Inverse relationship between body mass index and mitochondrial oxidative phosphorylation capacity in human subcutaneous adipocytes

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Fischer B, Schöttl T, Schempp C, Fromme T, Hauner H, Klingenspor M, Skurk T. Inverse relationship between body mass index and mitochondrial oxidative phosphorylation capacity in human subcutaneous adipocytes. Am J Physiol Endocrinol Metab 309: E380–E387, 2015. First published June 16, 2015; doi:10.1152/ajpendo.00524.2014.—Obesity is characterized by a substantial increase in adipose tissue that may contribute to energy balance. Recently, obesity was suggested to be associated with impaired mitochondrial function in adipocytes. In this study, we investigated the following: 1) the respiratory capacities of mitochondria isolated from mature adipocytes of female subjects whose body mass index (BMI) values were distributed over a wide range and 2) the amounts of electron transport chain complexes in these mitochondria. Fat cells were isolated from adipose tissue specimens by collagenase digestion. Mitochondria were isolated from these fat cells, and their respiratory capacity was determined using a Clark-type electrode. Fat cells were also sorted on the basis of their size into large and small fractions to assess their respiration. Western blot analyses were performed to quantify respiratory chain complex components. We also examined mitochondrial activity development during differentiation using human Simpson-Golabi-Behmel syndrome cells. Our results showed that mitochondrial respiratory capacities in adipocytes were inversely associated with BMI values but were independent of cell size. Western blot analyses revealed significantly fewer complex I and IV components in adipose tissues from obese compared with nonobese women. These results suggest that differences at the level of respiratory chain complexes might be responsible for the deterioration of respiratory capacity in obese individuals. In particular, electron transport at the level of complexes I and IV seems to be most affected.

ADIPOSE TISSUE IS AN IMPORTANT CONTRIBUTOR to the regulation of energy homeostasis. Although adipose tissue accounts for only about 4% of whole body energy turnover in normal-weight individuals (12), its contribution might increase considerably due to the increased adipose tissue mass associated with increasing obesity. It is well accepted that obesity is accompanied by adipocyte dysregulation, which is linked to abnormal adipokine secretion, an inflammatory status of adipose tissue, and ultimately to metabolic disorders like type 2 diabetes mellitus (reviewed in Ref. 11). Altered mitochondrial function in white adipose tissue has also been considered to be involved in abnormal metabolic states, as seen in obesity (37, 38).

Thermogenesis by adipocytes from obese donors was found to be reduced compared with that by adipocytes from lean donors (4). Moreover, obesity was found to be associated with the downregulation of transcription levels of genes that were involved in oxidative phosphorylation (OXPHOS) in white adipose tissue (25). However, other investigators did not find a link between the degree of obesity and electron transport chain gene transcription levels in adipose tissue (9). Nevertheless, basal oxygen consumption per gram of adipose tissue was found to be higher in lean subjects than in obese subjects (12).

In line with this, the maximal respiration rates of mitochondria isolated from small and large adipocytes were negatively correlated with body mass index (BMI) values (38). Furthermore, in vitro-differentiated preadipocytes from human subcutaneous adipose tissue of obese donors had lower oxygen consumption rates after isoproterenol stimulation compared with those from lean donors (37). However, the correlation between mitochondrial respiration in mature adipocytes and BMI remains largely unknown. Moreover, previous research did not address different mitochondrial respiration states and BMI values for unilocular fat cells.

In this study, we examined different OXPHOS states and variables associated with respiratory control of mitochondria that were isolated from subcutaneous abdominal adipocytes from women whose BMI values were distributed over a wide range. We also characterized mitochondrial respiration on the basis of different adipocyte sizes in a subpopulation. Because it is largely unknown which factors account for the differences in the respiratory rate in obese individuals, we also compared the amounts of respiratory chain component proteins in adipocytes from lean and obese women. To acquire further insights into mitochondrial function during adipocyte differentiation, we evaluated the respiratory of isolated mitochondria during Simpson-Golabi-Behmel syndrome (SGBS) cell differentiation, a human preadipocyte cell model.

Our results provide evidence for whether there are functional differences in the mitochondrial respiratory capacities of adipocytes from lean and obese subjects and between large and small adipocytes.

MATERIALS AND METHODS

Subjects. Subcutaneous adipose tissue was obtained from female patients who underwent elective abdominal surgery (Table 1). All subjects had no evidence of metabolic or infectious diseases and did not take any medication. Each subject provided written, informed consent and completed a detailed clinical and medical history.

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consent before these procedures. Our study protocol was approved by the ethics committee of the Technische Universität München. Adipose tissue from 16 subjects with an average age of 41 ± 13 yr was used for mitochondrial respiration experiments, adipose tissue specimens from five women with an average age of 41 ± 10 yr were used for mitochondrial respiration measurements based on cell size, and another 20 samples from female subjects with an average age of 43 ± 13 yr were used for Western blot analysis. Mitochondrial DNA (mtDNA) quantification was performed for a subsample of 17 subjects included in Western blot analyses.

Cell culture. Tissue samples were transported immediately from the operating room to our laboratory in Dulbecco’s modified Eagle’s medium (DMEM)-F-12 medium (Gibco, Thermo Fisher Scientific, Waltham, MA) supplemented with 1% penicillin-streptomycin (PAA Laboratories, Linz, Austria). For adipocyte isolation, tissues were dissected from connective tissue and visible blood vessels and minced with scissors. Fat pieces were digested in Krebs-Ringer phosphate (KRP) buffer that contained 100 U/ml collagenase (SERVA, Heidelberg, Germany) and 4% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO) at 37°C in a shaking water bath (60 cycles/min) for 60–90 min. Isolated cells were filtered twice through nylon meshes with pore sizes of 2,000 and 250 μm. Isolated adipocytes were washed three times with KRP + 0.1% BSA. The total cell fraction was either directly cultured in DMEM-F-12 (1:1 medium supplemented with 1% penicillin-streptomycin) or used for further fractionation based on cell size. Medium was changed 30 min later to reduce cell stress. Human SGBS preadipocytes (32) were cultured and differentiated as described previously (24).

Fractionation of mature fat cells based on cell size. Isolated adipocytes from lean and obese subcutaneous abdominal tissue (n = 5) were separated into very small cells (fraction I) and very large cells (fraction IV) based on their buoyancy in a separating funnel, as described previously (30). Briefly, 10–20 ml of a total cell suspension and 50 ml of buffer were gently mixed, and cells were allowed to float for 45 s to obtain fraction I. Intermediate-sized cells were collected and discarded after floating for 15 s. Cells that remained in the funnel were defined as fraction IV.

Determination of cell size and mature adipocyte culture. To determine the mean fat cell diameters for the total cell fraction and fractions I and IV, ~50 μl of cells from each fraction were pipetted onto a glass slide to determine the diameter of 100 cells under a light microscope. To isolate sufficient mitochondria from the fractionated cells on the next day, 20 ml of each fraction (total and fractions I and IV) was cultured in T75 culture flasks (TPP, Trasadingen, Switzerland) with a medium to cell ratio of 2:1 in DMEM-F-12 (1:1; vol/vol) supplemented with 1% penicillin-streptomycin. Medium was changed 30 min later to reduce cell stress.

Isolation of mitochondria. The next day, mitochondria were isolated. Cells were disrupted mechanically using a Potter homogenizer, and mitochondria were isolated by differential centrifugation. For the total cell fraction and fraction IV, a 30-ml glass-Teflon Potter homogenizer was used, and for fraction I and SGBS cells, a 15-ml glass-Teflon Potter homogenizer was used. A homogenate was transferred to a 50-ml tube and rinsed with 5–10 ml of STE buffer (250 mM sucrose, 5 mM Tris, 2 mM EGTA) + 4% BSA (fatty acid free; Roth, Karlsruhe, Germany). Subsequently, the homogenate was centrifuged at 800 g in a refrigerated centrifuge at 4°C for 10 min to pellet cell debris. The supernatants that contained mitochondria were collected and ultracentrifuged at 10,000 g at 4°C for 10 min. After the supernatant was discarded, the pellet was washed once in KHE buffer (120 mM KCl, 5 mM KH2PO4, 3 mM HEPES, 1 mM EGTA) and centrifuged again (10,000 g, 4°C, 10 min). After the supernatant was discarded, mitochondria were resuspended in ~100–500 μl of KHE buffer and then transferred to a 1.5-ml vial and stored on ice for subsequent analysis.

Mitochondrial respiration. Mitochondrial oxygen consumption was determined in 1 ml of KHE + 0.4% BSA (fatty acid free) at 37°C by measuring the partial oxygen pressure using a Clark-type oxygen electrode (Digital Model 10; Rank Brothers, Cambridge, UK). LabChart 6 software (ADInstruments, Dunedin, New Zealand) was used to record the data. A total of 300 μg of mitochondrial protein was used per measurement, and the following reagents were added in succession: 6 μM rotenone as a complex I inhibitor, 4 mM succinate as a substrate for complex II, 300 μM ADP for initiating ATP production, and 1 μg/ml oligomycin to inhibit ATP-synthase (all from Sigma-Aldrich). The complex II substrate succinate was used to obtain higher oxygen consumption rates than with NADH-based substrates to unravel even small differences in respiration. Respiration rates were expressed as nmol O2 consumed per minute per milligram of mitochondrial protein.

Western blot analysis. For protein analysis, isolated adipocytes were homogenized in Radio-Immunoprecipitation Assay buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 0.2% SDS, 1% Nonidet P-40, and 0.5% deoxycholate) and phosphatase (phosSTOP; Roche, Basel, Switzerland) and protease inhibitors (complete; Roche). After centrifugation (20,000 g, 4°C, 15 min), the fatty supernatant was removed, and protein amounts were determined using a bicinchoninic acid protein assay (Thermo Fisher Scientific). Equal amounts of protein (15 μg) were heated (55°C, 5 min) and subsequently loaded onto 15% acrylamide gels. After blotting, membranes were incubated with primary antibodies directed against citrate synthase (CS; Abcam, Cambridge, UK) and GAPDH (Ambion; Thermo Fisher Scientific) and with an antibody mixture for different subunits of respiratory chain complexes (MS601; Abcam). The antibodies in this mixture were directed against mitochondrial DNA copy number in dependency on BMI, probe-based quantitation.

Mitochondrial DNA quantification. DNA was isolated from adipocytes with a silica- and spin column-based DNA purification Kit (DNeasy Blood & Tissue Kit; Qiagen, Hilden, Germany). To quantify mtDNA copy number in dependency on BMI, probe-based quantitative real time PCR was performed on a LightCycler (LightCycler 480; Roche). Primers were directed against targets in the mitochondrial [forward 5′-TTC TGG CCA CAG CAC TTA AA-3′, reverse 5′-TGG

Table 1. Subject details

<table>
<thead>
<tr>
<th></th>
<th>Mitochondrial Respiration Measurements (n = 16)</th>
<th>Mitochondrial Respiration Measurements Based on Cell Size (n = 5)</th>
<th>Western Blot Analysis (n = 20)</th>
<th>Mitochondrial DNA Quantification (n = 17)</th>
</tr>
</thead>
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<tr>
<td>BMI, kg/m²</td>
<td>25 ± 5 [18, 36]</td>
<td>28 ± 6 [22, 36]</td>
<td>27 ± 6 [19, 41]</td>
<td>27 ± 6 [19, 41]</td>
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</tr>
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<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Hypertension</td>
<td>None</td>
<td>None</td>
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</table>

Values are expressed as means ± SD [minimum, maximum]. BMI, body mass index.
TTA GGC TGG TGT TAG GG-3', probe 53 (cat. no. 04688503001) and nuclear genome [forward 5'-GCA GGC ATT CCT GGA AGA G-3', reverse 5'-TGT GTG CCC TAC ACA ATG C-3', probe 75 (cat. no. 04688988001)]. The nuclear primer pair amplifies a 78-bp fragment of chromosome 12 (713,966–714,043) with no annotated genes within >30 kb in either direction. The mitochondrial primer pair amplifies a 71-bp fragment of the mitochondrial genome (318–388) close to the origin of replication and 257 bp upstream of the phenylalanine tRNA gene (human genome build GRCh38/hg18) (1). Per- taining to both primer pairs, we carefully excluded binding outside their respective unique target region with special attention to the multiple nuclear copies of mitochondrial genome fragments. Samples were run in triplicates. Subsequently, the ratio of mtDNA to nuclear DNA was calculated (mtDNA/nDNA).

Statistical analysis. R (A Language and Environment for Statistical Computing, Vienna, Austria) was used for multiple-regression analyses. All other statistical analyses were performed with GraphPad Prism version 4 (GraphPad Software, La Jolla, CA). P values of <0.05 were considered significant. Possible associations between different mitochondrial respiration states, BMI values, and adipocyte sizes were assessed by Pearson correlation analysis if not indicated differently. Results for nonobese and obese subjects were compared using two-tailed unpaired Mann-Whitney test. Friedman’s test was used to test for differences between cells in the total fraction (TF) and fraction I (FI) and FIV, followed by Dunn’s posttest. Results for mitochondrial yields and BMI values are given as means ± SD. Cell size diameters are given as median [minimum, maximum].

RESULTS

Influence of BMI on mitochondrial respiration of mature human adipocytes. Leak respiration (state 4) of mitochondria that were respiring on the complex II substrate succinate did not exhibit BMI-dependent O₂ consumption, although there was a trend toward an association (r = −0.46, P = 0.077; Fig. 1A). Adding ADP resulted in maximal ADP-stimulated respiration according to the supply of ATP synthase (complex V, state 3). State 3 respiration was significantly reduced in a BMI-dependent manner (r = −0.65, P < 0.01; Fig. 1B). In the case of reutilization of ATP to ADP or remaining ADP, state 4 might be overestimated (5). To determine leak respiration without any interference by remaining ADP, the ATP synthase inhibitor oligomycin was added (state 4o; Fig. 1C). Concordant with state 4, state 4o also did not show a significant association with BMI values (r = −0.45, P = 0.127), indicating that there
Table 2. Multiple-regression analyses (n = 13)

<table>
<thead>
<tr>
<th>Model</th>
<th>Coefficient BMI</th>
<th>P Value</th>
<th>Coefficient BMI Adjusted for Age</th>
<th>P Value Adjusted for Age</th>
<th>P Value Adjusted for Age</th>
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</thead>
<tbody>
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<td>State 4</td>
<td>−0.4732</td>
<td>0.0765</td>
<td>−0.4649</td>
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<td>State 3</td>
<td>−4.3926</td>
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<td>0.9219</td>
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<tr>
<td>State 4o</td>
<td>−0.3661</td>
<td>0.2323</td>
<td>−0.3592</td>
<td>0.2620</td>
<td>0.4170</td>
</tr>
<tr>
<td>logATP linked</td>
<td>−0.1716</td>
<td>&lt;0.001</td>
<td>−0.1756</td>
<td>&lt;0.001</td>
<td>0.1950</td>
</tr>
<tr>
<td>RCR</td>
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<td>−0.3785</td>
<td>0.0351</td>
<td>0.7296</td>
</tr>
</tbody>
</table>

RCR, respiratory control ratio.

were no differences in mitochondrial membrane integrity between the mitochondria from lean and obese subjects.

ATP-linked respiration was determined by subtracting state 4o from state 3 respiration values (Fig. 1D). A highly significant inverse correlation between BMI values and ATP-linked respiration rates was observed (r = −0.84, P < 0.001). The respiratory control ratio (RCR), the ratio of state 3 to state 4o respiration (2), was also determined to assess the efficiency of the coupling process in the mitochondria that were isolated from mature human adipocytes from different subjects. A significant, negative correlation between RCR and BMI values was detected (r = −0.55, P < 0.05; Fig. 1E). To exclude an influence of the subject’s age on the respiration measurements, the associations between O2 and BMI were adjusted for age in multiple-regression analyses. The adjusted analyses are consistent with the unadjusted analyses, with only minor differences in the estimated coefficients and P values. The results for the unadjusted and adjusted regression analyses are listed in Table 2 (data available for n = 13).

Respiratory capacities of large and small human fat cells. Because obesity is characterized by fat cell hypertrophy, we next determined whether large and small adipocytes differed in their mitochondrial respiratory capacity. Determination of the cell size of the fractionated adipocytes via light microscopy reveal the median diameter of 92 μm [27, 151] for the TF, 81 μm [22, 135] for FI, and 113 μm [43, 184] for FIV. The amount of mitochondrial proteins per cell was significantly greater in large compared with small fat cells (Fig. 2A). The TF of adipocytes had a mean mitochondrial yield of 98.7 ± 40.6 pg/cell, the yield of FI (small cells) was 51.7 ± 20.5 pg/cell, and that of FIV (large cells) was 306.4 ± 135.0 pg/cell (n = 5). The calculated RCR values of small and large adipocytes were not significantly different (Fig. 2B). This was also shown by the significantly positive correlation of RCR values of small and large adipocytes from the same subjects (r = 1, P < 0.05; Fig. 2C), which suggested that disturbances in respiration occurred independently of fat cell size. Furthermore, we evaluated whether SGBS cells were an appropriate model for evaluating mitochondrial function and found that the RCR values of mitochondria isolated from undifferentiated and lipid-laden SGBS cells were in a range comparable with that of mitochondria in mature adipocytes (Fig. 2B).

Respiratory chain complexes. We next examined the differences observed in OXPHOS of adipocyte mitochondria with regard to BMI values by evaluating the amounts of single respiratory chain complex components. We performed Western blot analyses using whole adipocyte protein lysates from obese (mean BMI = 32.38 ± 4.2; n = 9) and nonobese (mean BMI = 22.58 ± 2.3; n = 11) female subjects. The amounts of respiratory chain complex proteins were normalized using GAPDH as a loading control and CS as an indicator for the amount of mitochondria. Simultaneous detection of complexes I–V showed that there was a significant reduction in the protein amounts for complexes I (NDUFB8, P < 0.05) and IV (MT-CO2, P < 0.05; Fig. 3, A and D).

The mean protein amounts of complexes II, III, and V appeared to be slightly lower in adipocyte mitochondria from obese compared with those from lean subjects; how-
ever, these differences were not statistically significant (Fig. 3, B, C, and E).

Mitochondrial content in dependency on BMI. To gain insights about mitochondrial content in adipocytes in dependency on BMI, CS values from Western blot analyses as well as mtDNA in proportion to nDNA in isolated adipocytes were correlated with BMI values. In both analyses, a significant negative association with the subjects BMI could be observed (CS: \( r = -0.45, P < 0.05 \); mtDNA/nDNA: \( r = -0.59, P < 0.05 \); CS: \( r = -0.45, P < 0.05 \); Fig. 4, A and B).

DISCUSSION

Increasing evidence suggests that the accumulation of body fat causes alterations in adipocyte mitochondrial function (25, 37, 38). In addition, this has been linked to insulin resistance.
Although visceral adipose tissue has been considered to play a major role in insulin resistance (16), other reports have emphasized the predominant role of abdominal subcutaneous fat mass for the development of diabetes mellitus in men and women (22, 34) due to its depot size. Therefore, our main objective was to measure different respiration states of mitochondria isolated from human abdominal subcutaneous white fat cells with respect to donor BMI values. Our results showed that mitochondrial respiration was inversely associated with BMI values.

In particular, ADP-stimulated mitochondrial respiration (state 3) was significantly reduced with increasing BMI (Fig. 1B), whereas leak respiration was unrelated to BMI (Fig. 1A and C). Thus, ATP-linked respiration was concomitantly lower with higher BMI values (Fig. 1D). This inverse association was also accompanied by a steady decline in RCR values (Fig. 1E). Thus, our results indicate that altered mitochondrial function in obese individuals was due to a reduced OXPHOS capacity. Adipocytes require ATP for their metabolic activities, such as glyceroneogenesis and reesterification of free fatty acids (FFAs) (10, 21), and it can be speculated that a reduced OXPHOS capacity causes decreased ATP levels in adipocytes.

In this context it may be important to note that impaired reesterification may promote insulin resistance (36) by causing an elevation of circulating FFA levels (3, 29). Furthermore, ATP depletion might affect adipokine secretion and aggrivate adipose tissue inflammation. Thus, impaired mitochondrial function could induce endoplasmic reticulum (ER) stress and reactive oxygen species generation, which may account for reduced adiponectin levels (7, 17). In turn, low adiponectin levels are closely associated with insulin resistance and type 2 diabetes mellitus (23). It has also been demonstrated that ER stress in 3T3 cells induced the secretion of leptin as well as inflammatory cytokines (27). Altogether, impaired mitochondrial function could trigger adipose tissue dysfunction, which adversely affects obesity-related metabolic complications. The limitation of our study might be that the model of isolated mitochondria does not allow us to discriminate whether these changes go along with a reduction in ATP levels. However, a decreased mitochondrial respiratory capacity could be compensated by glycolysis, as suggested by Keuper et al. (15).

Expansion of body fat mass is characterized by hypertrophy (31). Thus, there is a question of whether a BMI-dependent decline in respiration is particularly related to altered mitochondrial function in hypertrophic adipocytes. However, we found that the respiratory capacity of large cells was not significantly different from that of smaller cells isolated from the same subjects, as reflected by the significant positive correlation with RCR values (Fig. 2C). Similar data from another group also showed that mitochondrial function impairment was independent of fat cell size (38). One interpretation might be that there is a compromise in the respiratory capacity of not only enlarged fat cells but all adipocytes from obese individuals. Comparable inverse associations were also found for other essential biochemical functions of adipocytes, like lipolysis and FFA storage (20, 28).

The mechanisms related to the reduced OXPHOS capacity of adipocytes from obese donors remain unclear. Our current results emphasize that differences in the electron transport chain might be responsible for BMI-dependent adipocyte mitochondrial impairment. To determine the molecular background for this diminished respiratory capacity, we measured the amounts of defined single respiratory chain complex proteins. To control for quantitative differences in the amounts of mitochondria in adipose tissue specimens depending on BMI, we chose the CS protein for normalization and found reduced amounts of NDUFB8 and MT-CO2, which are subunits of complex I and complex IV, respectively, in obese subjects compared with nonobese subjects. However, the protein amounts of selected subunits of the three other complexes were also lower in obese subjects compared with lean subjects, although these reductions were not significantly different.

MT-CO2, which was found to be significantly reduced in obese subjects, is one of the three catalytic core subunits encoded for by mitochondrial DNA and is rate-limiting for electron transport (6). Therefore, the reduced amounts of this subunit in our study may at least partly explain the reduced mitochondrial respiration capacity of adipocytes from obese subjects. Corresponding findings were made for fat-specific insulin receptor knockout mice. In this mouse model, a higher complex IV (subunit 4) protein level was associated with improved insulin sensitivity in high-fat diet-fed mice compared with wild-type high-fat diet-fed mice (14).

mtDNA as a measure for mitochondrial abundance is widely used (9a, 13, 14, 18). Previously, it was considered controversial if mtDNA copy number was a valuable biomarker of mitochondrial content (19). Therefore, we determined not only mtDNA abundance but also CS expression. Interestingly, mtDNA in relation to nDNA as well as the CS amounts was significantly inversely associated with BMI values. This indicated that mitochondrial content per se, as also shown previously (13), was reduced in adipocytes from obese subjects. Accordingly, reduced mitochondrial content could even increase the effect of impaired mitochondrial respiratory capacity in adipocytes of obese subjects.

One constraint of our study could be the exclusive use of complex II substrate that might cause an underestimation of the differences in respiration between lean and obese, as observations of Western blot analysis also indicate fewer complex I components in adipose tissue from obese compared with lean women. Although we monitored all respiratory complexes, another limitation of our study might be that only a small spectrum of subunits could be analyzed. Nevertheless, our data indicate that a qualitative change in complex composition and/or abundance could explain the differences in respiratory capacities. Further functional studies will be needed to address enzymatic activities of these different complexes with regard to variations in BMI values. Omental adipose tissue did not show any significant associations between enzyme activity and donor BMI values at the level of complexes I and II (8), which again possibly points to the critical involvement of subcutaneous fat in metabolic diseases. Thus, SGBS cells could be a valuable tool for investigating mitochondrial function under different metabolic conditions, as we showed that their respiratory capacities were comparable with those of mature primary adipocytes (TF, FI, and FIV; Fig. 2B).

In conclusion, we found an inverse relationship between the mitochondrial respiratory capacity of human white adipocytes and BMI. Nevertheless, we could not show any bioenergetic differences in isolated mitochondria from large and small fat cells from the same donor. Therefore, we suggest that impaired respiration is a general phenomenon of all adipocytes,
at least for those in the subcutaneous depot. To our knowledge, this is the first report to demonstrate a reduced amount of complex I and IV components in human obesity. Thus, these results indicate that there is inadequate respiratory chain protein formation relative to total mitochondrial protein in adipocytes from obese individuals.

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DISCLOSURES

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS


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1. No authors listed. UCSC Genome Bioinformatics [Online]. UCSC Genome Informatics Group, Center for Biomolecular Science & Engineering, University of California, Santa Cruz, CA. http://genome.ucsc.edu/ [June 2015]


