Myotubes from lean and severely obese subjects with and without type 2 diabetes respond differently to an in vitro model of exercise

Yuan Z. Feng,1 Nataša Nikolić,1 Siril S. Bakke,1 Eili T. Kase,1 Kari Guderud,1 Jøran Hjelmesæth,2,3 Vigdis Aas,4 Arild C. Rustan,1 and G. Hege Thoresen1,5

1Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway; 2The Morbid Obesity Center, Vestfold Hospital Trust, Tønsberg, Norway; 3Department of Endocrinology, Morbid Obesity and Preventive Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway; 4Faculty of Health, Oslo and Akershus University College of Applied Sciences, Oslo, Norway; and 5Department of Pharmacology, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo and Oslo University Hospital, Oslo, Norway

Submitted 17 September 2014; accepted in final form 20 January 2015

Physical activity plays an important role in both prevention and treatment of obesity and type 2 diabetes (T2D). Skeletal muscle is the largest insulin-sensitive organ in humans, accounting for >80% of insulin-stimulated glucose disposal (15). Physical training leads to extensive adaptations in skeletal muscles (14, 46), but the molecular mechanisms underlying these adaptations are still poorly understood, and there is increasing evidence that some individuals may be inherently less responsive to exercise (20, 53). Regular physical activity not only improves oxidative capacity, such as increased lipid and glucose oxidation (39), but is also known to increase insulin-stimulated glucose uptake (45). The effect of exercise on substrate oxidation is less clear in obese and type 2 diabetic subjects than in lean subjects (37). Impairments have been noted in skeletal muscle glucose and fatty acid oxidation in obesity and T2D in the resting state (6, 29, 30). It is therefore important to determine whether interventions, either exercise or pharmacologically, can effectively reverse this impairment.

Plasticity of skeletal muscle in response to regular exercise extends beyond the metabolic changes, and peroxisome proliferator-activated receptor-δ (PPARδ) activation is one of many pathways proposed to be involved in exercise adaptations (15, 35). PPARδ is an important regulator of skeletal muscle metabolism, in particular lipid oxidation. It is well known that endurance exercise increases mitochondrial content (46, 54), and that myosin heavy chain type I, slow-twitch oxidative (MHCI) muscle fibers are associated with higher mitochondrial content compared with MHCII (type II, fast-twitch glycolytic) fibers (32). On the other hand, it is debated whether conversion of muscle type II to type I phenotype actually happens in adult humans (22).

Moreover, skeletal muscle has been identified as a secretory organ that releases a diversity of biologically active proteins classified as myokines that can have autocrine, paracrine, or endocrine functions. Muscle contraction during exercise is a major stimulus of these endocrine functions, and the myokines are thought to mediate beneficial effects of exercise and may have a role in the protection against conditions associated with low-grade inflammation, such as T2D and metabolic syndrome (38, 42, 56). The myokines, interleukin-6 (IL-6) and IL-8, are markedly produced in contracting muscles and, at least for IL-6, released into plasma during the postexercise period (2, 51) when the insulin sensitivity is enhanced (23). It seems that IL-6 works as an energy sensor and preserves fuel availability during exercise (40) by enhancing insulin-stimulated glucose disposal and fatty acid metabolism (10, 55). Interestingly, while IL-6 released from skeletal muscle may promote insulin sensitivity (21), IL-6 secreted from adipose tissue may induce insulin resistance in muscle (41). Thus, while it has become evident that contracting skeletal muscle releases myokines that may influence metabolism and function of muscle tissue and other tissues and organs, the secretion of myokines from obese and/or diabetic muscle is yet to be fully clarified.

Previously, we have established an in vitro model of regular exercise of cultured human skeletal muscle cells (myotubes) by...
applying chronic, low-frequency electrical pulse stimulation (EPS) (36), thereby enabling the study of exercise-induced cellular mechanisms under controlled conditions. Human myotubes are considered a valid model for studying metabolic disorders as obesity and T2D because perturbances evident in vivo, such as depressed lipid oxidation and insulin resistance, are retained in myotubes in culture (1, 7, 19). After EPS, myotubes established from lean nondiabetic subjects showed an increase in glucose and lipid metabolism as well as indications of fast-twitch glycolytic muscle fiber transformation into slow-type oxidative fibers (36) and enhanced expression and release of IL-6 and IL-8 (31, 44, 48). In the present study, we wanted to explore whether there are differences in EPS response on insulin sensitivity, glucose and lipid metabolism, and gene expression in myotubes from three groups of donors—lean nondiabetic, severely obese nondiabetic, and severely obese subjects with established T2D—and whether these differences reflect the in vivo characteristics of the donor groups. Moreover, PPARα agonists and exercise training are shown to act synergistically to increase the content of oxidative muscle fibers, and PPARα agonists have been proposed as exercise mimetics (35). Therefore, we wanted to explore the combined effect of EPS together with the PPARα agonist, GW501516, on lipid metabolism as well.

MATERIALS AND METHODS

Human myotubes. Human myotubes grown from satellite cells were isolated as previously described (18) from the musculus obliquus internus abdominis from lean or severely obese subjects. The lean nondiabetic donor biopsies were obtained from subjects donating a kidney at Oslo University Hospital, Norway and the severely obese donor biopsies were obtained from subjects undergoing bariatric surgery at The Morbid Obesity Center, Vestfold Hospital Trust, Norway. The isolation of satellite cells from all biopsies was performed at the same location and by the same researchers. The biopsies were obtained with written informed consent and were approved by the National Committee for Research Ethics, Oslo, Norway (S-04133 and S-09078d).

Classification of the severely obese donors into the two groups, severely obese nondiabetic subjects (SO-nD) and severely obese subjects with T2D (SO-T2D), was based on fasting plasma glucose values (≥7.0 mmol/l), HbA1c (glycosylated hemoglobin, ≥6.5%), and/or use of one or more antidiabetic drugs. The cells were cultured in DMEM-Glutamax (5.5 mmol/l glucose) with supplements during proliferation and differentiation as previously described (25). Briefly, at ~80% confluence, growth medium was replaced by differentiation medium to induce fusion of myoblasts into multinucleated myotubes. Experiments were performed on cells from passage 2 to 5 and after 7 days of differentiation. There was no difference in mRNA expression levels of muscle fiber type 1 marker between the myotubes from lean nondiabetic and severely obese subjects or between the myotubes from severely obese nondiabetic and diabetic subjects (data not shown).

Electrical pulse stimulation of human myotubes. Human myotubes grown on Corning CellBIND six-well plates (Corning Life-Sciences, Schiphol-Rijk, The Netherlands) were stimulated via carbon electrodes by applying chronic, low-frequency EPS (single, bipolar pulses of 2 ms, 30 V, 1 Hz) continuously for the last 48 h of the differentiation period, as previously described (36). Culture media were changed every 12 or 24 h during EPS. Electrical pulses were generated by a muscle stimulator built at the Electronics Lab, Institute of Chemistry, University of Oslo, Norway. Neither applying EPS for 48 h to the myotubes nor changing the media every 12 or 24 h showed differences in floating cells evaluated by microscopic inspection or by measurement of cell protein content in the three donor groups (data not shown).

Immunoblotting. After EPS, cells were harvested in Laemmli buffer (0.5 mol/l Tris-HCL, 10% SDS, 20% glycerol 10% β-mercaptoethanol, and 5% Bromophenol blue). Total cell lysates were electrophoretically separated on 4–20% Mini-Protean TGX gels (Bio-Rad, Copenahgen, Denmark) with Tris/glycine buffer (pH 8.3) followed by blotting to nitrocellulose membrane and incubation with antibodies against total Akt kinase, Akt phosphorylated at Ser473, β-actin, α-tubulin (all from Cell Signaling Technology, Beverly, MA), MHCI (Millipore, Billerica, MA), and an OXPHOS antibody cocktail recognizing Complex I subunit NDUFB8, Complex II subunit, Complex III subunit core 2, Complex IV subunit II and ATP synthase subunit α (Abcam, Cambridge, UK). Immunoreactive bands were visualized with enhanced chemiluminescence (Chemidoc XRS, Bio-Rad) and quantified with Image Lab software (version 4.0). β-Actin or α-tubulin was used to normalize the protein-antibody signals versus protein loading.

Glucose metabolism. After EPS, myotubes were exposed to serum-free DMEM supplemented with [1-14C]oleic acid (0.5 μCi/ml, 1 mmol/l, PerkinElmer, Boston, MA) and 1 mmol/l pyruvate in the presence or absence of 100 mmol/l insulin for 3 h to study basal and insulin-stimulated glycogen synthesis as previously described (16). Glucose oxidation was measured after 3 h of incubation, 900 μl cell medium was transferred to airtight flasks, and 300 μl of phenyl ethylamine:ethanol (1:1, vol/vol) was added to a center well containing a folded filter paper. Subsequently, 200 μl of 1 mol/l perchloric acid was added to the cell medium through the stopper tops using a syringe, as previously described (36). The flasks were placed at room temperature to trap labeled CO2 for 2 h, and radioactivity was counted by liquid scintillation. All data were related to protein content.

Fatty acid oxidation. Myotubes were treated with 0.1% DMSO (vehicle) or 10 mmol/l GW501516 for 96 h, and EPS was applied to the cells for the last 48 h of the treatment period. After EPS, the myotubes were exposed to 1 ml of DPBS supplemented with HEPES (10 mmol/l), NaHCO3 (44 mmol/l), [1-14C]oleic acid (1 μCi/ml, 100 mmol/l, PerkinElmer), 40 μmol/l BSA, and 1 mmol/l l-carnitine in a 5% CO2 incubator at 37°C. After 2 h, CO2 and cell-associated radioactivity were measured as previously described (36). Briefly, 500 μl of cell medium was transferred to airtight flasks, and 300 μl of phenyl ethylamine:ethanol (1:1, vol/vol) was added to a center well containing a folded filter paper. Subsequently, 100 μl of 1 mol/l perchloric acid was added to the cell medium through the stopper tops using a syringe. The flasks were placed at room temperature to trap labeled CO2 for 2 h. The cells were placed on ice and washed twice with PBS, lysed with 0.1 mol/l NaOH, and cell-associated radioactivity was counted by liquid scintillation to determine uptake of oleic acid. All data were related to protein content.

Live imaging. After EPS, the myotubes were incubated at 37°C and 5% CO2 with MitoTracker Red FM (100 nmol/l, Invitrogen, Carlsbad, CA) for 15 min to stain mitochondria, Bodipy 493/503 (2 μg/ml, Invitrogen) for 15 min to stain neutral lipids and Hoechst 33258 (2.5 μg/ml, Invitrogen) for 15 min to stain nuclei and wash with PBS in between. Live imaging was carried out as previously described (25). Images were randomly taken in 25–36 positions per well with a ScanR platform (Olympus IX81 inverted fluorescence microscope) equipped with a temperature incubator for long-term live imaging. After the aggregates and dead cells were gated out, each parameter was determined from ~240 images per donor group (average of 38 ± 4 nuclei per image). MitoTracker Red FM and Bodipy 493/503 intensity per image was related to number of nuclei per image since for multinucleated myotubes the absolute number of cells cannot be determined. This is an accepted method to account for differences in cell numbers (13, 28, 34, 36). For the myotubes from lean nondiabetic subjects (L-nD), some of the cells were also treated with GW501516 for 96 h
and EPS was applied to the cells for the last 48 h of the treatment period.

**Gene expression analysis by qPCR.** After EPS, total RNA was isolated from the myotubes, and qPCR was performed as previously described (36). Briefly, total RNA from cells was isolated by Agilent Total RNA isolation kit according to the supplier’s protocol. RNA was reverse-transcribed with oligo primers using a PerkinElmer Thermal Cycler 9600, and qPCR was performed using an ABI PRISM 7000 Detection System (Applied Biosystems, Warrington, UK). Transcription levels were normalized to the housekeeping genes acidic ribosomal phosphoprotein P0 (36B4) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The following forward and reverse primers (Molecular Primes, Invitrogen, Carlsbad, CA) were used at a concentration of 30 μmol/l: 36B4 (accession no. M17885); GAPDH (accession no. NM_002046); IL-6 (accession no. NM_000600); IL-8 (accession no. NM_000584.2); and MYH7 (accession no. NM_000257.2).

**Data presentation and statistics.** Data are presented as means ± SE. Statistical analyses were performed using GraphPad Prism 5.0 for Windows (Graphpad Software, San Diego, CA). The value $n$ represents the number of different donors used, and each with at least duplicate observations. Two-tailed paired Student’s $t$-tests were performed to determine the effects of treatments, and unpaired Student’s $t$-tests (independent samples) were performed to determine the difference in effects of EPS between the donor groups. For correlation studies, Spearman correlation analysis was performed. Linear mixed model analysis (LMM, SPSS 20.0.0.1, IBM SPSS, Chicago, IL) was used to determine the effect of EPS in OXPHOS experiments. The linear mixed model includes all observations (complex I–V) in the statistical analyses and at the same time takes into account that not all observations are independent. $P < 0.05$ was considered statistically significant.

**RESULTS**

**Clinical characteristics of the biopsy donors.** Clinical characteristics of the donors are presented in Table 1. Fasting plasma glucose and HbA1c were significantly higher in severely obese subjects with T2D (SO-T2D) than in nondiabetic subjects with similar body mass index (SO-nD). Fasting plasma levels of insulin, triacylglycerol, and high-density lipoprotein were not different between the two obese groups. As expected, clinical characteristics of lean nondiabetic subjects (L-nD) were significantly different from severely obese subjects with similar body mass index; TAG, triacylglycerol; HDL, high-density lipoprotein.

<table>
<thead>
<tr>
<th>Clinical characteristics of biopsy donors</th>
<th>L-nD (n = 14)</th>
<th>SO-nD (n = 9)</th>
<th>SO-T2D (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>49 ± 4</td>
<td>41 ± 2</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.3 ± 0.9</td>
<td>42.0 ± 1.7*</td>
<td>44.6 ± 2.2*</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>5.6 ± 0.3</td>
<td>4.8 ± 0.1</td>
<td>8.2 ± 0.5†</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.5 ± 0.1</td>
<td>7.2 ± 0.6‡</td>
<td></td>
</tr>
<tr>
<td>Insulin, μmol/l</td>
<td>89.4 ± 16</td>
<td>62.4 ± 18</td>
<td></td>
</tr>
<tr>
<td>TAG, mmol/l</td>
<td>1.0 ± 0.1</td>
<td>1.6 ± 0.2*</td>
<td>2.0 ± 0.3*</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>1.6 ± 0.1</td>
<td>1.1 ± 0.1*</td>
<td>1.0 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Glycosylated hemoglobin (HbA1c) and plasma insulin were not measured in the lean nondiabetic cohort (L-nD). *Significantly different from the lean group; †significantly different from both of the nondiabetic groups; ‡significantly different from severely obese nondiabetic (SO-nD) ($P < 0.05$). SO-T2D, severely obese with type 2 diabetes; BMI, body mass index; TAG, triacylglycerol; HDL, high-density lipoprotein.

**Effect of EPS on insulin sensitivity in myotubes.** Myotubes from severely obese subjects with T2D maintained their diabetic phenotype in culture shown as absence of insulin-stimulated glucose uptake in myotubes from SO-T2D, and furthermore, phosphorylation of Akt tended to be lower in myotubes from SO-T2D compared with SO-nD (4). To examine whether EPS could increase insulin sensitivity, insulin-stimulated phosphorylation of Akt (Ser473) and glycogen synthesis were measured in myotubes established from L-nD, SO-nD, and SO-T2D after EPS stimulation. In absence of insulin, phosphorylation of Akt was not affected by EPS (Fig. 1A), whereas insulin-stimulated phosphorylation of Akt was significantly increased by EPS in myotubes from SO-T2D (Fig. 1B). In the absence of insulin, glycogen synthesis was not affected by EPS (Fig. 1C), whereas insulin-stimulated glycogen synthesis after EPS was increased in myotubes from both SO-nD and SO-T2D subjects (Fig. 1D). Furthermore, EPS-induced effect on insulin-stimulated phosphorylation of Akt and glycogen synthesis in the myotubes correlated positively with BMI of the subjects (Fig. 1, E and F), i.e., the EPS effect is increased with increasing BMI of the donors.

**Effect of EPS on glucose and oleic acid metabolism.**

We wanted to explore whether there were differences in the EPS-induced response on oxidative capacity in myotubes established from lean, nondiabetic, and severely obese subjects with and without T2D. Glucose oxidation after EPS was increased in myotubes from both lean and severely obese subjects (Fig. 2A), whereas EPS had an effect on oleic acid oxidation in myotubes from lean subjects only (Fig. 2B). Furthermore, oleic acid oxidation after EPS was significantly different in myotubes from lean subjects compared with myotubes from severely obese subjects (Fig. 2B), and the EPS-induced effect on oleic acid oxidation correlated also negatively with the BMI of the subjects irrespective of donor group (Fig. 2C). Mitochondrial content shown as MitoTracker intensity related to nuclei was significantly increased by EPS in myotubes from L-nD subjects (Fig. 2D), but not in myotubes from severely obese subjects (SO-nD, $P = 0.076$; SO-T2D, $P = 0.32$). Also the overall expression levels of proteins involved in mitochondrial oxidative phosphorylation (OXPHOS proteins) were significantly increased by EPS in myotubes from L-nD subjects (Fig. 2D), whereas no further differences in lipid droplet number between the groups (Fig. 2G). In line with this, cellular uptake of oleic acid, assessed as the sum of cell-associated and CO₂-trapped radioactivity, was not affected by EPS in either of the donor groups (Fig. 2H).

**Effect of EPS on oleic acid metabolism in the presence of PPARγ activation.** To examine whether the PPARγ agonist GW501516 and exercise could act synergistically in our cell model, myotubes were exposed to both EPS and GW501516. GW501516 increased oleic acid oxidation in myotubes from both lean and severely obese subjects, whereas no further increase with GW501516 and EPS in combination compared with GW501516 alone (Fig. 3A) was seen. Cellular uptake of oleic acid was increased after GW501516 treatment in myotubes from all three donor groups, whereas there was no further increase...
with GW501516 and EPS in combination (Fig. 3B). Although only studied in myotubes established from lean nondiabetic subjects, mitochondrial content measured as MitoTracker intensity was not further increased in cells treated with both GW501516 and EPS compared with EPS alone (Fig. 3C).

Effect of EPS on slow-oxidative (MHCI) fiber type. We wanted to investigate the effect of EPS on expression of genes and proteins for type I fibers (MYH7, the gene that regulates protein expression of MHCI). After EPS, MYH7 gene expression was reduced in myotubes from SO-T2D subjects (Fig. 4A). Furthermore, the EPS effect on gene expression of MYH7 in myotubes correlated negatively with fasting plasma glucose (Fig. 4B) as well as HbA1c (Fig. 4C) levels of the subjects. However, results obtained for protein expression of MHCI (Fig. 4D) showed no significant differences between the donor groups.

Effect of EPS on gene expression of IL-6 and IL-8. To investigate whether EPS-induced gene regulation of myokines seen in myotubes from lean nondiabetic subjects (48) also occurred in myotubes originating from severely obese subjects, qPCR on selected genes was performed. Gene expression of IL-6 was increased after EPS in myotubes established from L-nD and SO-nD subjects (Fig. 5A). Furthermore, EPS-induced effect on IL-6 gene expression in myotubes correlated negatively with fasting plasma glucose (Fig. 5B) as well as HbA1c (r = −0.60, P < 0.05) levels of the subjects. Similar results were also obtained for IL-8; although the EPS-increased IL-8 expression was not significant (Fig. 5C). In line with this, effect of EPS on gene expression of IL-8 also correlated negatively with fasting plasma glucose levels of the subjects (Fig. 5D).
DISCUSSION

In this study, we show that insulin sensitivity was increased in myotubes established from severely obese subjects after EPS. Furthermore, EPS enhanced oxidative capacity of glucose in myotubes from all subjects, while oleic acid oxidation and mitochondrial content were improved only in myotubes from lean subjects. Oleic acid oxidation and uptake were also increased after GW501516 treatment in myotubes from all subjects, whereas combination of GW501516 treatment and EPS showed no additional effect on oleic acid oxidation or uptake. Of particular interest, the impact of EPS on gene expression of $\text{IL-6}$ and $\text{IL-8}$ was different in myotubes originating from lean and severely obese nondiabetic subjects compared with myotubes from severely obese diabetic sub-

Fig. 2. EPS improved oxidative capacity of human myotubes. Myotubes were electrically stimulated for 48 h. Glucose and oleic acid oxidation, mitochondrial staining, OXPHOS, and lipid droplets were measured after termination of EPS. A: glucose oxidation. Myotubes were exposed to $[^{14}\text{C}]$glucose (1 mmol/l) for 3 h before CO$_2$ assessment ($n = 6-7$). B: oleic acid oxidation. Myotubes were exposed to $[^{14}\text{C}]$oleic acid (100 μmol/l) for 2 h before CO$_2$ assessment ($n = 7-8$). C: EPS-induced effect on oleic acid oxidation correlated negatively with BMI of the subjects ($n = 21$). D: myotubes were stained for mitochondrial content with MitoTracker Red FM, and nuclei with Hoechst 33258. Data are shown as MitoTracker intensity related to nuclei ($n = 5-6$). E: expression of proteins involved in mitochondrial oxidative phosphorylation, OXPHOS (Complex I subunit NDUFB8, Complex II subunit, Complex III subunit core 2, Complex IV subunit II, and ATP synthase subunit α). Expression levels were normalized to β-tubulin ($n = 4-6$). Linear mixed model was performed for OXPHOS experiments. F: representative immunoblots from one experiment (L-nD). Ctr, control. G: myotubes were stained for lipid droplets with Bodipy 493/503. Data are shown as lipid droplets related to nuclei. H: cellular uptake of oleic acid was assessed as the sum of cell-associated and CO$_2$-trapped radioactivity ($n = 7-8$). *$P < 0.05$ vs. control. #$P < 0.05$ vs. L-nD.
MYOTUBES FROM DIFFERENT DONORS RESPOND DIFFERENTLY TO EPS

Fig. 3. Effect of EPS combined with peroxisome proliferator-activated receptor-β (PPARβ) activation on oleic acid metabolism. Myotubes were treated with 10 nmol/l GW501516 for 96 h and EPS was applied to the cells for the last 48 h of the treatment period. A and B: after termination of EPS, myotubes were exposed to [14C]oleic acid (100 μmol/l) for 2 h. A: oleic acid oxidation (n = 5–10). B: cellular uptake of oleic acid, assessed as the sum of cell-associated and CO2-trapped radioactivity (n = 5–7). C: after termination of EPS, myotubes were stained for mitochondrial content with MitoTracker Red FM, and nuclei with Hoechst 33258. Data are shown as MitoTracker intensity related to nuclei and normalized to unstimulated control cells (n = 5–6). *P < 0.05 vs. control.

Fig. 4. Effect of EPS on gene and protein expression of myosin heavy chain type I (MHCI). The myotubes were electrically stimulated for 48 h. A: after termination of EPS, expression of MYH7, the gene that regulates protein expression of MHCI, was measured by qPCR and compared relative to the housekeeping genes 36B4 and GAPDH (n = 5–6). B and C: EPS effect on gene expression of MYH7 correlated negatively with fasting plasma glucose (B) and HbA1c (C) of the subjects (n = 16). D: immunoblot analysis with antibodies against MHCI, related to β-actin, presented relative to unstimulated control. *P < 0.05 vs. control.
insulin sensitivity in insulin-resistant myotubes from severely obese diabetic subjects. The observed effect on insulin sensitivity was more evident in myotubes from severely obese subjects, which is consistent with other studies reporting greater exercise-induced changes in insulin sensitivity in subjects who were more insulin resistant at baseline (5, 20), and more evident in obese men compared with lean men (20). This suggests that interventions to increase physical activity may be particularly effective at improving insulin sensitivity in population groups who are more insulin resistant or have an increased predisposition to insulin resistance. Lack of EPS-induced increase in insulin sensitivity in myotubes from lean nondiabetic subjects could also be explained by maximal responsiveness to insulin in those cells per se and limited capacity to respond further. In agreement with other studies reporting enhanced carbohydrate oxidation after exercise in obese subjects (9) and in subjects with T2D (50), we showed an increase in glucose oxidation after EPS in myotubes from all three donor groups. In contrast, we have previously (36) and in the present study shown that mitochondrial content was increased after EPS in myotubes from lean nondiabetic subjects, and this was followed by increased rate of oleic acid oxidation. In this study, however, we showed that this is not the case in myotubes from both groups of severely obese subjects. These data are in contrast to studies reporting an increase in muscle ex vivo palmitate oxidation in obese subjects after in vivo exercise (33). The discrepancy may to some extent be explained by differences between in vivo and in vitro exercise models. Without EPS, there was no difference in basal lipid content between myotubes from the three donor groups, excluding the possibility that the absence of EPS effect on oleic acid oxidation was due to label dilution when using a labeled fatty acid as a measurement of fatty acid oxidation. At resting state, the current consensus is that fatty acid oxidation is reduced in obese versus lean muscle (6, 26). However, some studies have also reported similar fatty acid oxidation in myotubes from lean and obese diabetic subjects (49) and even higher oxidation in obese than lean muscle (3). The reason behind this discrepancy is unclear but may be a result of different subgroups of obese populations studied. It is an ongoing debate whether the impairment in oxidative capacity in obese and insulin-resistant subjects is due to lower mitochondrial content or intrinsic mitochondrial dysfunction (54). Although it has been shown that exercise improves both mitochondrial content and intrinsic mitochondrial function in obese and insulin-resistant subjects (43), it has also been reported that there were no changes in key regulators of mitochondrial biogenesis in skeletal muscles in diabetic men after in vivo exercise (12). Consistent with this, in our exercise model, myotubes from subjects with higher BMI were less responsive to exercise-induced effects on fatty acid oxidation than myotubes from lean subjects. It has been suggested that insulin resistance in obesity and T2D is associated with intracellular accumulation of lipid in skeletal muscles (29). Consequently, much focus has been on the possibility of increasing lipid utilization by exercise to avoid lipid accumulation. However, evidence has emerged that increased lipid oxidation impairs glucose uptake by metabolic feedback, as suggested by the Randle cycle (reviewed in ref. 29). In line with this, we have shown that EPS was able to improve insulin sensitivity.

Fig. 5. Effect of EPS on gene expression of IL-6 and IL-8. The myotubes were electrically stimulated for 48 h and harvested after termination of EPS. Expression of IL-6 (A) and IL-8 (C) was measured by qPCR and studied relative to the housekeeping genes 36B4 and GAPDH. Fasting plasma glucose levels were inversely related to the effect of EPS on gene expression of IL-6 (B, n = 22) and IL-8 (D, n = 19). *P < 0.05 vs. control.
and glucose oxidation in myotubes from both groups of severely obese subjects without having impact on lipid oxidation.

Gene regulation involved in exercise adaptation is complex (14, 48), but genes regulated by PPARγ may be involved (15). It has also been proposed that PPARγ agonists and exercise training synergistically increase the proportion of type 1 fibers (MHC1) in adult mice (35). However, selective biomarkers for fatty acid β-oxidation were not further induced with exercise compared with GW501516 (35), which is consistent with our findings that oleic acid oxidation was not further increased when myotubes were treated with a combination of GW501516 and EPS.

In response to acute exercise, an increase of IL-6 mRNA expression has been detected in muscle during exercise and in the postexercise period in both lean and obese subjects (11, 51). In contrast to myotubes from lean and obese nondiabetic subjects, EPS did not increase mRNA expression of IL-6 in myotubes from obese diabetic subjects. In support of our findings, other studies have also reported no effect on IL-6 mRNA levels in muscle biopsies from obese diabetic subjects (52) and in plasma from diabetic mice after in vivo exercise (8). This may reflect an inherent abnormal IL-6 response in insulin-resistant skeletal muscle (27, 47). It has previously been shown that EPS increased gene expression of IL-8 in lean nondiabetic myotubes (48), and our current data indicate that the impact of EPS on gene expression of IL-8 differs in myotubes from severely obese nondiabetic subjects compared with diabetic subjects. Exercise-derived IL-8 is postulated to stimulate angiogenesis in skeletal muscles (39), but the biological role of muscle-derived IL-8 remains to be clarified. Despite vast research on myokines the past decade, more research is needed to clarify whether there is an altered myokine response in skeletal muscles from subjects with disturbed metabolic status.

In summary, by applying chronic, low-frequency EPS on cultured myotubes established from three different donor groups, lean nondiabetic subjects and severely obese subjects with and without T2D, we observed an increase in insulin sensitivity with no improvement of lipid oxidation after EPS in myotubes from both groups of severely obese subjects. These findings were associated with BMI of the subjects, suggesting an effect linked to obesity and indicating that improving insulin sensitivity is not solely dependent on combating lipid accumulation with increased oxidative capacity of lipids. Furthermore, we observed a reduced or no effect of EPS on gene expression of the myokines IL-6 and IL-8 in myotubes from severely obese diabetic subjects. These findings were associated with fasting plasma glucose levels of the subjects, suggesting an effect linked to the diabetic phenotype. Taken together, these data show that myotubes from different donor groups responded differently to EPS, suggesting that this effect may reflect the in vivo characteristics of the donor groups.

ACKNOWLEDGMENTS

The authors thank Terje Grønås (Electrons Lab, Institute of Chemistry, University of Oslo) for support and technical assistance. We are also thankful to Gerbrand Koster and Oddmund Bakke from the NORMIC-UIO imaging platform, Department of Molecular Biosciences, University of Oslo, for support, use of equipment, and excellent technical assistance.

GRANTS

This work was funded by the University of Oslo, Diabetesforbundet forskningsfond, and Anders Jahres fond til vitenskapsfremme.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


