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Skeletal muscle mitochondria exhibit decreased pyruvate oxidation capacity and increased ROS emission during surgery-induced acute insulin resistance

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Hagve M, Gjessing PF, Fuskevåg OM, Larsen TS, Irtun O. Skeletal muscle mitochondria exhibit decreased pyruvate oxidation capacity and increased ROS emission during surgery-induced acute insulin resistance. Am J Physiol Endocrinol Metab 308: E613–E620, 2015. First published February 10, 2015; doi:10.1152/ajpendo.00459.2014.—Development of acute insulin resistance represents a negative factor after surgery, but the underlying mechanisms are not fully understood. We investigated the postoperative changes in insulin sensitivity, mitochondrial function, enzyme activities, and release of reactive oxygen species (ROS) in skeletal muscle and liver in pigs on the 2nd postoperative day after major abdominal surgery. Peripheral and hepatic insulin sensitivity were assessed by D-[6,6-2H2]glucose infusion and subsequent hydrogen peroxide (H2O2) emission, and measured spectrophotometrically, and H2O2 was increased in SSM, IFM, and liver mitochondria (2.3-, 2.5-, and 2.5-fold, respectively, all P < 0.05). All other mitochondrial enzyme activities were unchanged. No changes in mitochondrial function in liver were observed. Mitochondrial H2O2 and O2− emission was measured spectrofluorometrically, and H2O2 was increased in SSM, IFM, and liver mitochondria (−2.3-, −2.5-, and −2.5-fold, respectively, all P < 0.05). We conclude that an impairment in skeletal muscle mitochondrial PDC activity and pyruvate oxidation capacity arises in the postoperative phase along with increased ROS emission, suggesting a link between mitochondrial function and development of acute postoperative insulin resistance.

reactive oxygen species; surgery; insulin resistance; mitochondria; skeletal muscle; liver

After surgery, the development of an acute transitory state of insulin resistance has been identified as a negative factor associated with increased morbidity and hospital stay (45), but the physiological and molecular mechanisms underlying this condition are not fully elucidated. The decline in insulin sensitivity in the postoperative phase has been thoroughly documented and is reflected mainly by impaired glucose disposal in peripheral tissues, primarily skeletal muscle (21, 36).

Oxidative glucose disposal in skeletal muscle is regulated by the pyruvate dehydrogenase complex (PDC), which is an enzyme complex that regulates the flux of glucose-borne pyruvate into the mitochondria by converting pyruvate to acetyl-CoA, which subsequently enters the tricarboxylic acid (TCA) cycle for oxidation. The activity of PDC is controlled by pyruvate dehydrogenase kinase 4 (PDK4) which phosphorylates and inhibits PDC, thereby also inhibiting glucose oxidation. PDK4 mRNA and protein levels are upregulated during conditions associated with acute onset of insulin resistance, such as fasting and burn injury (39, 48, 55). Regulation of PDC has been suggested as a key mechanism contributing to the protective effect of preoperative feeding (4), whereas mitochondrial dysfunction has been hypothesized to be central to the development of postoperative insulin resistance (3). Furthermore, we have demonstrated recently that following major abdominal surgery in pigs, an increase in PDK4 mRNA and protein expression in skeletal muscle leads to decreased insulin-stimulated oxidative glucose disposal and peripheral insulin resistance (19, 21). This indicates that impaired PDC activity after surgical trauma leads to reduced capacity for mitochondrial pyruvate oxidation and a reduced effect of insulin on oxidative glucose disposal (25, 43, 55).

Interestingly, these alterations are also seen during chronic insulin resistance (17, 24), which is further associated with increased mitochondrial release of reactive oxygen species (ROS), which is most likely due to nutritional overload on the mitochondria and alterations in the mitochondrial membrane structure (9, 41, 46). In both skeletal muscle and liver, mitochondria are thought to be the most important source of superoxide (O2−) and subsequent hydrogen peroxide (H2O2) release, and recent studies have shown that mitochondria and ROS have a causal role in various forms of insulin resistance (10, 27, 34, 53). Increased ROS release is also associated with activation of so-called uncoupling, a process where mitochondria exhibit increased leak oxygen consumption and reduced efficiency for ATP production. In this setting, activation of uncoupling is hypothesized to be a feedback mechanism for reducing ROS release at the cost of energy production (14, 54). To our knowledge, no study has yet investigated the role of mitochondrial function and ROS release in insulin-sensitive
tissues in relation to development of acute surgery-induced insulin resistance.

In this study, we assessed the change in mitochondrial functions, with special focus on pyruvate oxidation capacity, enzyme activities, levels of ROS release, and uncoupling in skeletal muscle and liver mitochondria during a state of acute insulin resistance after major abdominal surgery.

METHODS

Animals. The protocols were approved by the committee of the Norwegian Experimental Animal Board, and all experiments were conducted in compliance with the institutional animal care guidelines and the National Institutes of Health’s (NIH) Guide for the Care and Use of Laboratory Animals [Dept of Health and Human Services Publication no. 85-23 (NIH), revised 1985]. Yorkshire/Landrace hybrid pigs weighing 32.2 (27.8–37.4) kg were stalled and acclimatized in the animal research facilities for 1 wk before experiments. Animals were submitted to a 12:12-h light-dark cycle, a standardized diet, and ad libitum access to water. Experiments commenced between 7 and 8 AM.

Anesthesia and instrumentation. After sedation by intramuscular injection of 15 mg/kg ketamine, 1 mg/kg midazolam, and 1 mg atropine, the pigs were cleaned and weighed. Following mask inhalation of 4% isoflurane in 100% O2, the pigs were intubated orotracheally, gas anesthesia was mixed with nitrous oxide, and oxygen (40/60%) was continued throughout the experiments at a minimal alveolar concentration of 0.5–1.5%. Deep anesthesia was induced through an intravenous (iv) bolus of 0.01 mg/kg fentanyl and main-alveolar concentration of 0.5–1.5%. Deep anesthesia was induced by the addition of 4% isoflurane in 100% O2 the pigs were intubated orotracheally, gas anesthesia was mixed with nitrous oxide, and oxygen (40/60%) was continued throughout the experiments at a minimal alveolar concentration of 0.5–1.5%. Deep anesthesia was induced by the addition of 4% isoflurane in 100% O2 the pigs were intubated orotracheally, gas anesthesia was mixed with nitrous oxide, and oxygen (40/60%) was continued throughout the experiments at a minimal alveolar concentration of 0.5–1.5%. Deep anesthesia was induced through an intravenous (iv) bolus of 0.01 mg/kg fentanyl and main-alveolar concentration of 0.5–1.5%. Deep anesthesia was induced by the addition of 4% isoflurane in 100% O2 the pigs were intubated orotracheally, gas anesthesia was mixed with nitrous oxide, and oxygen (40/60%) was continued throughout the experiments at a minimal alveolar concentration of 0.5–1.5%. Deep anesthesia was induced through an intravenous (iv) bolus of 0.01 mg/kg fentanyl and main-alveolar concentration of 0.5–1.5%

Tracer methodology and hyperinsulinemic euglycemic step clamps. Basal glucose turnover was assessed during the last 30 min of a 90-min-long primed (6 mg/kg) continuous (0.12 mg·kg−1·min−1) infusion of 6-[6,6-3H2]glucose (basal period), followed by peripheral and hepatic insulin sensitivity measurements consisting of two consecutive 120-min-long hyperinsulinemic (~15 and 40 IU/ml) euglycemic (~4.5 mmol/l) clamps with labeled glucose infusate (2.1 atom %enrichment). Insulin was infused at rates of 0.4 (low-insulin clamp) and 1.2 mU·kg−1·min−1 (high-insulin clamp) to differentiate between hepatic and peripheral insulin sensitivity, as described previously (20).

Determination of tracer enrichment and calculations of glucose kinetics. Determination of tracer enrichment in arterial blood measured by LC-MS/MS and calculations of endogenous glucose release and whole body glucose disposal were performed based on modified versions of Steele’s equations (18, 51), as described previously (20).

Hormone analyses. Serum insulin, C-peptide, and plasma glucagon were measured by RIA (Linco Research, St. Charles, MO). Serum cortisol concentrations were determined by electrochemiluminescence immunoassay on a Roche Modular E 170 analyzer (Roche Diagnostics, Basel, Switzerland).

Free fatty acids, triglycerides, and lipid peroxides. Plasma free fatty acids (FFA) were measured using a colorometric assay kit (Wako Diagnostics, Richmond, VA) and a Wallac Victor2 1420 Multilabel Counter. For triglyceride measurements, lipids were extracted from skeletal muscle and liver tissue by the method of Folch, dried, and thereafter dissolved in a tert-butyl and Triton X-100/methyl alcohol mixture. The triglyceride concentration of the extracts was measured with a Triglyceride 25 kit from ABX Diagnostics (Montpellier, France). Lipid peroxides were measured in tissue homogenates as HNE protein adducts using a colorometric ELISA kit (Cell Biolabs).

Inflammatory biomarkers. Porcine-specific C-reactive protein (CRP) was measured with polyethylene glycol-enhanced immunoturbidimetric assay (Random). Samples were reacted with a specific antisemur to form a precipitate that was measured turbidimetrically at 340 nm. Haptoglobin was analyzed with an immunoturbidimetric assay in a Cobas 8000 autoanalyzer (Roche Diagnostics).

Isolation of skeletal muscle mitochondria. Isolation of skeletal muscle subsarcolemmal (SSM) and intramyofibrillar mitochondria (IFM) followed the procedure described by Rosca et al. (44). Briefly, biopsies (1–2 g) were harvested from the gluteus medius muscle and placed in buffer A (pH 7.4) containing (all in mmol/l) 100 KCl, 50 MOPS, 1 EGTA, 7 MgSO4, and 1 ATP. All visible connective tissue and fat were removed, and the biopsies were washed, weighed, minced with scissors, and incubated during constant stirring for 10 min in buffer A (4°C) supplemented with 1 mg/g protease disperse ( Worthington Biochemical). The concentration of disperse was diluted by the addition of buffer B (buffer A with 2 mg/ml bovine serum albumin), and the mixture was subjected thereafter to 10 min of centrifugation at 280 g. The buffer was discarded and the tissue homogenized in buffer B using Polytron and Potter-Elvehjem homogenizers. The homogenate was centrifuged at 1,100 g for 10 min at 4°C. The supernatant was filtered through a nylon mesh, resuspended, and centrifuged twice at 7,000 g for 10 min to collect SSM. The pellet containing IFM was resuspended in buffer A (10 ml/g) and homogenized. Trypsin (5 mg/g) and collagenase 2 (30 mg/g) were added, and the suspension was incubated on a rocker for 10 min at 4°C. After incubation, buffer B was added (10 ml/g) and the solution centrifuged at 12,000 g for 10 min for removal of proteases. The pellet was resuspended in buffer B (10 ml/g) and centrifuged twice at 1,100 g for 10 min. The supernatants were pooled and filtered, and the IFM pellet was collected by centrifugation at 12,000 g for 20 min. The final suspensions of both IFM and SSM were washed twice and resuspended (0.30 ml/g) in buffer C (pH 7.4) containing (all in mmol/l) 100 KCl, 0.5 EGTA, 5 MgSO4, and 1 ATP. The suspensions were left on ice for 45 min to allow stabilization of membranes.

Isolation of liver mitochondria. Liver mitochondria were isolated as described by Hoppel et al. (26) with slight modifications. Briefly, liver biopsies were harvested (~1 g) and placed in ice-cold MSM buffer (pH 7.4) containing (all in mmol/l) 220 mannitol, 70 sucrose, 5 MOPS, and 2 EDTA. They were rinsed, blotted, weighed, and minced with scissors before homogenization with a loose-fitted Potter-Elvehjem homogenizer. The homogenate was centrifuged at 580 g for 10 min, resuspended, and recentrifuged. The supernatant was filtered and centrifuged at 7,000 g for 10 min, and the pellet washed.
twice before final resuspension in buffer C. Protein content was estimated by the Bradford method for both skeletal muscle and liver mitochondria (8). One mitochondrial preparation from skeletal muscle and one from liver were excluded due to technical problems during the isolation process.

**Mitochondrial respiratory activity.** Measurement of mitochondrial oxygen consumption was performed by high-resolution respirometry, using an oxygraph (Oxygraph 2k; Oroboros Instruments) with a closed 2-ml chamber at 30°C. Respiration was performed in respiration medium (pH = 7.4) containing (all in mmol/l) 100 KCl, 50 MOPS, 1 EGTA, 5 KH2PO4, and 1 mg/ml BSA. Mitochondria were added to the medium to give a final concentration of ~0.1–0.3 mg protein/ml. Respiration was measured with 5 mmol/l glutamate + 2.5 mmol/l malate, 10 mmol/l pyruvate + 2.5 mmol/l malate, or 2.5 mmol/l palmitoyl-l-carnitine (PC) + 2.5 mmol/l malate in separate runs. ADP (200 μmol/l) was added to achieve ADP-stimulated respiration (VADP). Mitochondrial leak oxygen consumption (Vo_{ligo}) was recorded after depletion of ADP and addition of oligomycin (4 μg/ml). Mitochondrial ADP/O ratio was calculated as added ADP/ΔO2 (O2 tension recorded before and after ADP depletion). Respiration control ratio (RCR) was calculated as V_{ADP}/V_{ligo}. To estimate mitochondrial proton leakage through adenine nucleotide (ANT) and uncoupling protein 3 (UCP3), 25 μmol/l carboxyatractyslose (ATR) and 500 μmol/l guanosinediphosphate (GDP) were added, and V_{ATR} + GDP was recorded after the addition of oligomycin. To estimate the postoperative change in the effect of ATR + GDP on Vo_{ligo}, pre- and postoperative ΔV_{uncoupling} was calculated as Vo_{ligo} - V_{ATR} + GDP for comparison.

**Mitochondrial ROS emission.** H2O2 was estimated fluorometrically by oxidation of Amplex Red (nonfluorescent) to resofurin (fluorescent), as described by Schönfeld and Wojtczak (47), with modifications. Briefly, isolated mitochondria were added (~0.3 mg protein/ml) to a respiration buffer containing glutamate + malate or PC + malate and oligomycin (as in respiration measurements). For estimation of H2O2 release, 50 μmol/l Amplex Red and 2 μM/ml horseradish peroxidase were added to the suspension, allowing Amplex Red oxidation by H2O2. Superoxide (O2·-) release was estimated fluorometrically, as described by Johnson-Cadwell et al. (28). Briefly, the same mitochondria suspension as for H2O2 release was prepared, and 5 μmol/l MitoSOXred was added. All samples were then added to a 96-well microplate, and O2·- and H2O2 were measured simultaneously in separate wells. O2·- emission was monitored at excitation (ex) 510 nm/emission (em) at 580 nm, and H2O2 emission was monitored at ex 571 nm/em and nm/em 585 nm at 30°C, using a spectrofluorometer (Spectrmax Gemini EM). The emission of H2O2 was calculated as the increase in relative fluorescence (RF) per minute. The effect of GDP + ATR on O2·- emission was evaluated as percent increase in RF from Vo_{ligo}.

**Mitochondrial and antioxidant enzyme activities.** Pyruvate dehydrogenase and glutathione peroxidase activities were determined in tissue homogenates using colorimetric assay kits according to the manufacturer’s instructions (BioVision). NADH dehydrogenase and succinate-ubiquinone Q reductase activities were determined in tissue homogenates using precoated microplate ELISA assay kits (Abcam). Coenzyme Q (CoQ) cytochrome c oxidoreductase was estimated in tissue homogenate with a colorimetric assay kit (Cayman) according to the manufacturer’s instructions. Citrate synthase was determined spectrophotometrically according to the method of Srere (50). Superoxide dismutase (SOD) was measured as the inhibition activity on xanthine oxidase reaction with a commercial kit (Abcam) according to the manufacturer’s instructions and is displayed as percent inhibition rate of SOD. All assays were run on a Wallac Victor® 1420 Multilabel Counter, except for glutathione peroxidase, which was run on a VersaMax Microplate Reader.

**Statistics.** For all spectrofluorescence measurements, the preoperative levels were given the value of 1 and postoperative changes expressed as fold or percent changes from preoperative values. All data are presented as means ± SE. Comparisons between pre- and postoperative measurements were performed using two-way Student’s dependent t-test on absolute values. Wilcoxon signed-rank test was used where appropriate. Pearson’s production moment was used for correlation analysis. Differences were considered significant when P < 0.05.

**RESULTS**

**Basal glucose kinetics and glycemic response to surgery.** Coefficient of variation for serum glucose and tracer enrichment during the basal periods before (4.5 ± 0.4 and 10.0 ± 4.8%, respectively) and after surgery (5.7 ± 0.3 and 6.1 ± 1.3%) indicated steady-state conditions. A moderate elevation in blood glucose was seen after surgery compared with preoperative levels (4.52 ± 0.42 vs. 5.68 ± 0.25 mmol/l, P < 0.05), whereas no difference was observed in basal endogenous glucose release (4.70 ± 0.33 vs. 4.92 ± 0.43 mg·kg⁻¹·min⁻¹) or whole body glucose disposal (4.67 ± 0.32 vs. 4.90 ± 0.43 mg·kg⁻¹·min⁻¹).

**Glucose kinetics during two-step clamping.** Coefficient of variation before and after surgery during the last 40 min of each step clamp for serum glucose (low insulin: 4.5 ± 0.8 and 3.8 ± 1.0%; high insulin: 3.8 ± 0.5 and 2.8 ± 0.3%), tracer enrichment (low insulin: 7.2 ± 1.4 and 4.7 ± 0.9%; high insulin: 5.9 ± 1.3 and 3.9 ± 0.9%), and glucose infusion rate (low insulin: 22.6 ± 7.3 and 23.8 ± 8.7%; high insulin: 4.6 ± 0.7 and 4.8 ± 0.7%) indicated steady-state conditions, except for glucose infusion rate during the low-insulin clamp. Glucose infusion rates (GIR) are shown in Fig. 1A. Serum glucose, GIR during the last 40 min, tracer atom percent enrichment, endogenous glucose release, and whole body glucose disposal during two-step-clamping are shown in Table 1. The insulin-mediated response, measured as relative changes in endogenous glucose release and whole body glucose disposal from the basal period, is shown in Fig. 1B. Compared with preoperative levels, insulin-stimulated whole body glucose disposal was significantly reduced after surgery during both low- and high-insulin clamps (P < 0.05 and P < 0.001, respectively; Fig. 1B), indicating development of peripheral insulin resistance. Endogenous glucose release remained unchanged during both clamps, indicating unaltered hepatic insulin sensitivity ~48 h after surgical trauma.

**Insulin, C-peptide, and counterregulatory hormones.** Equal circulating insulin levels were seen before and after surgery during both low- and high-insulin infusion (Table 1). Glucagon levels during basal conditions did not change after surgery (20.0 ± 4.2 vs. 22.6 ± 3.4 pmol/l) and remained stable during clamping, whereas C-peptide was low or below detection level (<0.01 ng/ml) in both groups for all measurements (data not shown). There was no detectable difference in cortisol before or after surgery (90.3 ± 24.7 vs. 72.6 ± 18.0 nmol/l).

**FFA and triglycerides.** Free fatty acid (FFA) concentrations in arterial plasma were unchanged during the basal period before and after surgery (175 ± 34 vs. 215 ± 28 μmol/l) and were equally suppressed during low- (52 ± 9 vs. 52 ± 11 μmol/l) and high-insulin clamping (25 ± 4 vs. 23 ± 3 μmol/l). FFA plasma concentrations in venous blood sampled from conscious pigs were elevated from preoperative levels on the 1st day after surgery, although this increase did not reach statistical significance (236 ± 37 vs. 325 ± 48 μmol/l, P = 0.08). Triglycerides in arterial plasma were unchanged after......
surgery (0.24 ± 0.03 vs. 0.23 ± 0.03 mmol/l). Tissue triglyceride content was increased in liver postoperatively (7.88 ± 0.73 vs. 17.66 ± 5.00 vs. μmol/g, $P < 0.05$) but was unchanged in skeletal muscle (10.89 ± 1.26 vs. 14.63 ± 3.11 μmol/g).

Inflammatory biomarkers. Postoperatively, a significant increase in both CRP (14.2 ± 3.4 vs. 46.1 ± 4.7 mg/l, $P < 0.001$) and haptoglobin (88 ± 30 vs. 450 ± 18 mg/l, $P < 0.001$) indicated a surgery-induced inflammatory state.

Mitochondrial respiration. $V_{\text{ADP}}$, $V_{\text{o}_{\text{ligo}}}$, RCR, and ADP/O ratio calculations for IFM and SSM and liver mitochondria with malate in combination with glutamate, pyruvate, and PC as respiration substrates are shown in Table 2. No postoperative changes were seen in $V_{\text{ADP}}$ or $V_{\text{o}_{\text{ligo}}}$ for IFM or SSM with glutamate. During pyruvate-induced respiration, there was a significant postoperative reduction in $V_{\text{ADP}}$ in both IFM (~61%, $P < 0.05$) and SSM (~40%, $P < 0.001$) and a modest reduction of $V_{\text{o}_{\text{ligo}}}$ in IFM (~28% $P < 0.05$) but not in SSM. $V_{\text{ADP}}$ for PC was unchanged in skeletal muscle mitochondria, whereas $V_{\text{o}_{\text{ligo}}}$ was increased in both SSM (1.9-fold, $P < 0.05$) and IFM (2.5-fold, $P < 0.05$). No changes were observed in RCR postoperatively, except for a significant decrease when PC was used as substrate in SSM (~50%, $P < 0.05$). ADP/O ratio was significantly decreased during PC respiration in SSM (~20%, $P > 0.05$). In skeletal muscle mitochondria, the addition of GDP + ATR reduced $V_{\text{o}_{\text{ligo}}}$ no more than ~8–15% during PC respiration, whereas no changes were observed for the other substrates. With PC as substrate, $\Delta V_{\text{uncoupling}}$ after the addition of GDP + ATR was slightly higher in the postoperative phase in both IFM (3.04 ± 0.52 vs. 7.31 ± 1.33 nmol O$_2$-min$^{-1}$-mg$^{-1}$ protein, $P < 0.05$) and SSM (1.52 ± 0.82 vs. 3.31 ± 0.62 nmol O$_2$-min$^{-1}$-mg$^{-1}$ protein, $P = 0.05$). No postoperative changes in $V_{\text{ADP}}$, $V_{\text{o}_{\text{ligo}}}$, RCR, or ADP/O ratio for any of the three substrates were observed in liver mitochondria.

Mitochondrial enzyme activities. There was no change in NADH dehydrogenase (complex I) activity in skeletal muscle or liver (Fig. 2A), whereas the postoperative pyruvate dehydrogenase activity (PDCa) was significantly reduced in skeletal muscle (~32%, $P < 0.01$) but unchanged in liver (Fig. 2B). The postoperative reduction in PDCa was positively correlated to the decrease in WGD ($r = 0.748$, $P < 0.05$; Fig. 2C). Succinate CoQ reductase (complex II) was unchanged in skeletal muscle (0.79 ± 0.17 vs. 0.66 ± 0.18 mOD$_{550}$/min), as was CoQ cytochrome c oxidoreductase (complex III) in both skeletal muscle (1.36 ± 0.72 vs. 1.01 ± 0.50 mOD$_{550}$/min) and liver (2.75 ± 1.38 vs. 2.16 ± 0.63 mOD$_{550}$/min). Citrate synthase was unchanged in skeletal muscle (8.40 ± 0.44 vs. 8.76 ± 0.33 mM/mg dry wt) and minimally reduced in liver after surgery (6.14 ± 0.36 vs. 5.59 ± 0.21 mM/mg dry wt, $P < 0.05$).

Mitochondrial ROS release. Postoperatively, a significant increase in the rate of H$_2$O$_2$ release from SSM (~2.3-fold, $P < 0.05$), IFM (~2.5-fold, $P < 0.05$), and liver mitochondria (~2.3-fold, $P < 0.05$) was observed, using glutamate as substrate (Fig. 3). Both pre- and postoperatively, the addition of GDP + ATR did not result in any change in H$_2$O$_2$ emission using glutamate or PC in IFM, SSM, or liver mitochondria (data not shown). For O$_2^-$, the addition of ATR + GDP did not result in any change using glutamate as substrate, but when PC was applied, O$_2^-$ emission increased significantly in SSM (1.2 ± 1.1 vs. 47.6 ± 12.8%, $P < 0.01$) and IFM (7.4 ± 7.1 vs. 48.5 ± 16.3%, $P < 0.05$), but not in liver mitochondria (Fig. 4).

Antioxidant enzymes and 4-hydroxynonenal levels. There was no difference in SOD inhibition activity in either skeletal muscle (0.53 ± 0.11 vs. 0.50 ± 0.06, $P > 0.05$) and liver (0.63 ± 0.04 vs. 0.56 ± 0.03, $P > 0.05$) postoperatively, but a significant increase in hepatic 4-HNE (1.35 ± 0.83 vs. 3.53 ± 1.61 nM, $P < 0.05$). The hepatic CoQ levels decreased significantly postoperatively (0.74 ± 0.20 vs. 0.51 ± 0.18 μmol/g, $P < 0.05$).

Table 1. Glucose kinetics during clamps

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<tr>
<td>S-glucose, mmol/l</td>
<td>4.43 ± 0.06</td>
<td>4.51 ± 0.14</td>
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<td>Insulin, μU/ml</td>
<td>14.6 ± 1.3</td>
<td>14.9 ± 2.0</td>
<td>36.1 ± 2.3</td>
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<td>GIR, mg/kg/min</td>
<td>4.08 ± 0.29</td>
<td>4.17 ± 0.33</td>
<td>17.1 ± 1.53</td>
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<td>Tracer APE%</td>
<td>2.87 ± 0.11</td>
<td>5.11 ± 0.18</td>
<td>2.46 ± 0.08</td>
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<td>WGD, mg·kg$^{-1}$·min$^{-1}$</td>
<td>8.35 ± 0.80</td>
<td>6.08 ± 2.60$^*$</td>
<td>18.56 ± 1.77</td>
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<td>EGR, mg·kg$^{-1}$·min$^{-1}$</td>
<td>2.47 ± 0.43</td>
<td>2.62 ± 0.45</td>
<td>1.31 ± 0.21</td>
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Data are means ± SE for 8 pigs. GIR, glucose infusion rate; WGD, whole body glucose disposal; APE%, atom percent enrichment; EGR, endogenous glucose release. Serum glucose, insulin, steady-state GIR, tracer APE%, WGD, and EGR during pre- and postoperative hyperinsulinemic euglycemic step clamping. Significantly different vs. preoperative. *$P < 0.05$; †$P > 0.05$; ‡$P < 0.001$ (Student’s dependent t-test).
mitochondrial function after surgical trauma


discussion

In the present study, we show that impairment of the capacity of pyruvate oxidation (i.e., \( V_{\text{ADP}} \)) and pyruvate dehydrogenase activity in skeletal muscle mitochondria develops in concert with peripheral insulin resistance, inflammation, relative hyperglycemia, and a trend toward higher circulating free fatty acids after open major abdominal surgery. Furthermore, \( H_2O_2 \) emission was increased in both skeletal muscle and liver mitochondria on the 2nd postoperative day. These results demonstrate altered mitochondrial function after surgery, which we believe plays a role in the development of acute postoperative insulin resistance.

Glucose-borne pyruvate undergoes decarboxylation catalyzed by the PDC, yielding acetyl-CoA that enters the TCA cycle feeding complex I of the electron transport chain (ETC). Glutamate was also used as complex I substrate, which is thought to yield respiration conditions fairly identical to pyruvate (22) but is transported across the mitochondrial membrane by the electroneutral glutamate/OH exchanger and oxidized by glutamate dehydrogenase to feed complex I. With glutamate, the postoperative ADP-stimulated oxidative capacity (\( V_{\text{ADP}} \)) was unchanged, as was the \( V_{\text{ADP}} \) for PC that feeds ETC through β-oxidation. This indicates that the reduction in \( V_{\text{ADP}} \) for pyruvate is due to a reduced capacity of PDC rather than an overall impairment in the mitochondrial TCA cycle or ETC complexes. This contention is further supported by the postoperative reduction in PDC activity in skeletal muscle when assayed directly, whereas the activity of NADH dehydrogenase and other ETC enzymes was unchanged. A similar acute reduction in PDC activity is shown after fasting (49, 55) but has to our knowledge never before been demonstrated during acute postoperative insulin resistance. Based on the present data, we cannot rule out the involvement and/or impairment of other transporters such as the mitochondrial pyruvate carrier (23). However, the strong correlation between the decline in PDC activity and reduction in whole body glucose disposal.

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<td>Glutamate + Malate</td>
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Data are means ± SE and expressed as nmol O_2-min^-1-mg^-1 protein from mitochondrial preparations of 7 pigs. PC, palmitoyl-l-carnitine; SSM, subsarcolemmal mitochondria; IFM, intermyofibrillar mitochondria; \( V_{\text{ADP}} \), pre- and postoperative ADP-stimulated respiration; \( V_{\text{Voligo}} \), leak respiration; RCR, respiratory control ratio. \( V_{\text{ADP}} \), \( V_{\text{Voligo}} \), ADP/O ratio, and RCR (RCR = \( V_{\text{ADP}} \)/\( V_{\text{Voligo}} \)) in IFM, SSM, and liver mitochondria. Significantly different vs. preoperative. *\( P < 0.05; † \( P < 0.001 \) (Student’s dependent \( t \)-test).

Muscle (67 ± 5.7 vs. 58 ± 4.7%) or liver (90 ± 3.3 vs. 88 ± 4.9%). Glutathione peroxidase was reduced in muscle (2.06 ± 0.35 vs. 1.22 ± 0.31 U/ml, \( P < 0.05 \)) but unchanged in liver (5.06 ± 1.38 vs. 5.84 ± 1.62 U/ml). No statistically significant increase in 4-hydroxynonenal (4-HNE) levels was detected postoperatively (18.0 ± 2.23 vs. 27.0 ± 4.15 μg/mg protein, \( P = 0.16 \)).

Fig. 2. Enzyme activities in skeletal muscle (SM) and liver. A: NADH dehydrogenase (complex I) activity. B: pyruvate dehydrogenase activity (PDCa). Data are means ± SE from 8 pigs. Significantly different vs. preoperative. *\( P < 0.01 \) (Student’s dependent \( t \)-test). C: correlation between PDCa and WGD postoperatively (○). Pearson’s correlation was used (\( n = 8 \)).
after surgery indicates that regulation of this enzyme complex plays a predominant role in the development of insulin resistance.

The present data do not provide evidence to unravel the exact causal coherence and order of the events leading to the decrease in mitochondrial pyruvate oxidation capacity and peripheral insulin resistance. However, the impaired PDC activity and mitochondrial pyruvate respiration was developed along with reduced insulin sensitivity and increased inflammatory biomarkers. In this regard, we have in the same porcine model demonstrated a link between surgery-induced inflammation and postoperative impairment of insulin-mediated inhibition of PDK4 expression in muscle (19) resulting in increased PDK4 protein. This could, in concert with the present study, lead to reduced PDC activity and suppression of glucose oxidation. Furthermore, because skeletal muscle disposes ~80% of glucose during euglycemic insulin stimulation (5), these results collectively indicate that the decrease in pyruvate oxidation in muscle mitochondria is central to the impaired actions of insulin on glucose metabolism seen after surgery. In support of this contention, the postoperative decline in peripheral insulin sensitivity in our study is strikingly similar to what has been measured previously in humans after major abdominal surgery, and importantly, indirect calorimetry data in surgical patients have shown that in addition to a reduction in nonoxidative glucose disposal, a considerable postoperative decline in glucose oxidation occurs (36). The postoperative loss in insulin sensitivity measured ~48 h after surgery in the present study was due to a decrease in insulin-stimulated glucose disposal, whereas no decrease in hepatic insulin sensitivity was seen. This is in agreement with the finding that no significant changes in mitochondrial respiration measurements or PDC activity were seen in liver. It should be noted that anesthesia and antibiotics have been shown to affect mitochondrial function (29). However, they have been associated with dysfunction of the ETC complexes, whereas the measured complex activities in the present study were unchanged in both skeletal muscle and liver.

Increased FFA availability could also lead to a reduced flux of pyruvate from glycolysis into the mitochondria by actions of Randle’s glucose-fatty acid cycle (42) and peroxisome proliferator-activated receptor activation (24). Thus, although not statistically significant (P = 0.08), the general increase in plasma FFA after surgery could have contributed to the concomitant reduction of pyruvate respiration. Increased FFA would lead to increased deposition of tissue lipids. However, whereas an increased deposition of tissue triglycerides was seen in liver, triglyceride content in skeletal muscle was, somewhat unexpectedly, unchanged 2 days after surgery. Intriguingly, mitochondrial dysfunction is reported in skeletal muscle during the acute insulin-resistant state after burn injury, as demonstrated by reduced capacity for both pyruvate and PC respiration as well as reduced mitochondrial enzyme activities (11, 13, 37). More recently, onset of uncoupling was also demonstrated (40). Whereas the magnitude of reduction in pyruvate oxidation is in accord with our results, we found no reduction in the oxidation of other mitochondrial substrates, suggesting that more severe trauma is necessary for an overall impairment of mitochondrial oxidative capacity to develop. In support of this notion, burn injury is associated with more profound catabolism and markedly increased lipolysis, plasma FFA, and deposition of triglycerides in skeletal muscle (and liver) (2, 12).

The damaging effects of increased oxidative stress during metabolic conditions are well described (for reviews, see Refs. 9, 41, and 46). Recent evidence also supports the role of ROS as a regulator molecule and as an inductor of insulin resistance through activation of stress-induced serine/threonine kinases such as IκB kinase and c-Jun NH₂-terminal kinase (9, 16, 30) or by direct oxidative modification of the insulin receptor and/or phosphotyrosine phosphatases (10, 35), leading to impairment of insulin signaling. Although several studies have provided evidence supporting the existence of increased oxidative stress in different states of chronic insulin resistance (7, 27, 34), no study has to our knowledge directly looked at mitochondrial ROS release in acute insulin resistance after trauma or surgery. The levels of H₂O₂ and O₂− emission were measured directly in mitochondria respiring on glutamate, a substrate that in an experimental context induces fairly low but physiological levels of ROS (52). Interestingly, H₂O₂ emission was increased in both skeletal muscle and liver mitochondria after surgery. Such an increase in ROS during insulin resistance has previously been attributed to nutritional overload and excess substrate availability and alterations in mitochondrial membrane structure (9, 41, 46). Oxidative stress is often observed along with alterations in antioxidant mechanisms...
such as SOD and glutathione. We detected no difference in SOD activity, but there was a slightly reduced glutathione peroxidase activity in skeletal muscle indicating compromised antioxidant capacity, as reported previously after surgical trauma (33). Increased ROS release is also observed frequently along with increased levels of lipid peroxidation and product 4-HNE, which is further suggested to be a potent activator of mitochondrial uncoupling (14, 15). However, we failed to detect an increase in 4-HNE postoperatively in the present study.

Uncoupling is a process where mitochondria decrease their membrane potential and increase their oxygen consumption without production of ATP, and this process has been shown to reduce ROS release (14, 54). Leak oxygen consumption, an estimate of uncoupling, was measured by blocking the ATP synthase with oligomycin (V\text{oligo}) and furthermore by inhibiting the most essential mitochondrial uncouplers, uncoupling protein 3 (inhibited by GDP) and the adenine nucleotide translocase (inhibited by ATR) (1). Although no increase in V\text{oligo} was observed with glutamate or pyruvate, there was a significant increase in both SSM and IFM, using PC as substrate. This indicates a FFA-dependent activation of uncoupling and is in line with research showing that the presence of FFA derivatives is necessary for complete induction of uncoupling through a synergistic coactivation with ROS (15, 32). Surprisingly, the increase in V\text{oligo} postoperatively was only slightly lowered when mitochondria were exposed to ATR and GDP. However, the severe postoperative percentage increase in \textit{O}_2^- emission after the addition of GDP and ATR indicates that uncoupling was activated, leading to higher rates of ROS elimination after surgery. Why this was not reflected by reversal of mitochondrial leak respiration to preoperative levels when ATR and GDP were added is not clear to us, as we would have expected any increase in V\text{oligo} to be nearly completely eliminated by uncoupling inhibitors. However, this finding could be attributed to either the suggested lack of specificity of ATR and GDP (6, 31, 38), and thereby only partial inhibition of uncoupling, or increased leak respiration due to proton leakage through mitochondrial membrane proteins other than UCPs and ANT.

In conclusion, this study shows that impairment in mitochondrial pyruvate dehydrogenase activity and pyruvate oxidation capacity develops along with insulin resistance in skeletal muscle in the postoperative phase after major abdominal surgery. In addition, mitochondrial ROS release is increased in both skeletal muscle and liver. Skeletal muscle mitochondria express increased fatty acid-induced leak respiration, which could contribute to less effective mitochondrial oxidation. Collectively, these findings underpin the notion that mitochondrial alterations are involved in the acute decline in insulin sensitivity after surgical trauma.

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DISCLOSURES

The authors report no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

M.H., P.F.G., T.S.L., and O.I. conception and design of research; M.H., P.F.G., and O.M.F. performed experiments; M.H. and O.M.F. analyzed data; M.H., P.F.G., and O.I. interpreted results of experiments; M.H. prepared figures; M.H. drafted manuscript; M.H., P.F.G., O.M.F., T.S.L., and O.I. edited and revised manuscript; M.H., P.F.G., O.M.F., T.S.L., and O.I. approved final version of manuscript.

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