Oxidation of intramyocellular lipids is dependent on mitochondrial function and the availability of extracellular fatty acids

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SKELETAL MUSCLE INSULIN RESISTANCE is a central pathophysiological feature of type 2 diabetes (T2D) mellitus, and increasing evidence has shown that it is associated with increased intramyocellular lipid (IMCL) content. The precise molecular mechanisms underlying this relationship are not known but may involve the accumulation of lipid intermediates that either interfere with the insulin-signaling pathway and/or induce inflammatory pathways (1, 12, 22). The accumulation of IMCLs and lipid intermediates may be due to a mismatch between lipid storage, lipolysis, and oxidation (28). Elevation of plasma fatty acid concentration, so-called lipid overflow, may enhance IMCL accumulation and induce insulin resistance, as shown in lipid infusion studies (6, 36). Several animal models show that an improved fat oxidation can prevent high-fat diet-induced insulin resistance (8, 24). More specifically a reduced capacity to oxidize IMCLs may contribute to intracellular accumulation of lipids and lipid intermediates. It is unknown to what extent lipid overflow (i.e., increased extracellular fatty acid availability), mitochondrial function, and the oxidation of intracellular lipids contribute to IMCL accumulation.

Multiple studies have shown that fatty acid handling is impaired in skeletal muscle of obese, impaired glucose-tolerant, and T2D individuals (3, 9, 10, 25, 26), which may be due to a primary genetic component. A promoter polymorphism in the gene for the fatty acid transporter CD36 was associated with a decreased insulin sensitivity and an increased prevalence of T2D (11). Despite significant weight loss, a lack of improvement in the ability to increase fat oxidation during β-adrenergic stimulation in obese subjects was observed (4), and under similar conditions the ability to increase fat oxidation during exercise in T2D individuals remained impaired (5). A reduced ability to stimulate fat oxidation during exercise (26) and to switch between lipids and carbohydrates for fuel after a meal (9) was already present in “prediabetic” impaired glucose-tolerant subjects. In vitro experiments with myotubes established from diabetic patients show a lower baseline palmitate oxidation during acute palmitate exposure relative to cultured myotubes from body mass index-matched controls (19, 20, 40). Whether a primary defect in IMCL oxidation may play a role in the development of diabetes is unknown.

To study both primary and secondary impairments in fatty acid handling, cultured human myotubes represent a well-characterized in vitro model of skeletal muscle. The advantages of using human myotubes is that the extracellular environment can be controlled precisely and kept constant over time, which allows for studying the importance of the intrinsic characteristics without systemic homeostatic regulatory components from the nervous and endocrine systems. It has been used previously to study the separate contributions of intracellular and extracellular substrates on total substrate oxidation (13). Moreover, previous studies in these cultured myotubes have shown that the insulin-resistant phenotype is conserved (15, 18). In the present study, we took advantage of this model to investigate the effects of extracellular fatty acids, glucose, and mitochondrial uncoupling [carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP)] on oxidation of intracellular lipids (ICLOX) and to compare these effects in primary
myotubes established from healthy lean with those from obese T2D individuals.

MATERIALS AND METHODS

Materials. Dulbecco’s modified Eagle’s medium (DMEM-Glutamax), DMEM without phenol red (Sigma D5030), non-heat-inactivated fetal calf serum (FCS), and penicillin-streptomycin-amphotericin B were purchased from Gibco Invitrogen (GIBCO Life Technologies, Paisley, UK). Bovine serum albumin (BSA; essentially fatty acid free), L-carnitine, Dulbecco’s phosphate-buffered saline (DPBS; with Mg²⁺ and Ca²⁺), oleic acid (OA), extracellular matrix (ECM) gel, and HEPES were purchased from Sigma (St. Louis, MO). Ul- troser G was purchased from CIPHERGEN (Cergy-Saint-Christophe, France), and insulin (Actrapid) was purchased from Novo Nordisk (Bagsvaerd, Denmark). [1-¹⁴C]OA (55 mCi/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Corn ing CellBIND tissue culture plates were obtained from Corning Life Sciences (Schiphol-Rijk, The Netherlands). Glass bottom plates were from MatTek (Ashland, MA). OptiPHase Supermix, UniFilter-96 GF/B plates, and ScintiPlates were delivered by PerkinElmer (Shelton, CT). Protein assay kit was purchased from Bio-Rad (Copenhagen, Denmark). Bodipy 493/503 (4,4-difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-s-indacene), MitoTracker Red FM, and Hoechst 33258 were obtained from Molecular Probes (Invitrogen, Carlsbad, CA). All other chemicals were used of standard commercial high-purity quality.

Characterization of the donors. To establish primary myotube cultures, muscle biopsies were taken from M. vastus lateralis by needle biopsy according to Bergstrom (2) in 17 Caucasian participants, of which eight were lean healthy glucose-tolerant controls (5 males, 3 females) without family history of T2D and nine were obese subjects with T2D (5 males, 4 females). The characteristics of the participants are shown in Table 1. Initially, nine lean controls were included, but from one of the lean subjects (female) the myotubes showed insufficient growth for reliable measurements. Subjects refrained from strenuous physical activity ≥3 days before metabolic investigations. Diabetic subjects were treated with diet alone or in combination with sulfonylurea, metformin, or insulin, which was withdrawn 1 wk before biopsies were taken. None of the subjects suffered from diseases other than diabetes and hypertension. Insulin sensitivity was determined by hyperinsulinemic euglycemic clamp (40 mU·m⁻²·min⁻¹) (21). Respiratory quotients (RQ) were calculated from substrate oxidation rates measured by indirect calorimetry (Datex, Helsinki, Finland) with adjustments for protein oxidation. Metabolic flexibility was calculated as fasting RQ minus RQ during clamp. All subjects gave written, informed consent, and the local ethics committee of Funen and Vejle County approved the study.

Table 1. Clinical characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>All (n = 17)</th>
<th>Range</th>
<th>Lean (n = 8)</th>
<th>Obese T2D (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>50.7 ± 1.0</td>
<td>43–58</td>
<td>51.4 ± 0.9</td>
<td>50.0 ± 1.6</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>29.0 ± 1.4</td>
<td>21–39</td>
<td>23.7 ± 0.5</td>
<td>33.7 ± 1.3*</td>
</tr>
<tr>
<td>Fasting plasma glucose, mM</td>
<td>7.9 ± 0.6</td>
<td>5.3–12.9</td>
<td>5.7 ± 0.1</td>
<td>9.8 ± 0.7*</td>
</tr>
<tr>
<td>Fasting insulin, pM</td>
<td>50.0 ± 8.1</td>
<td>9–100</td>
<td>18.8 ± 3.0</td>
<td>75.0 ± 7.8*</td>
</tr>
<tr>
<td>Glucose infusion rate, mg·min⁻¹·m²</td>
<td>246 ± 34</td>
<td>55–487</td>
<td>378 ± 25</td>
<td>129 ± 16*</td>
</tr>
<tr>
<td>Hb A₁c, %</td>
<td>6.4 ± 0.4</td>
<td>5.1–10.6</td>
<td>5.6 ± 0.1</td>
<td>7.4 ± 0.6</td>
</tr>
<tr>
<td>Total cholesterol, mM</td>
<td>5.3 ± 0.2</td>
<td>3.9–7.2</td>
<td>5.3 ± 0.3</td>
<td>5.2 ± 0.4</td>
</tr>
<tr>
<td>LDL cholesterol, mM</td>
<td>3.1 ± 0.2</td>
<td>1.8–4.3</td>
<td>3.0 ± 0.3</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>HDL cholesterol, mM</td>
<td>1.6 ± 0.1</td>
<td>1.2–2.5</td>
<td>1.9 ± 0.1</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Fasting triacylglycerol, mM</td>
<td>1.5 ± 0.2</td>
<td>0.7–3.8</td>
<td>1.0 ± 0.1</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>RQfasting</td>
<td>0.79 ± 0.01</td>
<td>0.74–0.87</td>
<td>0.80 ± 0.01</td>
<td>0.78 ± 0.01</td>
</tr>
<tr>
<td>Metabolic flexibility (RQclamp – RQfasting)</td>
<td>0.00 ± 0.00</td>
<td>0.00–0.29</td>
<td>0.16 ± 0.03</td>
<td>0.05 ± 0.02</td>
</tr>
</tbody>
</table>

Values represent means ± SE and range (minimum to maximum) for all individuals together and lean and obese type 2 diabetic individuals separately. RQfasting, fasting respiratory quotient; RQclamp, clamp respiratory quotient. *P < 0.001; †P < 0.02; ‡P < 0.01 vs. lean subjects.
than on imitation of plasma values. The acute treatments were chosen to mimic various physiological conditions for skeletal muscle cells, such as the fed state (high glucose supply, low free fatty acid (FFA) availability) and fasting (high FFA uptake, low glucose transport), and other combinations to study the response on lipid oxidation (e.g., adaptability to increased fatty acid availability and suppressibility of glucose on fatty acid oxidation when being changed from low to high concentrations).

During the 4-h acute experiment, the production of 14CO2 was measured as described under the heading substrate oxidation assay. To distinguish between 14CO2 production from intracellular (ICLOX) or extracellular sources (ECLOX), labeling with [1-14C]OA was applied in the medium immediately at the start during pretreatment (14CO2 comes from intracellular lipids) or during the acute experiment (14CO2 is medium derived). The cells were washed twice with PBS after pretreatment, before addition of acute medium for CO2 trapping.

Substrate oxidation assay. Substrate oxidation was monitored by incubating cells grown on 96-well plates (CellBIND) with [14C]OA, with subsequent capture of liberated 14CO2 for 4 h at 37°C, using a “sandwich” clamp instrument, as described previously (41). Radioactivity in the filter wells was counted in a scintillation counter for multowell plates (MicroBeta; PerkinElmer). Immediately after 14CO2 trapping, the cells were washed twice with PBS and harvested with 0.1 M NaOH (200 μL/well). Protein was determined by Coomassie reagent (7). For total (cell-associated) accumulation of [14C]OA, 50 μl cell homogenate/sample was added to scintillation fluid and counted (MicroBeta).

Scintillation proximity assay. Radiolabeled substrates taken up and accumulated by adherent cells were concentrated close to the scintillator embedded in the plastic bottom of each well and thus provided a stronger signal than the radiolabel dissolved in the medium alone (41). Measurements of OA uptake by scintillation proximity assay (SPA) were performed in DME-medium, using 96-well ScintiPlates (http://www.perkinelmer.com for SPA technology; Perkin-Elmer) as described above without phenol red, with an additional 100 μM OA (1.5–2.2 μCi/ml [1-14C]OA) bound to BSA at a ratio of 2.5:1.

Lipid distribution. Myotubes grown on 12-well plates were pretreated with [1-14C]OA (100 μM) for 48 h. After the acute experiment (4 h), cells were washed twice with PBS, harvested in distilled water (200 μl), and stored at −20°C for extraction of cellular lipids (19). Briefly, the homogenized cell fraction was mixed with 20 volumes of chloroform-methanol (2:1), and FCS (30 μl) was added as a lipid carrier. After 30 min, four volumes of 0.9% NaCl (pH 2) was added and the mixture was centrifuged (1,000 g, 5 min). The organic phase was evaporated under a nitrogen stream at 40°C. The residual lipid extract was redissolved in 150 μl of n-hexane and separated by thin-layer chromatography (TLC) using hexane-diethyl ether-acetic acid (65:35:1) as mobile phase. The lipid bands were visualized with iodine and excised, with MitoTracker Red FM (100 nM) for 15 min to stain mitochondria, and stored at −80°C for long-term live imaging. We used a ×20 objective and standard filter sets with excitation window for Hoechst (350 nm), Bodipy 493/503 (488 nm), and Mitotracker Red FM (594 nm) and a triple band filter for emission. Images were taken in 25 positions/well, 2 wells/donor, and each image was the backdrop-subtracted maximal intensity projection from 12 images taken in z-direction (1 μm apart), where one image contained 52 ± 2 nuclei on average. Scion software was used for automated image analysis, using edge detection algorithm for object segmentation to quantify the number of nuclei, density, diameter, and number of LDs and total mitochondrial mass (total intensity of Mitotracker Red).

Presentation of data and statistics. All values are reported as means ± SE. The value n represents the number of different donors used. Linear mixed models were used to compare the differences between conditions with within-donor variation and simultaneously compare differences between groups with between-donor variation. The linear mixed models include all observations in the statistical analyses and at the same time take into account that not all observations are independent. Correlations are presented as Spearman’s correlation coefficient (r). Clinical data were compared with two-tailed, unpaired Student’s t-test. The statistical analyses were performed with SPSS version 12 (SPSS, Chicago, IL). P < 0.05 was considered significant.

RESULTS

Experimental setup: fatty acid uptake, accumulation, and prelabeling of myotubes. Isotopic equilibrium of the intracellular lipid pool (label entering the pool equals label leaving the pool) reached a plateau after ~48 h (Fig. 2A). Lipid accumulation measured as the cell-associated radioactivity and as the number of LDs (Fig. 3) per nucleus was not significantly different between myotubes from lean and obese T2D subjects (Fig. 2, B and C). The average LD sizes were 1.7 ± 0.1 and 1.8 ± 0.1 μm2 for T2D and lean, respectively (P = 0.31). The density of LDs was measured as fluorescence intensity per LD, showing no significant differences between T2D and lean subjects (data not shown). Furthermore, intracellular lipid distribution was assessed by TLC in myotubes from lean subjects (n = 4) after 48 h of pretreatment with labeled OA and 4 h of acute experiments (see Fig. 1 for conditions). Most of the cell-associated lipids were represented by triacylglycerols (53–61%), FFAs (25–33%), and to a lesser extent diacylglycerols (6–7%), phospholipids (2–4%), and cholesterol esters (3–4%). There was no significant effect of the different acute treatments on intracellular lipid distribution.

Mitochondrial mass by live imaging. After 48-h treatment with 100 μM OA, mitochondrial mass, measured as total intensity of Mitotracker Red FM per nucleus, was not different between myotubes from the two donor groups (fluorescence intensity: 611 ± 72 × 103 AU in T2D and 482 ± 34 × 103 AU in lean, P = 0.14; for images, see Fig. 3).

Oxidation of lipids. In myotubes from obese T2D subjects, the ICLOX (Fig. 4A), ECLOX (Fig. 4C), and total lipids (sum of
ICLOX and ECL0X; Fig. 4D) were systematically reduced in all four of the acute conditions compared with myotubes from lean subjects. Also, ICLOX adjusted for differences in total ICL content (cell-associated radioactivity) was systematically reduced in T2D myotubes (Fig. 4B). This indicates that a general defect in lipid oxidation can be extended to a reduced oxidation of intracellular lipids. Increasing the concentration of extracellular fatty acid markedly increased extracellular lipid oxidation (ECL0X) and total lipid oxidation (TLOX) (Fig. 4, C and D). The increase in ECL0X and TLOX upon increased fatty acid availability was smaller in myotubes from obese T2D subjects compared with myotubes from lean subjects at both 0.1 (g) and 5.0 (G) mM extracellular glucose (P < 0.05; Fig. 4, E and F, respectively). Cell-associated OA from the acute incubations was not different between myotubes from the two donor groups (data not shown).

Effects of extracellular fatty acids, glucose, and mitochondrial uncoupling on ICLOX. Figure 5 shows the relative change in substrate oxidation upon increases in glucose and fatty acid availability, taking the “gf” condition as the reference. Figure 5A shows that in the presence of extracellular fatty acids the ICLOX was lower (in the 100-μM condition “F” compared with the 5-μM condition “I”; P < 0.001) in myotubes from lean individuals (−32%) and in myotubes from T2D individuals (−46%). Figure 5B shows that a higher concentration of glucose has a similar but smaller effect and significantly suppressed ICLOX in the T2D cells (−24%, P < 0.05; Fig. 5B), but not in lean cells (−12%). Overall, the suppressive effects of extracellular OA and glucose on intracellular lipid utilization were significantly stronger in myotubes established from obese T2D subjects (P = 0.002). Figure 5C shows the effects after mitochondrial respiration was increased by addition of the mitochondrial uncoupler FCCP. The suppressive effect of increased extracellular fatty acid availability was still present when mitochondrial oxidative capacity (electron transport chain flux) was increased by FCCP. Figure 5C also shows that increased mitochondrial oxidative respiration by FCCP restored ICLOX in the presence of increased extracellular fatty acid availability (gf/FCPP and GF/FCPP) to the level of low fatty acid availability (gf/FCPP), at least in myotubes from lean subjects. In the presence of FCCP, ICLOX was increased significantly for both groups at all acute conditions examined. The relative effect of FCCP on ICLOX is presented in Fig. 5D. The uncoupling effect of FCCP overall was 14% lower in myotubes from T2D compared with lean donors (P = 0.04; Fig. 5D), suggesting a primary reduced mitochondrial capacity despite comparable mitochondrial mass.

Correlations with in vivo characteristics. To examine whether intrinsic characteristics of the myotubes in vitro were reflected by the phenotype of the donor in vivo, lipid oxidation...
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Fig. 4. Effects of substrate availability on lipid oxidation. Lipid oxidation after 4 h of CO₂ trapping under conditions that vary in glucose and fatty acid availability in myotubes from lean and T2D subjects. The cells were incubated with [14C]OA, as described in Fig. 1. Open bars represent lean subjects (n = 8), and black bars represent obese T2D subjects (n = 9). Values represent means ± SE, with each donor (n = 8–9) quantified from 8 wells/condition. *P < 0.05 for T2D vs. lean myotubes. A: CO₂ production over 4 h from intracellular lipids (ICL). *Group effect, P = 0.002; overall differences between treatments, P = 0.08. Post hoc analysis with Bonferroni correction for “gf” vs. “gF,” P = 0.02. B: fractional oxidation of intracellular lipids (ICLOX), i.e., the ICLs that are oxidized in relation to the total fatty acid availability in the cell [fatty acid oxidized/cell-associated fatty acids + fatty acids oxidized]. *Group effect, P = 0.002; overall differences between treatments not significant. C: CO₂ production over 4 h from extracellular lipids (ECL). *Group effect, P = 0.014; overall differences between treatments, P < 0.001. D: total CO₂ production (from intracellular and extracellular fatty acids). *Group effect, P = 0.02; overall differences between treatments, P = 0.003. E: bars represent the difference in ECL-OX at high fatty acid availability in the F condition (100 μM OA) and low fatty acid availability in the F condition (5 μM OA). Glucose availability is indicated by “g” (0.1 mM glucose) or “G” (5 mM glucose). Lean vs. T2D: *P = 0.04. F: bars represent the difference in total lipid oxidation (TL-OX) at high fatty acid availability in the F condition (100 μM OA) and low fatty acid availability in the F condition (5 μM OA). Glucose availability is indicated by g (0.1 mM glucose) or G (5 mM glucose). Lean vs. T2D: *P = 0.03. Differences between the 0.1 and 5.0 mM glucose were not significant (g vs. G), P > 0.50.

DISCUSSION

The findings of the present study suggest that elevated extracellular fatty acids suppress the oxidation of intramyocellular lipids. This is not because of an effect of extracellular lipids on ICL lipolysis. Mitochondrial uncoupling can counteract the effect and increase ICLOX to basal levels under conditions of high extracellular fatty acid availability. A reduced oxidation of ICL and reduced mitochondrial mass were shown to contribute independently to accumulation of intramyocellular lipids. Furthermore, the capacity to oxidize ICL and to increase ECLOX upon increased fatty acid availability is reduced in myotubes established from obese T2D individuals despite comparable mitochondrial mass. The finding that lipid oxidation in vitro was inversely related to fasting RQ in vivo supports the idea that the primary impairments observed in vitro are relevant for the in vivo phenotype.

An important observation is that when fatty acid availability is high, the oxidation of intracellular lipids is decreased. In general, most fatty acids that are taken up in skeletal muscle are shuttled toward oxidation (Fig. 8A). In subjects with a reduced capacity for lipid oxidation, a larger part of the fatty acids taken up may be shuttled toward storage (Fig. 8B). In the
In the case of an increased availability of extracellular fatty acids, the oxidation of extracellular fatty acids is increased due to a mass effect (Fig. 8C). This is demonstrated in the present in vitro experiment (Fig. 4C) as well as in in vivo in high-fat feeding studies (32, 33). However, we show that at the same time the oxidation of fatty acids derived from ICL is suppressed. In this way, increased extracellular fatty acid availability, also called lipid overflow, may enhance intramyocellular lipid accumulation despite an increase in ECL_{OX} (Fig. 8C). From a physiological point of view, this implies a phenotype in the initial phases of obesity that displays increased fat oxidation in parallel with increased IMTG and possibly increased insulin resistance. Other in vivo observations support the hypothesis that the oxidation of ICLs is increased when fatty acid availability is reduced and vice versa. Several human in vivo stable isotope studies have investigated the use of TAG for fuel (35, 36).
During exercise, an initial increase in the utilization of TAG-derived fatty acids was observed. For a while after the start of exercise, plasma FFA availability was increased due to increased epinephrine-induced lipolysis, and this was paralleled by a decrease in TAG-derived FFA oxidation in skeletal muscle. Inhibition of adipose tissue lipolysis with acipimox decreased FFA availability from adipose tissue and increased the oxidation of TAG-derived fatty acids during rest and exercise (38). What these studies further support is that, under restricted fatty acid availability, the contribution of ICLOX is relevant for total fat oxidation. In human myotubes, both exogenous and endogenous lipid sources are contributors to total lipid oxidation (13).

Differences in the oxidation of IMCL may be caused by differences in IMCL lipolysis. Some time ago, studies by Wicklmayr et al. (42) suggested that lipolysis of intramyocellular TAG, and therefore availability of fatty acids from the intracellular lipid pool, is to a lesser extent under hormonal control and may be more regulated by substrate supply. It was suggested that product inhibition of hormone-sensitive lipase by long-chain fatty acyl-CoA could lead to a decrease in IMCL oxidation. In the present study, no differences in total lipolysis were observed between high and low FFA availability, and thus the effect of extracellular OA on ICLOX could not be explained by changes in the ICL lipolysis rate.

When comparing myotubes from obese T2D individuals to lean controls, the oxidation of both intracellular and extracellular lipids was reduced, and the T2D myotubes showed a lower ability to increase ECLOX and total lipid oxidation upon increased fatty acid availability. It is suggested that the capacity to increase fatty acid oxidation in adaptation to a high-fat intake may be important to maintain insulin sensitivity, since it may limit the formation of potential lipotoxic intermediates (1, 12, 22, 23, 28). In this study, a lower ICLOX as well as a lower mitochondrial mass were independent factors related to a higher cell-associated lipid accumulation. A lower lipid oxidation, represented by a high-fasting RQ at the whole body level, predisposed Pima Indians and nonobese men to obesity and insulin resistance (34, 43). In myotubes established from young healthy subjects, the capacity to increase fat oxidation in vitro was positively related to insulin sensitivity in vivo (37). The increase in fat oxidation in vitro was also related to a change in RQ during a hyperinsulinemic euglycemic clamp. In the present study, this correlation with a change in RQ could not be replicated. Nevertheless, we also observed that a low lipid oxidation in vitro, represented by a high RQ and measured during fasting, was reflected by a low lipid oxidation in vitro.
in particular in myotubes from lean subjects. These observations suggest that the characteristics of the in vivo phenotype are to a certain extent carried over to the myotubes in vitro by either genetic or epigenetic mechanisms.

A molecular explanation for the impaired lipid oxidation in myotubes established from obese T2D subjects may be found in reduced mitochondrial function or number. Several studies demonstrate a reduced mitochondrial mass and function in muscle of lean, insulin-resistant offspring of T2D individuals (27, 31), which may be related to a downregulation of peroxisome proliferator-activated receptor-γ coactivator-1α, as shown in some (30) but not all (27) studies. In the present study, mitochondrial mass was not related to ICLOX in the myotubes established from obese T2D individuals. Moreover, the uncoupling effect of FCCP in myotubes from T2D subjects was slightly reduced compared with lean subjects. These findings support a reduced mitochondrial capacity in primary cultured myotubes. This is in line with a previous report where the rate of ICLOX contributed independently to IMCL accumulation during lipolysis of IMCL in our study. Mitochondrial mass and the oxidation of IMCL, but this could not be explained by changed extracellular lipid oxidation upon increased fatty acid availability in primary cultured myotubes from diabetic individuals compared with lean, whereas the mitochondrial mass was not different (14, 29).

In the present study, we compared myotubes established from lean subjects with myotubes from obese T2D subjects to optimize our conditions for finding significant differences. A limitation of the study is that myotubes established from obese nondiabetic subjects were not studied. Very recently, we showed that myotubes established from obese subjects express a reduced oxidation of endogenous lipids, whereas myotubes established from obese T2D subjects express a reduced oxidation of both endogenous and exogenous lipids (13).

In conclusion, extracellular lipid overflow may inhibit the oxidation of IMCL, but this could not be explained by changed lipolysis of IMCL in our study. Mitochondrial mass and the rate of ICLOX contributed independently to IMCL accumulation. Increased mitochondrial uncoupling by FCCP rescued the reduction in ICLOX. This implicates that improved mitochondrial function and a reduction in circulating fatty acids, e.g., by cultured myotubes from T2D subjects was slightly reduced compared with lean subjects. These findings support a reduced mitochondrial capacity in primary cultured myotubes. This is in line with a previous report where the rate of ICLOX contributed independently to IMCL accumulation during lipolysis of IMCL in our study. Mitochondrial mass and the oxidation of IMCL, but this could not be explained by changed extracellular lipid oxidation upon increased fatty acid availability in primary cultured myotubes from diabetic individuals compared with lean, whereas the mitochondrial mass was not different (14, 29).

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REFERENCES

None of the authors have a conflict of interest to declare.


