Exercise and Myocyte Enhancer Factor 2 Regulation in Human Skeletal Muscle

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Overexpression of GLUT4 in skeletal muscle enhances whole-body insulin action. Exercise increases GLUT4 gene and protein expression, and a binding site for the myocyte enhancer factor 2 (MEF-2) is required on the GLUT4 promoter for this response. However, the molecular mechanisms involved remain elusive. In various cell systems, MEF-2 regulation is a balance between transcriptional repression by histone deacetylases (HDACs) and transcriptional activation by the nuclear factor of activated T-cells (NFAT), peroxisome proliferator–activated receptor-γ coactivator 1 (PGC-1), and the p38 mitogen-activated protein kinase. The purpose of this study was to determine if these same mechanisms regulate MEF-2 in contracting human skeletal muscle. Seven subjects performed 60 min of cycling at ~70% of $V\dot{O}_{2peak}$. After exercise, HDAC5 was dissociated from MEF-2 and exported from the nucleus, whereas nuclear PGC-1 was associated with MEF-2. Exercise increased total and nuclear p38 phosphorylation and association with MEF-2, without changes in total or nuclear p38 protein abundance. This result was associated with p38 sequence-specific phosphorylation of MEF-2 and an increase in GLUT4 mRNA. Finally, we found no role for NFAT in MEF-2 regulation. From these data, it appears that HDAC5, PGC-1, and p38 regulate MEF-2 and could be potential targets for modulating GLUT4 expression.
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Skeletal muscle glucose transport is primarily mediated by the transmembrane glucose transporter GLUT4. The importance of this protein in maintaining glucose homeostasis is highlighted by studies disrupting skeletal muscle GLUT4, which results in severe insulin resistance and glucose intolerance (1). Furthermore, selective overexpression of GLUT4 in skeletal muscle improves whole-body insulin action and glucose homeostasis (2,3). As such, regulation of GLUT4 expression is seen as a potential therapeutic target for the management and treatment of insulin resistance in disorders such as type 2 diabetes. An acute bout of exercise increases GLUT4 transcription (4) and gene (5) and protein expression (6). A binding region on the GLUT4 promoter for the myocyte enhancer factor 2 (MEF-2) transcription factor is required for this response (7). Whereas stimuli such as increases in intracellular calcium and decreases in cellular energy balance have been implicated in the exercise-induced increase in GLUT4 (8), the molecular mechanisms regulating MEF-2 are unknown. In the basal state, DNA-bound MEF-2 is thought to be associated with, and inhibited by, the class II histone deacetylases (HDACs) (9). HDACs repress transcriptional activity by deacetylating the NH_2-terminal tails of histone proteins, resulting in chromatin condensation, which tightens the electrostatic interactions between the positively charged histone tails and negatively charged DNA backbone. The tightened, condensed histones prevent transcriptional coactivators from accessing their respective DNA binding regions and other transcriptional activators, such as MEF-2, thereby repressing gene transcription. The association between HDACs and MEF-2 appears to be broken by the calcium/calmodulin-dependent protein kinase (CaMK)-IV. Multiple serine residues on the HDACs are phosphorylated by CaMK-IV, which provides a binding site for the intracellular chaperone 14-3-3 and also masks a nuclear localization sequence (10). This results in the nuclear export of the HDAC and 14-3-3. To reverse the acetylation state of the surrounding histones, coactivators possessing histone acetyltransferase (HAT) activity must associate with MEF-2 (9). It is unclear if this is mediated by the calcineurin/nuclear factor of activated T-cells (NFAT) pathway or the peroxisome proliferator–activated receptor-γ coactivator 1 (PGC-1) pathway. Calcineurin dephosphorylates serine residues on the NFAT NH_2-terminus, unmasking two nuclear localization sequences, which results in NFAT nuclear translocation (11). Nuclear NFAT interacts with and recruits coactivators possessing intrinsic HAT activity to MEF-2, allowing MEF-2 dimerization and association with transcriptional coactivators such as MyoD (9). PGC-1 has been implicated in the expression of GLUT4 (12) and functions like NFAT in that it recruits coactivators with HAT activity to various transcription factors, including MEF-2 (13). Although MEF-2 dimerization and association with cofactors is sufficient to initiate MEF-2–mediated transcription, the rate of transcription dramatically increases after MEF-2 phosphorylation on its transcriptional activation domain, found toward the COOH-terminus (9). The p38 mitogen-activated protein kinase (MAPK) has been found to associate with and phosphorylate MEF-2 on various threonine residues in this domain, resulting in enhanced transcriptional activity (14). Although many studies have observed these individual

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AMPK, AMP kinase; CaMK, calcium/calmodulin-dependent protein kinase; HAT, histone acetyltransferase; HDAC, histone deacetylase; MAPK, mitogen-activated protein kinase; MEF-2, myocyte enhancer factor 2; MKK, MAPK kinase; NFAT, nuclear factor of activated T-cells; PGC-1, peroxisome proliferator–activated receptor-γ coactivator 1.

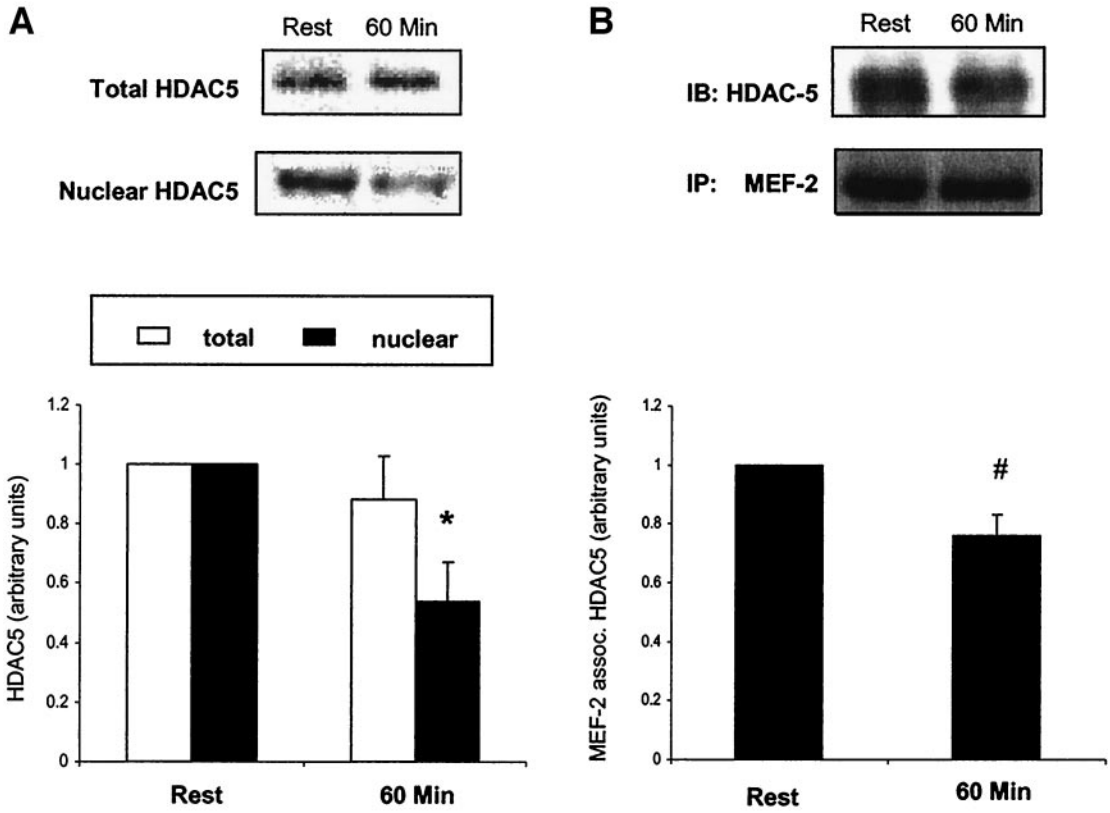

FIG. 1. *A*: Total and nuclear HDAC5 protein in response to exercise. *B*: MEF-2–associated HDAC5 in response to exercise. All values are calculated as the fold changes relative to rest and are reported as the means ± SE ($n = 7$). Significantly different from rest: *$P = 0.006$ and #$P = 0.009$. IB, immunoblot; IP, immunoprecipitate.

mechanisms in a variety of cell systems, it is unclear if these same mechanisms occur in contracting human skeletal muscle to regulate MEF-2 and ultimately control GLUT4 expression. The aim of this study was to determine if HDAC5, CaMK-IV, NFAT, PGC-1, and p38 MAPK were involved in the regulation of MEF-2 in human skeletal muscle during exercise.

RESEARCH DESIGN AND METHODS

Seven male subjects (aged 27 ± 3 years, 83 ± 4 kg, $V\text{O}_{2\text{peak}}$ 47 ± 2 ml · kg $^{-1}$ · min^{-1}) were recruited for the study after completing a medical questionnaire and giving their informed written consent. All experimental procedures were approved by the Deakin University Human Research Ethics Committee. At least 7 days before the experimental trial, all subjects performed an incremental cycling test (Lode, Groningen, the Netherlands) to fatigue to determine peak pulmonary oxygen uptake. This test was also used to select the power output for the experimental trial from the linear relationship between oxygen uptake and power output.

Exercise. Subjects performed a single bout of cycling for 60 min at 74 ± 2% of $V\text{O}_{2\text{peak}}$ after a 12-h overnight fast. Expired air was collected twice, between 15 and 20 min and 40 and 45 min to ensure that subjects were exercising at the expected exercise intensity.

Muscle biopsies. Muscle samples were obtained from the vastus lateralis before and immediately after exercise using the percutaneous needle biopsy technique with suction (15). Muscle samples were immediately frozen in liquid nitrogen and stored for later analysis.

Protein extraction. Nuclear proteins were isolated as previously described (16). For whole muscle proteins, ~15 mg muscle were homogenized in 10 volumes of homogenization buffer (25 mmol/l Tris, pH 6.8, 1% Triton X-100, 5 mmol/l EGTA, 50 mmol/l NaF, 1 mmol/l Na orthovanidate, 10% glycerol, 1% phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin) for ~30 s on ice. The homogenate was then spun in a centrifuge for 5 min at 1,000*g* at 4°C. The supernatant was extracted and stored for later analysis. Protein concentration was determined using the BCA method.

Co-immunoprecipitation. Nuclear protein (500 μg) was made up to 500 μl in immunoprecipitation wash buffer (50 mmol/l Tris, pH 7.5, 1 mmol/l EDTA, 1 mmol/l EGTA, 10% glycerol, 1% Triton X-100, 50 mmol/l NaF, 5 mmol/l Na pyrophosphate, 1 mmol/l dithiothreitol, and 1 mmol/l phenylmethylsulfonyl fluoride). Samples were precleared with 50 μl protein A Sepharose beads (Amersham Biosciences, Castle Hill, Australia) before being incubated with 2 μl anti–MEF-2 (Santa Cruz Biotechnology, Santa Cruz, CA) antibody overnight at 4°C. Samples were again incubated with 50 μl protein A Sepharose beads for 2 h, while rotating at 4°C. The Sepharose-bound immune complex was pelleted by centrifugation and washed four times with 1 ml immunoprecipitation wash buffer. Immune complexes were then resolved by SDS-PAGE, and the amounts of HDAC5, PGC-1, p38, and phospho-p38 present in the complex were determined by probing with antibodies directed toward those proteins. MEF-2 threonine phosphorylation was analyzed by immunoprecipitating MEF-2 from 100 μg nuclear protein and probing with a threonine-proline phosphospecific antibody (Cell Signaling Technology, Beverly, MA). A control lane of beads only was included on all gels to ensure that the signals observed were specific to the immune complex and not the beads themselves. Membranes were reprobed with the MEF-2 antibody to ensure that equal amounts of MEF-2 protein had been pulled down.

Immunoblotting. Proteins were separated and identified using SDS-PAGE. A total of 30 μg protein from the whole-cell homogenate and 50 μg nuclear protein from each sample were loaded onto 1.5-mm 8% acrylamide gels before undergoing electrophoresis for 75 min at 150 V. Proteins were wet transferred to a nitrocellulose membrane for 100 min at 100 V. Membranes were blocked for 1 h in blocking buffer (5% skim milk powder in Tris-buffered saline and 0.25% Tween) and exposed overnight, at 4°C, to primary antibodies for HDAC5 (Cell Signaling Technology), NFAT (Santa Cruz Biotechnology), PGC-1 (Chemicon, Temecula, CA), p38, and phospho-p38 (Cell Signaling Technology). Membranes were exposed to appropriate anti-species horseradish peroxidase–conjugated secondary antibodies at a concentration of 1 in 10,000 in blocking buffer for 60 min at room temperature. Antibody binding was viewed by incubating in enhanced chemiluminescence substrate (Pierce SuperSignal Chemiluminescent; Pierce Biotechnology, Rockford, IL) and exposing to a Kodak Image Station 440CF (NEN Life Science Products, Boston, MA). Bands were identified and quantified using Kodak 1D Image Analysis Software (Eastman Kodak, Rochester, NY).

Real-time RT-PCR. Total RNA was extracted from ~10 mg muscle using the acid guanidium thiocyanate-phenol-chloroform extraction technique with modifications (FastRNA Kit-Green; Qbiogene, Carlsbad, CA). Oligo dT single-

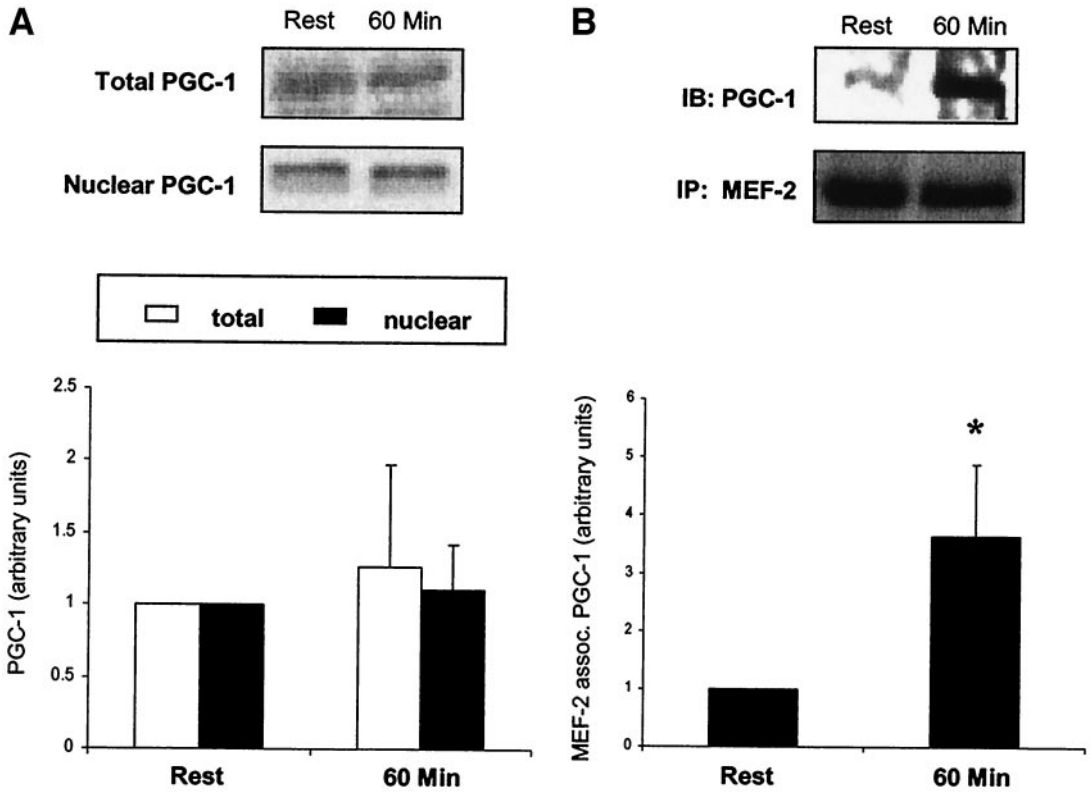

FIG. 2. ***A*: Total and nuclear PGC-1 protein in response to exercise.** ***B*: MEF-2–associated PGC-1 in response to exercise. All values are calculated as the fold changes relative to rest and are reported as the means ± SE (total $n = 7$; nuclear $n = 6$). Significantly different from rest: *$P = 0.041$. IB, immunoblot; IP, immunoprecipitate.**

stranded cDNA was synthesized using an AMV Reverse Transcriptase Kit (Promega A3500; Promega, Madison, WI). Forward and reverse primers complementary to the human GLUT4 gene (GenBank NM 001042) were designed using Primer Express software (Applied Biosystems, Foster City, CA). The GLUT4 forward primer sequence (5′ to 3′) was CTT CAT CAT TGG CAT GGG TTT, whereas the reverse primer sequence was AGG ACC GCA AAT AGA AGG AAG A. Real-time RT-PCR was performed using the GeneAmp 5700 sequence detector and software (Applied Biosystems, Scoresby, Australia). Changes in GLUT4 gene expression were normalized to the housekeeping gene cyclophilin.

Statistical analysis. All data are means ± SE, with resting samples assigned the arbitrary value of 1.0 and postexercise samples expressed relative to rest. Resting and postexercise means were compared using a *t* test with a significance level of 0.05.

RESULTS

GLUT4 mRNA. After exercise, GLUT4 mRNA was increased 2.1-fold ($P < 0.05$) when expressed as a ratio to the housekeeping gene cyclophilin. Cyclophilin mRNA expression did not change with exercise (data not shown).

HDAC5 association with MEF-2 and HDAC5 subcellular localization. HDAC5 association with MEF-2 decreased 26% ($P < 0.05$) after exercise (Fig. 1). This was associated with a 54% decrease ($P < 0.05$) in nuclear HDAC5 after exercise (Fig. 1), whereas there was no change in total HDAC5 protein content (Fig. 1).

Nuclear CaMK-IV and CaMK-II. While HDAC5 association with MEF-2 decreased after exercise and was associated with HDAC5 nuclear export, CaMK-IV was not detected in the nuclear fraction at either rest or after exercise (data not shown). Furthermore, the CaMK-IV detected in whole muscle homogenates was negligible (data not shown). Because we have recently found that CaMK-II activity is increased in contracting human skeletal muscle (17), we hypothesized that CaMK-II could possibly target nuclear HDACs. However, no CaMK-II was discovered in the nuclear fraction at either rest or after exercise (data not shown).

Nuclear NFAT. Nuclear NFAT content after exercise was similar to resting levels (rest, 50.7 ± 6.3 vs. 60 min, 51.1 ± 4.6 arbitrary units).

PGC-1 association with MEF-2 and PGC-1 subcellular localization. Exercise increased PGC-1 association with MEF-2 by 3.7-fold ($P < 0.05$; Fig. 2). However, exercise had no effect on total or nuclear PGC-1 abundance (Fig. 2).

p38 MAPK and phosphorylated p38 MAPK association with MEF-2 and subcellular localization. Total muscle p38 phosphorylation increased 4.8-fold ($P < 0.05$) and nuclear p38 phosphorylation increased 1.8-fold ($P < 0.05$) after exercise. However, there was no change in the abundance of total or nuclear p38 protein (Fig. 3). Association of p38 protein with MEF-2 increased 2.7-fold ($P < 0.05$) after exercise, whereas association of phosphorylated p38 with MEF-2 increased 1.8-fold ($P < 0.05$; Fig. 4).

MEF-2 threonine-proline phosphorylation. MEF-2 threonine-proline phosphorylation increased 2.7-fold ($P < 0.05$; Fig. 5) after exercise.

DISCUSSION

The initial step in the upregulation of MEF-2–mediated gene expression, including potentially that of GLUT4, is the dissociation of the class II HDACs from MEF-2 (9). It was found that HDAC5 association with MEF-2 significantly decreased 24% after exercise, suggesting that HDAC5 does regulate MEF-2 in human skeletal muscle and

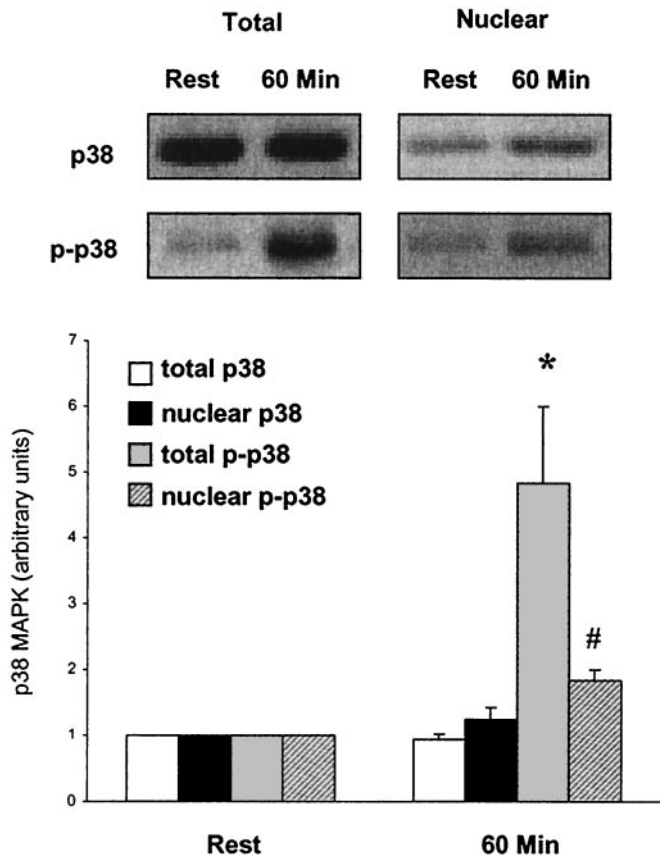

FIG. 3. Total and nuclear p38 and phosphorylated p38 (p-p38) in response to exercise. All values are calculated as the fold changes relative to rest and are reported as the means ± SE (n = 7). Significantly different from rest: *P = 0.016 and #P = 0.013.

is sensitive to exercise. Also observed was a 54% decrease in nuclear HDAC5 protein, whereas there was no change in total HDAC5 protein, suggesting that HDAC5 was exported from the nucleus. Although the assays used to generate these data are semiquantitative and nonstoichiometric, it does appear that more HDAC5 was exported from the nucleus than was dissociated from MEF-2. This could imply that HDAC5 regulates other exercise-responsive transcription factors in addition to MEF-2 and that HDAC5 could be an integral component in the molecular mechanisms leading to adaptations in response to exercise, such as enhanced muscle oxidative capacity and insulin sensitivity. Various studies have found that all of the class II HDACs (4, 5, 7, and 9) dissociate from MEF-2 and are exported from the nucleus in response to a variety of stimuli (18–20). This could imply that HDAC4, -5, -7, and -9 might all be responsive to exercise. Despite exercise dissociating HDAC5 from MEF-2 and inducing HDAC5 nuclear export, CaMK-IV was not detected in the nuclear fraction of human skeletal muscle either at rest or after exercise. Although various studies have suggested a direct role for CaMK-IV in phosphorylating HDAC5 and thereby causing dissociation from MEF-2 (10,19,21), it seems likely that other kinases with substrate specificities similar to CaMK-IV are able to phosphorylate HDAC5. This is supported by experiments using specific inhibitors of a variety of different kinases that have failed to block the phosphorylation of the class II HDACs in an in vitro kinase assay (22). Under these conditions, a broad specificity kinase inhibitor is needed to antagonize HDAC phosphorylation. Whereas CaMK-I has also been identified as a kinase capable of phosphorylating HDAC5 (19), there is little evidence to suggest that CaMK-I is present in human skeletal muscle. We have recently found that CaMK-II activity increases during exercise in human skeletal muscle (17). It has also been suggested that CaMK-II can phosphorylate cytosolic HDAC5 in neurons (23). However, no CaMK-II was detected in the nuclear fraction either at rest or after exercise, suggesting that CaMK-II was not responsible for the exercise-induced dissociation of HDAC5 from MEF-2 and subsequent HDAC5 nuclear export. A potential HDAC5 kinase could be AMP kinase (AMPK), which has also been implicated in the expression of the GLUT4 gene (24) and shares similar substrate specificity to the CaMKs. Furthermore, we have found that AMPK translocates to the nucleus in response to the same exercise protocol used in this study (16). Identification of the kinases involved in phosphorylating HDAC5 during exercise will be important in linking the molecular events regulating MEF-2 and the GLUT4 gene to a specific cellular stimulus generated during exercise, such as changes in energy balance or intracellular calcium (8).

Both the calcineurin/NFAT pathway (25) and PGC-1

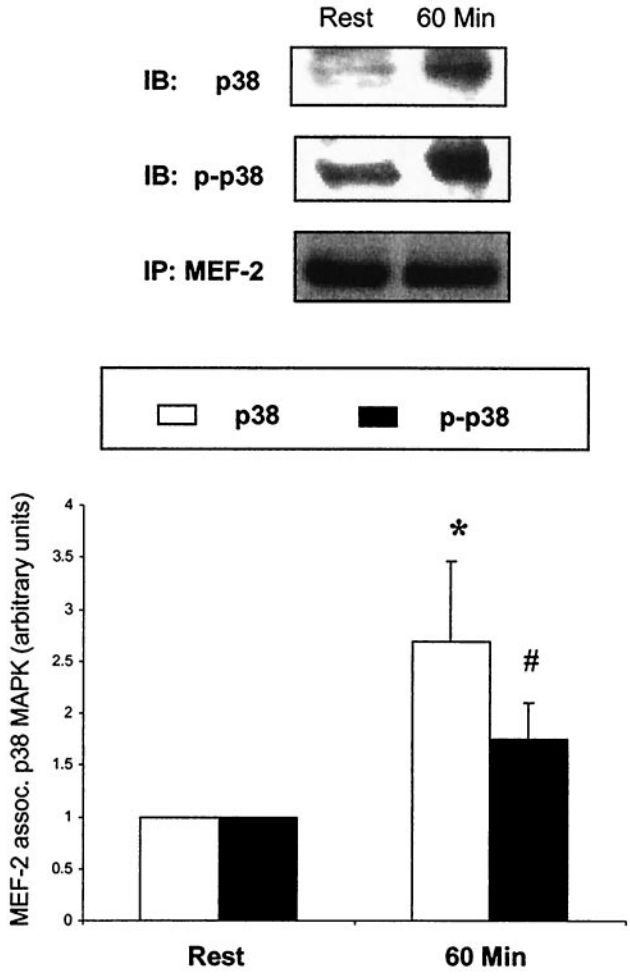

FIG. 4. MEF-2–associated p38 and phosphorylated p38 (p-p38) in response to exercise. All values are calculated as the fold changes relative to rest and are reported as the means ± SE (n = 7). Significantly different from rest: *P = 0.032 and #P = 0.035. IB, immunoblot; IP, immunoprecipitate.

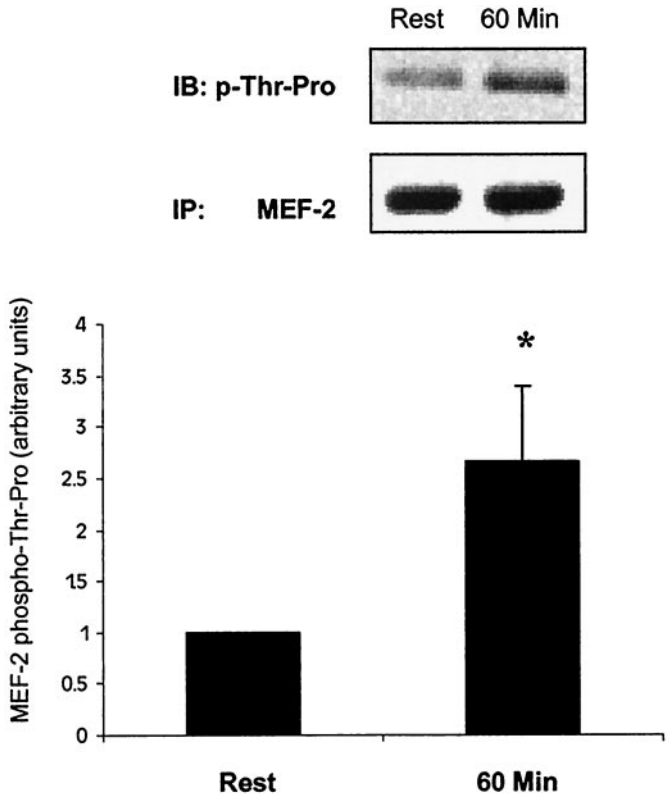

FIG. 5. MEF-2 threonine-proline phosphorylation in response to exercise. All values are calculated as the fold changes relative to rest and are reported as the mean ± SE (n = 7). Significantly different from rest: *P = 0.039. IB, immunoblot; IP, immunoprecipitate.

pathway (26) have been implicated in the regulation of the GLUT4 gene through recruitment of transcriptional coactivators possessing HAT activity to MEF-2. The critical regulatory step in the calcineurin pathway is the dephosphorylation and subsequent nuclear translocation of NFAT. Using an antibody specific for NFATc1 (NFAT2), the most abundant NFAT isoform in skeletal muscle (11), we found no nuclear translocation of NFAT after exercise. From these data, it appears that calcineurin does not regulate MEF-2 in humans during acute exercise. However, studies in mice have linked the calcineurin/NFAT pathway to MEF-2 activation in response to prolonged periods of contraction (27), and overexpression of constitutively active calcineurin results in many of the adaptations seen with exercise training (28), suggesting that calcineurin/NFAT might be more sensitive to prolonged exercise stimuli. It is also possible that an NFAT isoform other than NFATc1 could mediate effects of exercise on MEF-2. However, there is evidence that many signaling pathways activated during acute exercise are antagonistic to the calcineurin pathway, possibly supporting the data from the present study. Evidence exists that the p38 MAPK pathway is able to phosphorylate NFAT, retaining it to an extranuclear localization (29). Although the phosphorylation status of NFAT was not examined in the present study, p38 phosphorylation increased 4.8-fold after exercise. It has also been found that AMPK, which is activated during exercise of intensities >60% $V\text{O}_{2\text{peak}}$ (30), is able to antagonize transcriptional coactivators that are downstream of NFAT (31). Thus, although it seems that chronic or prolonged exercise is associated with changes in gene expression due to calcineurin activation, there is mounting evidence that the calcineurin pathway does not influence gene expression during acute exercise. Somewhat supporting this is evidence that NFAT is present in the nucleus only in response to prolonged low-amplitude calcium transients and is insensitive to high-amplitude transients (32). Although calcineurin has been implicated in changes in gene expression associated with fiber-type transformations with exercise (33), it seems likely that this occurs in the postexercise period. This makes evolutionary sense in that fiber-type transitions are not an immediate priority during exercise, as opposed to expression of exercise-responsive genes such as GLUT4, which could be critical to cell survival during exercise and in the immediate postexercise period. Although calcineurin could influence GLUT4 expression by switching fiber type from a fast to slow phenotype, because GLUT4 is more abundantly expressed in type 1 fibers (34), it appears that calcineurin may not be involved in the acute regulation of GLUT4 with exercise.

In contrast, we found that PGC-1 association with MEF-2 increased 3.7-fold after exercise, suggesting that it is PGC-1, rather than NFAT, that is responsible for recruiting coactivators with HAT activity to MEF-2 during exercise. Because we found no change in total or nuclear PGC-1 abundance, it seems that it is endogenous nuclear PGC-1 that is recruited to MEF-2. Once bound to a transcription factor, PGC-1 undergoes a conformational change that allows recruitment and binding of transcriptional coactivators (35). Although the localization of NFAT appears to be critical for its activity, the activity of PGC-1 appears to be regulated through association with a repressor (36,37). This association and the repression of PGC-1 appears to be broken by p38 MAPK (36,37). Although this was not assessed in this study, we did find that nuclear p38 phosphorylation increased 1.8-fold after exercise. It is thought that PGC-1 is primarily recruited to the MEF-2 isoforms C and D (38), whereas GLUT4 expression is primarily mediated by MEF-2A (39). Although this discrepancy could suggest that PGC-1 is not functionally involved in GLUT4 expression, it has been found that MEF-2A is required to form a heterodimer with MEF-2D to drive GLUT4 expression (39,40). The function of the MEF-2A/D heterodimer has not been established, but it is possible that the role of MEF-2D is to recruit transcriptional coactivators such as PGC-1 to MEF-2A. Supporting a role for PGC-1 in GLUT4 expression are data showing that overexpression of PGC-1 in GLUT4-deficient L6 myotubes restores GLUT4 mRNA levels to those observed in vivo (12). However, a recent study overexpressing PGC-1 in the skeletal muscle of mice found a downregulation of GLUT4 mRNA (41). Although these conflicting results are confounded by the different methods used in each study, the functional significance of PGC-1 binding to MEF-2 in contracting human skeletal muscle remains to be determined. Recent studies have found that PGC-1 transcription, mRNA, and protein are elevated after an acute bout of exercise (26,42). Whereas the recruitment of PGC-1 to transcription factors could determine PGC-1 function during exercise, the increase in PGC-1 after exercise could be important for postexercise adaptations such as mitochondrial biogenesis.

In the present study, total muscle p38 phosphorylation increased after exercise. This was associated with no change in total p38 protein. These findings are consistent with previous research (43). However, for the first time, we have demonstrated an increase in nuclear p38 phosphorylation after exercise. This was associated with no significant change in nuclear p38 protein, suggesting that the increase in nuclear p38 phosphorylation was largely due to phosphorylation of the endogenous nuclear p38 protein. Although not examined in this study, there are many kinases upstream that are capable of phosphorylating p38. The MAPK kinases (MKK)3, -4, and -6 are able to phosphorylate p38 (43), and it has also been observed that AMPK is upstream of p38 (44). It is unclear which, if any, of these kinases are responsible for the increase in nuclear p38 phosphorylation seen in the present study. We also observed that MEF-2–associated p38 and phosphorylated p38 increased after exercise. It is recognized that a distinct MEF-2A binding region on p38 exists and is required for p38 phosphorylation of MEF-2 (45). This study also found a binding domain for MKK3, indicating that MEF-2A, p38, and MKK3 can exist in a complex, possibly suggesting that it is MKK3 that is phosphorylating MEF-2–associated p38. Once associated, p38 can phosphorylate MEF-2A on its transcriptional activation domain on threonines 312 and 319 (14). As both of these threonine residues are followed by a proline residue, we immunoprecipitated MEF-2 from the nuclear fraction and probed with an antibody that only recognizes phosphorylated threonine residues that are followed by proline residues. Using this method, MEF-2 phosphorylation was found to increase 2.7-fold. Phosphorylation of MEF-2 on these residues is associated with an increase in MEF-2–mediated transcription (14), thus providing a functional link between p38 and MEF-2 regulation. This also suggests a potential role for p38 MAPK in the regulation of the GLUT4 gene.

Although many aspects of MEF-2 regulation were observed in this study, it was also found that GLUT4 mRNA was increased 2.1-fold after exercise, suggesting an increase in MEF-2 transcriptional activity. It is unclear if the dissociation of repressors, association of coactivators, and phosphorylation of MEF-2 are all required for enhanced MEF-2 transcriptional activity or whether each of these events are additive in nature. It is also unclear if regulation of MEF-2 through these mechanisms would increase MEF-2 DNA binding activity. Although increased MEF-2 DNA binding has been observed in humans after a marathon (46), studies investigating the class II HDACs found that the HDACs do not interfere with MEF-2 DNA binding, despite interacting with the DNA-binding MADS (MCMI, agamous, deficiens serum response factor) and MEF-2 domains of the MEF-2 proteins (10,47). Instead, these studies found that the HDACs repress MEF-2 transcriptional activity by inhibiting MEF-2 dimerization and associations with transcriptional coactivators. This could suggest that MEF-2 is DNA bound in the basal state. Furthermore, Wu and Olson (48) found that p38 activation is associated with enhanced MEF-2 transcriptional activity, without changes in MEF-2 DNA-binding activity. Additionally, others imply that MEF-2 DNA binding is a function of the abundance of the MEF-2 protein (7). In this study, we found that there was no change in the nuclear abundance of MEF-2 after exercise (data not shown). We have also conducted preliminary MEF-2 mobility shift assays on resting and exercised human skeletal muscle from this study and have been unable to detect differences in MEF-2 DNA binding. Whether this is a reflection of MEF-2 DNA binding in vivo or an issue with the sensitivity of the assay in our hands remains to be determined.

In conclusion, the results from the present study demonstrate that HDAC5, PGC-1, and p38 MAPK could regulate MEF-2 in response to exercise in human skeletal muscle, whereas we found no role for CaMK-IV, CaMK-II, and NFAT. These mechanisms could also regulate the expression of GLUT4 and other metabolic genes and might be therapeutic targets for the treatment and management of insulin resistance and type 2 diabetes.

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