Mitochondrial respiratory capacity and content are normal in young insulin-resistant obese humans.

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ABSTRACT

Considerable debate exists as to whether alterations in mitochondrial respiratory capacity and/or content play a causal role in the development of insulin resistance during obesity. The present study was undertaken in an effort to determine if such alterations are present during the initial stages of insulin resistance in humans. Young (~23 years) insulin sensitive-lean and insulin resistant-obese men and women were studied. Insulin resistance was confirmed via an intravenous glucose tolerance test. Measures of mitochondrial respiratory capacity and content, as well as $JH_2O_2$ emitting potential and the cellular redox environment were performed in permeabilized myofibers and primary myotubes prepared from vastus lateralis muscle biopsies. No differences in mitochondrial respiratory function or content were observed between lean and obese subjects, despite elevations in $JH_2O_2$ emission and reductions in cellular glutathione. These findings were apparent in permeabilized myofibers as well as primary myotubes. The results suggest that reductions in mitochondrial respiratory capacity/content are not required for the initial manifestation of peripheral insulin resistance.
INTRODUCTION

Despite an alarming increase in the prevalence of diet-induced insulin resistance or ‘pre-diabetes’, elucidation of the underlying etiology at the biochemical level remains unclear and heavily debated. With respect to skeletal muscle, reductions in ATP synthase activity/capacity, (2-7), and/or mitochondrial content (3; 8) have been suggested as potential causes of insulin resistance. Although several reports have indeed demonstrated lower ATP synthase flux/synthesis capacity (2-7) and/or mitochondrial content (2; 3; 8-12) in the presence of insulin resistance and/or overt type II diabetes, considerable debate remains as to whether such differences are causal, consequential, or unrelated to insulin resistance. Establishing causation within a given pathophysiological process requires adherence to certain general criteria; 1) associative data must consistently relate a particular stimulus with a disease, 2) the stimulus must precede disease onset 3) and removal of the stimulus must prevent/reverse the disease.

With respect to the first criterion, lower ATP synthase flux assessed in vivo using $^{31}$P magnetic resonance spectroscopy, as well as lower ATP generating capacity determined ex vivo in isolated mitochondria and/or permeabilized fibers in the presence of obesity-related insulin resistance has been reported by some (2-7), but not all (10-16) investigators. Discrepancies likely reflect differences in age (17) (age range ~ 23-60 years) and severity of fasting hyperglycemia/insulinemia between subject pools (18), as well as in the methodologies employed to quantify oxidative phosphorylation (19; 20). A similar degree of heterogeneity exists in relation to mitochondrial content with close to an equal number of investigations reporting lower (2; 3; 8-12) or no difference (4; 13-15; 21) between lean-insulin sensitive and obese-insulin resistant subjects. The few studies which have attempted to correlate indices of oxidative phosphorylation and/or mitochondrial content with insulin sensitivity have not found a significant relationship.
(13-15), with one exception (9). Regarding the second criteria, acutely elevating plasma free fatty acids via lipid infusion (3-6 hours) has been shown to transiently depress skeletal muscle insulin sensitivity in humans without affecting mitochondrial function and/or content (22; 23). Moreover, results from rodent models of high fat diet-induced insulin resistance have consistently demonstrated an initial up-regulation in mitochondrial capacity for oxidative phosphorylation as well as content despite the presence of insulin resistance (1; 24; 25). Lastly, administration of an iron-deficient diet in rodents failed to induce insulin resistance despite stark reductions in electron transport system protein content (26). Taken together, these data from humans, coupled with a large body of evidence in rodent models, do not support a role for altered capacity for oxidative phosphorylation and/or mitochondrial content as an underlying cause of diet-induced insulin resistance (1; 24-26).

Mitochondria contribute to the regulation of a number of cellular functions beyond energy provision, including cellular redox balance. Elevated mitochondrial oxidant emission stemming from nutrient overload has recently been put forth as a potential primary event in the etiology of diet-induced insulin resistance (27-29), based in part on observations of higher mitochondrial H$_2$O$_2$ emitting potential and oxidation of the cellular glutathione pool in skeletal muscle of obese-insulin resistant compared with lean-insulin sensitive subjects (10; 27). The present study was undertaken to determine if differences in mitochondrial respiratory capacity and content, as well as the cellular redox environment, are detectable during the early stages of impaired glucose tolerance in humans. Similar levels of mitochondrial respiratory capacity and content were found in permeabilized myofibers and cultured primary myotubes from young (~23 years), lean-insulin sensitive and obese-insulin resistant male and female subjects, despite higher mitochondrial H$_2$O$_2$ emitting potential and lower whole cell glutathione content in obese
subjects. Taken together, these data suggest that defects in mitochondrial capacity for oxidative phosphorylation (either inherent and/or acquired) are not required for the development of obesity-induced insulin resistance in humans, but are consistent with the proposed redox-regulated control of insulin sensitivity.

**RESEARCH DESIGN AND METHODS**

**Human subjects, tissue biopsy, IVGTT and primary human cell culture.**

Lean (n = 20, male/female = 10/10) and obese (n = 20, male/female = 10/10) subjects, 18-35 years of age were recruited from the faculty and student population of the University. All subjects were sedentary as defined by self-reported activity questionnaires (International Physical Activity Questionnaire; IPAQ) (30). Inclusion criteria for lean and obese subjects was BMI ≤ 25 BMI kg/m² (Lean) and ≥ 30 BMI kg/m² (Obese). Exclusion criteria included elevated fasting serum glucose (> 100 mg/dL) or total cholesterol (> 200 mg/dL), the presence of metabolic disease, diabetes, heart disease, or pregnancy. The University’s Institutional Review Board for human subjects approved all procedures in this study and all participants signed a written consent. All female participants were studied within the first 5 days of the follicular phase of their menstrual cycle to avoid the potential confounding influence of progesterone on mitochondrial function (31). On the day of the experiment, subjects reported to the clinical facility following an overnight fast (~10 hrs). After resting for 20 min, a catheter was placed in the antecubital vein and a baseline blood sample obtained. A skeletal muscle biopsy was then obtained from the vastus lateralis by the needle biopsy technique as described previously (27; 32) followed by an intravenous glucose tolerance test (IVGTT) to determine insulin sensitivity (33). Percent body fat was determined by dual-energy X-ray absorptiometry (GE Lunar Prodigy
Advanced). Blood insulin was assessed via electrochemiluminescence immunoassay (Labcorp, USA).

A second cohort of subjects consisting of young lean (21.1 ± 1 years; ≤ 25 BMI kg/m², n = 10) and obese (25.6 ± 3 years; ≥ 30 BMI kg/m², n = 10) males were recruited for primary human skeletal muscle cell culture. Following a 10 h overnight fast, approximately 50-100 mg of skeletal muscle from the vastus lateralis was obtained by percutaneous biopsy. The isolation and culturing of human primary skeletal muscle cells from biopsies was performed as previously described (34). On day 7 of differentiation, cells were incubated for 24 h in differentiation media (DMEM; 5 mM glucose; Control) or differentiation media supplemented with 10 mM galactose after which the cells were harvested for respirometry experiments. Separate aliquots of the same passage number were grown and treated similarly, then harvested for analysis of glutathione, mitochondrial proteins and citrate synthase activity.

**Preparation of permeabilized muscle fibers and primary myotubes.**

A portion of each muscle sample was separated for preparation of permeabilized fiber bundles as described previously (32) with the remainder quick frozen and stored in liquid nitrogen. Fiber bundles (0.2-0.8 mg dry wt.) were separated along their longitudinal axis using a pair of needle-tipped forceps under magnification (MX6 Stereoscope, Leica Microsystems, Buffalo Grove, IL, USA). Bundles were then treated with saponin [30 µg/ml] for 30 minutes at 4°C and subsequently washed in cold buffer Z [105 mM K-MES, 30 mM KCl, 1 mM EGTA, 10 mM K₂HPO₄, 5 mM MgCl₂·6H₂O, 0.005 mM glutamate and 0.002 mM malate with 5.0 or 0.5 mg/ml BSA; pH 7.1] until analysis (< 1 hr).

Myotubes were permeabilized as previously described (35). Cells were washed with PBS and lifted from culture flasks with 0.05% trypsin EDTA. This reaction was neutralized by
adding 10% FBS to the cell suspension and centrifuged for 10 min at 1000 rpm at room temperature. The cell pellet was then resuspended in growth media, counted using a hemocytometer, centrifuged again for 10 min, and re-suspended in room temperature respiration buffer [130 mM sucrose, 60 mM potassium gluconate, 1 mM EGTA, 3 mM magnesium chloride, 10 mM potassium phosphate, 20 mM HEPES, 0.1% BSA; pH 7.4]. Cells were then treated with 3 µg/10⁶ cells/ml digitonin (i.e., a mild, cholesterol-specific detergent) for 5 min at 37°C on an orbital shaker. Following permeabilization, myotubes were washed by centrifugation at 1000 rpm for 5 min to remove endogenous substrates. The cells were then resuspended in respiration buffer at a concentration of (1.5 × 10⁶ cells per 2 ml).

**Mitochondrial respiration measurements**

High-resolution O₂ consumption measurements were conducted at 30°C (fibers) or 37°C (myotubes) using the OROBOROS O2K Oxygraph (Oroboros Instruments, Innsbruck, Austria). Permeabilized fiber bundles were incubated for 5 minutes in 10 mM pyrophosphate prior to assay to deplete all endogenous adenine nucleotides and to inhibit contraction of the fibers during the assay. At the conclusion of each experiment, fibers were washed in double-distilled H₂O to remove salts, freeze-dried and weighed. Respiration measurements conducted using permeabilized myotubes were normalized to total protein.

For experiments utilizing intact (non-permeabilized) myotubes, cells were harvested as described above and resuspended in fresh differentiation media (2.0 × 10⁶ cells per 2 ml) that either did or did not contain galactose and loaded into the respiration chamber. Basal and trifluorocarbonylcyanide phenylhydrazone (FCCP; 5 µm)-induced respiration were assessed using the OROBOROS O2K Oxygraph with data expressed relative to cell count.

**Preparation of Muscle Protein.**
Homogenization buffer containing [50 mM HEPES, 10 mM EDTA, 1 mM EGTA, 100 mM NaF, and 50 mM NaPPi; pH 7.4] was prepared, de-gassed overnight and supplemented with anti-protease/phosphatase cocktails (Sigma, Saint Louis, MO). Muscle samples were homogenized on ice (1:20, w:v) under anaerobic conditions using an Anaerobic Chamber (Coy Laboratory Products, Grass Lake, Michigan). Homogenate was spun down at 10,000 RPM for 15 minutes and the supernatant was used for analysis.

**GSH and Western blot analysis.**

Total GSH was measured using a standard assay kit (Oxis International Inc.). Proteins for Western blotting were separated by SDS-PAGE and electrotransferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA) and probed overnight with a cocktail (1:1000) containing antibodies against the following proteins: Complex I subunit NDUFB8, Complex II subunit 30 kDa, Complex III subunit Core 2, Complex IV subunit I, and ATP synthase subunit alpha (Mitosciences, Eugene, OR). Following washing, membranes were incubated for 1 h at room temperature with a mouse secondary antibody and the immunoreactive proteins were detected using enhanced chemiluminescence (ChemiDoc XRS+ Imaging System, BioRad Laboratories, Inc., Hercules, CA). Samples were normalized to a crude muscle homogenate/cell lysate sample on each gel to normalize for blotting efficiency across gels.

**Citrate Synthase Activity.**

Citrate synthase activity was determined using a standard assay kit (Sigma CS0720, St. Louis, MO), which colorimetrically measures the reaction rate between acetyl coenzyme A and oxaloacetic acid.

**Glucose and Lactate.**
Media was collected after 24 h incubations and immediately frozen at –80° C for the subsequent determination of glucose and lactate. Glucose and lactate were determined by oxidation reactions (YSI model 2300 Stat Plus; Yellow Springs Instruments, Yellow Springs, OH). Glucose at the end of the incubation period was subtracted from the amount from stock differentiation media. Calculated glucose utilization and lactate levels were normalized to cell count.

Statistics

Data are presented as mean ± SEM. Statistical analyses were performed using t-tests or 1-way ANOVA with Student-Newman-Keuls methods for analysis of significance among groups. The level of significance was set at P < 0.05.

RESULTS

Subject characteristics.

Young men (22 ± 1 years) and women (23 ± 1 years) were recruited and subsequently grouped according to BMI as either lean (BMI = 23.8 ± 0.5) or obese (BMI = 36.7 ± 1.1). Baseline subject characteristics are provided in Table 1. Fasting blood glucose was similar between groups; however, corresponding insulin and HOMA-IR values were significantly elevated in the obese subjects regardless of gender. Insulin sensitivity index calculated from the IVGTT was significantly lower in both obese male and female subjects (Figure 1), confirming insulin resistance in the obese subjects.

Both gender and obesity do not impact mitochondrial respiratory capacity.

Mitochondrial oxygen consumption was assessed in permeabilized fiber bundles prepared from skeletal muscle biopsies obtained following an overnight fast. In the presence of saturating
concentrations of glutamate and malate, both basal (state 4) and maximal-ADP (state 3) supported respiration were not different based on gender or obesity (Figure 2A). Because no effect of gender was found for insulin sensitivity (Figure 1) or respiratory capacity (Figure 2A), all remaining data from male and female subjects were pooled based on BMI. The combination of glutamate and malate provides electrons exclusively at the level of complex-I. To assess electron transfer capacity throughout the entire system, saturating concentrations of substrates directed at β-oxidation (palmitoyl-L-carnitine; PC), complex-I (malate; M, glutamate; G) and complex-II (succinate; S) were added sequentially in the presence of maximal ADP. All respiration experiments were performed in the presence of 20 mM creatine in an effort to clamp ADP at the desired concentration throughout each experiment. In agreement with what was observed with GM, respiration rates were once again not different between lean and obese subjects (Figure 2B). Both maximal uncoupled respiration and protein content of various components of the oxidative phosphorylation system (OXPHOS) were not different between lean and obese subjects, suggesting that mitochondrial density was similar between groups (Figure 2C).

**Obesity does not impact respiratory capacity or mitochondrial content within primary human myotubes.**

As observed in the permeabilized fibers, respiratory capacity was also not different in permeabilized myotubes from young lean verses obese individuals (Figure 3A). Succinate supported respiration (electron entry via complex II exclusively) was also determined in the presence of rotenone under basal, maximal-ADP and uncoupled (FCCP) conditions (Figure 3B). No significant differences were observed between lean and obese myotubes. In agreement with
data from frozen muscle samples, neither OXPHOS protein content (Figure 3C) nor citrate synthase activity (Figure 3D) differed between lean and obese subjects.

**Acute exposure to galactose elevates respiratory capacity in primary myotubes: No impact of obesity.**

In contrast to skeletal muscle tissue, which in the basal state relies on oxidative phosphorylation to meet the majority of its energetic needs, myocytes in culture rely on glycolysis almost exclusively for ATP production. Replacing glucose with galactose in culture media has previously been shown to increase mitochondrial content, morphology and oxidative capacity, presumably as a consequence of increased reliance on oxidative phosphorylation within the galactose grown cells (36). To determine potential differences in adaptability between lean and obese cells, myotubes were incubated for 24 hours in the presence of galactose. Exposure to galactose elevated mitochondrial respiratory capacity (Figure 4B, pooled lean and obese data) within myotubes prepared from lean and obese subjects (Figure 4A). It should be noted that this galactose-induced elevation in respiratory capacity was evident despite the continual presence of glucose in the culture media. To confirm that the addition of galactose resulted in an increased reliance on oxidative metabolism, substrate incubation experiments were repeated, while simultaneously tracking lactate appearance and glucose disappearance within/from the culture media. As expected, glucose utilization (Figure 5A, pooled data from lean and obese) and lactate appearance (Figure 5B, pooled data from lean and obese) were lower in the presence of galactose compared to control conditions. Assessment of basal and FCCP-induced respiration within intact myotubes also revealed no differences between lean and obese subjects (Figure 5C). When data for lean and obese myotubes were pooled, elevations in respiration were evident in
the presence of galactose compared to controls; however, significance was only observed under FCCP-stimulated conditions (Figure 5D).

**Elevations in mitochondrial $JH_2O_2$ emission and reductions in cellular glutathione during obesity.**

To determine the impact of obesity on mitochondrial redox homeostasis, $JH_2O_2$ emitting potential was assessed in permeabilized myofibers prepared from human subjects under saturating substrate (palmitoyl-L-Carnitine; 25 µM, malate; 2 mM, glutamate; 5 mM and succinate; 10 mM) conditions in the absence of ADP. In agreement with previous findings (27), $JH_2O_2$ emission was higher in fibers prepared from obese subjects (Figure 6A). Reductions in total glutathione were also evident in the obese subjects, both in skeletal muscle homogenate (Figure 6B) and myotube lysate (Figure 6C).

**DISCUSSION**

The impetus for the present investigation stems from the ongoing debate within the field as to whether detriments in mitochondrial oxidative phosphorylation capacity and/or content are detectable under conditions of obesity-related insulin resistance, as well as if such derangements exist as a potential cause of the condition. Confirmation of this hypothesis would require impairments in mitochondrial phosphorylation capacity and/or content during obesity to be present at or near the onset of metabolic disease, (e.g., in young insulin-resistant obese subjects without substantial elevations in fasting blood glucose). The current study design was developed based on this concept. The present findings reveal no differences in mitochondrial respiratory capacity or content between young lean and obese subjects. This is supported by experiments conducted using permeabilized myofibers and primary myotubes, as well as intact myotubes in
Although indices of mitochondrial respiratory capacity were unaffected by obesity, higher mitochondrial H$_2$O$_2$ emitting potential was observed in the obese subjects. Moreover, total cellular glutathione was also found to be lower in both skeletal muscle homogenate and myotube lysate derived from obese subjects. Taken together, these data provide evidence that derangements in mitochondrial respiratory capacity are not required for insulin resistance, whereas total cellular redox buffering capacity appears to be impaired in humans at the early stages of obesity related insulin resistance consistent with the latter contributing to the etiology of metabolic disease.

The present findings agree with two recent investigations incorporating a similar study design in which rates of mitochondrial oxygen consumption from isolated mitochondria were not found to differ between lean and obese humans (10; 13). In contrast, Larsen et al (11) reported differences in respiratory capacity between lean and obese non-diabetic subjects; however, these differences were no longer evident when rates of respiration were normalized to citrate synthase activity. The findings of Larsen et al (11) are in agreement with a large body of evidence identifying lower mitochondrial content in obese compared to lean humans (2; 3; 8-12). In these studies, it is critical to point out the age range of the subject populations tested. The majority of lean versus obese comparisons have been made using subject populations older than 30 years of age (2; 3; 8-12). Reductions in ex vivo mitochondrial ATP production, citrate synthase activity, as well as mitochondrial protein abundance have been observed as a function of age in otherwise healthy humans (17). In the present investigation, depressions in mitochondrial content (assessed by western blotting, maximal FCCP-supported respiration and/or citrate synthase activity) were not evident within young (~23 years) obese participants. To our knowledge, only one other study has assessed mitochondrial content in an obese-insulin resistant ~22 year old
population (13). In agreement with the present study, no differences in mitochondrial DNA copy number were found between lean and obese subjects (13). It should be emphasized that while both citrate synthase activity and western blotting analysis of complex I-V have recently been shown to correlate strongly with transmission electron microscopy as surrogate indices of mitochondrial content (37), such measures do not rule out the possibility for reductions in specific mitochondrial proteins. In line with this notion, a recent study which incorporated tandem mass spectroscopy demonstrated lower abundance of specific mitochondrial proteins per mitochondrial mass within insulin-resistant obese subjects (10). Proteins included subunits within complex I, as well as enzymes involved in the oxidation of branched chain amino acids and fatty acids (10). The authors also reported elevations in mitochondrial H$_2$O$_2$ emitting potential within the obese subjects, which was suggested to result from elevated reducing pressure within the electron transport system (ETS) as a consequence of reduced ETS protein components relative to normal tricarboxylic acid cycle flux (10). Such conditions would be expected to favor higher NADH/NAD$^+$ for a given rate of respiration, thereby promoting accelerated electron leak (38; 39) and potentially explaining the increase in H$_2$O$_2$ emission observed ex vivo under saturating substrate conditions. Assessment of mitochondrial protein abundance via tandem mass spectroscopy was not performed in the present investigation; thus it remains to be seen if similar alterations in specific ETS proteins are apparent in young, obese-insulin resistant subject populations.

An alternative explanation for elevated JH$_2$O$_2$ emitting potential during obesity involves peroxide-mediated alterations to redox buffering integrity. Our group has previously reported higher H$_2$O$_2$ emitting potential in the presence of obesity, as well as in otherwise healthy humans 4 hours following a single high fat meal (27), thus demonstrating the sensitivity of the system to
positive metabolic balance. Elevations in substrate supply in the absence of a concomitant increase in demand for ATP generation (i.e., high caloric diet under sedentary conditions) is expected to increase NADH/NAD\(^+\), elevate reducing pressure within the ETS and thus accelerate electron leak \((38; 39)\). The glutathione and thioredoxin redox buffering systems operating within the matrix are responsible for degrading the \(\text{H}_2\text{O}_2\) produced. It is possible that prolonged exposure to increased \(\text{H}_2\text{O}_2\) may compromise redox buffering integrity, similar to that observed in the present study for whole cell reduced glutathione. It should be emphasized that the lack of a repeated measures design in the current investigation prevent the establishment of causation; however, because alterations in \(\text{H}_2\text{O}_2\) emitting potential and glutathione were evident during the early stages of obesity-induced insulin resistance these data support a potential causative role for altered cellular redox in contributing to disease etiology.

The present study was conducted in an effort to determine the relationship between insulin sensitivity and mitochondrial oxidative capacity/content, as well as redox homeostasis, specifically within skeletal muscle. Insulin sensitivity was determined by way of the IVGTT, which while it has been shown to correlate strongly with that of the hyperinsulinemic euglycemic clamp technique in humans \((40)\), the insulin sensitivity index measure is derived based on the combined effect of insulin on both skeletal muscle and liver \((33)\). This is a limitation of the current investigation as mitochondrial respiratory capacity and content were not determined in liver mitochondria.

The current results illustrating similar levels of mitochondrial respiratory capacity and content between primary human myotubes prepared from lean and obese subjects are in agreement with previously published reports \((41)\). In the present study, acute exposure of differentiated myotubes to galactose led to similar increases in maximal respiration in both lean
and obese myotubes. These results contrast with a recent report (41) in which the response to 24 hour lipid exposure was found to be blunted in obese compared to lean myotubes. This discrepancy is most likely a result of differences between permeabilization strategies between the two studies. In that study (41), permeabilization was carried out directly in the oxygraph chamber with 2-fold higher digitonin concentration and without the inclusion of a subsequent wash step. The inclusion of a wash step following digitonin-permeabilization is necessary to remove endogenous substrates which may interfere with rates recorded in response to exogenous substrate additions (42).

In conclusion, results of the present study do not provide support for the widely held hypothesis that diet-induced insulin resistance may be caused by alterations in either mitochondrial oxidative capacity or content. Rather, these findings in conjunction with other studies (10; 13-15), seem to suggest that any such changes in mitochondrial volume and/or function observed in response to diet-induced obesity are most likely secondary to the initial derangements in peripheral insulin sensitivity. This concept agrees well with data from rodent models in which high fat feeding has been shown to induce peripheral insulin resistance despite initial adaptive increases in mitochondrial respiratory capacity and content (1; 24). In contrast to that observed for indices of mitochondrial function/content, elevations in H$_2$O$_2$ emitting potential as well as alterations in the glutathione pool were readily apparent in young obese-insulin resistant subjects. At present, it would seem that therapeutic strategies directed at preventing the onset of insulin resistance would be best served by targeting the restoration of peripheral metabolic balance (via decreasing nutrient supply or increasing energetic demand) and/or the preservation of mitochondrial redox buffering integrity.
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FIGURE LEGENDS

**Figure 1.** Impact of gender and obesity on insulin sensitivity.

Insulin sensitivity index calculated in response to an intravenous glucose tolerance test (ML-male, lean (n = 13); MO-male, obese (n = 10); FL-female, lean (n = 11); FO-female, obese (n = 11)). Data represent mean ± SEM. *Different from ML or FL (P < 0.05)

**Figure 2.** Mitochondrial respiratory capacity is not different in permeabilized myofibers from young lean and obese humans.

Mitochondrial oxygen consumption was assessed in permeabilized myofibers prepared from vastus lateralis muscle of lean and obese human subjects (**A-B**). (**A**) Rates of O\(_2\) consumption in the presence of glutamate [10mM] and malate [2mM] under basal (state 4; GM\(_4\)) and maximal ADP [4mM]-stimulated (state 3; GM\(_3\)) conditions. (**B**) Rates of O\(_2\) consumption in response to PCM – palmitoyl carnitine [25µM], malate [2mM]; ADP [4mM]; Cyto C – cytochrome C [10µM]; G – glutamate [10mM]; S – succinate [10mM]; FCCP - Carbonyl cyanide p trifluoro methoxyphenylhydrazone [2µM]. With the exception of panel A, male and female data were pooled to compare lean versus obese. (**C**) Western blot analysis of mitochondrial OXPHOS proteins (MitoSciences) prepared from vastus lateralis frozen tissue homogenate. Data represent mean ± SEM; n = 7-10 (**A**), n = 16-17 (**B**), n = 10 (**C**).

**Figure 3.** Respiratory capacity and mitochondrial content in primary myotubes are not different in primary myotubes from young lean and obese humans.

Mitochondrial oxygen consumption was assessed in permeabilized primary human myotubes prepared from vastus lateralis muscle of lean and obese subjects (**A-B**). (**A**) Rates of O\(_2\)
consumption in response to PC – palmitoyl carnitine [25µM]; M - malate [2mM]; ADP [4mM]; Cyto C – cytochrome C [10µM]; G – glutamate [10mM]; S – succinate [10mM]; FCCP - Carbonyl cyanide p trifluoro methoxyphenylhydrazone [2µM].  (B) Rates of O₂ consumption in the presence of succinate [10mM] plus rotenone [1µM], under basal (S₄), ADP-stimulated (S₃), and uncoupled (FCCP) conditions.  (C) Western blot analysis of mitochondrial OXPHOS proteins (MitoSciences) prepared from cell lysate.  (D) Citrate synthase activity, expressed as µmol citrate/mg protein/min.  Data represent mean ± SEM; n = 9-10 (A), n = 5 (B), n = 10 (C-D).

**Figure 4.** Myotubes from young lean and obese humans show similar adaptive increases in respiratory capacity in response to metabolic challenge.

Fully differentiated myotubes were incubated for 24 hours in the presence of galactose (A-B), which was added directly to the differentiation media.  Following this 24 hour incubation, myotubes were harvested, permeabilized and oxygen consumption was assessed (A-B).  (B) Data from lean and obese subjects were pooled to illustrate the effects of galactose.  Data represent mean ± SEM; n = 9-10 (A), n = 19 (B). *Different from vehicle control (P < 0.05).

**Figure 5.** Basal and FCCP-stimulated respiration within intact primary human myotubes.

Primary human myotubes were incubated for 24 hours in differentiation media alone (control) or in differentiation media supplemented with galactose.  (A) Glucose utilization and (B) lactate production during the 24 hour incubations.  (C-D) Basal and FCCP [5µM]-stimulated respiration was assessed in intact primary human myotubes, following the 24 hour incubation.  (D) Pooled
data from lean and obese subjects. Data represent mean ± SEM; n = 7-8 (C), n = 14-16 (A, B, D). *Different from corresponding vehicle control condition (P < 0.05).

Figure 6. Elevations in $JH_2O_2$ emitting potential and depressed total GSH within young obese human subjects.

(A) Mitochondrial $JH_2O_2$ emission was assessed in permeabilized fibers in the presence of palmitoyl-L-Carnitine (25 µM), malate (2 mM), glutamate (5 mM) and succinate (10 mM). (B-C) Total glutathione (GSH₃) was assessed in tissue homogenate from vastus lateralis muscle of human subjects (B) and cell lysate from primary human myotubes (C). Data represent mean ± SEM; n = 11/15 (A), n = 10 (B-C). *Different from Lean (P < 0.05).

Table 1. Basic clinical characteristics of the study groups.

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Data are means ± SEM. BMI, body mass index, * Different from lean (P < 0.05), # Different from Lean Male
REFERENCES


capacity to produce ATP in association with severe insulin resistance. Diabetes 57:1166-1175, 2008
**Figure 1.** Impact of gender and obesity on insulin sensitivity.
Figure 2. Mitochondrial respiratory capacity is not different in permeabilized myofibers from young lean and obese humans.
Figure 3. Respiratory capacity and mitochondrial content in primary myotubes are not different in primary myotubes from young lean and obese humans.
Figure 4. Myotubes from young lean and obese humans show similar adaptive increases in respiratory capacity in response to metabolic challenge.
Figure 5. Basal and FCCP-stimulated respiration within intact primary human myotubes.
Figure 6. Elevations in $J_HO_2$ emitting potential and depressed total GSH within young obese human subjects.