Differential effects of exercise on insulin-signaling gene expression in human skeletal muscle

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Wadley, G. D., R. J. Tunstall, A. Sanigorski, G. R. Collier, M. Hargreaves, and D. Cameron-Smith. Differential effects of exercise on insulin-signaling gene expression in human skeletal muscle. J Appl Physiol 90: 436–440, 2001.—Skeletal muscle insulin sensitivity is enhanced after acute exercise and short-term endurance training. We investigated the impact of exercise on the gene expression of key insulin-signaling proteins in humans. Seven untrained subjects (4 women and 3 men) completed 9 days of cycling at 63 ± 2% of peak O2 uptake for 60 min/day. Muscle biopsies were taken before, immediately after, and 3 h after the exercise bouts (on days 1 and 9). The gene expression of insulin receptor substrate-2 and the p85α subunit of phosphatidylinositol 3-kinase was significantly higher 3 h after a single exercise bout, although short-term training ameliorated this effect. Gene expression of insulin receptor and insulin receptor substrate-1 was not significantly altered at any time point. These results suggest that exercise may have a transitory impact on the expression of insulin receptor substrate-2 and phosphatidylinositol 3-kinase; however, the predominant actions of exercise on insulin sensitivity appear not to reside in the transcriptional activation of the genes encoding major insulin-signaling proteins.

messenger ribonucleic acid; real-time polymerase chain reaction; transcription; insulin-receptor substrates; phosphatidylinositol 3-kinase

DESPITE THE EPIDEMIOLOGICAL evidence correlating regular physical activity with improvements in insulin action (9, 16, 17), there is a paucity of consistent data examining the cellular mechanisms of action, particularly in humans. In recent years, it has become apparent that transcriptional regulation of gene expression is an integral component of skeletal muscle adaptation to exercise (5, 23, 24). Although the mechanisms by which insulin signaling is enhanced after exercise remain unclear, the gene expression of key proteins involved in the insulin-signaling pathway could be mediated by endurance training and may account for some of the improvements in insulin signaling after chronic exercise.

The mRNAs of insulin receptor (IR), insulin receptor substrate-1 (IRS-1), and phosphatidylinositol 3-kinase (PI3-kinase) increase in response to endurance training in rats (13, 14), whereas skeletal muscle IR protein content is significantly elevated after both a single exercise bout and exercise training in rats (2). In humans, PI3-kinase activity during insulin infusion is increased after endurance training (11), although mRNA abundance was not reported. In addition to IRS-1, skeletal muscle contains an alternative substrate of the IR, insulin receptor substrate-2 (IRS-2). The response of IRS-2 appears to differ from IRS-1, with IRS-2 protein content increasing after a single bout of exercise, although this increase is abolished after several bouts of endurance exercise (2). The regulation of IRS-2 gene expression in human skeletal muscle by exercise has yet to be described.

In the present study, we hypothesized that a mechanism contributing to the enhancement of insulin action after endurance training in human subjects is upregulated gene expression of key members of the insulin-signaling cascade. To examine this hypothesis, the impact of a single bout of exercise and short-term endurance training on the mRNA levels of IR, IRS-1, IRS-2, and the p85α subunit PI3-kinase in untrained human subjects was determined.

MATERIALS AND METHODS

Experimental protocol. Seven healthy subjects volunteered to be involved in the study. Four were women and three were men. The age, peak pulmonary oxygen uptake (V̇O2 peak), and body mass index of the subjects before the study were 28.9 ± 3.1 (SE) yr, 37.1 ± 2.7 ml·kg⁻¹·min⁻¹, and 22.6 ± 1.4 kg·m⁻², respectively. Body mass did not change with the 9 days of exercise training (67.7 ± 5.4 vs. 67.2 ± 5.3 kg). The study was approved by the Deakin University Ethics Committee, and subjects gave their written consent to participate in this study after all procedures and the possible risks of participation were explained. Before testing, all subjects performed an incremental cycling test to exhaustion for the determination of V̇O2 peak. All cycling tests were performed on a cycle ergometer (Quinton Excalibur, Groningen, The Netherlands). Oxygen uptake was measured using indirect calorimetry (Gould Metabolic Systems, Dayton, OH).

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Triton X-100, 1 mM of each 2-deoxynucleotide 5'-phosphate, 20 U recombinant RNasin ribonuclease inhibitor (Promega, Madison, WI) from gene sequences obtained from GenBank (b-actin: X00351, IR: M10051, IRS-1: NM_005544, IRS-2: AF073310, PI3-kinase subunit: M61906). The primer sequences are shown in Table 1. Real-time PCR was used to quantify mRNA expression of these genes after 9 days of endurance training, absolute gene expression of b-actin is shown in Fig. 1. Fluorescent detection by real-time PCR demonstrated no significant training effect on the expression of b-actin. A small reduction in C_T was evident immediately after the completion of the first exercise bout, suggesting increased mRNA recovery or cDNA synthesis (reverse transcriptase) efficiency at this time point. Subsequent analysis of gene expression is normalized against b-actin (housekeeping gene) to account for the variability in cDNA abundance between samples.

The gene expression of IR (Fig. 2A) and IRS-1 (Fig. 2B) were not significantly altered either immediately after exercise or 3 h after a single bout of exercise. Furthermore, there was no significant change in the mRNA expression of these genes after 9 days of endurance training. The gene expression of IRS-2 was found to be 11 ± 4-fold higher (P < 0.01) 3 h after exercise.

All subjects reported to the laboratory in the morning after an overnight fast (10–12 h). For the 24 h preceding all test sessions, subjects abstained from alcohol, tobacco, caffeine, and exercise. Muscle samples were obtained before, immediately after, and 3 h after exercise (from the vastus lateralis) on the first and last days of a 9-day training program using the percutaneous needle biopsy technique. Excised muscle tissue from the biopsy was immediately frozen in liquid nitrogen for subsequent analysis. Exercise was 60 min on a cycle ergometer at 63% VO_2 peak, which was repeated for 9 consecutive days.

Analytic methods. Total RNA was isolated using FastRNA protocol (Q.BIOgene 101, Vista, CA) (3). RNA was reverse transcribed to synthesize first-strand cDNA using AMV reverse transcriptase (Promega, Madison, WI). Brieﬂy, the RNA was added to a mixture containing a ﬁnal concentration of 5 mM MgCl_2, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1% Triton X-100, 1 mM of each 2-deoxynucleotide 5’-triphosphate), 20 U recombinant RNasin ribonuclease inhibitor (40 U/μl), and 0.5 μg oligo(dT)_15. Primers were designed using the Primer Express software package version 1.0 (Perkin-Elmer, norwalk, CT) from gene sequences obtained from GenBank (b-actin: X00351, IR: M10051, IRS-1: NM_005544, IRS-2: AF073310, PI3-kinase p85α subunit: M61906). The primer sequences were validated using BLAST (1) to ensure each primer was homologous with the desired mRNA of human skeletal muscle. The primer sequences are shown in Table 1. Real-time PCR was used to quantify mRNA expression and has been described in detail previously (4). This technique has been modiﬁed to include SYBR Green chemistry rather than the oligonucleotide probe used elsewhere (4, 29). Direct detection of PCR product was monitored by measuring the increase in ﬂuorescence caused by the binding of SYBR Green to double-stranded DNA (Perkin-Elmer). mRNA levels were quantitated using the critical threshold (C_T) value, which is the cycle at which the ﬂuorescence emission increases above a threshold level (10 times the SD of the background). Therefore, C_T values are calculated at the initiation of the logarithmic phase of PCR amplification and provide accurate measurement of starting cDNA concentrations (4). Real-time PCR was performed in triplicate using the ABI PRISM 5700 sequence detection system (Perkin-Elmer). A real-time PCR mix of 2× SYBR Green Universal PCR Master Mix (Perkin-Elmer), forward and reverse primer (2 μM), and cDNA was run for 40 cycles of PCR in a volume of 25 μl. To compensate for variations in input RNA amounts, and efﬁciency of reverse transcription, b-actin mRNA was also quantitated, and results were normalized to these values. b-Actin mRNA levels have been reported not to change in response to 3, 6, and 12 wk of training in rat skeletal muscle (20) and therefore were considered adequate as an internal control for this study.

Samples were analyzed using two-way ANOVA with repeated measures. Post hoc analysis was performed to determine differences between groups using Newman-Keuls test, where appropriate.

RESULTS

The impact of acute exercise and exercise training was determined relative to the abundance of b-actin, a commonly used housekeeping gene. To confirm that the expression of b-actin is not modiﬁed in human muscle after exercise training, absolute gene expression of b-actin is shown in Fig. 1. Fluorescent detection by real-time PCR demonstrated no signiﬁcant training effect on the expression of b-actin. A small reduction in C_T was evident immediately after the completion of the first exercise bout, suggesting increased mRNA recovery or cDNA synthesis (reverse transcriptase) efﬁciency at this time point. Subsequent analysis of gene expression is normalized against b-actin (housekeeping gene) to account for the variability in cDNA abundance between samples.

The gene expression of IR (Fig. 2A) and IRS-1 (Fig. 2B) were not signiﬁcantly altered either immediately after exercise or 3 h after a single bout of exercise. Furthermore, there was no signiﬁcant change in the mRNA expression of these genes after 9 days of endurance training. The gene expression of IRS-2 was found to be 11 ± 4-fold higher (P < 0.01) 3 h after exercise.
compared with basal levels, although this effect was reduced to 4 ± 1-fold \((P < 0.05)\) after exercise training (Fig. 2C). Similarly, the mRNA levels of PI3-kinase were also higher \((P < 0.05)\) 3 h after exercise in the untrained state, but no changes in expression were found after exercise in the trained state (Fig. 2D).

**DISCUSSION**

The increased sensitivity of insulin-mediated glucose uptake after exercise persists for up to 48 h, during which time the acute effects of glucose on GLUT-4 translocation have been reversed and muscle glycogen concentrations are largely restored (6, 19). Our group and others have shown that a single bout of exercise is capable of eliciting increased gene expression of key components of the glucose disposal pathway, including GLUT-4 and hexokinase II (15, 22). Recent data have also shown increased levels of GLUT-4 protein 22 h after a single exercise bout in humans (7). In isolated muscle and fat cells, IRS-2 has been shown to have a similar role to IRS-1 in insulin-stimulated glucose transport (18, 30) and may act as an alternative pathway of insulin action. However, IRS-2 is not involved in glucose transport immediately after either a single exercise bout or short-term training (2, 10). A complex interplay between IRS-1 and IRS-2 is evident in the regulation of insulin action in skeletal muscle, with alterations in the balance of these proteins impacting on insulin action (27). Therefore, it is tempting to speculate of an early adaptive role of IRS-2 in the exercise-induced increases in insulin signaling in the hours after exercise. This role for IRS-2 might then become redundant after the more slowly initiated adaptive responses of other members of the insulin-signaling intermediates, such as IRS-1. In the present study, no changes in IRS-1 gene expression, following either a single exercise bout or short-term training were observed. However, insulin-stimulated function

**Fig. 2. Effect of a single bout of exercise (solid bars; untrained) and short-term endurance training (hatched bars; trained) on the gene expression of the insulin receptor (A), insulin receptor substrate-1 (B), insulin receptor substrate-2 (C), and p85α subunit of phosphatidylinositol 3-kinase (D). Values are means ± SE. \(^*P < 0.01\) vs. all other values. \(^*P < 0.05\) vs. trained values. \(^P < 0.05\) vs. Post untrained values.**
of IRS-1 has been observed to increase by 5 days of training (2). As tempting as it is to speculate on the role of IRS-2 to insulin signaling in the hours after exercise, further evidence is required to establish its contribution.

It was somewhat surprising that there were no observable changes in IR and IRS-1 after endurance training given the enhanced gene expression found previously in rodents after endurance training (13, 14). This may reflect species differences, the composition of fiber types analyzed and/or differences in training protocol. The present study used a relatively short (9 days and 60 min/day) training period, whereas the rodents were trained for 90 min/day over a 9-wk period. A further limitation of the present study was that subjects were relatively young and lean, without a family history of Type 2 diabetes. Thus any potential changes in insulin action may be small, such that large changes in the gene expression of IR and IRS-1 might not be expected. However, similar studies have demonstrated enhanced insulin sensitivity after only a single bout of exercise (28) and in training programs utilizing relatively short-term programs such as the one used in the present study (11). Thus it can be concluded that it is unlikely that enhanced gene expression of IR and IRS-1 are central components of the adaptive response to exercise. Exercise-induced improvements in translatable control such as RNA stability, protein-processing transport, and protein stability, as identified previously after exercise training (26) may exert a greater influence on IR and IRS-1 protein abundance and activity.

In the present study it is also possible that any significant changes in gene expression occurred outside or between the sampling periods. There are few data on induction of increased gene expression after an exercise stimulus or the half-lives of many mRNA species in humans (21). Therefore, in the present study, timing of the biopsy was aimed to maximize the likelihood of identifying altered gene expression in the target genes. A spectrum of responses in human skeletal muscle has been reported previously, with increased mRNA abundance demonstrated within minutes of exercise initiation (24), immediately after the completion of exercise (8, 15), and for up to 8 h postexercise (25). Indeed, significantly increased gene expression of IR, IRS-1, and PI3-kinase have been shown in rodents 48 h after the termination of a 9-wk training program (13, 14). In the present study, there were no changes in mRNA levels for IR and IRS-1 either immediately postexercise or when measured again 3 h postexercise. Furthermore, there was no evidence of a training effect on the gene expression of IR and IRS-1 because the second preexercise biopsy sample was obtained 24 h after the last training session. These results would therefore suggest that significantly altered gene expression to moderate exercise of the IR and IRS-1 genes is unlikely.

In summary, we have shown that in humans, skeletal muscle IRS-2 gene expression is significantly increased in the few hours after a single bout of exercise but that after training this effect is diminished. Similarly, the mRNA levels of PI3-kinase appear to be increased in the hours after a single bout of exercise; however, this effect was not observed again after short-term training. A single bout of exercise or short-term endurance training does not increase the gene expression of either the IR and IRS-1 genes. Therefore, in untrained human subjects undertaking moderate exercise training there is little evidence of sustained and substantial alterations in the gene expression of key members of the insulin-signaling pathway. Further studies are required to elucidate the mechanisms regulating the increased activity of the insulin-signaling cascade after exercise.

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REFERENCES


