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Mechanisms of metformin action on glucose transport and metabolism in human adipocytes

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ABSTRACT

The mechanisms of metformin effects on glucose transport and metabolism were investigated in human adipocytes. Human preadipocytes obtained from surgical biopsies were differentiated in vitro into adipocytes and the effects of metformin on glucose uptake, glucose oxidation and the involved signaling pathways were analyzed. Metformin (1 mM, 24 h) increased glucose uptake 2.3 ± 0.2-fold ($p < 0.001$ vs. basal) in human adipocytes, without altering cell viability and oxygen consumption. Metformin did not alter GLUT-1 mRNA expression and protein content but increased GLUT-4 mRNA expression and cellular protein content, leading to increased GLUT-4 protein content in the plasma membrane. Neither basal nor insulin-induced phosphorylation of Akt at Ser-473 and AS160 (Akt substrate of 160 kDa) at Thr-642 were enhanced by metformin. Suppression of metformin-induced AMP-activated protein kinase (AMPK) activity by AMPKα1 silencing, however, reduced metformin-associated GLUT-4 expression and stimulation of glucose uptake. In addition, metformin induced glucose oxidation. In conclusion, activation of AMPKα1 without impairment of cell respiration is crucial for metformin-mediated increase in GLUT-4 protein content and glucose uptake in human adipocytes.

1. Introduction

Metformin, the most frequently used antidiabetic drug worldwide, improves peripheral glucose uptake and reduces hepatic glucose in patients with type 2 diabetes mellitus (T2DM). Metformin is used as monotherapy or in combination with other antidiabetic agents including insulin [1,2]. AMP-activated protein kinase (AMPK) is a regulator of cellular and systemic energy homeostasis and the activation of this enzyme by metformin provides an explanation for the beneficial effects of this drug in inhibiting glucose output from hepatocytes and in inducing glucose uptake in myocytes [3,4].

GLUT-1 and GLUT-4, the two main glucose transporter isoforms expressed in adipose tissue, mediate glucose uptake. GLUT-1 is located predominantly in the plasma membrane. In the basal state, GLUT-4 is stored in storage vesicles but upon insulin stimulation it is translocated to the plasma membrane, inducing glucose uptake [5]. Insulin triggers GLUT-4 translocation by incompletely defined complex intracellular signaling pathways that include the classical phosphatidylinositol 3′ kinase (PI3K)/Akt/AS160 pathway [6].

Recently, we reported that metformin induces glucose uptake in human preadipocyte-derived adipocytes from obese and non-obese patients [7]. In the present study, we investigated the mechanisms of metformin action on the Akt signaling pathway, glucose transporters and glucose uptake, as well as its effect on cell viability, cell respiration and glucose oxidation in these human adipocytes.

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2. Materials and methods

2.1. Cell culture and treatment

The study was approved by the local Ethics Committee. Subcutaneous fat tissue samples were obtained from 12 non-diabetic, six non-obese (five males, one female; aged 64 ± 6 years; BMI 22.5 ± 0.8 kg/m^2) and six obese (two males, four females; aged 50 ± 8 years; BMI 32.8 ± 1.5 kg/m^2) patients undergoing abdominal surgery. Mature isolated adipocytes (floating cells) were directly used, whereas the stromal-vascular cell fraction containing pre-adipocytes was isolated and the preadipocytes were expanded in vitro in DMEM (Lonza, Verviers, Belgium) containing 10% FCS and 5 ng/ml 2-FGF (Lubio Science, Luzern, Switzerland) until confluence, and subjected to adipogenic differentiation medium for 14 days, as previously described [7].

After differentiation, adipocytes were washed twice with PBS and kept for 48 h in low-glucose (5 mM) medium: DMEM supplemented with nutrient mixture F-12 (1.8 g/l glucose), L-ascorbic acid (100 nM), biotin (8 μg/ml), D-pantothenate (15 mM, Sigma–Aldrich, Buchs, Switzerland), gentamycin (50 μg/ml), HEPES (15 nM) and 3% FCS (Lubio Science). Adipocytes were then left untreated or treated with metformin (from 0.001 to 10 mM, Sigma–Aldrich) for 24 h, followed by stimulation with phloretin (50 μM, Sigma–Aldrich) and/or insulin (100 nM, Actrapid, Novo-Nordisk Pharma, Küsnacht, Switzerland) for 20 min.

2.2. Assessment of cell viability using flow cytometry

After treatment, preadipocyte-derived adipocytes were incubated with 2 μg/ml BODIPY™ 493/503 (Lubio Science) diluted in PBS for 20 min at room temperature (RT), washed with PBS and harvested with 0.5 g/l EDTA (Sigma–Aldrich) diluted in PBS. Cells were resuspended in PBS + 0.5% BSA (Sigma–Aldrich) and propidium iodide (PI) (Lubio Science) was added to yield a final concentration of 4 ng/ml. Cells were analyzed by flow cytometry (Beckman Coulter's CYAN™ Flow Cytometer, Allschwil, Switzerland) using Summit 4.3 software (Beckman Coulter). A 488-nm Laser was used for excitation and emission was collected at 530/40 nm for BODIPY™ and at 613/20 nm for PI.

2.3. Oxygen consumption rate

Preadipocytes were cultured and if necessary differentiated in XF24 Seahorse Bioscience cell culture plates (Bucher Biotec AG, Basel, Switzerland). After 48 h in low-glucose medium followed by 24 h treatment with metformin, cell medium was changed to unbuffered low-glucose DMEM containing the same treatment as during the previous 24 h. The plate was placed in a CO_2-free incubator at 37 °C for 1 h. According to the manufacturer's instructions, the XF24 sensor cartridge was placed in a Seahorse 24-well plate containing Seahorse XF24 calibrant (pH 7.4) (Bucher Biotec AG) and stored overnight at 37 °C without CO_2 was loaded in the Seahorse XF24 analyzer (Bucher Biotec AG). After calibration, the cell plate was loaded in the analyzer and oxygen consumption was measured over 1 h. Metformin-treated wells were compared with control untreated wells in each plate analyzed.

2.4. Glucose uptake

After treatment, 1 μCi deoxy-D-glucose, [2-^{3}H(G)] (Perkin Elmer, Schwerzenbach, Switzerland) was added to each well and allowed to incubate for 15 min. The preadipocyte-derived adipocytes were then washed with PBS and lysed in 0.1% sodium dodecyl sulfate (Sigma–Aldrich). Radioactivity was measured in a betamatic liquid scintillation counter [7]. This protocol was also applied with minor modification to mature isolated adipocytes. In brief, these cells were incubated on a shaker (100 rpm) in Krebs Ringer Buffer (pH 7.4) supplemented with 1% BSA (Sigma–Aldrich) and 1 mM glucose (Sigma–Aldrich). 1 μCi deoxy-D-glucose, [2-^{3}H(G)] was added for 15 min as described above. After the addition of dinonyl phthalate (Sigma–Aldrich), the fat layer containing mature adipocytes was collected by centrifugation and the radioactivity measured.

2.5. Glucose oxidation rate

The glucose oxidation rate was measured by the method described in the literature [8]. Preadipocytes were cultured and differentiated into adipocytes in 25 cm^2 flasks. Four hours before the end of the treatment, 2.5 μCi of [U-^{14}C]-glucose (Hartmann Analytic, Göttingen, Germany) was added to each flask and a $^{14}CO_2$-collecting device was tightly attached to the culture flask. 1 ml of 5 N sulfuric acid was added to the flask through the septum to stop metabolism and drive off all the CO_2. 30 min later, the scintillation pad saturated with 150 μl of aminoethanol (Sigma–Aldrich) was removed from the filter paper rest and the adsorbed $^{14}CO_2$ radioactivity was measured with a liquid scintillation counter.

2.6. Confocal microscopy

Preadipocytes were grown and differentiated on sterile coverslips. Adipocytes were washed and fixed on ice for 15 min with 4% PFA (Sigma–Aldrich). After blocking (PBS + 1% BSA for 15 min), cells were incubated for 30 min at RT with TRITC-concanavalin A (1:500 in PBS + 1% BSA; Lubio Science). Adipocytes were incubated overnight at 4 °C with anti-GLUT-4 polyclonal antibody (1:200 in PBS + 1% BSA; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The coverslips were then washed and incubated for 1 h at RT with Alexa 488-conjugated anti-rabbit antibody (1:500 in PBS + 1% BSA) from Lubio Science and mounted in Vectashield mounting medium (Reactolab, Servion, Switzerland). Fluorescence was viewed under a confocal laser scanning Axiovert S100 microscope (Zeiss, Feldbach, Switzerland). Evaluation of GLUT-4/concanavalin A co-localization intensity was performed using LSM 510 software (Zeiss).

2.7. AMPKα1 silencing

Preadipocyte-derived adipocytes were transfected with 2 μM validated human ACCELL™ SMARTpool siRNA targeting the PRKAA1 (AMPKα1) gene using ACCELL™ delivery media (Thermo Scientific, Lafayette, CO, USA) and following the manufacturer's instructions. ACCELL™ non-targeting pool (Thermo Scientific) was used as control siRNA. Silencing was performed for 72 h and metformin treatment was added to the ACCELL™ delivery media (Thermo Scientific) for additional 24 h.

2.8. Quantitative analysis of GLUT-1, GLUT-4 and AMPK mRNA expression

RNA was isolated and 1 μg total RNA was subjected to reverse transcription-PCR. cDNA was subjected to quantitative real-time PCR analysis using the power SYBR™-Green PCR master mix (Applied Biosystems, Rotkreuz, Switzerland) and the ABI 7500 Sequence detection system [9]. Optimal sets of primers were designed for each studied gene using the "ProbeFinder" (Roche Applied Bioscience, Rotkreuz, Switzerland) website interface (http://