, 1% penicillin-streptomycin, 100mM human insulin (Actrapid, Novo Nordisk), 17µM pantothenate (Sigma), 33µM biotin (Sigma), 1µM cortisol (Sigma), 1µM rosiglitazone (Sigma), 250µM 3-isobutyl-1-methylxanthine (IBMX, Sigma), 10µg/mL transferrin human (Sigma), 2nM 3, 3, 5-triodo L-thyronine (T3, Sigma)) for 5 days with replacement of induction cocktail on day 3. On day 5, cells were then cultured in maintenance medium (composition is the same as the differentiation cocktail except for omitting IBMX) until day 14. Medium was replenished every 3 days.

**Gene Expression Analysis**

Gene expression was assessed using either RNA-Seq or quantitative polymerase chain reaction (qPCR).

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Table 1. Anthropometric and clinical characteristics of participating women

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<tr>
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<td>HbA1c (% NGSP)</td>
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<td>6.9 ± 1.0</td>
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<td>Serum insulin (mU/L)</td>
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<td>Total cholesterol (mmol/L)</td>
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<td>5.0 ± 0.8</td>
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<td>Plasma HDL cholesterol (mmol/L)</td>
<td>1.8 ± 0.7</td>
<td>1.1 ± 0.7</td>
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<td>Plasma LDL cholesterol (mmol/L)</td>
<td>3.4 ± 1.1</td>
<td>3.2 ± 0.7</td>
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<tr>
<td>Plasma triglycerides (mmol/L)</td>
<td>1.2 ± 0.4</td>
<td>1.9 ± 1.0</td>
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*Data are mean ± SD. Blood chemistry is fasting.*

**Abbreviations:** BME, body mass index; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; NGSP, National Glycohemoglobin Standardization Program; T2D, type 2 diabetes; WHR, waist to hip ratio.
Transcriptomics
A subgroup of SAT samples was used for RNA-Seq at Exiqon A/S (9), and Novogene (12). RNA-Seq data are presented as Fragment Per Kilobase of transcript per Million mapped reads (FPKM). FPKM is a unit used in RNA-Seq analysis, normalizing gene expression by accounting for both gene length and total number of mapped reads; thus, it is a measure of gene expression that allows for comparisons between different genes and across samples. The data were used to determine correlations between OCT3 (SLC22A3) mRNA levels and markers of lipid and glucose metabolism.

Quantitative PCR
Total RNA was extracted from whole adipose tissue (n = 8) using the RNeasy lipid tissue mini kit (Qiagen). The total RNA concentration and purity were measured with the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Rockford, IL, USA). RNA (400 ng) was then reverse-transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher Scientific, CA, USA). The protocols were carried out as per the manufacturers’ guidelines. TaqMan gene expression assays (Thermo Fisher Scientific) were used to study the expression of OCT3 (Hs00109571_m1). Gene expression was detected using the QuantStudio 3 sequence detection system (Applied Biosystem) and calculated using a 2^(-ΔΔCT). The gene expression levels were normalized to the housekeeping gene GUSB (Hs00939627_m1), and all samples were run in duplicates.

Isolation of Human Adipocytes and SVF for Immunoblotting
Adipocytes and SVF were isolated from SAT (n = 3) for analysis of OCT3 protein, as described previously (13, 14). Adipose tissue (500 mg) was digested using collagenase A (Roche) in a shaking water bath at 105 rpm for 1 hour at 37 °C in Medium 199 (Gibco, Life Technologies) supplemented with 6mM glucose, 4% bovine serum albumin (BSA, Sigma), 150mM adenosine (Sigma), pH = 7.4. The floating adipocytes were washed 4 times in Hank’s medium (6 mmol/L glucose, 4% BSA, 0.15 μmol/L adenosine, pH = 7.4) and were separated from the medium by filtration through dinonyl phthalate (Merck, Darmstadt, Germany). The SVF fraction was isolated from the collagenase medium by centrifugation for 3 minutes at 1200 rpm and washed with phosphate-buffered saline (PBS). The adipocytes and SVF were used for immunoblotting analysis of OCT3. Total protein levels of the adipocyte and SVF were measured using the BCA protein assay kit (Pierce, Thermo Scientific) and used to extrapolate the total OCT3 protein levels in the whole tissue sample.

Western Blot
Total protein was isolated from adipocytes, SVF, preadipocytes, and differentiated cells on day 14 of in vitro differentiation (n = 3), as previously reported (11). Cells were lysed in ice-cold lysis buffer: 25mM Tris-HCl (Sigma), pH 7.4; 0.5mM EGTA (Sigma); 25mM NaCl (Sigma); 1% Nonidet P-40; 10mM NaF; 100mM okadaic acid (Alexis Biochemicals), 1X Complete protease inhibitor cocktail (Roche, Indianapolis, IN, USA) and 1mM orthovanadate (Sigma). The lysate was vortexed and incubated on ice for 10 minutes and then centrifuged at 15 000g for 15 minutes at 4 °C. The infranatant was transferred into a new tube and saved at ~80 °C. Proteins (10-20 μg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 5%-8% gradient stain-free gels, Bio-rad), transferred to nitrocellulose membranes and blocked with 0.05% Tween-PBS (Medigaco) with 5% BSA (Sigma). Stain-free blot imaging was used to quantify the total protein for each sample and to normalize OCT3 protein levels as described by (15). Membranes were incubated overnight with the primary antibody anti-OCT3 (1:500, Abcam, ab124826, RRID:AB_10974589). Membranes were then washed with 0.05% Tween-PBS and incubated with horseradish peroxidase conjugated goat anti-rabbit secondary antibody (1:2000, Cell Signaling, 7074S, RRID:AB_2099233). Protein bands and stain-free blots were imaged using enhanced chemiluminescence with a high-resolution field and quantified with ChemiDocTM MP System (Bio-Rad) and quantification with Image Lab Software (software version).

Immunohistochemistry
The SAT was fixed overnight at room temperature in a paraffin solution, which was replaced with 70% ethanol the following day. Immunocytochemistry staining was performed on SAT sections (n = 8). Sections were deparaffinized and rehydrated, and this was followed by heat-induced antigen retrieval. In brief, slides were placed in Tris-EDTA buffer (10mM Tris base, 1mM EDTA, 0.05% Tween) and incubated in a water bath at 95 °C for 15 minutes. Slides were washed 2 x 5 minutes with PBS and 0.025% Triton-X-100 and then blocked in PBS with 5% goat serum and 1% BSA 2 hours at room temperature. Slides were incubated with the primary antibody OCT3 (1:100, Abcam, ab124826, RRID:AB_10974589) overnight in a humidified chamber, followed by the goat anti-rabbit secondary antibody conjugated AlexaFluor 488, (1:250, Thermo, A11008, RRID:AB_143165). SAT sections were imaged using the InCells Fluorescent microscope (Bertin Instruments) and 3 images were used for analysis from each histological section. Images were quantified using ImageJ Version 1.51 and the average mean fluorescence intensity relative to the total area corrected for background for the triplicate images was used as a measure for OCT3 protein levels.

Glucose Uptake
Glucose uptake in isolated adipocytes (n = 34) was performed as described previously (12, 16). In brief, SAT was digested using collagenase A (Roche) in a shaking water bath at 105 rpm for 1 hour at 37 °C in Medium 199 (Gibco, Life Technologies, Paisley, UK) supplemented with 6mM glucose, 4% bovine serum albumin (BSA, Sigma), 150mM adenosine (Sigma), pH = 7.4. The cell suspension was passed through a 250 μm nylon mesh and collected into a Falcon tube. Adipocytes were isolated from digestion media and washed 3 times at 5-minute intervals in glucose-free Krebs-Ringer bicarbonate medium (KRH) supplemented with 4% BSA, 150mM adenosine, pH = 7.4. Next, adipocytes were incubated at 37 °C in a shaking water bath at 65 rpm for 15 minutes in KRH medium without (basal), or with 25 or 1000 μM of insulin (Actrapid, Novo Nordisk, Bagsværd, Denmark). After 15 minutes, the cells were incubated with D-(U-14C) glucose (0.86 μM glucose, 0.26 mCi/mL, Perkin Elmer, Boston, USA) for 45 minutes. The reaction was stopped by transferring the reaction tubes onto ice, and cells were separated from the media by centrifugation using silicon fluid (WR...
Chemicals, Leuven, Belgium). Radioactivity associated with cells was then measured with a scintillation counter (Radiomatic series 500TR, Perkin Elmer Analytical Instruments). Glucose uptake was expressed as clearance of medium glucose per cell. Cell number was determined following measurements of triglyceride content (Doles extraction) (17) and cell size (Axiovision, München, DE) (18).

**Lipolysis**
Lipolysis was measured in isolated adipocytes from SAT (n = 31-32) as previously described (19). SAT was digested using collagenase and adipocytes were isolated as described above (glucose uptake section). The adipocyte suspension (lipocrit 3%-5%) was incubated in Hank’s medium containing 6 mM glucose, 4% BSA, 0.15 μM adenosine, pH 7.4, with or without 0.5 μM of isoproterenol (beta-adrenergic agonist) in a gently shaking water bath at 37 °C for 2 hours. Glycerol released into the medium was used as lipolytic index and normalized per cell number (nmol/10E5 cells/h).

**Single Nucleotide Polymorphism Analysis**
Associations of OCT3 SNPs with diabetes and obesity-related traits were assessed using Open Targets Genetics (https://genetics.opentargets.org/). We used publicly available results from the UK Biobank, Genetic Epidemiology Research on Adult and Aging (GERA), Genetic Investigation of ANthropometric Traits (GIANT), and the BioBank Japan, Million Veteran Program (MV). We identified associations with 8 metabolic traits for OCT3, and therefore, the P values were Bonferroni-corrected with a factor of 8. The significance threshold for the resulting P values in SNP analyses was P < 5 × 10^{-8}.

**Statistics**
All data are presented as mean ± standard error of the mean (SEM), unless otherwise stated. Normality of data was first assessed and a comparison between paired groups was made using a paired t test, or between 3 or more using one-way ANOVA with repeated measures or mixed model effects. For multiple comparison correction, the false discovery rate (FDR) method Benjamini, Krieger, and Yekutieli was used. Spearman’s correlation was used to test for bivariate analysis, and significant variables were included in a multilinear regression model to predict the impact of clinical variables on the OCT3 gene expression. A P value of less than .05 was considered statistically significant. All data were analyzed using GraphPad Prism 9.0.2, IBM SPSS version 23, or Visualizations for transcriptomics data were performed in the R statistical software version 4.3.0.

**Results**
**Human SAT Abundantly Expresses Machinery for Extraneuronal Catecholamine Transport and Degradation**
We assessed the mRNA levels of OCTs, which are known extraneuronal catecholamine transporters in SAT and found that OCT3 mRNA levels were higher than OCT1 and OCT2 mRNA levels (Fig. 1A). Furthermore, the mRNA levels of catecholamine degradation enzymes monoamine oxidase A (MAOA) and MAOB were high compared to catechol-O-methyltransferase (COMT) in SAT (Fig. 1B). Due to this, we focused on OCT3, (in addition to MAOA and MAOB) in this study. No significant differences in mRNA levels were found for OCT1, 2, and 3 and MAOA, MAOB, and COMT in subjects with or without T2D (data not shown).

**OCT3 in SAT Is Found Primarily in Mature Adipocytes**
Studies using mice adipocytes have shown that OCT3 was predominantly found close to the plasma membrane (3), therefore, we assessed whether this was reflected in human SAT. Immunofluorescence imaging of SAT showed that OCT3 was predominantly present close to the membrane of mature adipocytes (Fig. 2A). To quantitatively assess the proportion of OCT3 in adipocytes compared to whole adipose tissue, we measured OCT3 protein levels in mature adipocytes and in the SVF fraction of SAT. We found that OCT3 protein levels were approximately 15-fold higher in mature adipocytes than the SVF (Fig. 2B and 2C, P < .05). To account for the different proportions of adipocytes and SVF in the SAT sample, we measured total protein and after adjustment, we found that approximately 1% of OCT3 resides in the SVF fraction. Furthermore, we compared OCT3 protein levels in preadipocytes obtained from the SVF at day 0 of differentiation and after 14 days of preadipocyte differentiation, and we found that OCT3 was approximately 10^3-fold higher in differentiated mature adipocytes (Fig. 2D and 2E, P < .05).

**OCT3 mRNA and Protein Levels in SAT Are Increased With Aging and With Menopause**
We assessed the relationship between OCT3 mRNA levels and aging and found that OCT3 mRNA levels in SAT were positively correlated with age (Fig. 3A, P < .01), and this corresponded to increased OCT3 protein levels in SAT from individuals with ages in the upper tertile compared with the lower tertile (tertile 3 vs 1) (Fig. 3B and 3C, P < .01). These differences were also observed with immunofluorescence staining of OCT3 in SAT samples (Fig. 3D and 3E). Correspondingly, OCT3 mRNA and protein levels were higher in SAT from postmenopausal women than from premenopausal women (Fig. 3F, 3G, and 3H, P < .05 and P < .01, respectively). Estradiol has been reported to be a nonspecific inhibitor of
Therefore, to assess whether estradiol could account for changes in \textit{OCT3} mRNA levels with aging and menopause, SAT from postmenopausal women was incubated with E2 for 24 hours. The E2 treatment resulted in a dose-dependent reduction in \textit{OCT3} mRNA levels (\textit{Fig. 3I}, \textit{P} < .05). We also assessed whether mRNA levels of monoamine degradation enzymes were altered with aging and found that \textit{MAOA} and \textit{MAOB} mRNA levels displayed a positive trend with age (\textit{Fig. 3J} and 3K). Aldo-keto reductases or aldehyde dehydrogenases, which convert primary amines, such as catecholamines, to aldehydes, ammonia, and hydrogen peroxide were unchanged with aging in SAT from women (data not shown).

\textit{OCT3} mRNA Levels in SAT Are Negatively Associated With Markers of Insulin Resistance

The association between \textit{OCT3} mRNA levels and markers of insulin resistance, dyslipidemia, and adiposity was assessed. We found that \textit{OCT3} mRNA levels in SAT were negatively correlated with area under the curve (AUC) glycerol during an oral glucose tolerance test and markers of insulin resistance (eg, HbA1c, homeostatic model assessment for insulin resistance [HOMA-IR]), and positively associated with high-density lipoprotein (HDL) levels (Table 2, \textit{P} < .05). Levels of \textit{OCT3} mRNA did not significantly correlate to markers of obesity (eg, BMI, waist to hip ratio [WHR]) (Table 2).

Since age and menopausal status are collinear, we used 2 multiple regression models to assess which characteristics are stronger predictors of \textit{OCT3} mRNA levels in SAT. Model A consists of age, HbA1c, HOMA-IR, and T2D, while model B consists of menopausal status, HbA1c, HOMA-IR, and T2D. The inclusion of either age or menopausal status showed that age appeared to be a stronger predictor of \textit{OCT3} mRNA levels (\textit{β} = .476 vs \textit{β} = .305) (Table 3). Moreover, both age and HOMA-IR were independent predictors of \textit{OCT3} mRNA levels (Table 3).
mRNA Levels in SAT Are Negatively Associated With Adipocyte Lipolysis

Next, we assessed the relationship between OCT3 mRNA levels and ex vivo adipocyte lipolysis and glucose uptake. OCT3 mRNA levels were negatively associated with basal and isoprenaline-stimulated lipolysis (Fig. 4A and 4B, \( P < .05 \)). This corresponded to a negative association between OCT3 mRNA levels in SAT with gene markers of lipolysis activation (ATGL) and a positive association with lipolysis inhibition (PLIN1) (Table 4). Although OCT3 mRNA levels did not correlate to ex vivo adipocyte glucose uptake (Fig. 4C and 4D, \( P > .05 \)), OCT3 was positively associated with gene markers of insulin signaling and glucose transport markers (GLUT4 and AKT1, Table 4). In addition, OCT3 mRNA levels were positively correlated with markers of catecholamine degradation (Table 4, \( P < .001 \)).
Association of OCT3 Polymorphisms With Adiposity, Diabetes, and Dyslipidemia

Association between OCT3 SNPs and obesity and diabetes-related traits were assessed (Table 5). We found that SNPs in OCT3 were associated with BMI, body fat distribution, WHR, T2D, low-density lipoprotein (LDL), HDL, and triglycerides (Table 5).

Proposed Outcome Related to Increased OCT3 mRNA and Protein Levels With Aging in SAT From Women

Lastly, we use our data to propose a model of increased OCT3 mRNA and protein levels with aging (Fig. 5). Our results show that OCT3 is increased in SAT with aging, which may be due to reduced E2, which is an inhibitor of OCT3 (8). Increased catecholamine transport, through OCT3, and degradation, through MAOA and MAOB, would result in less available noradrenaline to stimulate adipocyte lipolysis, thus reducing the release of lipids from adipocytes. Reduced adipocyte lipolysis and release of lipids from adipocytes, in turn, may contribute to reduced peripheral insulin resistance.

Discussion

Aging is associated with a decrease in catecholamine-stimulated lipolysis of adipocytes, which is linked to an increase in adipose tissue accumulation, hypertrophy, and insulin resistance (7, 20, 21). In this study, we investigated the association between levels of OCT3 in SAT and markers of lipid and glucose metabolism in SAT. To our knowledge, this is the first study to show that in human SAT, OCT3 mRNA and protein levels are upregulated with aging, and OCT3 mRNA levels are negatively associated with markers of insulin resistance and ex vivo adipose tissue lipolysis. Moreover, we found that SAT specimens from postmenopausal women have higher mRNA and protein levels of OCT3 than SAT from premenopausal women, and SAT incubated with E2 for 24 hours has reduced OCT3 mRNA levels. Our results may indicate that increased OCT3 expression, and subsequent catecholamine degradation, results in reduced catecholamine-stimulated lipolysis observed with aging.

Recent studies have proposed that adipocytes are an important site of noradrenaline clearance in adipose tissue (3, 7). In mice, OCT3 is abundant in white adipose tissue and adipocytes display the greatest rate of noradrenaline clearance (3). Similarly, we found that OCT3 was the predominant extraneuronal catecholamine transporter gene expressed in human SAT, and its gene expression levels were comparable between subjects with and without T2D. Moreover, OCT3 was primarily found close to the membrane of mature adipocytes, which is in agreement with OCT3 localization in mouse primary inguinal adipocytes (3). Histologically, we localize OCT3 in close proximity of the cell surface of adipocytes, but the resolution of our assays does not enable confirmation of OCT3 being exclusively located at the adipocyte plasma membrane rather than in the intercellular space or in adjacent SVF cells. Furthermore, we found abundant mRNA levels of intracellular enzymes for catecholamine degradation, MAOA, and MAOB, which further indicates that SAT is likely an important site for catecholamine degradation. Our data on the mRNA levels of OCTs and catecholamine degradation enzymes are in accordance with protein levels found in the Human Protein Atlas (22) and previous studies (23–26).

Since aging is associated with a decrease in catecholamine-stimulated lipolysis, we assessed whether OCT3 mRNA and protein levels were altered with aging in SAT. OCT3 mRNA and protein levels in SAT were increased with aging, and correspondingly, in postmenopausal women. Since age and menopausal status are collinear, we performed 2 multiple regression analysis to determine if the differences we found with menopausal status were mainly due to age. We found that age was a stronger predictor than menopausal status of OCT3
mRNA levels in SAT. This is in agreement with previous studies showing that markers related to catecholamine degradation were shown to be upregulated in adipose tissue with age (7, 27).

OCT3 mRNA levels were inversely associated with markers of insulin resistance (ie, HOMA-IR, HbA1c), and our multilinear regression model demonstrated that HOMA-IR was an independent predictor of OCT3 mRNA levels. This corresponded to a positive association between OCT3 mRNA levels and gene markers of glucose transport and insulin sensitivity.

We hypothesized that high OCT3 mRNA levels in SAT correspond to reduced capacity to stimulate adipocyte lipolysis, due to increased transport and degradation of catecholamines. We found that OCT3 mRNA levels were negatively associated with both basal and stimulated lipolysis, and negatively associated with certain gene markers of lipolysis (eg, ATGL), emphasizing a possible link between increased OCT3 mRNA levels and reduced lipolysis. This agrees with a study by Song et al, which showed that adipose-specific OCT3 knockout mice have reduced catecholamine-stimulated lipolysis (3). The relationship between catecholamine-stimulated lipolysis and insulin resistance has been previously shown, and catecholamines are also known to directly impair insulin action by increasing cAMP levels, for example in adipose tissue and skeletal muscle (28–30). For instance, Mullins et al showed that catecholamine-stimulated lipolysis inhibits adipocyte glucose uptake through inhibition of rapamycin (mTOR) complexes 

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Table 5. Association of OCT3 polymorphisms with adiposity, diabetes, and dyslipidemia

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<td>T</td>
<td>Chr. 6:160353456</td>
<td>.012</td>
<td>.009 to .015</td>
<td>3.00E-09</td>
<td>2.40E-08</td>
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<td>WHR</td>
<td>rs668871</td>
<td>C</td>
<td>T</td>
<td>Chr. 6:160348779</td>
<td>−0.13</td>
<td>−0.17 to −0.01</td>
<td>7.00E-12</td>
<td>5.60E-11</td>
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<td>WHR adjusted for BMI</td>
<td>rs474513</td>
<td>A</td>
<td>G</td>
<td>Chr. 6:160349280</td>
<td>−0.12</td>
<td>−0.15 to −0.08</td>
<td>7.00E-11</td>
<td>5.60E-10</td>
<td>381 152</td>
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<tr>
<td></td>
<td>rs555754</td>
<td>G</td>
<td>A</td>
<td>Chr. 6:160348391</td>
<td>−0.23</td>
<td>−0.26 to −0.20</td>
<td>1.80E-30</td>
<td>1.44E-29</td>
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<td></td>
<td>rs501470</td>
<td>T</td>
<td>G</td>
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<td>−0.17</td>
<td>−0.21 to −0.14</td>
<td>2.00E-24</td>
<td>1.60E-23</td>
<td>379 501</td>
</tr>
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<td></td>
<td>rs622217</td>
<td>T</td>
<td>C</td>
<td>Chr. 6:160345738</td>
<td>−0.19</td>
<td>−0.23 to −0.15</td>
<td>4.00E-24</td>
<td>3.20E-23</td>
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<td>Weight</td>
<td>rs673736</td>
<td>G</td>
<td>A</td>
<td>Chr. 6:160355621</td>
<td>−0.26</td>
<td>−0.31 to −0.21</td>
<td>2.00E-20</td>
<td>1.60E-19</td>
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<td>Type 2 diabetes</td>
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<td>T</td>
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<td>.015 to .021</td>
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<td>G</td>
<td>Chr. 6:160346873</td>
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<td>C</td>
<td>A</td>
<td>Chr. 6:160354985</td>
<td>−0.32</td>
<td>−0.41 to −0.23</td>
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<td>T</td>
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<td>−0.063 to −0.048</td>
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Associations of OCT3 SNPs with diabetes and obesity-related traits were assessed using Open Targets Genetics. Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SNP, single nucleotide polymorphism.

MAOB, which correspond to a decrease in serum FFA (27). Since we found that OCT3 mRNA levels were negatively associated with adipocyte lipolysis, we assessed whether its expression was associated with circulating lipids, and we found that OCT3 gene expression was negatively associated with AUC glycerol, highlighting a possible link between OCT3 mRNA levels and circulating lipids. Several genome-wide studies have identified variants in OCT3 to be associated with lipid traits and cardiovascular risk (6, 31, 32). We assessed whether polymorphisms in OCT3 were associated with markers of adiposity and insulin resistance and found that OCT3 polymorphisms were associated with adiposity, diabetes status, and circulating lipids, strengthening the link between OCT3 transport and fat accumulation and insulin resistance.

We found that OCT3 mRNA and protein levels in SAT were positively associated with aging and higher in postmenopausal women. It has been shown that estradiol is a potent inhibitor of OCT3-mediated transport (8). Thus, we assessed whether estradiol treatment of SAT altered OCT3 mRNA levels. We found that estradiol treatment of SAT resulted in a dose-dependent reduction in OCT3 mRNA levels. Sex hormones have been shown to be important for the regulation of catecholamine catabolism. For instance, estrogen has been shown to downregulate the expression of the monoamine degradation enzyme MAOA in HeLa cells, through estrogen receptor signaling (33). Similarly, in mice, estradiol has been shown to downregulate other monoamine transporters such as PMAT, and correspondingly, serotonin reuptake, through estrogen receptors (34). Estradiol levels are reduced after menopause contributes to an increase in OCT3 mRNA and protein levels in SAT and results in increased catecholamine degradation. To our knowledge, there are no studies
investigating the levels of OCT3 in women taking hormone replacement therapy. There have been few studies investigating the direct role of estradiol or estrogen replacement therapy on adipocyte lipolysis, and these studies are often contradictory due to the use of different experimental models. For instance, in one study using ovariectomized mice (a model of menopause), estradiol treatment increased catecholamine-stimulated lipolysis (35), whereas, in a separate study local perfusion of the abdomen with estradiol blunted adipocyte lipolysis (36). Interestingly, in one study performed in humans, hormone replacement therapy (estradiol) increased catecholamine excretion in urine, which was hypothesized to be due to decreased synaptic uptake and decreased catecholamine degradation (37). It is unclear whether the increase in catecholamine excretion and a reduction in degradation may be due to an estrogen-induced reduction of peripheral OCT3 expression and thus decreased catecholamine degradation. Moreover, although it is known that aging and menopause are associated with an increased risk of insulin resistance and the development of T2D (38, 39), it is still not known whether an increase in OCT3 mRNA levels, and thus a decrease in stimulated lipolysis, could function as a compensatory mechanism to limit insulin resistance. Future studies are needed in order to functionally assess whether estrogen deficiency results in reduced stimulated lipolysis in SAT, such as estrogen treatment in OCT3 knockout ovariectomized mice.

This study has some limitations. This study is explorative in nature and future studies should address the functional role of OCT3 in human adipocytes. However, known inhibitors of OCT3 are nonspecific (40) and target multiple pathways, and are therefore not ideal to assess the role of OCT3 in SAT. Although the relationship between OCT3 and lipolysis has been studied in mice (3) functional studies are warranted in human mature adipocytes to specifically inhibit or knock-out OCT3 in order to confirm its importance in lipolysis in the context of aging. Moreover, studies are required to further investigate the role of estrogen on OCT3-mediated catecholamine clearance and lipolysis.

In conclusion, OCT3 mRNA and protein levels are increased with aging in SAT from women. Moreover, OCT3 mRNA levels are negatively associated with insulin resistance and ex vivo lipolysis. Estradiol treatment of SAT downregulates OCT3 mRNA levels, which may in part explain lower OCT3 mRNA and protein levels in premenopausal compared with postmenopausal women. This suggests that high OCT3 levels, resulting in increased catecholamine degradation, may partly account for the reduction in adipose tissue lipolysis observed with aging.

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Disclosures

All authors have no potential conflict of interest to declare.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References


