Exercise-Induced Protein Kinase C Isoform-Specific Activation in Human Skeletal Muscle

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We determined whether protein kinase C (PKC) isoforms are redistributed and phosphorylated in response to acute exercise in skeletal muscle. Muscle biopsies were obtained from six healthy subjects (four women, two men; age 25 ± 1 years) before, during, and after 60 min of one-leg cycle ergometry at ~70% $V\text{O}_{2\text{peak}}$. Exercise for 30 and 60 min was associated with a three- and fourfold increase in PKC-ζ/λ abundance and a four- and threefold increase in phosphorylation, respectively, in total membranes ($P < 0.05$) and a decrease in PKC-ζ/λ phosphorylation in cytosolic fractions. During exercise recovery, PKC-ζ/λ abundance and phosphorylation remained elevated. PKC-ζ/λ abundance and phosphorylation were increased in nonexercised muscle upon cessation of exercise, indicating a systemic response may contribute to changes in PKC abundance and phosphorylation. Exercise did not change PKC-δ or -ε abundance or phosphorylation in either the cytosolic or total membrane fraction. In conclusion, exercise is associated with an isoform-specific effect on PKC. PKC-ζ/λ are candidate PKC isoforms that may play a role in the regulation of exercise-related changes in metabolic and gene-regulatory responses. *Diabetes* 53:21–24, 2004

Physical activity has profound effects on skeletal muscle metabolism and morphology. Acute exercise leads to increased skeletal muscle glucose uptake, changes in glycogen metabolism, and improved insulin sensitivity. Furthermore, a single bout of exercise induces changes in gene transcription and protein synthesis, constituting a possible mechanism for the chronic adaptations to regular physical exercise (1). There is a growing body of evidence to suggest that mitogen-activated protein kinase (MAPK) cascades (2,3) and AMP-activated protein kinase (AMPK) (4,5) are important cellular signaling mechanisms mediating exercise-induced adaptations in skeletal muscle. In addition, Ca^{2+}-dependent protein kinase C (PKC) isoforms are signaling intermediates that may be activated in response to muscle contraction, and thereby constitute an additional exercise-responsive signaling pathway regulating metabolic and gene-regulatory responses in skeletal muscle (6–8).

PKC constitutes a family of important regulators of metabolism, differentiation, and cell growth that are classified into subfamilies based on amino acid similarity and mode of activation (9). Conventional isoforms (cPKC; α, β_1, β_2, and γ) are dependent on both Ca^{2+} and diacylglycerol (DAG) for stimulation, novel isoforms (nPKC; δ, ε, θ, η) are dependent on DAG, and atypical isoforms (aPKC; ζ, λ/ι) are independent of Ca^{2+} and DAG (10). Upon activation, PKCs are redistributed within the cell, such that the membrane-associated kinase is considered to be the active form. Early studies support the involvement of the PKC pathway in exercise-mediated signaling to glucose transport. Inhibition of PKC by polymyxin B is associated with decreased contraction-stimulated glucose transport (7). Furthermore, the PKC inhibitor calphostin C reduces contraction- but not insulin-stimulated glucose uptake (11,12). Recently, treadmill exercise in mice has been shown to increase PKC-ζ/λ activity in skeletal muscle (13), an effect that was additive to insulin stimulation. However, whether specific PKC isoforms are activated by exercise in human skeletal muscle is unknown.

The aim of this study was to determine the effects of acute exercise on different PKC isoforms in human skeletal muscle. We assessed whether exercise leads to redistribution and/or phosphorylation of novel (δ and ε), and atypical (ζ/λ) PKC isoforms in cytosol and total membrane fractions in exercised and rested skeletal muscle.

RESEARCH DESIGN AND METHODS

Six healthy, moderately trained volunteers (four women and two men) participated in the study (age 25 ± 1 years; weight 67.8 ± 4.4 kg; height 171 ± 3 cm). The procedures were explained to and informed consent was obtained from each subject. The Regional Ethical Committee at Karolinska Institutet approved the study protocol. The study was conducted according to the principles expressed in the Declaration of Helsinki. Maximal oxygen uptake ($V\text{O}_{2\text{peak}}$) for one- and two-leg cycle ergometry was determined at least 3 days before the experimental protocol was undertaken. $V\text{O}_{2\text{peak}}$ was 3.4 ± 0.4 and 2.6 ± 0.3 l/min for one- and two-leg exercise, respectively. Plasma glucose, lactate, and serum insulin, IGF-I, and IGF-I binding protein levels were assessed at rest, during exercise, and at recovery (Table 1), as previously reported (14). Subjects were asked to avoid any kind of strenuous exercise during the 48 h before the experimental day.

Exercise protocol. Subjects performed one-leg cycle ergometry for 60 min at a load corresponding to ~70% of one-leg $V\text{O}_{2\text{peak}}$, as previously described (14). After local anesthesia, an incision was made in the skin and underlying muscle fascia, and a muscle biopsy (20–100 mg) was obtained from the vastus lateralis portion of the quadriceps femoris by means of a Weil-Blakesley's conchotome. Each biopsy was removed from a separate incision site ~3 cm apart. Muscle specimens were obtained from the nonexercised (rested) leg at 0 and 63 min and from the exercised leg at 30 and 60 min during cycle ergometry and 15 min after exercise (Fig. 1). Muscle tissue was immediately frozen in liquid nitrogen and stored at −80°C.

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AMPK, AMP-activated protein kinase; aPKC, atypical protein kinase C isoform; DAG, diacylglycerol; ERK, extracellular signal−regulated kinase; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; TBST, Tris-buffered saline with Tween.

TABLE 1
Substrate and hormone concentration at rest, during exercise, and at recovery.

	Rest	Exercise 30 min	Exercise 60 min	Recovery 15 min
Glucose (mmol/l)	4.4 ± 0.3	4.6 ± 0.1	4.7 ± 0.2	4.3 ± 0.2
Lactate (mmol/l)	1.1 ± 0.1	4.8 ± 0.5*†	2.7 ± 0.5*‡	1.5 ± 0.2
Insulin (pmol/l)	120 ± 23.7	74 ± 15.1§	41.6 ± 5.7‖	46.0 ± 5.0‖
IGF-I (μg/l)	258 ± 31	286 ± 34	264 ± 30	254 ± 28
IGF-I binding protein (ng/ml)	14 ± 5.8	9.8 ± 5.0	11.2 ± 2.7	21.4 ± 6.3

Data are means ± SE. *$P < 0.01$ vs. rest, †$P < 0.001$ vs. recovery, ‡$P < 0.005$ vs. exercise for 30 min, §$P < 0.05$ vs. rest, ‖$P < 0.005$ vs. rest.

Tissue processing. Portions of skeletal muscle biopsies (20–50 mg) were homogenized in ice-cold buffer (1:25) containing 20 mmol/l Tris-HCl (pH 7.4), 1 mmol/l EGTA, 1 mmol/l EDTA, 0.5 mmol/l MgCl2, 0.5 mmol/l phenylmethylsulfonyl fluoride, 0.1 μg/ml aprotinin, 0.1 μg/ml leupeptin, and 10 mmol/l 2-mercaptoethanol. The homogenate was centrifuged (100,000*g* for 30 min at 4°C), and the supernatant was collected as cytosol fraction and stored at −80°C. The remaining pellet was resuspended in 100 μl ice-cold homogenization buffer, to which 0.5% Triton X-100 was added and solubilized by rotation for 60 min at 4°C. Thereafter, the resuspended pellet was centrifuged for 10 min at 15,000*g* at 4°C. The supernatant, representing the total membrane fraction, was collected and stored at −80°C until analysis.

Immunoblotting. Protein concentration was determined in cytosol and total membrane fractions according to Bradford (Bio-Rad Protein Kit; Bio-Rad, Richmond, CA). Aliquots (40 μg) were solubilized in Laemmli buffer, separated by SDS-PAGE (10% resolving gel), cytosol, and membrane fractions run on separate gels. Thereafter proteins were electrophoretically transferred to polyvinylidine difluoride membrane and blocked in 7.5% nonfat dried milk and 0.1% Tween 20 in Tris-buffered saline (TBST) for 2 h at room temperature. Membranes were incubated overnight at 4°C with phospho-specific antibodies for PKC-ζ/λ or PKC-δ (1:1,000; Cell Signaling, Beverly, MA). Membranes were washed (5 × 15 min in TBST) and incubated with horseradish peroxidase−conjugated anti-rabbit IgG (1:25,000; Bio-Rad, Hercules, CA) for 1 h at room temperature, followed by additional washes (5 × 15 min in TBST). Proteins were visualized by enhanced chemiluminescence (ECL Plus; Amersham, Arlington Heights, IL) and quantified by densitometry (Bio-Rad, Richmond, CA). After being immunoblotted with phospho-specific PKC antibodies, membranes were stripped as previously described (14) and immunoblotted for total PKC-ζ/λ, PKC-δ, or PKC-ε protein (Transduction Laboratories, Kensington, NY).

Statistical analysis. Data were analyzed by ANOVA, repeated measures design, with one dependent factor (time) with five levels (at rest, exercise 30 min, exercise 60 min, rest after, and postexercise recovery). Statistical significance was considered at $P < 0.05$. The planned comparison post hoc test was used to detect the systematic effects. Data are expressed as means ± SE.

RESULTS

PKC-ζ/λ abundance and phosphorylation in total membrane and cytosolic fractions of rested and exercised skeletal muscle. Acute exercise was associated with changes in aPKC abundance and phosphorylation in skeletal muscle. Exercise (60 min) led to a marked increase (4.2-fold) in PKC-ζ/λ abundance ($P < 0.05$) in the total membrane fraction (Fig. 2). A tendency for increased PKC-ζ/λ abundance was present in the total membrane fraction after only 30 min of exercise ($P = 0.065$). PKC-ζ/λ abundance in the cytosolic fraction was not altered in response to exercise (Fig. 3). The exercise-induced increase in PKC-ζ/λ abundance in total membranes was associated with a concomitant increase in PKC-ζ/λ phosphorylation in total membrane fractions (Fig. 4), which was maintained throughout the exercise bout ($P < 0.05$). PKC-ζ/λ phosphorylation was decreased ($P < 0.05$) in the cytosolic fraction in skeletal muscle from exercised and nonexercised legs (Fig. 5). After recovery from exercise (15 min postexercise), PKC-ζ/λ abundance (Fig. 2) and phosphorylation (Fig. 4) in total membranes remained elevated. Furthermore, PKC-ζ/λ abundance and phosphorylation in total membranes was increased and PKC-ζ/λ phosphorylation in cytosol was decreased in nonexercised muscle after cessation of exercise, indicating a systemic response may contribute in mediating these effects.

PKC-δ and -ε abundance and phosphorylation in total membrane and cytosolic fractions of rested and exercised skeletal muscle. In contrast to the finding with PKC-ζ/λ, exercise did not alter abundance or phosphorylation of PKC-δ or -ε in either the cytosolic fraction or total membranes (data not shown).

DISCUSSION

To date, there has been limited information regarding the regulation and activation of PKCs in human skeletal muscle. Activation of different PKC isoforms by physical

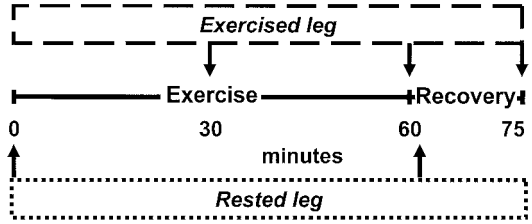

FIG. 1. Schematic representation of the study protocol. Skeletal muscle biopsies were obtained before, during, and after 60 min of one-legged cycle ergometry at ~70% Vo_{2peak}. Arrows indicate biopsy sampling.

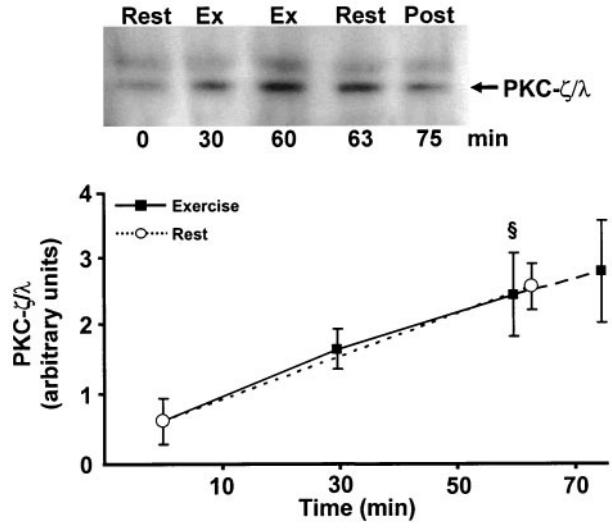

FIG. 2. Exercise-induced PKC-ζ/λ abundance in total membranes. Biopsies were obtained as indicated in Fig. 1 from nonexercised and exercised muscle. Data in graph represent means ± SE arbitrary units ($n = 4–6$). §$P < 0.05$ rest vs. 60 min exercise. Representative immunoblot is also shown.

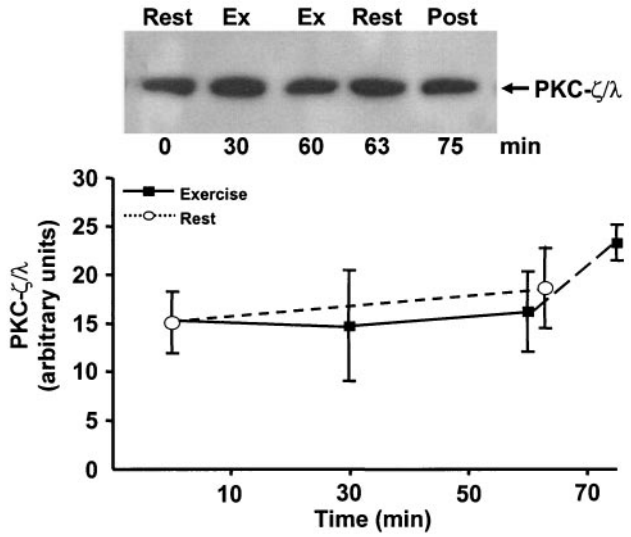

FIG. 3. Exercise-induced PKC-ζ/λ abundance in cytosol fractions. Biopsies were obtained as indicated in Fig. 1 from nonexercised and exercised muscle. Data in graph represent means ± SE arbitrary units (n = 4–6). Representative immunoblot is also shown.

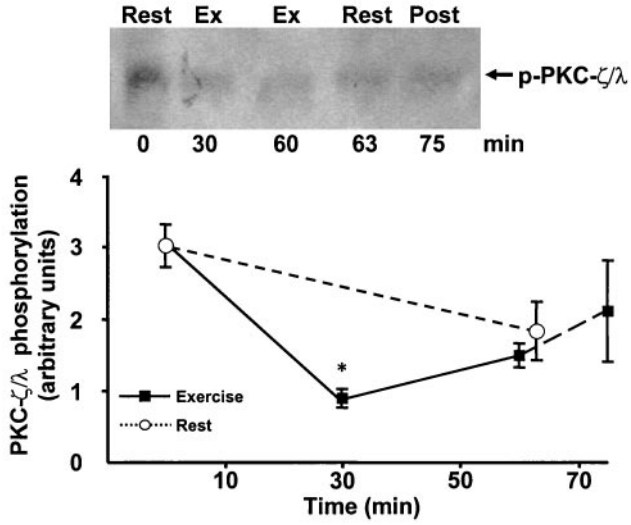

FIG. 5. Exercise-induced PKC-ζ/λ phosphorylation in cytosol fractions. Biopsies were obtained as indicated in Fig. 1 from nonexercised and exercised muscle. Data in graph represent means ± SE arbitrary units (n = 4–6). *P < 0.05 rest vs. 30 min exercise. Representative immunoblot is also shown.

exercise could be important in the regulation of glucose transport and transcriptional events in skeletal muscle. In this study, we provide evidence for an isoform-specific exercise response of PKC. Exercise was associated with an increase in phosphorylation and abundance of atypical (ζ/λ) PKC isoforms in skeletal muscle. In contrast, exercise had no effect on novel PKC (δ and ϵ) isoforms. To our knowledge, this is the first evidence that PKC isoforms are differentially regulated in response to exercise in human skeletal muscle. Furthermore, using a one-leg exercise protocol, we provide evidence that systemic factors also elicit effects on PKC-ζ/λ. Our finding of isoform-specific effects on PKCs provides a putative mechanism to explain downstream exercise-mediated responses.

Previous studies in mice (treadmill running) and humans (bicycle exercise) have shown that exercise is associated with an increase in aPKC activity in vastus lateralis skeletal muscle (13,15). We have characterized the effects of exercise on aPKC abundance and phosphorylation in human skeletal muscle and have extended this study to consider additional PKC isoforms. Exercise was associated with an increase in PKC-ζ/λ abundance and phosphorylation in total membranes and a decrease in PKC-ζ/λ phosphorylation in the cytosol fractions. However, we did not detect a decrease in PKC-ζ/λ abundance in the cytosol fractions after exercise. This latter observation may be expected as the cytosolic fraction represents a major proportion of the total PKC-ζ/λ pool and the amount translocated to total membranes is likely to account for only a minor depletion of the cytosolic pool of PKC-ζ/λ. Accordingly, in rat hepatocytes, stimulation with C2-ceramide induces translocation of PKC-ζ to nuclear and membrane fractions, with no measurable decrease of the enzyme observed in cytosolic fractions (16).

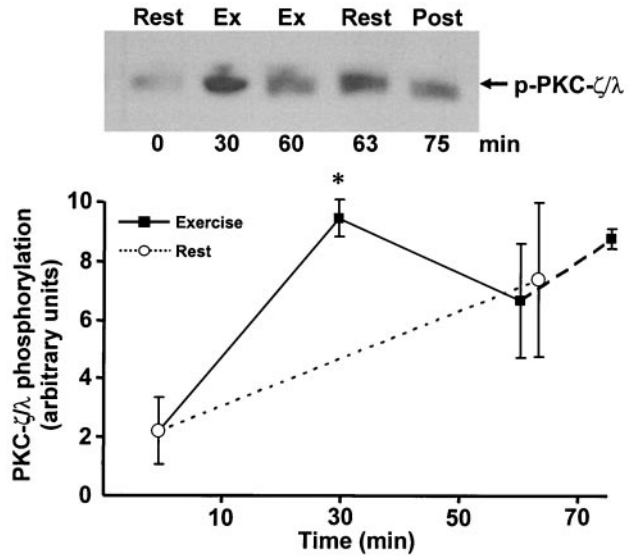

FIG. 4. Exercise-induced PKC-ζ/λ phosphorylation in total membranes. Biopsies were obtained as indicated in Fig. 1 from nonexercised and exercised muscle. Data in graph represent means ± SE arbitrary units (n = 4–6). *P < 0.05 rest vs. 30 min exercise. Representative immunoblot is also shown.

PKC isoforms are likely to have unique, as well as redundant roles, in mediating cellular events. PKC-ζ/λ has been proposed to mediate both insulin- and exercise-induced glucose uptake through separate signaling pathways (13). Insulin-action on PKC-ζ/λ is dependent on phosphatidylinositol 3-kinase (17), whereas the exercise effects on this isoform are partly mediated via AMPK and extracellular signal−regulated kinase (ERK) (13). These studies implicate PKC isoforms in mediating glucose transport. In addition to these metabolic actions, PKC isoforms are involved in the regulation of mitogenic signaling and gene expression. Transfection studies, whereby constitutively active mutants of conventional, novel, and atypical PKC isoforms are expressed in Cos-7 cells, have shown that PKC isoforms activate the ERK/MAPK pathway (18). Furthermore, increased expression and activity of PKC-β modulates gene expression and hypertrophic processes in cardiac muscle (19,20). These studies implicate PKC isoforms in mediating mitogenic responses. Similar mechanisms could be envisaged in skeletal muscle; however, this

remains to be determined. Although the physiological importance of PKC activation can be revealed in human studies, definitive conclusions regarding the precise interaction between exercise-responsive PKC isoforms and downstream metabolic or gene-regulatory responses cannot be fully resolved.

Somewhat surprising was the observation that the exercise was associated with a systemic effect on PKC-ζ/λ. The nature of the systemic factor is unknown. The effects of exercise on PKC-ζ/λ abundance and phosphorylation persisted into the 15-min postexercise period, consistent with an earlier report in rat skeletal muscle (8) in which electrical stimulation (contraction), followed by a period of rest (30 min), was associated with an acute direct, as well as a persistent effect on PKC activity. This response for PKC is in contrast to the exercise-mediated response for ERK/MAPK (14), which is rapidly suppressed after cessation of exercise. However, the finding of a persistent effect of exercise on PKC parallels the exercise response on p38 MAPK in these subjects (14). Both p38 MAPK and PKC have been implicated in the regulation of insulin-stimulated glucose transport (17,21). Thus the persistent effect of exercise on p38 MAPK and PKC may provide evidence to suggest these kinases participate in mediating the increase in insulin sensitivity on glucose transport in skeletal muscle observed after exercise (22); however, this remains to be determined.

In conclusion, acute exercise has an isoform-specific effect on PKC. The increased abundance and phosphorylation of atypical (ζ/λ) PKC isoforms describes a potential signaling mechanism by which exercise mediates metabolic and gene-regulatory responses in skeletal muscle. Further studies to directly link specific PKC isoforms with exercise-mediated responses are required. Increased knowledge of the mechanisms involved in the exercise-induced adaptations in skeletal muscle may identify new targets for therapeutic approaches to combat several diseases including type 2 diabetes.

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