

J.O. HOLLOSZY

## REGULATION BY EXERCISE OF SKELETAL MUSCLE CONTENT OF MITOCHONDRIA AND GLUT4

Division of Geriatrics and Nutritional Sciences, Department of Medicine,  
Washington University School of Medicine, St. Louis, Missouri, USA

Endurance exercise-training results in an increase in the size and number of mitochondria in the skeletal muscles that are involved in the exercise. In early studies of this phenomenon, long-term training programs of progressively increasing intensity and duration were used. These studies gave the impression that the adaptive increase in mitochondria is a slow process. Recent advances in the understanding of how mitochondrial biogenesis is regulated, have made it possible to study the mechanisms by which exercise regulates mitochondrial biogenesis. These studies have shown that a single bout of exercise induces a rapid increase in mitochondrial biogenesis that is mediated both by activation and by increased expression of a transcription coactivator, peroxisome proliferator-activated receptor-gamma coactivator 1 $\alpha$  (PGC-1 $\alpha$ ). PGC-1 $\alpha$  docks on and coactivates transcription factors that regulate expression of nuclear genes that encode mitochondrial proteins and also of the nuclear gene that encodes mitochondrial transcription factor A (TFAM). TFAM regulates mitochondrial DNA transcription. Thus, PGC-1 $\alpha$  regulates the coordinated expression of mitochondrial proteins encoded in both nuclear and mitochondrial genes. In addition to an increase in mitochondrial biogenesis, exercise induces an increase in the GLUT4 isoform of the glucose transporter. This increase in GLUT4 occurs in parallel with, and is mediated by, the same signals and some of the same transcription factors as the increase in mitochondrial biogenesis. Two signals generated during exercise, the increase in cytosolic Ca<sup>2+</sup> and the decrease in high energy phosphates, mediate the activation and increased expression of PGC-1 $\alpha$ . The purpose of this article is to present an overview of what is known regarding these phenomena.

Key words: *AMP kinase, calcium, mitochondrial biogenesis, PGC-1 $\alpha$*

## INTRODUCTION

Skeletal muscle has the ability to undergo major adaptations in response to exercise-training. The adaptive response of muscle to training differs dramatically depending on the nature of the adaptive stimulus. Heavy resistance exercise, also referred to as strength training, results in hypertrophy of the muscle cells with an increase in strength, without major changes in biochemical makeup. Endurance exercise increases the capacity of muscle for aerobic metabolism that is mediated by an increase in mitochondria, without muscle hypertrophy or an increase in strength. This adaptation makes it possible for previously untrained individuals to markedly increase their ability to exercise for prolonged periods at exercise intensities that could be maintained for only a few minutes in the untrained state. Exercise also induces an increase in the GLUT4 isoform of the glucose transporter, which makes possible more rapid glucose uptake and greater glycogen storage after glycogen depleting exercise. This review will deal with these adaptive response to endurance exercise.

Early during development of the field of exercise physiology, research interest was largely focused on the factors that determine and limit maximal oxygen uptake ( $\dot{V}_{O_2 \max}$ ), and on the mechanisms responsible for, and the biological effects of, lactate production by exercising muscle. It was thought that lactate production is mediated by development of muscle hypoxia, and that the improvement in the ability to perform prolonged submaximal exercise in response to training is mediated by increases in  $\dot{V}_{O_2 \max}$  and the ability to deliver  $O_2$  to the working muscles. Endurance exercise training does induce major cardiovascular adaptations that result in increases in  $\dot{V}_{O_2 \max}$  and the ability to provide the working muscles with oxygen. This adaptation makes it possible to exercise at higher work rates in the trained than in the untrained state. However, the oxygen uptake by the working muscles at a given submaximal exercise intensity is the same in the trained and untrained states. Training does not result in increased delivery of blood and oxygen to the working muscles during exercise of an intensity that could be sustained in the untrained state. Actually, blood flow to the working muscles tends to be lower in the trained state, and the trained muscles compensate for the decreased oxygen delivery with increased oxygen extraction.

The evidence that training does not result in improved oxygen delivery to the working muscles at the same submaximal exercise intensity suggested that the training induced increases in endurance for, and decreased lactate production during, submaximal exercise is due to biochemical adaptations in the muscles and not to the increase in  $\dot{V}_{O_2 \max}$ . A clue regarding what this adaptive response might involve came from comparative studies showing that the skeletal muscles of active wild animals, such as ducks and rabbits, have a much higher content of mitochondria than the same muscles of sedentary domestic animals of the same species. To evaluate the possibility that this difference in mitochondrial content was due to an exercise-training effect, rather than genetically mediated, a series

of studies was conducted on rats that were trained by means of a treadmill running program of progressively increasing speed and duration until after 12 weeks they were running for two hours per day at 31 meters per min up an 8° incline five days per week. This exercise program resulted in ~2-fold increases in the capacity of the rats' hindlimb muscles to oxidize pyruvate, fatty acids, and ketones (1-3). The mitochondria from the trained muscles exhibited tightly coupled oxidative phosphorylation providing evidence for a proportional, *i.e.* 2-fold, increase in the capacity to generate ATP (1). This increase in the capacity to generate ATP *via* oxidative metabolism is mediated by increases in the levels of the mitochondrial enzymes of the fatty acid oxidation pathway, citrate cycle, respiratory chain, and ATP synthesis (1, 2, 4, 5). These findings have been confirmed in numerous studies on laboratory rodents (6, 7) and shown to also occur in humans (8-10). Electron microscopic studies have shown that increases in both the size and number of mitochondria are involved in this adaptive response (11). All of the muscle fiber types are involved in this adaptation (12).

#### BIOLOGICAL CONSEQUENCES OF THE EXERCISE-INDUCED INCREASE IN MUSCLE MITOCHONDRIA

The endurance exercise-induced increase in mitochondria results in increase in the maximal capacity of muscle to generate ATP *via* oxidative phosphorylation. The increase in mitochondria also results in less disturbance of homeostasis during submaximal exercise, as evidenced by smaller decreases in creatine phosphate and ATP, smaller increases in ADP, AMP, inorganic phosphate ( $P_i$ ) and lactate, less glycogenolysis and increased fatigue resistance (13, 14). There is also a change in substrate utilization, with decreased utilization of glucose and glycogen and increased oxidation of fat (13, 15 - 17). Because oxygen utilization is the same in the trained and untrained state at the same submaximal work rate, the same work rate requires a smaller proportion of a muscles' maximal oxygen uptake capacity in the trained than in the untrained state.

When a muscle contracts repetitively, ATP is utilized resulting in decreases in the concentrations of ATP and creatine phosphate and increases in the concentrations of ADP,  $P_i$  and AMP. Mitochondrial electron transport is tightly coupled to ATP synthesis and is limited by the availability of ADP. The steady state concentrations of ADP, ATP and  $P_i$  attained in muscle during submaximal exercise are determined by the work rate and the muscles' content of mitochondria. Thus, substrate oxidation and ATP synthesis are geared to the work rate by the availability/concentration of ADP and the ratio of [ATP] to [ADP][ $P_i$ ]. During exercise ATP concentration falls and the concentrations of ADP and  $P_i$  rise until respiration is activated sufficiently for the rate of ATP synthesis *via* oxidative phosphorylation to balance the rate of ATP utilization during muscle contractions. As a result of the increase in mitochondria, oxygen consumption and

ATP production per mitochondrion are less at the same submaximal work rate in trained than in untrained muscle. In other words, with more mitochondrial respiratory chains, the rate of electron transport per respiratory chain has to be "turned on" to a smaller extent in order to result in the same rates of oxygen utilization and ATP production per gram of muscle at the same work rate in the trained than in the untrained state. As a consequence, ATP and PC concentrations decrease less, and ADP, AMP and  $P_i$  increase to lower steady state levels in the trained than in the untrained state in response to the same submaximal work rate (13, 14). The availability of  $P_i$  is rate-limiting for glycogenolysis by phosphorylase, while the inhibition of phosphofructokinase by ATP is countered by AMP,  $P_i$  and ADP (18-21). Therefore, as a consequence of lower  $P_i$ , AMP and ADP levels, glycogenolysis and glycolysis are turned on to a smaller extent in the trained than in the untrained state at the same submaximal work rate.

#### MECHANISMS BY WHICH THE EXERCISE-INDUCED INCREASE IN SKELETAL MUSCLE MITOCHONDRIA IS MEDIATED

Although the discovery that endurance exercise induces an increase in muscle mitochondria was reported in 1967, there was no significant progress in elucidating the mechanisms involved until the beginning of the 21<sup>st</sup> century. The major reason for this lack of progress was a complete lack of information regarding how mitochondrial biogenesis, which requires the orchestrated expression of the genes encoded in the mitochondrial genome and the nuclear genes encoding mitochondrial proteins, is regulated. The initial breakthrough in the elucidation of how mitochondrial biogenesis is regulated was the discovery by Scarpulla and coworkers (22-25) of the transcription factors nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2), which regulate transcription of nuclear genes that encode many of the proteins of the mitochondrial respiratory chain. This finding was followed by the discovery of a number of other transcription factors that regulate expression of a range of mitochondrial proteins (26-29).

The second major breakthrough, which explained how the signals generated by adaptive stimuli result in a coordinated increase in expression of the many genes encoding mitochondrial proteins, was the discovery by Spiegelman's group of an inducible coactivator that activates the transcription factors that regulate genes encoding mitochondrial proteins. Spiegelman's group named this protein peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 $\alpha$  (PGC-1 $\alpha$ ). Overexpression of PGC-1 $\alpha$  in skeletal muscle and myotubes results in large increases in functional mitochondria (30-32) and in GLUT4 expression (33). PGC-1 $\alpha$  mediates its effect on mitochondrial biogenesis by docking on and activating the transcription factors that regulate nuclear genes encoding mitochondrial proteins and that induce expression of mitochondrial transcription

factor A, which regulates mitochondrial DNA transcription, thus activating the coordinated expression of mitochondrial proteins (34).

Soon after the discovery of PGC-1 $\alpha$  a number of studies showed that a single bout of exercise or stimulation of muscle contractions induces rapid increases in PGC-1 $\alpha$  transcription, *i.e.* mRNA (35 - 39), and expression, *i.e.* PGC-1 $\alpha$  protein (35, 40), in skeletal muscle, both in rodents and humans. It was generally assumed that this increase in PGC-1 $\alpha$  protein mediates the increase in mitochondrial biogenesis that follows a bout of exercise. However, an examination of the time course of the adaptive response showed that the mRNA levels of a number of mitochondrial constituents, such as cytochrome c and citrate synthase, as well as mitochondrial proteins with very short half-lives, were increased in rat skeletal muscle immediately after 6h of swimming, while PGC-1 $\alpha$  protein did not increase until 3 hr after exercise (41). The binding of the NRF-1 and NRF-2 transcription factors to their binding sites on the cytochrome c and cytochrome oxidase subunit IV promoters respectively, was markedly increased after 2h of exercise. NRF-1 and NRF-2 are activated by PGC-1 $\alpha$  (30, 42), so this finding provided evidence that PGC-1 $\alpha$  activity is increased rapidly by exercise. Further evidence for the rapid activation of PGC-1 $\alpha$  was provided by the finding that some PGC-1 $\alpha$  protein, most of which is in the cytosol in resting skeletal muscle, had moved into the nucleus after 3h of exercise (41).

It has been shown that p38 mitogen-activated protein kinase (MAPK) phosphorylates and activates PGC-1 $\alpha$  (43, 44). Exercise results in rapid activation of p38 MAPK (45, 46), which mediates both the activation and increased expression of PGC-1 $\alpha$  (41, 47). Activation of p38 MAPK mediates the increase in PGC-1 $\alpha$  expression by phosphorylating ATF-2, a member of the cyclic AMP response element binding (CREB) proteins, which binds to and activates the CREB site on the PGC-1 $\alpha$  promoter and induces increased PGC-1 $\alpha$  expression (47, 48). Based on these findings, we have proposed the following sequence of events (41): Exercise results in activation of p38 MAPK which phosphorylates and activates PGC-1 $\alpha$  in the cytosol. The activated PGC-1 $\alpha$  moves into the nucleus and coactivates the transcription factors that regulate expression of mitochondrial proteins. Thus, the first phase of the exercise-induced increase in mitochondrial biogenesis is mediated by PGC-1 activation. The second phase is mediated by increased PGC-1 $\alpha$  protein expression (41), which results from binding of the transcription factors ATF-2 and MEF-2 to their recognition sites on the PGC-1 $\alpha$  promoter (49, 50). ATF-2, a member of the CREB family of transcription factors, is activated by phosphorylation by p38 MAPK (50). The activity of MEF2 is repressed by histone deacetylase 5 (49). Yan's group has recently provided evidence that phosphorylation of HDAC5 by protein kinase D releases MEF2 from HDAC5 inhibition during muscle contractile activity (51). In addition MEF2 is coactivated by PGC-1 $\alpha$  (50). It seems probable, based on this information, that the exercise-induced increase in

PGC-1 $\alpha$  expression is mediated by activation and increased binding of ATF-2 and MEF2 to the PGC-1 $\alpha$  promoter.

In addition to the increase in mitochondrial biogenesis, exercise induces increased expression of the GLUT4 glucose transporter (52-55) and of pyruvate dehydrogenase kinase (PDK) (56). The GLUT4 protein is recruited from intracellular sites by insulin and exercise and moves to the cell surface, where it mediates the transport of glucose into the muscle cells (57). The adaptive increase in GLUT explains the increase in the responsiveness of glucose transport to insulin and muscle contractions induced by exercise (58, 59). The increase in GLUT4 expression is mediated by the transcription factors MEF2A and D and a GLUT4 enhancer factor (GEF) (60, 61). PGC-1 $\alpha$  coactivates MEF2A (50) and also increases MEF2A protein expression by activating NRF-1 (62, 63), and possibly, other transcription factors that regulate MEF2A expression. GLUT4 protein has a short half-life and increases rapidly after a bout of exercise. A single prolonged bout of exercise results in as much as 2-fold increases in both GLUT4 protein and insulin-stimulated glucose transport 18 hr post exercise (59). As a consequence of this adaptive increase in GLUT4, muscle glycogen storage following glycogen depleting exercise occurs more rapidly and to a greater extent in the trained than in the untrained state (64-66). The increase in GLUT4 reverses rapidly (67).

The enzyme PDK phosphorylates pyruvate dehydrogenase and decreases its activity, thus slowing glucose oxidation by decreasing the rate of pyruvate entry into the citrate cycle. PGC-1 $\alpha$  activates the transcription factor estrogen related receptor  $\alpha$  (ERR $\alpha$ ) which regulates PDK expression. The exercise induced increase in PDK expression (56) helps to explain the carbohydrate-sparing effect of exercise-training, with an increased reliance on fat oxidation for energy production during exercise in the trained state (15, 16).

#### EXERCISE-GENERATED SIGNALS THAT MEDIATE INCREASED MITOCHONDRIAL BIOGENESIS IN SKELETAL MUSCLE

Exercise causes numerous disturbances in cellular homeostasis in skeletal muscle, making it impossible to use contracting muscle to determine which of the many signals generated during exercise are responsible for inducing the increase in mitochondrial biogenesis. It was, therefore, necessary to use experimental models in which the signals generated in muscle during exercise can be studied individually. The models that have been used to study the signals and signaling pathways that lead to increased mitochondrial biogenesis include myotubes in culture, the small, very thin rat epitrochlearis muscle and laboratory rodents. Two exercise generated signals that mediate mitochondrial biogenesis have been identified. One is the decrease in ATP and phosphocreatine concentrations and increase in AMP concentration in muscle during exercise. The other is the

increase in cytosolic  $\text{Ca}^{2+}$  that occurs as a result of release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum during excitation-contraction coupling.

It is not feasible to evaluate the effects of lowering of high energy phosphates and increasing AMP on mitochondrial biogenesis directly, because these perturbations and the associated increase in inorganic phosphate result in major changes in homeostasis, including activation of glycogenolysis and respiration. However, it is well established that many of the effects of decreases in high energy phosphates are mediated by AMP-activated protein kinase (AMPK). It is possible to activate AMPK by treatment of muscles with AICAR. AICAR is taken up by muscle cells and converted to the AMP analog ZMP which activates AMPK. Studies on rodents, on isolated epitrochlearis muscles and on myotubes in culture have shown that activation of AMPK with AICAR results in increased mitochondrial biogenesis (68, 69) and increased expression of GLUT4 (70-74). These effects of AMPK activation appear to be mediated by phosphorylation and activation of PGC-1 $\alpha$  (75), which leads to increased mitochondrial biogenesis by coactivation of the transcription factors that regulate expression of mitochondrial proteins. PGC-1 $\alpha$  also increases GLUT4 expression by activating NRF-1, which increases MEF-2A expression (62, 63), and by coactivating MEF-2A, which further increases GLUT4 transcription. Furthermore, AMPK phosphorylates GLUT4 enhancer factor (GEF) (73) and the transcription repressor histone deacetylase 5 (HDAC5) (74) resulting in activation of GEF and release of MEF2 from repression by HDAC5, resulting in increased GLUT4 transcription.

Studies on myotubes in culture have shown that raising cytosolic  $\text{Ca}^{2+}$  by exposing them to  $\text{Ca}^{2+}$  ionophores, or to caffeine, which releases  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum, induces increases in mitochondrial biogenesis (76-78) and GLUT4 (72), that are mediated by an increase in PGC-1 $\alpha$  (77). L6 and C<sub>2</sub>C<sub>12</sub> myotubes do not contract in response to increases in cytosolic  $\text{Ca}^{2+}$ , making this approach feasible. The increase in  $\text{Ca}^{2+}$ , like exercise, induces a rapid increase in PGC-1 $\alpha$ , resulting in activation of transcription factors, as evidenced by rapid binding of NRF-1 and NRF-2 to their recognition sites on promoters of genes that they regulate (77).

The first steps in two signaling pathways that mediate  $\text{Ca}^{2+}$ -induced mitochondrial biogenesis in muscle cells are catalyzed by the  $\text{Ca}^{2+}$ -activated enzymes calcium/calmodulin dependent protein kinase (CAMK) and calcineurin. Overexpression of either CAMKIV or calcineurin in myotubes and skeletal muscle results in increases in PGC-1 $\alpha$  expression and mitochondrial biogenesis (79-82). These findings and other less direct evidence was interpreted as evidence that activation of calcineurin and CAMKIV mediate the increase in mitochondrial biogenesis induced by exercise. A problem with this interpretation is that the isoform of CAMK that is expressed in skeletal muscle is CAMKII, which is directly activated by  $\text{Ca}^{2+}$ , not CAMKIV, which is activated by a CAMK kinase. However, it seems likely that the mechanism by which overexpression of

constitutively active CAMKIV induces PGC-1 $\alpha$  expression is the same as that mediated by activation of CAMKII by Ca<sup>2+</sup>.

Inhibition of CAMKII activity completely prevents the Ca<sup>2+</sup> induced increases in PGC-1 $\alpha$  expression and mitochondrial biogenesis in L6 myotubes (77) and in rat epitrochlearis muscles incubated *in vitro* (83), providing direct evidence that CAMKII catalyzes the initial step in the pathway by which Ca<sup>2+</sup> activates mitochondrial biogenesis. On the other hand, complete inhibition of calcineurin activity does not prevent the exercise-induced increase in mitochondrial biogenesis (84) or GLUT4 expression (85). It, therefore, appears that Ca<sup>2+</sup> mediates its effects on mitochondrial biogenesis *via* activation of CAMKII with calcineurin playing no role in this adaptive response. This finding seems somewhat surprising in light of the evidence that calcineurin can mediate mitochondrial biogenesis in muscle, and it is not known why it does not play a role in the adaptive response to exercise.

As a follow-up to the studies on myotubes in culture, additional studies have been conducted on a biologically more relevant model, rat epitrochlearis muscles maintained in oxygenated culture medium. This muscle, obtained from young rats, is sufficiently thin to be oxygenated adequately by diffusion and can be maintained in good condition *in vitro* for 24 hr (83). Using this preparation it was possible to raise cytosolic Ca<sup>2+</sup> sufficiently to stimulate mitochondrial biogenesis while using a concentration of caffeine that is too low to cause a contraction or a decrease in high energy phosphates (83). Raising cytosolic Ca<sup>2+</sup> in epitrochlearis muscle induces increases in PGC-1 $\alpha$  expression and mitochondrial biogenesis. This adaptation is prevented by inhibiting CaMKII. Like exercise, which mediates both activation and increased expression of PGC-1 by activating p38 MAPK, raising cytosolic Ca<sup>2+</sup> results in p38 MAPK activation (83). p38 MAPK mediates phosphorylation of ATF-2, a member of the CREB transcription factor family, that binds to the CREB binding site on the PGC-1 $\alpha$  promoter and induces increased PGC-1 $\alpha$  expression (47, 48). Inhibition of p38 MAPK blocks the increases in PGC-1 $\alpha$  and mitochondrial biogenesis which, as mentioned earlier, are also blocked by inhibition of CAMKII (83). These findings led to the conclusion that p38 MAPK is downstream of CAMKII in a signaling pathway by which increases in cytosolic Ca<sup>2+</sup> lead to increased expression of PGC-1 $\alpha$  and mitochondrial biogenesis. As was also found for the acute response to exercise (41), the increase in mitochondrial biogenesis induced by Ca<sup>2+</sup>, begins before the increase in PGC-1 $\alpha$  protein expression (83). PGC-1 $\alpha$  activation by p38 MAPK appears to mediate the early phase of the adaptive increase in mitochondrial biogenesis, which is then maintained and amplified by the increase in PGC-1 $\alpha$  protein (41, 83).

Another signal that may play a role in mediating the exercise-induced adaptive increase in mitochondria is the large increase in plasma free fatty acids (FFA) that occurs in response to prolonged exercise. In contrast to the roles of AMPK and Ca<sup>2+</sup>, which are well established, the role of FFA is still speculative and would at most play a secondary, amplifying role. Overexpression of the nuclear receptor PPAR $\delta$  in muscle results in an increase in mitochondria, and activation of PPAR $\delta$  with the

chemical GW501516 also increases mitochondrial biogenesis (86, 87). In these studies, mitochondrial biogenesis increased in the absence of an increase in PGC-1 $\alpha$  mRNA, leading to the conclusion that the increase in mitochondria is mediated directly by PPAR $\delta$  (86, 87). However, PPAR $\delta$  is the transcription factor for a limited number of mitochondrial proteins, including the UCPs and fatty acid oxidation enzymes. It does not, therefore, seem possible that PPAR $\delta$  could directly mediate mitochondrial biogenesis, which requires coordinated expression of a large number of proteins that are regulated by other transcription factor. The coordinated activation of these transcription factors and, thus, of mitochondrial biogenesis is mediated by PGC-1 $\alpha$ . It was subsequently shown that overexpression or activation of PPAR $\delta$  results in an increase in PGC-1 $\alpha$  protein *via* a post-transcriptional mechanism (88). The reason that the increase in PGC-1 $\alpha$  protein was missed is that it is now common practice to just measure mRNA level and refer to increases in gene transcription as increased gene expression. This practice does not take into consideration that genes are expressed as proteins, not mRNA, and that gene expression can be regulated during translation and post translationally.

Fatty acids activate PPAR $\delta$ , and raising plasma FFA to high levels by giving rats a high fat diet plus a daily injection of heparin results in large, intermittent increases in serum FFA levels similar to the FFA response during prolonged exercise (89). Raising FFA using this approach resulted in activation in PPAR $\delta$ , as evidenced by a large increase in binding of PPAR $\delta$  to the PPAR response element of the carnitine palmitoyl transferase-1 promoter (89). Raising plasma FFA to very high levels intermittently with a high fat diet plus heparin (89) or a more modest increase in FFA induced by feeding a high fat diet alone (88, 90, 91) results in highly significant increases in PGC-1 $\alpha$  protein (88, 90, 91), proteins of the mitochondrial FFA oxidation pathway, citrate cycle and respiratory chain (88-90). There is also an increase in the capacity for fat oxidation, providing evidence for an increase in functional mitochondria (88-90). In contrast to the adaptive responses induced by exercise, Ca<sup>2+</sup> and AMPK, the increase in mitochondrial biogenesis induced by FFA occurs very slowly (88). The only proteins that increase significantly during the first two weeks on a high fat diet are those that are directly regulated by PPAR $\delta$  such as fatty acyl CoA dehydrogenase and UCP3. PGC-1 $\alpha$  protein and mitochondrial marker proteins begin to increase after 2 wk and are significantly elevated after 4 wk. In this context, it seems possible that during the course of prolonged training large increases in FFA during exercise might potentiate the effects of the increases in cytosolic Ca<sup>2+</sup> and activation of AMPK on PGC-1 $\alpha$  expression and mitochondrial biogenesis.

The rapid progress in elucidation of the mechanisms by which exercise induces an increase in mitochondrial biogenesis in muscle over the past 8 years has resulted in a reasonably complete picture of how this process is regulated. There are, of course, a number of details that are still needed to make this picture complete. One of these, which is proving to be a difficult problem to solve, is how activation of PPAR $\delta$  by fatty acids results in a delayed and gradual post-

transcriptional increase in PGC-1 $\alpha$  protein expression. However, it seems probable that much of the future research activity in this area will be focused on pharmacological approaches to inducing increased mitochondrial biogenesis in skeletal muscle and thus improving endurance performance without the need for training. An example of such research is the report by Lagouge *et al.* (92) claiming that giving Resveratrol to mice on a high fat diet results in increases in muscle mitochondria and running performance.

Conflicts of interest statement: None declared.

#### REFERENCES

1. Holloszy JO. Biochemical adaptations in muscle. Effects of exercise on mitochondrial O<sub>2</sub> uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem* 1967; 242: 2278-2282.
2. Mole PA, Oscai LB, Holloszy JO. Adaptation of muscle to exercise. Increase in levels of palmitoyl CoA synthetase, and in the capacity to oxidize fatty acids. *J Clin Invest* 1971; 50: 2323-2330.
3. Winder WW, Baldwin KM, Holloszy JO. Enzymes involved in ketone utilization in different types of muscle: Adaptation to exercise. *Eur J Biochem* 1974; 47: 461-467.
4. Holloszy JO, Oscai LB, Don IJ, Mole PA. Mitochondrial citric acid cycle and related enzymes: Adaptive response to exercise. *Biochem Biophys Res Commun* 1970; 40: 1368-1373.
5. Oscai LB, Holloszy JO. Biochemical adaptations in muscle II. Response of mitochondrial ATPase, creatine phosphokinase, and adenylate kinase activities in skeletal muscle to exercise. *J Biol Chem* 1971; 246: 6968-6972.
6. Booth FW, Baldwin KM. Muscle plasticity: energy demanding and supply processes. In: Handbook of Physiology, Section 12, Exercise Regulation and Integration of Multiple Systems, LB Lowell, JT Shepherd (eds). New York: Oxford University Press, 1997, pp. 1075-1123.
7. Hood DA. Plasticity in skeletal, cardiac and smooth muscle. Invited Review: Contractile activity-induced mitochondrial biogenesis in skeletal muscle. *J Appl Physiol* 2001; 90: 1137-1157.
8. Saltin B, Gollnick PD. Skeletal muscle adaptability: significance for metabolism and performance. In: Handbook of Physiology, Section 10, 1983, pp. 555-631.
9. Gollnick PD, Armstrong RB, Saltin B, Saubert CW, IV, Sembrowich WL, Shepherd RE. Effect of training on enzyme activity and fiber composition of human skeletal muscle. *J Appl Physiol* 1973; 34: 107-111.
10. Spina RJ, Chi MMY, Hopkins MG, Nemeth PM, Lowry OH, Holloszy JO. Mitochondrial enzymes increase in muscle in response to 7-10 days of cycle exercise. *J Appl Physiol* 1996; 80: 2250-2254.
11. Hoppeler H, Luthi P, Claassen H, Weibel ER, Howald H. The ultrastructure of normal human skeletal muscle. A morphometric analysis on untrained men, women and well-trained orienteers. *Pflugers Arch* 1973; 344: 217-232.
12. Baldwin KM, Klinkerfuss GH, Terjung RL, Mole PA, Holloszy JO. Respiratory capacity of white, red, and intermediate muscle: Adaptive response to exercise. *Am J Physiol* 1972; 222: 373-378.
13. Constable SH, Favier RJ, McLane JA, Fell RD, Chen M, Holloszy JO. Energy metabolism in contracting rat skeletal muscle: adaptation to exercise training. *Am J Physiol* 1987; 253: C316-C322.
14. Dudley GA, Tullson PC, Terjung RL. Influence of mitochondrial content on the sensitivity of respiratory control. *J Biol Chem* 1987; 262: 9109-9114.

15. Coggan AR, Kohrt WM, Spina RJ, Bier DM, Holloszy JO. Endurance training decreases plasma glucose turnover and oxidation during moderate-intensity exercise in men. *J Appl Physiol* 1990; 68: 990-996.
16. Hurley BF, Nemeth PM, Martin WH, III, Hagberg JM, Dalsky GP, Holloszy JO. Muscle triglyceride utilization during exercise: Effect of training. *J Appl Physiol* 1986; 60: 562-567.
17. Martin WH, Dalsky GP, Hurley BF, et al. Effect of endurance-training on plasma free fatty acid turnover and oxidation during exercise. *Am J Physiol* 1993; 265: E708-E714.
18. Chasiotis D, Sahlin K, Hultman E. Regulation of glycogenolysis in human skeletal muscle at rest and during exercise. *J Appl Physiol* 1982; 53: 708-715.
19. Ren J-M, Hultman E. Regulation of glycogenolysis in human skeletal muscle. *J Appl Physiol* 1989; 67: 2243-2248.
20. Ren JM, Gulve EA, Cartee GD, Holloszy JO. Hypoxia causes glycogenolysis without an increase in percent phosphorylase a in rat skeletal muscle. *Am J Physiol* 1992; 263: E1086-E1091.
21. Uyeda K. Phosphofructokinase. *Adv Enzymol Relat Areas Mol Biol* 1979; 48: 193-244.
22. Evans MJ, Scarpulla RC. NRF-1: a trans-activator of nuclear-encoded respiratory genes in animal cells. *Genes Dev* 1990; 4: 1023-1034.
23. Virbasius JV, Virbasius CA, Scarpulla RC. Identity of GABP with NRF-2, a multisubunit activator of cytochrome oxidase expression, reveals a cellular role for an ETS domain activator of viral promoters. *Genes Dev* 1993; 7: 380-392.
24. Virbasius JV, Scarpulla RC. Activation of the human mitochondrial transcription factor A gene by nuclear respiratory factors: A potential regulatory link between nuclear and mitochondrial gene expression in organelle biogenesis. *Proc Natl Acad Sci USA* 1994; 91: 1309-1313.
25. Scarpulla RC. Transcriptional activators and coactivators in the nuclear control of mitochondrial function in mammalian cells. *Gene* 2002; 286: 81-89.
26. Gulick T, Cresci S, Caira T, Moore DD, Kelly DP. The peroxisome proliferator activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc Natl Acad Sci USA* 1994; 91: 11012-11016.
27. Huss JM, Kopp RP, Kelly DP. Peroxisome proliferator-activated receptor coactivator-1 $\alpha$  (PGC-1 $\alpha$ ) coactivates the cardiac-enriched nuclear receptors estrogen-related receptor- $\alpha$  and - $\gamma$ . *J Biol Chem* 2002; 277: 40265-40274.
28. Vega R, Huss JM, Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor  $\alpha$  in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol* 2000; 20: 1868-1876.
29. Schreiber SN, Knutti D, Brogli K, Uhlmann T, Kralli A. The transcriptional coactivator PGC-1 regulates the expression and activity of the orphan nuclear receptor estrogen-related receptor  $\alpha$  (ERR $\alpha$ ). *J Biol Chem* 2003; 278: 9013-9018.
30. Wu Z, Puigserver P, Andersson U, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 1999; 98: 115-124.
31. Lehman JJ, Barger PM, Kovacs A, Saffitz J, Medeiros DM, Kelly DP. Peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest* 2000; 106: 847-856.
32. Lin J, Wu H, Tarr PT, et al. Transcriptional co-activator PGC-1 $\alpha$  drives the formation of slow-twitch muscle fibres. *Nature* 2002; 418: 797-801.
33. Wende AR, Schaeffer PJ, Parker GJ, et al. A role for the transcriptional coactivator PGC-1 $\alpha$  in muscle refueling. *J Biol Chem* 2007; 282: 36642-36651.
34. Lin J, Handschin C, Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metabolism* 2005; 1: 361-370.
35. Baar K, Wende AR, Jones TE, et al. Adaptations of skeletal muscle to exercise: Rapid increase in the transcriptional coactivator PGC-1. *FASEB J* 2002; 16: 1879-1886.

36. Goto M, Terada S, Kato M, et al. cDNA cloning and mRNA analysis of PGC-1 in epitrochlearis muscle in swimming-exercised rats. *Biochem Biophys Res Commun* 2000; 274: 350-354.
37. Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T, Tabata I. Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. *Biochem Biophys Res Commun* 2002; 296: 350-354.
38. Pilegaard H, Saltin B, Neufer PD. Exercise induces transient transcriptional activation of the PGC-1 $\alpha$  gene in human skeletal muscle. *J Physiol* 2003; 546: 851-858.
39. Norrbom J, Sundberg CJ, Ameln H, Kraus WE, Jansson E, Gustafsson T. PGC-1 $\alpha$  mRNA expression is influenced by metabolic perturbation in exercising human skeletal muscle. *J Appl Physiol* 2004; 96: 189-194.
40. Irrcher I, Adhihetty PJ, Sheehan T, Joseph A-M, Hood DA. PPAR $\gamma$  coactivator-1 $\alpha$  expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. *Am J Physiol* 2003; 284: C1669-C1677.
41. Wright DC, Han D-H, Garcia-Roves PM, Geiger PC, Jones TE, Holloszy JO. Exercise-induced mitochondrial biogenesis begins before the increase in muscle PGC-1 $\alpha$  expression. *J Biol Chem* 2007; 282: 194-199.
42. Mootha V, Handschin C, Arlow D, et al. Err $\alpha$  and Gabpa/b specify PGC-1 $\alpha$ -dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. *Proc Natl Acad Sci USA* 2004; 101: 6570-6575.
43. Puigserver P, Rhee J, Lin J, et al. Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPAR $\gamma$  coactivator-1. *Mol Cell* 2001; 8: 971-982.
44. 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* 2001; 98: 9713-9718.
45. Boppart MD, Asp S, Wojtaszewski JFP, Fielding RA, Mohr T, Goodyear LJ. Marathon running transiently increases c-Jun NH $_2$ -terminal kinase and p38 activities in human skeletal muscle. *J Physiol* 2000; 526.3: 663-669.
46. Widegren U, Jiang XJ, Krook A, et al. Divergent effects of exercise on metabolic and mitogenic signaling pathways in human skeletal muscle. *FASEB J* 1998; 12: 1379-1389.
47. Akimoto T, Pohnert SC, Li P, et al. Exercise stimulates Pgc-1 $\alpha$  transcription in skeletal muscle through activation of the p38 MAPK pathway. *J Biol Chem* 2005; 280: 19587-19593.
48. Cao W, Daniel KW, Robidoux J, et al. p38 mitogen-activated protein kinase is the central regulator of cyclic AMP-dependent transcription of the brown fat uncoupling protein 1 gene. *Mol Cell Biol* 2004; 24: 3057-3067.
49. Czubyrt MP, McAnnally J, Fishman GI, Olson EN. Regulation of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1alpha) and mitochondrial function by MEF2 and HDAC5. *Proc Natl Acad Sci USA* 2003; 100: 1711-1716.
50. Handschin C, Rhee J, Lin J, Tarr PT, Spiegelman BM. An autoregulatory loop controls peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  expression in muscle. *Proc Natl Acad Sci USA* 2003; 100: 7111-7116.
51. Akimoto T, Li P, Yan Z. Functional interaction of regulatory factors with the Pgc-1 $\alpha$  promoter in response to exercise by in vivo imaging. *Am J Physiol Cell Physiol* 2008; 295: C288-C292.
52. Rodnick KJ, Holloszy JO, Mondon CE, James DE. Effects of exercise-training on insulin-regulatable glucose-transporter protein levels in rat skeletal muscle. *Diabetes* 1990; 39: 1425-1429.
53. Ploug T, Stallknecht BM, Pedersen O, et al. Effect of endurance-training on glucose transport capacity and glucose transporter expression in rat skeletal muscle. *Am J Physiol* 1990; 259: E778-E786.
54. Friedman JE, Sherman WM, Reed MJ, Elton CW, Dohm GL. Exercise-training increases glucose transporter protein GLUT4 in skeletal muscle of obese Zucker (fa/fa) rats. *FEBS Lett* 1990; 268: 13-16.

55. Goodyear LJ, Hirshman MF, Valyou PM, Horton ES. Glucose transporter number, function, and subcellular distribution in rat skeletal muscle after exercise training. *Diabetes* 1992; 41: 1091-1099.
56. Wende AR, Huss JM, Schaeffer PJ, Giguere V, Kelly DP. PGC-1 $\alpha$  coactivates PDK4 gene expression via the orphan nuclear receptor ERR $\alpha$ : a mechanism for transcriptional control of muscle glucose metabolism. *Mol Cell Biol* 2005; 25: 10684-10694.
57. Holloszy JO. A forty-year memoir of research on the regulation of glucose transport into muscle. *Am J Physiol* 2003; 284: E453-E467.
58. Rodnick KJ, Henriksen EJ, James DE, Holloszy JO. Exercise-training, glucose transporters and glucose transport in rat skeletal muscles. *Am J Physiol* 1992; 262: C9-C14.
59. Ren J-M, Semenkovich CF, Gulve EA, Gao J, Holloszy JO. Exercise induces rapid increases in GLUT4 expression, glucose transport capacity, and insulin-stimulated glycogen storage in muscle. *J Biol Chem* 1994; 269: 14396-14401.
60. Mora S, Pessin JE. The MEF2A isoform is required for striated muscle-specific expression of the insulin-responsive GLUT4 glucose transporter. *J Biol Chem* 2000; 275: 16323-16328.
61. McGee J, Sparling D, Olson AL, Hargreaves M. Exercise increases MEF2- and GEF DNA-binding activity in human skeletal muscle. *FASEB J* 2006; 20: 348-349.
62. Baar K, Song Z, Semenkovich CF, et al. Skeletal muscle overexpression of nuclear respiratory factor 1 increases glucose transport capacity. *FASEB J* 2003; 17: 1666-1673.
63. Ramachandran B, Yu G, Gulick T. Nuclear respiratory factor 1 controls myocyte enhancer factor 2A transcription to provide a mechanism for coordinate expression of respiratory chain subunits. *J Biol Chem* 2008; 283: 11935-11946.
64. Greiwe JS, Hickner RC, Hansen PA, Racette SB, Chen MM, Holloszy JO. Effects of endurance exercise training on muscle glycogen accumulation in humans. *J Appl Physiol* 1999; 87: 222-226.
65. Hickner RC, Fisher JS, Hansen PA, et al. Muscle glycogen accumulation after endurance exercise in trained and untrained individuals. *J Appl Physiol* 1997; 83: 897-903.
66. Nakatani A, Han D-H, Hansen PA, et al. Effect of endurance exercise training on muscle glycogen supercompensation in rats. *J Appl Physiol* 1997; 82: 711-715.
67. Garcia-Roves PM, Han D-H, Song Z, Jones TE, Hucker KA, Holloszy JO. Prevention of glycogen supercompensation prolongs the increase in muscle GLUT4 after exercise. *Am J Physiol* 2003; 285: E729-E736.
68. Winder WW, Holmes BF, Rudink DS, Jensen EB, Chen M, Holloszy JO. Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol* 2000; 88: 2219-2226.
69. Jorgensen SB, Trebak JT, Viollet B, et al. Role of AMPK $\alpha$ 2 in basal, training-, and AICAR-induced GLUT4, hexokinase II, and mitochondrial protein expression in mouse muscle. *Am J Physiol* 2007; 292: E331-E339.
70. Holmes BF, Kurth-Kraczek EJ, Winder WW. Chronic activation of 5'-AMP-activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. *J Appl Physiol* 1999; 87: 1990-1995.
71. Ojuka EO, Nolte LA, Holloszy JO. Increased expression of GLUT-4 and hexokinase in rat epitrochlearis muscles exposed to AICAR in vitro. *J Appl Physiol* 2000; 88: 1072-1075.
72. Ojuka EO, Jones TE, Nolte LA, et al. Regulation of GLUT4 biogenesis in muscle: Evidence for involvement of AMPK and Ca<sup>2+</sup>. *Am J Physiol* 2002; 282: E1008-E1013.
73. Holmes BF, Sparling DP, Olson AL, Winder WW, Dohm GL. Regulation of muscle GLUT4 enhancer factor and myocyte enhancer factor 2 by AMP-activated protein kinase. *Am J Physiol* 2008; 289: E1071-E1076.
74. McGee SL, van Denderen BJ, Howlett KF, et al. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes* 2008; 57: 860-867.

75. Jager S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 $\alpha$ . *Proc Natl Acad Sci USA* 2007; 104: 12017-12022.
76. Ojuka EO, Jones TE, Han D-H, et al. Intermittent increases in cytosolic Ca<sup>2+</sup> stimulate mitochondrial biogenesis in muscle cells. *Am J Physiol* 2002; 283: E1040-E1045.
77. Ojuka EO, Jones TE, Han D-H, Chen M, Holloszy JO. Raising Ca<sup>2+</sup> in L6 myotubes mimics effects of exercise on mitochondrial biogenesis in muscle. *FASEB J* 2003; 17: 675-681.
78. Freyssenet D, Irrcher I, Connor MK, Di Carlo M, Hood DA. Calcium-regulated changes in mitochondrial phenotype in skeletal muscle cells. *Am J Physiol* 2004; 286: C1053-C1061.
79. Ryder JW, Bassel-Duby R, Olson EN, Zierath JR. Skeletal muscle reprogramming by activation of calcineurin improves insulin action on metabolic pathways. *J Biol Chem* 2003; 278: 44298-44304.
80. Schaeffer PJ, Wende AR, Magee CJ, et al. Calcineurin and calcium/calmodulin-dependent protein kinase activate distinct metabolic gene regulatory programs in cardiac muscle. *J Biol Chem* 2004; 279: 39593-39603.
81. Wu H, Kanatous SB, Thurmond FA, et al. Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science* 2002; 296: 349-352.
82. Long YC, Glund S, Garcia-Roves PM, Zierath JR. Calcineurin regulates skeletal muscle metabolism via coordinated changes in gene expression. *J Biol Chem* 2007; 282: 1607-1614.
83. Wright DC, Geiger PC, Han D-H, Jones TE, Holloszy JO. Calcium induces increases in peroxisome proliferator-activated receptor  $\gamma$  coactivator-1 $\alpha$  and mitochondrial biogenesis by a pathway leading to p38 mitogen-activated protein kinase activation. *J Biol Chem* 2007; 282: 18793-18799.
84. Garcia-Roves PM, Huss J, Holloszy JO. Role of calcineurin in exercise-induced mitochondrial biogenesis. *Am J Physiol Endocrin Metab* 2006; 290: 1172-1179.
85. Garcia-Roves PM, Jones TE, Otani K, Han D-H, Holloszy JO. Calcineurin does not mediate the exercise-induced increase in muscle GLUT4. *Diabetes* 2005; 54: 624-628.
86. Luquet S, Lopez-Soriano J, Holst D, et al. Peroxisome proliferator-activated receptor  $\delta$  controls muscle development and oxidative capability. *FASEB J* 2003; 17: 2299-2301.
87. Wang Y-X, Zhang C-L, Yu RT, et al. Regulation of muscle fiber type and running endurance by PPAR $\delta$ . *PLOS Biology* 2004; 2: 1532-1539.
88. Hancock CR, Han D-H, Chen M, et al. High fat diets cause insulin resistance despite an increase in muscle mitochondria. *Proc Natl Acad Sci USA* 2008; 105: 7815-7820.
89. Garcia-Roves PM, Huss JM, Han D-H, et al. Raising plasma fatty acid concentration induces increased biogenesis of mitochondria in skeletal muscle. *Proc Natl Acad Sci USA* 2007; 104: 10709-10713.
90. Turner N, Bruce CR, Beale SM, et al. Excess lipid availability increases mitochondrial fatty acid oxidative capacity in muscle. Evidence against a role for reduced fatty acid oxidation in lipid-induced insulin resistance in rodents. *Diabetes* 2007; 56: 2085-2092.
91. Hoeks J, Briede JJ, de Vogel J, et al. Mitochondrial function, content and ROS production in rat skeletal muscle: Effect of high-fat feeding. *FEBS Letts* 2008; 582: 510-516.
92. Lagouge M, Argmann C, Gerhart-Hines Z, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 $\alpha$ . *Cell* 2006; 127: 1109-1122.

Received: October 1, 2008

Accepted: December 3, 2008

Author's address: John O. Holloszy, MD, Professor of Medicine, Washington University School of Medicine, 4566 Scott Avenue, Campus Box 8113, St. Louis, MO 63110, Phone: +1-314-362-3506, Fax: +1-314-362-7657; e-mail: jhollosz@dom.wustl.edu