Ramping up the signal: Promoting endurance training adaptation in skeletal muscle by nutritional manipulation

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Summary

1. Mitochondrial biogenesis in skeletal muscle results from the cumulative effect of transient increases in mRNA transcripts encoding mitochondrial proteins in response to repeated exercise sessions. This process requires the coordinated expression of both nuclear and mitochondrial (mtDNA) genomes and is regulated, for the most part, by the peroxisome proliferator-activated receptor γ co-activator (PGC-1α).

2. Several other exercise-inducible proteins also play important roles in promoting an endurance phenotype including the AMP-activated protein kinase (AMPK), the p38 mitogen-activated protein kinase (p38 MAPK) and the tumour suppressor protein p53.

3. Commencing endurance-based exercise with low muscle glycogen availability results in a greater activation of many of these signalling proteins compared to when the same exercise is undertaken with normal glycogen concentration, suggesting that nutrient availability is a potent signal that can modulate the acute cellular responses to a single bout of exercise.

4. When exercise sessions are repeated in the face of low glycogen availability (*i.e.* chronic training), the phenotypic adaptations resulting from such interventions are also augmented.

Introduction

Almost 50 years ago, groundbreaking work by John Holloszy demonstrated that endurance-based exercise induced an increase in skeletal muscle mitochondrial number in rats subjected to an intense protocol of treadmill running.[1] In that study, mitochondria from muscles of exercise trained animals exhibited higher levels of respiratory control and tightly coupled oxidative phosphorylation compared to muscle from untrained animals. This increase in electron transport capacity was associated with a concomitant rise in the ability to produce adenosine triphosphate (ATP) and was, in large part, responsible for the prolonged endurance running time observed after training.[1] Since publication of that landmark study, developments of our understanding of the mechanisms responsible for exercise-induced mitochondrial biogenesis have progressed rather slowly. Indeed, only with recent advances in molecular biology has it been possible to elucidate the mechanisms regulating mitochondrial biogenesis and how exercise training promotes an increase in mitochondria. In this regard, contemporary studies have demonstrated that exercise increases the transcription of an array of genes with putative roles in training adaptation.[2] Furthermore, a growing body of evidence shows that the glycogen content of skeletal muscle is a major determinant regulating the transcriptional activation of many genes involved in this adaptive response.[3] Collectively, these discoveries have stimulated current interest into the effects of manipulating substrate availability to modulate both acute and chronic responses to exercise. In this brief review, we will examine how exercise-nutrient interactions impact on gene expression and cell signalling, and ultimately how these manipulations can modulate processes implicated in training adaptation.

Exercise induces mitochondrial biogenesis

Endurance exercise induces an increase in muscle mitochondria.[4-6] A single bout of exercise stimulates mitochondrial biogenesis, as evidenced by increases in the expression of mitochondrial proteins.[7] Repeated bouts of exercise (*i.e.* training), maintain this effect. Training improves exercise capacity and endurance, making it possible to work at higher intensities for longer time periods. An increase in the exercise stimulus results in a greater increase in mitochondrial biogenesis. As a result, exercise training programs in which the stimulus is progressively increased result in a proliferation in muscle mitochondria up to the point where a further increase in training stimulus causes no further increase in mitochondria.

Mitochondrial biogenesis in skeletal muscle results from the cumulative effects of transient increases in mRNA transcripts encoding mitochondrial proteins after successive exercise sessions.[8] This process requires the coordinated expression of both nuclear and mitochondrial (mtDNA) genomes through various co-factors specifically dedicated to families of genes encoding distinct categories of mitochondrial proteins.[9] During the past decade it has been established that overall coordination of these two processes is regulated by the peroxisome proliferator-activated receptor γ co-activator (PGC-1α).[6,10] This transcriptional co-activator influences the transcriptional activity of nuclear respiratory factors (NRFs)[9] and peroxisome proliferator-activated receptors (PPARs).[11] NRF-1 activates expression of the nuclear gene that encodes mitochondrial transcription factor A (Tfam), which moves to the mitochondria where it regulates transcription of the mitochondrial DNA[12] which

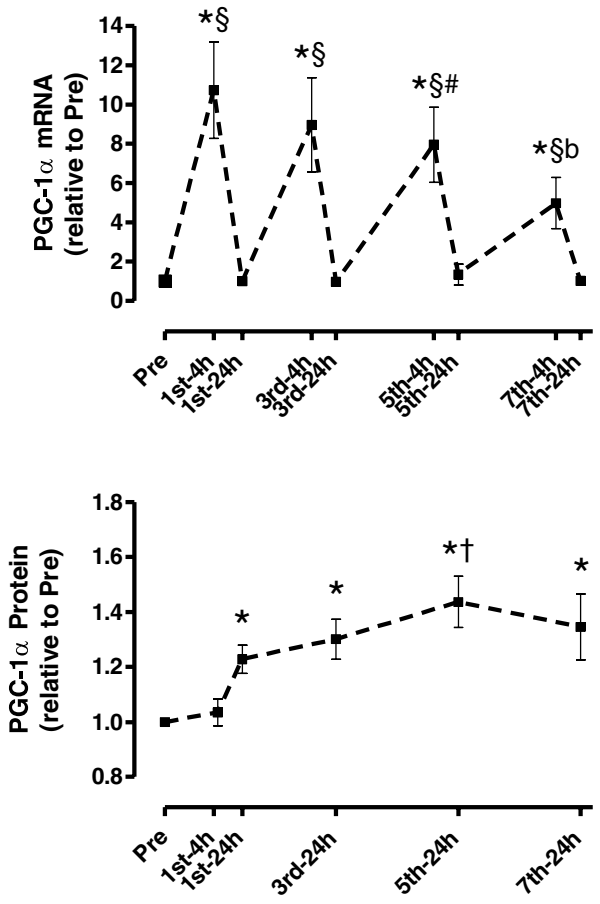

Figure 1. Early time-course of signalling events involved in the mitochondrial adaptive response to exercise in human skeletal muscle*. Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. PGC-1α mRNA (top panel) and protein content (lower panel) during two weeks of high-intensity interval training. *Significantly different from Pre; § Significantly different from all 24 h time points; # Significantly different from 1st 4 h; ♭ Significantly different from all 4 h time points; †Significantly different from 1st 24 h (all* $P < 0.05$*). Values are mean ± SEM for 8-9 subjects per time point.*

encodes fundamental respiratory proteins and other factors involved in mtDNA transcription and replication.[6] The PPARs regulate genes for lipid transport and metabolism and lipid catabolism.[13] By interacting with these transcription factors, PGC-1α directs a coordinated up-regulation of muscle mitochondrial content and the capacity for substrate metabolism and oxidative phosphorylation.

The results of Perry *et al.*[8] provide important mechanistic insight into the time course of the mitochondrial adaptive response after a single bout of exercise in human skeletal muscle. These workers had subjects undertake 7 intense endurance exercise sessions over a 2 wk training block. Their results (Figure 1) demonstrate that daily bouts of exercise result in transient increases in mRNA, and that these 'bursts' of increased mRNA abundance preceded increases in transcriptional and mitochondrial proteins during the training intervention. Over time, these transient 'pulses' of increased mRNA expression eventually lead to an accumulation of new muscle proteins, which constitute the 'training adaptation' and subsequent improvements in metabolic and endurance capacity.

Perry *et al.*[8] also observed that with each successive training session, the acute increases in PGC-1α mRNA was attenuated (Figure 1), supporting the hypothesis that reduced transcriptional responses with repeated training bouts may play a role in the so-called 'plateau effect' that occurs over time with continued exercise pulses (*i.e.* elite athletes with a prolonged history of training). Alternatively, it could be that as an individual accumulates a 'history' of training, the primary mechanism behind the adaptive response shifts from transcriptional regulation to post-translational modification of existing proteins. Further research into these exercise-induced mechanisms will provide greater insight into the complex control of mitochondrial biogenesis.

While considerable research has focused on the role of PGC-1α as a 'master regulator' of training adaptation, several other signalling proteins have important roles in this process. One exercise-induced signal that leads to increased mitochondrial biogenesis is the increase in AMP concentration in muscle during exercise that results in activation of the AMP-activated protein kinase (AMPK). AMPK functions as a 'fuel gauge' in skeletal muscle because when it becomes activated in response to decreased energy levels (*i.e.* muscle contraction, or energy restriction), it inhibits ATP-consuming pathways and activates pathways involved in carbohydrate and fatty acid catabolism to restore ATP levels. AMPK promotes fatty acid (FA) oxidation in skeletal muscle during exercise by inhibiting acetyl-CoA carboxylase (ACC-β) and activation of malonyl-CoA, thus removing inhibition of mitochondrial fatty acyl-CoA translocation by carnitine palmitoyltransferase-1 (CPT-1). Numerous studies have reported that these exercise-induced effects on ACC-β and malonyl-CoA are closely paralleled by activation of AMPK.[14,15] The mechanism by which activation of AMPK induces increased mitochondrial biogenesis is explained by the recent finding that AMPK directly phosphorylates and activates PGC-1α.[16] The AMPK also phosphorylates and inactivates histone deacetylase 5 (HDAC5)[17] which, in turn, leads to the removal of HDAC5 from the nucleus, allowing the myocyte enhancer factor 2 (MEF2) to bind and activate the PGC-1α gene.

The p38 mitogen-activated protein kinase (p38 MAPK) phosphorylates and activates PGC-1α[18,19] and can also increase PGC-1α expression by phosphorylating the transcription factor ATF-2. Exercise results in rapid activation of p38 MAPK, which mediates both the activation and increased expression of PGC-1α.[20] Another transcription factor that may be regulated by AMPK and/or p38 MAPK, and is emerging as a potent regulator of mitochondrial content and function is the tumour

suppressor protein p53. Preliminary evidence in support of a role for p53 in regulating mitochondrial capacity has been provided from several animal studies demonstrating that p53 knock-out (KO) mice display reduced endurance exercise capacity compared with wild type animals.[21,22] Skeletal muscles from p53 KO mice also exhibit reduced subsarcolemmal and intermyofibrillar mitochondrial content (and reduced state 3 respiration in the latter) as well as reduced cytochrome oxidase (COX) activity and PGC-1α expression.[23] p53 may regulate exercise-induced mitochondrial biogenesis through interactions with Tfam in the mitochondria where it functions to co-ordinate regulation of the mitochondrial genome. Indeed, the absence of p53 reduces Tfam mRNA and protein levels as well as mtDNA content in resting muscle.[22] Recently, Saleem & Hood[24] found that that a single bout of endurance exercise can signal to localise p53 to the mitochondria where it may serve to positively modulate the activity of the mitochondrial transcription factor Tfam.

Ramping up the signal: Nutritional manipulation to increase the training impulse

The notion that nutrient availability could interact with contractile stimuli to modulate acute responses to exercise can be traced back to the work of Chan and colleagues.[25] These workers determined the effect of muscle glycogen availability and contraction on intracellular signalling and gene transcription in humans who performed 60 min of submaximal exercise under two different exercise-diet conditions: either with prior ingestion of a normal carbohydrate diet or after a low carbohydrate diet intended to reduce pre-exercise muscle glycogen content. Their data showed that reduced glycogen availability increased phosphorylation of nuclear p38 MAPK compared to when the same exercise bout was commenced with normal glycogen stores.[25] Subsequent studies confirmed that commencing exercise when muscle glycogen concentration was low resulted in a greater transcriptional activation of interleukin-6, pyruvate dehydrogenase kinase 4 (PDK4), hexokinase, and heat shock protein 72 compared with when muscle glycogen concentration was high or normal at the start of exercise.[26]

It was not until the pioneering study of Hansen *et al*.[27] however, that the overall hypothesis that endurance exercise commenced with low muscle glycogen level could enhance training adaptation came into existence. These workers tested their hypothesis using an experimental design in which both legs of the same individual were trained according to different protocols: one leg performed one training session each day, whereas the other leg performed two training sessions separated by a brief recovery every second day. This latter training schedule resulted in a marked decrease in muscle glycogen content after the first bout of exercise and when the second bout of exercise was performed on the same day, it was commenced with very low starting muscle glycogen availability. This protocol allowed Hansen *et al*.[27] to compare training with low muscle glycogen *vs.* training with high muscle glycogen content. Citrate synthase activity along with resting muscle glycogen concentration and exercise time until exhaustion were all enhanced by training twice every second day (*i.e.* with low glycogen availability) when compared with training once daily (with high glycogen availability), thus confirming their hypothesis that 'train-low' could enhance training adaptation and, under the conditions of that experiment, exercise capacity.

Since the work of Hansen *et al*.[27] the results of several studies demonstrate that, independent of prior training status, a single bout of exercise, or short-term (3-10 wk) training programs in which a portion of workouts are commenced with either low muscle glycogen (and/or low exogenous glucose availability) augment training adaptation (*i.e.* they increase the maximal activities of selected genes and proteins involved in carbohydrate and/or lipid metabolism and promote mitochondrial biogenesis) to a greater extent than when all workouts are undertaken with normal or elevated glycogen stores.[28-34] An overview of the putative cell signalling pathways with roles in regulating the enhanced mitochondrial adaptations observed when endurance-based training is undertaken under conditions of reduced carbohydrate availability is depicted in Figure 2.

We investigated acute skeletal muscle signalling events in well-trained endurance cyclists in response to a single bout of high-intensity interval training (HIT; 8 × 5 min at 85% of VO_{2max}) commenced with either low or normal muscle glycogen stores.[33] AMPK phosphorylation was greater when HIT was commenced with low compared with normal muscle glycogen availability. We have also observed that a single bout of endurance-based exercise (HIT or moderate-intensity continuous running) increases $p53^{Ser15}$ phosphorylation during the early recovery from exercise in a temporal time-course that was associated with upstream signaling through either AMPK or p38MAPK[35] and further demonstrated that the exercise-induced activation of p53 was glycogen-dependent (*i.e.* low carbohydrate availability was associated with a three-fold increases in p53 phosphorylation 3 h post-exercise, while high carbohydrate availability completely suppressed p53 signalling.[28] The enhanced p53 response with low carbohydrate availability was also associated with enhanced ACC^{Ser79} phosphorylation immediately post exercise (but not p38MAPK phosphorylation), suggesting that AMPK may be the dominant upstream signalling kinase regulating contractile-induced p53 activity.

Exercise commenced with low glycogen has also been demonstrated to increases PGC-1α gene expression in human skeletal muscle. Psilander *et al*.[31] studied 10 highly trained cyclists who rode for 60 min at ~64% of maximal aerobic power (VO_{2max}) with either low or normal muscle glycogen levels (achieved by prior exercise/diet intervention). The mRNA of PGC-1α was enhanced to a greater extent when exercise was performed with low compared with normal glycogen availability (8.1-fold *vs.* 2.5-fold increase), while cytochrome c oxidase subunit I and pyruvate dehydrogenase kinase isozyme 4 mRNA were increased after low (1.3- and 114-fold increase, respectively), but not normal glycogen conditions.[31]

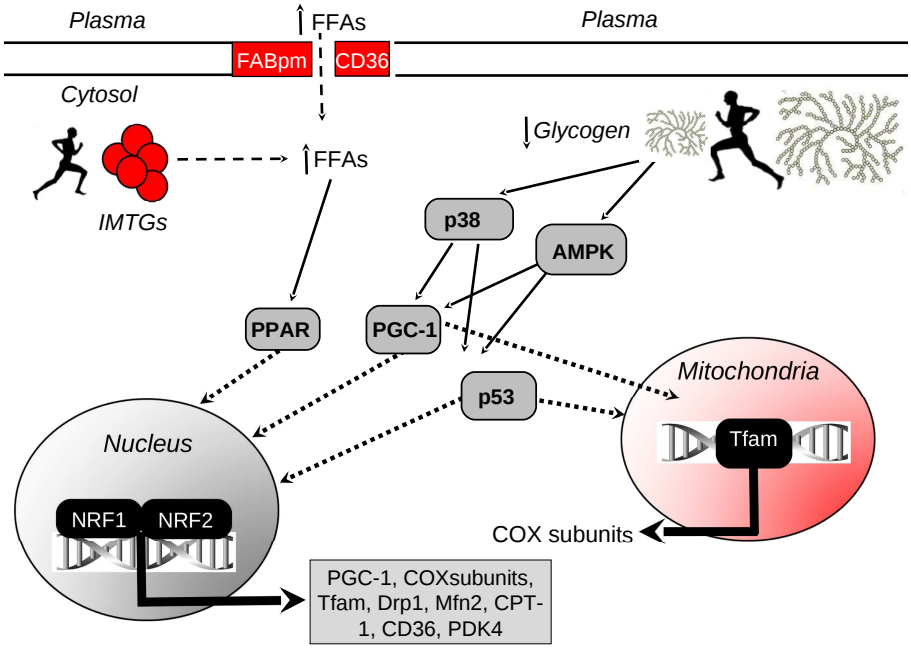

Figure 2. Schematic overview of the potential cell signalling pathways with roles in regulating the enhanced mitochondrial adaptations observed when commencing endurance-based training under conditions of reduced carbohydrate availability*. Reduced muscle glycogen availability enhances both AMPK and p38MAPK phosphorylation that subsequently results in increased PGC-1α activation and translocation to both the nucleus and mitochondria. In the nucleus, PGC-1α co-activates the transcription factors NRF1 and NRF2 to increase the expression of COX subunits and Tfam as well auto-regulating its own expression. In the mitochondria, PGC-1α co-activates Tfam to co-ordinate regulation of mtDNA and induces the expression of key mitochondrial proteins of the electron transport chain (*i.e. *COX subunits). Similar to PGC-1α, p53 activity is also increased when exercising under conditions of low carbohydrate availability (possibly due to upstream signaling through AMPK) where it translocates to mitochondria to modulate Tfam activity and mtDNA expression. Additionally, nuclear p53 abundance may also be enhanced where it functions to increase expression of Drp1 and Mfn2 to later assist in mitochondrial fission and fusion, respectively. Finally, reduced carbohydrate availability increases both adipose tissue and intramuscular lipolysis* via *decreased circulating insulin and increased adrenaline concentrations. The resulting elevations in free fatty acids activate the nuclear transcription factor, PPARδ, to increase expression of key proteins involved in regulation of lipid oxidation such as CPT-1, PDK4 and CD36. Thin solid lines represent activation, dashed lines represent translocation.*

While there is growing evidence to show that acute signaling events are generally augmented when endurance training is commenced with low carbohydrate availability, several disconnects exist between upstream signaling events and selected downstream substrates. For example, we hypothesized that the greater AMPK activation in response to intense training with low glycogen availability[33] would result in concomitant increases in the localization and/or phosphorylation of some of these downstream targets of AMPK, but this was not the case: there were no differences in the localization of HDAC5 and the phosphorylation state of CREB when subjects commenced intense exercise with either low or normal muscle glycogen levels.[33] In that study we speculated that the magnitude of increase in AMPK phosphorylation may not have been sufficient to specifically increase its activity towards HDAC5 and CREB. In support of this contention, it has recently been reported that the mitochondrial content and oxidative capacity of skeletal muscle are key determinants of the activation of signalling proteins important to muscle plasticity.[36] The attenuation of kinase phosphorylation in muscle with high mitochondrial content suggests that these proteins may require a greater stimulus input for activation to propagate these cues downstream to evoke phenotypic adaptations, as was previously found by Yu *et al.*[15]

Alternatively, there may be other mechanisms by which low muscle glycogen availability increases the acute training response. Indeed, recent evidence demonstrates that glycogen binding to the glycogen-binding domain on the AMPK β subunit allosterically inhibits AMPK activity and phosphorylation by upstream kinases.[37] McBride *et al.*[37] reported that AMPK is inhibited by glycogen, particularly preparations with high branching content. Moreover, these workers also showed that this inhibition of AMPK activation by several different types of carbohydrates was dependent on the glycogen-binding domain being abolished

by mutation of residues required for carbohydrate binding. Collectively, these results strongly suggest that glycogen is a potent regulator of AMPK activity through its association with the glycogen-binding domain on the AMPK β subunit.[37]

Finally, carbohydrate availability is not the only variable manipulated in many of these investigations. Under conditions of low muscle glycogen, circulating free fatty acids and catecholamine concentrations are also elevated (Figure 2) and it seems reasonable to consider that these perturbations may contribute to the promotion of exercise training-induced mitochondrial biogenesis. Evidence in support of this contention comes from studies in animals where chronically raising plasma FFA concentration induces increased biogenesis of mitochondria in skeletal muscle.[38] However, it should be noted that when the post-exercise rise in plasma fatty acid concentration is abolished (by administration of acipimox, a pharmacological inhibitor of adipose tissue lipolysis), the normal exercise-induced increase in mRNA abundance of selected metabolic genes (*i.e.* PDK4 and PGC-1α) persists.[39] These results clearly demonstrate that the increase in circulating fatty acid concentrations during the later stages of exercise and subsequent recovery are not essential to induce skeletal muscle mRNA expression of several proteins involved in regulating substrate metabolism.

Finally, the various studies reviewed here have employed different exercise modes, a diverse range of fitness levels and athletic abilities in the subjects tested, and have sometimes altered both endogenous and exogenous carbohydrate availability (reviewed in Hawley *et al.*, 2011[26] and Tunstall *et al.*, 2007[39]). It is quite possible that some of the results are not directly attributable to differences in carbohydrate availability *per se* but rather to the effects of the exercise training protocol itself (*i.e.* differences in recovery time between workouts, training once/day *vs.* twice every 2nd day). Human studies are also limited by practical issues, such as the number of muscle biopsies and the time points at which these are taken; in reality, most investigations provide only a 'snapshot' of the major cellular events taking place, thus precluding our ability to make meaningfully interpretations. Clearly, the interaction of muscle fuel stores and the concomitant perturbations in the hormonal milieu during and after exercise commenced with low glycogen availability play major roles in modulating the training response and subsequent adaptation, and this is a fertile area for future research.

Conclusions

'Train-low' has become a catchphrase in athletic circles as a generic term that describes the planned manipulation of nutrient availability to promote the training response-adaptation. While it should be stressed that commencing endurance exercise with low muscle glycogen concentration is only one strategy to alter carbohydrate availability[40] the possibility that several common exercise-induced signalling pathways may be sensitive to endogenous glycogen stores is attractive on theoretical grounds. Just how low glycogen availability modulates the activities of proteins with putative roles in promoting mitochondrial biogenesis is not precisely clear. However, the time-course data of Perry *et al*.[8] may offer some insight into this conundrum. The normal time-course of activation of the majority of exercise-induced genes is that their activity peaks early (*i.e.* 1-4 h) during recovery and has typically returned to resting (pre-exercise) values within 16-24 h. Commencing exercise with low muscle glycogen stores and/or withholding carbohydrate during recovery from strenuous endurance exercise may 'prolong' the transient increases in mRNA transcription, resulting in a greater or more sustained signal.[41] Low glycogen availability after exercise may be important for the structural integrity of the power-generating capacity of the previously exercised musculature. Certainly further work is warranted to define the time-course of post-transcriptional regulation and if withholding carbohydrate during recovery from exercise is associated with functionally meaningful changes in the activity of key proteins that can further promote the endurance phenotype.

References

1. Holloszy JO. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J. Biol. Chem*. 1967; **242**: 2278-82.
2. Mahoney DJ, Parise G, Melov S, Safdar A, Tarnopolsky MA. Analysis of global mRNA expression in human skeletal muscle during recovery from endurance exercise. *FASEB J.* 2005; **19**: 1498-500.
3. Pilegaard H, Keller C, Steensberg A, Helge JW, Pedersen BK, Saltin B, and Neufer PD. Influence of pre-exercise muscle glycogen content on exercise-induced transcriptional regulation of metabolic genes. *J. Physiol.* 2002; **541**:261-71.
4. Hawley JA. Adaptations of skeletal muscle to prolonged, intense endurance training. *Clin. Exp. Pharmacol. Physiol*. 2002; **29**: 218-222.
5. Hawley JA, Holloszy JO. Exercise: it's the real thing! *Nutr. Rev.* 2009; **67**:172-8.
6. Joseph AM, Pilegaard H, Litvintsev A, Leick L, Hood DA. Control of gene expression and mitochondrial biogenesis in the muscular adaptation to endurance exercise. *Essays Biochem*. 2006; **42**:13-29.
7. Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, Holloszy JO. Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J.* 2002; **16**:1879-86.
8. Perry CG, Lally J, Holloway GP, Heigenhauser GJ, Bonen A, Spriet LL. Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J. Physiol*. 2010; **588**: 4795-810.
9. Scarpulla RC. Transcriptional activators and coactivators in the nuclear control of mitochondrial function in mammalian cells. *Gene* 2002; **286**: 81-89.

10. Lin J, Handschin C, Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab*. **1**: 361-370, 2005.
11. Wang Y-X, Zhang C-L, Yu RT, Cho HK, Nelson MC, Bayuga-Ocampo CR, Ham J, Kang H and Evans RM. Regulation of muscle fiber type and running endurance by PPARd. *PLoS Biol*. 2004; **2**: 1532-39.
12. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999; **98**: 115-24.
13. Gilde AJ, Van Bilsen M. Peroxisome proliferator-activated receptors (PPARS): regulators of gene expression in heart and skeletal muscle. *Acta Physiol. Scand*. 2003; **178**: 425-34.
14. Rasmussen BB, Winder WW. Effect of exercise intensity on skeletal muscle malonyl-CoA and acetyl-CoA carboxylase. *J. Appl. Physiol*. 1997: **83**: 1104-9.
15. Yu M, Stepto NK, Chibalin AV *et al.* Metabolic and mitogenic signal transduction in human skeletal muscle after intense cycling exercise. *J. Physiol.* 2002: **546**: 327-35.
16. Jäger S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1a. *Proc. Natl. Acad. Sci. USA*. 2007: **104**: 12017-22.
17. McGee SL, van Denderen BJW, Howlett KF, Mollica J, Schertzer JD, Kemp BE, Hargreaves M. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes* 2008; **57**: 860–867.
18. Knutti D, Kressler D, Kralli A. Regulation of the transcriptional coactivator PGC-1 via MAPK-sensitive interaction with a repressor. *Proc. Natl. Acad. Sci. USA* 98: 9713-9718, 2001.
19. Puigserver P, Rhee J, Lin J, Wu Z, Yoon JC, Zhang CY, Krauss S, Mootha VK, Lowell BB, Spiegelman BM. Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPARγ coactivator-1. *Mol. Cell* 2001; **8**: 971-82.
20. Akimoto T, Pohnert SC, Li P, Zhang M, Gumbs C, Rosenberg PB, Williams RS, Yan Z. Exercise stimulates Pgc-1α transcription in skeletal muscle through activation of the p38 MAPK pathway. *J. Biol. Chem*. 2005; **280**: 19587-93.
21. Matoba S, Kang J-G, Patino WD, Wragg A, Boehm M, Gavrilova O, Hurley PJ, Bunz F, Hwang PM. p53 regulates mitochondrial respiration. *Science* 2006; **312**: 1650-53.
22. Park J-Y, Wang P-Y, Matsumoto T, Sung HJ, Ma W, Choi JW, Anderson SA, Leary SC, Balaban RS, Kang J-G, Hwang PM. p53 improves aerobic exercise capacity and augments skeletal muscle mitochondrial DNA content. *Circ. Res*. 2009; **105**: 705–12.
23. Saleem A, Adhihetty PJ, Hood DA. Role of p53 in mitochondrial biogenesis and apoptosis in skeletal muscle. *Physiol. Genom*. **37**: 58–66, 2009.
24. Saleem A, Hood DA. Acute Exercise induces p53 translocation to the mitochondria and promotes a p53-Tfam-mtDNA complex in skeletal muscle. *J. Physiol*. **591:** 3625-36.
25. Chan MH, McGee SL, Watt MJ, Hargreaves M, Febbraio MA. Altering dietary nutrient intake that reduces glycogen content leads to phosphorylation of nuclear p38 MAP kinase in human skeletal muscle: association with IL-6 gene transcription during contraction. *FASEB J.* 2004; **18**: 1785-7.
26. Hawley JA, Burke LM, Phillips SM, Spriet LL. Nutritional modulation of training-induced skeletal muscle adaptations. *J. Appl. Physiol.* 2011; **110**: 834-845.
27. Hansen AK, Fischer CP, Plomgaard P, Andersen JL, Saltin B, Pedersen BK. Skeletal muscle adaptation: training twice every second day vs. training once daily. *J. Appl. Physiol*. 2005; **98**: 93-9.
28. Bartlett JD, Louhelainen J, Iqbal Z, Cochran AJ, Gibala MJ, Gregson W, Close GL, Drust B, Morton JP. Reduced carbohydrate availability enhances exercise-induced p53 signaling in human skeletal muscle: implications for mitochondrial biogenesis. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 2013; **304**: R450-58.
29. Hulston CJ, Venables MC, Mann CH, Martin C, Philp A, Baar K, Jeukendrup AE. Training with low muscle glycogen enhances fat metabolism in well-trained cyclists. *Med. Sci. Sports Exerc*. 2010; **42**: 2046-55.
30. Morton JP, Croft L, Bartlett JD, Maclaren DP, Reilly T, Evans L, McArdle A, Drust B. Reduced carbohydrate availability does not modulate training-induced heat shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle. *J. Appl. Physiol*. 2009; **106**: 1513-1521.
31. Psilander N, Frank P, Flockhart M, Sahlin K.Exercise with low glycogen increases PGC-1α gene expression in human skeletal muscle. *Eur. J. Appl. Physiol*. 2013; **113**: 951-63.
32. Steinberg GR, Watt MJ, McGee SL, Chan S, Hargreaves M, Febbraio MA, Stapleton D, Kemp BE. Reduced glycogen availability is associated with increased AMPKα2 activity, nuclear AMPKα2 protein abundance, and GLUT4 mRNA expression in contracting human skeletal muscle. *Appl. Physiol. Nutr. Metab*. 2006; **31**: 302-12.
33. Yeo WK, McGee SL, Carey AL, Paton CD, Garnham AP, Hargreaves M, Hawley JA. Acute signalling responses to intense endurance training commenced with low or normal muscle glycogen. *Exp Physiol.* 2010; **95**: 351-8.
34. Yeo WK, Paton CD, Garnham AP, Burke LM, Carey AL, Hawley JA. Skeletal muscle adaptation and

performance responses to once a day versus twice every second day endurance training regimens. *J. Appl. Physiol*. 2008; **105**: 1462-1470.
35. Bartlett JD, Hwa Joo C, Jeong TS, Louhelainen J, Cochran AJ, Gibala MJ, Gregson W, Close GL, Drust B, Morton JP. Matched work high-intensity interval and continuous running induce similar increases in PGC-1α mRNA, AMPK, p38, and p53 phosphorylation in human skeletal muscle. *J. Appl. Physiol.* 2012; 112: 1135-43.
36. Ljubicic V, Hood DA. Specific attenuation of protein kinase phosphorylation in muscle with a high mitochondrial content. *Am. J. Physiol. Endocrinol. Metab*. 2009; **297**: E749–58.
37. McBride A, Ghilagaber S, Nikolaev A, Hardie DG. The glycogen-binding domain on the AMPK β subunit allows the kinase to act as a glycogen sensor. *Cell Metab*. 2009; **9**: 23–34.
38. Garcia-Roves P, Huss JM, Han DH, Hancock CR, Iglesias-Gutierrez E, Chen M, Holloszy JO. Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. *Proc. Natl. Acad. Sci. USA*. 2007; **19:** 104:10709-13.
39. Tunstall RJ, McAinch AJ, Hargreaves M, van Loon LJ, Cameron-Smith D. Reduced plasma free fatty acid availability during exercise: effect on gene expression. *Eur. J. Appl. Physiol.* 2007; **99**: 485-93.
40. Hawley JA, Burke LM. Carbohydrate availability and training adaptation: effects on cell metabolism. *Exerc. Sport Sci. Rev*. 2010; **38**:152-60.
41. Hawley JA. Nutritional strategies to modulate the adaptive response to endurance training. *Nestle Nutr. Inst. Workshop Ser*. 2013; **75**: 1-14.

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