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Muscle Damage Impairs Insulin Stimulation of IRS-1, PI3-Kinase and Akt-Kinase in Human Skeletal Muscle

Luis F. del Aguila*, Raj K. Krishnan*, Jan S. Ulbrecht‡, Peter A. Farrell*, Pamela H. Correll§, Charles H. Lang‖, Juleen R. Zierath¶ and John P. Kirwan*.

*Noll Physiological Research Center and Departments of ‡Biobehavioral Health and Clinical Medicine, §Veterinary Science, ‖Molecular and Cellular Physiology, The Pennsylvania State University, PA. 16802, USA. ¶Department of Clinical Physiology, Karolinska Hospital, Karolinska Institute, SE 171-76, Stockholm, Sweden.

Address for correspondence: John P. Kirwan, Ph.D.
Case Western Reserve University School of Medicine at MetroHealth Medical Center
Departments of Reproductive Biology & Nutrition
Bell Greve Bldg., Room G-231B
2500 MetroHealth Drive
Cleveland, Ohio 44109-1998
E-mail: jkirwan@metrohealth.org

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ABSTRACT

Physiological stress associated with muscle damage results in systemic insulin resistance. However, the mechanisms responsible for the insulin resistance are not known. Therefore, present study was conducted to elucidate the molecular mechanisms associated with insulin resistance following muscle damage. Muscle biopsies were obtained before (BASE) and at 1 h during a hyperinsulinemic-euglycemic clamp (INS) (40 mU•kg^{-1}•min^{-1}) in 8 young (age 24±1 yr), healthy sedentary (VO_{2max} 49.7±2.4 ml•kg^{-1}•min^{-1}) males before (CTRL) and 24 h after eccentric exercise-induced muscle damage (ECC). In order to determine the role of cytokines in ECC-induced insulin resistance, venous blood samples were obtained before and 24 h after ECC to evaluate *ex vivo* endotoxin-induced mononuclear cell secretion of TNF-α, IL-6 and IL-1β. Glucose disposal was 19% lower after ECC ($P<0.05$). Insulin-stimulated IRS-1 tyrosine phosphorylation was 45% lower after ECC ($P<0.05$). Insulin-stimulated PI3-kinase, Akt serine phosphorylation and Akt activity were reduced 34, 65 and 20%, respectively after ECC ($P<0.05$). TNF-α, but not IL-6 or IL-1β production, increased 2.4-fold 24 h after ECC ($P<0.05$). TNF-α production was positively correlated with reduced insulin-action on PI3-kinase ($r=0.77$, $P=0.04$). In summary, the physiological stress associated with muscle damage impairs insulin stimulation of IRS-1, PI3-kinase and Akt-kinase, presumably leading to decreased insulin-mediated glucose uptake. Although more research is needed in relation to the potential role for TNF-α inhibition of insulin action, elevated TNF-α production after muscle damage may impair insulin-signal transduction.

Key words: TNF-α, cytokines, signal-transduction, "stress diabetes", glucose uptake

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INTRODUCTION

Previous studies performed in our laboratory (27, 28, 31) and in others (2, 3), have shown that the physiological stress associated with muscle damage results in transient insulin resistance. This phenomenon of stress-induced insulin resistance, or "stress diabetes", has also been shown following musculoskeletal injury (19) and surgical trauma (4), though the underlying mechanisms could be different for each type of stress. Impaired insulin action following muscle damage has been linked to decreased GLUT-4 protein content (2, 3), but the molecular mechanisms by which the physiological stress associated with muscle damage induces insulin resistance have not been determined. In the present study we provide the first evidence for the molecular mechanisms associated with impaired insulin action following the stress of muscle damage in human subjects.

The pleiotrophic effects of insulin on metabolism and cellular growth are initiated by insulin binding to its receptor at the cell membrane (7, 17). Insulin signaling from the insulin receptor is transmitted through the insulin receptor substrate (IRS)-1 (7). IRS-1 tyrosine phosphorylation has been implicated in signal transduction from the insulin receptor to phosphatidylinositol (PI) 3-kinase (37), leading to GLUT-4 translocation (30) and subsequent glucose uptake. Furthermore, preliminary studies in humans demonstrate that insulin-stimulated PI3-kinase is correlated with whole-body glucose uptake, suggesting that PI3-kinase plays an important role in the regulation of insulin-mediated glucose uptake in human skeletal muscle. In addition, Akt-kinase, also known as PKB (Protein Kinase B), has been proposed as a key step in the insulin signaling pathway linking the activation of PI3-kinase to glucose uptake (13). Furthermore, human type 2 diabetes is accompanied by impaired insulin-signal transduction at the level of IRS-1-associated PI3-kinase (43) and Akt-kinase (32). Thus, the insulin-signaling pathway plays a critical role in the regulation of insulin action in health, and abnormalities in insulin-signal transduction likely underlie important

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disease processes. In the present study we explored which changes in the insulin-signaling pathway are associated with muscle damage-induced whole body insulin resistance.

Previous studies have shown that limited skeletal muscle damage results in systemic insulin resistance (2). Therefore, it is postulated that a systemic factor must be responsible for the significant decrease in insulin action in skeletal muscle in general, after muscle damage. Skeletal muscle damage initiates a series of immune reactions known as the acute phase immune response (25). The acute phase immune response following muscle damage has been associated with elevated production of mononuclear cell (MNC)-derived cytokines, including tumor necrosis factor (TNF)-α (6), interleukin (IL)-6 (38) and IL-1β (5). TNF-α has been shown to impair insulin-signal transduction in cultured muscle cells (12) and in cultured adipocytes (22). Moreover, *in vivo* administration of TNF-α in animals has been shown to impair glucose uptake by the whole-body and skeletal muscle (33). In support of these data on the TNF-α-insulin resistance link, neutralization of TNF-α in animal models of insulin resistance resulted in a marked increase in insulin action (21). Thus, there has been intense speculation that TNF-α may play a role in type 2 diabetes (23, 40). However, the effects of cytokines, especially TNF-α, on insulin action in human skeletal muscle have not been determined. In the present study we evaluated the potential role played by TNF-α in the inhibition of insulin action following limited human muscle damage.

In summary, the purpose of this investigation was to determine the effects of muscle damage on insulin-signal transduction at the level of IRS-1, PI3-kinase and Akt-kinase, critical steps in the regulation of insulin-action and insulin-mediated glucose uptake. In addition, we made a first attempt in evaluating the extent to which elevated cytokines, particularly TNF-α, are associated with transient insulin resistance following muscle damage in human subjects.

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METHODS

Subjects: Eight young healthy sedentary male subjects participated in the study. Physical characteristics of the subjects are shown in Table I. All subjects signed an informed consent in accordance with the Institutional Review Board for human research at The Pennsylvania State University. All subjects had a normal plasma glucose response to a 75-g oral glucose tolerance test (35) and were not using any medications. Body composition was determined by hydrostatic weighing (1) and percentage body fat was calculated using the Siri equation (39). Maximal oxygen consumption (VO_{2max}) was determined by an incremental treadmill test, and the concentrations of O_2 and CO_2 were measured on an electrochemical O_2 analyzer (Applied Electrochemistry, S-3A) and infrared CO_2 analyzer (Beckman LB-2), respectively.

Study design: All subjects performed 2 trials: 1) a non-exercise control trial (CTRL) and 2) a single bout of eccentric exercise (ECC) to induce muscle damage (5, 28). Concentric exercise (CONC) was not included in the protocol because our previous studies have shown no effect of acute CONC on insulin action (28, 31). ECC consists of a predominance of muscle fiber lengthening contractions and has been shown to induce marked myofibrillar damage (18) and an inflammatory response similar to the response present after other types of muscle injury (41). The CTRL trial was performed prior to the ECC trial. The ECC trial (day 1) consisted of a single bout of downhill treadmill running (-17% grade; 30 min; 80% of HR_{max}, determined from VO_{2max}). No exercise was performed on day 1 of the CTRL trial. Hyperinsulinemic (40 $mU \bullet kg^{-1} \bullet min^{-1}$) euglycemic (5.0 mM) clamps were performed 24 h (day 2) following the CTRL and ECC trials to determine whole-body insulin action (11).

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Ratings of perceived soreness: Measurements of muscle soreness were used as a manifestation of muscle damage. Ratings of perceived soreness were obtained while a constant pressure of 4.1 kg was applied on different muscle sites in the upper and lower body using a spring loaded pressure indicator with a 2-cm-diameter probe end as previously described (31). The scale for perceived soreness ranged from 0 ("absence of soreness") up to 9 ("unbearable soreness") arbitrary units.

Diet: The subjects consumed an eucaloric balanced diet (55% carbohydrate, 30% fat and 15% protein) provided by the General Clinical Research Center for two days prior to the clamps. The diet was similar for the CTRL and ECC trials. All subjects consumed an eucaloric balanced diet during the days between the CTRL and ECC trials.

Hyperinsulinemic-euglycemic clamp: A polyethylene catheter was inserted into an antecubital vein for infusion of insulin, glucose (20% dextrose) and [6,6-^{2}H] glucose. A second catheter was positioned in retrograde fashion in a dorsal hand vein, and the hand was warmed in a heated box at 60°C for sampling arterialized blood (34). A primed infusion of [6,6-^{2}H] glucose (Tracer Technology, Somerville, MA), followed by a continuous infusion throughout the clamp was used to measure hepatic glucose output (28). Blood samples for glucose kinetics were collected after tracer equilibration and during the last 30 min of insulin. Glucose disposal rates (GDR) were calculated as described previously (28). After tracer equilibration, a primed continuous infusion (40 mU•m^{-2}•min^{-1}) of human insulin (Humulin, Eli Lilly & Co., Indianapolis, IN) was initiated and maintained for 2 h. Plasma glucose levels were clamped at 5 mM (euglycemia) by a variable rate of 20% dextrose infusion. Samples for plasma glucose were drawn every 5 min during the clamp, and plasma glucose concentrations were determined with a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA).

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Samples for plasma insulin were drawn every 15 min during the clamp, and assayed in duplicate by double antibody radioimmunoassay (Linco Research, St. Charles, MO, USA). Indirect calorimetry was performed before the clamp and during the last 2 h of hyperinsulinemia to measure carbohydrate oxidation rates and non-oxidative carbohydrate metabolism (16).

Muscle biopsy: Muscle biopsies were obtained from the vastus lateralis muscle using the needle biopsy procedure (14). Biopsies were performed before the clamp and at 1 h of INS, since PI3-kinase activation in human muscle has been shown to peak at 60 min of INS (20). Muscle tissue was immediately homogenized for subsequent determination of insulin action on IRS-1, PI3-kinase and Akt-kinase. Protein was determined using a commercially available kit from Bio-Rad Laboratories (Hercules, CA, USA).

IRS-1 tyrosine phosphorylation and Akt-kinase serine phosphorylation: Western Blot analysis was used to determine IRS-1 tyrosine phosphorylation as previously described (12). Additional aliquots (50 μg) of the original supernatant were saved for the determination of Akt-kinase serine phosphorylation using a polyclonal Akt-α antibody (New England Biolabs, Beverly, MA, USA). Immunodetection was performed by enhanced chemiluminescence (ECL; Amersham, Arlington, IL, USA) following the manufacturer's instructions. The immunoblots were quantified by densitometry.

IRS-1 associated PI3-kinase activity: A total of 1 mg of protein was immunoprecipitated with 4 μg of IRS-1 polyclonal antibody to determine IRS-1 associated PI3-kinase activity as

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previously described (12). Quantification of enzymatic activity was determined by phosphoImaging.

Akt-kinase activity: A total of 500 μg of protein was immunoprecipitated with anti-Akt-α antibody (generous gift from Richard A. Roth, Stanford University, Stanford CA), and Akt-kinase activity was determined as previously described (32). Quantification of the kinase activity was performed using a phosphoImager.

Mononuclear cell (MNC) isolation and cytokine determination: Forty milliliters of venous blood was drawn before (CTRL) and 24 h following ECC for MNC isolation by density gradient centrifugation (400 g for 45 min) on histopaque-1077. Whole blood was diluted 1:2 with pyrogen free saline and underlayered with histopaque-1077. After centrifugation, the MNC layer was removed and washed twice with saline. The pellet was obtained from the last wash and resuspended in RPMI 1640 culture medium (2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin) to yield a final concentration of $5 \cdot 10^6$ cells/ml. The MNC were then cultured for 24 h (5% CO_2 at 37°C) with 1 ng/ml lipopolysaccharide (LPS) endotoxin (Serotype 005:B5, Sigma, St. Louis, MO). Cell supernatants were obtained to determine TNF-α, IL-1β and IL-6 concentrations by enzyme linked immunosorbent assay (ELISA).

Statistical Analysis: The MIXED procedure for the Statistical Analysis System (SAS Institute Inc., Cary, NC) was used for ANOVA by rank transformation (nonparametric) approach to identify statistical differences in the data. Glucose disposal rates, IRS-1, PI3-kinase, Akt-kinase and cytokine concentrations were the variables of interest. Spearman

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product-moment correlations were used to evaluate associations between insulin-signal transduction and cytokine production following muscle damage. All values are expressed as mean ± standard error (SE). An *a priori* alpha level of 0.05 was used to determine statistical significance.

RESULTS

Exercise and ratings of perceived soreness: The subjects performed the eccentric exercise bout at 82±1% of HR_{max}. The exercise bout resulted in a marked increase in perceived muscle soreness (P=0.001) for the upper and lower body at 24 h post-exercise compared to pre-exercise. The greatest soreness ratings were obtained in the quadriceps, biceps and trapezius muscle groups (6.9±0.5, 5.3±0.7 and 5.0±0.4 arbitrary units, respectively).

Hyperinsulinemic-euglycemic clamp: Fasting plasma glucose and fasting plasma insulin were not different between trials (Table II). Mean glucose concentrations during the last 30-min (90-120) of the clamp were not different between CTRL and ECC trials (Table II). GDR were lower ($p<0.05$) in ECC compared with CTRL (Table III). Furthermore, GDR decreased in all the subjects in ECC compared to CTRL. Hepatic glucose output was completely suppressed by insulin in both trials. Rates of carbohydrate oxidation (C_{OX}) at 2 h of hyperinsulinemia were not significantly different between trials (Table III). However, non-oxidative carbohydrate rates (C_{NONOX}) were significantly decreased ($p<0.05$) with muscle damage.

Insulin signaling assays: IRS-1 tyrosine phosphorylation, Akt-kinase serine phosphorylation, PI3-kinase activity and Akt-kinase activity were expressed as a fold increase at 1 h of insulin infusion with respect to baseline activity. The time point of 1 h for

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enzymatic analysis was chosen because insulin-signal transduction in human muscle has been shown to peak at 60 min of insulin stimulation (20). Insulin-induced IRS-1 tyrosine phosphorylation was significantly elevated above BASE ($P<0.01$) in both CTRL and ECC trials (Fig. 1). However, insulin-stimulated IRS-1 tyrosine phosphorylation was lower ($P<0.001$) in ECC compared to CTRL trials (5.8±1.0 vs 3.2±1.3 fold increase above BASE, CTRL vs ECC) (Fig. 1). IRS-1 total protein expression in baseline and insulin-stimulated biopsies were similar between trials. Insulin-stimulated IRS-1 associated PI3-kinase activity was significantly increased above BASE in the CTRL trial ($P=0.01$), but not in the ECC trial (Fig. 2). The magnitude of PI3-kinase activation with insulin was lower ($P<0.01$) in the ECC trial compared to CTRL (4.4±1.4 vs 2.9±1.3 fold increase above BASE, CTRL vs ECC) (Fig. 2). However, the average PI3-kinase activity at BASE of the 8 subjects was not altered by ECC (22±3 vs. 20±4 arbitrary phosphoimager units, CTRL vs. ECC) (figure not shown). Insulin-induced serine phosphorylation of Akt-kinase was significantly elevated above BASE ($P<0.05$) in both CTRL and ECC trials (Fig. 3). Insulin-stimulated serine phosphorylation of Akt-kinase was lower ($P<0.05$) in ECC compared to CTRL (25.0±5.9 vs 8.7±1.3 fold increase above BASE, CTRL vs ECC) (Fig. 3, panel B). There were no significant changes in Akt protein expression (13.1±0.9 vs 17.1±3.1 arbitrary densitometry values, CTRL vs ECC) (Fig. 3, panel A). Insulin stimulated Akt-kinase activity was significantly elevated above BASE ($P<0.05$) in both CTRL and ECC trials (Fig. 4). However, insulin-stimulated Akt-kinase activity was significantly lower ($P<0.05$) in ECC compared to CTRL (1.5±0.1 vs 1.2±0.1 fold increase above BASE, CTRL vs ECC) (Fig. 4)

Cytokine production: Endotoxin-induced MNC secretion of TNF-α was significantly increased ($P<0.05$) 24 h following muscle damage (1.1±0.3 vs 2.6±0.9 $ng \cdot ml^{-1}$) (Fig. 5). In

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contrast, IL-6 and IL-1β secretion were not significantly different to the respective baseline values at 24 h after ECC (0.9±0.3 vs 3.8±1.3 ng•ml^{-1}; P=0.12 and 2.7±0.9 vs 2.1±0.7 ng•ml^{-1}; P=0.90, respectively). Furthermore, increased MNC secretion of TNF-α, but not IL-6 or IL-1β, was positively correlated with impaired insulin-stimulated IRS-1 associated PI3-kinase activity (r=0.77, P=0.04; r=0.21, P=0.64; r=-0.12, P=0.78; TNF-α, IL-6 and IL-1β, respectively) (Fig. 6). TNF-α production after ECC was not significantly correlated with GDR, insulin-stimulated Akt-kinase serine phosphorylation, or insulin-induced Akt-kinase activity.

DISCUSSION

The purpose of the present study was to investigate the molecular mechanisms leading to transient insulin resistance affecting skeletal muscle in general following limited muscle damage in human subjects. Specifically, we examined the effects of muscle damage on insulin-signal transduction at the level of IRS-1, PI3-kinase and Akt-kinase. These proteins are key regulatory steps in the insulin-signaling pathway leading to glucose uptake. We provide the first evidence that decreased insulin action following muscle damage is associated with impaired insulin-signal transduction at the level of IRS-1, PI3-kinase and Akt-kinase in human skeletal muscle, presumably leading to impair insulin-mediated glucose uptake.

Downhill treadmill running consists of a predominance of dynamic forced-lengthening eccentric contractions that result in muscle damage (6, 18, 28). Downhill treadmill running (28) and other ECC modes such as resistance exercise (31) and exhaustive exercise protocols (27) result in transient insulin resistance. Our findings reinforce the effectiveness of the ECC-muscle damage stress model to induce transient insulin resistance in humans. Since hepatic

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glucose output was completely suppressed by insulin in both trials, it appears that insulin resistance after muscle damage occurs in peripheral tissues rather than at the liver. Furthermore, the data from indirect calorimetry suggest that defects in non-oxidative carbohydrate metabolism, rather than in oxidative pathways, lead to decreased glucose disposal after muscle damage. Therefore, defects in non-oxidative glucose metabolism resulting in an impaired "pulling effect" of glycogen on glucose uptake could play an important role in the development of insulin resistance following muscle damage. These changes at the level of the body as a whole are similar to the impairment in non-oxidative glucose disposal resulting from infectious-like states (15).

The insulin-signaling pathway is comprised of a complex array of protein interactions that regulate insulin-mediated glucose uptake. IRS-1 and PI3-kinase are critical intermediate steps in transmitting the signal from the insulin receptor leading to GLUT-4 translocation and a subsequent increase in glucose uptake (8). In the present study, we show that following muscle damage, insulin-signal transduction is impaired at the level of IRS-1 and PI3-kinase. As expected, tyrosine phosphorylation of IRS-1 and IRS-1-associated PI3-kinase activity was significantly elevated after insulin-stimulation in the control trial. However, although insulin-stimulated IRS-1 tyrosine phosphorylation was increased after ECC-induced muscle damage, the magnitude of the IRS-1 increase was severely blunted compared to the control insulin-stimulated condition. Furthermore, IRS-1-associated PI3-kinase activity was not significantly increased after ECC in response to the insulin-stimulation. Indeed, insulin-stimulated PI3-kinase was reduced following muscle damage when compared to control. In addition, IRS-1 protein content was not altered by muscle damage. Thus, impaired insulin-signal transduction after muscle damage is not due to changes in IRS-1 protein expression, but rather occur as a consequence of functional defects in insulin signal activation.

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Akt-kinase has been identified as a downstream target of PI3-kinase (10) and has been shown to play an important role in insulin-signal transduction to glucose uptake in *in vivo* animal models (42) and *in vitro* human skeletal muscle (32). However, recently the requirement for Akt-kinase in the activation of glucose transport has been challenged (24, 29). Here, we provide the first evidence that Akt-kinase is stimulated in human skeletal muscle *in vivo* in the presence of physiological levels of plasma insulin. Furthermore, we show that insulin-stimulated Akt serine phosphorylation and Akt-kinase activity are reduced in human skeletal muscle following the stress of muscle damage. These functional changes at the level of Akt-kinase cannot be attributed to decreases in Akt-kinase protein expression. In fact, we observed a trend toward increased Akt-kinase protein expression following muscle damage. This phenomenon could be a compensatory response to the decrease in insulin action following muscle damage. Interestingly, a similar trend for increased Akt-kinase protein expression has been reported for skeletal muscle from people with type 2 diabetes (32). Thus, the profound impairments in insulin-signal transduction at the level of IRS-1, PI3-kinase and Akt-kinase are likely to be the cellular changes underlying the mechanisms of insulin resistance following the physiological stress of skeletal muscle damage in human subjects.

An additional purpose of the present investigation was to determine potential mechanisms of insulin resistance following muscle damage. Electron microscopy studies performed in injured human skeletal muscle after one-legged eccentric exercise contractions revealed that only a small percentage of muscle fibers were severely damaged (36). Furthermore, studies have also shown that within the damaged muscle fiber, only a small portion of the fiber is actually damaged (18). Thus, based on the fact that the reductions in glucose uptake in muscle damage models is of the order of 20-30% (2, 28), it is postulated that a systemic factor must be responsible for the significant decrease in insulin action in

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skeletal muscle in general, after limited muscle damage. The cytokines TNF-α, IL-6 and IL-1β have been shown to be involved in the acute phase immune response following muscle damage (5, 6, 38). Therefore, we measured MNC secretion of TNF-α, IL-6 and IL-1β after ECC to investigate the extent to which these cytokines may link focal muscle damage to the more widespread muscle resistance noted in this model. We observed that *ex vivo* MNC secretion of TNF-α was significantly elevated 24 h after muscle damaging exercise. Furthermore, TNF-α production was positively correlated with the impairment in insulin stimulated IRS-1-associated PI3-kinase activity following muscle damage. We (12) and others (22, 26) have previously provided evidence for a link between TNF-α and impaired insulin-signal activation in cultured cells. Although it is not possible from these *in vitro* studies (12, 22, 26) to provide evidence of a direct effect of TNF-α on insulin-signal transduction *in vivo*, the present data are consistent with the hypothesis that TNF-α may impair insulin-signal transduction at the level of PI3-kinase in the *in vivo* ECC-induced muscle damage stress model in human subjects. The absence of a correlation between TNF-α and GDR, or between TNF-α and Akt-kinase activity may be due to a quicker activation of PI3-kinase, a step which precedes glucose uptake (12, 20). Although an increase in mean concentrations of IL-6 was observed after ECC compared to CTRL, this observation was not statistically significant (data not shown). The absence of statistical significance for the increase in IL-6 after ECC could be due to the low statistical power of our study (9). However, it is very unlikely that the increase in IL-6 after muscle damage plays a role in the development of insulin resistance after ECC because 1) the individual changes in IL-6 were not significantly correlated with impaired insulin-signal transduction after ECC, and 2) Preliminary studies from our laboratories have shown that, in contrast to TNF-α (12, 22, 26), IL-6 has no effect on insulin action in cultured muscle cells. Thus, TNF-α, rather than IL-6

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and IL-1β may be involved in down-regulation of insulin-signal transduction following ECC-induced muscle damage. Although the present TNF-α data *in vivo* support previous investigations on the effect of this cytokine on insulin action *in vitro* (12, 22, 26), more research is needed to confirm the potential role the TNF-α may play in the regulation of insulin action in humans.

In summary, we provide the first evidence for a molecular mechanism that may account for the transient insulin resistance following the stress of muscle damage in human subjects. We found specific defects in insulin-signal transduction at the level of IRS-1, PI3-kinase and Akt-kinase following muscle damage. In addition, the present study is the first to show that physiological hyperinsulinemia is sufficient to activate Akt-kinase *in vivo* in human subjects. Furthermore, marked increases in *ex vivo* TNF-α production following muscle damage were associated with impaired insulin-stimulated IRS-1-associated PI3-kinase activity. These results suggest that elevations in TNF-α during the acute phase immune response might be associated with decreased insulin-signal transduction and impaired insulin action following the physiological stress of muscle damage in human subjects. However, more studies are needed to confirm the involvement of TNF-α in the down-regulation of insulin action following muscle damage.

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LEGENDS FOR FIGURES

Figure 1. **Insulin receptor substrate (IRS)-1 tyrosine phosphorylation after 1 h of hyperinsulinemia during hyperinsulinemic-euglycemic clamps performed under CTRL and 24 h following ECC.** Panel A is a representative immunoblot of IRS-1 protein expression from one subject. Panel B is a representative immunoblot of IRS-1 tyrosine phosphorylation from one subject, and the graph represents the mean±SE of the densitometry values (expressed as fold increase above BASE) from the 8 subjects. *Significantly different from BASE ($P<0.01$). ‡, Significantly different response to ECC compared with CTRL, $P<0.001$.

Figure 2. **Phosphatidylinositol (PI)3-kinase activity after 1 h of INS during hyperinsulinemic-euglycemic clamps performed under CTRL and 24 h following ECC.** Representative phosphoImage of IRS-1-associated PI3-kinase activity from one subject, and the graph represents the mean±SE of the phosphoImaging values (expressed as fold increase above BASE) from the 8 subjects. *Significantly different from BASE ($P=0.01$). ‡, Significantly different response to ECC compared with CTRL, $P<0.01$.

Figure 3. **Akt-kinase serine phosphorylation after 1 h of hyperinsulinemia during hyperinsulinemic-euglycemic clamps performed under CTRL and 24 h following ECC.** Panel A is a representative immunoblot of Akt-kinase protein expression from one subject. Panel B is a representative immunoblot of Akt-kinase serine phosphorylation from one subject, and the graph represents the mean±SE of the densitometry values (expressed as fold increase above BASE) from the 8 subjects. *Significantly different from BASE ($P<0.05$). ‡, Significantly different response to ECC compared with CTRL, $P<0.05$.

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Figure 4. **Akt-kinase activity after 1 h of INS during hyperinsulinemic-euglycemic clamps performed under CTRL and 24 h following ECC.** The graph represents the mean ±SE of the phosphoImaging values (expressed as fold increase above BASE) from the 8 subjects. *Significantly different from BASE ($P<0.05$). ‡, significantly different response to ECC compared with CTRL, $P<0.05$.

Figure 5. **Endotoxin-induced secretion of TNF-α by cultured mononuclear cells (MNC).** Blood samples were obtained before (CTRL) and 24 h following ECC. MNC were isolated as described in Methods. MNC were cultured overnight with 1 ng•ml^{-1} lipopolysaccharide, and TNF-α concentrations were measured from cultured supernatants by ELISA. *Significantly increased from CTRL, $P<0.05$.

Figure 6. **Relationship between increased TNF-α levels and impaired insulin-stimulated IRS-1-associated PI3-kinase activity following muscle damage.** TNF-α was expressed as fold increase 24 h following ECC (post-ECC) with respect to pre-exercise (pre-ECC). IRS-1-associated PI3-kinase activity is expressed as the difference between the fold increase observed in the CTRL trial and the fold increase in the ECC trial. The correlation analysis was obtained from 7 subjects as technical difficulties were encountered during the clamp procedure for one of the subjects.

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Table 1. Subjects characteristics

Age, yr	24 ±1
Height, cm	178.3±2.1
Body Weight, kg	76.8±2.4
Body Mass Index, $kg \cdot m^{-2}$	24.2±0.5
Body Fat, %	17.1±1.6
VO_{2max}, $ml \cdot kg^{-1} \cdot min^{-1}$	49.7±2.4

Values are means±SE of 8 subjects

VO_{2max}, maximal oxygen consumption

Table 2. Plasma glucose and insulin concentration at BASE and at 2h INS

	CTRL		ECC	
	BASE	INS	BASE	INS
Glucose(mM)	5.2±0.1	4.9±0.1	5.2±0.1	4.8±0.1
Insulin (pM)	56±6	288±12	60±8	281±22

Values represent means±SE

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Table 3. Clamp results and indirect calorimetry measurements

	CTRL	ECC
GDR	4.8±0.9	3.9±0.7*
C_{OX}	1.8±0.7	2.1±0.9
C_{NONOX}	3.0±1.1	1.8±0.7*

Glucose disposal rates (GDR), oxidative (C_{OX}) and non-oxidative (C_{NONOX}) carbohydrate oxidation rates ($mg \cdot kg^{-1} \cdot min^{-1}$) at 2h of hyperinsulinemia (INS) for CTRL and ECC trials. Values represent means±SE. * Significantly different from CTRL ($P<0.05$)

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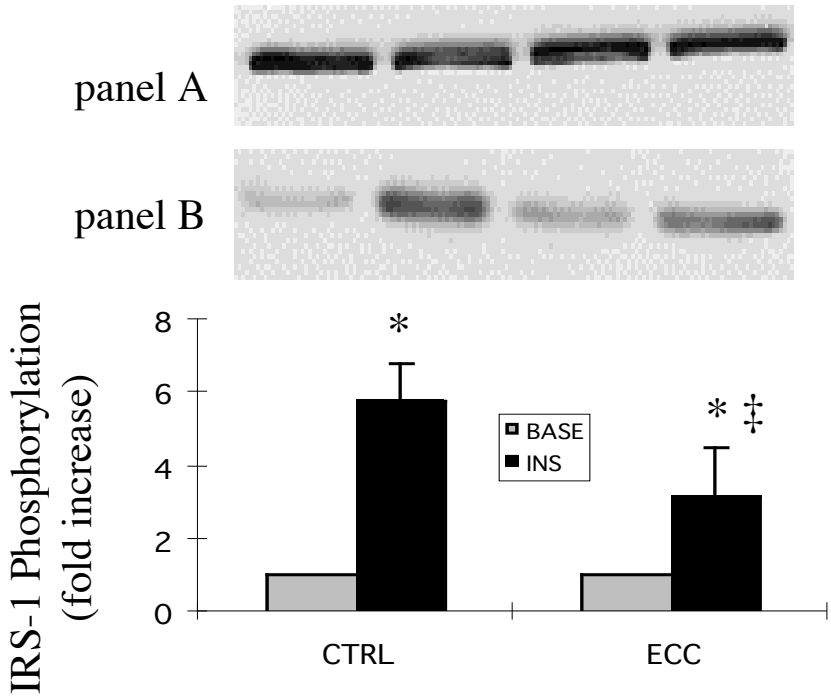

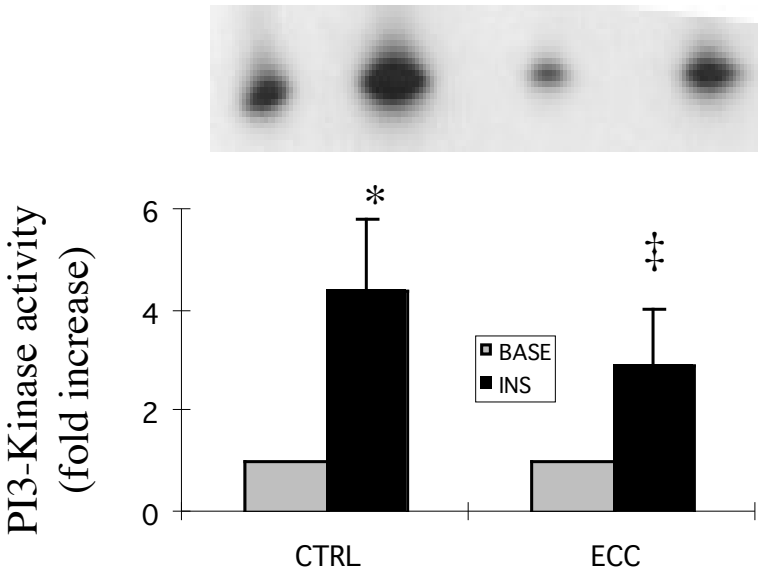

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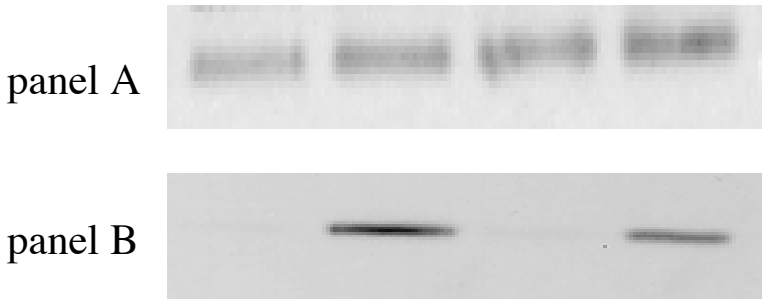

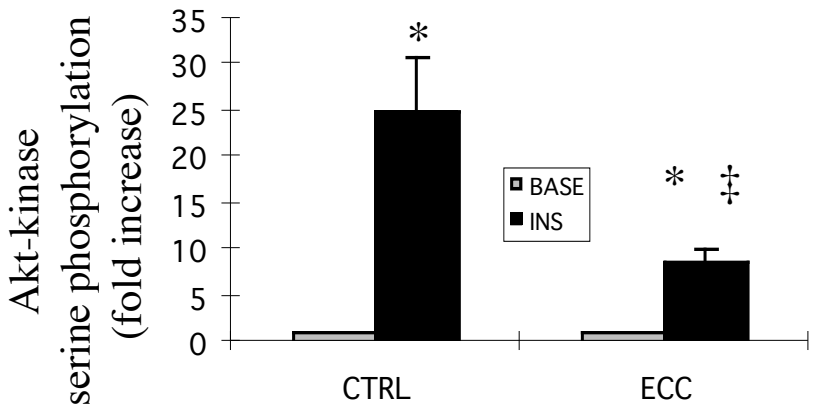

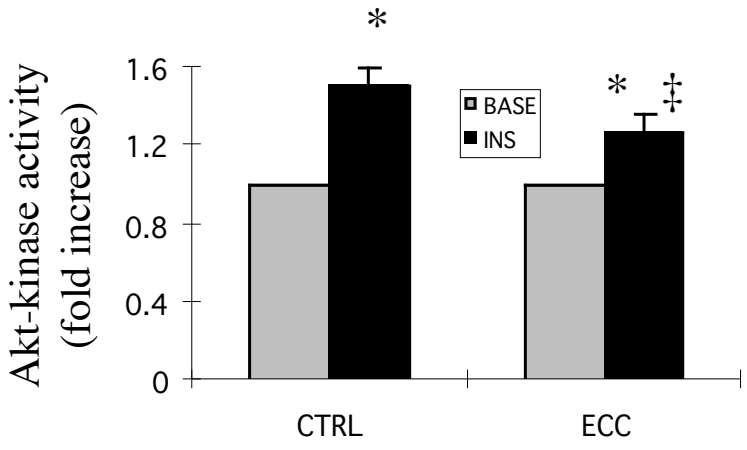

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