Trends in Endocrinology & Metabolism

CellPress
REVIEWS

Review

Epigenetics and Exercise

Sean L. McGee[1,*] and Mark Hargreaves[2,*]

Epigenetics can be defined as 'the structural adaptation of chromosomal regions so as to register, signal, or perpetuate altered activity states.' Increased transcription of key regulatory, metabolic, and myogenic genes is an early response to exercise and is important in mediating subsequent adaptations in skeletal muscle. DNA hypomethylation and histone hyperacetylation are emerging as important crucial events for increased transcription. The complex interactions between multiple epigenetic modifications and their regulation by metabolic changes and signaling events during exercise, with implications for enhanced understanding of the acute and chronic adaptations to exercise, are questions for further investigation.

Highlights

Alterations in epigenetic marks, including DNA methylation and histone modifications, are linked to the skeletal muscle transcriptional responses to exercise that ultimately mediate the exercise adaptations.

Signaling pathways activated by exercise control the activity of epigenetic modifying enzymes, providing specificity to the exercise-induced transcriptional response.

Flux through metabolic pathways can impact on epigenetics directly by modulating the availability of the metabolic intermediates that are required for epigenetics marks, such as acetyl-CoA for acetylation and S-adenosyl-L-methionine for methylation. Furthermore, some metabolites, such as lactate, can directly regulate the activity of epigenetic modifying enzymes.

Emerging evidence suggests that epigenetic modifications can mediate the intergenerational transmission of exercise and diet effects on physiology.

Background

The Oxford dictionary defines epigenetics as 'the study of changes in organisms caused by the modification of gene expression rather than alteration of the genetic code itself.' Over the years in biology it has had several meanings, from how genotypes give rise to phenotypes during development, to heritable changes in gene function not attributable to changes in gene sequence [1]. A unifying definition that sought to reconcile contemporary usages with the requirement of heritability has been proposed [1]: 'the structural adaptation of chromosomal regions so as to register, signal, or perpetuate altered activity states.' The two most studied epigenetic events are DNA methylation and post-translational modification of histones, the major protein component of chromatin responsible for organizing DNA within nucleosomes (Figure 1). Other potential epigenetic modifications are those mediated by noncoding RNAs, notably microRNAs. These microRNAs can alter gene expression via post-transcriptional modulation and may also influence translational events. Identification of circulating microRNAs has raised the possibility that they are involved in cell–cell and tissue–tissue communication.

Exercise has profound effects on all physiological systems within the body [2] and regular physical activity/exercise training results in enhanced functional capacity and improved health and wellbeing. Much attention has focused on skeletal muscle responses and adaptations to acute and chronic exercise stimuli, since these adaptations contribute significantly to improved athletic performance and health. A complex array of molecular mechanisms, involving various signaling pathways and downstream mediators, has been identified in skeletal muscle [2,3]. In addition, contracting skeletal muscle releases a number of biologically active proteins, nucleic acids, and metabolites ('myokines') that may mediate some of the systemic effects of exercise [4]. Over the years, a paradigm has emerged that highlights the importance of transient increases in the mRNA levels of various metabolic, myogenic, and regulatory genes in response to a single bout of exercise, which when repeated on a regular basis as occurs during exercise training, leads to increased levels of key proteins that mediate many of the functional benefits of such training [3,5,6]. Experimental evidence in support of this paradigm has been reported [7–9]. Of course, enhanced translational capacity and proteome abundance are also key elements of the increased skeletal muscle metabolic function and mass following exercise training [10]. Nevertheless, much attention has focused on the early transcriptional events and their regulation in skeletal muscle in response to a single exercise bout and the potential contribution of epigenetic mechanisms. This

[1]Metabolic Research Unit, School of Medicine and Centre for Molecular and Medical Research, Deakin University, Geelong Waurn Ponds, VIC 3216, Australia
[2]Department of Physiology, The University of Melbourne, VIC 3010, Australia

*Correspondence:
sean.mcgee@deakin.edu.au (S.L. McGee) and
m.hargreaves@unimelb.edu.au (M. Hargreaves).

 https://doi.org/10.1016/j.tem.2019.06.002

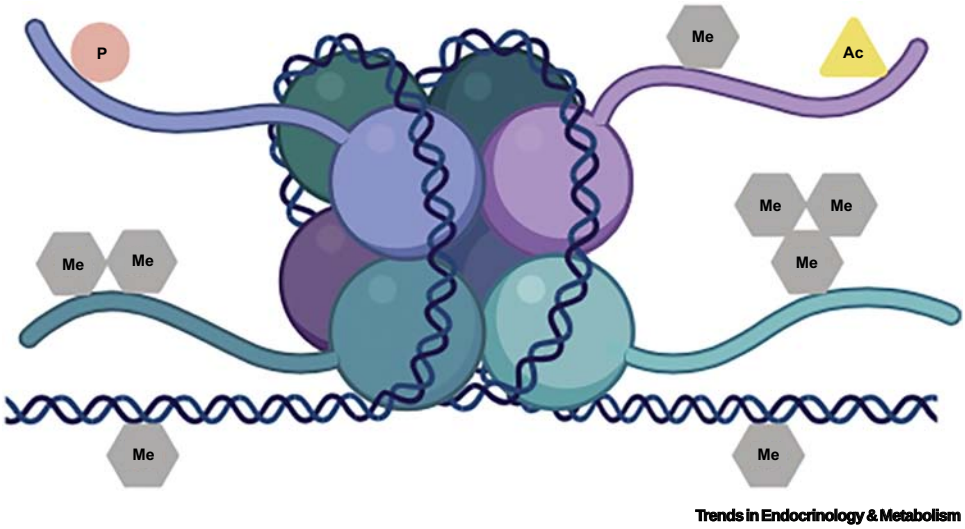

Figure 1. Major Types of Epigenetic Regulation. DNA methylation and histone (blue, green, and purple circles) post-translational modifications are capable of regulating gene transcription. Abbreviations: Me, methylation (mono-, di-, and tri-methylated lysine residues; mono- and di-methylated arginine residues); P, phosphorylation; Ac, acetylation. Figure prepared using BioRender.

review provides a brief overview of exercise effects on DNA methylation and histone modifications, predominantly from studies in humans. It will not address the contribution of microRNAs and readers are referred to a recent review on this topic [11].

DNA Methylation

DNA methylation involves the addition of a methyl group to the 5-carbon position of cytosine bases through the action of a family of DNA methyltransferases (DNMT). The cytosine bases most susceptible to methylation are often found in the cytosine-phosphate-guanine (CpG) dinucleotide sequences of DNA, referred to as CpG islands. Studies from both plants and animals have shown that the major biological consequence of DNA methylation is gene silencing [12]. It is also likely to be involved in the etiology of human disease. As an example, cytosine hypermethylation of peroxisome proliferator-activated receptor γ coactivator-1α (*PGC-1α*) promoter region is associated with reduced skeletal muscle mitochondrial content in patients with type 2 diabetes [13]. DNA methylation can directly prevent the binding of basal transcriptional machinery or of most mammalian transcription factors that require interactions with cytosine. These transcription factors, such as Sp1, have GC-rich binding sites and CpGs in their DNA recognition elements [12]. Methylated CpGs can also influence nucleosome stability or positioning and core histone access, thereby modifying access of transcription factors to promoter regions.

Given the increase in skeletal muscle mRNA levels of selected genes following a single bout of exercise, it is possible that changes in DNA methylation play a role in exercise-induced gene expression. Indeed, acute exercise in human subjects resulted in a reduction in global DNA methylation [14]. Further analysis of gene-specific DNA methylation indicated variable responses; however, for key metabolic and regulatory genes, such as *PGC-1α*, peroxisome proliferator-activated receptor δ (*PPAR-δ*), mitochondrial transcription factor A (*TFAM*), and myocyte enhancer factor 2 (*MEF2*), exercise-induced DNA hypomethylation was generally associated with increased gene expression, with effects being greater at higher exercise intensities [14]. Consistent with this

finding was the observation that acute exercise resulted in hypomethylation of the skeletal muscle *PGC-1α* promoter and altered nucleosome structure around this hypomethylated site, which correlated with the fold increase in *PGC-1α* gene expression and the postexercise decrease in intramuscular lipid content [15]. The underlying mechanisms mediating exercise-induced changes in DNA methylation remain to be fully elucidated. *Ex vivo* contraction in isolated rat soleus resulted in DNA hypomethylation and increased gene expression, suggesting intramuscular factors may be primary [14]. Caffeine produced similar responses in L6 myotubes, implicating calcium in the signaling between exercise and DNA methylation [14]. A single exercise bout produced dynamic changes in DNA methylation, with 130 hypomethylated and 110 hypermethylated regions in human adipose tissue [16]. There was only a modest association between DNA methylation and gene expression, suggesting that gene expression is only partly regulated by DNA methylation. Furthermore, despite acute exercise-induced gene expression changes being smaller in magnitude following 6 weeks of endurance training, the number of differentially methylated regions was more pronounced [16]. Again, there was not a clear association between the changes in DNA methylation and gene expression following training, which supports the idea that gene expression is only partly regulated by DNA methylation. In contrast, in skeletal muscle endurance, exercise training does appear to be associated with a relative DNA hypomethylation of key metabolic and regulatory genes, such as NADH:ubiquinone oxidoreductase subunit C2 (*NDUFC2*) and *MEF2A* [17,18]. An interesting observation after a second period of resistance training, following an initial resistance training program and subsequent detraining/unloading, was an increased frequency of hypomethylation with reloading (~18 800 CpG sites) compared with the initial loading (~9100 CpG sites), suggesting the potential of an 'epigenetic memory' of hypertrophy in human skeletal muscle [19]. The significance of this remains to be determined, although the concept of an exercise 'epigenetic memory' is not supported by well-controlled experiments examining the transcriptional response to endurance exercise [20]. Finally, a cross-sectional comparison of elite athletes and sedentary control subjects demonstrated polymorphisms in a number of enzymes involved in DNA methylation and synthesis in the elite athletes that contributed to a relative DNA hypomethylation and perhaps a greater potential to increase muscle mass [21]. Collectively, the results suggest that exercise generally results in DNA hypomethylation of key genes in skeletal muscle, and this represents an early response mediating skeletal muscle adaptations to exercise.

Histone Modifications

Although the skeletal muscle transcriptional responses to different exercise modalities has been well defined over the past decade, our understanding of the epigenetic mechanisms involved in regulating these responses is still rudimentary (Box 1). Exercise increases skeletal muscle histone 3 (H3) serine phosphorylation in both untrained and trained subjects [22]. Our own early work in this area identified global increases in H3 lysine (K36) acetylation in human skeletal muscle following a single 60 min bout of cycling at ~70% VO_{2peak} [23]. However, these studies did not examine the chromatin regions at which these histone modifications occurred. Indeed, there are only a handful of studies to date that have examined exercise-induced histone modifications at specific gene loci by chromatin immunoprecipitation (ChIP). Swimming exercise in rats increased H3 K9/14 acetylation, a histone mark associated with transcriptional activation, at the glucose transporter type 4 (*Glut4*) promoter in triceps muscle [24]. Similar data has been observed for the nuclear respiratory factor 1 (*Nrf-1*) and *Mef2A* promoter regions [25]. A recent study has examined histone acetylation and other epigenetic regulatory mechanisms across different regions of the *Pgc-1α* gene in response to treadmill running in rats. Interestingly, histone acetylation and RNA polymerase II (pol II) abundance across different regions of the *Pgc-1α* gene correlated poorly with the exercise-induced increase in *Pgc-1α* gene expression in both plantaris and soleus skeletal muscles, which are comprised of predominantly fast and slow fibers, respectively. However,

Box 1. DNA, Histones, and Nucleosome.

DNA is wrapped around an octamer of histones 2A, 2B, 3, and 4, which is referred to as a nucleosome [78]. Repeating nucleosomes together form chromatin, which has a complex three-dimensional structure that allows vast lengths of DNA to be efficiently packaged into the nucleus [78]. The structure of chromatin also plays a critical role in transcriptional regulation. In the transcriptionally repressed state, chromatin is densely compacted such that the transcriptional machinery, including polymerase II (Pol II), cannot gain access to promoter and gene regions to initiate transcription [79]. Specific post-translational modifications of histone proteins can decompact chromatin, which also requires histone exclusion or exchange from the nucleosome by chromatin remodeling enzymes and histone chaperones [80]. This state is generally permissive for transcriptional activation by exposing promoter and gene regions to the transcriptional machinery that initiates gene expression [79]. Histone modifications that activate transcription include: phosphorylation, acetylation, and methylation, as well as other acylation marks, which are emerging as novel histone post-translational modifications [81]. Lysine residues play an important role in the regulation of chromatin structure and function, as in the unmodified state the lysine side chain carries a positive charge that is the basis for histone–DNA and nucleosome–nucleosome interactions. Histone modifications, particularly acetylation by histone acetyltransferases (HATs), neutralizes the charge on the lysine side chain and is sufficient to break these electrostatic interactions in what is the first step of histone exclusion and transcriptional initiation [80]. However, not all histone modifications confer transcriptional activation and some instead favor transcriptional repression, including methylation of certain histone regions [82]. An overview of characterized histone modifications and their generalized effect on transcription has been reviewed recently [83]. These histone modifications are regulated by a network of histone modifying enzymes, the activity of which is regulated by intracellular signaling mechanisms that link extracellular stimuli with appropriate transcriptional responses. Conversely, transcriptional deactivation is controlled by transcriptional repressors, including histone deacetylases (HDACs), which promote the remodeling of chromatin back into its compacted state by removing histone modifications that initiate transcription. These modifications are transient, with the half-life of acetylated histones being ~3 min and phosphorylated histones ~20 min [84].

the actual abundance of H3 across the *Pgc-1α* gene was the best predictor of *Pgc-1α* gene expression, suggesting that histone exchange patterns could play in important role in regulating exercise-induced transcriptional responses. Furthermore, an increase in H3 K4 trimethylation during exercise at the alternative upstream *Pgc-1α* promoter was associated with expression of the alternative *Pgc-1α* transcript [26]. This same histone modification was not observed at the canonical promoter and this study remains the only one to examine a specific histone methylation mark at a promoter level following exercise. Intriguingly, a number of studies have examined histone modifications in various brain regions in response to exercise. Forced swimming induces H3 K14 acetylation and serine 10 phosphorylation in spatially distinct regions of the dentate gyrus in a time-dependent manner [27]. Furthermore, voluntary exercise in rats increases H3 acetylation at the brain-derived neurotrophic factor (*Bdnf*) promoter in the hippocampus [28]. These studies were among the first to associate changes in histone modifications with alterations in exercise-induced gene expression. It is possible that the endocrine functions of skeletal muscle during exercise that link muscle contraction with adaptive responses to exercise in tissues, such as the liver and adipose tissue, could also mediate an exercise transcriptional response in the brain.

Although there is a paucity of information describing the specific histone modifications regulating gene expression responses to exercise, the signaling mechanisms involved in the adaptive response to exercise in skeletal muscle are better defined. Pathways, including the AMP-activated protein kinase (AMPK), mitogen activated protein kinases (MAPK), protein kinase A (PKA), protein kinase C (PKC), and the calcium/calmodulin protein kinase II (CaMKII), are all important for phosphorylation-dependent signaling in skeletal muscle during exercise [29]. A number of studies have linked these signaling pathways with specific histone modifications. A critical stimulus driving exercise adaptations is reduced cellular energy balance, as muscle contraction markedly increases ATP requirements. The heterotrimeric AMPK complex is an important sensor of low cellular energy balance, and complexes containing the α_2 catalytic subunit are activated by endurance-based exercise of moderate intensity [30]. Activation of AMPK induces transcriptional responses that provide protection against future energy perturbations, including mitochondrial biogenesis and increased transport and handling capacity of glucose and fatty acids [31–33].

An extensive compendium of AMPK substrates now exists and a number of important regulators of histone modifications have been identified as bona fide AMPK substrates [34,35]. Although much of this work has not been verified in skeletal muscle or in the exercise adaptive response, these mechanisms give insights into potentially important pathways by which epigenetics and transcription are controlled by exercise. Our own work has established the class IIa histone deacetylases (HDACs) as AMPK substrates, with phosphorylation resulting in their nuclear export and derepression of the MEF2 transcription factor [36,37]. Class IIa HDAC phosphorylation following AMPK activation results in acetylation of H3 at K9 and 14 [36], modifications that are associated with transcriptional initiation. The class IIa HDACs do not possess catalytic activity against acetyl-lysine due to a critical amino acid substitution in the otherwise homologous HDAC active site [38], but instead recruit a corepressor complex that includes HDAC3 [39]. Indeed, genetic and pharmacological disruption of the class IIa HDAC corepressor complex induces exercise-like transcriptional responses in skeletal muscle [40]. Regulation of class IIa HDAC phosphorylation during exercise also highlights the redundancy in the signaling mechanisms controlling important exercise adaptive responses. In AMPK α_2 dominant negative mice, class IIa HDAC phosphorylation was maintained during exercise by a compensatory increase in protein kinase D (PKD) activation [41], an alternative class IIa HDAC kinase [42]. Although PKD is not normally activated by exercise [23], expression of constitutively active PKD in myogenic cells increases the expression of exercise-responsive genes in a class IIa HDAC phosphorylation-dependent manner, including *PGC-1α, PPARα*, and *GLUT4* [41]. Furthermore, CaMKII is also a class IIa HDAC kinase [43] that has been linked to gene expression responses to exercise. Administration of a moderately specific CaMKII inhibitor to rats prior to an acute bout of exercise is sufficient to prevent histone acetylation at the *GLUT4* promoter around its MEF2 binding site [24]. In addition to kinase control of HDAC function, a number of metabolites produced during exercise, including lactate [44] by skeletal muscle and β-hydroxybutyrate [45] by the liver, can act as direct inhibitors of HDACs at high physiological concentrations. For example, it has been reported that the increase in BDNF expression in the brain following exercise is due to the inhibitory action of β-hydroxybutyrate on HDAC2 and 3 at the *Bdnf* promoter [46]. The contribution of lactate to epigenetic control during exercise remains to be effectively quantified.

Interestingly, both AMPK and CaMKII also phosphorylate H3 directly [34,47]. It is thought that phosphorylation of this site is required before H3 can be acetylated [48], highlighting the stepwise control of chromatin decompaction and histone exclusion mechanisms that are required to initiate transcription. It also highlights that signaling pathways mediating exercise adaptations, and transcriptional control more broadly, typically regulate multiple epigenetic mechanisms. Redundancy could also explain many of the findings from exercise studies in histone acetyltransferase (HAT) loss of function in animal models, which collectively have failed to definitively identify HATs that mediate chromatin remodeling during exercise. For example, knockout of p300 has no effect on the skeletal muscle adaptations to exercise training [49], despite p300 being one of the most important acetyltransferases mediating histone acetylation [50]. Indeed, there appears to be considerable overlap in substrate specificity between the five major acetyltransferase families [51]. Similar findings have been observed for the transcriptional coactivator PGC-1α, which was thought to be an essential contributor to the transcriptional response to exercise through its ability to recruit HATs to specific transcription factors [52,53]. However, skeletal muscle-specific *Pgc-1α* knockout mice have normal skeletal muscle transcriptional responses to acute exercise [54,55]. These studies highlight the importance of the adaptive response to exercise and the apparent redundancy designed to protect this response. Indeed, organismal survival throughout evolution was highly dependent on the ability to adapt to the energetics demands imparted by the need for safety, food, and shelter. So fundamental for survival is this trait, it

appears that molecular redundancy has evolved to protect it. Given this redundancy, new approaches beyond simple genetic manipulation will be required to tease out the signaling and epigenetic mechanisms controlling the exercise adaptive response.

Metabolism and Epigenetic Modifications

Investigations over the past decade are beginning to detail interactions between metabolic pathway flux and the regulation of epigenetic modifications (Figure 2). For example, acetylation and methylation reactions require metabolite intermediates from specific metabolic pathways in the form of acetyl-CoA and S-adenosyl-L-methionine (SAMe), respectively. It appears that a variety of substrates can provide the acetyl-CoA required for histone acetylation in different cell types and under different energetic contexts. Initial studies in transformed cells suggested that glucose was the predominant substrate providing the nucleocytosolic acetyl-CoA required for histone acetylation [56]. Although glucose-derived acetyl-CoA is produced in the mitochondria, mitochondrial citrate is exported to the cytosol via the citrate transporter before being converted

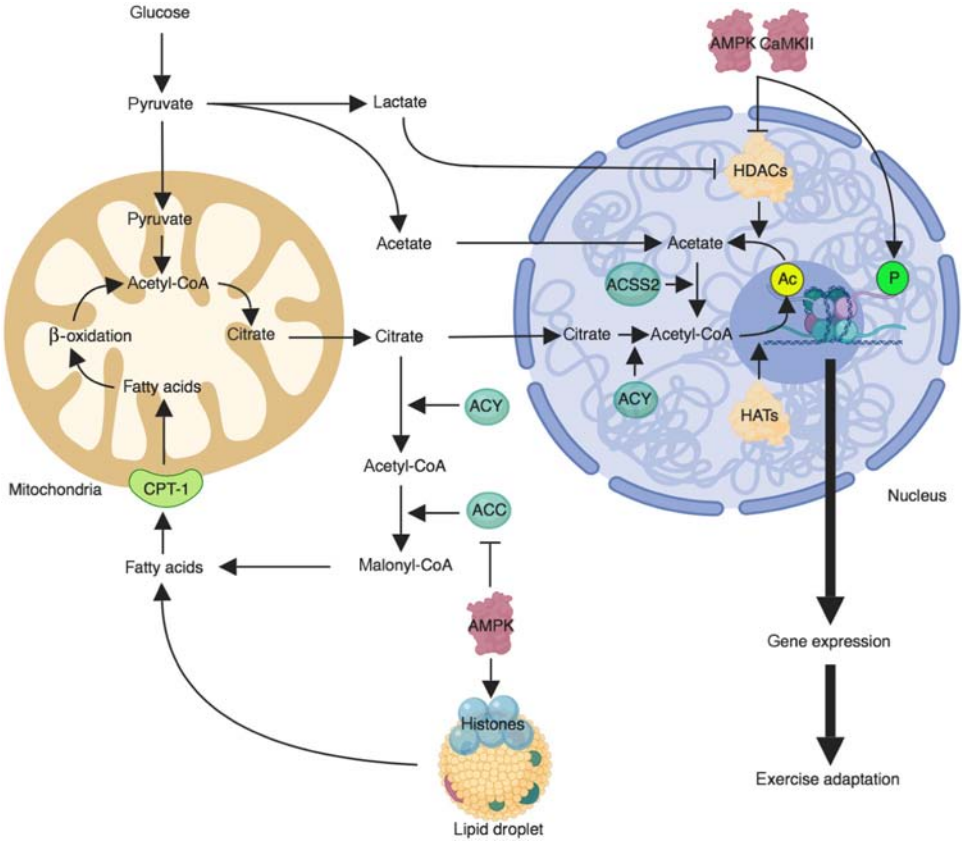

Figure 2. Established and Potential Interactions between Intermediary Metabolism and Histone Modifications. Metabolic pathways that produce acetyl-CoA are linked to histone acetylation (Ac) through the actions of histone acetyltransferases (HATs), while histone deacetylases (HDACs) oppose this process. Key enzymes that produce acetyl-CoA from citrate and acetate are ATP-citrate lyase (ACY) and acetyl-CoA synthetase 2 (ACSS2). Exercise-responsive kinases, including the AMP-activated protein kinase (AMPK) and the calcium/calmodulin-dependent protein kinase II (CaMKII), phosphorylate (P) histones. Regulation of histones associated with lipid droplets might also play a role in the supply of fatty acids. Abbreviations: ACC, acetyl-CoA carboxylase; CPT-1, carnitine palmitoyl-transferase 1. Figure prepared using BioRender.

back to acetyl-CoA by ATP-citrate lyase (ACY) [56]. A recent study has also suggested that acetyl-CoA synthetase (ACSS2), which converts acetate to acetyl-CoA, is also important for histone acetylation [57]. *De novo* acetate production was thought be negligible in mammalian cells until a recent study identified a new pathway of acetate synthesis directly from pyruvate, through two distinct mechanisms that involve either reactive oxygen species or keto acid dehydrogenases [58], further linking glucose utilization and histone acetylation. Studies in nontransformed cells suggest that the majority of acetyl-CoA required for histone acetylation is derived from fatty acid oxidation [59]. Intriguingly, using stable isotope tracking of fatty acid-derived carbon, fatty acid-mediated histone acetylation was linked to specific transcriptional reprogramming of lipid metabolism [59]. It is noteworthy that AMPK also regulates histone acetylation through the control of metabolic pathway flux. AMPK is a well-established inhibitor of the acetyl-CoA carboxylase (ACC) enzyme that converts acetyl-CoA to malonyl-CoA, the first step in fatty acid synthesis. Malonyl-CoA is an allosteric inhibitor of the carnitine palmitoyl-transferase 1 (CPT-1) transporter of fatty acids into the mitochondria. While phosphorylation-dependent regulation of ACC is the major mechanism by which AMPK increases β-oxidation of fatty acids, including during exercise [60], ACC inhibition also increases the amount of acetyl-CoA available for histone acetylation [61,62]. This highlights the coordinated control of multiple epigenetic regulatory mechanisms by signaling mediators of the epigenetic responses.

It has also been speculated that the interaction between metabolism and histone acetylation could be bidirectional. Based on the number of known histone acetylation sites, chromatin can consume up to 3 mM of acetyl-CoA, while the cellular concentration of acetyl-CoA is maintained around 20 μM [63]. As deacetylation reactions produce acetate, this suggests that histones are a large reservoir of this metabolite, which can be used to regenerate nucleocytosolic acetyl-CoA for metabolic or signaling purposes. Acetate also has important roles in the regulation of cellular pH [64]. Histones have also been identified in the proteome of lipid droplets in a variety of cell types [65–68]. While these initial findings focused on the ability of lipid droplets to control the supply of histones throughout development [66] and as a store that can release histones that act as antimicrobial agents [68], it is also possible that control of histone function is important for regulation of fatty acid metabolism. The interaction between metabolism and histone acetylation raises a number of important questions regarding exercise-mediated epigenetic regulation that remain to be resolved. For example, how is histone acetylation increased at exercise-responsive gene regions during exercise when there is high energetic demand? What substrates supply this acetyl-CoA? Does histone acetylation during exercise require acetyl-CoA production, or is it simply dependent on acetate redistribution within distinct chromatin regions? Does the histone acetate reservoir play a role in skeletal muscle metabolism or pH regulation during exercise? Is the regulation of histones at lipid droplets important for fatty acid metabolism during exercise? These are just a few of the questions that will shape research within the field in the coming years. Similarly, the DNA demethylation events that have been characterized during exercise could also have important metabolic implications. Demethylation reactions produce formaldehyde from the liberated methyl group, which through its detoxification produces reducing equivalents in the form of NADH [63], which could be coupled to mitochondrial ATP production. Chromatin can consume up to 11 mM of SAMe [63] and could therefore provide a significant energy source during exercise. It is clear that the field is only just scratching the surface with regard to the important interactions between epigenetics and metabolism and how they influence exercise adaptive responses.

Intergenerational Transmission

A key question is whether the effects of exercise in parents can be transmitted to offspring and, if so, whether this is mediated by epigenetic modifications. It is well recognized that the parental, intrauterine, and postnatal environments can have profound effects on the health outcomes of

offspring and this has led to testing of the 'developmental origins of health and disease hypothesis.' Perinatal, maternal exercise has been shown to improve the metabolic health of offspring [69–71] and to prevent the deleterious effects of a maternal high-fat diet on the metabolic health of offspring [72]. Paternal exercise has also been shown to improve glucose metabolism in offspring, related to improved sperm motility and alterations in multiple classes of small RNAs in sperm [73]. Preconception exercise in obese fathers has been shown to normalize the sperm microRNA profile and improve the metabolic health of offspring [74]. Other studies have observed exercise-induced changes in sperm microRNA expression and methylation in relation to genes associated with neurological function [75,76]. Finally, exercise prevented hypermethylation of the *Pgc-1α* promoter in offspring of mothers on a high-fat diet, which resulted in enhanced *Pgc-1α* and its target gene expression and improved metabolic function in offspring [77]. Collectively, these results implicate epigenetic modifications as mediators of intergenerational transmission of exercise and diet effects. Given the increasing prevalence of obesity and metabolic dysfunction and the impact that they have on the development of a number of chronic diseases, this is an important area for future investigation. Defining the specific mechanisms mediating the benefits of maternal and paternal exercise on offspring will be an important step in developing optimized exercise interventions to improve the health and wellbeing of potential parents and to mitigate transmission of such characteristics to their offspring. Such endeavors will be vital to attenuate the increasing prevalence of chronic disease in modern society.

Concluding Remarks

Increased transcription of key regulatory, metabolic, and myogenic genes is an early response to exercise and is important in mediating subsequent adaptations in skeletal muscle. DNA hypomethylation and histone hyperacetylation are emerging as important crucial events for increased transcription. There are likely to be complex interactions between these epigenetic modifications and this is an area for future research, together with investigation of the metabolic changes and signaling events during exercise that may influence such modifications. Enhanced understanding of these interactions will inform optimization of exercise interventions and the development of novel therapeutic strategies to manage metabolic disease (see Outstanding Questions).

Outstanding Questions

What are the specific epigenetic marks that activate transcription of genes during exercise, and what are the epigenetic modifying enzymes involved?

How is histone acetylation increased at exercise-responsive gene regions during exercise when there is high energetic demand? What substrates supply this acetyl-CoA? Does histone acetylation during exercise require acetyl-CoA production, or is it simply dependent on acetate redistribution within distinct chromatin regions?

Does the histone acetate reservoir play a role in skeletal muscle metabolism or pH regulation during exercise?

Is the regulation of histones at lipid droplets important for fatty acid metabolism during exercise?

What are the specific epigenetic mechanisms involved in the intergenerational transmission of exercise adaptations? Are these adaptations persistent, or are they lost with inactivity?

References

1. Bird, A. (2007) Perceptions of epigenetics. *Nature* 447, 396–398
2. Hawley, J.A. *et al.* (2014) Integrative biology of exercise. *Cell* 159, 738–749
3. Egan, B. and Zierath, J.R. (2013) Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab.* 17, 162–184
4. Whitham, M. and Febbraio, M.A. (2016) The ever-expanding myokinome: discovery challenges and therapeutic implications. *Nat. Rev. Drug Discov.* 15, 719–729
5. Booth, F.W. and Neufer, P.D. (2012) Exercise genomics and proteomics. In *ACSM's Advanced Exercise Physiology* (2nd edn) (Farrell, P.A., *et al.*, eds), pp. 669–698, Lippincott Williams & Wilkins
6. Williams, R.S. and Neufer, P.D. (1996) Regulation of gene expression in skeletal muscle by contractile activity. In *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems* (1st edn) (Rowell, L.B. and Shepherd, J.T., eds), pp. 1124–1150, Oxford University Press
7. Egan, B. *et al.* (2013) Time course analysis reveals gene-specific transcript and protein kinetics of adaptation to short-term aerobic exercise training in human skeletal muscle. *PLoS One* 8, e74098
8. Kraniou, G.N. *et al.* (2004) Effect of short-term training on GLUT-4 mRNA and protein expression in human skeletal muscle. *Exp. Physiol.* 89, 559–563
9. Perry, C.G. *et al.* (2010) Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J. Physiol.* 588, 4795–4810
10. Robinson, M.M. *et al.* (2017) Enhanced protein translation underlies improved metabolic and physical adaptations to different exercise training modes in young and old humans. *Cell Metab.* 25, 581–592
11. Russell, A.P. and Lamon, S. (2015) Exercise, skeletal muscle and circulating microRNAs. *Prog. Mol. Biol. Transl. Sci.* 135, 471–496
12. Bird, A.P. and Wolffe, A.P. (1999) Methylation-induced repression – belts, braces, and chromatin. *Cell* 99, 451–454
13. Barres, R. *et al.* (2009) Non-CpG methylation of the PGC-1α promoter through DNMT3B controls mitochondrial density. *Cell Metab.* 10, 189–198
14. Barres, R. *et al.* (2012) Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metab.* 15, 405–411
15. Bajpeyi, S. *et al.* (2017) Skeletal muscle PGC1α -1 nucleosome position and -260 nt DNA methylation determine exercise response and prevent ectopic lipid accumulation in men. *Endocrinology* 158, 2190–2199
16. Fabre, O. *et al.* (2018) Exercise training alters the genomic response to acute exercise in human adipose tissue. *Epigenomics* 10, 1033–1050
17. Lindholm, M.E. *et al.* (2014) An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. *Epigenetics* 9, 1557–1569

18. Nitert, M.D. *et al.* (2012) Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. *Diabetes* 61, 3322–3332
19. Seaborne, R.A. *et al.* (2018) Human skeletal muscle possesses an epigenetic memory of hypertrophy. *Sci. Rep.* 8, 1898
20. Lindholm, M.E. *et al.* (2016) The impact of endurance training on human skeletal muscle memory, global isoform expression and novel transcripts. *PLoS Genet.* 12, e1006294
21. Terruzzi, I. *et al.* (2011) Genetic polymorphisms of the enzymes involved in DNA methylation and synthesis in elite athletes. *Physiol. Genomics* 43, 965–973
22. Yu, M. *et al.* (2003) Metabolic and mitogenic signal transduction in human skeletal muscle after intense cycling exercise. *J. Physiol.* 546, 327–335
23. McGee, S.L. *et al.* (2009) Exercise-induced histone modifications in human skeletal muscle. *J. Physiol.* 587, 5951–5958
24. Smith, J.A. *et al.* (2008) CaMK activation during exercise is required for histone hyperacetylation and MEF2A binding at the MEF2 site on the Glut4 gene. *Am. J. Physiol. Endocrinol. Metab.* 295, E698–E704
25. Joseph, J.S. *et al.* (2017) Exercise increases hyper-acetylation of histones on the *cis*-element of NRF-1 binding to the *Mef2a* promoter: implications on type 2 diabetes. *Biochem. Biophys. Res. Commun.* 486, 83–87
26. Lochmann, T.L. *et al.* (2015) Epigenetic modifications of the PGC-1α promoter during exercise induced expression in mice. *PLoS One* 10, e0129647
27. Chandramohan, Y. *et al.* (2008) The forced swimming-induced behavioural immobility response involves histone H3 phosphoacetylation and c-Fos induction in dentate gyrus granule neurons via activation of the *N*-methyl-D-aspartate/extracellular signal-regulated kinase/mitogen- and stress-activated kinase signaling pathway. *Eur. J. Neurosci.* 27, 2701–2713
28. Gomez-Pinilla, F. *et al.* (2011) Exercise impacts brain-derived neurotrophic factor plasticity by engaging mechanisms of epigenetic regulation. *Eur. J. Neurosci.* 33, 383–390
29. Hoffman, N.J. *et al.* (2015) Global phosphoproteomic analysis of human skeletal muscle reveals a network of exercise-regulated kinases and AMPK substrates. *Cell Metab.* 22, 922–935
30. Chen, Z.P. *et al.* (2003) Effect of exercise intensity on skeletal muscle AMPK signaling in humans. *Diabetes* 52, 2205–2212
31. McGee, S.L. and Hargreaves, M. (2010) AMPK-mediated regulation of transcription in skeletal muscle. *Clin. Sci. (Lond.)* 118, 507–518
32. McGee, S.L. and Hargreaves, M. (2008) AMPK and transcriptional regulation. *Front. Biosci.* 13, 3022–3033
33. Herzig, S. and Shaw, R.J. (2018) AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat. Rev. Mol. Cell Biol.* 19, 121–135
34. Schaffer, B.E. *et al.* (2015) Identification of AMPK phosphorylation sites reveals a network of proteins involved in cell invasion and facilitates large-scale substrate prediction. *Cell Metab.* 22, 907–921
35. Ducommun, S. *et al.* (2015) Motif affinity and mass spectrometry proteomic approach for the discovery of cellular AMPK targets: identification of mitochondrial fission factor as a new AMPK substrate. *Cell. Signal.* 27, 978–988
36. McGee, S.L. *et al.* (2008) AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes* 57, 860–867
37. McKinsey, T.A. *et al.* (2000) Signal-dependent nuclear export of a histone deacetylase regulates muscle differentiation. *Nature* 408, 106–111
38. Lahm, A. *et al.* (2007) Unraveling the hidden catalytic activity of vertebrate class IIa histone deacetylases. *Proc. Natl. Acad. Sci. U. S. A.* 104, 17335–17340
39. Fischle, W. *et al.* (2002) Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. *Mol. Cell* 9, 45–57
40. Gaur, V. *et al.* (2016) Disruption of the class IIa HDAC corepressor complex increases energy expenditure and lipid oxidation. *Cell Rep.* 16, 2802–2810
41. McGee, S.L. *et al.* (2014) Compensatory regulation of HDAC5 in muscle maintains metabolic adaptive responses and metabolism in response to energetic stress. *FASEB J.* 28, 3384–3395
42. Chang, S. *et al.* (2005) An expression screen reveals modulators of class II histone deacetylase phosphorylation. *Proc. Natl. Acad. Sci. U. S. A.* 102, 8120–8125
43. Backs, J. *et al.* (2006) CaM kinase II selectively signals to histone deacetylase 4 during cardiomyocyte hypertrophy. *J. Clin. Invest.* 116, 1853–1864
44. Latham, T. *et al.* (2012) Lactate, a product of glycolytic metabolism, inhibits histone deacetylase activity and promotes changes in gene expression. *Nucleic Acids Res.* 40, 4794–4803
45. Shimazu, T. *et al.* (2013) Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science* 339, 211–214
46. Sleiman, S.F. *et al.* (2016) Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body β-hydroxybutyrate. *Elife* 5, e15092
47. Awad, S. *et al.* (2013) Nuclear CaMKII enhances histone H3 phosphorylation and remodels chromatin during cardiac hypertrophy. *Nucleic Acids Res.* 41, 7656–7672
48. Lo, W.S. *et al.* (2000) Phosphorylation of serine 10 in histone H3 is functionally linked *in vitro* and *in vivo* to Gcn5-mediated acetylation at lysine 14. *Mol. Cell* 5, 917–926
49. LaBarge, S.A. *et al.* (2016) p300 is not required for metabolic adaptation to endurance exercise training. *FASEB J.* 30, 1623–1633
50. Lee, K.K. and Workman, J.L. (2007) Histone acetyltransferase complexes: one size doesn't fit all. *Nat. Rev. Mol. Cell Biol.* 8, 284–295
51. McCullough, C.E. and Marmorstein, R. (2016) Molecular basis for histone acetyltransferase regulation by binding partners, associated domains, and autoacetylation. *ACS Chem. Biol.* 11, 632–642
52. Puigserver, P. *et al.* (1999) Activation of PPARγ coactivator-1 through transcription factor docking. *Science* 286, 1368–1371
53. Lerin, C. *et al.* (2006) GCN5 acetyltransferase complex controls glucose metabolism through transcriptional repression of PGC-1α. *Cell Metab.* 3, 429–438
54. Leick, L. *et al.* (2008) PGC-1α is not mandatory for exercise- and training-induced adaptive gene responses in mouse skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 294, E463–E474
55. Rowe, G.C. *et al.* (2012) PGC-1α is dispensable for exercise-induced mitochondrial biogenesis in skeletal muscle. *PLoS One* 7, e41817
56. Wellen, K.E. *et al.* (2009) ATP-citrate lyase links cellular metabolism to histone acetylation. *Science* 324, 1076–1080
57. Mews, P. *et al.* (2017) Acetyl-CoA synthetase regulates histone acetylation and hippocampal memory. *Nature* 546, 381–386
58. Liu, X. *et al.* (2018) Acetate production from glucose and coupling to mitochondrial metabolism in mammals. *Cell* 175, 502–513
59. McDonnell, E. *et al.* (2016) Lipids reprogram metabolism to become a major carbon source for histone acetylation. *Cell Rep.* 17, 1463–1472
60. Winder, W.W. and Hardie, D.G. (1996) Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am. J. Phys.* 270, E299–E304
61. Galdieri, L. *et al.* (2016) Activation of AMP-activated protein kinase by metformin induces protein acetylation in prostate and ovarian cancer cells. *J. Biol. Chem.* 291, 25154–25166
62. Galdieri, L. and Vancura, A. (2012) Acetyl-CoA carboxylase regulates global histone acetylation. *J. Biol. Chem.* 287, 23865–23876
63. Ye, C. and Tu, B.P. (2018) Sink into the epigenome: histones as repositories that influence cellular metabolism. *Trends Endocrinol. Metab.* 29, 626–637
64. McBrian, M.A. *et al.* (2013) Histone acetylation regulates intracellular pH. *Mol. Cell* 49, 310–321
65. Bersuker, K. *et al.* (2018) A proximity labeling strategy provides insights into the composition and dynamics of lipid droplet proteomes. *Dev. Cell* 44, 97–112
66. Li, Z. *et al.* (2012) Lipid droplets control the maternal histone supply of *Drosophila* embryos. *Curr. Biol.* 22, 2104–2113
67. Cermelli, S. *et al.* (2006) The lipid-droplet proteome reveals that droplets are a protein-storage depot. *Curr. Biol.* 16, 1783–1795

68. Anand, P. *et al.* (2012) A novel role for lipid droplets in the organismal antibacterial response. *Elife* 1, e00003
69. Carter, L.G. *et al.* (2012) Perinatal exercise improves glucose homeostasis in adult offspring. *Am. J. Physiol. Endocrinol. Metab.* 303, E1061–E1068
70. Carter, L.G. *et al.* (2013) Maternal exercise improves insulin sensitivity in mature rat offspring. *Med. Sci. Sports Exerc.* 45, 832–840
71. Stanford, K.I. *et al.* (2017) Maternal exercise improves glucose tolerance in female offspring. *Diabetes* 66, 2124–2136
72. Stanford, K.I. *et al.* (2015) Exercise before and during pregnancy prevents the deleterious effects of maternal high-fat feeding on metabolic health of male offspring. *Diabetes* 64, 427–433
73. Stanford, K.I. *et al.* (2018) Paternal exercise improves glucose metabolism in adult offspring. *Diabetes* 67, 2530–2540
74. McPherson, N.O. *et al.* (2015) Preconception diet or exercise intervention in obese fathers normalizes sperm microRNA profile and metabolic syndrome in female offspring. *Am. J. Physiol. Endocrinol. Metab.* 308, E805–E821
75. Ingerslev, L.R. *et al.* (2018) Endurance training remodels sperm-borne small RNA expression and methylation at neurological gene hotspots. *Clin. Epigenetics* 10, 12
76. Short, A.K. *et al.* (2017) Exercise alters mouse sperm small noncoding RNAs and induces a transgenerational modification of male offspring conditioned fear and anxiety. *Transl. Psychiatry* 7, e1114
77. Laker, R.C. *et al.* (2014) Exercise prevents maternal high-fat diet-induced hypermethylation of the Pgc-1α gene and age-dependent metabolic dysfunction in the offspring. *Diabetes* 63, 1605–1611
78. Even-Faitelson, L. *et al.* (2016) Coming to terms with chromatin structure. *Chromosoma* 125, 95–110
79. Dultz, E. *et al.* (2018) Quantitative imaging of chromatin decompaction in living cells. *Mol. Biol. Cell* 29, 1763–1777
80. Venkatesh, S. and Workman, J.L. (2015) Histone exchange, chromatin structure and the regulation of transcription. *Nat. Rev. Mol. Cell Biol.* 16, 178–189
81. Rousseaux, S. and Khochbin, S. (2015) Histone acylation beyond acetylation: terra incognita in chromatin biology. *Cell J.* 17, 1–6
82. Simone, C. (2006) SWI/SNF: the crossroads where extracellular signaling pathways meet chromatin. *J. Cell. Physiol.* 207, 309–314
83. Su, Z. and Denu, J.M. (2016) Reading the combinatorial histone language. *ACS Chem. Biol.* 11, 564–574
84. Jackson, V. *et al.* (1975) Studies on highly metabolically active acetylation and phosphorylation of histones. *J. Biol. Chem.* 250, 4856–4863