



# NEW IDEAS ABOUT NUTRITION AND THE ADAPTATION TO ENDURANCE TRAINING

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## KEY POINTS

- Classic endurance training increases the number of blood vessels (to deliver more oxygen) and the mitochondrial volume (to produce more energy) in skeletal muscle, with the largest changes occurring in type I fibers, and smaller changes in type II fibers.
- Since most skeletal muscles are ~50% type I and 50% type II fibers, increasing the power/velocity at lactate threshold to a greater extent could be achieved by increasing the mitochondrial volume and number of blood vessels in type II fibers.
- This could be attained by increasing the metabolic stress of endurance exercise, and from a molecular biology point of view, increasing the activity of PGC-1 $\alpha$ , a protein that has been called the master regulator of increased mitochondria and blood vessels.
- Increasing the activity of PGC-1 $\alpha$  can be achieved by increasing the amount of PGC-1 $\alpha$  protein or its charge, allowing it to move to the nucleus and bind to its partners to increase transcription (production) of genes that ultimately lead to more mitochondria and blood vessels.
- The enzymes that increase the charge and transcription of PGC-1 $\alpha$  are regulated by oxygen free radicals, duration of exercise, ATP and glycogen depletion, rate of lactate production and activation of the fight or flight response.
- A simple nutritional strategy is presented that can be used to maximize this adaptive response to endurance training.

## FUELING FOR COMPETITION VS. TRAINING

Every athlete knows that on race day he or she needs to be properly fueled in order to perform at his/her best. However, in the months of training leading up to the event, does the same rule apply, or is there a better way to reach maximal performance? As we learn more about how the body responds to training, it is becoming increasingly clear that in some instances the athlete might be able to get more adaptation if not fully fueled during certain periods of training. This Sports Science Exchange article will discuss the concept of how nutrition affects our adaptation to endurance training.

The common goal for an endurance athlete is to maximize his power/velocity at lactate threshold since this is the best determinant of endurance performance (Coyle, 1999). One of the reasons that lactate begins to accumulate is that as exercise intensity increases we recruit larger motor units, whose fibers tend to be type II fibers with fewer mitochondria (Gollnick et al., 1974). Power/velocity at lactate threshold is therefore partially determined by the number of mitochondria and blood vessels in the largest motor units. Abundant vessels are necessary to maximize the ability to deliver oxygen to mitochondria for aerobic energy production and the fibers in the smaller motor units have already maximized these adaptations. Interestingly, the genetic profile of an individual who does not increase his aerobic fitness in response to endurance training is characterized by the inability to increase blood vessels and mitochondria in his muscles (Timmons et al., 2010). Clearly then, the goal of the endurance athlete is to maximize the number of mitochondria and blood vessels within his larger motor units.

## HOW THE MOLECULAR BIOLOGY WORKS

From the perspective of a molecular biologist, maximizing mitochondria and blood vessels in large type II fibers is the role of peroxisome proliferator-activated receptor gamma co-activator 1-alpha (PGC-1 $\alpha$ ) and its binding partners. It has been known for more than 10 years that PGC-1 $\alpha$  can increase the number of mitochondria within a muscle (Wu et al., 1999) and that PGC-1 $\alpha$  is activated by endurance exercise (Baar et al., 2002; Pilegaard et al., 2000, 2003). More recently, research has shown that PGC-1 $\alpha$ , together with its binding partner estrogen related receptor  $\alpha$  (ERR $\alpha$ ), can also drive the increase in blood vessels that occurs with endurance training (Chinsomboon et al., 2009). Therefore, from a molecular perspective the key to endurance adaptations is to maximize PGC-1 $\alpha$  activity with training.

Since the key to endurance adaptations is PGC-1 $\alpha$ , it is important to understand how this protein works. PGC-1 $\alpha$  is what is called a transcriptional co-activator. This complicated title simply means that its job is to increase transcription (the production of new mRNA from a gene), but it doesn't do this job by itself. The "co-activator" part of the name means that PGC-1 $\alpha$  doesn't identify the genes that it turns on. Instead, it works by binding to a number of partners and increasing their activity. Put another way, the binding partners identify which genes to turn on, whereas PGC-1 $\alpha$  determines the volume. For example, by interacting with the nuclear response factors, PGC-1 $\alpha$  can increase mitochondrial proteins (Wu et al., 1999); by interacting with the peroxisome proliferator-activated receptors (PPARs), PGC-1 $\alpha$

can increase fat oxidation proteins (Narkar et al., 2008); and by interacting with  $ERR\alpha$  PGC-1 $\alpha$  can increase blood vessels (Chinsomboon et al., 2009). Thus, in many ways, together with its binding partners, PGC-1 $\alpha$  can induce all of the adaptations to endurance exercise.

So, if the key to performance in the future is repeated activation of PGC-1 $\alpha$  in training, the question becomes how can PGC-1 $\alpha$  activity be maximized? PGC-1 $\alpha$  is activated in two ways. First, existing PGC-1 $\alpha$  protein can be modified to either make it go into the nucleus (where transcription takes place) and interact better with its binding partners. There are two ways that PGC-1 $\alpha$  is modified: phosphorylation and acetylation. PGC-1 $\alpha$  is most active when it is more phosphorylated and less acetylated. Phosphorylation means that enzymes within muscle add a negative charge (a phosphate group) to the PGC-1 $\alpha$  protein. Acetylation means that a different set of enzymes remove a positive charge from the PGC-1 $\alpha$  protein by adding a neutral acetyl group to a positively charged lysine residue. As a result, it may be easier to remember that PGC-1 $\alpha$  is most active when it has more regions with positive and negative charges on them. This makes sense once you know that proteins bind to one another largely as the result of charge interactions (positive amino acids in one protein bind to negative amino acids in another protein). Therefore, more phosphorylation and less acetylation means more negative and positive charges and therefore better binding between PGC-1 $\alpha$  and its partners to turn on genes.

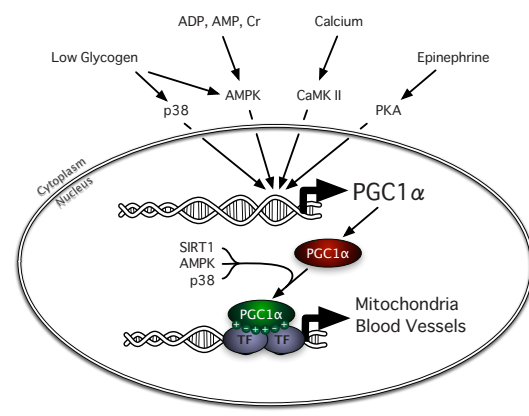
The second way to increase PGC-1 $\alpha$  activity is to make more of it. The amount of PGC-1 $\alpha$  is regulated by how much of its mRNA is made at any one time. Therefore, in order to make more PGC-1 $\alpha$  protein, the transcription of the PGC-1 $\alpha$  gene has to increase. The complex regulation of PGC-1 $\alpha$  transcription has been elegantly detailed by Akimoto and colleagues (2004) and will be summarized below.

### Modulation of PGC-1 $\alpha$

From the data presented above, a coach with an eye on molecular biology would be looking to increase the charge and the transcription of PGC-1 $\alpha$  in larger motor units in order to maximize endurance performance. The big question is: How can the charge and the transcription of PGC-1 $\alpha$  be increased? All of the research to date suggests that this is controlled by metabolic intermediates such as: ADP/AMP,  $NAD^+$ , reactive oxygen (ROS), cAMP and calcium (Figure 1). The effects of calcium will be discussed first and this will be followed by a discussion of the others.

### Calcium

Every time muscles contract, calcium is released from an intracellular store. Therefore, when cycling at a cadence of 100 rpm, this means that muscles are releasing calcium 100 times every minute. While the majority of that calcium is used to initiate contraction, some of it activates a family of calcium-binding proteins that are important in the adaptation to endurance training. One of these calcium-binding proteins is an enzyme called calcium/calmodulin-activated kinase II (CaMKII). CaMKII is a powerful activator of PGC-1 $\alpha$  transcription. Therefore, calcium release increases the amount of PGC-1 $\alpha$  in part by activating CaMKII. Since the amount of calcium released in a contracting skeletal muscle fiber generally doesn't change from contraction to contraction, the only way to increase the effects of calcium is to train that muscle fiber longer. This is the molecular rationale behind the idea of long slow training. The longer an athlete is on the bike, in the pool, running, etc., the longer the calcium levels will be high in his muscle fibers and the more PGC-1 $\alpha$  transcription will occur. When this type of long slow training is started, slower fibers (type I) and small groups of faster fibers (type II) are used. As work continues, glycogen in these fibers decreases and larger groups of fast muscle fibers must be used. Therefore, near the end of a long training bout, calcium release will occur in the larger motor units, providing the signal to increase mitochondria and blood vessels in these muscle fibers and improve the power/velocity at lactate threshold.



**Figure 1.** At times of muscle continuous contraction (rising intracellular calcium), high metabolic stress (ATP turnover, lactate production, glycogen depletion, caloric insufficiency) and whole body stress (rising epinephrine), a group of kinases and deacetylases are activated that, 1) increase the transcription of the PGC-1 $\alpha$  gene (making more PGC-1 $\alpha$  mRNA) and 2) increase the charge of PGC-1 $\alpha$ , making it more active. The result of these changes is an increase in mitochondrial mass, blood vessels and, therefore, aerobic capacity.

If calcium increases PGC-1 $\alpha$  transcription, what increases its charge? The charge of PGC-1 $\alpha$  (phosphorylation and acetylation) is regulated by stress and stress is highest when training at high intensities. When training at high intensities, four things occur that affect PGC-1 $\alpha$  activity: 1) increased ADP and AMP; 2) depleted muscle glycogen; 3) increased lactate (NAD<sup>+</sup>) production; and 4) increased fight or flight response (e.g., release of epinephrine).

### AMPK

During high-intensity training, ATP and phosphocreatine (PCr) are rapidly used. In order to continue to train, ATP and PCr need to be regenerated either through glycolysis or aerobic metabolism. In the process of regenerating ATP and PCr, three other metabolites are made that affect PGC-1 $\alpha$  activity: ADP, AMP and creatine (Cr). As ADP, AMP and Cr rise, this activates a protein called the AMP-activated protein kinase (AMPK). AMPK is one of the most potent regulators of PGC-1 $\alpha$  activity and can increase both the charge of PGC-1 $\alpha$  by phosphorylating it (Jager et al., 2007) as well as increase its transcription (McGee et al., 2008). In this way, AMPK can regulate PGC-1 $\alpha$  and our power/velocity at lactate threshold by increasing the blood vessels and mitochondria in larger motor units.

### Glycogen

During exercise, glycogen stored within our muscle fibers is used to produce the needed energy. As the glycogen levels in the contracting muscles fall, the loss of glycogen is sensed and the muscles respond by activating AMPK and another important protein called the p38 mitogen activated protein kinase (Chan et al., 2004). Like AMPK, p38 increases both the charge of PGC-1 $\alpha$  by phosphorylating it (Puigserver et al., 2001) and its transcription (Pogozelski et al., 2009). Therefore, decreasing muscle glycogen is a powerful nutritional tool to increase the activity of PGC-1 $\alpha$ . Muscle glycogen can be decreased either by training at high intensity or training for a long period of time.

### NAD<sup>+</sup>

Training above the lactate threshold results in the accumulation of lactate within contracting muscle fibers. Lactate rises at high intensities due to 1) recruitment of large motor units with fewer mitochondria; 2) production of more of the fight or flight hormone epinephrine (also called adrenaline), and the metabolites calcium, ADP and AMP, which all directly increase glycogen breakdown and drive glycolysis in muscles; and 3) decreasing lactate clearance in the liver and kidneys by redirecting blood flow away from these tissues to working muscles.

The rise in lactate occurs in an effort to regenerate NAD<sup>+</sup> so that glycolysis can continue. NAD<sup>+</sup> is required for glycolysis, but also serves another very important role in activating the NAD<sup>+</sup>-dependent deacetylases. The deacetylases are a family of enzymes that remove

acetyl groups from proteins making them more positive. The most famous member of this family, sirtuin (SIRT1), is known to deacetylate and increase the charge of PGC-1 $\alpha$ . Even though we have shown that SIRT1 is not required for the adaptation to endurance exercise (Philp et al., 2011), it can increase the activity of PGC-1 $\alpha$  and increase mitochondrial mass when it is present (Rodgers et al., 2005). Therefore, activating SIRT1 should increase our endurance adaptation.

SIRT1 is well known, because it is activated by caloric restriction, and in the long-term, is thought to increase lifespan in lower organisms (Ghosh, 2008). It was originally thought that SIRT1 was activated by resveratrol, a component of the skins of the red grapes that red wine is made from. However, it is now known that resveratrol does not directly activate SIRT1 (Park et al., 2012). However, SIRT1 does have important metabolic roles. For instance, it is required for the positive effects of caloric restriction on muscle metabolism (Schenk et al., 2011). This fact, together with the above data, suggests that in order to increase the activity of SIRT1, and therefore PGC-1 $\alpha$ , caloric intake should be limited before endurance training. This type of training has been validated in humans (Van Proeyen et al., 2011a, b) with men responding better than women (Stannard et al., 2010).

### Epinephrine

As mentioned above, training at intensities above our lactate threshold produces a dramatic increase in the fight or flight hormone epinephrine. Epinephrine also increases when exercising for long periods without consuming carbohydrates. Epinephrine has many functions in the body that allow exercise at high intensities. This hormone also plays an important role in the activation of PGC-1 $\alpha$  (Chinsomboon et al., 2009). In mice, simply injecting a drug that mimics the effects of epinephrine increases the transcription of PGC-1 $\alpha$  through the second messenger cAMP, and this is enough to increase mitochondria and the formation of new blood vessels in muscle (Chinsomboon et al., 2009).

### Reactive Oxygen Species

The last important factor in the control of PGC-1 $\alpha$  is reactive oxygen species (ROS). ROS, in the form of oxygen free radicals, are produced in mitochondria during aerobic exercise. During exercise, the rate of ROS production increases. Most of these ROS are quenched naturally by a series of cellular scavengers and antioxidants. However, it seems that some ROS are necessary to increase the transcription of PGC-1 $\alpha$  (Irrcher et al., 2009). In fact, supplementation with high levels of synthetic antioxidants can blunt the normal increase in mitochondria with endurance training (Strobel et al., 2011), suggesting that consumption of high levels of synthetic antioxidants prior to training will blunt the training response. In contrast, there is currently no evidence to suggest that the natural occurring levels of antioxidants present in fruits and vegetables have any negative effects on PGC-1 $\alpha$  modulated training adaptations.

### SCIENCE-BASED RECOMMENDATIONS FOR TRAINING TO MAXIMIZE ENDURANCE ADAPTATIONS AND SUMMARY

Using the molecular and metabolic information provided above, some simple nutritional strategies can be provided to make sure that we get the most out of our endurance training. In order to do this, our goal as athletes and coaches is to maximize calcium, ADP/AMP, NAD<sup>+</sup>, cAMP and ROS while minimizing injury. Below is a recommendation based on our understanding of the molecular biology of endurance adaptations. There are no studies yet to support superior training effects but this is the recommendation for endurance athletes based on current knowledge of molecular biology in relation to training adaptation:

- a) Once or twice a week, start an “adaptive” session in a caloric deficit (i.e., in a fasted state such as in the morning before breakfast). This will make sure that SIRT1 activity would be high.
- b) Use a pre-training drink containing a low dose of caffeine (3mg/kg body weight) but free of synthetic antioxidants so that perceived exertion would be decreased and the antioxidant scavenger levels would remain low. Caffeine decreases perceived exertion, helping to maintain exercise at a high intensity even when carbohydrate-depleted.
- c) Use a pre-training drink low in carbohydrate in an effort to maximize AMPK activity and epinephrine levels as a result of training.
- d) Train at a low absolute intensity for a long time to maximize the amount of time all of these signals are on while minimizing the mechanical strain on our bodies.
- e) Alternatively, use double sessions where the first session depletes glycogen and the second session is performed at a high intensity in a glycogen-depleted state.

It is important to remember that this nutritional strategy is designed to maximize the adaptive response to training and is likely to decrease performance during training. Of course such training strategies will affect recovery as well, and athletes will have to find ways to balance training and recovery. Too many low glycogen sessions will increase the risk of overtraining despite lowering of the quality of training.

It is also very important to remember that the job of a molecular biologist is to reduce complex processes to simple genetic models. In reality, endurance performance is dependent on far more than PGC-1 $\alpha$ .

The nutritional strategy presented here uses the most recent scientific data to maximize the metabolic stress that leads to the increase in mitochondria and blood vessels that are required to increase power/velocity at lactate threshold. However, this stress would also decrease immune function, and therefore, if used too

often may increase infections and decrease training. This type of “adaptive” training should simply be seen as another tool that can be used 2-3 times per week to help an athlete build his endurance capacity. What the athlete does with high-endurance capacity will depend on those high-quality training sessions that come when the athlete is completely fueled.

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