Altering Endogenous Carbohydrate Availability to Support Training Adaptations

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Abstract
Glycogen was first identified in muscle over a century and a half ago. Even though we have known of its existence and its role in metabolism for a long time, recognition of its ability to directly and indirectly modulate signaling and the adaptation to exercise is far more recent. Acute exercise induces a number of changes within the body (i.e. sympathetic nervous system activation and elevation of plasma free fatty acids) and muscle (increased AMP-activated protein kinase activity and fat metabolism) that may underlie the long-term adaptation to training. These changes are also affected by glycogen depletion. This review discusses the effect of exercise in a glycogen-depleted state on metabolism and signaling and how this affects the adaptation to exercise. Although ‘training low’ may increase cellular markers associated with training and enhance functions such as fat oxidation at sub-maximal exercise intensities, how this translates to performance is unclear. Further research is warranted to identify situations both in health and athletic performance where training with low glycogen levels may be beneficial. In the meantime, athletes and coaches need to weigh the pros and cons of training with low carbohydrate within a periodized training program.

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Introduction

In 1858 [1], Claude Bernard reported the successful isolation of carbohydrate from liver and muscle. This discovery began a century and half of research aimed at determining the function and clinical relevance of these cellular carbohydrate stores. The principal storage form of carbohydrate in mammals is
glycogen, a polymer of d-glycosyl units joined by 1:4 or 1:6 bonds to produce a polysaccharide ‘tree’.

The structure and metabolic regulation of glycogen has been the focus of researchers in many different fields [2]. Prior to the 1930s, glycogenolysis was thought to occur by simple hydrolysis of glycogen to glucose [2]. However, in 1936, Parnas and Ostern [3] demonstrated that it was more complex, requiring inorganic phosphate and resulting in the release of a hexose monophosphate 90% of the time and free glucose only 10% of the time. Carl and Gerty Cori identified the hexose monophosphate as glucose-1-phosphate, identified the enzyme that catalyzed this reaction as glycogen phosphorylase, and showed that the breakdown of glycogen was controlled by a series of reactions that required phosphate transfer [4]. For this work, which revolutionized our understanding of the physiological regulation of glycogen and introduced the first signaling cascade, the Coris, along with Prof. Bernardo Houssay, received the Nobel Prize in 1947.

Almost a century on from Bernard’s landmark paper, Bergstrom and Hultman began to investigate the relationship between glycogen and exercise performance, and the mechanism of glycogen resynthesis following depletion [5]. These early studies demonstrated that the glycogen content of the working muscle is a major determinant of the capacity to sustain long-duration exercise [5]. Importantly, Hultman and Bergstrom [6] also demonstrated that alterations in diet and exercise could greatly vary the glycogen content in skeletal muscle, which then affected exercise sustainability. Finally, they observed that ingestion of a high-carbohydrate diet following exercise increased the recovery of muscle glycogen stores compared to a diet containing mainly fat and protein, providing direct evidence that dietary glucose was the precursor for muscle glycogen [7]. The increase in exercise performance with increasing glycogen has been replicated on numerous occasions [8] and has led to widespread changes in the nutrition of athletes.

**Training with Low Glycogen: The Molecular Viewpoint**

When glycogen availability decreases, whole body metabolism shifts dramatically. In humans, glycogen depletion results in reduced pyruvate oxidation, increased systemic release of amino acids from muscle proteolysis, and increased fat metabolism [9]. The decrease in glycogen content with exercise occurs concomitant with an increase in fatty acid oxidation, suggesting that lower glycogen is directly sensed by the body and leads to a shift in metabolism from carbohydrate to fatty acids. Furthermore, the transient decrease in muscle glycogen with exercise may be directly involved in the cellular adaptation to training by controlling signaling pathways within the active muscle [10].
To directly test the postulate that glycogen depletion is related to the muscular adaptation to training, Hansen et al. [11], hypothesized that training in a low glycogen state would provide greater muscle adaptation than the equivalent training in a normal or high glycogen state. In support of the hypothesis, a leg that started half of its sessions with low muscle glycogen concentrations over 10 weeks of training showed a greater increase in time to exhaustion and citrate synthase activity, and a trend towards higher 3-hydroxyacyl-CoA dehydrogenase activity (βHAD) than the subject’s other leg that trained with high glycogen. The results of this study have since been extended to trained athletes [12, 13]. As would be expected, athletes who commenced high-intensity intervals with ~50% lower muscle glycogen showed significantly lower power outputs during each training bout than a cohort who exercised with higher glycogen content. However, 60-min time trial performance improved to the same degree following 3 weeks of training in both the cyclists who ‘trained low’ for half their sessions and cyclists who refueled between their training sessions. The major difference between the two training groups was in submaximal exercise, where following training, the low glycogen group used a significantly higher percentage of fatty acids and a significantly smaller amount of glycogen. Interestingly, tracer analysis indicated that the greater fat oxidation was the result of the increased oxidation of intramuscular triglycerides (IMTG), facilitated by increases in cluster of differentiation (CD36) protein and βHAD protein and activity. Together, these data indicate that regardless of training state, high-intensity exercise with low muscle glycogen improves the capacity for fatty acid oxidation to a greater degree than training with normal glycogen levels.

Manipulating glycogen stores creates a number of alterations that together are likely to underpin the improved adaptive response to training. These include changes in sympathetic nervous system activity, changes in the activity of proteins that contain a glycogen-binding domain, and alterations in fat and carbohydrate metabolism. Even though the exact molecular mechanism has yet to be determined, the existing data are sufficient to develop salient hypotheses as to how exercise in a low glycogen state drives improved fatty acid oxidation.

The key components of this paradigm (described below) are the peroxisome proliferator-activated receptor-γ coactivator (PGC)1α, the 5’AMP-activated protein kinase (AMPK), and the peroxisome proliferator-activated receptors (PPAR) α and δ (fig. 1). PGC1α is a coactivator of transcription factors involved in mitochondrial biogenesis, angiogenesis, and fat metabolism. AMPK is activated by metabolic stress, and acutely controls the rate of fat metabolism through its regulation of malonyl CoA levels and in the long-term alters transcription of genes involved in mitochondrial biogenesis and metabolism. The PPARs are fatty acid-activated transcription factors that, together with PGC1α, control the expression of enzymes of fatty acid metabolism. How the activity of these factors might be altered in a low glycogen state and work together to produce the enhanced training adaptation will be discussed below.
Exercise in a low glycogen state is perceived as a greater stress to the body resulting in elevations in circulating catecholamine (epinephrine and norepinephrine) levels, which can affect the adaptive response to training in two obvious ways. First, catecholamines can affect the adaptive response through the phosphorylation and activation of the cAMP response element-binding protein (CREB). Exercise can increase the activation state of CREB in both exercised muscle and muscles that were not recruited during the exercise [14] due to the elevated sympathetic nervous system activity. One of the targets of CREB is PGC1α. Akimoto et al. [15] demonstrated that the CREB site within the PGC1α promoter is required for the increase in PGC1α induced by exercise. Miura et al. [16] extended this work to show that blocking β-adrenergic receptors with ICI 118,551 prevented 69% of the exercise-induced increase in PGC1α.
Further, the induction of PGC1α following exercise was lower in mice lacking β-receptors than in wild-type mice [16]. Not only is PGC1α mRNA increased by catecholamines, catecholamines drive the expression of a splice variant of PGC1α made from a different promoter that may have a higher activity [17]. Together, these data suggest that catecholamines acting through β-adrenergic receptors may play a significant role in the increase in fatty acid oxidation following endurance training in the glycogen-depleted state. However, it should be noted that Mortensen et al. [18] showed that training in a low glycogen state did not alter the expression of the PGC1α family members in response to exercise, and Robinson et al. [19] did not see an increase in PGC1α expression or mitochondrial protein synthesis within the first 5 h after a 1-hour infusion of isoproterenol. However, it is not surprising that PGC1α mRNA is not changed after training (more likely after acute exercise) [18], and whether the primers used in these studies would identify the splice variant is unclear. Therefore, whether catecholamines can acutely regulate PGC1α in humans remains to be determined.

The second way catecholamines can increase the adaptive response to training is by altering fatty acid metabolism. Catecholamines alter fat metabolism by activating hormone-sensitive lipase (HSL) through protein kinase A (PKA). HSL is phosphorylated by PKA on three sites (Ser563, Ser659 and Ser660). Even though it is not clear how these sites regulate HSL activity, increased HSL activity drives lipolysis both in adipose tissue and skeletal muscle. The result is the liberation of free fatty acids from both fat and intramuscular depots. The increase in fatty acids delivered to the muscle during exercise results in an increase in the fatty acids oxidized for energy and an increase in the activity of the PPARs, transcription factors that are activated by binding to fatty acids. In muscle, PPARα and -δ, together with PGC1α, bind to the promoters of key enzymes of fat metabolism including carnitine palmytol transferase 1 (CPT1), CD36/fatty acid translocase (CD36/FAT), and HSL itself. We have recently found, in rats, that exercise in a glycogen-depleted state causes a greater increase in binding of PPARδ to the promoter of the CPT1 gene [unpubl. results], suggesting that PPARδ activity is increased and may underlie the improved phenotype following glycogen depletion training.

Mammalian AMPK is an αβγ heterotrimer with multiple genes encoding each of the subunits [20]. The regulation of the catalytic α-subunit is mediated by its phosphorylation, a glycogen-binding domain located on the β-subunit and the binding of AMP or ATP to the γ-subunit [20]. High-intensity endurance exercise increases α2-AMPK activity up to 7-fold, whereas α1-activity increases only ~50%. Beyond its greater activation by exercise, α2-AMPK controls the metabolic adaptations associated with endurance exercise training. Mice that overexpress dominant negative α2 do not undergo mitochondrial biogenesis in response to energy deprivation [21] and ablation of α2-AMPK decreases basal and AICAR-stimulated expression of several metabolic genes in skeletal muscle and results in insulin resistance [22], suggesting that activation of α2-AMPK promotes an
endurance training phenotype. The tumor suppressor LKB1 appears to be the principal α2-AMPK kinase in skeletal muscle since muscle-specific deletion of LKB1 reduces α2-AMPK activity by ~95% [23]. We [unpubl. results] and others [24] have observed that both the basal and postexercise activity of α2-AMPK is higher in the glycogen-depleted state. Furthermore, ingestion of sufficient glucose to spare glycogen depletion attenuates AMPK activation ~50% compared to a placebo trial [25], whereas a similar glucose ingestion protocol during cycling that did not result in glycogen sparing has no effect on α2-AMPK activity [26]. This suggests that the amount of glycogen within the muscle directly modulates AMPK activity. In fact, McBride et al. [27] have shown that incubation of AMPK with isomaltose, which mimics the branch points of glycogen, inhibited the AMPK activity by 33%. Steinberg et al. [28] demonstrated that exercise in a glycogen-depleted state also led to nuclear translocation of α2-AMPK and subsequent increases in GLUT4 mRNA expression. Nuclear translocation of α2-AMPK has been suggested to be an important factor in the regulation of two important transcription factors: myocyte-enhancing factor 2 (MEF2) and nuclear respiratory factor 1 (NRF1). MEF2 and NRF1 play an important role in the control of a variety of genes for fiber type, carbohydrate metabolism, and mitochondrial biogenesis [29]. However, we demonstrated that the training-induced increase in GLUT4 protein was blunted when half of the training occurred in the low glycogen state [12], suggesting that AMPK alone does not underlie the low glycogen training effect.

Pharmacological studies by Narkar et al. [30] may provide an important clue as to how low-glycogen training can affect enzymes of fat metabolism. These authors showed that training rats on a treadmill while at the same time giving them GW1516, a drug that activates PPARδ, resulted in an increased capacity to use fat as a fuel. The GW compound alone had no effect on endurance or fat metabolism, whereas mice that were exposed to both the drug and training increased CPT-1, CD36/FAT and lipoprotein lipase, as well as their ability to run at ~50% of VO2max more than those that just undertook training. Interestingly, when the GW compound was given together with AICAR, a drug that activates AMPK, the authors saw an increase in enzymes of fat metabolism. They also observed a direct interaction between α2-AMPK and PPARδ and showed that the activity of AMPK and PPARδ could be increased a further 2.5-fold by PGC1α. Together, these data suggest that PPARδ interacts with AMPK and PGC-1α to increase the enzymes of fatty acid metabolism (fig. 1). When extrapolated to exercise in the glycogen-depleted state, these data suggest a possible mechanism for the improved adaptation: (1) exercise in the glycogen-depleted state increases circulating catecholamines; (2) catecholamines drive the transcripational activation of PGC1α from its alternative promoter resulting in a more active form of PGC1α in muscle; (3) catecholamines also drive the breakdown of triglycerides to fatty acids resulting in higher plasma nonesterified fatty acids (NEFA); (4) increased NEFA and the breakdown of IMTGs
results in greater uptake and oxidation of fatty acids; (5) the greater uptake and/or oxidation of fatty acids results in increased activation of PPARα and -δ; (6) high-intensity exercise in the glycogen-depleted state increases AMPK activity more than the equivalent exercise in a glycogen-replete state; (7) together, the highly active PGC1α, PPARs and AMPK bind to the promoters of genes involved in fatty acid metabolism and increase the expression of these genes; (8) when repeated, this results in an increased capacity to use fat as a fuel during exercise at 70% of VO₂max, sparing muscle glycogen, and potentially improving exercise capacity.

**Training with Low Glycogen: The Athlete’s Viewpoint**

Clearly, the muscular adaptations achieved by training are an important part of improving athletic performance. However, changes in muscle physiology or cellular markers of metabolism are not, per se, a proxy for performance. Sports scientists are continually looking for strategies to enhance performance, and the elite athletes and coaches with whom they work stay up to date on cutting-edge research. Therefore, it is not surprising that news of the benefits of training with low glycogen [11] created a level of excitement in sports circles. Unfortunately, there is anecdotal evidence of misunderstandings by athletes, coaches and even sports scientists of the principles and practice of ‘train low’ techniques. Ill informed athletes are consuming carbohydrate-restricted diets for prolonged periods as a result of misunderstandings surrounding the train low strategy. However, the seminal train low study [11] neither implemented a carbohydrate-restricted diet nor exposed all exercise sessions to a low carbohydrate environment. Instead, their protocol and a series of follow-up studies have involved a ‘two a day’ training program supported by a carbohydrate-rich diet. The second training session in a day is undertaken a few hours following a first exercise bout whose goal is to deplete glycogen, with a short recovery period with negligible carbohydrate intake ensuring that there is minimal refueling during the recovery period. The second training session is then started with approximately 40% less muscle glycogen. However, in the recovery from the glycogen depleted session it is likely that there is super-compensation of muscle glycogen due to the subjects’ high carbohydrate diet and the ~40 h of recovery before the next training session [31]. Other studies have used: (1) exercise after an overnight fast; (2) water only during prolonged training sessions; (3) carbohydrate-free periods in the hours after exercise, or (4) carbohydrate intakes below the fuel requirements of the training load [32]. These strategies differ in the duration of exposure to the low-carbohydrate environment as well as whether the glycogen is depleted locally (in the active muscle) or centrally (in the liver).

The clever design of the original study by Hansen et al. [11] involved previously untrained people who exercised one leg with a two a day training protocol
every second day, while their contralateral leg undertook the same workouts spread over a daily training schedule. Ten weeks of training increased maximal power output equally in each leg, but the ‘two a day’ leg, which commenced half the training sessions in a low glycogen state, showed a greater enhancement of its capacity to work at ~90% of pretraining maximal power output. These findings have significant scientific merit and possible application for exercise programs targeting metabolic improvements and health outcomes. However, the relevance to athletic populations has been questioned on various grounds: (1) the large increase in metabolic capacity in previously untrained individuals compared with what would be expected from a well-trained population; (2) the relevance of the peripherally limited one-legged kicking exercise to sport, and (3) the use of a ‘clamped’ training program (each leg trained at the same absolute intensity in each session) in comparison to the principles of progressive overload and self-pacing that are incorporated into the training programs of athletes [32, 33].

Three further studies [12, 13, 34] have been undertaken utilizing the two a day model of training with low glycogen in athletic populations ranging from active to well trained. A variety of different parameters have been tested in these studies, including the type and volume of training, whether training was performed at a fixed intensity or according to a self-selected pace, and the protocol used to measure performance. It will take a large number of studies to cover all the potential areas of interest and application to sport. However, the general findings of this train low literature are consistent across all of the studies, namely that undertaking some exercise sessions with low muscle glycogen concentrations can enhance the metabolic adaptations associated with training, even in well-trained individuals. Muscle markers of aerobic metabolism are typically increased [12, 13, 34], and there is upregulation of fat utilization during steady state exercise [12, 13]. However, these benefits have not translated into a detectable performance improvement over conventional training with higher muscle glycogen concentrations [12, 13, 34]. Furthermore, the two studies in which the sessions completed with low muscle glycogen content were self-paced high-intensity interval workouts, self-selected power outputs were lower in the train low group than in the cohort undertaking a conventional training program [12, 13].

The possibility that train low protocols reduce the capacity to train at higher intensities/workloads requires attention. Most coaches prescribe training programs in which athletes are required to work at specific intensities/speeds/power outputs that are higher than their ‘race pace’ since success in most sports, including endurance and ultra-endurance events, is defined by high end speed (e.g. the sprint to the ball or finish line, the breakaway, the uphill climb, the surge). Intuitively, sacrificing the capacity to train at high intensities should be made with reluctance until there is evidence that this does not impair performance. Indeed, the performance benefits of training at moderate altitude,
another strategy used to amplify training adaptations, were questioned when it was realized that high-speed training was compromised. As a result, altitude training is now performed differently: only during the base phase of training in which high-intensity training is less important, using ‘live high, train low’ (sleep at high altitude and train at lower altitudes) protocol, selecting a training location that provides access to a lower altitude venue where high-speed training sessions can be completed, or simply taking advantage of slight downhill sections that allow high-speed training at a lower metabolic cost. It should also be noted that unlike the endurance studies described above, the adaptations to resistance exercise are attenuated when the session is undertaken with low muscle glycogen content [35].

An important question from these studies is why the metabolic advantages in the muscle achieved by train low protocols have, in trained individuals at least, failed to transfer into performance benefits. Explanations for this apparent disconnect include the brevity of the study period, the length of the performance test (60 min or less) not benefiting from glycogen sparing, the possibility that performance is not reliant or quantitatively linked to the markers that have been measured [32] and the fact that as the intensity of exercise increases above 75–80% VO$_{2\text{max}}$ during the performance test, there is a rise in epinephrine and a shift to carbohydrate metabolism regardless of the capacity to oxidize fat. Finally, there is the possibility that we may be unable to measure performance well enough in the lab to detect changes that would be significant in the world of sport [36]. It should also be noted that the performance trials used in the two studies most relevant to athletic training practices [12, 13] were undertaken following an overnight fast and without carbohydrate supplementation. This does not reflect the ‘compete high’ model originally proposed [11], or the current practices or sports nutrition guidelines for athletes [37].

Even though much of the discussion about low-glycogen training has focused on the benefits, it is important to consider the potential for side effects of this strategy as well. Apart from the potential loss of training at high power outputs/work rates, there is the possibility that upregulation of fat utilization during exercise is associated with downregulation of carbohydrate utilization. Indeed, in adapting to high-fat diets prior to carbohydrate loading for endurance and ultra-endurance events, the glycogen ‘sparing’ during exercise now appears to be the result of impaired glycogen use due to reduced pyruvate dehydrogenase activity, resulting in decreased carbohydrate entry into the TCA cycle [38]. This impairment in carbohydrate oxidation can impair the performance of sustained high-intensity activities during endurance events [39]. Finally, the effect of repeated training with low carbohydrate status on the risk of illness [40], injury [41] and overtraining [42] also needs to be considered.

Most elite athletes practice an intricate periodization of both diet and exercise loads within their training program that varies within a microcycle, e.g. over week(s) as well as over the year. Either by intent or happenstance, some
Training sessions are undertaken with low carbohydrate availability (overnight fasting, high volume training involving several sessions in the day, little carbohydrate intake during the workout), whereas others are undertaken with high carbohydrate status (more recovery time, post-meal, carbohydrate intake during the session) [32]. Therefore, in real-life, elite athletes already undertake a proportion of their training with low-glycogen content. It makes sense that sessions undertaken at lower intensity or at the beginning of a training cycle are most suited or perhaps least disadvantaged by train low strategies. Conversely, ‘quality’ sessions done at higher intensities or in the transition to peaking for competition are best undertaken with better fuel support. Athletes will, by accident or design, develop a nutrition strategy that suits their lifestyle and resources and maximizes their training and competition performances [32]. Finding this optimum balance is the art of coaching, and also the difficulty for sports scientists who need to conduct their studies with far more rigorous control than exists in real life. Furthermore, the milliseconds that differentiate the winners may result from things that cannot be measured in the lab.

**Remaining Questions**

The hypothesis that manipulating glycogen can optimize training adaptations is relatively new. As a result, there are a number of important questions that remain to be answered.

1. Is sympathetic nervous system activation required for the improvement in fatty acid oxidation following low-glycogen training? If catecholamines are important in regulating the expression of PGC1α from the alternative promoter, then high-intensity exercise will be a stronger stimulus for adaptation than the equivalent work completed at a lower intensity.

2. Are the PPARs, together with AMPK and PGC1α central to the improvement in fatty acid oxidation following low glycogen training? Is the response dependent on PPARα or -δ or do both isoforms function equivalently? These questions can be addressed using the PPAR-specific agonists: LY518674, fenofibrate, gemfibrozil, or troglitazone for PPARα and GW1516 for PPARδ. If, as expected, the PPARs play an important role in this adaptation, these agonists will have to be added to the banned agents list by WADA and other governing bodies for endurance competitions.

3. Is the increase in fatty acid uptake and oxidation key to increasing PPAR activity? If so, which endogenous ligands (fatty acids) best activate the PPARs? Are there nutritional strategies that can legally maximize the activation of the PPARs? For instance, green tea extract increases circulating fatty acid levels. Can green tea increase PPAR activation and the adaptive response to training?
4 What are the current training and nutrition practices of the world's best athletes, and how does this influence carbohydrate availability for each session in the microcycles and macrocycles of the periodized training program?

5 How can research protocols be designed to systematically study the range of possible permutations and combinations of train high and train low strategies within the periodized training programs of highly trained athletes, and thus identify ways to promote superior performance outcomes in specific sports?

Conclusions

The manipulation of pretraining muscle glycogen is a very easy way to optimize the capacity for fat oxidation following endurance exercise training. Low-glycogen training has the potential to not only provide a novel training technique for athletes, but it may also provide fundamental information regarding the mechanism of muscle adaptation to exercise. For instance, determining the molecular mechanism underlying the low-glycogen training effect may lead to the development of novel treatments for diseases such as diabetes that result from altered muscle metabolic function. Before it can be successfully applied to the athletic situation, however, more questions need to be answered about the potential for negative outcomes, and the optimum way to integrate some specific train low sessions into the periodized training program. It may prove difficult to undertake the sophisticated studies needed to define the optimal protocols, just as it is difficult for sports scientists to educate athletes and coaches about the true interpretations and applications of their work.

References


Dr. Burke: The opportunity to work with you on this topic has been interesting because we come to it from different perspectives. If I can use a gross generalization: I come from a world where success is measured in terms of gold medals, while the academic world is driven by ‘publish or perish.’ So, while we can appreciate each other’s work, it doesn’t always mean we are pursuing the same ends or speaking the same language. The new techniques in molecular biology provide fascinating insights into what happens when a stimulus is applied acutely or repeatedly to the muscle, but I am interested in intact humans and the application of a variety of changing stimuli. How do we take the ‘black and white’ details of these complex molecular insights into my world? How do we set up a molecular model with all the variables associated with the periodized training and nutrition programs undertaken by real-life athletes? And how can we decide if the outcomes make a difference to sports performance, or even on systems outside the muscle?

Dr. Baar: What I did here is talk exclusively about muscle. There is no reason that you need to periodize for strength as far as the muscle is concerned. But what happens outside the muscle, in the connective tissue, is much different from what is happening in...
muscle, and there is a much slower adaptation. The same thing is true in an endurance individual, where the base phase is the perfect time to do low glycogen training to maximize endurance capacity. But as you increase the speed, using more high-quality sessions, you are actually decreasing the focus on the muscular component and increasing the stress on the connective tissue system. Although I didn't talk about it, we should consider how these things interact. We know that myostatin has a negative effect on muscle mass, but it has a very positive effect on the connective tissue. If you talk to Pfizer, they think the best thing you can do for an old person is to get rid of myostatin but I think that's the worst thing to do, because they will have a bigger muscle but it will weaken the connective tissue. It is very similar to when people started using steroids without growth hormone or anything to help the connective tissue. The steroids targeted skeletal muscle exclusively, the connective tissue didn't improve at all, and the rate of injury went through the roof.

In the discussion here, we are somewhat blinded because we focused on the muscle and specifically metabolism within the muscle. When you step back and you look at the whole organism, it's much more difficult because you're trying to integrate a signal that is positive for the skeletal muscle but perhaps negative for other issues. As far as low glycogen training is concerned, having only looked at muscle, it's very difficult for me to say that there is a certain training benefit or that there is one molecule that is going to be the key. But I think it would be impossible to look only at the whole organism with an intervention as complex as exercise and try to make significant scientific advances. You do that with performance. But to actually understand the mechanism of how performance is improved, I don't think that's possible using only the whole organism.

If we came back to talk about connective tissue, we would have a whole different group of things to consider or a whole different way to look at our intervention. With a positive effect on skeletal muscle, and a negative effect on connective tissue, we need to find the best balance to get optimal performance. That's what you are trying to do with periodization. For example, we believe that the best thing we can do for muscle performance is to train at a high cadence so if you are a runner train at a high speed. However, that is the worst thing you can do for the connective tissue because it's going to be extremely damaging, especially if there is not enough recovery time, since connective tissue recovers more slowly. As a result, the runners will periodize their workouts to try to balance the positives for skeletal muscle with the negatives for connective tissue to come up with the best combination of those two for performance. When we boil down low glycogen training and look at only the skeletal muscle, I can say that PPARs are playing a role – not necessarily, the only thing that is happening – that seems to be positive in certain situations. Whether the PPARs are going to be positive in all the cell types in the body, or whether they will have a negative effect on other tissues, I can't tell you just by looking at the muscle.

Dr. Zemel: It's very difficult to manipulate glycogen without changing everything else as well, including fatty acid concentrations. You also linked fatty acids with PPARs, so how much do you think the findings of your work have to do with low glycogen and how much do they have to do with fatty acids?

Dr. Baar: Right now I would say that, at least on the PPAR side, the adaptation that we see for enzymes involved with fatty acid uptake and oxidation does not have anything to do with the low glycogen directly. We are manipulating glycogen because that's what we can control, but in the background the fat is going up and compensating. We end up
with a situation where we can acutely do what the people who do fat manipulation (increased dietary fat) do over a longer term. In either case, PPAR is activated and that's one of the key adaptations. So all we are doing is instead of having to go through a week or a number of days of chronic changes to the diet, in a single day with the two sessions we are activating the same transcription factor that probably underlies the fat adaptation response. So, at least as far as PPAR activation is concerned, I think that it's all dependent on the fat.

Dr. Zemel: What were the fatty acid concentrations in your animal work, and is this realistically achievable in humans?

Dr. Baar: Our cell culture work uses 250 μm fatty acids because we are trying to reach a high physiological level. Of course, at the moment, this is a single fatty acid, since we perform our experiments in a serum-free medium and add only one fatty acid each time. In the future, we will combine the best individual fatty acids to see whether there is an additive effect. We can also test other metabolites that seem to have a positive effect in parallel. We have already done it with the nutraceutical ECGC because it was supposed to have a similar effect to low glycogen training, but we don't see any effect of ECGC in isolated muscle. Rather, we think there is a whole body effect where ECGC causes the release of fat from the adipose which trickles down to the muscle, resulting in more delivery of fatty acids, and the same increase in PPAR activity that we see with the low-glycogen training.

Dr. van Loon: I would like to explore your work on the anaplerotic amino acids. We have some overlapping data, but there are all sorts of striking differences that may be related to timing and dose. If we look at leucine, we see something similar to what you see: an increase in PGC1α, an increase in mitochondrial biogenesis and an increase in fat oxidation, but it takes some time – it's a structure-independent effect. When we do the same thing with C2C12 myotubes and the other branch chain amino acids, we see absolutely nothing when using a low-glucose medium.

Dr. Baar: We don't see a great effect in a low- or normal-glucose environment, but we see anaplerotic effects when we give a carbohydrate or high-fat challenge. For example, if we increase the fatty acid or the sugar content of the media we would normally see an inhibition of metabolic function, whereas if at the same time you give an anaplerotic amino acid you reverse that entirely. That's how we have done the experiments: we have given pyruvate or glucose or fructose, and then we supplement so you have got two entry points to the TCA cycle.

We have done everything from 0.5 to 50 mm. The 50 mm has a huge effect, but this is way outside the physiological range. However, we still get the effects within the physiological range – a 0.5 mm range. Of course, the glucose within the medium will decline over time. We refresh the medium every 12 h to try to get around the normal decline in substrate and build up of waste, since it is also important to remember that the cells are producing a lot of lactate and lactate accumulation may cause adaptations as well.

Dr. van Loon: Are the effects of using high glucose and any of the anaplerotic amino acids fairly rapid?

Dr. Baar: The PGC1α transcriptional data that I showed are the effects of 3 h of treatment, so relatively rapid. We have done 3 days of treatment and see increases in oxygen consumption rate, but these effects take longer because they represent wholesale changes within the muscle cells.
**Dr. Maughan:** You talked about the historical developments in the understanding of glycogen metabolism. You said, initially people thought it was broken down to glucose, but then they realized that it was glucose phosphate. Of course, about 10% is liberated as free glucose, and if you do high-intensity exercise with high glycogen content, you have the unique situation of very high glycolysis rates that will achieve very high intracellular free glucose. What's the role of that free glucose in all of these things?

**Dr. Baar:** I don't know about the free glucose per se, but the position of those free glucose moieties is very interesting and may be important. If you look at proteins like AMP kinase that have glycogen-binding domains, they seem to have a preference for the branch points, and it's specifically the branch points that lead to increased arborization. People like Graham Hardie think that as glycogen is broken down, cleavage of the branch point sugars results in a signal that there is a serious decrease in glycogen. The proteins that bind to these sites, specifically AMP kinase and glycogen synthase, are released and activated and they serve to put a brake on metabolic processes in the muscle: decreasing anabolic and increasing catabolic processes. So whether it's a signal and whether free glucose could be a measure of the activation of proteins that have a glycogen binding domain, I don't know but it is an interesting observation.

**Dr. Spriet:** In your cell work, do you include insulin? Most people argue that the initial upregulation of PDK4 that occurs whenever you limit the carbohydrate source is due to low levels of insulin.

**Dr. Baar:** The data that I presented here were mainly the pyruvate data where the effects occur independent of insulin. When we use glucose or fructose for the carbon overload, we add a small amount of insulin.

**Dr. Spriet:** With the ‘two a day training’ in humans, you are putting quite a bit of stock on the increase in catecholamines associated with low glycogen. But is that something that persists day after day or does it decrease as you continue the training?

**Dr. Hawley:** We haven't measured that in our studies but it's a good question – do you adapt to it or does it just drop? I suspect it drops.

**Dr. Spriet:** It's a strange thing because with very high-intensity exercise you get very high epinephrine levels, and yet your ability to mobilize fat either from adipose tissue or the muscle seems to be limited, indicating that something else is overriding it. Epinephrine levels are higher when working at 85% versus 60% of maximal aerobic capacity, yet you don't get as much free fatty acids out of the adipose tissue, and you don't break down as much intramuscular fat. So, in spite of those signals being present, they are overridden by other regulators.

**Dr. Baar:** The data of Romijn and colleagues suggest that the lack of release of fatty acids from the fat is due to constriction of the blood vessels to the adipose tissue and that if you increase circulating fatty acids you can oxidize approximately 30% more fatty acids.

**Dr. Spriet:** Yes, the lipolytic rate stays up in adipose tissue, and interestingly inside the muscle the high levels of AMPK are actually overriding the effects of contraction and the effects of the epinephrine at higher exercise intensities.

**Dr. Baar:** It's a possibility. Thinking back to some of the early work that Larry Oscai did with HSL and lipoprotein lipase in skeletal muscle during exercise and following training, I wonder whether part of the response and a component of improving fat oxidation is actually to increase the amount and the activity of these lipases. That could have a positive effect on being able to import more triglyceride from the circulation and free more triglyceride out of the intramuscular stores.
Dr. Spriet: When you measure the amount of HSL in the ‘a’ or active form, it’s definitely down at higher exercise intensities. However, little work has been done in muscle on the newly discovered enzyme, adipose tissue triacylglycerol lipase (ATGL).

Dr. Baar: Again, that suggests that HSL and ATGL could be a key limitation in this situation. Increasing HSL or ATGL could increase the amount of fat that you could oxidize at a high intensity, but I don’t know.

Dr. van Loon: It has previously been reported that free fatty acid concentrations in the cytosol increase during high intensity exercise, so free intracellular free fatty acid availability is unlikely to be a limitation to fat oxidation.

Dr. Baar: If there is an increase in free fatty acids within the cytosol at high intensity, you would expect that they either have a signaling role, which is entirely possible, or that there is another limitation, and the obvious limitation is CPT1. Work from Clinton Bruce from Australia has shown that when they increase CPT1 in muscle, by overexpression, they see a positive effect on fatty acid oxidation. This would suggest that CPT1 does limit fat oxidation as well. Interestingly, CPT1 and HSL levels are both transcriptionally controlled by PPARs.

Of course, overexpression experiments can cause many side effects. However, the Bruce experiment was a transient overexpression using electroporation in adult muscle, so fewer side effects would occur. If we view training through the lens of molecular biology, what we are trying to do is get the body to produce more of the key proteins responsible for adaptation: a transient overexpression if you will. By repeating this training stimulus, we are trying to establish a new steady state where we have increased proteins like HSL and CPT1 and this will result in greater fat oxidation.

Dr. Hoppeler: You mentioned that it’s not only muscle tissue but connective tissue—and, of course, capillaries are very important. Do you have any comments on these alternate promoters of PGC1α and the capillary vessel?

Dr. Baar: Zoltan Arany’s work showed quite nicely that the capillarization is dependent on PGC1α and the estrogen receptor-related (ERR) α-protein. ERRα is one of the proteins that PGC1α coactivates. If I remember correctly, his group showed that neither the PGC1α muscle-specific knockout nor the ERRα knockout mice show angiogenesis following exercise. That would indicate to me that the angiogenic response is dependent on PGC1α. So, PGC1α plays a role in angiogenesis, but there may be other factors involved as well including angiogenic growth factors. How those are influenced by nutrient interventions has yet to be determined.

Dr. Gibala: My bias is that anaplerosis, or at least the exercise-induced expansion of the TCA cycle intermediate pool during exercise, has absolutely nothing to do with the regulation of oxidative metabolism in humans. We have conducted a number of studies where we tried to manipulate TCA cycle intermediates during exercise but found no effect on performance or markers of oxidative metabolism. How do you measure anaplerosis in your studies, and how does it impact TCA cycle flux? Do you measure TCA cycle intermediates?

Dr. Baar: The way we have done this is using a Seahorse metabolic flux machine, and we use acetate as a measure of TCA flux. We don’t have the capacity to measure the TCA intermediates. We add acetate and look to see what happens to oxygen consumption. If TCA flux is higher, there is a bigger increase in oxygen consumption when we give acetate. The experiments involve treating muscle cells for 3 days with the anaplerotic amino acids or non-anaplerotic amino acids and then measuring the change in acetate flux.
**Dr. Gibala:** Is it due to the increase in the intermediates, or could they be going out through cataplerosis and then entering back into the cycle at the level of the acetyl-CoA, so it's just additional substrate.

**Dr. Baar:** We don't think it is additional substrate, because what we see in glycolytic overload is a decrease in ATP consumption. We see an increase in fatty acid oxidation enzymes but no increase in fat uptake or oxidation even though PDH phosphorylation is high. So, it doesn't look to us like providing more acetyl-CoA would have a positive effect. We have also used anaplerotic amino acids that enter the TCA cycle at different points, and it seems like the amino acids that enter at SDH have the strongest effect. So, to us this suggests that having substrates enter the TCA cycle at more than one point can overcome a block in acetyl-CoA entry. The result is an increase in flux through the TCA cycle.

That brings up the possibility that what exercise does is increase the flux through the TCA cycle, and that is one of the signals to increase PGC1α. When we looked at all of the standard candidates to signal to PGC1α, not a single one of them was changing. But we know that there is more flux and this creates a rapid change in PGC1α, and so all we can conclude is that the flux itself increases PGC1α. In fact, it could be that anything that increases TCA flux increases PGC1α and improves muscle metabolism, whereas anything that decreases flux leads to insulin resistance and metabolic disease. The difference between your work and ours is that in our systems there was metabolic overload, whereas you looked at exercising people in a normal metabolic state. We see this a lot, if you have a deficiency and you supplement there is a positive effect, but the same positive effect is not seen when you give the same supplement to a complete system. So, that's what we have in the glycolytic or fat overload situation: we have a diseased system. When we give the anaplerotic amino acids in these 'disease' states, we have a beneficial effect, whereas in the normal situation this would not be the case.

**Dr. Hawley:** I know that the human model I am using is a 'dirty model', and it seems like your reductionist approach is very good to tease out mechanisms, but to bring it back to the practical side, we use a different model from the Hansen studies. We get our guys to exercise maximally at the highest intensity possible, so presumably catecholamines and other hormones are high, but the problem is that our subjects feel terrible and train at a lower intensity. So, we are now addressing in a series of studies whether we can alleviate some of the central and peripheral fatigue by giving them caffeine or a little bit of glucose. This might allow subjects to feel better and train with low glycogen at a higher intensity. We have preliminary data that caffeine supplementation partially rescues training capacity, but doesn't rescue up to the point of training with normal glycogen. The further question to ask is whether that level of rescue can change the overall performance outcome from training with low glycogen.

**Dr. Baar:** That will be the key aspect. The other issue with caffeine is that you are looking at something that might also affect calcium signaling, which might have an additional effect. All you have to do is to get around a lot of this central limitation or the negative feelings of fatigue, is to fool the brain into thinking that it will be getting sugars soon. Carter and colleagues showed that rinsing the mouth with a sugar drink improves performance. So, I think the more precise way might be give a mouth wash with a sweet solution to give them a performance boost without a metabolic interference.

**Dr. Burke:** It's great to see what's happening at the level of the muscle, but to me the functional outcome is what's most important. So even if caffeine adds some additional
factors to the mixture, at the end of the day if the athlete who trains with caffeine can perform better, do we really care?

*Dr. Baar:* The reason we need to understand the mechanism is so we can maximize it. If I know what I want to maximize in the muscle, I can produce a high throughput test to see which nutrients maximize it. For example, with the PPAR activation, I can test 40 compounds at a time and know the result in 9 h, whereas with an athlete you will have to train for 3–6 weeks before you know the effects of a single intervention. In order for you to go through all of the different interventions, it would take much longer, and that’s the difference. If you can boil it down to a very basic thing; if you understand which molecules to look at, you can quickly look at thousands of compounds. This is the power of molecular biology. If a few look really good in the lab, we can then move to translate those to the athlete. This is what I think is lost in the translation from people who are talking about the molecules to the people who are really only focused on performance. We need to meet in the middle. I can identify a handful of foods, fats, sugars, etc. that maximize the response in the lab, and then it is up to the performance scientist to determine whether any of these are beneficial to performance.

*Dr. Burke:* I guess that’s the bottom line. We need to work together because you can efficiently identify what could be important, getting it to the point where I can take over and do the performance studies. By ourselves, we haven’t got the complete picture. I think that’s a problem at the moment because some people, even educated athletes and coaches with access to the internet, can go straight to the molecular work and consider this a proxy for performance. We need to keep pushing the value of the performance study to make sure that it actually translates.