Reduced insulin-mediated citrate synthase activity in cultured skeletal muscle cells from patients with type 2 diabetes: Evidence for an intrinsic oxidative enzyme defect

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Abstract

In myotubes established from patients with type 2 diabetes (T2D), lipid oxidation and insulin-mediated glucose oxidation are reduced, whereas in myotubes from obese non-diabetic subjects, exposure to palmitate impairs insulin-mediated glucose oxidation. To determine the underlying mechanisms of these metabolic malfunctions, we studied mitochondrial respiration, uncoupled respiration and oxidative enzyme activities (citrate synthase (CS), 3-hydroxy-acyl-CoA-dehydrogenase activity (HAD)) before and after acute exposure to insulin and/or palmitate in myotubes established from healthy lean and obese subjects and T2D patients. Basal CS activity was lower (14%) in diabetic myotubes compared with myotubes from lean controls (P = 0.03). Incubation with insulin (1 μM) for 4 h increased the CS activity (26–33%) in myotubes from both lean (P = 0.02) and obese controls (P < 0.001), but not from diabetic subjects. Co-incubation with palmitate (0.6 mM) for 4 h abolished the stimulatory effect of insulin on CS activity in non-diabetic myotubes. No differences were detected in mitochondrial respiration and HAD activity between myotubes from non-diabetic subjects and T2D patients, and none of these measures responded to high levels of insulin and/or palmitate. These results provide evidence for an intrinsic defect in CS activity, which may play a role in the pathogenesis of T2D. Moreover, the data suggest that insulin resistance at the CS level can be induced by exposure to high free fatty acid levels.

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1. Introduction

Type 2 diabetes (T2D) mellitus is characterized by alterations in both lipid and glucose metabolism, and there is an increasing body of evidence to suggest an association between insulin resistance, type 2 diabetes and mitochondrial dysfunction. Initial in vivo studies have reported a diminished citrate synthase (CS)/hexokinase (HK) ratio in diabetic skeletal muscles, where the oxidative/glycolytic ratio was shown to correlate with insulin sensitivity [1]. Mitochondrial size and activity have also been shown to be reduced in obese and T2D subjects and to correlate with the degree of insulin resistance [2]. Stump et al. [3] showed that insulin has a direct stimulatory effect on mitochondrial ATP production and mRNA transcripts of mitochondrial enzymes in skeletal muscle of non-diabetic subjects, whereas no change in mitochondrial ATP production was observed in T2D subjects. Moreover, recent analysis of gene expression patterns have demonstrated a coordinated reduction in mRNA transcripts of nuclear-encoded genes involved in mitochondrial biology.
oxidative phosphorylation, including the catalytic beta-subunit of the ATP synthase in both prediabetic and T2D subjects [4–6]. Consistently, we have observed a reduced protein content and phosphorylation of the ATP-synthase beta-subunit in skeletal muscle of T2D patients using a proteomic approach [7]. Interestingly, insulin treatment for 10 weeks of T2D patients under poor glycemic control normalized the mRNA levels of ATP-synthase beta-subunit in muscle [6]. In addition, the reduction in ATP-synthase beta-subunit protein levels in the muscle of T2D patients correlated with fasting plasma glucose levels [7]. Taken together, these observations suggest that some of these alterations in mitochondrial function may be adaptive in nature, and it could be speculated that many of the changes in expression would normalize under normophysiological conditions. However, more recently, Petersen et al. [8] have provided evidence for a possible inherited defect in mitochondrial oxidative phosphorylation the skeletal muscle of insulin-resistant offspring of patients with T2D, which was associated with dysregulation of intracellular fatty acid metabolism. Our current knowledge on oxidative enzyme activities in the tricarboxylic acid (TCA) cycle, mitochondrial beta-oxidation and electron transport chain in relation to insulin resistance and T2D originates, however, mainly from in vivo studies. The oxidative capacity of skeletal muscle is highly influenced by physical activity, ageing and fiber type composition, which makes it difficult to determine the contribution of genetic and environmental factors to mitochondrial dysfunction in T2D. Cultured myotubes offer a unique model to separate the genetic influence on insulin resistance and type 2 diabetes from environmental factors [9–11]. In myotubes established from patients with type 2 diabetes, lipid oxidation and insulin-mediated glucose oxidation are reduced; in addition, palmitate impairs insulin-mediated glucose oxidation in myotubes of obese non-diabetic subjects [12,13]. These data suggest that abnormalities in oxidative function in vivo, in part, is of genetic origin.

In order to determine to which extent abnormalities in oxidative phosphorylation are intrinsic or acquired in obesity and T2D, we studied mitochondrial respiration (ADP stimulated and uncoupled respiration) and oxidative enzyme activities (citrate synthase (CS), 3-hydroxy-acyl-CoA-dehydrogenase activity (HAD)) before and after acute exposure to insulin and/or palmitate in myotubes established from healthy lean and obese subjects and patients with type 2 diabetes. Hexokinase (HK) activity was determined to evaluate the ratio between oxidative and glycolytic enzyme activities.

2. Methods

2.1. Human study subjects

Ten lean and 10 obese control subjects carefully matched to 10 obese type 2 diabetic patients participated in the study (Table 1). Muscle biopsies were obtained from the vastus lateralis muscle by needle biopsy under local anaesthesia. Diabetic patients were treated with either diet alone or in combination with sulfonylurea, metformin or insulin withdrawn 1 week before the study. The patients suffered from no diabetic complications except from simplex retinopathy. The control subjects had normal glucose tolerance and no family history of diabetes. All subjects gave written informed consent, and the local ethics committee of Funen and Vejle County approved the study.

2.2. Materials

Dulbecco’s modified Eagle’s medium, foetal calf serum (FCS), Ultroser G, penicillin-streptomycin-amphotericin B and trypsin–EDTA were obtained from Life Technology (Scotland, UK). The protein assay kit was purchased from BioRad (Copenhagen, DK). Palmitic acid (PA), bovine serum albumin (BSA) (essentially fatty acid free), L-carnitine and ECM-gel were purchased from Sigma Chemical Co. (St. Louis, USA). Insulin Actrapid was from Novo Nordisk (Bagsvaerd, DK).

2.3. Cell culture

Cell cultures were established as previously described [14,15]. In brief, muscle tissue was minced, washed and dissociated for 60 min by three treatments with 0.05% trypsin–EDTA. The cells harvested were pooled and FCS was added to stop trypsination. The cells obtained were

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**Clinical characteristics of the study subject**

<table>
<thead>
<tr>
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<th>Control, lean</th>
<th>Control, obese</th>
<th>T2D</th>
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<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51 ± 1</td>
<td>49 ± 1</td>
<td>50 ± 1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.6 ± 3.0</td>
<td>105.5 ± 6.4*</td>
<td>102.2 ± 4.1*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2 ± 0.5</td>
<td>33.7 ± 1.4*</td>
<td>33.5 ± 1.1*</td>
</tr>
<tr>
<td>Fasting plasma glucose (mM)</td>
<td>5.7 ± 0.1</td>
<td>5.7 ± 0.2</td>
<td>10.0 ± 0.7*</td>
</tr>
<tr>
<td>Fasting serum insulin (pM)</td>
<td>24.3 ± 5.7</td>
<td>52.7 ± 5.0*</td>
<td>94.6 ± 10.1*</td>
</tr>
<tr>
<td>Glucose infusion rate (mg/min)</td>
<td>383.3 ± 20.4</td>
<td>257.9 ± 28.3*</td>
<td>117.8 ± 18.6*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.5 ± 0.1</td>
<td>5.4 ± 0.1</td>
<td>7.7 ± 0.5*</td>
</tr>
<tr>
<td>Fasting total cholesterol (mM)</td>
<td>5.29 ± 0.22</td>
<td>5.43 ± 0.41</td>
<td>5.42 ± 0.37</td>
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<tr>
<td>Fasting LDL cholesterol (mM)</td>
<td>2.94 ± 0.22</td>
<td>3.33 ± 0.33</td>
<td>3.20 ± 0.27</td>
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<tr>
<td>Fasting HDL cholesterol (mM)</td>
<td>1.85 ± 0.15</td>
<td>1.48 ± 0.15</td>
<td>1.36 ± 0.03*</td>
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<tr>
<td>Fasting plasma triglyceride (mM)</td>
<td>1.12 ± 0.16</td>
<td>1.35 ± 0.18</td>
<td>1.93 ± 0.40</td>
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Data are means ± S.E.

* Significantly different from the lean and obese controls (P < 0.05).

* Significantly different from the lean controls (P < 0.05).
seeded for up-scaling on ECM-gel coated dishes after 30 min of preplating. Cell cultures were established in DMEM medium supplemented with 10% FCS, 50 U/ml penicillin, 50 µg/ml streptomycin and 1.25 µg/ml amphotericin B. After 24 h, cell debris and non-adherent cells were removed by change of growth medium to DMEM supplemented with 2% FCS, 2% Ultroser G, 50 U/ml penicillin, 50 µg/ml streptomycin and 1.25 µg/ml amphotericin B. Cells were subcultured twice before final seeding. At 75% confluence, the growth medium was replaced by basal medium (DMEM supplemented with 2% FCS, 2% Ultroser G, 50 U/ml penicillin, 50 µg/ml streptomycin and 1.25 µg/ml amphotericin B and 25 pmol/l insulin) in order to induce differentiation. The cells were cultured in humidified 5% CO2 atmosphere at 37 °C, and the medium was changed every 2–3 days.

2.4. Experimental design

Human myotubes established from controls and T2D subjects were allowed to differentiate for 8 days under physiological conditions, i.e. 0.24 mmol/l fatty acid free albumin (BSA), 0.5 mmol/l L-carnitine, 20 mmol/l HEPES, 25 pmol/l insulin and 5.5 mmol/l glucose. All myocyte cultures were used for analysis 8 days after the onset of differentiation. Myotubes were rinsed twice and incubated under basal conditions or 4 h of high insulin stimulation (1 µM), high fatty acid (palmitate, 0.6 mM) or both. Corresponding activities of HK, CS, HAD and total protein, recovered in the mitochondria-rich pellet averaged 87 ± 5% for CS and 26 ± 2% for protein and there were no difference between groups.

2.6. Measurement of oxygen consumption

The respiration rates of the suspended mitochondria-rich pellet were measured with a Clark-type electrode (Hansatech Instruments DW1) in a water-jacket glass chamber maintained at 25 °C and equipped with magnetic stirring. The measurements were carried out in 0.3 ml aliquots of a reaction medium containing 225 mM mannitol, 75 mM sucrose, 10 mM Tris, 10 mM KCl, 10 mM K2HPO4, 0.1 mM EDTA, 4.8 mM pyruvate, 1.9 mM malate, 0.25 mM ADP and pH 7.0. The ADP-stimulated respiration was initiated by the addition of 30 µl of the mitochondrial suspension to the reaction medium. When the respiration reached a stable rate, the maximal uncoupled respiration was measured by adding 5 µM carbonyl cyanide m-chlorophenylhydrazone (CCCP). When a steady state uncoupled respiration was achieved, the non-mitochondrial oxygen consumption was assessed by blocking mitochondrial respiration with 10 mM azide. The mitochondrial ADP-stimulated and uncoupled respiration was measured as the steady state respiration rates before and after adding CCCP minus the respiration in the presence of azide. As a marker of myotube total mitochondrial content, the maximal respiration of the electron transport chain was determined in triton X-100 (0.3%, 15 min) permeabilised mitochondria in the presence of NADH (450 μM) and cytochrome C (2 μM), i.e. electron transport chain quantity.

2.7. Enzyme activity and protein content

The maximal activities of HAD, a marker of beta-oxidation, and CS, a marker of the tricarboxylic acid (TCA) cycle, were determined fluorometrically [16] in the mitochondria-rich pellet. Acetoacetyl-CoA was used as substrate for HAD and prepared in a fresh solution on the day of analysis. HK activity was determined spectrophotometrically in accordance with the method described by Vestergaard et al. [17]. Mitochondrial protein content was assessed using a standard kit (Pierce BCA protein reagent No, 23225).

2.8. Statistical analysis

All values are given as means ± S.E. for all experiments run in duplicate or triplicate. For all statistical evaluations, significance was established at the 0.05 level. Statistical comparisons were performed with one way variance analysis (ANOVA) performed with StatView. When the ANOVA resulted in a significant F value (P < 0.05), the significant difference between means was located by Fisher’s PLSD post hoc test. n is the number of experiments.
3. Results

3.1. Subject characteristics

The clinical characteristics of the lean and obese controls and T2D patients are shown in Table 1. Fasting plasma glucose, serum insulin and HbA1c levels were significantly higher in the diabetic group compared with both the lean and obese controls. Fasting HDL cholesterol concentrations were lower in T2D patient compared with lean controls. The obese controls showed higher fasting serum insulin levels compared with lean controls. The glucose infusion rates (GIR) during the steady state of the hyperinsulinaemic euglycaemic clamp period were significantly lower in T2D patients compared with both lean and obese control subjects, and GIR was significantly lower in obese controls compared with lean control subjects.

3.2. CS activity

CS activity under baseline conditions averaged 24.1 ± 0.7 in myotubes established from T2D, 27.9 ± 1.5 in lean controls and 26.7 ± 1.0 unit/g protein in obese controls (Fig. 1). The CS activity was significantly lower (14%) in myotubes established from T2D patients compared with myotubes from lean controls (P < 0.05, Fig. 1). Incubation with insulin (1 μM) for 4 h increased the CS activity by 26% (P < 0.05) in myotubes from lean controls and 33% (P < 0.0001) in obese controls, whereas, in myotubes from T2D subjects, CS activity remained unaffected by insulin incubation (Fig. 2). Incubation with high extracellular fatty acid (palmitate, 0.6 mM) for 4 h showed no effect on CS activity in neither of the groups. However, when incubating palmitate in combination with insulin, the stimulatory insulin effect on CS activity was abolished (Fig. 3).

3.3. Myotube oxidative function

ADP-stimulated oxygen consumption was similar in the different groups, averaging 23.0 ± 0.6 μmol O2 protein⁻¹ min⁻¹, and was not influenced by treatment. The CCCP uncoupled respiration averaged 35.5 ± 0.5 μmol O2 g protein⁻¹ min⁻¹ with no differences between neither groups nor treatments (Fig. 3A). In addition, no differences were found in the maximal mitochondrial uncoupled respiration under basal conditions when related to CS activity, averaging 1.33 ± 0.03 μmol O2 unit CS⁻¹ min⁻¹ (Fig. 3B). Given the insulin stimulated increase in CS activity, the CCCP uncoupled respiration per CS activity was significantly lower in the obese (P < 0.05) and there was a tendency to lower respiration in the lean control (P = 0.14) following insulin incubation. In both lean and obese controls, there was a lower CCCP uncoupled respiration per CS activity compared with T2D when incubation with insulin and FA in combination. The CCCP uncoupled respiration was significantly correlated with CS activity under basal conditions for controls (r² = 0.96, P < 0.001).

To verify if the lower CS in T2D could be explained by a lower mitochondrial content, the NADH respiration in permeabilised mitochondria was measured as a marker of mitochondrial content. There were no significant difference in the mitochondrial NADH respiration under basal conditions between groups, averaging 35.4 ± 3.4, 27.6 ± 4.9, and 23.6 ± 3.5 μmol O2 g protein⁻¹ min⁻¹, in the control (n = 8), obese (n = 5) and T2D (n = 5), respectively. However, there was a tendency (P = 0.06) to a lower NADH respiration in the T2D myotubes. There were no difference in mitochondrial NADH respiration in the control myotubes following incubation with high insulin (1 μM) for 4 h (35.4 ± 3.4 vs. 36.2 ± 5.0 μmol O2 g protein⁻¹ min⁻¹, basal (n = 8) and ins (n = 6), respectively).

3.4. HAD and HK activity

No group differences in myotube HAD activity were found (Fig. 4). Furthermore, no effect of either insulin, palmitate or both on HAD enzyme activity was revealed. Moreover, no differences in HK activity or HK/CS activity ratio were detected between groups, and the activity and ratio showed no significant response on either insulin, palmitate or both (data not shown).

4. Discussion

The main findings of the present study are the following: (1) myotubes established from T2D subjects express a primarily reduced CS activity insensitive to insulin stim-
ulation; and (2) insulin stimulates CS activity by 26–33% in myotubes established from lean and obese control subjects. The stimulatory insulin effect was abolished in the presence of high palmitate concentrations. No primary defects or direct insulin or fatty acid effects were localized to HK and HAD activities or oxidative phosphorylation abnormalities and there were no difference in the total mitochondrial content. These results provide evidence for an intrinsic defect in CS activity, which may play a role in the pathogenesis of T2D. Moreover, the data suggest that insulin resistance at the CS level can be induced by exposure to high free fatty acid levels in lean and obese subjects. The electron transport chain does not seem to be primarily affected during insulin resistance.

Several studies have shown an association between obesity, insulin resistance and reduced oxidative capacity in skeletal muscle [1,18,19], as demonstrated by the finding of decreased mitochondrial number, altered mitochondrial morphology and decreased levels of mitochondrial enzymes, i.e. succinate dehydrogenase [18,20], CS [1,2,21,22] and carnitine palmitoyltransferase-1 (CPT1) [21,22]. To gain further insight into the mechanism underlying these alterations, we investigated to which extent these alterations could be explained by inherited factors by using a model of cultured human myotubes. Recently, we showed that basal lipid oxidation and insulin-mediated glucose oxidation are reduced and are therefore possibly inherited defects in diabetic myotubes [12,13]. In the present study, we extended these findings by showing that diabetic myotubes have an intrinsic defect in CS activity with a lower basal activity and an attenuated response to insulin stimulation. These data suggest that previous reports of reduced CS activity in vivo may be of primary origin and the CS activity is impaired irrespective of the mitochondrial content. An intriguing new observation was that myotubes established from lean and obese control subjects, but not from T2D subjects, express an insulin-mediated increased CS activity. The mechanism of the increased CS activity is elusive. No polymorphism in the human CS gene have been reported yet, and de novo CS protein synthesis is also an unlikely explanation based on the fact that co-incubation with palmitate for 4 h completely abolished this effect of insulin on CS activity. Furthermore, allosteric modulation seems doubtful since CS was assayed in vitro under similar assay conditions. It could, however, be speculated that the insulin-induced CS activation is due to a covalent modification. In fact, citrate synthase was recently identified as a phosphorylated protein in a global analysis of phosphoproteins in bacteria (Corynebacterium glutamicum) [23]. The regulation of proteins by phosphorylation is far more typical for eukaryotes than for prokaryotes, particularly in response to insulin. Thus, phosphorylation/dephosphorylation of citrate synthase represents a likely mechanism for the observed change in activity in response to insulin. However, other covalent modifications could be involved, and so far no experimental evidence exists for such a covalent CS modification in animals, and the pathways responsible for these findings are at present unknown. Recently, it has been reported that acute exercise for 75 min at 70% of VO2max induced a 43% increase in CS activity and that the elevated CS remains 3 h after exercise [24]. Plasma insulin is reduced during long-term exercise, demonstrating that exercise is an additional stimulus for CS activation, independent of insulin, very similar to the translocation of GLUT4-containing vesicles to the membrane in skeletal muscle.

Fig. 2. Effects of insulin and high fatty acid on CS activity in myotubes from lean controls, obese controls and T2D patients. CS activity was determined in the mitochondria-rich suspensions of fully differentiated myotubes established from lean, obese and T2D subjects precultured under basal conditions (bas), with high insulin (1 μM, ins), high fatty acid (palmitate, 0.6 mM, FA) or both for 4 h, as described in Methods. Data are means ± S.E., n = 10 in each group. *P < 0.05 vs. basal.
We previously showed that free fatty acids (FFA) abolish the insulin-mediated increase in glucose oxidation in myotubes [13] and in line with this, the present finding verifies that FFA impairs the insulin-mediated CS activation in myotubes from non-diabetic subjects. This observation indicates that the increased FFA associated with T2D and obesity may operate through CS, and thereby may contribute to the pathogenesis of lipid-induced insulin resistance. We hypothesise that FFA, in some way, may abolish the covalent CS modification. Palmitate has been shown to exert effects on insulin-signalling pathways without conversion to palmitoyl-CoA [25]. FFA-induced insulin resistance is associated with an impairment of IRS-1 tyrosine phosphorylation and IRS-1-associated PI3K activation [26–28]. Ceramide is a palmitate derivative and a second messenger produced by sphingomyelinase activation, and it is known to be increased in insulin-resistant cell systems [29,30]. Studies have shown that PKB activation can be reduced in the presence of ceramide [31]. Other mechanisms responsible for palmitate-induced reduced glucose metabolism include activation by saturated fatty acids of the hexosamine pathway [32], and increased proportions of saturated fatty acids in the membrane have been shown to reduce insulin action [33]. However, the
precise mechanism by which FFA inhibits insulin action on CS remains at present unknown.

The mitochondrion has long been known as the powerhouse of the eukaryotic cell, supplying most of the necessary ATP. Recently, functional mitochondria impairment has been linked to the pathogenesis of insulin resistance in skeletal muscle. To support the hypothesis of an existing link between impaired mitochondrial function and T2D, patients with the autosomal disorder Friedreich’s ataxia, resulting in an impaired oxidative phosphorylation, frequently develop diabetes [5]. Petersen et al. [8] revealed that the skeletal muscle of insulin-resistant T2D patient offspring have a possibly inherited defect in mitochondrial oxidative phosphorylation, which may account for the dysregulation of intracellular fatty acid metabolism. A crucial observation made in muscle tissue is that the mitochondrial content is reduced in diabetic muscle [2], as well as the expression the expression of mitochondrial genes of oxidative metabolism [4]. In addition, the activity of rotenone sensitive NADH:O2 oxidoreductase, reflecting the activity of the respiratory chain, was reported to be impaired in T2D subjects and, to a lesser degree, in obesity [2]. These observed impairment in mitochondrial content or function could be both inherited and/or acquired. In order to determine to which extent oxidative phosphorylation abnormalities are intrinsic or acquired in obesity and T2D, we studied mitochondrial uncoupled respiration before and after acute exposure to insulin and/or palmitate in myotubes established from healthy lean and obese subjects and T2D patients. When measuring the mitochondrial respiration, the rate will be determined by the enzyme showing the lowest activity. Using pyruvate and malate as substrates, iso-citrate will be the end product, and the enzyme with the lowest activity in the production of NADH will be PDH [34]. Rasmussen et al. [34] observed that when using pyruvate and malate, the ATP synthesis exerted the major flux control over the respiration revealed as an increased respiration when an uncoupler was added to the measurement suspension. In line with this, the present study demonstrated an increased respiration after uncoupling of the proton gradient, in myotubes established from lean, obese and T2D subjects. This strongly suggests that the respiration is limited by the down-stream complex IV, i.e. the ATP synthesis or alternatively the adenine–nuclotide–translocase. Furthermore, the uncoupled respiration rate was not significantly changed between the groups, suggesting that the electron transport chain expresses no intrinsic defects. To verify the possibility of a lower mitochondrial content in T2D myotubes, we investigated the mitochondrial content in the myotubes by three different parameters: (1) differences between groups for mitochondrial protein yield, (2) CCCP uncoupled respiration, and (3) NADH respiration of the electron transport chain determined in permeabilised mitochondria. All 3 measures of mitochondrial content did not show any significantly differences in mitochondrial content between groups. The reduced mitochondrial content of diabetic muscle tissue seems in our hand to be adaptive. Furthermore, a reduced mitochondrial content seems not to be the explanation for the lower basal CS activity in diabetic myotubes.

We previously showed that diabetic myotubes express a primarily reduced lipid oxidation [12]. In the present study, we investigated the activity of HAD, the rate-limiting enzyme in the mitochondrial beta-oxidation, and were not able to verify significant differences between the different groups, thus indicating that the beta-oxidation is not primarily affected in T2D and that the controls may be at the CPT1 level. In line with this, we recently showed that etomoxir, an inhibitor of CPT1, decreased lipid oxidation more in control than in diabetic myotubes [12], thus suggesting that CPT1 activity is reduced or that CPT1 is already inhibited i.e. by malonyl-CoA in diabetic myotubes. Cultured myotubes offer a unique model for separating the genetic influence on the disease from...
environmental factors. Our model system is not influenced by differences in fibre type level or physical training status, which influence the oxidative capacity and are confounding factors in the interpretation of data from muscle biopsies.

In conclusion, we showed that myotubes established from T2D subjects express a primarily reduced CS activity and that insulin stimulates CS activity in myotubes established from lean and obese control subjects, but not in myotubes from T2D patients. The stimulatory insulin effect on CS activity in control myotubes was absent in the presence of high palmitate concentrations. Electron transport does not seem primarily or secondarily affected by insulin or FFA in myotubes established from lean and obese control and T2D subjects. Our data furthermore focus on mitochondrial dysfunction as a part of the pathogenesis of insulin resistance and T2D through primary and adaptive alterations shown by CS at present.

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References


