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# Muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase, and Akt-kinase in human skeletal muscle

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**Del Aguila, Luis F., Raj K. Krishnan, Jan S. Ulbrecht, Peter A. Farrell, Pamela H. Correll, Charles H. Lang, Juleen R. Zierath, and John P. Kirwan.** Muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase, and Akt-kinase in human skeletal muscle. *Am J Physiol Endocrinol Metab* 279: E206–E212, 2000.—Physiological stress associated with muscle damage results in systemic insulin resistance. However, the mechanisms responsible for the insulin resistance are not known; therefore, the present study was conducted to elucidate the molecular mechanisms associated with insulin resistance after muscle damage. Muscle biopsies were obtained before (base) and at 1 h during a hyperinsulinemic-euglycemic clamp ( $40 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) in eight young (age  $24 \pm 1$  yr) healthy sedentary (maximal  $\text{O}_2$  consumption,  $49.7 \pm 2.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) males before and 24 h after eccentric exercise (ECC)-induced muscle damage. To determine the role of cytokines in ECC-induced insulin resistance, venous blood samples were obtained before (control) and 24 h after ECC to evaluate ex vivo endotoxin-induced mononuclear cell secretion of tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6, and IL-1 $\beta$ . Glucose disposal was 19% lower after ECC ( $P < 0.05$ ). Insulin-stimulated insulin receptor substrate (IRS)-1 tyrosine phosphorylation was 45% lower after ECC ( $P < 0.05$ ). Insulin-stimulated phosphatidylinositol (PI) 3-kinase, Akt (protein kinase B) serine phosphorylation, and Akt activity were reduced 34, 65, and 20%, respectively, after ECC ( $P < 0.05$ ). TNF- $\alpha$ , but not IL-6 or IL-1 $\beta$  production, increased 2.4-fold 24 h after ECC ( $P < 0.05$ ). TNF- $\alpha$  production was positively correlated with reduced insulin action on PI 3-kinase ( $r = 0.77$ ,  $P = 0.04$ ). In summary, the physiological stress associated with muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase, and Akt-kinase, presumably leading to decreased insulin-mediated glucose uptake. Although more research is needed on the potential role for TNF- $\alpha$  inhibition of insulin action, elevated TNF- $\alpha$  production after muscle damage may impair insulin signal transduction.

tumor necrosis factor- $\alpha$ ; cytokines; signal transduction; stress diabetes; glucose uptake

IN PREVIOUS STUDIES we (27, 28, 31) and 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 after musculoskeletal injury (19) and surgical trauma (4), although the underlying mechanisms could be different for each type of stress. Impaired insulin action after 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 after the stress of muscle damage in human subjects.

The pleiotropic 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 PI 3-kinase is correlated with whole body glucose uptake, suggesting that PI 3-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 PI 3-kinase to glucose uptake (13). Furthermore, human type 2 diabetes is accompanied by impaired insulin signal transduction at the level of IRS-1-associated PI 3-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 disease processes. In the present study, we explored which

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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/m <sup>2</sup>                             | 24.2 ± 0.5  |
| Body fat, %  | 17.1 ± 1.6  |
| $\dot{V}O_{2\max}$ , ml · kg <sup>-1</sup> · min <sup>-1</sup> | 49.7 ± 2.4  |

Values are means ± SE of 8 subjects.  $\dot{V}O_{2\max}$ , maximal oxygen consumption.

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, 28). 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 after muscle damage has been associated with elevated production of mononuclear cell (MNC)-derived cytokines, including tumor necrosis factor (TNF)- $\alpha$  (6), interleukin (IL)-6 (38), and IL-1 $\beta$  (5). TNF- $\alpha$  has been shown to impair insulin signal transduction in cultured muscle cells (12) and in cultured adipocytes (22). Moreover, in vivo administration of TNF- $\alpha$  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- $\alpha$ -insulin resistance link, neutralization of TNF- $\alpha$  in animal models of insulin resistance resulted in a marked increase in insulin action (21). Thus there has been intense speculation that TNF- $\alpha$  may play a role in type 2 diabetes (23, 40). However, the effects of cytokines, especially TNF- $\alpha$ , on insulin action in human skeletal muscle have not been determined. In the present study, we evaluated the potential role of TNF- $\alpha$  in the inhibition of insulin action after limited human muscle damage.

In brief, the purpose of this investigation was to determine the effects of muscle damage on insulin signal transduction at the level of IRS-1, PI 3-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- $\alpha$ , are associated with transient insulin resistance after muscle damage in human subjects.

## METHODS

**Subjects.** Eight young healthy sedentary male subjects participated in the study. Physical characteristics of the subjects are shown in Table 1. 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 of body fat was calculated with the Siri equation (39). Maximal oxygen consumption

( $\dot{V}O_{2\max}$ ) was determined by an incremental treadmill test, and the concentrations of O<sub>2</sub> and CO<sub>2</sub> were measured on an electrochemical O<sub>2</sub> analyzer (Applied Electrochemistry, S-3A) and an infrared CO<sub>2</sub> analyzer (Beckman LB-2), respectively.

**Study design.** All subjects performed two 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 was not included in the protocol, because our previous studies have shown no effect of acute concentric exercise 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 before the ECC trial. The ECC trial (*day 1*) consisted of a single bout of downhill treadmill running [−17% grade; 30 min; 80% of maximal heart rate (HR<sub>max</sub>), determined from  $\dot{V}O_{2\max}$ ]. No exercise was performed on *day 1* of the CTRL trial. Hyperinsulinemic (40 mU · kg<sup>-1</sup> · min<sup>-1</sup>) euglycemic (5.0 mM) clamps were performed 24 h (*day 2*) after the CTRL and ECC trials to determine whole body insulin action (11).

**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 with 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”) to 9 (“unbearable soreness”) arbitrary units.

**Diet.** The subjects consumed a eucaloric balanced diet (55% carbohydrate, 30% fat, and 15% protein) provided by the General Clinical Research Center for two days before the clamps. The diet was similar for the CTRL and ECC trials. All subjects consumed a 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-<sup>2</sup>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-<sup>2</sup>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<sup>-2</sup> · min<sup>-1</sup>) of human insulin (Humulin, Eli Lilly, 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). Samples for plasma insulin were drawn every 15 min during the clamp and were assayed in duplicate by double antibody radioimmunoassay (Linco Research, St. Charles, MO). Indirect calorimetry was performed before the clamp and during the last 2 h of hyperinsulinemia to measure carbohydrate oxidation rates and nonoxidative carbohydrate metabolism (16).

**Muscle biopsy.** Muscle biopsies were obtained from the vastus lateralis muscle by use of the needle biopsy procedure (14). Biopsies were performed before the clamp and at 1 h of INS, because PI 3-kinase activation in human muscle has

Table 2. Plasma glucose and insulin concentrations at BASE and at 2 h 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. CTRL, control, i.e., no exercise; ECC, eccentric exercise-induced muscle damage; BASE, before hyperinsulinemic-euglycemic clamp (INS).

been shown to peak at 60 min of INS (20). Muscle tissue was immediately homogenized for subsequent determination of insulin action on IRS-1, PI 3-kinase, and Akt-kinase. Protein was determined with a commercially available kit from Bio-Rad Laboratories (Hercules, CA).

**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 by means of a polyclonal Akt-α antibody (New England Biolabs, Beverly, MA). Immunodetection was performed by enhanced chemiluminescence (Amersham, Arlington, IL), following the manufacturer's instructions. The immunoblots were quantified by densitometry.

**IRS-1-associated PI 3-kinase activity.** A total of 1 mg of protein was immunoprecipitated with 4 µg of IRS-1 polyclonal antibody to determine IRS-1-associated PI 3-kinase activity, as 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 with a phosphoImager.

**MNC isolation and cytokine determination.** Forty milliliters of venous blood were drawn before (CTRL) and 24 h after 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  $5 \times 10^6$  cells/ml. The MNC were then cultured for 24 h (5% CO<sub>2</sub> at 37°C) with 1 ng/ml lipopolysaccharide endotoxin (Serotype 005:B5, Sigma, St. Louis, MO). Cell supernatants were obtained to determine TNF-α, IL-1β, and IL-6 concentrations by ELISA.

**Statistical analysis.** The MIXED procedure for the Statistical Analysis System (SAS Institute, Cary, NC) was used for ANOVA by rank transformation (nonparametric) approach to identify statistical differences in the data. Glucose disposal rates, IRS-1, PI 3-kinase, Akt-kinase, and cytokine concentrations were the variables of interest. Spearman product-moment correlations were used to evaluate associations between insulin signal transduction and cytokine production after muscle damage. All values are expressed as means ± SE. An a priori α-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  $82 \pm 1\%$  of HR<sub>max</sub>. The exercise bout resulted in a marked increase in perceived muscle soreness ( $P = 0.001$ ) for the upper and lower body at 24 h postexercise compared with preexercise. The greatest soreness ratings were obtained in the quadriceps, biceps, and trapezius muscle groups ( $6.9 \pm 0.5$ ,  $5.3 \pm 0.7$  and  $5.0 \pm 0.4$  arbitrary units, respectively).

**Hyperinsulinemic-euglycemic clamp.** Fasting plasma glucose and fasting plasma insulin were not different between trials (Table 2). Mean glucose concentrations during the last 30 min (*minutes 90-120*) of the clamp were not different between CTRL and ECC trials (Table 2). GDR were lower ( $P < 0.05$ ) in ECC compared with CTRL (Table 3). Furthermore, GDR decreased in all the subjects in ECC compared with CTRL. Hepatic glucose output was completely suppressed by insulin in both trials. Rates of carbohydrate oxidation at 2 h of hyperinsulinemia were not significantly different between trials (Table 3). However, nonoxidative carbohydrate rates were significantly decreased ( $P < 0.05$ ) with muscle damage.

**Insulin signaling assays.** IRS-1 tyrosine phosphorylation, Akt-kinase serine phosphorylation, PI 3-kinase activity, and Akt-kinase activity were expressed as a multiple increase at 1 h of insulin infusion with respect to baseline activity. The time point of 1 h for 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 with CTRL trials ( $5.8 \pm 1.0$ - vs.  $3.2 \pm 1.3$ -fold increase above BASE, CTRL vs. ECC; Fig. 1). IRS-1 total protein expression in baseline and insulin-stimulated biopsies was similar between trials. Insulin-stimulated IRS-1-associated PI 3-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 PI 3-kinase activation with insulin was lower ( $P < 0.01$ ) in the ECC trial compared with CTRL ( $4.4 \pm 1.4$ - vs.  $2.9 \pm 1.3$ -fold increase above BASE, CTRL vs. ECC; Fig. 2). However, the average PI 3-kinase activity at BASE for all eight subjects was not

Table 3. Clamp results and indirect calorimetry measurements

|                    | CTRL      | ECC        |
|--------------------|-----------|------------|
| GDR                | 4.8 ± 0.9 | 3.9 ± 0.7* |
| C <sub>OX</sub>    | 1.8 ± 0.7 | 2.1 ± 0.9* |
| C <sub>NONOX</sub> | 3.0 ± 1.1 | 1.8 ± 0.7* |

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

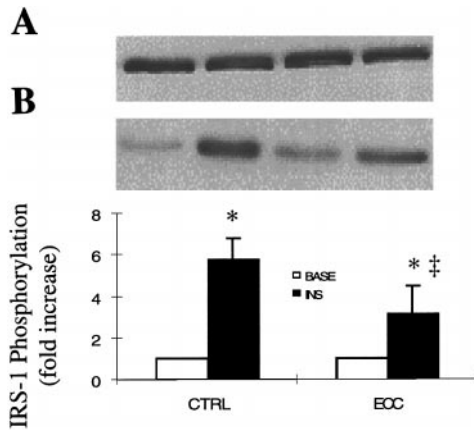


Fig. 1. Insulin receptor substrate (IRS)-1 tyrosine phosphorylation after 1 h of hyperinsulinemia (INS) during hyperinsulinemic-euglycemic clamps performed under control (CTRL) and 24 h after eccentric exercise (ECC). A: representative immunoblot of IRS-1 protein expression from one subject. B: representative immunoblot of IRS-1 tyrosine phosphorylation from one subject. The graph represents the means  $\pm$  SE of the densitometry values [expressed as multiples of increase above BASE (before clamp)] from the 8 subjects. \*Significantly different from BASE ( $P < 0.01$ ); ‡significantly different response to ECC compared with CTRL ( $P < 0.001$ ).

altered by ECC ( $22 \pm 3$  vs.  $20 \pm 4$  arbitrary phosphoImager units, CTRL vs. ECC). 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 with CTRL ( $25.0 \pm 5.9$ - vs.  $8.7 \pm 1.3$ -fold increase above BASE, CTRL vs. ECC; Fig. 3B). There were no significant changes in Akt protein expression ( $13.1 \pm 0.9$  vs.  $17.1 \pm 3.1$  arbitrary densitometry values, CTRL vs. ECC; Fig. 3A). 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 with

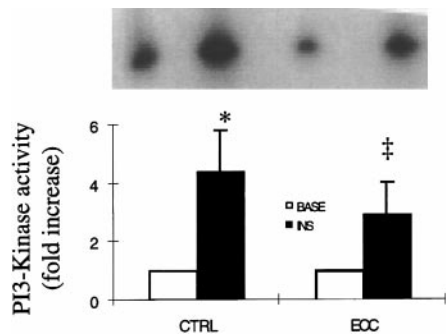


Fig. 2. Phosphatidylinositol (PI) 3-kinase activity after 1 h of INS during hyperinsulinemic-euglycemic clamps performed under CTRL and 24 h after ECC. Shown is a representative phosphoImage of IRS-1-associated PI 3-kinase activity from one subject. The graph represents the means  $\pm$  SE of the phosphoImaging values (expressed as multiples of increase above BASE) from the 8 subjects. \*Significantly different from BASE ( $P = 0.01$ ); ‡significantly different response to ECC compared with CTRL ( $P < 0.01$ ).

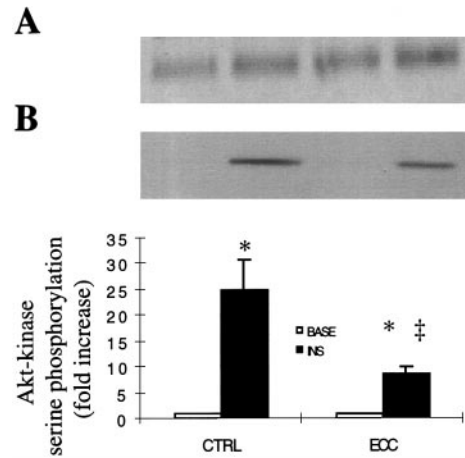


Fig. 3. Akt-kinase serine phosphorylation after 1 h of INS during hyperinsulinemic-euglycemic clamps performed under CTRL and 24 h after ECC. A: a representative immunoblot of Akt-kinase protein expression from one subject. B: a representative immunoblot of Akt-kinase serine phosphorylation from one subject. The graph represents the means  $\pm$  SE of the densitometry values (expressed as multiples of increase above BASE) from the 8 subjects. \*Significantly different from BASE ( $P < 0.05$ ); ‡significantly different response to ECC compared with CTRL ( $P < 0.05$ ).

CTRL ( $1.5 \pm 0.1$ - vs.  $1.2 \pm 0.1$ -fold increase above BASE, CTRL vs. ECC; Fig. 4).

**Cytokine production.** Endotoxin-induced MNC secretion of TNF- $\alpha$  was significantly increased ( $P < 0.05$ ) 24 h after muscle damage ( $1.1 \pm 0.3$  vs.  $2.6 \pm 0.9$  ng/ml) (Fig. 5). In contrast, IL-6 and IL-1 $\beta$  secretions were not significantly different from the respective baseline values at 24 h after ECC ( $0.9 \pm 0.3$  vs.  $3.8 \pm 1.3$  ng/ml,  $P = 0.12$ , and  $2.7 \pm 0.9$  vs.  $2.1 \pm 0.7$  ng/ml,  $P = 0.90$ , respectively). Furthermore, increased MNC secretion of TNF- $\alpha$ , but not IL-6 or IL-1 $\beta$ , was positively correlated with impaired insulin-stimulated IRS-1-associated PI 3-kinase activity ( $r = 0.77$ ,  $P = 0.04$ ;  $r = 0.21$ ,  $P = 0.64$ ;  $r = -0.12$ ,  $P = 0.78$ ; TNF- $\alpha$ , IL-6, and IL-1 $\beta$ , respectively; Fig. 6). TNF- $\alpha$  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

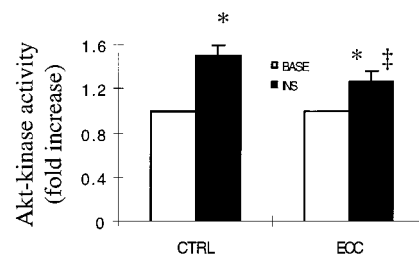


Fig. 4. Akt-kinase activity after 1 h of INS during hyperinsulinemic-euglycemic clamps performed under CTRL and 24 h after ECC. The graph represents the means  $\pm$  SE of the phosphoImaging values (expressed as multiples of increase above BASE) from the 8 subjects. \*Significantly different from BASE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

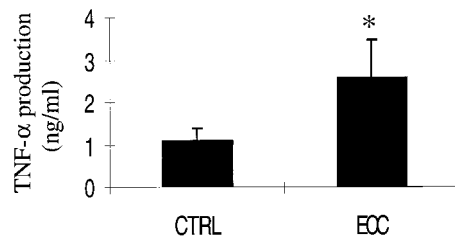


Fig. 5. Endotoxin-induced secretion of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) by cultured mononuclear cells (MNC). Blood samples were obtained before (CTRL) and 24 h after ECC. MNC were isolated as described in METHODS. MNC were cultured overnight with 1 ng/ml lipopolysaccharide, and TNF- $\alpha$  concentrations were measured from cultured supernatants by ELISA. \*Significantly increased from CTRL ( $P < 0.05$ ).

resistance affecting skeletal muscle after limited muscle damage in human subjects. Specifically, we examined the effects of muscle damage on insulin signal transduction at the level of IRS-1, PI 3-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 after muscle damage is associated with impaired insulin signal transduction at the level of IRS-1, PI 3-kinase, and Akt-kinase in human skeletal muscle, presumably leading to impairment of 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-induced muscle damage stress model to induce transient insulin resistance in humans. Because hepatic 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 nonoxidative carbohydrate metabolism, rather than in oxidative pathways, lead to decreased glucose disposal after muscle damage. Therefore, defects in nonoxidative glucose metabolism resulting in an impaired “pulling effect” of glycogen on glucose uptake could play an important role in the development of insulin resistance after muscle damage. These changes at the level of the body as a whole are similar to the impairment in nonoxidative 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 PI 3-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 after muscle damage, insulin signal transduction is impaired at the level of IRS-1 and PI 3-kinase. As expected, tyrosine phosphorylation of IRS-1 and IRS-1-

associated PI 3-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 with the control insulin-stimulated condition. Furthermore, IRS-1-associated PI 3-kinase activity was not significantly increased after ECC in response to the insulin stimulation. Indeed, insulin-stimulated PI 3-kinase was reduced after muscle damage compared with control. In addition, IRS-1 protein content was not altered by muscle damage. Thus impaired insulin signal transduction after muscle damage is not caused by changes in IRS-1 protein expression; rather it occurs as a consequence of functional defects in insulin signal activation.

Akt-kinase has been identified as a downstream target of PI 3-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). Recently, however, 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 after 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 after muscle damage. This phenomenon could be a compensatory response to the decrease in insulin action after 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, PI 3-kinase, and

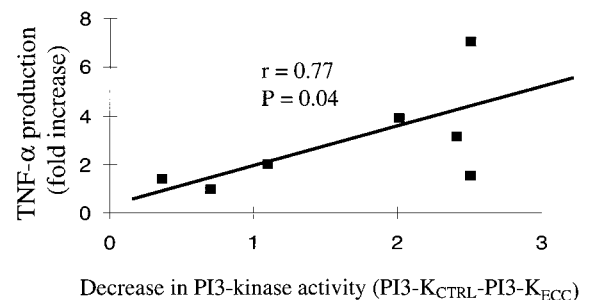


Fig. 6. Relationship between increased TNF- $\alpha$  levels and impaired insulin-stimulated IRS-1-associated PI 3-kinase activity after muscle damage. TNF- $\alpha$  was expressed as a multiple of increase 24 h after ECC with respect to preexercise. IRS-1-associated PI 3-kinase activity is expressed as the difference between the multiple of increase observed in the CTRL trial (PI 3-K<sub>CTRL</sub>) and that in the ECC trial (PI 3-K<sub>ECC</sub>). The correlation analysis was obtained from 7 subjects, because technical difficulties were encountered during the clamp procedure for 1 of the subjects.

Akt-kinase are likely to be the cellular changes underlying the mechanisms of insulin resistance after 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 after muscle damage. Electron microscopy studies performed on injured human skeletal muscle after one-legged eccentric exercise contractions revealed that only a small percentage of muscle fibers was 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 skeletal muscle after limited muscle damage. The cytokines TNF- $\alpha$ , IL-6, and IL-1 $\beta$  have been shown to be involved in the acute phase immune response after muscle damage (5, 6, 38). Therefore, we measured MNC secretion of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  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- $\alpha$  was significantly elevated 24 h after muscle damaging exercise. Furthermore, TNF- $\alpha$  production was positively correlated with the impairment in insulin-stimulated IRS-1-associated PI 3-kinase activity after muscle damage. We (12) and others (22, 26) have previously provided evidence for a link between TNF- $\alpha$  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- $\alpha$  on insulin signal transduction in vivo, the present data are consistent with the hypothesis that TNF- $\alpha$  may impair insulin signal transduction at the level of PI 3-kinase in the in vivo ECC-induced muscle damage stress model in human subjects. The absence of a correlation between TNF- $\alpha$  and GDR, or between TNF- $\alpha$  and Akt-kinase activity, may be caused by a quicker activation of PI 3-kinase, a step which precedes glucose uptake (12, 20). Although an increase in mean concentrations of IL-6 was observed after ECC compared with 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 caused by 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- $\alpha$  (12, 22, 26), IL-6 has no effect on insulin action in cultured muscle cells. Thus TNF- $\alpha$ , rather than IL-6 and IL-1 $\beta$ , may be involved in downregulation of insulin signal transduction after ECC-induced muscle damage. Although the present TNF- $\alpha$  data in vivo support previous investi-

gations on the effect of this cytokine on insulin action in vitro (12, 22, 26), more research is needed to confirm the potential role that TNF- $\alpha$  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 after the stress of muscle damage in human subjects. We found specific defects in insulin signal transduction at the level of IRS-1, PI 3-kinase, and Akt-kinase after 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- $\alpha$  production after muscle damage were associated with impaired insulin-stimulated IRS-1-associated PI 3-kinase activity. These results suggest that elevations in TNF- $\alpha$  during the acute phase immune response might be associated with decreased insulin signal transduction and impaired insulin action after the physiological stress of muscle damage in human subjects. However, more studies are needed to confirm the involvement of TNF- $\alpha$  in the downregulation of insulin action after muscle damage.

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