Biochemical and Biophysical Research Communications 402 (2010) 70–74

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

ATP synthesis is impaired in isolated mitochondria from myotubes established from type 2 diabetic subjects

Ariane D. Minet, Michael Gaster *

Department of Endocrinology and Department of Pathology, Odense University Hospital, Denmark

ARTICLE INFO

Article history:
Received 20 September 2010
Available online 25 October 2010

Keywords:
ATP
Human skeletal muscle
Myotubes
Mitochondria
Mitochondrial mass

ABSTRACT

To date, it is unknown whether mitochondrial dysfunction in skeletal muscle from subjects with type 2 diabetes is based on primarily reduced mitochondrial mass and/or a primarily decreased mitochondrial ATP synthesis. Mitochondrial mass were determined in myotubes established from eight lean, eight obese and eight subjects with type 2 diabetes precultured under normophysiological conditions. Furthermore, mitochondria were isolated and ATP production was measured by luminescence at baseline and during acute insulin stimulation with or without concomitant ATP utilization by hexokinase. Mitochondrial mass and the ATP synthesis rate, neither at baseline nor during acute insulin stimulation, were not different between groups. The ratio of ATP synthesis rate at hexokinase versus ATP synthesis rate at baseline was lower in diabetic mitochondria compared to lean mitochondria. Thus the lower content of muscle mitochondria in type 2 diabetes in vivo is an adaptive trait and mitochondrial dysfunction in type 2 diabetes in vivo is based both on primarily impaired ATP synthesis and an adaptive loss of mitochondrial mass.

1. Introduction

Studies suggest that mitochondrial dysfunction in type 2 diabetes is due to a lower content of muscle mitochondria [1,2], whereas a role for decreased functional capacity per mitochondrion needs to be further explored [3–6]. Studies using magnetic resonance spectroscopy have shown lower rates of mitochondrial ATP production and substrate oxidation in lean first degree relatives with severe insulin resistance [7,8]. Using high-resolution respirometry, Boushel et al. [4] found that ADP-stimulated state 3 respiration was normal in permeabilized skinned muscle fibers from type 2 diabetic patients. In contrast, Mogensen et al. [5] reported that maximal ADP-stimulated respiration was reduced in mitochondria isolated from diabetic patients but could not reproduce the finding in a recent study [6]. At present there is no clear evidence for whether the intrinsic ATP production is primarily impaired or not in mitochondria in skeletal muscle from type 2 diabetic subjects.

The oxidative capacity of skeletal muscle is highly influenced by physical activity, aging, hormonal status, and fiber type composition, rendering it difficult to determine the contribution of single factors to the alteration in oxidative metabolism. Cultured myotubes offer a unique model to distinguish between genetic and environmental factors in the etiology of insulin resistance and type 2 diabetes [9–18]. In the present study, we took advantage of this model to investigate whether the mitochondrial mass is primary reduced in myotubes established from type 2 diabetic subjects and whether mitochondrial ATP production at baseline or during insulin stimulation is primary impaired in mitochondria isolated from myotubes established from lean and obese subjects, and patients with type 2 diabetes. Furthermore, ATP synthesis was studied under conditions of maximal ATP utilizations by the hexokinase reaction.

2. Materials and Methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), 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 Bio-Rad (Copenhagen, DK). Palmitic acid, L-carnitine, cytochrome c, and ECM-gel were purchased from Sigma Chemical Co. (St. Louis, USA). Bovine serum albumin (BSA) (essentially fatty acid free) was from Calbiochem (VWR, Roskilde, DK). Insulin Actrapid was from Novo Nordisk (Bagsvaerd, DK).

2.2. Human study subjects

Eight lean, eight obese control subjects, and eight obese patients with type 2 diabetes participated in the study (Table 1). Their

* Corresponding author. Address: Department of Endocrinology, Odense University Hospital, DK-5000 Odense, Denmark. Fax: +45 65919653.
E-mail address: michael.gaster@ouh.regionsyddanmark.dk (M. Gaster).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.09.115

Table 1
Clinical characteristics of the study subject.

	Control, lean	Control, obese	Type 2 diabetic
n	8	8	8
Age (years)	53 ± 2	55 ± 2	52 ± 2
BMI (kg/m^2)	23.2 ± 0.7	30.79 ± 0.9*	30.0 ± 1.5*
Fasting plasma glucose (mM)	5.7 ± 0.2	5.9 ± 0.2	9.3 ± 0.8#
Fasting serum insulin (pM)	34.9 ± 5.1	34.4 ± 3.9	87.6 ± 19.81#
Glucose infusion rate (mg/min)	316 ± 22	287 ± 21	118 ± 25#
HbA_{1c} (%)	5.4 ± 0.1	5.4 ± 0.1	7.0 ± 0.5#

Data are means ± SE.
* Significant different from the lean controls ($P < 0.05$).
# Significant different from the lean and obese controls ($P < 0.05$).

clinical characteristics have partly been published [6]. Muscle biopsies were obtained from the *vastus lateralis* muscle by needle biopsy under local anesthesia. Diabetic patients were treated either with diet alone or in combination with sulfonylurea, metformin or insulin, withdrawn one 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.

2.3. Myotube cultures

Cell cultures were established as previously described [11,19]. In brief, muscle tissue was minced, washed and dissociated for 60 min by three treatments with 0.05% trypsin–EDTA. The cells obtained were seeded for up-scaling on ECM-gel coated dishes after 30 min of pre-plating. Growth medium contained 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.

3. Experimental

Human myotubes established from lean, obese and diabetic patients were allowed to differentiate under physiological conditions of insulin (25 pmol/l) and glucose (5.5 mmol/l) for 4 days before half of each group were exposed for a high insulin concentration (1 μmol/l) for 1 h while the rest remained in basal conditions, followed by subsequent mitochondria isolation. In purified mitochondria from baseline and acute insulin stimulated myotubes, ATP synthesis rate was measured by luminescence with or without ATP utilization by the hexokinase reaction catalyzing glucose 6-phosphate production from glucose and ATP. The mitochondrial substrate buffer contains 300 mM sucrose, 10 mM KCl, 10 mM KH_2PO_4, 10 mM Tris–Base, 0.1 mM EDTA, 1 mM pyruvate, 1 mM malate and 0.35 mM ADP, and was supplemented by 3 U/ml hexokinase and 1 mM glucose during conditions of ATP utilization. ATP production was measured after 0, 5, 10, 15, 30 and 45 min (Fig. 1). ATP synthesis is constant over the first 15 min and free ATP continues to increase during the whole time of measurement. In the presence of hexokinase, much less free ATP is present and after 15 min, ATP production reaches equilibrium with the ATP consumption of the hexokinase reaction. Mitochondrial ATP production was corrected for fading of luminescence of the luciferase used in the ATP assay and normalized per μg of mitochondria protein and per minute. Moreover, mitochondrial mass were determined in

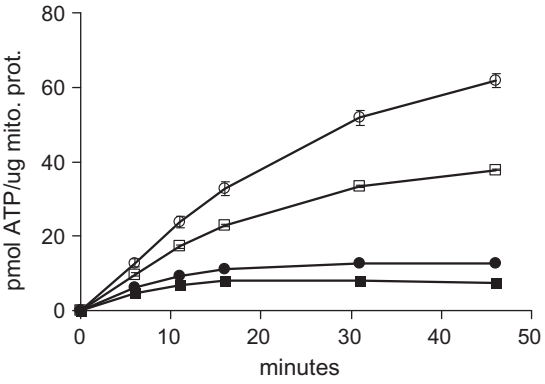

Fig. 1. ATP synthesis at baseline and during ATP utilization. Isolated mitochondria were incubated with substrate buffer without (baseline) and with hexokinase (HK) to simulate ATP utilization (see Section 3). Total ATP was measured at the indicated time points. Open circle: lean at baseline; closed circle: lean with HK; open square: diabetic at baseline; closed square: diabetic with HK.

myotubes established from eight lean, eight obese and eight subjects with type 2 diabetes precultured under normophysiological conditions.

3.1. Mitochondria preparation

Mitochondria were isolated from cultured human myotubes using the MACS mitochondria isolation kit from Miltenyi Biotech, Germany, following the protocol of Hornig-Do et al. [20]. Mitochondria extracts were examined by Western blot. Glycogen synthase (GS), used as cytosolic marker, was present in myotubes but completely absent in purified mitochondria while the mitochondrial marker prohibitin was strongly enriched in mitochondrial samples (data not shown).

In order to test mitochondrial integrity isolated mitochondria were incubated with 100 μM cytochrome C (Sigma–Aldrich/Fluka) which is expected to increase their ATP production in the case of damaged mitochondrial membrane, ATP production was not increase in our experiment, indicating that the isolated mitochondria were intact (data not shown).

3.2. ATP

ATP was determined with the ATP monitoring reagent (ATPlite from PerkinElmer, Turku, Finland) in 96-well plates and determined by luminescence on a Microbeta counter (PerkinElmer, Finland) as previous described [21].

3.3. SDS–PAGE and Western blot

Proteins were separated by SDS–PAGE using the NuPAGE system of Invitrogen with 4–12% Bis–Tris gradient gels and MOPS SDS running buffer according to the manufacturer's instructions. After running, gels were transferred onto nitrecellulose membranes and blotted using the iBlot dry blotting system from Invitrogen. Blots were probed with prohibitin (Lab Vision/NeoMarkers, Fremont, Canada) and glycogen synthase (GS) antibody (a gift from Dr. H. Vestergaard, Copenhagen, Denmark) [22] as primary antibodies and detected with chromogen (Invitrogen).

3.4. Mitochondrial mass

For quantification of mitochondrial mass, we used MitoTracker Green Probe (Molecular Probes, Eugene, OR) which preferentially accumulates in mitochondria regardless of the mitochondrial membrane potential and gives an assessment of the mitochondrial mass.

Myotubes were incubated at 37 °C for 30 min with 100 nM MitoTracker Green in PBS and subsequently washed with PBS. Fluorescence intensity was determined on a VICTOR plate reader model 1420-050 (PerkinElmer, Turku, Finland) with excitation and emission wavelength of 485 and 535, respectively as described previously [18]. Values were corrected for protein, and indicated as arbitrary units.

3.5. Statistical analysis

The data in the text, tables, and figures are given as mean ± SEM. Statistical analysis were performed with INSTAT 2.01 (GraphPad, USA). $P < 0.05$ was considered to be significant.

3.6. Methodological considerations

In the present study, cultured myotubes rather than skeletal muscle tissue were used to verify intrinsic ATP synthesis impairments in diabetic mitochondria. Advantageously, isolated satellite cells replicated for 4–6 weeks and differentiated under normophysiological conditions, excluding other metabolic inducing factors, thus allowing our approach to determine heritable traits.

4. Results

4.1. Subject characteristics

Clinical characteristics of lean and obese controls and patients with type 2 diabetes are shown in Table 1. BMI was significantly higher in obese controls and diabetic subjects compared with lean control subjects. Fasting plasma glucose, serum insulin and HbA_{1c} levels were significantly higher in the diabetic group compared with both the lean and obese controls. The glucose infusion rates (GIR) during the steady state of the hyperinsulinemic euglycemic clamp period were significantly lower in diabetic patients compared with both lean and obese control subjects.

4.2. Myotube cultures

Myotubes established from control and diabetic subjects were indistinguishable under phase-contrast microscopy. Differentiated cultures contained many multinucleated myofibers. Mitochondrial protein was 7, 9% ± 0, 29 (means ± SEM) of total protein independent of group.

4.3. Intrinsic mitochondrial ATP synthesis

Mitochondria isolated from myotubes established from lean, obese and diabetic subjects did not express a different ATP production rate neither at baseline nor during acute insulin stimulation (Fig. 2A).

4.4. Intrinsic mitochondrial ATP synthesis during ATP utilization

The ATP synthesis rate during ATP utilization by hexokinase decreased in all mitochondria at least 48% ($P < 0.05$) and were not insulin sensitive (Fig. 2A). There was trend towards less ATP production during hexokinase exposure in mitochondria isolated from diabetic myotubes compared to lean myotubes ($P = 0.07$). The ratio, ATP synthesis rate at hexokinase divided by ATP synthesis rate at baseline, was 24% lower in diabetic mitochondria compared to lean mitochondria ($P < 0.05$) and was not insulin sensitive (Fig. 2B).

4.5. Mitochondrial mass

Prior observations indicate that skeletal muscle from type 2 diabetic subjects show a lower mitochondrial mass [1,2]. We measured the mitochondrial mass in myotubes established from lean, obese and diabetic subjects and could not detect any significant differences ($P > 0.80$) in mitochondrial mass between groups (Fig. 3).

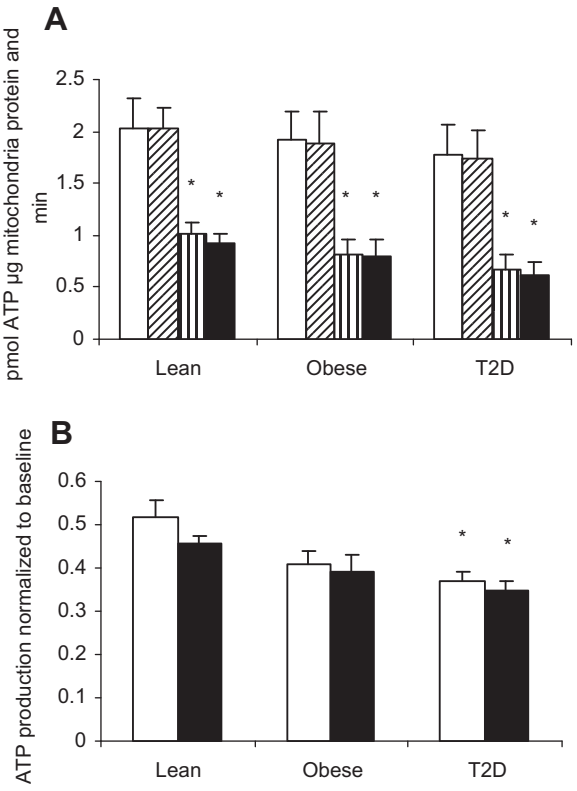

Fig. 2. Rates of ATP synthesis in isolated mitochondria. (A) The ATP synthesis rate was determined in isolated mitochondria of differentiated myotubes established from lean, obese and type 2 diabetic subjects. Myotubes were precultured under baseline conditions and during insulin stimulation and their purified mitochondria were incubated with or without hexokinase as described in Section 3. Open bars: baseline without hexokinase; cross hatched bars: insulin stimulation without hexokinase; longitudinally hatched bars: baseline with hexokinase; black bars: insulin stimulation with hexokinase. Data are means ± SE, $n = 8$ in each group. $^*P < 0.05$ vs. baseline. (B) The ATP synthesis rate during hexokinase exposure were normalized to the corresponding ATP synthesis rate at baseline or acute insulin stimulation respectively in mitochondria isolated from myotubes established from lean, obese and diabetic subjects as described in Section 3. Data are means ± SE, $n = 8$ in each group. $^*P < 0.05$ vs. corresponding lean.

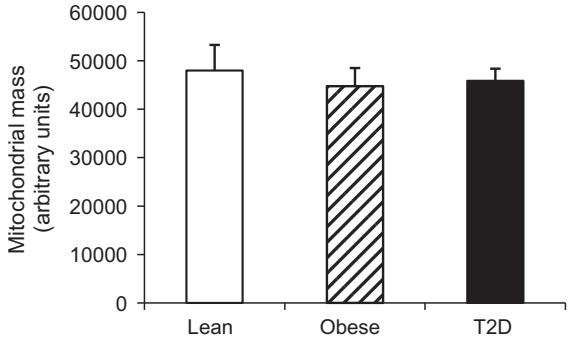

Fig. 3. Mitochondrial mass. Mitochondrial mass determined by MitoTracker Green fluorescence in myotubes established from lean ($n = 8$; white bars), obese ($n = 8$; cross hatched bars) and T2D subjects ($n = 8$; black bars). All data are given as mean ± SEM. There were no significant differences between groups.

5. Discussion

Cultured human myotubes represent a well-characterized in vitro model system of skeletal muscle in which the extracellular

environment can be controlled precisely and kept consistent over time [19]. In the present study, we used this model to compare the metabolic characteristics of isolated mitochondria from myotubes established from lean, obese and diabetic subjects cultured under normoglycemic and normoinsulinemic conditions and after acute insulin stimulation. We aimed to investigate whether the intrinsic ATP synthesis in mitochondria isolated from diabetic patients was primarily reduced. We could not find evidence for primarily reduced ATP synthesis at baseline in isolated mitochondria of myotubes established from diabetic patients suggesting that the previous in vivo findings of reduced ATP flux by magnetic resonance spectroscopy (MRS) is based either on a reduced number of mitochondria and/or adaptive mitochondrial dysfunction. Our data are supported by recent studies of ADP-stimulated respiration of mitochondria of skeletal muscle from type 2 diabetic subjects, showing no differences between groups [4,6]. Moreover, we assessed the ATP synthesis rate during ATP utilization by the hexokinase reaction i.e. when producing glucose 6-phosphate from glucose and ATP. Notably, we found that diabetic mitochondria's ATP synthesis rate during ATP utilization was significantly impaired compared to lean mitochondria. In accordance, Schrauwen-Hinderling et al. [2] showed that in vivo mitochondrial function was compromised by 45% in type 2 diabetic patients evaluated by measurement of phosphocreatine recovery after exercise detected by ^{31}P-MRS. The mechanism responsible for the impaired ATP synthesis during ATP consumption is at present unknown. Besides being a defect of the electron transport chain, an impaired ATP synthesis could reflect several other causes i.e. flaws in regulation of ATP synthase, ADP/ATP translocase defects, or failure to establish an adequate proton gradient. Recently, abnormal site-specific phosphorylation of ATP synthase-β has been described in insulin resistant human muscle [23]. Further studies are needed to clarify the molecular background for the impaired ATP synthesis in diabetic mitochondria during metabolic demand but also its impact in the pathogenesis of insulin resistance and type 2 diabetes.

Previous investigations suggest that mitochondrial dysfunction in type 2 diabetes is primarily due to a lower content of muscle mitochondria [1,2]. We addressed the same question by measuring the mitochondrial mass in myotubes established from lean, obese and type 2 diabetic subjects and could not show significant differences between groups, thus indicating that the reduced mitochondrial mass in vivo is an adaptive trait. In line, Bonnard et al. [24] showed that 1 month of high-fat, high-sucrose diet feeding induce glucose intolerance in mice, without any evidence of mitochondrial dysfunction. However, when the diet intervention was extended up to 16 weeks, they observed altered mitochondrial biogenesis, structure, and function in mouse muscle tissue, accompanied by the induction of a diabetic state. Thus combining above findings shows that mitochondrial dysfunction in type 2 diabetes in vivo is based both on primarily impaired ATP synthesis and an adaptive loss of mitochondrial mass.

Diabetic myotubes expressed primary insulin resistance at the level of glucose uptake, oxidation and storage [9–14]. Although mitochondrial respiration under baseline conditions or during acute insulin stimulation was equivalent for both diabetic and lean mitochondria, citrate synthase activity was reduced by 14% in the diabetic group [17]. Moreover, transcriptional profiling of diabetic myotubes showed no evidence for a primary defect in oxidative phosphorylation genes or mitochondrial mass at normophysiological (baseline) conditions [18]. Diabetic myotubes show several signs of impaired mitochondrial function, including a reduced complete palmitate oxidation with a concomitant increased release of acid-soluble metabolites [12–14] and a primary reduction of TCA flux [15]. Thus, diabetic mitochondria express impaired functions localized to the TCA cycle and β-oxidation, while ATP synthesis and respiration was unaffected. We conclude that primary insulin resistance under normophysiological conditions is not dependent on defects in the oxidative phosphorylation but rather other mitochondrial dysfunction. In agreement, Koves et al. [25] showed that incomplete fatty acid oxidation contributed to skeletal muscle insulin resistance. The physiological impact of a heritable impairment of ATP synthesis during metabolic energy demand with regard to the pathogenesis of insulin resistance in skeletal muscle needs further clarification.

Acknowledgments

I. Lynfort and J. Agergaard provided excellent technical assistance. B.F. Vind, Department of Endocrinology, Diabetes Research Centre, Odense University Hospital, Odense, Denmark, is thanked for muscle biopsies. The Danish Medical Research Council, the Novo Nordisk Foundation are thanked for financial support. MG is a member of the Cost action FA602.

References

[1] D.E. Kelley, J. He, E.V. Menshikova, Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes, Diabetes 51 (2002) 2944–2950.
[2] V.B. Schrauwen-Hinderling, M.E. Kooi, M.K. Hesselink, J.A. Jeneson, W.H. Backes, C.J. van Echteld, J.M. van Engelshoven, M. Mensink, P. Schrauwen, Impaired in vivo mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus and BMI-matched control subjects, Diabetologia 50 (2007) 113–120.
[3] V.B. Ritov, E.V. Menshikova, J. He, R.E. Ferrell, B.H. Goodpaster, D.E. Kelley, Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes, Diabetes 54 (2005) 8–14.
[4] R. Boushel, E. Gnaiger, P. Schjerling, M. Skovbro, R. Kraunsøe, F. Dela, Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle, Diabetologia 50 (2007) 790–796.
[5] M. Mogensen, K. Sahlin, M. Fernström, D. Glintborg, B.F. Vind, H. Beck-Nielsen, K. Højlund, Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes, Diabetes 56 (2007) 1592–1599.
[6] M. Hey-Mogensen, K. Højlund, B.F. Vind, L. Wang, F. Dela, H. Beck-Nielsen, M. Fernström, K. Sahlin, Effect of physical training on mitochondrial respiration and reactive oxygen species release in skeletal muscle in patients with obesity and type 2 diabetes. Diabetologia 2010. doi:10.1007/s00125-010-1813-x.
[7] K.F. Petersen, S. Dufour, D. Befroy, R. Garcia, G.I. Shulman, Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes, N. Engl. J. Med. 350 (2004) 664–671.
[8] D.E. Befroy, K.F. Petersen, S. Dufour, G.F. Mason, R.A. de Graaf, D.L. Rothman, G.I. Shulman, Impaired mitochondrial substrate oxidation in muscle of insulin-resistant offspring of type 2 diabetic patients, Diabetes 56 (2007) 1376–1381.
[9] R.R. Henry, L. Abrams, S. Nikoulina, T.P. Ciaraldi, Insulin action and glucose metabolism in nondiabetic control and NIDDM subjects. Comparison using human skeletal muscle cell cultures, Diabetes 44 (1995) 936–946.
[10] R.R. Henry, T.P. Ciaraldi, S. Mudaliar, L. Abrams, S.E. Nikoulina, Acquired defects of glycogen synthase activity in cultured human skeletal muscle cells: influence of high glucose and insulin levels, Diabetes 45 (1996) 400–407.
[11] M. Gaster, I. Petersen, K. Hojlund, P. Poulsen, H. Beck-Nielsen, The diabetic phenotype is conserved in myotubes established from diabetic subjects: evidence for primary defects in glucose transport and glycogen synthase activity, Diabetes 51 (4) (2002) 921–927.
[12] M. Gaster, A.C. Rustan, V. Aas, H. Beck-Nielsen, Reduced lipid oxidation in skeletal muscle from type 2 diabetic subjects may be of genetic origin: evidence from cultured myotubes, Diabetes 53 (3) (2004) 542–548.
[13] M. Gaster, A.C. Rustan, H. Beck-Nielsen, Differential utilization of saturated palmitate and unsaturated oleate: evidence from cultured myotubes, Diabetes 54 (3) (2005) 648–656.
[14] A.J. Wensaas, A.C. Rustan, M. Just, R.K. Berge, C.A. Drevon, M. Gaster, Fatty acid incubation of myotubes from humans with type 2 diabetes leads to enhanced release of beta oxidation products due to impaired fatty acid oxidation. Effects of tetradecylthioacetic acid and eicosapentaenoic acid, Diabetes 58 (2009) 527–535.
[15] M. Gaster, Reduced TCA-flux in diabetic myotubes: a governing influence on the diabetic phenotype?, Biochem Biophys. Res. Commun. 387 (4) (2009) 651–655.
[16] L. Hansen, M. Gaster, E.J. Oakeley, K. Brusgaard, E.M. Damsgaard-Nielsen, H. Beck-Nielsen, O. Pedersen, B.A. Hemmings, Expression profiling of insulin action in human myotubes: induction of inflammatory and pro-angiogenic pathways in relationship with glycogen synthesis and type 2 diabetes, Biochem. Biophys. Res.Commun. 323 (2) (2004) 685–695.
[17] N. Ortenblad, M. Mogensen, I. Petersen, K. Hojlund, K. Levin, K. Sahlin, H. Beck-Nielsen, M. Gaster, Reduced insulin-mediated citrate synthase activity in cultured skeletal muscle cells from patients with type 2 diabetes: evidence for an intrinsic oxidative enzyme defect, Biochim. Biophys. Acta 1741 (1-2) (2005) 206–214.

[18] C.M. Frederiksen, K. Højlund, L. Hansen, E.J. Oakeley, B. Hemmings, B.M. Abdallah, K. Brusgaard, H. Beck-Nielsen, M. Gaster, Transcriptional profiling of myotubes from patients with type 2 diabetes. No evidence for a primary defect in oxidative phosphorylation genes, Diabetologia 51 (2008) 2068–2077.
[19] M. Gaster, S.R. Kristensen, H. Beck-Nielsen, H.D. Schroder, A cellular model system of differentiated human myotubes, APMIS 109 (11) (2001) 735–744.
[20] H.T. Hornig-Do, G. Günther, M. Bust, P. Lehnartz, A. Bosio, R.J. Wiesner, Isolation of functional pure mitochondria by superparamagnetic microbeads, Anal. Biochem. 389 (1) (2009) 1–5.
[21] M. Gaster, Insulin resistance and the mitochondrial link. Lessons from cultured human myotubes, Biochim. Biophys. Acta 1772 (7) (2007) 755–765.
[22] M. Gaster, H.D. Schroder, A. Handberg, H. Beck-Nielsen, The basal kinetic parameters of glycogen synthase in human myotube cultures are not affected by chronic high insulin exposure, Biochim. Biophys. Acta 1537 (3) (2001) 211–221.
[23] K. Højlund, Z. Yi, N. Lefort, P. Langlais, B. Bowen, K. Levin, H. Beck-Nielsen, L.J. Mandarino, Human ATP synthase beta is phosphorylated at multiple sites and shows abnormal phosphorylation at specific sites in insulin-resistant muscle, Diabetologia 53 (3) (2010) 541–551.
[24] C. Bonnard, A. Durand, S. Peyrol, E. Chanseaume, M.A. Chauvin, B. Morio, H. Vidal, J. Rieusset, Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice, J. Clin. Invest. 118 (2008) 789–800.
[25] T.R. Koves, J.R. Ussher, R.C. Noland, D. Slentz, M. Mosedale, O. Ilkayeva, J. Bain, R. Stevens, J.R. Dyck, C.B. Newgard, G.D. Lopaschuk, D.M. Muoio, Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance, Cell Metab. 7 (2008) 45–56.