A SINGLE BOUT OF EXERCISE INDUCES A TRANSIENT ALTERATION IN GENE EXPRESSION OF THE INSULIN SIGNALING PATHWAY

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Abstract

The present study was conducted to examine whether gene expression of the insulin signaling pathway is activated in response to a single bout of exercise. Adult male rats underwent a single bout of treadmill exercise for 90 min at 22 m/min on a 6°C incline. The animals were sacrificed immediately after exercise or after a predetermined post-exercise recovery period of up to 48 h, and the level of mRNA in the gastrocnemius muscle was measured by the RT-PCR method. Immediately after exercise, mRNA levels of insulin signal transduction intermediates in the gastrocnemius muscle were lower in the exercise group than in the controls. Following this tendency reduction, mRNA levels of insulin receptor, IRS-1, PI 3-kinase, SH-PTP2 and Nck were higher in the exercise group than in the controls. The levels of PI 3-kinase mRNA was significantly higher in the exercise group than in the controls within 6 h after exercise, while levels of mRNA for insulin receptor, IRS-1, SH-PTP2 and Nck were significantly higher in the the exercise group than in the controls at 48 h after exercise. Gene expression of oncogenes was also affected by a single bout of exercise. Levels of c-fos and c-myc mRNA were significantly higher than in the controls immediately after exercise, while the level of c-jun mRNA was lower in the exercise group at 3 h after exercise. Thus, a single bout of exercise affects the gene expression of insulin signal transduction pathway intermediates and oncogenes in rat gastrocnemius muscle. The present findings suggest that an exercise-induced adaptive increase of skeletal muscle insulin sensitivity is a cumulative effect of a single bout of exercise on the gene expression of insulin signal transduction pathway intermediates.

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key words : mRNA, oncogene, insulin sensitivity, insulin signaling pathway, exercise

Endurance training has been reported to enhance insulin sensitivity with increasing insulin receptor number in skeletal muscle¹⁻⁵⁾. Enhanced gene expression of post receptor insulin signal transduction pathway may also contribute to increased insulin sensitivity of endurance trained skeletal muscle, since mRNA levels of insulin receptor, insulin receptor substrate-1 (IRS-1)⁶⁾, phosphatidylinositol 3-kinase (PI 3-kinase)⁷⁾ and GLUT4^{8,9)} were increased in skeletal muscle of endurance trained rats. Increase in gene expression of GLUT4 was detected 16 h after one pro-

longed exercise session¹⁰⁾, suggesting that enhanced gene expression observed in trained skeletal muscle reflects an effect of a single bout of exercise.

Insulin elicits normal cellular effects by first binding to receptor, resulting in activation of the receptor tyrosine kinase. Activated insulin receptor tyrosine kinase causes tyrosine phosphorylation of IRS-1. Phosphorylated IRS-1 subsequently binds cytoplasmic signaling proteins containing SH2 domains, such as PI 3-kinase, GRB2, Nck and SH-PTP2. GRB2 transmits insulin signal on the

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receptor to ras complex and MAP kinase pathway^{11,12)}. In addition to gene expression of insulin receptor, that of IRS-1, PI3-kinase, Nck, GRB2, SH-PTP2, ras and GLUT4 are also affected in endurance trained skeletal muscle although physiological function of some of these signaling molecules is less well known⁷⁾.

Effect of a single bout of exercise on gene expression is transient. For example, gene expression of hexokinase in rat skeletal muscle increased 8 h after a single bout of exercise but returned to basal level by 24 h¹³⁾. One prolonged exercise session increased gene expression of GLUT4 within 16 h but a time course of the change in gene expression after an exercise session has not been studied¹⁰⁾. In this study, we examined a time course of changes in gene expression of insulin signal transduction pathway intermediates in rat gastrocnemius muscle during 48 h after a single bout of exercise.

METHODS

Animal care. Sixty male Sprague-Dawley rats (6 week old) were obtained from CLEA Japan, Tokyo. All procedures involving animals were approved by the experimental animal care committee of University of Tsukuba. The rats were housed at 22°C with light from 7:00 to 19:00 h and with free access to water and food (CE-2, CLEA Japan, Tokyo). To familiarize the animals with the motor-driven treadmill (model III: Autome Kogyo, Tokyo)¹⁴⁾, all animals were exercised for 10 minutes daily for 4 days.

Experimental procedures. The exercise protocol employed in the present study was based on the work of O'Doherty et al.¹³⁾. Rats undertook a single bout of treadmill exercise for 90 min at 22 m/min on 6° incline. Animals were sacrificed by decapitation before (control value), immediately, 3, 6, 9, 12, 24 or 48 h after exercise. Gastrocnemius muscle was immediately removed, freeze-

Table 1. Sequences of PCR primers.

Gene	Nucleotide number	Size of PCR product (bp)	GenBank accession no.
Insulin receptor	2474-2493 2743-2724	270, 234	M 29014
IRS-1	1382-1401 1718-1699	337	X 58375
Shc	1-24 416-383	416	D 83015
SH-PTP 2	1-24 449-426	449	D 83016
GRB 2	191-214 667-644	477	X 62853
Nck	21-44 251-228	251	H 32835
PI 3-kinase	1297-1382 1527-1504	231	M 61904, M 61746
GLUT 4	221-240 613-594	393	X 14771
ERK 1	499-518 758-739	260	M 61177
ERK 2	234-253 530-511	297	M 64300
β-Actin	2846-2865 3222-3203	377	J 00691

Sequences of each primer pair and their location in sequences cited in GenBank database are noted.

clamped at liquid nitrogen temperature, and stored at -80°C until use. Body weight of animals at the time of sacrifice ranged from 200 ~ 260 g and it was not different among the groups.

Analytical methods. Total RNA was extracted from skeletal muscle with a guanidium thiocyanate water-saturated phenol extraction method¹⁵⁾. First-strand cDNA synthesis was performed on 5 µg total RNA using oligo (dT) as described by manufacturer's instructions (BRL SuperScript Kit, Life Technologies). The sequences of the primers¹⁶⁻¹⁹⁾ used for amplification are shown in Table 1. Before PCR amplification, the primers were labeled with γ-[³²P] ATP (Amersham) by using T4 polynucleotide kinase (Takara, Tokyo, Japan). PCR reactions were carried out in a DNA Thermal Cycler (Perkin-Elmer, Cetus) using the

following cycle conditions: initial denaturation at 94°C for 6 min, denaturation at 94°C for 1 min, annealing at 60°C for 2 min and extension at 72°C for 3 min²⁰. As an exception, annealing at 63°C for PI3-kinase, 55°C for oncogenes and 58°C for β -actin. There were 25 cycles for insulin receptor IRS-1, Nck, SH-PTP2 and oncogenes, 23 cycles for PI3-kinase and GRB2, 22 cycles for MAP kinase isoforms (ERK1, ERK2) and GLUT4 and 18 cycles for β -actin. PCR products (10 μ l) were analyzed by electrophoresis in 7.5% polyacrylamide gels. The amounts of mRNA in each sample were quantitated by a laser image analyzer (Fujix BAS2000, Fuji Film). The PCR cycles and imaging plate times were kept within their respective

exponential and linear range.

Statistics. Changes in mRNA levels after the exercise are expressed as % of control value. Values shown are means $\pm$ SE. Comparison of the mean values was performed with one-way ANOVA with a post hoc Dunnett's test. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Immediately after a single bout of exercise, mRNA levels of all insulin signal transduction pathway intermediates were lower than the control values and the difference in insulin receptor and SH-PTP2 mRNA was statistically significant.

Fig. 1. Time course of changes in mRNA levels of insulin signal transduction intermediates in gastrocnemius muscle after 90 min of exercise (▨). Values are mean $\pm$ SE. * $P < 0.05$ and ** $P < 0.01$ (statistical significance from initial value).

Following this lowering change, mRNA levels of insulin receptor, IRS-1, PI3-kinase, SH-PTP2 and Nck were higher in exercise group than those in the control during the 48 h after the cessation of exercise. Changes in mRNA levels of GRB2, ras, ERK1, ERK2 and GLUT4 were not statistically significant (Fig. 1).

Gene expression of oncogenes was also affected by a single bout of exercise. mRNA levels of *c-fos* and *c-myc* were significantly higher than those in the control immediately after exercise, and the increase in *c-fos* was still statistically significant at 3 and 12 h after exercise. Conversely, mRNA level of *c-jun* was lower in exercise group than those in the control at 3 and 6 h after exercise (Fig. 2). Gene expression of β -actin was not affected by exercise (data not shown).

Fig. 2. Time course of changes in mRNA levels of oncogenes in gastrocnemius muscle after 90 min of exercise (■). Values are mean $\pm$ SE. * $P < 0.05$ and ** $P < 0.01$ (statistical significance from initial value).

DISCUSSION

The novel finding from this study is that a single bout of exercise elicits increase in gene expression of insulin signal transduction intermediates in rat gastrocnemius muscle. mRNA

levels of insulin receptor, IRS-1 and SH2 domain containing signal molecules that interact with IRS-1 (PI3-kinase, SH-PTP2, Nck) increased during the 48 h after the exercise (Fig. 1). Physiological significance of this finding could be related to not only an acute but also a chronic effect of endurance exercise on insulin sensitivity. Enhanced mRNA levels of insulin receptor, IRS-1, PI3-kinase and ras were also observed in endurance trained skeletal muscle⁷⁾. The transient changes in mRNA level of insulin signal transduction intermediates, such as insulin receptor, IRS-1, PI3-kinase, Nck and SH-PTP2, after a single bout of exercise did not complete within 24 h. Present results suggest the possibility that enhanced gene expression observed in trained skeletal muscle reflects a cumulative effect of a single bout of exercise. MAP kinase pathway is suggested to be involved in regulation of gene transcription, protein synthesis, glycogen metabolism and glucose transport^{11,12)}. Recent study demonstrated that MAP kinase isoforms (ERK1, 2) were activated immediately after a single bout of exercise²¹⁾ but gene expression of ERK1, 2 was not affected under our experimental condition. Altered gene expression of Nck, SH-PTP2 and GRB2 was also found in adipose tissue and liver of diabetic animals^{22,23)}, but physiological significance of these alterations remains to be elucidated.

Enhanced gene expression of GLUT4 was detected at 16 h after a single bout of exhaustive swimming lasting 6 h¹⁰⁾. In the present experiment, mRNA level of GLUT4 increased 12 h after cessation of exercise although the increase was not statistically significant and our result is therefore consistent with the previous report which examined GLUT 4 gene expression at 3, 8 and 24 h after a single bout of treadmill running lasting 90 min¹³⁾. Failure to detect a significant increase in GLUT4 gene expression after a single bout of exercise can be explained by the difference in

experimental conditions such as type and duration of exercise.

An increase in mRNA levels of insulin receptor, IRS-1, Nck and SH-PTP2 were first detected at 48 h while that of PI 3-kinase was already detected within 24 h after exercise, suggesting different mechanisms of exercise to modify gene expression. Since the increase in gene expression of insulin signal transduction pathway was preceded by a transient decrease in gene expression immediately after exercise, it is likely that exercise itself does not directly elicit upregulation of the genes. Insulin receptor and IRS-1 gene possess responsive elements for AP-1 complex, consisted with *c-fos* and *c-jun*, in their promoter region and gene expression of these immediate early genes in skeletal muscle is enhanced by a combination of stretch and electrical stimulation^{24,25}. Considering available knowledge of their physiological function, it was conceivable that oncogene products, as AP-1 transcriptional factor might mediate the effect of a single bout of exercise to stimulate gene expression of insulin signal transduction pathway. Consistent with the observation after in situ electrical stimulation, a single bout of exhaustive running enhanced gene expression of *c-fos* and *c-myc* immediately after exercise but decreased *c-jun* expression. Thus changes in expression of oncogenes preceded the increase in gene expression of insulin signal transduction pathway, but the physiological role of oncogene products as a transcriptional factor for gene expression of insulin signal transduction pathway remained to be clarified.

Thus a single bout of exercise affects gene expression of insulin signal transduction pathway intermediates and oncogenes in rat gastrocnemius muscle. It is conceivable that the effect of a single bout of exercise to increase mRNAs of insulin signal transduction pathway intermediates accumulates and results in enhanced insulin sensitivity in

trained skeletal muscle.

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