Fat Adaptation Science: Low-Carbohydrate, High-Fat Diets to Alter Fuel Utilization and Promote Training Adaptation

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Abstract

The effect of manipulating an individual's habitual diet on skeletal muscle fuel utilization has been of longstanding interest to scientists, and it is now well established that changes in dietary intake that alter the concentration of blood-borne substrates and hormones cause substantial perturbations in the macronutrient storage profile of muscle and exert profound effects on rates of substrate oxidation during exercise. Only recently, however, has it become appreciated that nutrient-exercise interventions can modulate many contraction-induced responses in muscle, and that fuel availability per se provides a 'trigger' for adaptation. Consumption of low-carbohydrate, high-fat diets in the face of endurance training alters patterns of fuel utilization and subsequent exercise responses. Human studies show how low-carbohydrate, fat-rich diets interact with specific contractile stimulus to modulate many of the acute responses to exercise, thereby promoting or inhibiting subsequent training adaptation.

Introduction

The impact of manipulating an individual's habitual diet on skeletal muscle fuel utilization during exercise has been of longstanding interest to scientists. Classic studies conducted at the turn of the 20th century provided the first comprehensive information on the interactive effects of diet and contractile activity on muscle metabolism. The main findings from these investigations were that: (1) both fat- and carbohydrate (CHO)-based fuels were oxidized by the working
muscles during submaximal exercise, with the contribution from fat decreasing as exercise intensity increased; (2) changes in an individual’s preceding diet altered resting muscle metabolism and patterns of fuel oxidation during subsequent exercise, and (3) humans have a low capacity for exercise when fat-based fuels are the major energy source. In the 1930s, researchers in Scandinavia provided further evidence of diet-exercise interactions, specifically how training status and exercise duration affected patterns of fuel utilization during strenuous physical activity. The findings from these pioneering studies underpin our current understanding of muscle fuel metabolism during exercise [1].

It is now well established that changes in dietary intake that alter the concentration of blood-borne substrates and hormones cause large perturbations in the macronutrient storage profile of skeletal muscle (and other insulin-sensitive tissues) and exert profound effects on fuel use during exercise. Only recently, however, has it been appreciated that nutrient-exercise interventions can modulate many contraction-induced responses in muscle [2–4] and that fuel availability per se provides a ‘trigger’ for adaptation. This chapter provides a contemporary perspective on how consumption of a low-CHO, high-fat diet combined with strenuous physical activity alters patterns of fuel utilization and subsequent exercise responses. Emphasis will be on the results of human studies and how fat-rich diets interact with specific contractile stimulus to modulate many of the acute responses to exercise, thereby promoting or inhibiting subsequent training adaptation.

**Effects of High-Fat Diets on Fuel Utilization during Exercise**

The effect of consuming a high-fat (60–65% of energy intake, 4–5 g/kg body mass per day), low-CHO (<20% of energy intake, 2 g/kg body mass per day) diet for less than 3 days is to lower resting muscle and liver glycogen concentrations and increase rates of whole-body fat oxidation during moderate intensity (60–75% of maximal O$_2$ uptake, VO$_{2\text{max}}$) exercise. It should be noted, though, that muscle glycogen levels are well preserved even during fasting in the absence of exercise. Such short-term exposure to a low-CHO, fat-rich diet is detrimental to training capacity and endurance performance [5, 6], presumably due to a combination of lowered muscle glycogen stores in the absence of any worthwhile increase in the capacity of the muscle to oxidize fat to compensate for the reduction in endogenous CHO availability. However, consumption of high-fat, low-CHO diets for longer periods (i.e. 1–7 weeks) elicits metabolic adaptations that significantly enhance rates of fat oxidation during both low and intense (>85% of VO$_{2\text{max}}$) exercise and, to a large extent, compensate for the diet-induced reduction in CHO availability.

In one of the longest exposures to a fat-rich diet, Helge et al. [7] studied 13 untrained male subjects who performed 7 weeks of endurance training (3–4
sessions per week) while consuming either a high-fat (n = 7) or a high-CHO diet (n = 6). Before and after the training-diet intervention, subjects performed 60 min of submaximal cycling during which stable isotopes (1-13C palmitate), combined with whole-body gas exchange and muscle and blood measures were employed to determine substrate kinetics. RER values were lower in subjects who had consumed the high-fat diet than those consuming the high-CHO diet (0.86 vs. 0.93 units; p < 0.05), with the majority of this increased fat oxidation being derived from a higher uptake of plasma fatty acids (FAs) by muscle and also a large uptake of very low-density lipoprotein triacylglycerol. The decreased CHO oxidation (so-called ‘carbohydrate sparing’) during the 60-min exercise bout was entirely due to a reduction in the rate of muscle glycogenolysis (2.6 ± 0.5 vs. 4.8 ± 0.5 mmol/kg dry mass per minute, p < 0.05) and not to a diminished plasma glucose uptake. However, it should be noted that the high-fat diet reduced resting (i.e. pre-exercise) muscle glycogen levels compared to the high-CHO diet (480 ± 29 vs. 683 ± 46 mmol/kg dry mass, p < 0.05). Glycogen ‘sparing’ can only be substantiated if subjects commence standardized exercise with similar muscle glycogen stores after both high-fat and high-CHO diets [5].

**Effects of a High-Fat Diet and Carbohydrate Restoration on Fuel Utilization during Exercise**

In 1995, Hawley and Hopkins [8] proposed that 'optimal endurance and ultra-endurance performance may be attained if an athlete trains for most of the year on a high-CHO diet, then, one week before a major event, undergoes a short (5-day) period of fat adaptation, followed by 24–36 h of a high-CHO intake to restore muscle and liver glycogen stores.' Such 'dietary periodization' could, in theory, allow athletes to maximize endogenous fuel stores (muscle glycogen and triacylglycerol, and liver glycogen), optimize the contribution from both aerobic lipolytic and glycolytic pathways to oxidative metabolism, and 'spare' muscle glycogen [8]. To test this hypothesis, we have undertaken a series of independent but related studies using a standardized dietary-exercise intervention protocol (a 5- to 6-day period of either a high-fat or isoenergetic high-CHO diet consumed while subjects undertake strenuous training followed by 24 h of rest and high CHO intake) in association with prolonged, steady-state cycling (to allow for measures of substrate oxidation) and training and performance responses [9–15].

Figure 1 summarizes metabolic data from Burke et al. [9], and shows RER values along with rates of whole-body CHO and fat oxidation measured during 20 min of submaximal cycling at baseline (day 1), after 5 days of fat adaptation (day 6), and finally during 120 min of steady-state exercise after one day of rest and a high-CHO diet (day 7) or 6 days of an energy-matched high-CHO diet.
Five days of strenuous training while consuming either diet reduced RER values, so that on day 6 values were lower than on day one. However, the RER after fat adaptation was lower than with the high-CHO diet (0.82 ± 0.01 vs. 0.88 ± 0.01; p < 0.05). Muscle glycogen levels after 5 days of a high-fat diet were significantly lower than on day 1 (255 ± 24 vs. 451 ± 32 mmol/kg dry weight; p < 0.05) and also lower than after 5 days on a high-CHO diet (464 ± 42 mmol/kg dry weight; p < 0.05). One day of high-CHO diet and rest increased muscle glycogen values for both dietary treatments (to 554 ± 45 and 608 ± 51 mmol/kg dry mass for fat adaptation and high CHO, respectively). This ‘carbohydrate restoration’ was associated with an increase in RER values for both treatments, but RER values were still lower than values on day 1 after fat adaptation (0.87 ± 0.01 vs. 0.90 ± 0.01; p < 0.05) and below the corresponding values for the high-CHO diet (0.93 ± 0.01, p < 0.05). Although there was a progressive decline in RER values during the 120 min of exercise undertaken on day 7 with both treatments,
RER values after fat adaptation remained lower than during the high-CHO trial at all corresponding time points [9].

Five days of low-CHO, high-fat intake increased rates of fat oxidation during submaximal exercise (fig. 1). On day 6 of fat adaptation, rates of fat oxidation were almost twofold higher than values observed on day 1 (1.04 ± 0.07 vs. 0.57 ± 0.07 g/min; p < 0.05) and were greater than the corresponding values for the high-CHO trial (0.63 ± 0.06 g/min; p < 0.05). Although one day of rest and high-CHO intake attenuated the rates of fat oxidation during the first 20 min of exercise on day 7, fat oxidation remained elevated above baseline (day 1; p < 0.05) and above rates measured in the high-CHO trial (0.70 ± 0.05 vs. 0.37 ± 0.04 g/min; p < 0.05). Fat oxidation increased over 120 min of cycling with both treatments, but was higher after fat adaptation at all time points [9].

It is interesting to observe that despite the brevity of the adaptation period (5 days), a low-CHO, energy-sufficient diet induces substantially higher rates of FA oxidation (compared to baseline) in trained athletes who already have a high capacity for fat oxidation and may have been expected to have maximized training-induced adaptations for FA metabolism. Of note was that a subsequent investigation revealed that elevated rates of fat oxidation persist even under conditions in which CHO availability was increased by either having athletes consume a high-CHO meal before commencing exercise [10] and/or ingesting glucose solutions during exercise [10, 13]. Thus, 5 days of a high-fat diet consumed in the face of vigorous training induces powerful metabolic adaptations within skeletal muscle that upregulate FA oxidation independent of both endogenous and exogenous CHO availability. Using a combination of serial muscle biopsies and glucose tracer methodology, we were able to show that the reduction in CHO oxidation during submaximal exercise was almost entirely accounted for by a true ‘sparing’ of muscle glycogen stores: both total blood glucose oxidation and rates of ingested glucose oxidation were similar during exercise after both high-fat and high-CHO interventions [9, 10, 15].

The investigations of the effects of low-CHO, high-fat diets from our lab [9–15] have all utilized competitive athletes who continue to train intensely while consuming diets containing a higher proportion of fat (4 g/kg per day, 65% of energy) than they would habitually ingest. Vogt et al. [16] determined whether some of the metabolic adaptations that result from such extreme diets can be obtained if athletes ingest diets in which the fat content is within the range typically chosen by trained individuals. These workers [16] studied 11 duathletes who ingested a low-CHO, high-fat (50–55% of energy) diet for 5 weeks while undertaking their normal training regimen. Resting muscle glycogen content was not different between the two diets (488 ± 38 vs. 534 ± 33 mmol/kg dry weight for high-fat and high-CHO diet, respectively), but the high-fat diet resulted in a twofold increase in resting muscle triacylglycerol content. Blood lactate concentrations and RER values were lower after the low-CHO, high-fat diet at rest and during a range of submaximal exercise tests. However, in
accordance with previous findings [9, 10, 12], a variety of performance tests were not different after either diet intervention [16].

**What Mechanisms Explain the Increased Rates of Fat Oxidation after High-Fat Diets?**

Several metabolic adaptations in skeletal muscle contribute to the increased rates of whole-body fat oxidation and decreased rates of CHO oxidation observed after fat adaptation strategies. With regard to FA metabolism, fat-rich diets facilitate an increase in rates of whole-body lipolysis and an increased FA response at rest and during exercise [16], in part due to higher circulating catecholamine concentrations [7]. The elevated FA levels at rest and during exercise, in association with lower muscle glycogen stores are likely to underlie the elevated muscle triacylglycerol content seen after fat adaptation [7, 15, 16]. Elevated circulating FA levels are also likely to drive increased enzymatic activities such as greater concentrations of β-hydroxyacyl-CoA-dehydrogenase, carnitine palmitoyltransferase I (CPT I) and hormone-sensitive lipase [14, 17, 18] as well as modulating the expression of mRNA-encoding proteins necessary for FA transport, such as FA translocase [11].

As would be expected, there were reciprocal changes in CHO metabolism, with high-fat diets reducing resting muscle glycogen concentration [10, 15] and presumably liver glycogen stores to some degree. As noted earlier, the reduction in CHO oxidation observed after fat adaptation and CHO restoration is due to a sparing of muscle glycogen [10, 15]. While such ‘sparing’ should, in theory, be beneficial for endurance exercise capacity, fat adaptation strategies have not been demonstrated to provide a clear benefit to the performance of prolonged exercise [5, 6]. One reason to explain this paradox is that fat adaptation protocols directly inhibit rates of muscle glycogenolysis during exercise. In this regard, Stellingwerff et al. [14] reported that 5 days of a high-fat diet followed by one day of ‘carbohydrate restoration’ suppressed resting levels of pyruvate dehydrogenase (PDHa) activity by ~60% compared to an isoenergetic high-CHO diet for 6 days (0.39 ± 0.10 vs. 0.82 ± 0.18 mmol/kg wet weight per minute). In response to a bout of submaximal cycling undertaken on day 7 (20 min at 70% of VO₂max), there was a rapid increase in PDHa activity which, although similar after both diet interventions, remained 29% lower at the end of exercise after fat adaptation (1.69 ± 0.25 vs. 2.39 ± 0.19 mmol/kg wet weight per minute; p = 0.003). Even in a 60-second sprint, an exercise challenge that would be expected to maximally activate muscle glycogenolysis, PDHa activity remained suppressed after fat adaptation (p < 0.05), even though the relative increase in PDH activation during the 1-min sprint was similar between dietary treatments (~35%). As a result of less pyruvate oxidation (via a reduced PDH flux), estimates of glycogenolysis during both the first minute of submaximal exercise
and the supramaximal sprint were also lower after fat adaptation [14]. While the precise metabolic signals responsible for the shift in muscle substrate use during submaximal and supra-maximal cycling after fat adaptation are not known, it is clear that they are likely to involve both an upregulation of processes involved in FA metabolism, as well as downregulation of CHO oxidation pathways.

**Interaction of Training and High-Fat Diets on Training Adaptation**

Helge et al. [19] were the first to study the interaction of training and a high-fat diet on metabolism and training capacity. These workers studied 20 young, healthy males who ingested either a high-fat (n = 10) or a high-CHO (n = 10) diet while performing endurance training 3–4 times a week for 7 weeks. During the 8th week, both groups ingested a high-CHO diet. Endurance performance conducted at the completion of the 7th week of the training program revealed a significantly greater improvement after a CHO-rich diet has been consumed during training compared to a fat-rich diet (56%). Even when the high-fat diet was replaced by a high-CHO diet for the 8th week of training, endurance performance was still significantly lower than in subjects who had trained for the complete duration on a high-CHO diet. They [19] concluded that ‘ingesting a fat-rich diet during an endurance training programme is detrimental to endurance performance (. . .) due to suboptimal adaptations that are not remedied by the short-term increase in carbohydrate availability.’

From a practical perspective, it seems unlikely that fat oxidation can match the energy demands of the strenuous training undertaken by competitive endurance and ultra-endurance athletes. To test this hypothesis, Stepto et al. [13] determined the effect of consuming a high-fat diet for 4 days on the training responses of competitive ultra-endurance cyclists/triathletes with a history of prolonged endurance training. These subjects were either national or international level athletes, with 3 of them completing the Hawaii Ironman World Championships within weeks of study completion. During the 4-day intervention period, subjects performed two high-intensity cycle interval training sessions in the laboratory (consisting of a 20-min warm up at 65% of VO$_{2\text{max}}$ followed by 8 × 5 min work bouts at 85% of VO$_{2\text{max}}$ with 60 s recovery at 100 W), during which pulmonary gas exchange measures were taken at regular intervals to allow for estimates of fuel oxidation. The other two training sessions consisted of 2- to 4-hour easy rides on the road (fig. 2).

Rates of fat oxidation during the 20-min warm up (undertaken at a power output of 232 W) were 69 ± 25 μmol/kg per minute [14]. These values are in close agreement with the 57 μmol/kg per minute we have previously reported for competitive endurance athletes cycling at 70% of VO$_{2\text{max}}$ (234 W) after 5 days of a fat-rich diet [10]. However, despite an increase in both the relative and absolute exercise intensity when undertaking the intense interval workout
(ridden at an average power output of 323 ± 32 W), rates of fat oxidation did not decline [14]. This indicates that after 4 days of a high-fat diet, well-trained athletes were better able to oxidize fat during high-intensity exercise to compensate for their low muscle glycogen stores. Although subjects in that study [14] were able to complete the prescribed training regimen while consuming a high-fat diet, this was associated with a significant increase in perceived effort for both laboratory and on-road sessions. This suggests that consumption of a fat-rich diet for a longer intervention period would limit the ability of well-trained athletes to undertake strenuous training.

**Does Training with Increased Fat Availability Augment Skeletal Muscle Adaptation?**

Endurance training is associated with an increase in the activities of key enzymes of the mitochondrial electron transport chain and a concomitant increase in mitochondrial protein content. These biochemical and morphological changes, along with an increased capillary supply and blood flow, result in a shift in trained muscle to a greater reliance on fat as a fuel with a concomitant reduction in glycolytic flux and tighter control of acid-base status. High-fat diets and interventions that chronically elevate plasma FA concentrations (i.e. heparin injections) induce adaptations in skeletal muscle that mimic endurance
training (e.g. activate transcription of genes encoding enzymes involved in FA oxidation and increase the capacity of muscle to oxidize FA). The mechanisms by which exercise and increased FA availability activate mitochondrial biogenesis appear to be distinct [20], but it has been proposed that the 5’ adenosine monophosphate-activated protein kinase (AMPK) may provide a link between training- and nutrient-induced adaptations in skeletal muscle [21]. Evidence for such a premise comes from the results of both human and animal studies in which AMPK activity has been shown to be modulated by both contraction (i.e. exercise) and substrate availability (i.e. muscle glycogen and triacylglycerol stores and circulating FA levels).

AMPK is a member of a metabolite-sensing protein kinase family that exists as a heterotrimer formed by three subunits α, β and γ. The α catalytic and β regulatory subunits exist in two isoforms (α₁, α₂, β₁, β₂) while the γ subunit exists in three (γ₁, γ₂ and γ₃). This structure allows for twelve possible combinations of the AMPK complex due to the fact that each subunit is encoded by multiple genes subjected to alternative splicing [20]. AMPK functions as a metabolic ‘fuel gauge’ in skeletal muscle; activation in response to decreased energy levels (i.e. muscle contraction) inhibits ATP-consuming pathways and activates pathways involved in CHO and FA catabolism to restore ATP levels. AMPK promotes FA oxidation in skeletal muscle during exercise by inhibiting acetyl-CoA carboxylase (ACC-β) and activating malonyl-CoA, thus removing inhibition of mitochondrial fatty acyl-CoA translocation by CPT I. Numerous studies have reported that these exercise-induced effects on ACC-β and malonyl-CoA are closely paralleled by activation of AMPK.

AMPK induces mitochondrial biogenesis by increasing the ability of the peroxisome proliferator-activated receptor-γ coactivator (PGC-1α) to coactivate transcription factors that regulate the coordinated expression of mitochondrial proteins encoded in the nuclear and mitochondrial genomes. Indeed, AMPK directly phosphorylates and activates PGC-1α. Overexpression of PGC-1α in muscle results in a large increase in functional mitochondria, while a single bout of exercise induces a rapid increase in PGC-1α in skeletal muscle [22]. In contrast, high-fat feeding and/or increased circulating FAs are thought to promote mitochondrial gene transcription by regulating PGC-1α via different mechanisms. Raising circulating FAs increase the peroxisome proliferator-activated receptor co-activators (PPARs) which regulate expression of the mitochondrial FA oxidative enzymes [23] and lead to a posttranscriptional increase in PGC-1α protein [24]. Specifically, PPARα induces an increase in the expression of proteins involved in transport and metabolism of FA [25], while activation or overexpression of the PPARδ has been reported to result in an increase in mitochondrial biogenesis in muscle [26].

A single bout of prolonged exercise is associated with an increase in both AMPK activity and elevations in circulating FAs; so, an important question is
whether chronic exercise undertaken with elevated FA availability has an additive effect on skeletal muscle training adaptation (i.e. mitochondrial biogenesis). We recently reported an increase in AMPK-α2 but not -α1 activity in rodent skeletal muscle in response to chronic high-fat feeding [27]. In that study, AMPK-α1 activity was only increased when animals concurrently undertook an intense endurance training program while consuming a high-fat diet [27]. At the time, we suggested distinct roles for the AMPK-α subunit in skeletal muscle, with AMPK-α1 activity being linked to exercise training-induced adaptations and CHO availability (i.e. muscle glycogen storage), and AMPK-α2 activity being responsive to increased lipid availability (i.e. muscle triacylglycerol stores and circulating FAs). Further evidence in support of this hypothesis comes from the results of Yeo et al. [15]. These workers reported a strong relationship between resting AMPK-α2 activity (but not -α1) and muscle triglyceride content in humans consuming a fat-rich diet and undertaking strenuous training. In contrast, AMPK-α1 (but not -α2) was associated with increased muscle glycogen content in that study [15].

Recently, Fillmore et al. [20] determined if chronic AMPK activation in skeletal muscle (via a chemical activator of AMPK, 5-aminoimidazole-4-carboxamide riboside [AICAR]), and high-fat diet-induced elevations in blood FAs had an additive effect on mitochondrial content of skeletal muscle. Rats were treated for 6 weeks with AICAR injections, a high-fat diet, or both AICAR injections and a high-fat diet. They reported additive effects of AICAR and high-fat feeding on markers of FA metabolism, the citric acid cycle, the electron transport chain, and transcriptional regulation. However, these changes were confined to muscles with low oxidative capacity [20]. Such an observation is of note, because prolonged endurance training in humans is typically associated with a reliance on slow-twitch muscle fibers with a high oxidative capacity. This suggests that there are species-specific differences with regard to the interactive effects of training and high-fat diets. Indeed, the majority of human studies that have examined the interaction of training and a high-fat diet fail to demonstrate a beneficial effect on either enzymatic responses and/or endurance capacity [10, 12, 18, 19]. Part of the difference in results between rodent and human studies may be due to differences in habitual macronutrient intake of fat: the typical Western diet consists of ~35–40% fat, and it is possible that further elevations in dietary lipid intake would be less likely to enhance mitochondrial biogenesis than if the fat content of the regular diet was closer to the typical ‘control’ diets consumed by rats [20]. Alternatively, few human studies examine fiber type-specific responses to diet-training interventions, and when sampling mixed muscle (usually the vastus lateralis), any potential differential effects are likely to have been overlooked.

As noted previously, other signaling proteins regulate expression of the mitochondrial FA oxidative enzymes in response to changes in circulating FAs. In humans, Horowitz et al. [28] reported an increased PPARα skeletal
muscle protein expression in 5 untrained middle aged women after ~3 months of endurance training, while Russell et al. [29] found that PPARα protein was higher in 7 healthy males after 6 weeks of endurance training. Both of these studies were undertaken while subjects consumed moderate- to high-CHO diets. In contrast, Helge et al. [30] studied 13 men who trained one leg for 4 weeks (~30 h of total exercise), while the other leg remained untrained. During the intervention period, 6 subjects consumed a high-fat diet (58% of energy intake as fat, 24% CHO), while the remainder maintained their normal diet (33% fat, 56% CHO). Muscle biopsies were obtained from vastus lateralis in both legs before and after training. Despite a lower RER during standardized submaximal exercise in both untrained and trained legs in subjects who consumed the high-fat diet, PPARα mRNA abundance and protein expression remained unchanged [30]. They [30] concluded that ‘a longer and more intense training programme may be required to induce changes in PPARα expression’. However, we reported that in rat muscle, PPARα and PPARγ protein expression is unaltered after 8 weeks exposure to a high-fat diet [31]. Taken collectively, the results from these studies suggest that the PPARs may play only a permissive role in modulating training adaptation in the face of increased fat availability.

Conclusions

The consumption of a fat-rich diet for less than 3 days is associated with reduced muscle glycogen stores in subjects who continue to train and increased rates of FA oxidation during low- to moderate-intensity exercise. Such short-term fat diets are detrimental to endurance capacity and the performance of prolonged exercise. In contrast, longer (1–7 weeks) periods of high-fat intake in combination with regular endurance training elicit metabolic adaptations in muscle that markedly increase rates of fat oxidation during both low- and high-intensity exercise and, to a large extent, compensate for the diet-induced reduction in CHO availability. Yet, despite marked changes in metabolism that favor fat oxidation and ‘spare’ muscle glycogen oxidation, fat adaptation strategies do not provide clear benefits to training capacity or endurance performance. The mechanisms by which exercise and increased FA availability activate mitochondrial biogenesis appear to be different, but likely involve the AMP kinase and possibly the PPARs. While fat adaptation, CHO restoration protocols offer scientists a unique human model to investigate the interactive effects of alterations in fuel availability on muscle metabolism during exercise, high-fat diets are associated with the development of muscle insulin resistance, a condition that is not compensated for by the diet-induced increases in fat oxidation and/or mitochondrial biogenesis. Accordingly, caution should be exercised when recommending high-fat diets to athletes.
Acknowledgements

Studies of fat adaptation undertaken in the author's laboratory have been funded by grants from the Australian Sports Commission and Nestlé Australia. The author would like to thank Drs. Trent Stellingwerff, Lawrence Spriet and Ronald Maughan for constructive comments in the preparation of the manuscript.

References

Dr. Maughan: It’s perhaps worth thinking hard about whether we are really talking about high-fat diets or whether the most important aspect of these diets is that they are low in carbohydrate. Supposing you fed your volunteers a diet with a normal (for them) amount of carbohydrate and supplemented the diet with fat so you had normal carbohydrate plus high fat, would you see the same effect?

Dr. Hawley: It’s important to know whether these are trained or untrained subjects, because the absolute capacity for fat oxidation differs greatly. It probably also depends on the subjects’ training load. Training sessions that deplete muscle glycogen may give a very different response from studies where subjects do just 30 min a day of light- to moderate-intensity aerobic exercise. Exercise with low muscle glycogen stores will increase the absolute and relative contribution from lipid-based fuels to oxidative metabolism. So to answer your question, I believe you would see an increase in fat metabolism when a ‘normal’ carbohydrate diet is supplemented with fat, at least in trained individuals. A study from Dr. Hoppeler’s lab shows just this effect [1].

Discussion

Dr. Maughan: But can we look at your studies in more detail? The papers by Stepto et al. [2] and Burke et al. [3], for example. Were the diets fed in these studies high-fat diets or low-carbohydrate diets?

Dr. Hawley: They were both! In these two investigations, the diets were low in carbohydrate (2.5 g/kg body mass per day) and high in fat (4 g/kg body mass per day). We gave that amount of carbohydrate because it was sufficient to maintain glycemia, yet resulted in low muscle glycogen levels at the onset of the intervention.

Dr. Maughan: But that is a low-carbohydrate diet. If you gave a normal diet with added fat – the same amount of fat – would training still be impaired? I am asking you to speculate as to the mechanism that is driving these effects. Feeding a low-carbohydrate diet has far greater metabolic and hormonal effects than feeding a high-fat diet.

Dr. Hawley: You are correct in that carbohydrate restriction is likely to have a more pronounced effect on the hormonal milieu than a high-fat diet. There is more than one candidate for the mechanism driving the training adaptation, but muscle glycogen concentration is a major factor determining transcription of some regulatory genes. As we discussed in a recent review [4], commencing selected training sessions when muscle glycogen concentration is low results in greater transcriptional activation of several genes with key roles in the adaptation process compared to undertaking the same exercise bout when muscle glycogen concentration is normal or high. As noted by Hansen et al. [5], this is probably because several transcription factors include glycogen-binding domains: when muscle glycogen is low, these factors are released and become free to associate with different targeting proteins. However, in some of the studies we recently reviewed [4], low-glycogen was not the only variable to be manipulated. For example, in several investigations, subjects trained twice daily on the low-glycogen treatment compared to daily when glycogen was normal: it may be that training frequency or indeed the recovery between workouts is also an important factor driving adaptation.

Dr. Phillips: What if you just took away some of the carbohydrate and replaced it with protein, or what if you just cut back total energy intake so that the subjects weren’t getting sufficient carbohydrate from the standpoint of full repletion. Then you wouldn’t have to manipulate fat.

Dr. Hawley: That’s something we haven’t considered. Of course, if you change energy intake, there is a whole new set of issues concerning the ability to train anyway! When carbohydrate intake is very low (e.g. 2–3 g/kg body mass per day), intense training sessions just go out the window. In the study by Stepto et al. [2], it was clear that unless we had used highly trained, highly motivated subjects, they would have quit the training sessions we prescribed fairly early on: they were having trouble sustaining any intensity over 70–75% of VO2max after just 4 days of a low-carbohydrate, high-fat diet; so, it’s my feeling that it would be impossible to go any longer than that.

Dr. Phillips: But let’s go back to the original two times a day training model that created this whole high fat paradigm. It would seem that some of the adaptations taking place in muscle are quite beneficial for somebody who is overweight or who has metabolic syndrome or type 2 diabetes.

Dr. Hawley: Let’s clarify something here. The original study by Hansen et al. [5] was actually entitled ‘Skeletal muscle adaptation: training twice every second day vs. training once daily’. If you read that paper, they proposed the paradigm that some training sessions should be commenced with low muscle glycogen stores, not necessarily high fat.
availability (at least in their minds). And clearly, they thought that twice-a-day training was superior for promoting adaptation than once-a-day training. If you get motivated people with insulin resistance and/or type 2 diabetes to exercise twice a day three times a week rather than once a day five times a week (for the same total exercise time), you may well see a greater adaptation. I don't know if anyone has looked at the effects of this experimental model in obese individuals or in people with insulin resistant metabolic syndrome. Those are logical studies to do.

Dr. Maughan: But the trouble with changing the protein content of the diet is it’s not the same experimental model because you shift acid-base status.

Dr. Phillips: That would depend in part on the protein that you used. Whey is very neutral, it’s low in sulphur-containing amino acids, so you don’t get much sulphuric acid production, and therefore you don’t do a lot to pH.

Dr. Zemel: Can we clarify the nature of these high-fat diets? Just as when we are talking about protein, we talk about the nature of the protein and its amino acid composition, so when we raise the notion of high-fat diet, we should think about the nature of these fats. We have already addressed the issue of medium chain fatty acids, but there are several other issues related to the composition of fat. For example, α-linolenic acid is oxidized differently, or I should say preferentially, compared to other fatty acids.

Dr. Hawley: Prof. Louise Burke was responsible for the formulation of the diets in the human studies we did, so perhaps she can address your question. However, in a recent study in rats we fed diets containing fatty acids with different chain lengths [6]. As someone alluded to earlier in this meeting, changing the fatty acid composition of the diet changes the membrane phospholipids and many other aspects of cell metabolism. That is well known. We didn’t measure acid-base parameters after the different diets, but these would probably change too.

Dr. Burke: We did think about changing the fatty acid composition of the diet. Work from Dr. Peters’ lab [7] has examined this very question. They showed that elevated n-3 fatty acids in a high-fat diet attenuate the increase in PDH kinase activity but not PDH activity in human skeletal muscle. So, clearly changing the fatty acid composition of the diet has metabolic consequences. One thing that stopped us from taking this further was just the practicality of constructing diets that would give 250 g of fat while also providing an obligatory amount of carbohydrate. This is just unworkable; so, when we found that there was impairment of the training intensity and the capacity to train, we just gave up the battle. The information is interesting in the metabolic sense, but does not seem to change sports performance, so we moved on.

Dr. Hawley: That might be interesting in a clinical setting. These subjects have a tremendous capacity to oxidize fat. And these high-fat diets do not induce insulin resistance. We have done that study [8], and the insulin sensitivity of well-trained subjects consuming our high-fat diets doesn’t change at all, at least in the short-term. As Dr. Burke noted, the modified diet that we used gave the metabolic effects that we were interested in, so we just kept the same diet for subsequent investigations.

Dr. Montain: Historically, there was always the concept that when you are on a high-fat diet you feel sluggish and don’t want to move. What has been your observation of your subject population – did they experience the same sort of symptoms?

Dr. Hawley: Certainly, they felt sluggish and they didn’t want to move: we had to encourage them to complete the training regimen. The training was very hard, and they wouldn’t have done it voluntarily. The ratings of perceived exertion are high, although
perhaps not as high as you might expect, and the high-intensity training is just agony. There is only a small amount of carbohydrate available at the onset of the interval training sessions and when you are doing these intense efforts (8 times 5-min work bouts at 85% of VO$_{2\text{max}}$) we know from previous studies that they deplete approximately 50% of muscle glycogen stores when you start with values around 400–500 mmol/kg dry mass [9]. So when you start the same session with muscle glycogen values of around 200 mmol/kg dry weight, you are obviously going to be in trouble.

Dr. Burke: In one of those studies, we blinded the diets. We were able to produce foods that looked identical and that tasted very similar, but by day 3, all of the subjects just felt so terrible with the low-carbohydrate high-fat diet that there was nothing we could do to disguise it.

Dr. Hawley: I had forgotten that study with the so-called 'decoy foods'. We gave them lots of strawberries and lots of lettuce: subjects perceived this as a healthy diet and associated it with carbohydrate but at the end of the intervention, they weren't fooled.

Dr. Hoppeler: What is really high-fat diet? We used an intervention on highly-trained subjects where we just changed the diet composition by going down to 20% fat, or up to 50% fat still changed RER values by about 20% [1]. Substrate metabolism changes significantly, but you can't maintain the glycogen levels. It is also clear that people are very different in their reaction to these diets. When you report the mean values, it seems that nobody improves on a high-fat diet, but when you look at the individuals, it's completely different. These observations are usually not reported because it is not at all clear what is happening in these individuals, but our bias is that people who like high-fat diets work better on those and vice versa.

Dr. Hawley: I tried to bring that point out in the paper, and you will have noted that we always plot the individual subjects' data in our papers. We do know from subjects who took part in more than one study that if you are a 'responder' then you are always a 'responder' in subsequent studies. In animal models for high-fat diets, where some of the enzymatic changes are much more profound than in humans, the extreme diets used are probably the main factor. In the human studies, the diet manipulations are not as extreme as in the animal models. You have shown that [1].

Dr. Hoppeler: Rats, dogs and also mice produce mitochondria when you feed them diets that are high in fat. This is well known. Humans just don't respond in the same way.

Dr. Hawley: The work from John Holloszy's lab clearly shows that increasing fatty acid availability induces mitochondrial biogenesis in rodent skeletal muscle [10]. However, I want to stress that mitochondrial biogenesis and insulin sensitivity don't go hand in hand. Again, work from Holloszy's lab clearly shows that high-fat diets cause insulin resistance despite an increase in mitochondrial biogenesis [11]. I think this whole issue about mitochondrial deficiency causing insulin resistance and type 2 diabetes is nonsense [12]. Although the response of humans to a high-fat diet is not as robust as rodents when it comes to induction of mitochondrial biogenesis, it's not totally absent.

Dr. Hoppeler: When we changed the fat content of the diet by going up to only 50% lipid, there was absolutely no mitochondrial biogenesis, but still they got the higher fat oxidation: they lowered RQ by 20 at 75% of VO$_{2\text{max}}$, so I think it really depends on what you call a high fat diet and it may be different when you don't go to the extreme.

Dr. Hawley: But again, I would still like to make the point quite clearly that you can induce mitochondrial biogenesis and still remain insulin resistant. There are five or six papers I can quote to support this.
Dr. Maughan: One of the differences may be that laboratory animals are typically fed a very constant diet at baseline, but human volunteers are different. The composition of the volunteers' normal diet at entry to the study can vary quite considerably in terms of the fat, protein and carbohydrate content. If you then put them all on a fixed experimental diet, this will be a much bigger change for some than for others. You said there were some people who responded differently from others. Was that related to the degree of change from their habitual diet?

Dr. Hawley: We looked at the subjects' habitual diet but couldn't see any relationship to whether they were 'responders' or not. The freely selected diets of these well-trained athletes were quite homogenous, and were high in carbohydrate (about 6 g/kg body mass per day). So, clearly their habitual diet does not predict the response. We couldn't see anything in the food questionnaires that would predict yes or no. We thought about looking at the response to a single high-fat meal: would one high-fat meal turn on different genes in different individuals and then predict adaptation? So we have got that in store but it may not show much. I would like to think it would.

Dr. Baar: What about the possibility of changing the timing of fat intake, rather than a drastic change in composition? If you gave a bolus of the right fat just at the end of an endurance exercise bout, it could then be used during the period of high fat uptake and oxidation. This might drive the adaptive response in the same way that we can do with leucine or protein meals following resistance exercise.

Dr. Hawley: It's a great idea. I don't know of any experimental evidence, but if you look at the discussion in the recent paper from Will Winder's lab [13], they think that the postexercise elevation in free fatty acids is an important factor driving training adaptation. I am sure it plays a part but, as discussed in my paper, it's probably not enough to drive all the things that we see.

Dr. Baar: It may depend on what markers you use for mitochondrial biogenesis. Do you use a specific marker for mitochondria or are you looking at fat oxidation enzymes that are within the mitochondria but might vary independently of total mitochondrial protein? It could be that when some investigators say there is no mitochondrial biogenesis and others say that there is, they are looking at different things. Another consideration is whether the proteins have different half-lives and therefore might change at different rates. All of these factors make it difficult to address what really is happening.

Dr. Hoppeler: When I say mitochondria, I mean mitochondrial volume actually measured with electron microscopy techniques, which is sort of a global protein assay for a functional entity. Within that, you can obviously change the protein composition so that you can modify substrate selection independent of the volume.

Dr. Baar: We think of mitochondrial biogenesis as an increase in mitochondrial DNA (mtDNA). For us, that's the gold standard even though the protein level is important. There should be some proportionality between the amount of mitochondrial protein and the DNA number. You want to have something that's relevant, and so we tend to measure mtDNA number, but we also measure a couple of protein markers: we look at fat oxidation enzymes independently to get an idea of whether these enzymes all shift in the same direction, or one is shifting to a greater extent.

Dr. Hawley: In our recent paper on low carbohydrate, high fat availability [14], we measured mtDNA by real-time PCR. mtDNA is a marker of mitochondrial volume and increases with training. In contrast to the increases in mitochondrial enzyme activities,
we saw after training twice a day on the low-carbohydrate, high-fat regimen [14], mtDNA content and PGC-1α protein content were unchanged after both diet-training interventions. Of course, the small (approximately 15–20%) increases in maximum enzyme activity observed after training twice every second day could coincide with a small increase in mitochondrial volume that is not detectable through mtDNA analysis.

**Dr. Baar:** But you still saw the adaptation with fat oxidation, which means that it doesn't take more mitochondria to get more fat oxidation enzymes into those mitochondria.

**Dr. Hawley:** And as Dr. Spriet has pointed out, measuring maximum enzyme activity (such as β-hydroxy-acyl-CoA) is probably not the way to go. We need to look at the rate-limiting enzyme in the transfer of long-chain fatty acids into the mitochondria, CPT I. As we have recently noted [15], most previous studies have been limited to total muscle measures that provide no information regarding the compartment or location where the protein changes occurred. This is important for subsequent work in this area.

**Dr. Maughan:** There was a whole series of studies done in the early 1900s in the preparations that were going on for Polar expeditions. Nansen used sled dogs, so he did studies feeding the dogs and the men different diets because high-fat foods are much easier to carry than high carbohydrate foods. My recollection of these studies is that he found the dogs could perform well on a diet that was pretty much 100% fat (with a bit of protein) for many weeks, but the men could not walk for more than 12 h a day unless the diet contained some carbohydrate. Mike Stroud and Ranulph Fiennes recently tried to walk across the Antarctic, but they took a higher fat content to save weight and had some problems in the later stages. When we talk about endurance, maybe we should remember those very long endurance activities, with up to 12 h of fairly hard exercise per day. Possibly you can cope with a high fat in events like that if you have muscles that are well adapted to fat oxidation.

**Dr. Baar:** So, notwithstanding the interest of this type of model for helping to understand the mechanisms that underpin adaptation in the muscle, do you see any point in pursuing this for any type of benefit?

**Dr. Hawley:** Yes, if you are one of those people who respond positively, I do definitely. However, from the perspective of an athlete or coach, the picture is less certain. It doesn’t enhance performance and you feel worse during training. The athlete and coach will close the door when they hear that. It’s a useful model to look at some of the factors around cell and metabolic regulation.

**Dr. Burke:** We have to remember the conditions of that experiment, though. The subjects were fasted, and no carbohydrate intake was allowed during the exercise. This is not what happens in the real world, at least with the conventional sports that we work with. It may be different for some of the adventure ultra-endurance events.

**Dr. Hawley:** Again, as Dr. Maughan has pointed out, if you looked at a different ‘performance’ measure such as submaximal exercise time to exhaustion, you may get different results to those obtained after more intense (shorter duration) lab performance measures. If we had looked at exercise time to exhaustion in our studies, I am pretty sure that fat adaptation would have prolonged it. So, perhaps with different exercise models, we might have reached different conclusions.
References


