Acute exercise reverses starvation-mediated insulin resistance in humans

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1The Swedish School of Sport and Health Sciences, Åstrand Laboratory of Work Physiology, Stockholm, Sweden; 2Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; and 3Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

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Frank P. Katz A. Andersson E. Sahlin K. Acute exercise reverses starvation-mediated insulin resistance in humans. Am J Physiol Endocrinol Metab 304: E436–E443, 2013. First published December 26, 2012; doi:10.1152/ajpendo.00416.2012.—Within 2–3 days of starvation, pronounced insulin resistance develops, possibly mediated by increased lipid load. Here, we show that one exercise bout increases mitochondrial fatty acid (FA) oxidation and reverses starvation-induced insulin resistance. Nine healthy subjects underwent 75-h starvation on two occasions: with no exercise (NE) or with one exercise session at the end of the starvation period (EX). Muscle biopsies were analyzed for mitochondrial function, contents of glycogen, and phosphorylation of regulatory proteins. Glucose tolerance and insulin sensitivity, measured with an intravenous glucose tolerance test (IVGTT), were impaired after starvation, but in EX the response was attenuated or abolished. Glycogen stores were reduced, and plasma FA was increased in both conditions, with a more pronounced effect in EX. After starvation, mitochondrial respiration decreased with complex I substrate (NE and EX), but in EX there was an increased respiration with complex I + II substrate. EX altered regulatory proteins associated with increases in glucose disposal (decreased phosphorylation of glycogen synthase), glucose transport (increased phosphorylation of Akt substrate of 160 kDa (AS160)). In conclusion, exercise reversed starvation-induced insulin resistance and was accompanied by reduced glycogen stores, increased lipid oxidation capacity, and activation of signaling proteins involved in glucose transport and FA metabolism.

IT IS WELL ESTABLISHED THAT SHORT-TERM STARVATION in humans induces insulin resistance (32). Starvation may well be one of the earliest, if not the first, forms of insulin resistance in man. Therefore, elucidation of the mechanisms involved in this form of insulin resistance could have implications for understanding the development of insulin resistance under other conditions, such as obesity and type 2 diabetes.

Within 24 h of starvation liver glycogen is depleted (35), and thereafter insulin resistance develops in peripheral tissues. The reduced insulin sensitivity is an important physiological response to prioritize glucose for the central nervous system. Since exercise acutely stimulates both insulin-dependent and insulin-independent muscle glucose uptake (18, 41), there is a potential danger that exercise during a hypoglycemic state may compromise metabolic homeostasis. However, the effect of acute exercise on insulin sensitivity during starvation is unclear, and further studies are required.

Starvation is associated with increased lipolysis and increases in plasma fatty acid (FA) and ketone bodies as well as intramyocellular lipids (8, 13, 48). Increased lipid load is also a common feature of many conditions associated with reduced insulin sensitivity [i.e., obesity, type 2 diabetes, lipid infusion (5), high-fat diet (5, 28), and starvation (28)]. Therefore, dysfunctional lipid metabolism is considered to be involved in the pathogenesis of insulin resistance, but the mechanism remains unclear.

It has long been known that increased FA levels inhibit glucose oxidation (38). Overload of lipids also leads to increases in intramyocellular lipids and in toxic lipid intermediates (e.g., ceramide, diacylglycerol, fatty acyl-CoA), which can potentially interfere with insulin signal transduction (44). Another possibility is that lipid-derived ketone bodies, which increase markedly during both starvation and untreated type 2 diabetes, play a role in the development of insulin resistance (1, 58). The increased energy demand and fuel utilization during exercise will most likely remove toxic lipid intermediates and thus potentially reverse lipid-induced insulin resistance. Exercise will also stimulate muscle glucose uptake by activation of glycogen synthase (GS) and by phosphorylation of Akt substrate of 160 kDa (AS160).

Mitochondrial oxidation of lipids is essential for maintaining an adequate balance between supply and utilization of lipids, and a high mitochondrial capacity to oxidize lipids may be essential for prevention of insulin resistance (43). In fact, conditions of increased lipid load and insulin resistance are associated with mitochondrial dysfunction, such as reduced mitochondrial respiration and electron chain activity (25, 33, 39). In a recent study, it was shown that both insulin sensitivity and mitochondrial respiratory capacity were reduced after a 60-h fast (25). However, it remains unclear whether exercise can counteract the starvation-induced decrement in mitochondrial respiration. Furthermore, mitochondrial production of reactive oxygen species (ROS) has been implicated in insulin resistance (24, 43), but the effects of starvation and exercise on ROS production are unclear.

The purpose of this study was to investigate whether acute exercise can reverse starvation-induced insulin resistance and whether this is associated with altered mitochondrial function and capacity to oxidize lipids.

RESEARCH DESIGN AND METHODS

Subjects. Nine healthy untrained subjects (5 men and 4 women) participated in this study. Average age, weight, and maximal oxygen uptake (V̇O₂max) were 23.2 ± 0.5 yr, 71.1 ± 3.5 kg, and 3.3 ± 0.2 l/min, respectively (men: 23.4 ± 0.8 yr, 76.0 ± 2.0 kg, and 3.8 l/min; women: 23.0 ± 0.8 yr, 65.1 ± 4.4 kg, and 2.7 ± 0.1 l/min). All subjects were fully informed of the possible risks of the study and signed an informed consent document. The study design was approved by the Regional
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freeze-dried, dissected free of blood and connective tissue, and stored at nitrogen and stored at

was immediately permeabilized and the other rapidly frozen in liquid nitrogen. All muscle samples were separated into two parts; one was obtained using the percutaneous needle biopsy technique with 2–4 ml Carbocain 20 mg/ml; Astra Zeneca, Södertälje, Sweden), an

following morning at 0900 a biopsy was obtained; thereafter, an IVGTT was performed. On the 4th day, subjects who had one exercise session at the end of the starvation period (EX) came in at 0800 in the morning and performed one exercise session consisting of 5 × 10 min intervals separated by 2–4 min rest starting at 70% \( V_{O2\max} \). To avoid fatigue and be able to complete the exercise session, the intensity had to be gradually reduced to 50% \( V_{O2\max} \) on the last interval. The final biopsy was taken prior to IVGTT 3 h postexercise. Blood samples were taken prior to muscle biopsy and within 3 min after termination of exercise. To control food deprivation, subjects were instructed to collect a urine sample every morning, freeze it, and bring it to the laboratory for ketone body analysis. Ketone body levels in urine were elevated in all subjects from day 2 and onward (results not shown).

Muscle and blood sample. Muscle samples were obtained from the middle portion of the vastus lateralis muscle. After local anaesthesia (2–4 ml Carbocain 20 mg/ml; Astra Zeneca, Södertälje, Sweden), an incision was made through the skin and fascia at one-third the distance between the patella and anterior superior iliac spine. A muscle sample was obtained using the percutaneous needle biopsy technique with suction (7). All muscle samples were separated into two parts; one was immediately permeabilized and the other rapidly frozen in liquid nitrogen and stored at −80°C. Thereafter, the frozen samples were freeze-dried, dissected free of blood and connective tissue, and stored at −80°C for later determination of glycogen and immunoblotting. Blood (4 ml) was centrifuged at 1,500 g at 4°C for 10 min, and plasma was stored at −20°C for later analysis (see below).

IVGTT. Venous cannulae were inserted into the antecubital vein of each arm. One arm was used for glucose infusion and the other for blood sampling. Basal samples were collected at 15 and 5 min prior to glucose infusion. Glucose (300 mg/ml; Apl, Stockholm, Sweden) was infused with a continuous flow for 2 min (0.3 g/kg body wt), after which blood samples were taken frequently for 90 min. The cannula was flushed frequently with saline to avoid blood clotting. Glucose tolerance was calculated as the area under the glucose curve above basal (glucose\(_{AUC}\)) and glucose disappearance rate (\(K_g\)) as the slope of the logarithmic glucose concentrations between 10 and 40 min. \(S_{Ig\text{baseline}}\) was used as a measure of whole body insulin sensitivity and was calculated as the ratio of \(K_g\) over the area under the insulin curve from 0 to 40 min above basal. Insulin release was measured as the area under the plotted curve above basal between 0 and 40 min (insulin\(_{AUC}\) from 0 to 40 min). Acute insulin response was calculated as the ratio between the areas under the curves for insulin and glucose above basal during the initial period (0–10 min).

Analysis of blood samples. Blood glucose was analyzed in all samples using an automated analyzer (Biosen 5140; EKF Diagnostics, Barleben, Germany), and insulin was analyzed in plasma using an insulin ELISA kit (Mercodia, Uppsala, Sweden). Plasma concentration of FA was determined with a commercially available colorimetric enzymatic procedure (NEFA C test kit; Wako Chemicals, Neuss, Germany).

The concentration of β-hydroxybutyrate (BOH) in blood was analyzed with an enzymatic technique (55). BOH was mixed (2:1) with perchloric acid (0.65 M) and stored on ice. The sample was centrifuged at 3,000 g for 15 min, and the blood supernatant was stored at −80°C. One milliliter of supernatant was mixed with 0.1 ml K2CO3 (3.6 M) and incubated at 0°C for 5 min. The sample was centrifuged at 1,400 g for 30 s, the supernatant was transferred to another eppendorf tube, and pH was checked (pH 9.5). A 96-well plate was loaded with the samples and mixed with a reagent solution (4:1) consisting of glycine (330 mM), NAD (7.9 mM), and malate dehydrogenase (82 μg/ml). The absorbance was measured after 10 min at 340 nm with a plate reader (Tecan infinite F200 Pro; Tecan, Männedorf, Switzerland). Then, 5 μl of sodium β-OH dehydrogenase (17 U/ml) was added to each well, and the absorbance was measured after 30 min when the reaction was finished. The concentration of BOH was calculated from the change in absorbance using a concentration curve.

Analysis of muscle samples. Glycogen was analyzed in 1–2 mg of freeze-dried muscle according to the method previously described by Harris et al. (23), which includes enzymatic hydrolysis of glycogen followed by enzymatic analysis of glucose.

Mitochondrial respiration and ROS emission were measured in permeabilized muscle fiber bundles, as described previously (21, 42, 50), with minor changes. Muscle samples (10–25 mg wet wt) were stored in an ice-cold medium with the following composition (in mM): 2.8 CaK2EGTA, 7.2 K2EGTA, 5.8 Na2 ATP, 6.6 MgCl2, 20 taurine, 15 Na2 phosphocreatine, 20 imidazole, 0.5 diithiothreitol and, 50 MES adjusted to pH 7.1. The specimen was split into 2- to 5-mg fiber bundles, and each bundle was mechanically separated, using surgical needles in a network formation to expose fiber membranes to the surrounding medium. The bundles were incubated with saponin (50 μg/ml), washed twice, and stored in a medium with the following composition (in mM): 0.5 EGTA, 3 MgCl2, 60 K-lactobionate, 20 taurine, 10 KH2PO4, 20 HEPES, 110 sucrose, and 1 g/l BSA adjusted to pH 7.1. Mitochondrial respiration was measured using an oxygraph (Oroboros Instruments, Innsbruck, Austria) at 37°C in the storage medium. Oxygen level in the chamber was adjusted to 400 nmol/ml with H2O2 and 280 U/m catalase. To reduce muscle contractions, 45 μM benzyltoluene sulfonamide was added (36). Oxygen consumption was measured using two different protocols. In the first, the following substances were added in sequence (in mM): 2 malate, 0.5 octanoylcarnitine, 2.5 ADP, 10 glutamate, 10 succinate, 10 μM cytochrome c, 0.05 μM FCCP, and 0.5 μM rotenone. In the second protocol, additions were in (mM): 2 malate, 10 glutamate, 2.5 ADP, 10 succinate, 10 μM cytochrome c, and 2 μg/ml oligomycin. All fiber bundles with a >10% increase in respiration following addition of cytochrome c (indicating damage of the outer mitochondrial membrane) were excluded (4 measurements in protocol 1 and 2 measurements in protocol 2), and missing values were accounted for in the statistical analysis. The rate of mitochondrial H2O2 production was measured with Amplex Red (Invitrogen, Eugene, OR), which, in the presence of peroxidase enzyme, reacts with H2O2 and produces the red fluorescent compound.
resorufin. Permeabilized fiber bundles (n = 9) were added to the measuring medium (in mM: 225 mannitol, 75 sucrose, 10 Tris base, 10 K2HPO4, 0.1 EDTA, 0.08 MgCl2, 2 g/l BSA, 13.5 U/ml horseradish peroxidase, 45 μM benzyltoluene sulfonamide, 45 U/ml SOD, and 2 μg/ml oligomycin adjusted to pH 7.1) and kept at 30°C. The change in fluorescence was recorded (Hitachi fluorescence spectrophotometer F-2500 with magnetic stirrer; Hitachi, Tokyo, Japan) after subsequent additions of (in mM) 0.5 octanoyl-carnitine, 10 glutamate, 5 succinate, and 0.5 μM rotenone.

For immunoblotting, freeze-dried muscle tissue (n = 8) was homogenized using a bullet blender (Bullet Blender 1.5; Next Advance) and prepared as described elsewhere (3). Primary antibodies used were phosphorylated AMP-activated protein kinase (p-AMPK Thr172), phosphorylated acetyl-CoA carboxylase (p-ACC Ser 79), p-Akt Ser 473, phosphorylated AMP-activated protein kinase (p-AMPK Thr172), phosphorylated GSK-3 (p-GSK3 Ser21/9) (Cell Signaling Technology), α-tubulin (Sigma-Aldrich), uncoupling protein 3 (UCP3), p-GS Ser641/645, GLUT1, and GLUT4 (Millipore). Anti-rabbit or anti-mouse HRP (Cell Signaling Technology) was used as secondary antibody.

Statistical analysis. Data are presented as means ± SE. Statistical significance was evaluated with two-way or one-way repeated-measures ANOVA (plasma BOH and FA) using Statistica (Stat Soft, Tulsa, OK). When a significant main effect or interaction was observed, post hoc analyses (Fisher’s least significant difference test) were performed to locate the differences. Statistical significance was accepted at P < 0.05.

RESULTS

Physiological and metabolic responses to starvation and exercise. Body weight decreased from 71.1 ± 3.5 to 68.0 ± 3.6 after starvation (P < 0.001), without any difference between EX and subjects with no exercise (NE).

Plasma levels of BOH increased 50-fold after starvation, and although BOH decreased immediately following exercise, it increased to the same levels as NE 3 h postexercise (Table 1). FA increased 3.3-fold in NE and 4.1-fold in EX, with a significant difference between conditions. Muscle glycogen was reduced in both conditions (P < 0.001) but was lower in EX than in NE (31 and 72% of initial values, respectively; P < 0.001 EX vs. NE) (Table 1).

Responses to IVGTT. Starvation resulted in marked decreases in basal plasma glucose and insulin, without differences between NE and EX (Table 1). Plasma glucose peaked within 10 min following the glucose infusion but was higher after starvation in both NE and EX (between 30 and 90 min following glucose infusion) and higher in NE than in EX (Fig. 2A). Ks decreased by 65% in NE and 37% in EX, and glucoseAUC increased by 250 and 181% in NE and EX, respectively (Table 2). Both parameters demonstrate reduced glucose tolerance after starvation but a better glucose tolerance in EX compared with NE. Plasma insulin levels were lower 2–20 min into the IVGTT in both conditions after starvation, without differences between conditions (Fig. 2B).

Whole body insulin sensitivity, measured as SIgalvin, decreased after starvation in NE (P < 0.05) but was not changed in EX. SIgalvin was significantly higher in EX compared with NE, indicating that exercise improves insulin sensitivity. Determination of the glucose and insulin AUCs demonstrated that insulin sensitivity was increased in EX compared with NE after starvation (Table 2).

Table 1. Metabolic responses to starvation and exercise

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<th>Basal</th>
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<td></td>
<td>NE</td>
<td>EX</td>
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<tr>
<td></td>
<td>(0 h)</td>
<td>(3 h)</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
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<tr>
<td>Glycogen, mmol/kg dry wt</td>
<td>425 ± 32</td>
<td>436 ± 30</td>
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<tr>
<td>FA, mmol/l</td>
<td>0.42 ± 0.05</td>
<td>0.44 ± 0.06</td>
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<tr>
<td>Insulin, mU/l</td>
<td>3.5 ± 1.4</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>Whole blood</td>
<td></td>
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<tr>
<td>β-Hydroxybutyrate, mmol/l</td>
<td>0.13 ± 0.07</td>
<td>0.13 ± 0.05</td>
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<tr>
<td>Glucose, mmol/l</td>
<td>5.0 ± 0.1</td>
<td>5.1 ± 0.2</td>
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<tr>
<td>NE</td>
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<td></td>
<td>308 ± 27**</td>
<td>133 ± 22###</td>
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Values are means ± SE. NE, without exercise; EX, with exercise 3 h prior to intravenous glucose tolerance test (IVGTT); FA, fatty acids. Muscles and blood samples were taken after an overnight fast prior to IVGTT (basal) and after 75 h of starvation. Additional blood samples were taken immediately after exercise [(EX (0 h)]. *P < 0.05 and **P < 0.001 vs. prestarvation; #P < 0.05 and ##P < 0.001 vs. NE.

Fig. 2. Plasma glucose and insulin concentrations during IVGTT. Tests were performed before and after 75-h starvation with (EX) or without exercise (NE) (n = 9). A: plasma glucose. B: plasma insulin. For clarity only, mean values are shown. *P < 0.05, postexercise (post) vs. preexercise (pre); #P < 0.05, EX post vs. NE post.

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and after inhibition of complex I with rotenone. Mitochondrial respiration (state 3) with complex I substrate (octanoyl-carnitine) and complex II substrate (succinate), respiration in EX was increased significantly (P < 0.05 vs. prestarvation and vs. NE; Fig. 3A). The elevated respiration in EX remained when the electron flux was uncoupled from ATP production (FCCP). The elevated respiration in EX remained when the electron flux was uncoupled from ATP production (FCCP).

Mitochondrial respiration and ROS formation. ADP-stimulated mitochondrial respiration (state 3) with complex I substrate (malate + glutamate) decreased after starvation in both NE and EX (main effect of starvation; Fig. 3B). When malate and glutamate were used with FA-derived complex I substrate, ADP-stimulated respiration (state 3) with complex II substrate (octanoyl-carnitine) and complex II substrate (succinate), respiration in EX was increased significantly (P < 0.05 vs. prestarvation and vs. NE; Fig. 3A). The elevated respiration in EX remained when the electron flux was uncoupled from ATP production (FCCP) and after inhibition of complex I with rotenone. Mitochondrial H$_2$O$_2$ production was reduced after starvation under both conditions (main effect of starvation; Fig. 3C).

Protein expression and phosphorylation. Phosphorylated AS160 Thr$_{642}$ (p-AS160) and ACC Ser$_{79}$ (p-ACC) were not changed after starvation in NE but increased in EX by 138 (P = 0.01; Figs. 4A and 5) and 24% (P < 0.05; Figs. 4B and 5), respectively. Phosphorylated GS Ser$_{641/645}$ (p-GS) increased by 20% following starvation in NE (P < 0.05) and decreased by 26% in the EX condition (P < 0.01; Figs. 4C and 5). Expression of UCP3, GLUT1, and GLUT4 or phosphorylation of GSK3$\alpha$ Ser$_{21}$ and Akt Ser$_{473}$ were not affected under any condition (Table 3 and Fig. 5).

### DISCUSSION

The main findings of this study are that the impaired whole body insulin sensitivity and glucose tolerance after starvation...
were reversed or attenuated by exercise. Exercise was accompanied by reduced glycogen stores, increased lipid oxidation capacity, and alterations in signaling proteins involved in glucose and FA metabolism.

Consistent with previous studies, starvation resulted in hypoglycemia and reductions in plasma insulin, insulin sensitivity (13), and acute insulin response (17). To our knowledge, only one study has previously investigated the effect of exercise on starvation-induced insulin resistance (22). In contrast to the present findings, Green et al. (22) found no improvement in insulin sensitivity when 67-h starvation was combined with endurance exercise. The difference between studies may relate to a more pronounced depletion of muscle glycogen in the present study (due to higher exercise intensity and less-trained subjects) and/or the shorter time elapsed between exercise and IVGTT (3 vs. 15 h).

Lipid oxidation and insulin sensitivity. Surplus of lipids is associated with reduced insulin sensitivity, and the mechanism has been attributed to accumulation of toxic lipid intermediates, interfering with insulin signal transduction (44). Consistent with previous studies (8, 16), plasma levels of FA and ketone bodies (i.e., BOH) increased markedly after starvation and demonstrate a metabolic state with increased lipid load. Plasma BOH was reduced immediately postexercise (vs. NE), but both BOH and FA levels were similar to (BOH) or higher (FA) than NE at 3 h postexercise (i.e., prior to IVGTT). This would suggest that elevated levels of blood lipids were not the direct cause of starvation-induced insulin resistance. However, plasma lipids give limited information about intracellular lipid levels. Exercise will, due to the increased energy demand, have a major influence on intracellular lipid metabolism, which possibly persists and overrides the effect of high blood lipids.

Lipid oxidation is in part controlled by mitochondrial uptake of long-chained FA via carnitine palmitoyltransferase 1 (CPT 1). Malonyl-CoA inhibits CPT 1, and formation of malonyl-CoA is controlled by the activity of ACC. We observed an increased
phosphorylation, i.e., inactivation of ACC in EX, suggesting decreased formation of malonyl-CoA, which would stimulate mitochondrial uptake of FA (56). Since octanoyl-carnitine is a medium-chain FA and therefore not transported by CPT I (10), increased p-ACC cannot explain the observed increase in mitochondrial respiration with complex I + II substrate in vitro. However, both the increased phosphorylation of ACC and the increased mitochondrial respiration with complex I + II substrates suggest that there is an increased capacity for lipid oxidation after exercise.

The increased phosphorylation of ACC after exercise (Fig. 4B) is consistent with previous studies (31, 52) and is likely related to upstream activation (i.e., phosphorylation) of AMPK by energy deficiency and glycogen depletion (30). Despite marked reductions in muscle glycogen, p-AMPK was unchanged 3 h postexercise in this study (Table 3), but since exercise-induced phosphorylation of AMPK is reversed within 1–2 h of recovery (14, 57), we cannot exclude that the increased ACC phosphorylation is related to AMPK activation.

Mitochondrial dysfunction and insulin sensitivity. Starvation resulted in reduced mitochondrial ADP-stimulated respiration with complex I substrate, and in a recent study 60-h fasting resulted in decreased respiration with complex I + II substrates as well as during maximal uncoupled conditions (25). Mitochondrial respiration has also been shown to be reduced during other conditions with reduced insulin sensitivity, such as type 2 diabetes (33), obesity (2), aging (37), and preoperative starvation (4). Therefore, mitochondrial dysfunction has been implicated in the pathogenesis of insulin resistance (29). In type 2 diabetics, plasma FA was found to correlate negatively with ATP synthesis rate (49). Furthermore, acute lipid infusion reduces skeletal muscle ATP synthesis (9). Therefore, the starvation-induced reduction in mitochondrial respiration could be related to the elevation in plasma FA levels, which is a common feature of these conditions.

A novel interesting finding in this study is the robust increase in mitochondrial complex I + II substrate oxidation when starvation was followed by exercise. Since respiration with complex I substrates was unaffected by exercise, the results demonstrate a substrate-specific mitochondrial adaption. In a previous study, we observed an increased mitochondrial respiration with FA after ultraendurance exercise concomitant with pronounced elevation of plasma FA (15). Similarly, mitochondrial lipid oxidation was increased when exercise was performed after 2.5 wk of high-fat diet (46). The exercise-induced increase in the capacity of mitochondrial lipid oxidation appears to be a general phenomenon that occurs during exercise with increased lipid availability. The mechanism cannot simply be explained by increased substrate concentration since mitochondrial respiration was measured under standardized conditions. Further studies are required to elucidate the molecular mechanism for the increased capacity of mitochondria to oxidize lipids after exercise in a hyperlipidemic state.

**Glycogen depletion and insulin sensitivity.** Muscle glycogen decreased significantly during starvation (Table 1), which is in agreement with earlier studies (13, 26). Impaired glycogen storage is considered to have a major role in insulin resistance (6, 43). GS is the rate-limiting enzyme for glycogen synthesis, and the activity is controlled by phosphorylation (inactivation) and dephosphorylation (activation), with insulin and low glycogen levels being factors that promote activation (40). Consistent with previous findings (13), this study shows that starvation caused a significant increase in GS phosphorylation (i.e., inactivation). However, it was demonstrated previously that activation of GS during euglycemic hyperinsulinemia was not impaired after a 3-day fast (13), indicating that starvation-induced insulin resistance is not caused by reduced GS activation. Prolonged exercise resulted in a marked dephosphorylation (i.e., activation) of GS, and this has been shown previously by measurements of GS fractional activity as well (27). None of the changes in GS phosphorylation were associated with changes in GSK-3 phosphorylation, and it is likely that the effect of starvation and exercise occurred through altered activities of phosphatases (59) and/or other kinases possibly influenced by lipid overload (6). Thus it is possible that exercise-mediated activation of GS contributed to improved insulin action following starvation.

Transmembrane glucose transport is limited by the amount of GLUT4 protein or the translocation of the protein to surface membranes. Recently, Vendelbo et al. (54) showed that the total tissue level of GLUT4 protein was not affected by a 3-day fast. Our results support these findings and extend them by the observation that GLUT1 protein was also not affected. GLUT4 translocation to surface membrane is stimulated synergistically by insulin and exercise, and the signaling cascades converge at Rab-GTPase activating proteins, which include AS160 and TBC1D1 (11). Consistent with previous studies (47), starvation did not affect the basal levels of the insulin-signaling molecules p-Akt and p-AS160 (using p-AS160 Thr442). However, starvation significantly decreased insulin-stimulated phosphorylation of Akt and AS160 Thr442 (47), suggesting that starvation induces insulin resistance in human skeletal muscle by interfering with insulin signaling. In the present study, AS160 phosphorylation was increased markedly (+138%) 3 h after exercise, suggesting that the exercise-mediated enhancement of glucose tolerance and insulin sensitivity after starvation was

### Table 3. Expression and phosphorylation of proteins

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<td></td>
<td>NE</td>
<td>EX</td>
<td>NE</td>
<td>EX</td>
</tr>
<tr>
<td>p-AMPK Thr172</td>
<td>0.95 ± 0.07</td>
<td>0.84 ± 0.12</td>
<td>0.99 ± 0.12</td>
<td>0.96 ± 0.16</td>
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<tr>
<td>p-Akt Ser473</td>
<td>0.50 ± 0.11</td>
<td>0.81 ± 0.12</td>
<td>0.47 ± 0.07</td>
<td>0.71 ± 0.11</td>
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<tr>
<td>UCP3</td>
<td>1.24 ± 0.14</td>
<td>1.28 ± 0.22</td>
<td>1.38 ± 0.14</td>
<td>1.22 ± 0.15</td>
</tr>
<tr>
<td>p-GSK-3a Ser21</td>
<td>0.41 ± 0.02</td>
<td>0.38 ± 0.02</td>
<td>0.39 ± 0.03</td>
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<tr>
<td>p-GSK-3B Ser47</td>
<td>0.95 ± 0.03</td>
<td>0.92 ± 0.06</td>
<td>0.88 ± 0.05</td>
<td>0.99 ± 0.05</td>
</tr>
<tr>
<td>GLUT1 (total)</td>
<td>0.84 ± 0.05</td>
<td>0.88 ± 0.08</td>
<td>0.95 ± 0.11</td>
<td>0.94 ± 0.16</td>
</tr>
<tr>
<td>GLUT4 (total)</td>
<td>0.25 ± 0.05</td>
<td>0.28 ± 0.04</td>
<td>0.24 ± 0.03</td>
<td>0.29 ± 0.04</td>
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Values are reported as means ± SE. Subjects (n = 8) participated in 2 experimental sessions with 75 h of starvation. All proteins are expressed relative to the reference protein α-tubulin (arbitrary units).
related to enhanced glucose transport. It is likely that the increased AS160 phosphorylation was mediated by AMPK (although p-AMPK was unaltered 3-h postexercise), as judged by elevated levels of the AMPK target p-ACC.

**Methodological considerations.** Insulin sensitivity was assessed with IVGTT in the present study. IVGTT is a well-established technique to measure insulin sensitivity, which correlates well with that obtained during euglycemic hyperinsulinemic clamp (19, 51). However, there are certain limitations with IVGTT, such as unstable insulin levels and the inability to distinguish between insulin-dependent and insulin-independent glucose removal (34). The exercise-induced increase in insulin-independent glucose uptake is short-lived and lasts only a few hours postexercise (12, 20, 60). Therefore, by delaying the IVGTT until 3 h postexercise, the acute effects of exercise were minimized. In contrast to the hyperinsulinemic euglycemic clamp, IVGTT has the benefit to provide information about the release of insulin in response to hyperglycemia. The present study shows that the increase in plasma insulin was reduced after starvation and not affected by exercise.

**Perspectives and conclusions.** Insulin resistance is a physiological response to starvation in humans that likely contributes to channeling glucose to critical organs such as the brain. Food intake and insulin secretion following starvation would in the early state channel glucose to critical organs other than muscle despite hyperinsulinemia to restore deficits incurred by starvation. However, exercise would favor an increased glucose uptake by muscle and thereby limit glucose uptake by brain. In prehistoric man, such an event might have been an appropriate flight or fight response, and thus increased muscle glucose uptake during exercise would in the short term serve as a survival mechanism. Our results show that exercise reverses starvation-induced insulin resistance. The finding that exercise can reverse yet another mode of insulin resistance (starvation) further supports the general usefulness of exercise as a treatment for insulin-resistant conditions, including type 2 diabetes.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

P.F., A.K., E.A., and K.S. contributed to the conception and design of the research; P.F. performed the experiments; P.F. analyzed the data; P.F., A.K., E.A., and K.S. interpreted the results of the experiments; P.F. prepared the figures; P.F., A.K., E.A., and K.S. drafted the manuscript; P.F., A.K., E.A., and K.S. edited and revised the manuscript; P.F., A.K., E.A., and K.S. approved the final version of the manuscript.

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