REVIEW / SYNTHÈSE

Exercise and MEF2–HDAC interactions

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Abstract: Exercise increases the metabolic capacity of skeletal muscle, which improves whole-body energy homeostasis and contributes to the positive health benefits of exercise. This is, in part, mediated by increases in the expression of a number of metabolic enzymes, regulated largely at the level of transcription. At a molecular level, many of these genes are regulated by the class II histone deacetylase (HDAC) family of transcriptional repressors, in particular HDAC5, through their interaction with myocyte enhancer factor 2 transcription factors. HDAC5 kinases, including 5′-AMP-activated protein kinase and protein kinase D, appear to regulate skeletal muscle metabolic gene transcription by inactivating HDAC5 and inducing HDAC5 nuclear export. These mechanisms appear to participate in exercise-induced gene expression and could be important for skeletal muscle adaptations to exercise.

Key words: gene expression, skeletal muscle, HDAC5, AMPK, PKD.

Résumé : L'activité physique augmente la capacité métabolique du muscle squelettique et, de ce fait, améliore l'homéostasie énergétique de tout l'organisme et contribue aux bienfaits apportés pour la santé. Ces adaptations de l'organisme sont en partie médiées par l'augmentation de l'expression d'un certain nombre d'enzymes du métabolisme dont la régulation s'exerce dans la transcription. Au niveau moléculaire, plusieurs de ces gènes sont sous le contrôle de la classe II des histone-désacétylases (HDAC) qui agissent comme des répresseurs de la transcription, et notamment la HDAC5 kinase qui agit de concert avec les facteurs de transcription MEF2. Les HDAC kinases (AMPK et PKD) semblent réguler la transcription du gène métabolique dans le muscle squelettique par l'inactivation de HDAC5 et son exportation nucléaire. Ces mécanismes sont probablement impliqués dans l'expression des gènes causée par l'exercice physique et jouent probablement un rôle important dans les adaptations du muscle squelettique à l'exercice physique.

Mots-clés : expression génique, muscle squelettique, HDAC5, AMPK, PKD.

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Introduction

Exercise produces a number of bioenergetic adaptations in skeletal muscle that ultimately increase the capacity of this tissue to produce adenosine triphosphate (ATP). Such adaptations result in improved maintenance of energy homeostasis at a whole-body level and contribute to the positive health benefits of exercise. The mechanism by which exercise induces these adaptations is, in part, mediated by acute transcriptional regulation of a number of metabolic enzymes in skeletal muscle. Exercise is known to increase the expression of enzymes involved in substrate transport, carbohydrate metabolism, beta oxidation, and the electron transport chain. This is mediated largely at the level of transcription (Hood et al. 2006). As these pathways play key roles in both performance and metabolic disease, there has been considerable interest in understanding how these gene networks are regulated. A number of putative stimuli have been implicated in the adaptive response of skeletal muscle to exercise. These include perturbations in cellular energy balance associated with increased ATP turnover, and also elevations in intracellular calcium concentration as a result of excitation-contraction coupling (Ojuka 2004). However, few studies have identified molecular mechanisms involved in these responses. Analysis of the regulatory promoter regions of various exercise responsive genes reveals that many possess a binding region for the myocyte enhancer factor 2 (MEF2) family of transcription factors. Beyond embryogenesis, the MEF2 family includes isoforms A, C, and D, and although expressed in a number of tissues, they are most highly expressed in muscle cells. They form homodimers and heterodimers that bind AT-rich DNA sequences to regulate numerous biological processes (McKinsey et al. 2002*a*). Metabolic genes that possess confirmed MEF2 binding sites on their promoters include the peroxisome proliferator activated receptor gamma coactivator-1 (PGC-1) (Czubryt et al. 2003), glucose transporter isoform 4 (GLUT4) (Thai et al. 1998), uncoupling protein 3 (Acin et al. 1999), carnitine palmatoyl transferase 1 (Moore et al. 2003), and various cytochrome *c* oxidase subunits (Lenka et al. 1998). Given the important role that MEF2 plays in myoblast differentiation into myotubes, it might seem obvious that many of these genes expressed in muscle are regulated by MEF2. However, it is clear that acute regulation of MEF2 in the post-differentiated state following cell

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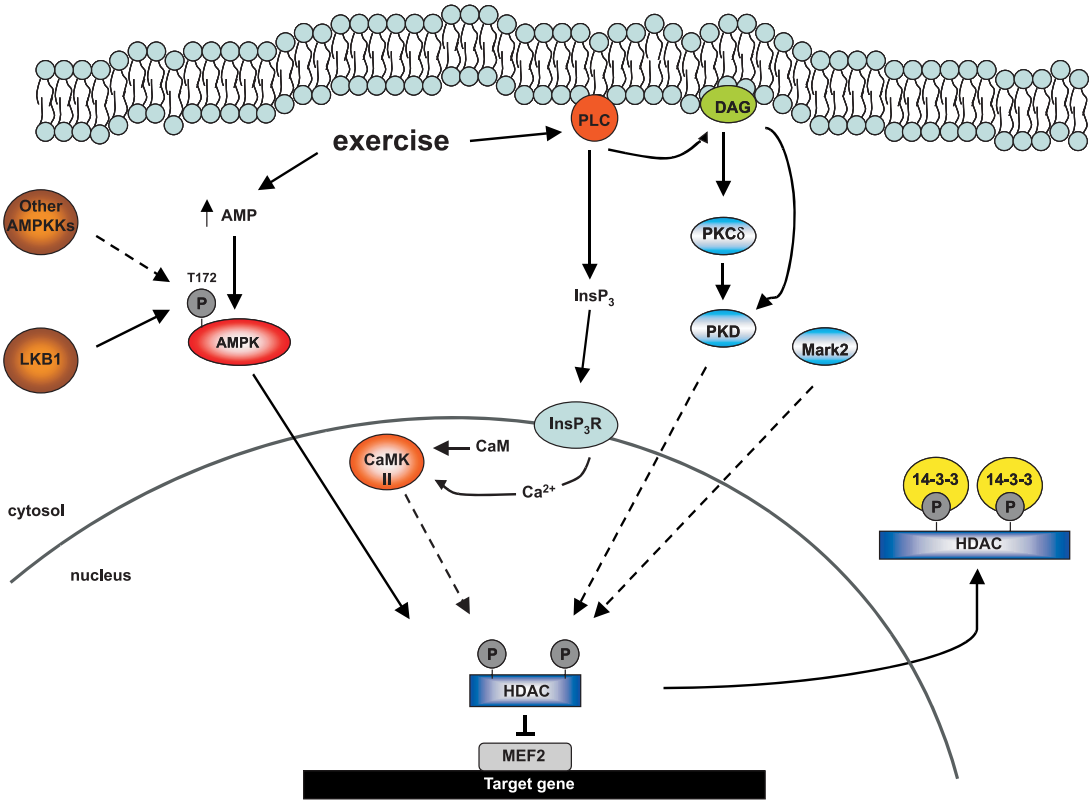
Fig. 1. Exercise-induced gene expression through regulation of HDAC5. Exercise increases intramuscular AMP concentrations that allow activation and phosphorylation of AMPK by LKB1 and other AMPK kinases. AMPK translocates to the nucleus, where it can then phosphorylate S259 and S498 on HDAC5. This results in HDAC5 dissociating from MEF2 and HDAC5 nuclear export via 14–3-3 proteins. Other alternate HDAC5 kinases include PKD, MARK2, and CaMKII (through local nuclear calcium signaling). Solid lines denote established steps, whereas steps signified by dashed lines are yet to be validated in the regulation of metabolic genes in skeletal muscle. Abbreviations: AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; AMPKK, AMP-activated protein kinase kinase; CaMKII, calmodulin-dependent protein kinase II; DAG, diacylglycerol; HDAC, histone deacetylase; $InsP_3$, inositol 1,4,5 triphosphate; LKB1, serine/threonine kinase; MARK2, microtubule affinity regulated kinase 2; MEF2, myocyte enhancer factor 2; P, phosphorus; PKC, protein kinase C; PKD, protein kinase D; PLC, phospholipase C.



cycle withdrawal can regulate MEF2-dependent gene expression (McKinsey et al. 2002*a*) and could be an important control point for skeletal muscle adaptation in response to exercise.

Regulation of MEF2

MEF2 regulation has been extensively studied in models of cardiac hypertrophy and T-cell proliferation. In the basal state, MEF2 is physically associated with members of the class IIa histone deacetylases (HDACs), which inhibit MEF2-dependent transcription via remodeling of surrounding chromatin. The class IIa HDACs catalyze the deacetylation of lysine residues within the chromatin-forming histone proteins, creating positively charged side chains that form tight electrostatic interactions with the negatively charged DNA phosphate backbone. This ensures that transcriptional coactivators and other enzymes required for transcription, such as RNA polymerase, are denied access to the surrounding region of DNA, resulting in repression of MEF2-dependent transcription (McKinsey et al. 2002*a*). This process is opposed by histone acetyltransferases (HATs), which acetylate histone lysine side chains and remove their positive charge, effectively disrupting the electrostatic and physical histone and DNA interaction. This grants transcriptional machinery access to DNA and allows MEF2-mediated transcription to proceed. The rate of transcription at any given moment appears to be a balance of HDAC and HAT activities (McKinsey et al. 2001). The numerous roles of HDACs in biological function are only just emerging; however, aberrant regulation of HDAC activity is associated with cardiac hypertrophy and cardiac sudden death (Olson et al. 2006). Furthermore, HDAC inhibitors are currently being tested in clinical trials for certain cancers (Kim et al. 2003). Initial characterization of HDAC5, a class IIa HDAC highly expressed in skeletal muscle, showed that HDAC activity is highly controlled by phosphorylation. Specifically, phosphorylation of S259 and S498 on HDAC5 dissociates HDAC5 from MEF2 and provides binding sites for 14–3-3 chaperone proteins that export HDAC5 from the nucleus via a chromosome region maintenance 1-dependent mechanism (McKinsey et al. 2000). The subsequent reduction in MEF2-associated HDAC activity allows MEF2-dependent transcription to

proceed. HDAC5 activity can also be regulated by allosteric interactions with activated calmodulin (Berger et al. 2003) and Gβγ proteins (Spiegelberg and Hamm 2005). Analysis of the other class IIa HDAC isoforms 4, 7, and 9 suggests that these regulatory mechanisms are conserved among this family of transcriptional repressors. However, the gene specificity of these isoforms and their interactions are yet to be elucidated.

HDAC regulation of metabolic gene transcription

These mechanisms appear to regulate a number of metabolic genes in skeletal muscle, as was recently demonstrated in vitro, where human primary myotubes were acutely treated with Scriptaid™, a HDAC inhibitor. This resulted in 6- and 7-fold increases ($p < 0.05$) in PGC-1 and GLUT4 mRNA, respectively (S.L. McGee and M. Hargreaves, unpublished). This not only demonstrates that some skeletal muscle metabolic genes are regulated by HDACs, but also reinforces that their regulation is a balance of HDAC and HAT activities. Indeed, this is supported by the fact that PGC-1 plays a large role in adaptive bioenergetics as a transcriptional coactivator that recruits HAT enzymes to numerous gene promoters (Knutti and Kralli 2001). Further evidence that HDACs regulate metabolic gene expression come from recent observations that overexpression of HDAC5 in L6 myotubes results in repression of a number of metabolic genes including GLUT4, PGC-1, cytochrome *c* oxidase subunit 4, and carnitine palmitoyltransferase 1 (S.L. McGee and K. Baar, unpublished). Furthermore, gene reporter assays using the human GLUT4 promoter region tagged to the firefly luciferase gene show that HDAC5 represses MEF2A-activated GLUT4 reporter gene activity. Together, these data indicate that metabolic gene expression is regulated by HDAC, and in particular HDAC5, activity. This paradigm also appears to operate in human skeletal muscle in response to exercise. Following 60 min of cycling at ~70% of $VO_{2\ peak}$, HDAC5 is dissociated from MEF2 and is exported from the nucleus (McGee and Hargreaves 2004). This is also associated with an increase in GLUT4 gene expression immediately after exercise. These data suggest that HDAC5 could be a key regulator of exercise-induced gene expression.

Regulation of HDAC5

As aberrant regulation of HDAC activity has become implicated in an increasing number of disease states, efforts to identify kinases capable of phosphorylating and regulating the class II HDACs have also increased. In this regard, most work has been performed on HDAC5. However, owing to the high sequence homology between the regulatory phosphorylation sites on all class II HDACs, it is thought that HDAC5 kinases are likely class II HDAC kinases (Chang et al. 2005). Initially, the calcium/calmodulin-dependent protein kinases (CaMK) I and IV were found to phosphorylate S259 and S498 on HDAC5 and to regulate HDAC5 activity and localization (McKinsey et al. 2000). However, pharmacological inhibition of CaMK activity fails to totally abolish HDAC5 kinase activity (McKinsey et al. 2002*b*), suggesting redundancy in signaling to HDAC5. Furthermore, skeletal muscle does not express CaMKI or IV (Rose et al. 2006). This led us to hypothesize that the 5′-AMP-activated protein kinase (AMPK) could be a potential HDAC5 kinase. AMPK is a heterotrimeric protein consisting of a catalytic α and regulatory β and γ subunits that are activated in response to metabolic stress via increases in the AMP to ATP ratio and a number of hormones (Kahn et al. 2005). Allosteric activation of AMPK by AMP allows phosphorylation of T172 on the catalytic α subunit by upstream kinases, such as the LKB1–STRAD–MO25 complex, and augmentation of AMPK activity. Exercise increases AMPK activity, and it is thought that AMPK plays a key role in exercise metabolism by increasing fatty acid oxidation and glucose uptake. Activation of AMPK has also been linked to exercise adaptations in skeletal muscle (Jorgensen et al. 2006). Although we have previously observed nuclear translocation of AMPK during exercise in human skeletal muscle (McGee et al. 2003), its physiological function in the nucleus was not determined. In vitro kinase assays revealed that AMPK is a HDAC5 kinase, and that S259 and S498 were required for this response (S.L. McGee et al. unpublished). Furthermore, this appears to be a functional relationship, as AMPK and HDAC5 associate in vivo and treatment of human primary myotubes with 5-aminoimidazole-4-carboxamide-β-D-ribofuranoside (AICAR), a pharmacological activator of AMPK, induces HDAC5 nuclear export, which is consistent with AMPK being a HDAC5 kinase. It also appears that AMPK regulates metabolic gene expression through this mechanism, as serine to alanine mutations of residues 259 and 498 on HDAC5 blocks induction of GLUT4 reporter gene expression in response to AICAR treatment. This was also assessed in an endogenous system through chromatin immunoprecipitation assays on AICAR-treated human primary myotubes. In these experiments, the increase in AMPK activity associated with AICAR treatment decreased the abundance of HDAC5 found at the MEF2 binding region on the GLUT4 promoter (S.L. McGee et al. unpublished). Collectively, these data suggest that AMPK regulation of HDAC5 could be an important mechanism regulating exercise-induced gene expression in skeletal muscle and reinforce the important role of HDAC5 in this process.

Other HDAC kinases

Despite these findings, multiple research groups, using a number of different animal models, have found that AMPK is dispensable for exercise-induced expression of various metabolic genes, thereby questioning the relevance of AMPK in this response. However, in an elegant screen for class II HDAC kinases, protein kinase D (PKD; also referred to as PKCμ) and microtubule affinity regulated kinase 2 (MARK2) were recently identified as novel class II HDAC kinases (Chang et al. 2005), illustrating further redundancy in HDAC signaling. Although originally categorized as a member of the PKC family of kinases, the catalytic domain of PKD shares high homology with the catalytic domains of the CaMK family (Rozengurt et al. 2005). It does, however, interact with diacylglycerol, produced at the plasma membrane by a variety of stimuli, similar to PKCs. Upon interacting with diacylglycerols, the catalytic domain of PKD is exposed and phosphorylated by novel PKCs on S744 and S748, resulting in autophosphorylation of serine 916. Phos-

phorylation of this residue correlates well with PKD activity. Once activated, PKD translocates to the nucleus, where it can participate in transcriptional regulatory processes. PKD has been implicated in membrane trafficking, cell survival, differentiation and proliferation, and diseases such as cardiac hypertrophy (Rozengurt et al. 2005), consistent with its role as a class II HDAC kinase. MARK2 is a member of the AMPK family of kinases and, like AMPK, is phosphorylated on its activation loop (T211) by upstream kinases, including the serine/threonine kinase LKB1 (Alessi et al. 2006). MARK2 appears to regulate cell polarity and has been implicated in the hyperphosphorylation of tau and therefore Alzheimer's disease (Kosuga et al. 2005). However, MARK2 appears to be constitutively active, and recent analysis of MARK3 (Goransson et al. 2006) would suggest that phosphorylation by atypical PKCs on multiple serine and threonine residues provides binding sites for 14–3-3 proteins that tether MARK2 to the cytosol to provide control over MARK2 activity.

The contribution of these kinases to exercise-induced gene expression is unclear; however, skeletal muscle MARK2 activity has been assessed in response to in vitro contractions, and consistent with previous studies in other tissues, MARK2 activity was unchanged (Sakamoto et al. 2004). Although MARK2 localization was not assessed in this study, it could suggest that MARK2 is not important for transcriptional control during exercise. Nonetheless, we are currently investigating the potential roles of PKD and MARK2 on skeletal muscle metabolic gene regulation. Furthermore, the potential role of CaMKII, the major CaMK isoform expressed in muscle, also warrants investigation. Exercise is known to increase skeletal muscle CaMKII activity (Rose et al. 2006), and although it is not a HDAC5 kinase, CaMKII does phosphorylate HDAC4 (Backs et al. 2006) and could be the mechanism by which calcium signaling participates in metabolic adaptation. Indeed, a recent paper examining the regulation of hypertrophy genes in cardiac tissue suggests a role for CaMKII in regulating HDACs (Wu et al. 2006). It was found that nuclear CaMKII was regulated by nuclear calcium fluxes that are dependent on phospholipase C-derived inositol 1,4,5-triphosphate ($InsP_3$) and $InsP_3$ receptors found at the nuclear envelope (Wu et al. 2006). This mechanism for calcium sensing at the nucleus is also independent of global calcium transients that occur during excitation-contraction coupling, and presents a potential mechanism for calcium-regulated gene expression in skeletal muscle during exercise. Finally, the role of phosphorylation-independent regulatory mechanisms on metabolic gene regulation also needs to be elucidated.

Summary

Control of skeletal muscle metabolic gene transcription appears to be regulated by the class IIa HDAC family of transcriptional repressors, and in particular HDAC5. AMPK and other HDAC5 kinases can regulate metabolic gene expression via regulation of HDAC5 activity and localization. As HDAC5 dissociation with MEF2 and HDAC5 nuclear export is observed during exercise, along with activation of HDAC5 kinases such as AMPK (Fig. 1), it is possible that these mechanisms participate in skeletal muscle adaptations to exercise.

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