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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.

Keywords: Citrate synthase; Insulin resistance; Free fatty acid; Myotube; Obese; Type 2 diabetes; Uncoupled respiration

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

Abbreviations: CPT-1, carnitine palmitoyltransferase-1; CS, citrate synthase; HAD, 3-hydroxy-acyl-CoA-dehydrogenase; HK, hexokinase; T2D, Type 2 diabetes; CCCP, carbonyl cyanide *m*-chloro phenyl hydrazone; TCA, tricarboxylic acid; FCS, foetal calf serum; PA, palmitic acid; BSA, bovine serum albumin; GIR, glucose infusion rates; PDH, pyruvate dehydrogenase; G6P, glucose-6-phosphate; FFA, free fatty acid

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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 in 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

Table 1
Clinical characteristics of the study subject

	Control, lean	Control, obese	T2D
n	10	10	10
Age (years)	51±1	49±1	50±1
Weight (kg)	71.6±3.0	105.5±6.4*	102.2±4.1*
BMI (kg/m^2)	24.2±0.5	33.7±1.4*	33.5±1.1*
Fasting plasma glucose (mM)	5.7±0.1	5.7±0.2	10.0±0.7[a]
Fasting serum insulin (pM)	24.3±5.7	52.7±5.0*	94.6±10.1[a]
Glucose infusion rate (mg/min)	383.3±20.4	257.9±28.3*	117.8±18.6[a]
HbA_{1c} (%)	5.5±0.1	5.4±0.1	7.7±0.5[a]
Fasting total cholesterol (mM)	5.29±0.22	5.43±0.41	5.42±0.37
Fasting LDL cholesterol (mM)	2.94±0.22	3.33±0.33	3.20±0.27
Fasting HDL cholesterol (mM)	1.85±0.15	1.48±0.15	1.36±0.03*
Fasting plasma triglyceride (mM)	1.12±0.16	1.35±0.18	1.93±0.40

Data are means±S.E.
[a] Significantly different from the lean and obese controls ($P<0.05$).
* Significantly different from the lean controls ($P<0.05$).