Metabolic flexibility is conserved in diabetic myotubes

Michael Gaster[1]

Molecular Endocrinology Unit, Department of Endocrinology, Odense University Hospital, Odense, Denmark

Abstract   The purpose of this study was to test the hypothesis that metabolic inflexibility is an intrinsic defect. Glucose and lipid oxidation were studied in human myotubes established from healthy lean and obese subjects and patients with type 2 diabetes (T2D). In lean myotubes, glucose oxidation is raised by increasing glucose concentrations (0–20 mmol/l) and acute insulin stimulation ($P < 0.05$), whereas it is inhibited by palmitate (PA). PA oxidation is raised by increasing PA concentrations (0–0.6 mmol/l), whereas 1.0 mmol/l PA inhibits its own oxidation ($P < 0.05$). Furthermore, PA oxidation is increased by acute insulin stimulation ($P < 0.05$) and inhibited by glucose. Even 0.05 mM PA and 2.5 mM glucose significantly reduce glucose and PA oxidation ($P < 0.05$), respectively. Glucose and PA oxidation are insulin-sensitive in myotubes established from lean (46% and 17% glucose and PA oxidation, respectively; $P < 0.05$ vs. basal), obese (31% and 14%; $P < 0.05$), and T2D (17% and 8%; $P < 0.05$) subjects. PA supplementation reduces both basal and insulin-stimulated glucose oxidation by 33–44% ($P < 0.05$), and myotubes are still insulin-sensitive in all three groups ($P < 0.05$).▮▮ Therefore, the metabolic inflexibility described in obese and diabetic patients is not an intrinsic defect; rather, it is based on an extramuscular mechanism (i.e., the inability to vary extracellular fatty acid concentrations during insulin stimulation). Thus, skeletal muscles are metabolic-flexible per se.—Gaster, M. **Metabolic flexibility is conserved in diabetic myotubes.** *J. Lipid Res.* 2007. **48:** 207–217.

Supplementary key words   fuel selection • glucose oxidation • insulin resistance • lipid oxidation • metabolic inflexibility • skeletal muscle • type 2 diabetes

Metabolic inflexibility describes the inability of diabetic patients to shift between lipid and glucose oxidation during insulin stimulation and from carbohydrate to lipid oxidation during fasting (1, 2). Even though skeletal muscle substrate oxidation has been investigated for many years, the molecular background of metabolic inflexibility remains unclear. Ukropcova et al. (3) evaluated metabolic switching in human myotubes established from young, healthy subjects based on their ability to increase FA oxidation by increasing the FA concentration and the susceptibility of glucose to reduce lipid oxidation, hypothesizing that metabolic switching was an intrinsic property of skeletal muscle. The effect of insulin was not studied. Previous studies of myotubes established from patients with type 2 diabetes (T2D) revealed primarily reduced insulin-stimulated glucose uptake, oxidation, and glycogen synthesis, whereas palmitate (PA) exposure impaired insulin-stimulated glucose oxidation and insulin-stimulated citrate synthase activity in control myotubes (4–9). The interplay between glucose and FAs on substrate oxidation in skeletal muscle in vivo has been studied for many years. First, the glucose-FA cycle was studied by Randle et al. (10), showing the ability of exogenous FAs to reduce glucose oxidation. Second came the observation that hyperglycemia can reduce FA oxidation in skeletal muscle, designated the reverse Randle cycle (11–13). Both high plasma glucose and/or plasma FFA are seen in obese and T2D subjects; therefore, increased substrate levels may be part of the mechanism responsible for metabolic inflexibility. Thus, metabolic inflexibility could be based on both a primary and an induced mechanism.

Our current knowledge of oxidative metabolism in skeletal muscle originates mainly from in vivo studies. The oxidative capacity of skeletal muscle is highly influenced by physical activity, aging, hormonal status, and fiber type composition, making it difficult to determine the contribution of genetic or individual environmental factors to the alteration in oxidative metabolism. It is especially difficult to estimate the impact of insulin stimulation on oxidative metabolism in vivo, as insulin stimulation is followed by changes in the level of glucose and FFAs in plasma. Cultured myotubes offer a unique model in which to separate the genetic influence on substrate oxidation from environmental factors and allow study of the interaction of various substrates on their own oxidation (4, 6, 14). The purpose of this study was to test the hypothesis that metabolic inflexibility is an intrinsic defect in myotubes established from obese and T2D subjects. Glucose and lipid oxidation were studied in human myotubes

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Abbreviations: CPT1, carnitine palmitoyltransferase-1; FCS, fetal calf serum; PA, palmitate; PDH, pyruvate dehydrogenase; T2D, type 2 diabetes.

[1] To whom correspondence should be addressed.
  e-mail: michael.gaster@ouh.fyns-amt.dk

established from healthy lean and obese subjects and patients with T2D under various conditions of glucose, FA, and insulin stimulation.

METHODS

Human study subjects

Ten lean and 10 obese control subjects and 10 obese T2D patients participated in the study (**Table 1**), and their clinical characteristics have been published previously (9). Muscle biopsies (200–300 mg) 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.

Materials

Dulbecco's modified Eagle's medium, fetal calf serum (FCS), penicillin-streptomycin-amphotericin B, and trypsin-EDTA were obtained from Invitrogen. Ultroser G was purchased from Pall Biosepra (Cergy-Saint-Christophe, France). The protein assay kit was purchased from Bio-Rad (Copenhagen, Denmark). Palmitic acid, L-carnitine, antimycin a, oligomycin, rottlerin, and ECM gel were purchased from Sigma Chemical Co. (St. Louis, MO). BSA (essentially FA free) was from Calbiochem (VWR, Roskilde, Denmark). Insulin Actrapid was from Novo Nordisk (Bagsvaerd, Denmark). Etoximir was a gift from Rolf K. Berge (Bergen, Norway).

Cell culture

Cell cultures were established as described previously (15, 16). 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 upscaling on ECM gel-coated dishes after 30 min of preplating. Cell cultures were established in DMEM 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 nonadherent cells were removed by changing the 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 (4–6 weeks). 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) to induce differentiation. The cells were cultured in a humidified 5% CO_2 atmosphere at 37°C, and medium was changed every 2–3 days.

Experimental design

Human myotubes established from lean, obese, and T2D subjects were allowed to differentiate under physiological conditions of insulin (25 pmol/l) and glucose (5.5 mmol/l). All myotube cultures were used for analysis at day 8 after the onset of differentiation. Myotubes were exposed to different protocols, as follows. For protocol Ia, control cultures were exposed to increasing PA concentrations (0, 0.05, 0.3, 0.6, and 1.0 mmol/l, and 0.6 mmol/l PA preincubated with etoximir) at 5.0 mmol/l glucose for 4 h to determine basal and insulin-stimulated PA oxidation and protein content. In studies with etoximir, myotubes were preexposed to 1 μmol/l etoximir for 30 min, washed, and allowed to recover for 30 min before measurement of lipid oxidation as described above. For protocol Ib, control cultures were exposed to 0.6 mmol/l PA supplemented with increasing glucose concentrations (0, 2.5, 5.0, 10.0, and 20.0 mmol/l, and 5.0 mmol/l glucose with 3 μg/ml rottlerin) for 4 h to determine basal and insulin-stimulated PA oxidation and protein content. For protocol IIa, control cultures were exposed to increasing glucose concentrations (0, 2.5, 5.0, 10.0, and 20.0 mmol/l with or without 0.6 mmol/l PA, and 5.0 mmol/l glucose with 3 μg/ml rottlerin) for 4 h to determine basal and insulin-stimulated glucose oxidation and protein content. For protocol IIb, control cultures were exposed to 5.0 mmol/l glucose supplemented with increasing PA concentrations (0, 0.05, 0.3, 0.6, and 1.0 mmol/l, and 0.6 mmol/l PA preincubated with etoximir) for 4 h to determine basal and insulin-stimulated glucose oxidation and protein content. In studies with etoximir, myotubes were preexposed to 1 μmol/l etoximir for 30 min, washed, and allowed to recover for 30 min before the measurement of lipid oxidation as described above. For protocol III, myotubes established from lean, obese, and T2D subjects were exposed to 0.6 mmol/l PA followed by determination of basal and insulin-stimulated PA oxidation and protein content (IIIa) or to 5.0 mmol/l glucose with or without

TABLE 1. Clinical characteristics of the study subjects

Variable	Control, Lean	Control, Obese	Type 2 Diabetes
Number	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]
Body mass index (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]
Hemoglobin A$_{1c}$ (%)	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 ± SEM.
[a] Significantly different from the lean controls ($P < 0.05$).
[b] Significantly different from the lean and obese controls ($P < 0.05$).

0.6 mmol/l PA followed by determination of basal and insulin-stimulated glucose oxidation and protein content (IIIb). The PA/BSA ratio was 2.5:1. Control and treated myotubes were exposed to equal amounts of fatty acid-free BSA.

Glucose oxidation

Cells were cultured in 12.5 cm^2 flasks and differentiated as described above. Cultures were exposed to DMEM with 0.24 mmol/l fat-free albumin, 50 U/ml penicillin, 50 μg/ml streptomycin, 1.25 μg/ml amphotericin B, and D-[^{14}C]glucose (2.0 μCi/ml) and supplemented with glucose and PA as indicated above and with 25 pmol/l insulin under basal conditions or with 1 μmol/l insulin during insulin stimulation. Rottlerin, a protein kinase C inhibitor, has been described to inhibit insulin-stimulated pyruvate dehydrogenase (PDH) activity (17) and was added (3 μg/ml) in some experiments to study whether the glucose oxidation could be inhibited and whether the effect of glucose on PA oxidation could be reversible. Flasks were air-tightened with a rubber stopper. After 4 h, 300 μl of phenyl ethylamine-methanol (1:1, v/v) was added with a syringe to a center well containing a folded filter paper. Perchloric acid (300 μl of 1 M) was subsequently added to the cells through the stopper tops by means of a syringe. The flasks were placed for a minimum of 1 h at room temperature to trap labeled CO_2. Cell-free flasks (no-cell controls) went through the same procedure to correct for unspecific CO_2 trapping.

FA oxidation

Cells were cultured in 12.5 cm^2 flasks, differentiated, and exposed as described above. Myotubes were exposed to DMEM with 0.24 mmol/l FA-free BSA, 0.5 mmol/l L-carnitine, 20 mmol/l HEPES, and [1-^{14}C]palmitic acid (2.0 μCi/ml) and supplemented with glucose and PA as indicated above and with 25 pmol/l insulin under basal conditions or 1 μmol/l insulin during insulin stimulation. Etoximir, a carnitine palmitoyltransferase-1 (CPT1) inhibitor, was added in some experiments to study whether PA oxidation could be inhibited and whether the effect of PA on glucose oxidation was reversible. Myotubes were exposed to 1 μmol/l etoximir for 30 min (18), washed, and allowed to recover for 30 min before measuring lipid oxidation, as described above. Flasks were air-tightened with stopper tops. After 4 h, 300 μl of phenyl ethylamine-methanol (1:1, v/v) was added with a syringe to a center well containing a folded filter paper. Perchloric acid (300 μl of 1 mol/l) was subsequently added to the cells through the stopper tops using a syringe. The flasks were placed for a minimum of 1 h at room temperature to trap labeled CO_2. Cell-free flasks (no-cell controls) went through the same procedure to correct for unspecific CO_2 trapping.

Statistical analysis

Data in text, tables, and figures are given as means ± SEM. Statistical analyses were performed with INSTAT 2.01 (Graph-Pad). The Kruskal-Wallis test or Friedman's test was used to assess nonpaired and paired significant differences between multiple treatments. The Pearson correlation coefficient was used for co-variance analysis. $P \leq 0.05$ was considered significant.

RESULTS

Subject characteristics

Clinical characteristics of the lean and obese controls and T2D patients are shown in Table 1. Fasting plasma glu-cose, serum insulin, and hemoglobin A1c levels were significantly higher in the diabetic group compared with both the lean and obese controls. Fasting HDL cholesterol concentrations were lower in T2D patients compared with lean controls. The obese controls showed higher fasting serum insulin levels compared with lean controls. During the steady state of the hyperinsulinemic euglycemic clamp period, the glucose infusion rates were significantly lower in T2D patients compared with both lean and obese control subjects, and glucose infusion rates were significantly lower in obese controls compared with lean control subjects.

Cell culture

Established cultures form many myotubes, as shown by phase-contrast microscopy (data not shown). We could not differentiate between study groups by visual inspection. The various treatments used in this study proved not to be toxic for the cells, as verified by measurements of cellular protein content (data not shown). The insulin-stimulated glycogen synthesis rate was significantly lower in diabetic myotubes compared with lean myotubes (19).

Glucose oxidation is raised by increasing glucose concentration and insulin stimulation

Myotubes exposed to increasing glucose concentrations expressed a concentration-dependent increase in glucose oxidation, which was sensitive to acute insulin stimulation at all concentrations investigated (**Fig. 1A**). This glucose oxidation pattern was not changed by PA supplementation but reduced the absolute rates of glucose oxidation. The insulin-mediated increments in glucose oxidation (fold change) were not altered by increased glucose concentrations, whereas the insulin-mediated increments in glucose oxidation tended to be lower during PA supplementation but were not significantly reduced under all conditions investigated (Fig. 1B). Furthermore, we studied the effect of rottlerin, which has previously been described to inhibit insulin-stimulated PDH activity (17), but we showed no significant effect on basal (288 ± 29 vs. 237 ± 16 pmol/min/mg; $P = 0.15$ with vs. without rottlerin) or insulin-stimulated (365 ± 26 vs. 327 ± 25 pmol/min/mg; $P = 0.48$ with vs. without rottlerin) glucose oxidation (Fig. 1A).

Glucose oxidation is reduced by increasing PA concentration, which can be partly inhibited by etoximir

Glucose oxidation was significantly reduced by increasing PA concentration in a dose-dependent pattern (**Fig. 2A**). Even a concentration of 0.05 mmol/l PA significantly reduced glucose oxidation. Increasing the PA concentration from 0.3 to 0.6 mmol/l did not reduce glucose oxidation further. The insulin-mediated increments in glucose oxidation (fold change) tended to be lower during PA exposure but were not reduced significantly (Fig. 2B). To show that the effect is mediated through PA, we exposed myotubes to etoximir, an inhibitor of CPT1, thereby reducing the uptake of PA into the mitochondria. Pretreatment of myotubes with etoximir significantly increased glucose oxidation (Fig. 2A).
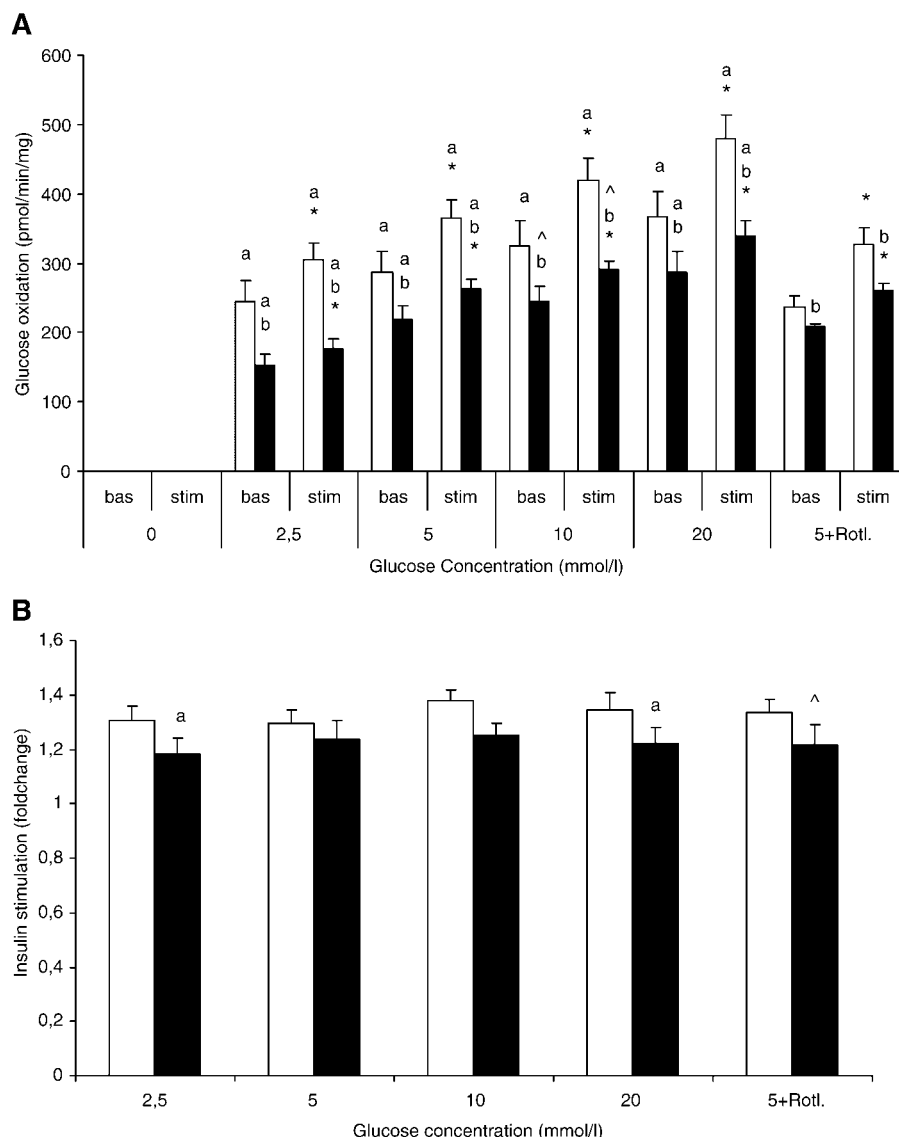
Fig. 1. Glucose oxidation in lean human myotubes with increasing glucose concentrations. Human satellite cell cultures were established from lean subjects, grown and differentiated under physiological conditions. Glucose oxidation was determined by radiotracer technique, as described in Methods. A: Basal (bas) and insulin-stimulated (stim) glucose oxidation at increasing glucose concentrations supplemented with and without (closed and open bars) 0.6 mmol/l palmitate (PA). The effect of rottlerin (Rotl.) on glucose oxidation at a glucose concentration of 5.0 mmol/l is indicated. * $P < 0.05$, insulin-stimulated versus basal. [a] $P < 0.05$ versus previously lower glucose concentration. [^] $P = 0.09$ versus previously lower glucose concentration. [b] $P < 0.05$ versus without PA. B: Insulin-mediated increment (fold change) in glucose oxidation determined as the ratio between insulin-stimulated and basal glucose oxidation at the indicated glucose concentrations with and without (open and closed bars) PA supplementation. [a] $P < 0.05$ versus without PA. [^] $P = 0.06$ versus without PA. Data shown are means ± SEM (n = 8).

PA oxidation is raised by increasing PA concentration and insulin stimulation and partly inhibited by etoximir

Myotubes exposed to increasing PA concentrations expressed a concentration-dependent increase in PA oxidation up to 0.6 mmol/l PA, which was sensitive to acute insulin stimulation at all concentrations investigated (**Fig. 3A**). Moreover, the insulin-mediated increments in PA oxidation were not changed significantly by increasing PA concentration (Fig. 3B). Pretreatment of myotubes with etoximir significantly reduced PA oxidation (Fig. 3A). Increasing the PA concentration to 1.0 mmol/l was followed by a significant reduction in basal and insulin-stimulated PA oxidation.

PA oxidation is reduced by increasing glucose concentration

The PA oxidation was reduced significantly by increasing glucose concentration in a dose-dependent pattern
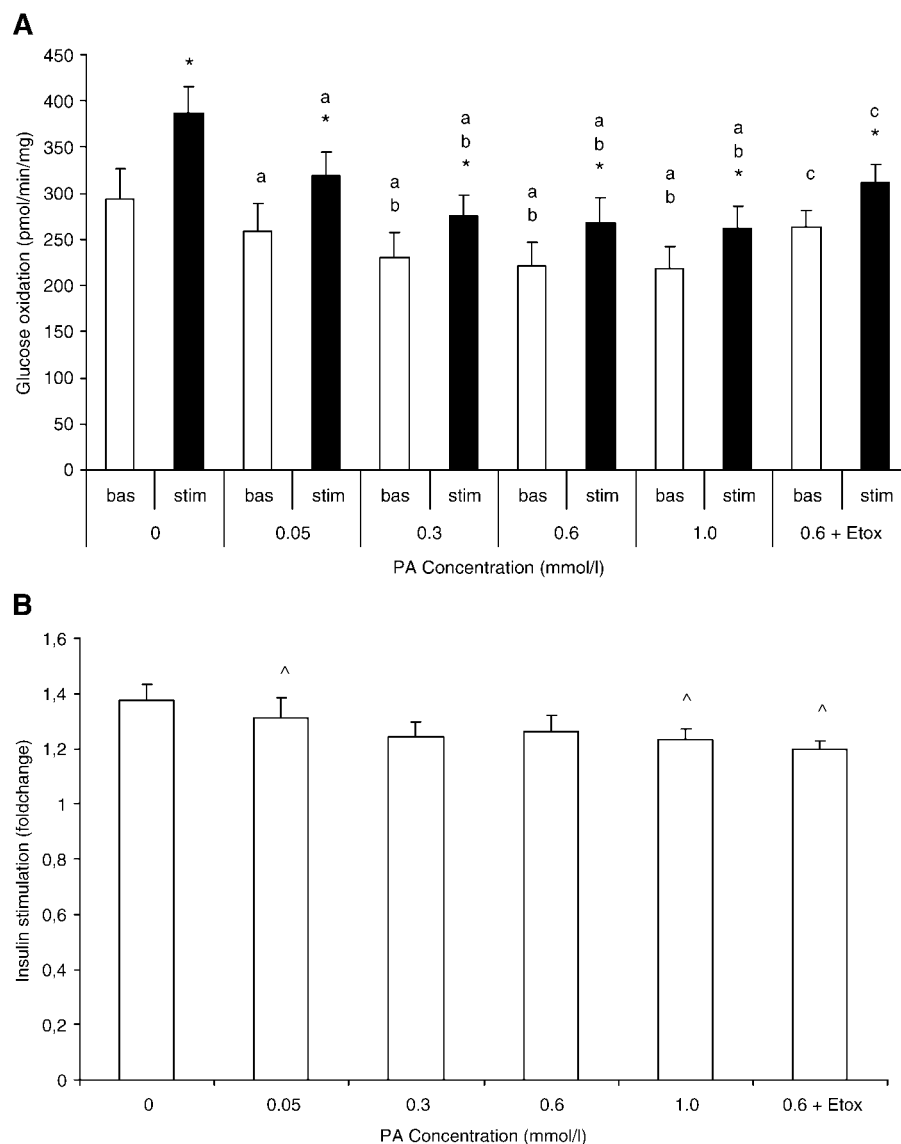
A

B

Fig. 2. Glucose oxidation in lean human myotubes exposed to increasing PA concentrations. Human satellite cell cultures were established from lean subjects, grown and differentiated under physiological conditions. Glucose oxidation was determined by radiotracer technique, as described in Methods. A: Basal (bas; open bars) and insulin-stimulated (stim; closed bars) glucose oxidation at 5.0 mmol/l glucose with increasing PA supplementation (0, 0.05, 0.3, 0.6, and 1.0 mmol/l). The effect of preincubating myotubes with etoximir (Etox) on glucose oxidation at a PA concentration of 0.6 mmol/l is shown. * $P < 0.05$, insulin-stimulated versus basal. [a] $P < 0.05$ versus without PA. [b] $P < 0.05$ versus 0.05 mmol/l PA. [c] $P < 0.05$ versus without etoximir. B: Insulin-mediated increment (fold change) in glucose oxidation determined as the ratio between insulin-stimulated and basal glucose oxidation at the indicated PA supplementation. [^] $P = 0.06$ versus without PA. Data shown are means ± SEM (n = 8).

(Fig. 4A). Increasing glucose concentrations to >5 mmol/l did not reduce PA oxidation further. The insulin-mediated increments in PA oxidation tended to increase during glucose exposure but were not increased significantly under all conditions investigated (Fig. 4B). To show that the effect is mediated through glucose, we exposed myotubes to rottlerin. Treating myotubes with rottlerin, however, did not significantly increase basal (10.3 ± 0.5 vs. 10.6 ± 0.7 nmol/mg; $P = 0.42$ with vs. without rottlerin)

or insulin-stimulated (11.9 ± 0.7 vs. 12.3 ± 0.8 nmol/mg; $P = 0.40$ with vs. without rottlerin) PA oxidation (Fig. 4A).

Glucose oxidation is insulin-sensitive in myotubes established from lean, obese, and T2D subjects and is reduced in diabetic myotubes

We investigated whether glucose oxidation was insulin-sensitive in myotubes established from lean, obese, and T2D subjects and were able to show that glucose oxida-
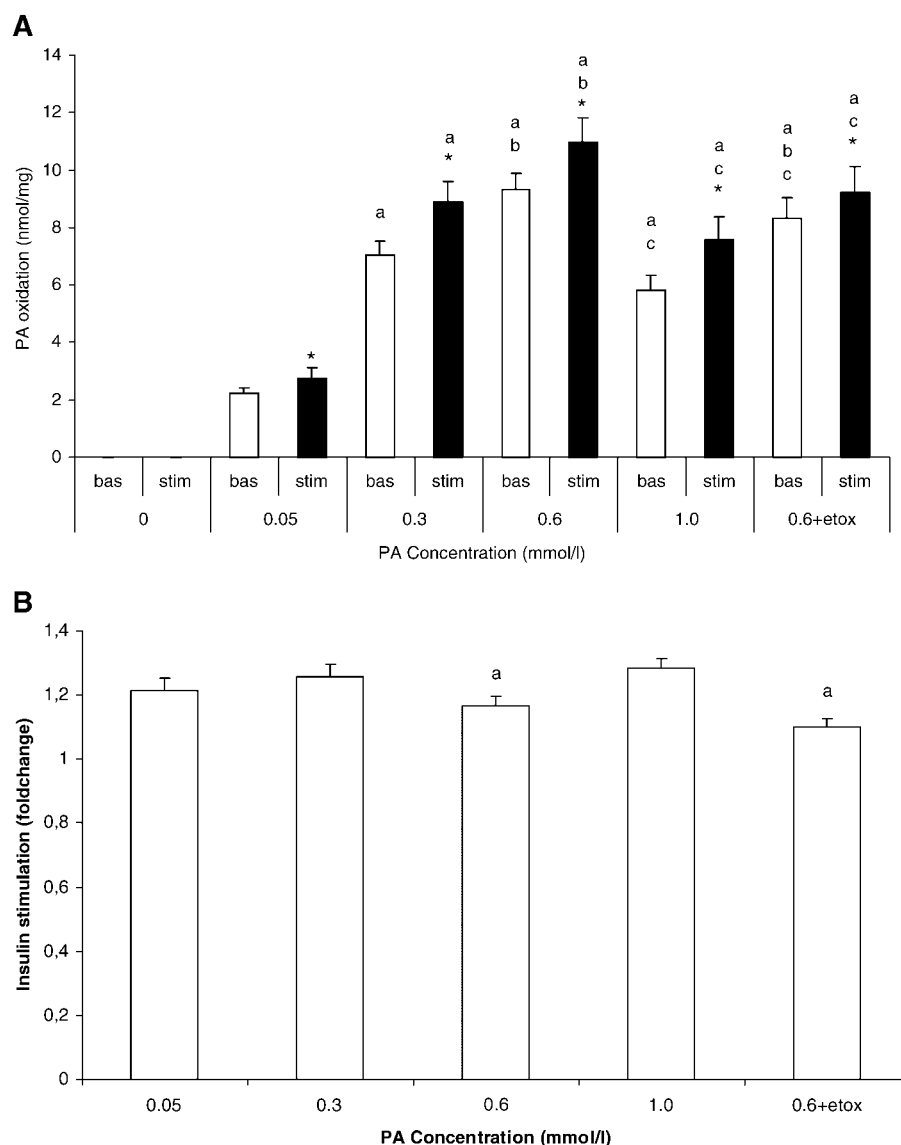
A

B

Fig. 3. PA oxidation in lean human myotubes with increasing PA concentrations. Human satellite cell cultures were established from lean subjects, grown and differentiated under physiological conditions. PA oxidation was determined by radiotracer technique, as described in Methods. A: Basal (bas; open bars) and insulin-stimulated (stim; closed bars) PA oxidation with increasing PA concentration at 5.0 mmol/l glucose. The effect of preincubating myotubes with etoximir (etox) on PA oxidation at a PA concentration of 0.6 mmol/l is shown. * $P < 0.05$, insulin-stimulated versus basal. [a] $P < 0.05$ versus 0.05 mmol/l PA. [b] $P < 0.05$ versus 0.3 mmol/l PA. [c] $P < 0.05$ versus 0.6 mmol/l PA without etoximir. B: Insulin-mediated increment (fold change) in PA oxidation determined as the ratio between insulin-stimulated and basal PA oxidation at the indicated PA concentrations. [a] $P < 0.05$ versus 0.05 mmol/l PA. Data shown are means ± SEM (n = 8).

tion was insulin-sensitive in myotubes established from lean (46%; $P < 0.05$ vs. basal), obese (31%; $P < 0.05$ vs. basal), and T2D (17%; $P < 0.05$ vs. basal) subjects (**Fig. 5A**). Basal glucose oxidation was found to be increased significantly in diabetic myotubes. Insulin-mediated increments in glucose oxidation (fold change) in diabetic myotubes were reduced significantly ($P < 0.05$) compared with those in lean myotubes (Fig. 5B). Insulin-mediated increments in glucose oxidation were significantly negatively correlated with corresponding basal glucose oxidation ($\rho = -0.67$, $P < 0.001$, n = 24; data not shown).

PA oxidation is insulin-sensitive in myotubes established from lean, obese, and T2D subjects

We investigated whether PA oxidation was insulin-sensitive in myotubes established from lean, obese, and T2D subjects and showed that PA oxidation was insulin-sensitive in myotubes established from lean (17%; $P < 0.05$ vs. basal), obese (14%; $P < 0.05$ vs. basal), and T2D (8%; $P < 0.05$ vs. basal) subjects (**Fig. 6A**). Basal PA oxidation seems lower in diabetic myotubes, but this was not statistically significant. Insulin-mediated increments in PA oxidation in diabetic myotubes were reduced compared with those in lean myotubes, but these did not reach sta-
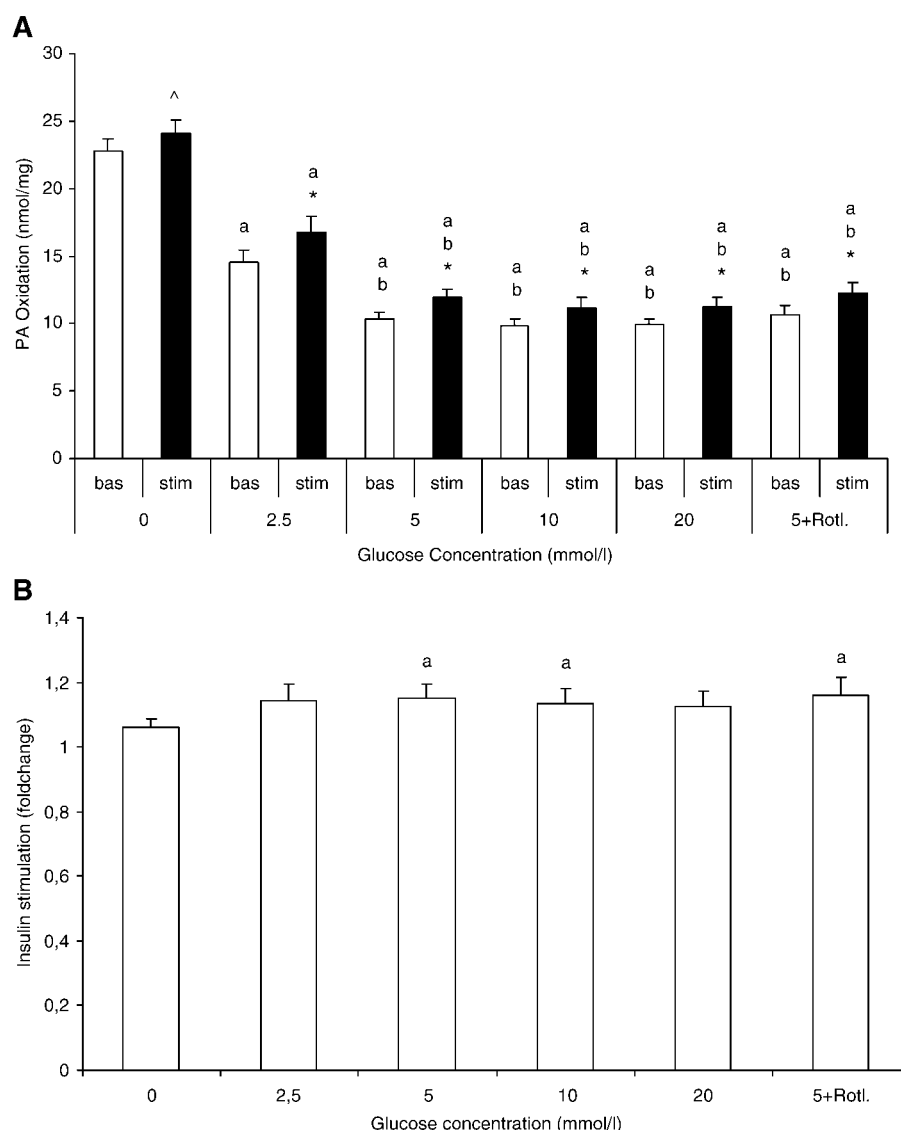
Fig. 4. PA oxidation in lean human myotubes exposed to increasing glucose concentrations. Human satellite cell cultures were established from lean subjects, grown and differentiated under physiological conditions. PA oxidation was determined by radiotracer technique, as described in Methods. A: Basal (bas; open bars) and insulin-stimulated (stim; closed bars) PA oxidation at 0.6 mmol/l PA supplemented with increasing glucose concentrations (0, 2.5, 5.0, 10.0, and 20 mmol/l). The effect of rottlerin (Rotl.) on PA oxidation at a glucose concentration of 5.0 mmol/l is indicated. * $P < 0.05$, insulin-stimulated versus basal. [a] $P < 0.05$ versus without glucose. [b] $P < 0.05$ versus 2.5 mmol/l glucose. B: Insulin-mediated increment (fold change) in PA oxidation determined as the ratio between insulin-stimulated and basal PA oxidation at the indicated glucose supplementation. [a] $P < 0.05$ versus without glucose. Data shown are means ± SEM (n = 8).

tistical significance (Fig. 6B). Insulin-mediated increments in PA oxidation were significantly positively correlated with basal PA oxidation ($\rho = 0.88$, $P < 0.001$, n = 24; data not shown).

Substrate oxidation

Basal glucose oxidation did not correlate significantly with basal PA oxidation ($\rho = -0.14$, $P = 0.50$, n = 24; data not shown), whereas insulin-mediated increments in glucose and PA oxidation were significantly positively correlated ($\rho = 0.53$, $P < 0.001$, n = 24; data not shown) in the three groups.

PA exposure reduced glucose oxidation in all three groups

To study the impact of PA on glucose oxidation in myotubes established from lean, obese, and T2D subjects, we exposed cultures to glucose with and without 0.6 mmol/l PA. PA supplementation reduced both basal and insulin-stimulated glucose oxidation by 33–44% ($P < 0.05$), and myotubes were still insulin-sensitive in all three groups ($P < 0.05$) (**Fig. 7A**). PA exposure significantly reduced only the insulin-mediated increments in glucose oxidation in lean myotubes ($P < 0.05$) (Fig. 7B). Insulin-mediated increments in glucose oxidation in diabetic myotubes were significantly reduced compared with those in lean myotubes when incubated without PA.
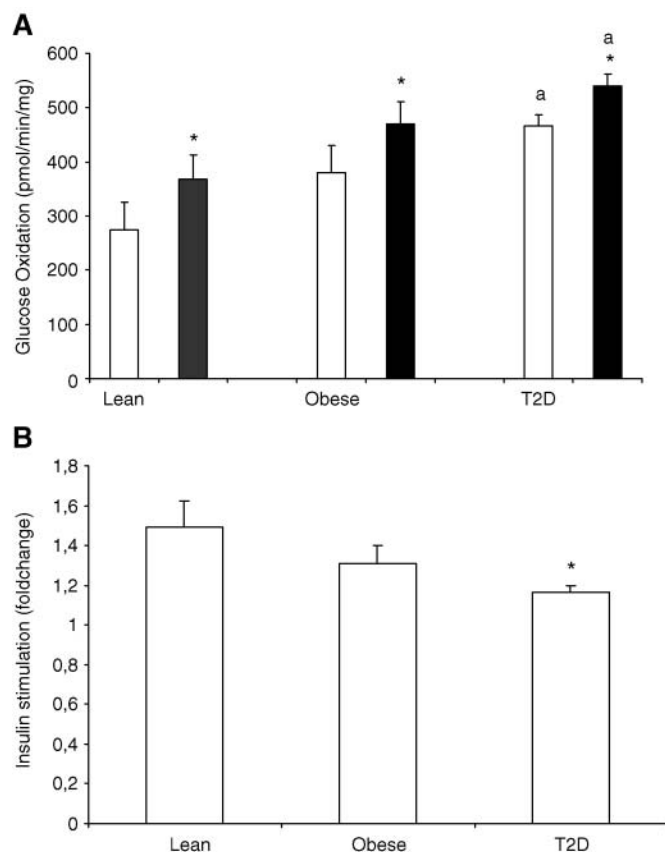
Fig. 5. Glucose oxidation in myotubes from lean controls, obese controls, and type 2 diabetes (T2D) patients. Human satellite cell cultures were established from lean, obese, and T2D subjects, grown and differentiated under physiological conditions. Glucose oxidation was determined by radiotracer technique, as described in Methods. A: Basal (open bars) and insulin-stimulated (closed bars) glucose oxidation for the three study groups. * $P < 0.05$, insulin-stimulated versus basal. [a] $P < 0.05$ versus corresponding lean. B: Insulin-mediated increment in glucose oxidation determined as the ratio between insulin-stimulated and basal glucose oxidation in the three groups. * $P < 0.05$ versus lean. Data shown are means $\pm$ SEM (n = 8).

Fig. 6. PA oxidation in myotubes from lean controls, obese controls, and T2D patients. Human satellite cell cultures were established from lean, obese, and T2D subjects, grown and differentiated under physiological conditions. PA oxidation was determined by radiotracer technique, as described in Methods. A: Basal (open bars) and insulin-stimulated (closed bars) PA oxidation for the three study groups. * $P < 0.05$, insulin-stimulated versus basal. B: Insulin-mediated increment in PA oxidation determined as the ratio between insulin-stimulated and basal PA oxidation in the three groups. Data shown are means $\pm$ SEM (n = 8).

DISCUSSION

The impetus for this study was to obtain a better understanding of the metabolic inflexibility in skeletal muscles described in obesity and T2D. Kelley and Mandarino (1) previously reviewed metabolic inflexibility but without clarifying whether inflexibility may be an intrinsic trait of obese and T2D skeletal muscles. Glucose and lipid oxidation were studied in human myotubes under various conditions of glucose, PA, and acute insulin stimulation to answer the question whether skeletal muscle from obese and T2D subjects may be metabolic-flexible per se. The advantages of using human myotubes is that the extracellular environment can be controlled precisely and kept constant over time, which allows studying the importance of the genetic background without systemic homeostatic regulatory components from the nervous and endocrine systems. Lipid and glucose m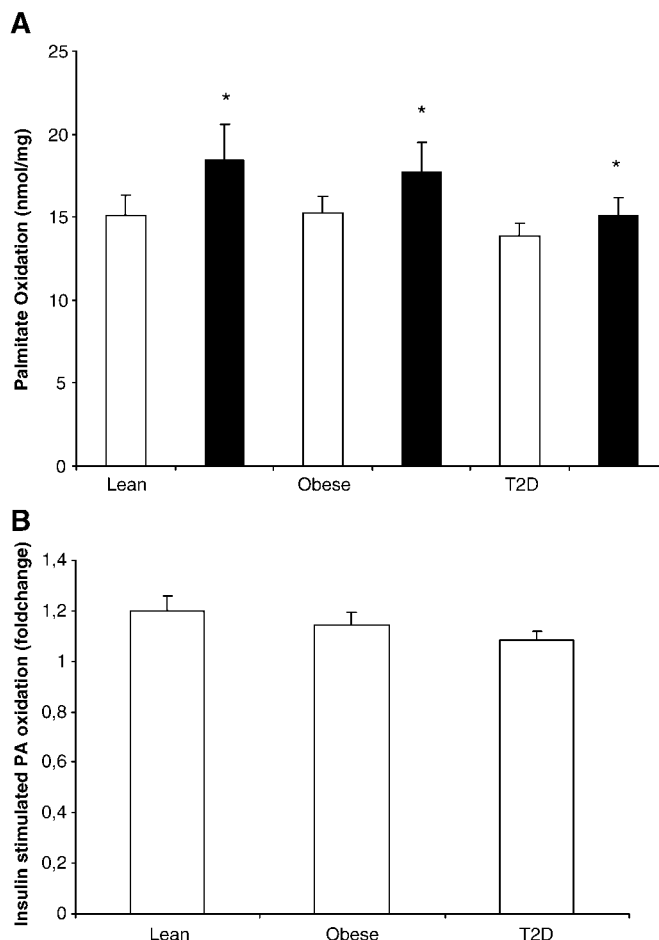etabolism in vivo is fiber type-dependent, as slow-twitch fibers express a higher rate of lipid and glucose uptake, lipid oxidation, and triacylglycerol content compared with glycolytic fibers. Myotubes in culture all express fast heavy myosin (15). Single-fiber analysis by He, Watkins, and Kelley (20) showed that skeletal muscle in obese and T2D subjects had a decreased oxidative capacity independent of the fiber type. Thus, human myotubes are suitable to detect alterations in oxidative metabolism.

The study of the interplay between glucose and PA on substrate oxidation in lean myotubes showed that both glucose and PA partly inhibit each other's oxidation. Surprisingly, even a low concentration, such as 0.05 mM PA and 2.5 mM glucose, significantly reduced glucose and PA oxidation, indicating that during normophysiological conditions both substrates actively depress the other's oxidation. Both PA oxidation and the suppression of glucose
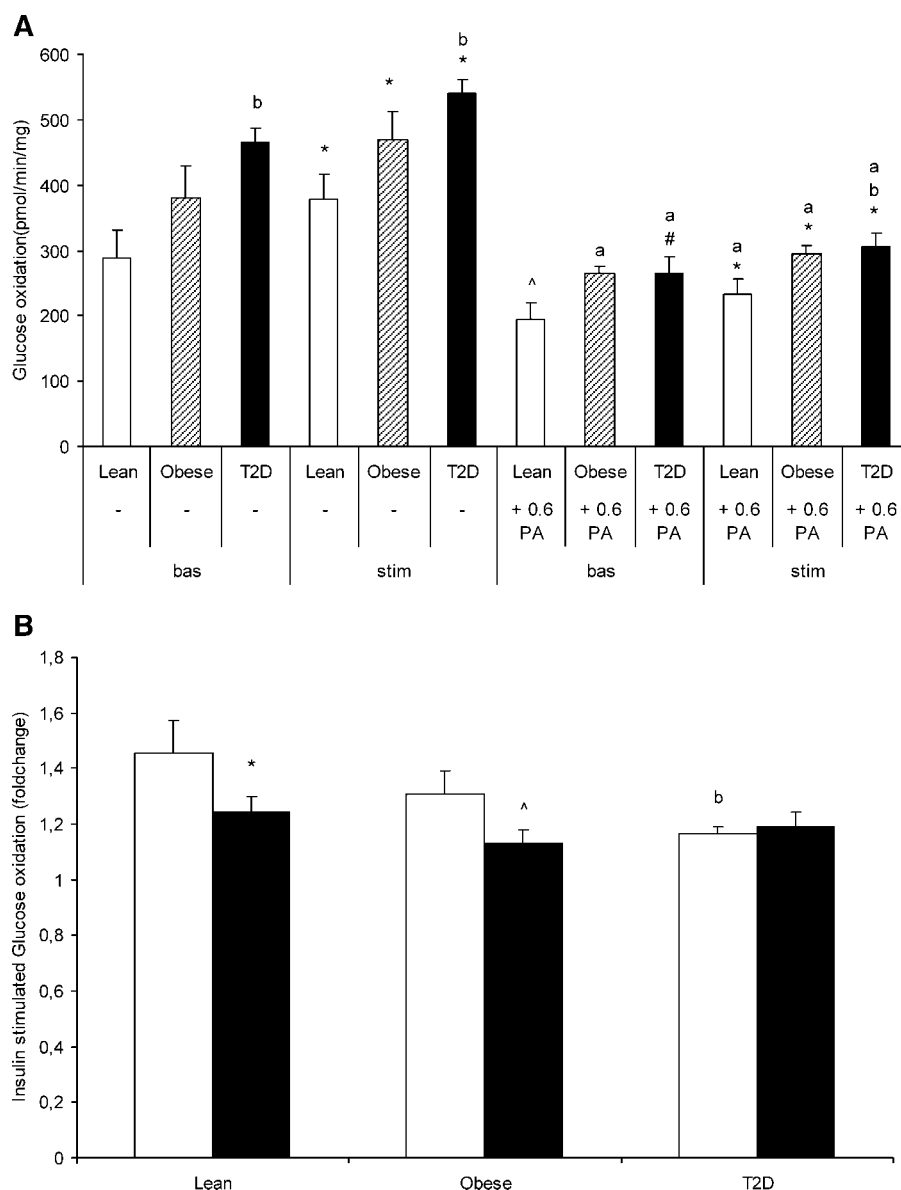
Fig. 7. Glucose oxidation in myotubes established from lean, obese, and T2D subjects supplemented with and without 0.6 mmol/l PA. Human satellite cell cultures were established from lean (open bars), obese (hatched bars), and T2D (closed bars) subjects, grown and differentiated under physiological conditions. Glucose oxidation was determined by radiotracer technique, as described in Methods. A: Basal (bas) and insulin-stimulated (stim) glucose oxidation with and without PA in the three study groups. * $P < 0.05$, insulin-stimulated versus basal. [a] $P < 0.05$ versus without PA. [b] $P < 0.05$ versus corresponding lean. [#] $P = 0.06$ versus corresponding lean. [^] $P = 0.06$ versus without PA. B: Insulin-mediated increment in glucose oxidation determined as the ratio between insulin-stimulated and basal glucose oxidation in the three groups without PA (open bars) and with PA (closed bars). * $P < 0.05$ versus PA. [^] $P = 0.09$ versus PA. [b] $P < 0.05$ versus lean. Data shown are means ± SEM (n = 8).

oxidation by PA could be partly inhibited when the entrance of palmitoyl-CoA into the mitochondria was inhibited by the CPT1 inhibitor etoximir, indicating that the PA inhibition is mediated through the operation of the glucose fatty cycle in human myotubes (10). The Randle effect is suggested to be mediated through the inhibition of the PDH complex by end products of fatty acid oxidation, such as acetyl-CoA and NADH. The reverse process, the decrease in lipid oxidation by the increase of

glucose use, is not fully understood. The depression of lipid oxidation by high glucose concentration is suggested to be mediated through an increase of malonyl-CoA, which is a potent inhibitor of acyl-CoA uptake into mitochondria by the inhibition of CPT1 (21, 22). In contrast to in vivo, maximal suppression of PA oxidation by glucose in human myotubes was obtained at normophysiological glucose concentrations under stable exogenous PA conditions. Moreover, the inhibition of glucose oxidation by

PA was not suppressed as expected by increasing glucose concentrations. This discrepancy between the vivo and in vitro effects of hyperglycemia on lipid oxidation is not clear. Myotube cultures are deprived in the neuroendocrine environment, as seen in vivo; therefore, these differences in sensitivity to hyperglycemia may be based on induced alterations in the reactivity to high glucose concentrations. The mechanism by which neuroendocrine factors elicit a differential effect on high glucose concentrations in lipid oxidation requires further investigation. Rottlerin, a protein kinase C inhibitor, was used to reduce glucose oxidation, as it has been suggested to be an inhibitor of insulin-stimulated PDH activity in mitochondria (17). Rottlerin was used to study the reverse Randle cycle, but it had no significant effect on either decreased basal or insulin-stimulated glucose oxidation or indirectly on increased PA oxidation. The minimal effect of rottlerin may be based on the fact that the effect seems to be transient in myotubes and liver cells (17). Thus, the glucose fatty acid cycle and the inverse Randle cycle are cooperating.

Both glucose oxidation and PA oxidation were insulin-sensitive in myotubes established from all three groups. Insulin-raised oxidation increased most in myotubes expressing high basal lipid oxidation and low basal glucose oxidation, as seen in most lean myotubes. The insulin-mediated increment in glucose oxidation was lower in diabetic myotubes and seems predictive of a reduced insulin-mediated increment in PA oxidation, as shown by the positive correlation between the ability of the individual myotubes to increase glucose oxidation and the corresponding PA oxidation in response to insulin. Thus, skeletal muscle is insulin-sensitive per se, although the insulin sensitivity may be reduced in skeletal muscle of obese and T2D subjects. This is in agreement with previous studies showing that glucose uptake, glycogen synthesis, and glycogen synthase activity are insulin-sensitive in diabetic myotubes, but at a lower magnitude compared with control myotubes, further emphasizing the fact that diabetic myotubes may express a changed metabolic setting compared with controls (4–8, 18). The physiological importance of the small effect of insulin stimulation on PA oxidation can be questioned. Insulin does not stimulate fatty acid oxidation in vivo, even under conditions in which clamps are used while FFAs are maintained by lipid infusion (23). The primary source of circulating lipids is lipolysis in adipose tissue. In healthy subjects, increased insulin presence is followed by reduced lipolysis in adipose tissue, decreasing plasma FFAs and thereby promoting a reduction in intracellular lipid oxidation (24).

In myotubes established from lean, obese, and T2D subjects, PA exposure was followed by reduced basal and insulin-stimulated glucose oxidation, in accordance with the Randle cycle, but all myotubes remained insulin-sensitive. In fact, FA release from adipose tissue in obesity and T2D during insulin stimulation is not suppressed, explaining the inability of obese and diabetic patients to shift between glucose and lipid oxidation during insulin stimulation by the failed reduction in plasma FFA. Moreover, PA exposure induced insulin resistance at the level

of glucose oxidation in lean myotubes, in agreement with previous observations of the ability of PA to induce insulin resistance in lean, but not in diabetic, myotubes. These results show that during fasting-like conditions, the metabolism of diabetic myotubes relies more on glucose oxidation than does that of lean myotubes, even if the metabolism is stimulated by insulin with or without PA pressure; thus, the respiratory quotient will be increased in diabetic myotubes during fasting conditions and during insulin stimulation, when PA is changed equally in study groups. However, if the PA concentration is decreased in lean but not in diabetic myotubes, the respiratory quotient will increase in lean myotubes, whereas that in diabetic myotubes will be nearly unchanged, and diabetic myotubes are described as metabolic-inflexible. The metabolic inflexibility described in obese and diabetic patients is not an intrinsic defect; rather, it is based on an extramuscular mechanism, the inability to vary extracellular fatty acid concentrations. Interestingly, although PA induces insulin resistance at the level of glucose oxidation in lean myotubes, abolishing this effect seems only minor compared with the effect of changes in PA concentration. Thus, although diabetic myotubes may have reduced insulin sensitivity and an increased basal respiratory quotient, metabolic inflexibility is not a primary defect of skeletal muscle.

A new finding is that PA suppresses its own oxidation at high concentrations. A cell-toxic effect seems less probable, as human myotubes have previously been exposed to 1.0 mM for up to 4 days (8). The regulation of FA oxidation is multifaceted (i.e., at the level of lipid uptake, fatty acid activation, binding to various transport proteins, transport over the mitochondrial membrane, and the rate of β-oxidation) (25, 26). The reduction in PA oxidation by increasing PA concentrations cannot be explained by malonyl-CoA generated from PA, as increasing palmitoyl-CoA concentrations will depress malonyl-CoA synthesis by inhibition of acetyl-CoA carboxylase (27). Theoretically, a reduction in NAD^+ may explain the inhibition, as NAD^+ can be the limiting factor when both TCA and β-oxidation operate at high levels. This will reduce PA oxidation without increasing glucose oxidation. However, additional studies are necessary to clarify the exact mechanism by which PA inhibits its own oxidation and especially whether a reduction in NAD^+ may be responsible for the PA-mediated inhibition of PA oxidation. The FFA concentration seems to be an important regulator of the balance between glucose and lipid oxidation in skeletal muscles, as low PA concentrations reduce glucose oxidation, whereas high PA concentrations suppress their own oxidation, favoring glucose oxidation. The reduced PA oxidation at increasing PA concentrations may promote the accumulation of acyl-CoA and its derivatives and thereby may be part of the pathogenesis of increased triacylglycerol content, lipotoxicity, and insulin resistance in skeletal muscle of obese and T2D subjects.

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