Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1772 (2007) 755–765
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbadis

# Insulin resistance and the mitochondrial link. Lessons from cultured human myotubes

Michael Gaster *

*KMEB, Department of Endocrinology, Odense University Hospital, DK-5000 Odense, Denmark*

Received 7 December 2006; received in revised form 5 March 2007; accepted 23 March 2007
Available online 1 April 2007

**Abstract**

In order to better understand the impact of reduced mitochondrial function for the development of insulin resistance and cellular metabolism, human myotubes were established from lean, obese, and T2D subjects and exposed to mitochondrial inhibitors, either affecting the electron transport chain (Antimycin A), the ATP synthase (oligomycin) or respiratory uncoupling (2,4-dinitrophenol). Direct inhibition of the electron transport chain or the ATP synthase was followed by increased glucose uptake and lactate production, reduced glycogen synthesis, reduced lipid and glucose oxidation and unchanged lipid uptake. The metabolic phenotype during respiratory uncoupling resembled the above picture, except for an increase in glucose and palmitate oxidation. Antimycin A and oligomycin treatment induced insulin resistance at the level of glucose and palmitate uptake in all three study groups while, at the level of glycogen synthesis, insulin resistance was only seen in lean myotubes. Primary insulin resistance in diabetic myotubes was significantly worsened at the level of glucose and lipid uptake. The present study is the first convincing data linking functional mitochondrial impairment per se and insulin resistance. Taken together functional mitochondrial impairment could be part of the pathophysiology of insulin resistance in vivo.


*Keywords:* Glucose metabolism; Insulin resistance; Lipid metabolism; Metabolic inhibition; Mitochondrial function; Myotube; Skeletal muscle; Type 2 diabetes

## 1. Introduction

Type 2 diabetes (T2D) mellitus is characterized by alterations in both lipid and glucose metabolism, and an increasing body of evidence suggests an association between insulin resistance, T2D, and mitochondrial dysfunction. Initial *in vivo* studies reported a diminished citrate synthase (CS)/hexokinase (HK) ratio in diabetic skeletal muscles, showing that the oxidative/glycolytic ratio correlated with insulin sensitivity [1]. Mitochondrial size and activity were also 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 analyses of gene expression patterns demonstrated a coordinated reduction in mRNA transcripts of nuclear-encoded genes involved in mitochondrial oxidative phosphorylation including the catalytic beta-subunit of the ATP synthase in both prediabetic and T2D subjects [4–6]. Petersen et al. [7] provided evidence for a possible, inherited defect in mitochondrial oxidative phosphorylation in skeletal muscle of insulin-resistant offspring of T2D patients. This defect was associated with dysregulation of intracellular fatty acid metabolism. In myotubes established from T2D patients, lipid oxidation, and insulin-mediated glucose oxidation and insulin-mediated increase in citrate synthase activity were reduced. In addition, palmitate (PA) impaired insulin-mediated glucose oxidation and insulin-mediated increase in citrate synthase activity in myotubes of obese non-diabetic subjects [8–10]. Taken together,

*Abbreviations:* AA, antimycin A; BSA, bovine serum albumin; DNP, 2,4-Dinitrophenol; FCS, foetal calf serum; FFA, Free fatty acids; GIR, glucose infusion rates; OA, oligomycin A; PA, palmitate; TAG, Triacylglycerol; T2D, Type 2 diabetic/type 2 diabetes

* Tel.: +45 65411769; fax: +45 65919653.
*E-mail address:* Michael.Gaster@ouh.fyns-amt.dk.

0925-4439/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbadis.2007.03.007

these data suggest that abnormalities in oxidative function may be associated with insulin resistance. Whether a direct link exists between reduced mitochondrial function and insulin resistance in skeletal muscles, can be questioned. However, our current knowledge of oxidative enzyme activities in the tricarboxylic acid cycle, mitochondrial beta-oxidation, and electron transport chain in relation to insulin resistance and T2D originates mainly from *in vivo* studies. Both the mitochondrial oxidative capacity and insulin resistance of skeletal muscle are highly influenced by physical activity, ageing, and fiber type composition rendering it difficult to clarify whether a direct link exists between mitochondrial dysfunction and insulin resistance in T2D. Cultured myotubes offer a unique model to separate the genetic influence on insulin resistance and T2D from environmental factors [11–13], but also the impact of single factors on the metabolism on various genetic backgrounds. In order to better understand the impact of reduced mitochondrial function for the development of insulin resistance and cellular metabolism, human myotubes were established from lean, obese, and T2D subjects exposed to mitochondrial inhibitors either affecting the electron transport chain (Antimycin A (AA), Complex III inhibitor), the ATP synthase (oligomycin (OL), F0–F1 inhibitor) or by respiratory uncoupling (2,4-dinitrophenol, ionophor) in order to evaluate if exposure to these inhibitors changes the intermediary metabolism and whether they may induce insulin resistance in exposed myotubes.

## 2. Methods

### 2.1. Human study subjects

Ten lean, ten obese control subjects, and ten obese T2D patients participated in the study (Table 1), and clinical characteristics have previously been published [10]. Muscle biopsies were obtained from the *vastus lateralis* muscle by needle biopsy under local anesthesia. 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 for *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.

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[a] | 102.2±4.1[a] |
| BMI (kg/m$^2$) | 24.2±0.5 | 33.7±1.4[a] | 33.5±1.1[a] |
| Fasting plasma glucose (mM) | 5.7±0.1 | 5.7±0.2 | 10.0±0.7[b] |
| Fasting serum insulin (pM) | 24.3±5.7 | 52.7±5.0[a] | 94.6±10.1[b] |
| Glucose infusion rate (mg/min) | 383.3±20.4 | 257.9±28.3[a] | 117.8±18.6[b] |
| $HbA_{1c}$ (%) | 5.5±0.1 | 5.4±0.1 | 7.7±0.5[b] |
| 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[a] |
| Fasting plasma triglyceride (mM) | 1.12±0.16 | 1.35±0.18 | 1.93±0.40 |

Data are means±SE.
[a] Significant different from the lean controls ($p<0.05$).
[b] Significant different from the lean and obese controls ($p<0.05$).

### 2.2. Materials

Dulbecco's modified Eagle's medium, fetal calf serum (FCS), penicillin–streptomycin–amphotericin B, and trypsin–EDTA were obtained from Invitrogen (Invitrogen, Scotland, UK). Ultroser G was purchased from Pall Biosepra (Cergy-Saint-Christophe, France). Protein assay kit was purchased from BioRad (Copenhagen, DK). Palmitic acid, L-carnitine, AA, OL, and ECM-gel were purchased from Sigma Chemical Co. (St. Louis, USA). Bovine serum albumin (BSA) (essentially FA free), were from Calbiochem (VWR, Roskilde, DK). 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 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, 50 U/ml penicillin, 50 μg/ml streptomycin, 1.25 μg/ml amphotericin B, and 25 pmol/l insulin) in order to induce differentiation. The cells were cultured in humidified 5% $CO_2$ atmosphere at 37°C, and medium was changed every 2–3 days.

### 2.4. Experimental design

Human myotubes established from lean, obese, and T2D subjects were allowed to differentiate under physiological conditions of insulin (25pmol/l) and glucose (5.5 mmol/l) for 8 days. All myotube cultures were used for analysis day eight after onset of differentiation. At day eight, myotubes were exposed to four different protocols: (a) basal medium (5.5 mmol/l Glucose, 25 pmol/l Insulin); (b) basal medium supplemented with 1.0 μg/ml AA; (c) basal medium supplemented with 5.0 μg/ml OL; (d) basal medium supplemented with 1.0 mmol/l 2,4-Dinitrophenol, and subsequently basal and insulin-stimulated glucose uptake, glucose oxidation, glycogen synthesis (GS), lipid uptake and lipid oxidation were determined. Furthermore, the lactate concentration was determined in the media after terminating the experiments.

### 2.5. Mitochondrial inhibition

In order to verify that the inhibitors were inhibiting the mitochondria the $O_2$-utilisation rate of trypsinated myotubes in suspension 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 in DMEM containing 5.5 mmol/l glucose [10]. Myotube cultures were used for analysis day eight after onset of differentiation. When the $O_2$-respiration reached the stable rate, the uncoupled respiration was measured after adding increased concentrations of DNP (1 pM to 1 mM). The maximal uncoupled respiration was measured after adding 0.1 mmol/l DNP (final concentration) without further increase, thereby immediately (30–60 s) enhancing the respiration rate 2.8 times. In parallel the effect of AA (1 pg/ml to 1 μg/ml) and OA (5 pg/ml to 5 μg/ml) on the respiration of myotubes were studied after a steady state respiration was achieved. Maximal inhibition of the respiration of myotubes was achieved at a concentration of 0.5 mg/ml and 1.25 mg/ml (final concentration) respective for AA and OA blocking the respiration rate to 5% and 3% of basal respiration rate (Data not shown, $N=2$). Furthermore, the total ATP content in cell extract was measured with the ATP monitoring reagent (ATPlite from PerkinElmer, Turku, Finland). Cells were grown and differentiated in 12 well plates, as described above. At day eight, myotubes were exposed to four different protocols: (a) basal medium (5.5 mmol/l Glucose, 25 pmol/l Insulin); (b) basal medium supplemented with 0.01, 0.1 and 1.0 μg/ml AA; (c) basal medium supplemented with 0.05, 0.5 and 5.0 μg/ml OL; d) basal medium supplemented