OCT1 expression in adipocytes could contribute to increased metformin action in obese subjects

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Objective: Metformin has been well characterized in vitro as a substrate of liver-expressed organic cation transporters (OCTs). We investigated the gene expression and protein levels of OCT-1 and OCT-2 in adipose tissue and during adipogenesis and evaluated their possible role in metformin action on adipocytes.

Research Design and Methods: OCT1 and OCT2 gene expressions were analyzed in 118 adipose tissue samples (57 visceral and 61 subcutaneous depots) and during human pre-adipocyte differentiation. To test the possible role of OCT1 mediating the response of adipocytes to metformin, co-treatments with cimetidine (OCT blocker, 0.5 and 5 mM) and metformin were made on human pre-adipocytes and subcutaneous adipose tissue (SAT).

Results: OCT1 gene was expressed in both subcutaneous and visceral adipose tissue. In both fat depots, OCT1 gene expression and protein levels were significantly increased in obese subjects. OCT1 gene expression in isolated preadipocytes significantly increased during differentiation in parallel to adipogenic genes. Metformin (5mM) decreased the expression of lipogenic genes and lipid droplets accumulation while increasing AMPK activation, preventing differentiation of human pre-adipocytes. Co-treatment with cimetidine restored adipogenesis. Furthermore, metformin decreased IL-6 and MCP-1 gene expression in comparison with differentiated adipocytes. Metformin (0.1 and 1 mM) decreased adipogenic and inflammatory genes in SAT. OCT2 gene expression was not detected in adipose tissue and was very small in isolated preadipocytes, disappearing during adipogenesis.

Conclusions: OCT1 gene expression and protein levels are detectable in adipose tissue. Increased OCT1 gene expression in adipose tissue of obese subjects might contribute to increased metformin action in these subjects.

Metformin (dimethylbiguanide) is the most widely used drug for the treatment of type 2 diabetes mellitus (1,2). This insulin-sensitizing agent has well known beneficial effects not only on glycemic control but also on the cardiovascular system. In the Diabetes Prevention Program, treatment with metformin reduced the incidence of type 2 diabetes by 31 percent. Interestingly, metformin was less effective in persons with a lower baseline body mass index (BMI) than in those with obesity (3). The reason for this observation is unknown. Shikata et al. also found that BMI was a strong predictor of metformin effects: the higher the BMI, the higher the response (4).

Metformin has been well characterized in vitro as a substrate of organic cation transporters (OCTs) (5-9). Members of the OCT family play essential roles in the handling of cationic drugs and endogenously synthesized organic cations. Human solute carrier family 22 (organic cation transporter), member 1 (OCT1) is expressed primarily in the liver, localized in the basolateral membrane of hepatocytes, mediating the hepatic uptake of several cationic drugs (metformin, as well as cimetidine, desipramine, midazolam, citalopram, or clonidine). OCT1 has been reported to be necessary for metabolic activities of metformin in liver cell lines (10). In fact, different polymorphisms in the OCT1 gene have been associated with metformin action (3).

To our knowledge, OCT1-dependent metformin activity on other cells has not been
previously studied. In addition to the liver-specific \textit{OCT1}, its paralog, human solute carrier family 22 (organic cation transporter), member 2 (OCT2) is a transporter expressed in abundance in the kidney (7,8).

The most prominent feature of obesity is increased fat mass. Despite the important observed effects of metformin in obesity, there is relatively scarce information in \textit{in vitro} models. Metformin effects have been evaluated in the murine 3T3-L1 cell line, in which an inhibition of adipogenesis was found (11-13). To our knowledge, the effects of this drug on adipogenesis has not been tested in human preadipocytes despite the potentially important mechanistic effects. Even though, the effects of metformin (1 mM, during 24h) increasing glucose intake in subcutaneous and visceral human adipocytes has been reported (14).

We first observed that metformin inhibited the differentiation of human adipocytes, decreasing the expression of different lipogenic genes. For this reason, we hypothesized that \textit{OCT1} was mediating these effects. In fact, the inhibition of this transporter using cimetidine reversed the blunted differentiation induced by metformin.

Lastly, we evaluated the potential \textit{in vivo} importance of these observations by studying the expression of \textit{OCT1} in human adipose tissue.

\textbf{RESEARCH DESIGN AND METHODS}

\textbf{Differentiation of human subcutaneous pre-adipocytes.} Isolated subcutaneous preadipocytes from lean (BMI<25) and obese (BMI<30) subjects (\textit{Zen-Bio Inc.}, Research Triangle Park, NC, USA) were plated on T-75 cell culture flasks and cultured at 37°C and 5\% CO2 in DMEM/Nutrient Mix F-12 medium (1:1, v/v) supplemented with 10U/mL P/S, fetal bovine serum (FBS) 10\%, HEPES 1\% and Glutamine 1\% (all from \textit{GIBCO}, Invitrogen S.A, Barcelona, Spain). One week later the isolated and expanded human subcutaneous pre-adipocytes were cultured (~40,000 cells/cm2) in 12-well plates with pre-adipocytes medium (PM, \textit{Zen-Bio Inc.}, Research Triangle Park, NC, USA) composed of DMEM/Nutrient Mix F-12 medium (1:1, v/v), HEPES, FBS, Penicillin and Streptomycin in a humidified 37°C incubator with 5\% CO2. Twenty-four hours after plating, cells were checked for complete confluence (day 0th) and differentiation was induced using differentiation medium (DM, \textit{Zen-Bio Inc. Research Triangle Park, NC, USA}) composed of PM, human insulin, Dexamethasone (DXM), Isobutylmethylxanthine (IBMX) and PPAR\textsubscript{\gamma} agonists (rosiglitazone). After 7 days (day 7th), DM was replaced with fresh adipocyte medium (AM, \textit{Zen-Bio Inc. Research Triangle Park, NC, USA}) composed of DMEM/Nutrient Mix F-12 medium (1:1, v/v), HEPES, FBS, Biotin, Panthothenate, human insulin, Dexamethasone (DXM), Penicillin, Streptomycin and Amphotericin. Negative control (non-differentiated cell) was performed with pre-adipocyte medium during all differentiation process. Fourteen days after the initiation of differentiation, cells appeared rounded with large lipid droplets apparent in the cytoplasm. Cells were then considered mature adipocytes, harvested and stored at -80°C for RNA extraction to study \textit{OCT1} and \textit{OCT2} gene expression levels after human adipocyte differentiation. Non-differentiated control was performed using proliferation medium along 14 days. Metformin (Sigma, Barcelona, Spain) (5 mM), Cimetidine (Sigma, Barcelona, Spain) (0.5 mM and 5 mM) and metformin and cimetidine co-incubations were performed with differentiation medium along 14 days. The experiment was performed in triplicate for each sample. The differentiation was monitored with the fatty acid synthase (FASN, \textit{Hs00188012_m1}, Applied Biosystems Inc, Madrid, Spain), adiponectin (\textit{Adipoq}, \textit{Hs00605917_m1}, Applied Biosystems Inc,
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Madrid, Spain), acetyl-Coenzyme A carboxylase alpha (ACCI, Hs00167385_m1, Applied Biosystems Inc, Madrid, Spain), peroxisome proliferator-activated receptor gamma (PPARγ, Hs00234592_m1, Applied Biosystems Inc, Madrid, Spain), fatty acid binding protein 4, adipocyte (FABP4, HS00609791_m1, Applied Biosystems Inc, Madrid, Spain), interleukin 6 (IL6, Hs00985639_m1, Applied Biosystems Inc, Madrid, Spain), monocyte chemoattractant protein-1 (MCP1, Hs00234140_m1, Applied Biosystems Inc, Madrid, Spain) expression. Ex-vivo experiments using subcutaneous adipose tissue explants. Subcutaneous adipose tissue was obtained from 6 obese subjects undergoing open abdominal surgery (gastrointestinal by-pass) under anesthesia after an overnight fast. The mean age was 46 ± 6.4 years (range 39 to 58 years) and the BMI 44.9 ± 12.4 Kg/m^2. Medical histories, physical examinations, electrocardiogram and blood screening showed that all patients were in good health. None of the subjects had a history of hepatic or renal disorders. The study had the approval of the Ecitcal Committee and all patients gave informed written consent.

Samples of subcutaneous adipose tissue were immediately transported to the laboratory (5-10 min). The handling of tissue was carried out under strictly aseptic conditions. The tissue was cut with scissors into small pieces (5-10 mg), and incubated in buffer plus albumin (3ml/g of tissue) for approximately 5-30 min. After incubation, the tissue explants were centrifuged for 30 s at 400 g. Then 100 mg of minced tissue was placed into 1 ml M199 (Gibco, Invitrogen) containing 10 % fetal bovine serum (Hyclone, Thermo Fisher Scientific Inc.), 100 unit/ml penicillin (Gibco, Invitrogen), 100 ug/ml streptomycin (Gibco, Invitrogen) and incubated for 48h in suspension culture under aseptic conditions (15). Control treatment (M199) and metformin (Sigma, Barcelona, Spain) (0.1 and 1 mM) were compared. After 48 h, all samples were immediately flash-frozen in liquid nitrogen before stored at -80°C. To evaluate cell integrity, lactate dehydrogenase activity released from damaged cells was analyzed by Cytotoxicity Detection Kit (lactate dehydrogenase, LDH) (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions in all treatments. OCT1, ACC1, FASN, Adipoq, PPARγ, IL-6 and MCP-1 relative gene expression were analysed using TaqMan® technology suitable for relative genetic expression quantification (described below). Human adipose tissue samples. A group of 118 adipose tissue samples (57 visceral and 61 subcutaneous depots), from participants with a body mass index (BMI) within 20 and 68 kg/m^2, who were recruited at the Endocrinology Service of the Hospital Universitari Dr. Josep Trueta (Girona, Spain), were analyzed. All subjects were of Caucasian origin and reported that their body weight had been stable for at least three months before the study. Liver and renal diseases were specifically excluded by biochemical work-up. All subjects gave written informed consent after the purpose of the study was explained to them. Adipose tissue samples were obtained from subcutaneous and visceral depots during elective surgical procedures (cholecystectomy, surgery of abdominal hernia and gastric by-pass surgery). All samples were washed, fragmented and immediately flash-frozen in liquid nitrogen before stored at -80°C. To perform the isolation of adipocyte and SVF, tissues were washed three to four times with phosphate-buffered saline (PBS) and suspended in an equal volume of PBS supplemented with 1% bovine serum and 0.1% collagenase type I prewarmed to 37°C. The tissue was placed in a shaking water bath at 37°C with continuous agitation for 60 minutes and centrifuged for 5 minutes at 300
to 500g at room temperature. The supernatant, containing mature adipocytes, was recollected. The pellet was identified as the SVF cell. The adipose tissue fractionation was performed from 8 subcutaneous fat depots.

**RNA expression.** To study gene expressions, RNA was prepared from these samples using RNeasy Lipid Tissue Mini Kit (QIAgen, Izasa SA, Barcelona, Spain). The integrity of each RNA sample was checked by Agilent Bioanalyzer (Agilent Technologies, Palo Alto, CA). Total RNA was quantified by means of spectrophotometer (GeneQuant, GE Health Care, Piscataway NJ) reverse transcribed to cDNA using High Capacity cDNA Archive Kit (Applied Biosystems Inc, Madrid, Spain) according to the manufacturer’s protocol.

Gene expression was assessed by real time PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems Inc, Madrid, Spain), using TaqMan® technology suitable for relative genetic expression quantification.

The commercially available and pre-validated TaqMan® primer/probe sets used were as follows: endogenous control PPIA (4333763, cyclophilin A, Applied Biosystems Inc, Madrid, Spain) and target gene Human Organic Cationic Transporter – 1 and Human Organic Cationic Transporter – 2 (OCT1, Hs00427552_m1; and OCT2, Hs00161893_m1, Applied Biosystems Inc, Madrid, Spain). The RT-PCR TaqMan® reaction was performed in a final volume of 25µl. The cycle program consisted of an initial denaturing of 10min at 95°C then 40 cycles of 15 sec denaturizing phase at 95°C and 1min annealing and extension phase at 60°C. A threshold cycle (Ct value) was obtained for each amplification curve and a ΔCt value was first calculated by subtracting the Ct value for human *Cyclophilin A* (PPIA) RNA from the Ct value for each sample. Fold changes compared with the endogenous control were then determined by calculating $2^{-\Delta\text{Ct}}$, so gene expression results are expressed as expression ratio relative to PPIA gene expression according to manufacturers’ guidelines.

**Western blot analysis.** Adipose tissue lysates (from 8 subcutaneous fat depots) were washed in ice-cold PBS followed by homogenization assay using RIPA lysis buffer (Millipore, Madrid, Spain) supplemented with a protease inhibitor cocktail (Sigma-Aldrich, Madrid, Spain) at 4°C for 30 min. Cellular debris were eliminated by centrifugation of the diluted samples at 14000 g for 30 min (4°C). Protein concentration was determined using Lowry assay. RIPA protein extracts (50 µg) were separated by SDS-PAGE and transferred to nitrocellulose membranes by conventional procedures. Membranes were immunoblotted with OCT1, FASN and β-actin antibodies (Santa Cruz Biotechnology,inc., CA, U.S.A). Anti-rabbit IgG and anti-mouse IgG coupled to horseradish peroxidase (HRP) was used as secondary antibody. HRP activity was detected by chemiluminescence and quantification of protein expression was performed using scion image software.

**AMPK and ACC activity.** AMPK activity was determined measuring pThr172AMPK by ELISA (KHO0651, Invitrogen, Barcelona, Spain). pThr172AMPK is directly associated with AMPK activity. According to the manufacturer, the analytical sensitivity of this assay is <1 Unit/mL of AMPKa [pT172]. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times. The average recovery was 90% The specificity of this assay for phosphorylated AMPKa [pT172] was confirmed by peptide competition. Intra- and interassay coefficients of variation for all these determinations were between 5-10%.

ACC activity was calculated measuring pSer79ACC1 and ACC1 (Total) by ELISA (KHO1061 and KHO1071, respectively,
Invitrogen, Barcelona, Spain). pSer79ACC1 is inversely associated with ACC activity. The analytical sensitivity of these assays are < 0.5 Units/mL of human ACC1 [pS79] and human ACC1, respectively. These were determined by adding two standard deviations to the mean O.D. obtained when the zero standards was assayed 30 times. The percent recovery was calculated as an average of 93% and 97 %, respectively. The specificity of this assay for phosphorylated ACC1 [pS79] was confirmed by peptide competition. Intra- and interassay coefficients of variation for all these determinations were between 5-10%.

**Oil Red staining and analysis.** Differentiation was monitored by morphological assessment and Oil red O staining. For oil red staining, cells were washed twice with PBS, fixed in 4 % formaldehyde for 1 h, and stained for 30 min with 0.2 % oil red O solution in 60 % isopropanol. Cells were then washed several times with water, and excess water was evaporated by placing the stained cultures at approximately 32 ºC. In order to determine the extent of adipose conversion, 0.2 ml of isopropanol was added to the stained culture dish. The extracted dye was immediately removed by gentle pipeting and its optical density was monitored spectrophotometrically at 500 nm using a multi-well plate reader (Model Anthos Labtec 2010 1.7 reader).

**Cell counting and LDH activity assay.** Cell counting was assessed by trypan blue dye exclusion using a Neubauer hemacytometer, after 14 days differentiation of human subcutaneous preadipocytes, in triplicate. To evaluate cell integrity, LDH activity released from damaged cells was analyzed by Cytotoxicity Detection Kit (LDH) (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions.

**Statistical analyses.** Statistical analyses were performed using SPSS 12.0 software. Unless otherwise stated, descriptive results of continuous variables are expressed as mean and SD for Gaussian variables, or median and interquartile range. Parameters that did not fulfill normal distribution were mathematically transformed to improve symmetry for subsequent analyses. The relation between variables was analyzed by simple correlation (Spearman’s test). Unpaired and paired t-test was used to compare clinical variables and OCT-1 and -2 gene expressions according to obesity status. Non-parametric tests, Mann Whitney U and Wilcoxon’s tests, were used to evaluate the effects of metformin and cimetidine treatments in vitro and ex vivo. The experiments were performed in triplicate.

**RESULTS**

**OCT-1 and OCT-2 expression during differentiation of human pre-adipocytes.** The differentiation process was monitored through FASN, ACC1, PPARγ and Adipoq gene expression (Figure 1), with accumulation of lipid droplets in the cytoplasm.

In isolated preadipocytes from lean and obese subjects, OCT1 gene expression was significantly increased during the differentiation process in parallel to adipogenic genes (Figure 1). In differentiated pre-adipocytes OCT1 protein levels were significantly higher than in non-differentiated pre-adipocytes (Figure 2). OCT2 gene expression was very small and disappeared during adipogenesis.

**Metformin effects on adipogenesis and AMPK activity.** Metformin (5 mM) decreased the expression of lipogenic genes (Figure 3) and lipid droplets accumulation (Figure 4), leading to impaired differentiation of human
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pre-adipocytes. Co-treatment with cimetidine restored adipogenesis (Figure 3). Proinflammatory molecules, like as IL-6 and MCP-1, decreased significantly during adipocyte differentiation. Even though, metformin administration did not increase IL-6 and MCP-1 gene expression in comparison with differentiated adipocyte. The decreased levels of expression of proinflammatory molecules were maintained with cimetidine cotreatment. High doses of cimetidine (5mM) decreased significantly adiponectin gene expression (Figure 3). OCT1 gene expression did not change significantly after metformin or cimetidine treatments (Figure 3).

Metformin treatment increased significantly AMPK activity, increasing \( p^{172\text{Thr}} \)AMPK and consequently \( p^{79\text{Ser}} \)ACC1. In cimetidine coinubation, the increase of AMPK activity was blunted (Figure 5). The total ACC1 quantification showed a similar expression pattern than \( ACC1 \) gene expression.

LDH activity was measured to evaluate the cytotoxicity in each of the treatments. No significant difference was found comparing undifferenciated and differenciated adipocytes, whether treated or untreated with cimetidine (0.5 mM and 5 mM). After metformin (5 mM) treatment, LDH activity tended to be higher compared with differentiated adipocytes (Figure 4).

**Metformin effects in subcutaneous adipose tissue explants.** Metformin (0.1 and 1 mM) led to decreased adipogenic and inflammatory gene expression (Figure 6). OCT1 gene expression was not affected by metformin administration (Figure 6).

In addition, baseline OCT1 gene expression was associated with the metformin-induced adipogenic gene expression reduction (\( ACC1 \), Adipoq, FASN and \( PPAR \gamma \) ) , suggesting that the higher the OCT-1 gene expression, the higher the effects of metformin (for 0.1 mM, \( r= -0.76 \), \( p=0.07 \), \( r= -0.94 \), \( p=0.004 \), \( r= -0.88 \), \( p=0.02 \) and \( r= -0.64 \), \( p=0.17 \), respectively).

Treatment of adipose tissue with metformin (0.1 and 1 mM) did not change lactate dehydrogenase activity (cell integrity) in comparison with control treatment (Figure 6).

**OCT1 and OCT2 expression in human adipose tissue.** Anthropometric and clinical characteristics of all participants are shown in Table 1. OCT1 gene was similarly expressed in subcutaneous and visceral adipose tissue [0.002 (0.0005-0.0034) vs. 0.0015 (0.0004-0.003) R.U., \( p=0.4 \), \( n=31 \)]. In fact, the expression of \( OCT1 \) in both fat depots correlated significantly (\( r= 0.54 \), \( p<0.01 \)). The relative OCT1 gene expression was lower compared with lipogenic genes (100- and 10-fold decreased in comparison with FASN and \( ACC1 \) gene expression). In both subcutaneous and visceral fat depots, OCT1 gene expression correlated significantly with body mass index (BMI) (\( r=0.46 \), \( p<0.01 \) and \( r=0.47 \), \( p<0.01 \), respectively) and percent fat mass (\( r=0.36 \), \( p=0.05 \) and \( r=0.49 \), \( p<0.01 \), respectively) (Figure 7). In addition, OCT1 gene expression correlated significantly with diastolic blood pressure (\( r=0.35 \), \( p=0.04 \)) in visceral adipose tissue. No associations were detected with other metabolic parameters (age, systolic blood pressure, fasting glucose, fasting triglycerides, HDL-cholesterol, and LDL-cholesterol).

There is evidence indicating that mRNA levels may not necessarily predict the translated protein levels. In this regard, we measured OCT1 protein in adipose tissue by western blot. OCT1 protein was significantly increased in obese subjects (Figure 8A).

To gain insight in the type of cells from adipose tissue that expressed OCT1, we analysed OCT1 gene expression in stromal-vascular cells (SVCs) and mature adipocytes from subcutaneous adipose tissue. In SVCs OCT1 gene expression was significantly
higher than in mature adipocytes (1.8-fold increased, p=0.01) (Figure 8B).

\( \text{OCT2} \) gene was not significantly expressed in human adipose tissue.

**DISCUSSION**

Metformin has been described to be more effective in obese subjects and the degree of obesity has been found to constitute a strong predictor of metformin effects (3,4). The main findings of this study are: 1) \( \text{OCT1} \) gene expression was detectable in whole adipose tissue (similarly in the subcutaneous and visceral fat depots) and in isolated adipocytes; 2) this expression increased significantly with adipocyte differentiation in association with lipogenic (\( \text{FASN, ACC, PPAR}_\gamma \)) and adipogenic (\( \text{Adipoq} \)) genes; 3) metformin (5 mM) blunted the adipocyte differentiation of human preadipocytes in parallel to decreasing significantly the expression of proinflammatory mediators; 4) blocking metformin action using cimetidine reversed these effects. 5) In ex-vivo experiments, metformin led to decreased adipogenic and proinflammatory gene expression. To the best of our knowledge, this is the first study evaluating metformin effects and \( \text{OCT1} \) gene expression in human adipocytes. The increased \( \text{OCT1} \) gene expression in SVCs compared with adipocytes suggests that metformin action on SVCs might contribute to systemic effects. Considering the importance of \( \text{OCT1} \) in the metformin response (9), we suggest that the higher \( \text{OCT1} \) gene expression in obese subjects is behind the increased metformin effects in these subjects. Interestingly we found that baseline \( \text{OCT1} \) gene expression was associated with the metformin-induced adipogenic gene expression reduction (\( \text{ACC1, Adipoq, FASN} \) and \( \text{PPAR}_\gamma \)), suggesting that the higher the \( \text{OCT-1} \) gene expression, the higher the effects of metformin.

The inhibitory effects of metformin on adipogenesis have been previously shown in the 3T3-L1 cell line (11-13) via AMPK activation. However, to our knowledge, these actions have not been explored in human preadipocytes. In the current study, metformin (5 mM) decreased significantly the expression of adipogenic (\( \text{FASN, ACC, PPAR}_\gamma, \text{adipoq} \)) genes and the formation of lipid droplets, increasing AMPK activity (\( \text{p}^{172} \text{Thr}\text{AMPK and consequently p}^{795} \text{Ser} \text{ACC1} \)). The adipocyte differentiation (increasing lipogenic gene expression and decreasing AMPK activity) was restored dose-dependently when the \( \text{OCT1} \) blocker agent cimetidine was used as a co-treatment.

In agreement with our data, the response to metformin was inhibited in mice models in which the \( \text{OCT1} \) gene was deleted (\( \text{OCT1}^{-/-} \)). Subjects carrying a single nucleotid polymorphism associated with a decrease in \( \text{OCT1} \) gene expression also showed decreased metformin effects (10,16).

Importantly, metformin administration decreased significantly IL-6 and MCP-1 gene expression in adipocytes and in adipose tissue explants. Recently, metformin has been shown to display anti-inflammatory effects in endothelial cells by inhibiting TNF-\( \alpha \)-induced IKK\( \alpha/\beta \) phosphorylation, IkappaB-\( \alpha \) degradation and IL-6 production (17,18).

The mode of action of metformin has yet to be fully established. In muscle, liver and endothelial cells, the metabolic changes induced by metformin appear to be mediated by the AMP-activated protein kinase (AMPK). AMPK acts as a sensor of the cellular energy status, being switched on by an increased ATP demand or by processes that interfere with ATP production like ischaemia. The activated form of AMPK switches on catabolic pathways while switching off ATP-consuming processes (19).

It has been reported that metformin binds to complex I of the mitochondrial respiratory chain and this could in part explain how this drug acts (20). The inhibition of complex I would cause a decrease in energy supply that
would in turn lead to a higher AMP/ATP ratio, and the concomitant activation of AMPK. It seems counterintuitive that metformin is providing a beneficial effect by attenuating adipocyte differentiation. However, while metformin has been associated with weight loss, glitazones lead to increased adipocyte differentiation and weight gain. Metformin stimulates catabolic pathways in white adipose tissue through the activation of AMPK, reducing the triglyceride stores as reflected by the smaller size of the adipocytes (13,21). These effects are achieved through an increase in lipolysis and β-oxidation, which would imply that there is no release of fatty acids and that they are oxidized within the adipocyte. Other authors have described that metformin inhibited adipocyte differentiation (using rat mesenchymal stem cells) (22). We here propose that OCT1 density in adipose tissue is a factor that can significantly influence all these effects of metformin. Furthermore, Fisher et al. have shown that metformin induces glucose uptake independent of insulin in subcutaneous and visceral human adipocytes (14).

In conclusion, OCT1 gene expression and protein levels are detectable in adipose tissue. The increased OCT1 gene expression in adipose tissue of obese subjects might contribute to increased metformin action in these subjects.


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Table 1. Anthropometrical and biochemical variables of study participants.

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Values are mean ± SD
*Comparing obese subjects vs. non-obese. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

FIGURE LEGENDS

Figure 1. OCT1, FASN, ACC1, PPARγ and adiponectin gene expression during differentiation of human preadipocytes from obese (A) and lean subjects (B) *P< 0.05 vs. day 0.

Figure 2. OCT1 and FASN protein levels in differentiated and non-differentiated preadipocytes at day 14. *P<0.05 vs non-differentiated preadipocytes.

Figure 3. FASN, ACC1, PPARγ, adiponectin, OCT1, FABP4, IL-6, MCP-1 gene expressions after metformin (5 mM) and cimetidine (0.5 and 5 mM) co-treatments in differentiated human adipocytes. *P< 0.05 vs. differentiated adipocytes; +P<0.05 vs. metformin (5 mM) treatment.

Figure 4. Oil red O staining and LDH activity in differentiated human adipocytes, and after metformin (5 mM) and cimetidine (0.5 and 5 mM) co-treatment. *P< 0.05 vs. differentiated adipocytes; +P<0.05 vs. metformin (5 mM) treatment.

Figure 5. Effects of metformin (5 mM) and cimetidine (0.5 and 5 mM) co-treatment on p172Thr AMPK, p79Ser ACC1 and ACC1 concentrations. *P< 0.05 vs. differentiated adipocytes; +P<0.05 vs. metformin (5 mM) treatment.

Figure 6. FASN, ACC1, PPARγ, adiponectin, OCT1, IL-6, MCP-1 gene expressions after metformin (0.1 and 1 mM) in subcutaneous adipose tissue explants. *P< 0.05 vs. Control (vehicle) treatment.

Figure 7. OCT1 relative gene expression in both visceral and subcutaneous adipose tissue according to obesity status.

Figure 8. A) OCT1 protein levels in subcutaneous adipose tissue according to obesity status. Non-normalized OCT1 protein and normalized for β-Actin values (relative levels) are shown. B) OCT1 gene expression in stromal-vascular cells and mature adipocytes from subcutaneous adipose tissue.
Figure 1

A

B

Figure 2

FASN protein levels (β-Actin (R.U.))

Differentiated preadipocytes  Non-differentiated preadipocytes

β-Actin (45 kDa)

OCT-1 (60 kDa)

FASN (272 kDa)
Figure 3
Figure 4

OCT-1 and metformin action in adipocytes
Figure 5

[Bar chart showing pThr172 AMPK (U/ml), pSer79 ACC (U/ml), and total ACC (ng/ml) with different conditions: Differentiation, Metformin (5 mM), Cimetidine (0.5 mM), Cimetidine (5 mM).]
Figure 6
OCT-1 and metformin action in adipocytes

Figure 7

Figure 8

A

B