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Jonathan D. Bartlett[a], John A. Hawley[ab] & James P. Morton[a]

[a] Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK

[b] Exercise & Nutrition Research Group, School of Exercise Sciences, Australian Catholic University (ACU), Fitzroy, VIC, Australia

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REVIEW ARTICLE

Carbohydrate availability and exercise training adaptation: Too much of a good thing?

JONATHAN D. BARTLETT[1], JOHN A. HAWLEY[1,2], & JAMES P. MORTON[1]

[1]*Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK,* [2]*Exercise & Nutrition Research Group, School of Exercise Sciences, Australian Catholic University (ACU), Fitzroy, VIC, Australia*

Abstract
Traditional nutritional approaches to endurance training have typically promoted high carbohydrate (CHO) availability before, during and after training sessions to ensure adequate muscle substrate to meet the demands of high daily training intensities and volumes. However, during the past decade, data from our laboratories and others have demonstrated that deliberately training in conditions of reduced CHO availability can promote training-induced adaptations of human skeletal muscle (i.e. increased maximal mitochondrial enzyme activities and/or mitochondrial content, increased rates of lipid oxidation and, in some instances, improved exercise capacity). Such data have led to the concept of 'training low, but competing high' whereby selected training sessions are completed in conditions of reduced CHO availability (so as to promote training adaptation), but CHO reserves are restored immediately prior to an important competition. The augmented training response observed with training-low strategies is likely regulated by enhanced activation of key cell signalling kinases (e.g. AMPK, p38MAPK), transcription factors (e.g. p53, PPARδ) and transcriptional co-activators (e.g. PGC-1α), such that a co-ordinated up-regulation of both the nuclear and mitochondrial genomes occurs. Although the optimal practical strategies to train low are not currently known, consuming additional caffeine, protein, and practising CHO mouth-rinsing before and/or during training may help to rescue the reduced training intensities that typically occur when 'training low', in addition to preventing protein breakdown and maintaining optimal immune function. Finally, athletes should practise 'train-low' workouts in conjunction with sessions undertaken with normal or high CHO availability so that their capacity to oxidise CHO is not blunted on race day.

Keywords: *Mitochondrial biogenesis, p53, PPAR*δ*, PGC-1*α*, AMPK*

Introduction

The effect of high carbohydrate (CHO) availability on prolonged exercise performance is well established (Cermak & van Loon, 2013; Hawley, Schabort, Noakes, & Dennis, 1997). Indeed, the practice of CHO loading to super-compensate muscle and liver glycogen stores in the days immediately prior to a major endurance competition is the foundation on which many other dietary interventions are based (i.e. increasing CHO availability during exercise through the ingestion of drinks, bars and gels) and is one of the most consistent nutritional messages that are conveyed to athletes and coaches. In addition to promoting competition performance, it is also recommended that endurance athletes promote high CHO availability before, during and after training sessions in order to support high daily training volumes and intensities and promote optimal recovery. Although the effects of altering CHO availability on intensive periods of training are reasonably well characterised (Achten et al., 2004; Cermak & van Loon, 2013; Hawley et al., 1997), it is difficult to provide definitive guidelines for CHO intake in relation to fuelling specific training sessions. This is because the metabolic demands (in terms of both total energy expenditure and CHO needs) of the real-world training programmes undertaken by elite athletes have not been systematically determined and, as a result, practical recommendations are often made based on feedback to sports nutritionists from coaches and the athletes themselves

Correspondence: J. P. Morton, Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Tom Reilly Building, Byrom St Campus, Liverpool L3 3AF, UK. E-mail: J.P.Morton@ljmu.ac.uk
Present address: Jonathan D. Bartlett, Institute for Sport, Exercise and Active Living (ISEAL), School of Sport and Exercise Science Victoria University, Melbourne, VIC, Australia

(Burke, Hawley, Wong, & Jeukendrup, 2011). As such, the most recent guidelines for CHO intake for training and competition (Burke et al., 2011) recognise the need for flexibility and individual differences among athletes and recommend CHO intake be based on a sliding scale that is dependent on training intensity and duration (see Table I).

In contrast to the effects of high CHO availability on augmenting exercise performance and training capacity, a growing body of evidence has consistently demonstrated a potent effect of *reduced* CHO availability (both endogenous and exogenous sources) on modulating training-induced adaptations in skeletal muscle (Philp, Hargreaves, & Baar, 2012). Indeed, the emergence of molecular biology techniques in the sport and exercise sciences has provided researchers with the tools to evaluate the effects of specific training interventions on training adaptation by elucidating many of the cellular and molecular markers that are considered essential for underpinning exercise performance. In this regard, the results of investigations that have systematically manipulated endogenous and/or exogenous CHO availability during short-term (3–10 weeks) training interventions have consistently demonstrated that commencing endurance-based exercise sessions with reduced CHO availability enhances the activation of cell signalling pathways (Bartlett et al., 2013; Cochran, Little, Tarnopolsky, & Gibala, 2010; Yeo et al., 2010), up-regulates oxidative enzymes (Morton et al., 2009; Yeo et al., 2008), increases whole-body (Yeo et al., 2008) and intramuscular lipid oxidation (Hulston et al., 2010), and in some instances, improves exercise capacity (Hansen et al., 2005). These data have underpinned the current 'train-low, compete-high' paradigm in which selected training sessions are deliberately commenced with reduced CHO availability, but competition is commenced with high CHO availability (Burke, 2010).

In this paper, we provide a concise review of our current understanding of the role of CHO availability in modulating fatigue during endurance exercise, and how a 'cycling' of CHO availability may promote optimal endurance training adaptation. We conclude by providing some practical recommendations of how the manipulation of CHO availability can be incorporated into an athlete's training programme, as well as outlining some potential directions for future research.

Muscle glycogen stores, performance and fatigue

The glycogen content of skeletal muscle of untrained individuals consuming a mixed diet is ~80–90 mmol kg^{-1} w.w., but for athletes involved in regular endurance training, this figure is somewhat higher at around 125 mmol kg^{-1} w.w. (Hawley et al., 1997). After 3–5 days of a high-CHO (>8 g of CHO kg^{-1} BM) diet and a reduction in training volume, muscle glycogen stores can be elevated to values in excess of 200 mmol kg^{-1} w.w. As 1 g of glycogen is typically stored with 2–3 g of

Table I. Overview of the current recommendations of CHO intake for athletes for training and competition

Daily fuelling and recovery: these CHO intakes should be optimised to meet the necessary training requirements and energy costs of individual athletes		
Activity	Intensity/duration	Carbohydrate targets
Low	Low-intensity and/or skill-based sports	3–5 g kg^{-1} day^{-1}
Moderate	Moderate intensity of durations ~1–2 h day^{-1}	5–7 g kg^{-1} day^{-1}
High	Moderate- to high-intensity activity of durations ~1–3 h day^{-1}	6–10 g kg^{-1} day^{-1}
Very high	Moderate- to high-intensity activity of durations >4–5 h day^{-1}	8–12 g kg^{-1} day^{-1}
Acute fuelling strategies: the CHO intakes are designed to provide high CHO availability for optimal performance during competition or key training sessions		
Activity	Duration	Carbohydrate targets
Pre		
General fuelling	<90 min	7–12 g kg^{-1} per 24 h
Carbohydrate loading	>90 min/intermittent exercise	10–12 g kg^{-1} per 24 h for 36–48 h
Pre-exercise fuelling	>60 min	1–4 g kg^{-1} 1–4 h prior to commencement of exercise
During		
Brief exercise periods	<45 min	No CHO needed
Sustained high intensity	45–75 min	Small amounts including CHO mouth rinse
Endurance including team sports	~60–150 min	30–60 g h^{-1}
Ultra-endurance exercise	>150 min	Up to 90 g h^{-1} (achieved via intake of multiple transportable CHO, i.e. glucose + fructose
Post		
Quick refuelling	<8 h recovery between two fuel-demanding training sessions	1.0–1.2 g kg^{-1} h^{-1} for first 1–4 h, then normal daily fuel needs thereafter

Source: Adapted from Burke et al. (2011).

water, a consequence of glycogen loading is that an athlete's BM may increase by 1–2% after several days of 'loading'. The normalised muscle glycogen stores of a well-trained athlete are generally sufficient to fuel sporting activities of up to 60–90 min duration. However, in events involving prolonged (~90 min) steady-state exercise, or intermittent high-intensity exercise lasting >60 min, athletes may experience fatigue and a reduced capacity for work due to depletion of glycogen stores in those muscle fibres specifically recruited during the exercise task. As such, 'carbohydrate loading' to super-compensate glycogen stores prior to competition was found to be an effective way to reduce the effects of muscle glycogen depletion on fatigue and exercise capacity (Bergström, Hermansen, Hultman, & Saltin, 1967). CHO loading was first described as a 7-day programme, which involved a phase to deplete muscle glycogen stores (low-CHO diet and training) followed by 3 days of refuelling (high-CHO diet and tapered exercise or rest). More recently, it has been shown that these elevated muscle glycogen stores may be achieved in as little as 24–36 h of rest and high CHO intake (8–12 g kg day^{-1}) (Bussau, Fairchild, Rao, Steele, & Fournier, 2002), thus providing a practical strategy for many athletes involved in weekly cycles of competition.

There is little effect of elevating pre-exercise muscle glycogen contents above normal resting values (i.e. 120 mmol kg^{-1} w.w.) on a single exhaustive bout of high-intensity exercise lasting < 5 min. Nor is there any benefit of increasing pre-exercise muscle glycogen content on maximal running or cycling events lasting 60–90 min. After such exercise, substantial quantities of glycogen remain in the working muscles (Hawley et al., 1997). However, elevated starting muscle glycogen content delays the onset of fatigue by ~10–20% in endurance events lasting more than 90 min. Indeed, during this type of exercise, exhaustion (defined as a 10% decline in power or speed below a fixed, submaximal work rate) usually coincides with critically low (25 mmol kg^{-1} w.w.) muscle glycogen content, suggesting that the supply of energy from glycogen cannot be replaced by an increased oxidation of blood glucose (Coyle, Coggan, Hemmert, & Ivy, 1986). Furthermore, glycogen super-compensation may also improve endurance performance in which a set distance is covered as quickly as possible. In such circumstances, high-CHO diets have been reported to improve performance by 2–3% (Hawley et al., 1997).

Recent studies on CHO loading strategies involve 'real life' angles from the world of sport. The first issue is the athlete's ability to repeat glycogen super-compensation protocols; this is of practical interest to elite competitors such as professional cyclists and team sport athletes who commonly perform multiple sessions of competition each week. As such, well-trained cyclists who undertook two consecutive periods of exercise depletion followed by 48 h of rest and high CHO intake (12 g kg day^{-1}) were able to elevate glycogen stores above resting levels on the first occasion but not the next (McInerney et al., 2005). Nevertheless, exercise capacity was increased on days 3 and 5 compared with day 1. More studies are needed to determine why glycogen storage is attenuated with repeated CHO loading and its relationship to exercise capacity and performance. Other workers have suggested that muscle glycogen may play a signalling role from the periphery in helping to determine pacing strategies during exercise (Rauch, St Clair Gibson, Lambert, & Noakes, 2005). The results of several studies have provided evidence that prior 'creatine loading' (using dietary creatine supplements to increase muscle creatine and creatine phosphate content) also increases the muscle capacity to store glycogen (van Loon et al., 2004). Although creatine supplementation is considered to be of little benefit to endurance athletes and may even be disadvantageous due to the associated gain in body mass, new studies should be conducted to see if these findings offer performance advantages to events where glycogen would otherwise be limiting. Finally, there is some evidence that CHO ingestion can reduce muscle glycogen utilisation during exercise in well-trained individuals with high resting muscle glycogen levels. Increasing exogenous CHO availability before and/or during exercise by feeding solutions of glucose or other sugars may result in a 'sparing' of endogenous muscle glycogen stores, which appears to be confined to the type I 'slow twitch' fibres (Tsintzas & Williams, 1998).

The effects of CHO availability on training adaptation

Endurance exercise training induces a number of adaptations in skeletal muscle that function to improve subsequent exercise capacity. The most important of these adaptations is the increase in mitochondrial mass (i.e. mitochondrial biogenesis) that ultimately, permits individuals to exercise at higher absolute intensities for a longer duration. The increased mitochondrial content that accompanies exercise training ensures that exercise in the trained state induces less perturbations to metabolic homeostasis for any given absolute exercise intensity, that is smaller decreases in adenosine triphosphate (ATP), phosphocreatine and muscle glycogen utilisation and smaller increases in adenosine diphosphate (ADP), adenosine monophosphate (AMP), inorganic phosphate and muscle lactate.

Although the phenomenon of training-induced increases in skeletal muscle mitochondria was first recognised over 40 years ago (Holloszy, 1967), the

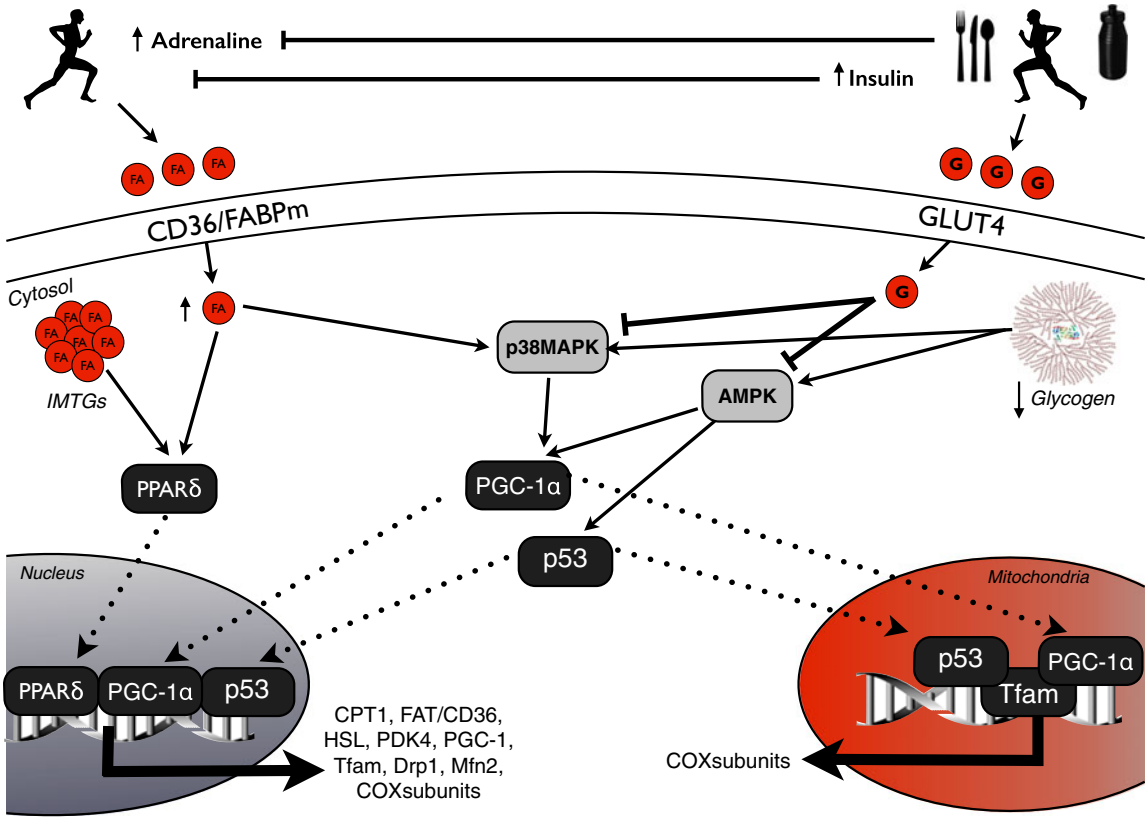

Figure 1. Schematic overview of the potential cell-signalling pathways with roles regulating mitochondrial biogenesis when commencing endurance-based exercise training in conditions of reduced CHO availability. Exercising in conditions of reduced muscle glycogen enhances both AMPK and p38MAPK phosphorylation that subsequently results in activation and translocation of PGC-1α to the mitochondria and nucleus. Upon entry into the nucleus, PGC-1α co-activates additional transcription factors (i.e. NRF1/2) to increase the expression of COX subunits and Tfam as well as auto-regulating its own expression. In the mitochondria, PGC-1α co-activates Tfam to coordinate regulation of mtDNA and induces expression of key mitochondrial proteins of the electron transport chain, for example COX subunits. Similar to PGC-1α, p53 activity is also increased in response to exercise in conditions of low CHO availability, upon which it translocates to the mitochondria to modulate Tfam activity and mtDNA expression and to the nucleus where it functions to increase expression of proteins involved in mitochondrial fission and fusion (DRP-1 and MFN-2) and electron transport chain proteins. Exercising in conditions of reduced CHO availability increases adipose tissue and intramuscular lipolysis via increased circulating adrenaline concentrations. The resulting elevation in FFA activates the nuclear transcription factor, PPARδ, to increase expression of proteins involved in lipid metabolism, such as CPT-1, PDK4, CD36 and HSL. However, consuming pre-exercise meals rich in CHO and/or CHO during exercise can down-regulate lipolysis (thereby negating FFA-mediated signalling) as well as reduce both AMPK and p38MAPK activity, thus having negative implications for downstream regulators.

precise molecular mechanisms underpinning mitochondrial biogenesis are only beginning to be understood. In order to provide a theoretical platform for the subsequent discussion, we provide an initial overview of our current understanding of the molecular pathways that regulate the promotion of an oxidative phenotype. We then proceed to discuss the effects of both endogenous and exogenous CHO availability in modifying the training response. A schematic overview of the proposed signalling mechanisms underpinning the augmented training adaptation that is observed during periods of reduced CHO availability is also presented in Figure 1.

Overview of mitochondrial biogenesis

Training-induced increases in mitochondrial biogenesis are thought to be due to the accumulative responses of transient changes in gene expression that occur in the hours during recovery from each training session (Perry et al., 2010). During muscle contraction, a number of contractile-induced stressors (e.g. increased AMP/ATP ratio, reactive oxygen species, Ca^{2+} flux, lactate, hypoxia and decreased energy availability) collectively alter the post-translational status of key cell signalling kinases, the most-well studied of which are the calmodulin-dependent protein kinase II (CaMKII), p38 mitogen-activated protein kinase (p38MAPK) and AMP-activated protein kinase (AMPK). These kinases, acting alone or in combination with each other, subsequently activate downstream transcription factors and co-activators that exert regulatory roles in co-ordinating the expression of both nuclear- and mitochondrial-encoded proteins.

Both p38MAPK and AMPK can directly phosphorylate PGC-1α on different sites, thereby resulting in

increased activity and subsequent translocation to both the nucleus and mitochondria during acute exercise (Safdar et al., 2011). In the nucleus, PGC-1α binds to and activates a host of transcription factors, such as NRF1, NRF2, peroxisome proliferator-activated receptor (PPARδ), oestrogen-related receptor (EERα) and myocyte enhancer factor 2 (MEF2), to collectively induce up-regulation of a variety of proteins involved in the transport and oxidation of glucose and fatty acids. In the mitochondria, PGC-1α modulates the activity of mitochondrial transcription factor A (Tfam), a nuclear-encoded protein that is, itself, regulated by PGC-1α and subsequently incorporated into the mitochondria, so as to increase transcription of mitochondrial-encoded proteins, notably the cytochrome *c*-oxidase (COX) sub-units. In this way, PGC-1α is therefore recognised as a major point of control in regulating both nuclear and mitochondrial DNA (mtDNA) expression.

In addition to AMPK and MAPK, the tumour suppressor protein p53 has recently emerged as a potent regulator of mitochondrial content, function and biogenesis. Indeed, studies employing p53 knock-out (KO) rodents provide supporting evidence for a regulatory role of p53 in modulating mitochondrial content and exercise performance (Saleem, Adhihetty, & Hood, 2009). In addition, acute exercise also induces post-translational modifications (Bartlett et al., 2012) and promotes both nuclear (Philp et al., 2011) and mitochondrial translocation (Saleem & Hood, 2013), which is consistent with the notion that p53 contributes to the regulatory network controlling contractile-induced mitochondrial biogenesis.

The role of muscle glycogen availability

The hypothesis that training with reduced CHO availability augments training adaptation originated from a series of studies demonstrating that when an acute bout of exercise was undertaken with reduced muscle glycogen, there was an enhanced expression of a number of genes related to the stress response, substrate utilisation and mitochondrial biogenesis. For example, the exercise-induced increases in mRNA expression of PDK4, UCP3, HKII and LPL (Pilegaard et al., 2002) are all augmented in conditions of reduced pre-exercise muscle glycogen. Pilegaard et al. (2005) also demonstrated that attenuating post-exercise muscle glycogen re-synthesis in the short-term recovery phase (24 h) further amplifies the transcription and mRNA expression of PDK4, UCP3, LPL, and CPT1 as well as the proposed master regulator of mitochondrial biogenesis, PGC-1α. On the basis of this molecular background, Hansen et al., (2005) subsequently demonstrated that undertaking repeated exercise sessions (i.e. exercise training) in the face of low muscle glycogen availability augmented skeletal muscle oxidative capacity compared to when the same training was commenced with normal (or elevated) glycogen content. Using a two-legged knee extensor model, these researchers subjected untrained males to a 10-week training (5 days per week) protocol during which one limb was trained twice every second day (interspersed by a recovery period of 2 h during which no CHO was consumed) whereas the contralateral limb was trained once daily. Each training session consisted of a set workload of 1 h at 75% peak power output. In this way, both limbs performed an identical amount of work done during training, yet the twice per day protocol resulted in 50% of the training sessions being completed with reduced pre-exercise muscle glycogen stores. Training-induced increases in resting muscle glycogen content and citrate synthase (CS) activity and exercise capacity were superior in the limb that trained twice every second day with low muscle glycogen availability.

Using a matched group design, Yeo et al., (2008) subsequently studied two groups of highly trained male cyclists undertaking a similar twice per day (i.e. low glycogen availability) versus once per day (i.e. high glycogen availability) training model that consisted of a 3-week training block and 6 training sessions per week. In this investigation, the high group trained once per day alternating 100-min of steady-state cycling at 70% $\dot{V}O_{2peak}$ (SS) followed 24 h later with high-intensity interval cycling (HIT; 8 × 5 min bouts at maximal effort with 1-min recovery in between bouts). In contrast, the low group trained twice per day every second day, performing SS in the morning followed by the HIT protocol after a 1–2 h recovery period. Despite the absolute training intensity being reduced by ~8% in the low group for the first six HIT sessions, training-induced increases in resting muscle glycogen stores, CS and β-hydroxyacyl-CoA dehydrogenase (β-HAD) activity, cytochrome *c* oxidase (COX) subunit IV and whole-body rates of lipid oxidation (during 60 min of SS cycling at 70% $\dot{V}O_{2peak}$) were all increased in low compared to high. However, although train-low augmented these markers of training adaptation, exercise performance (1-h time trial) was not improved accordingly. In an identical experimental design but which employed stable isotope tracers, Hulston et al., (2010) demonstrated that training-low specifically increases intramuscular triglyceride oxidation during habitual exercise, likely due in part to superior increases in protein content of both β-HAD and the lipid transporter, FAT/CD36. Nevertheless, these researchers also did not observe superior improvements in exercise performance.

Although the precise mechanisms underpinning the apparent enhanced training adaptation associated with low muscle glycogen are not currently known, much of the attention has focused on the AMPK, p38MAPK and PGC-1α cascades. Indeed, the exercise-induced activity (Wojtaszewski et al., 2003) and nuclear abundance of AMPKα2 (Steinberg et al., 2006) are all enhanced with low pre-exercise muscle glycogen levels, likely attributable to the presence of a glycogen-binding domain on the β-subunit (McBride, Ghilagaber, Nikolaev, & Hardie, 2009). Similarly, both resting and exercise-induced nuclear abundance of p38MAPK is also increased with reduced muscle glycogen stores (Chan, McGee, Watt, Hargreaves, & Febbraio, 2004). Given the increase in both AMPK and p38MAPK signalling, it is unsurprising that the exercise-induced increase in PGC-1α mRNA expression is augmented when pre-exercise glycogen levels are low (Psilander, Frank, Flockhart, & Sahlin, 2013). In addition to PGC-1α, the tumour suppressor p53 has also emerged as a potential regulator of the enhanced mitochondrial adaptations associated with CHO restriction. Indeed, we recently demonstrated completing an acute exercise bout in accordance with conventional sports nutrition guidelines (i.e. glycogen-loaded, pre-exercise meal and both CHO provision during exercise and in the recovery period) attenuated p53 signalling (Bartlett et al., 2013). In contrast, commencing and recovering from the exercise bout with low CHO availability induced threefold increases in p53 phosphorylation at 3 h post-exercise. The enhanced p53 response with low CHO availability was also associated with enhanced acetyl CoA carboxylase (ACC) phosphorylation on serine 79 immediately post-exercise (but not p38MAPK phosphorylation), thus suggesting that AMPK may be the dominant upstream signalling kinase-regulating contractile-induced p53 phosphorylation.

The role of exogenous CHO availability

In addition to the role of endogenous CHO in modifying the training stimulus, there are several exercise-nutrient protocols that manipulate exogenous CHO availability (e.g. morning fasted training or withholding CHO intake immediately before and/or during exercise). In this regard, Civitarese, Hesselink, Russell, Ravussin, and Schrauwen (2005) demonstrated that CHO feeding before, during and after 2-h low-intensity cycling (50% of Wmax) attenuated GLUT-4, PDK4, AMPK, CD36, CPT-1 and UCP3 mRNA abundance. Feeding CHO (1.4–2.0 g kg^{-1} BW) prior to 2 h of one-legged knee extensor exercise has also been reported to attenuate AMPKα2 activity (Akerstrom et al., 2006). Accordingly, we also observed that the enhanced oxidative adaptations that are apparent when individuals train twice a day are actually blunted when exogenous glucose is consumed before and during the second training session, despite reduced muscle glycogen availability (Morton et al., 2009). Furthermore, performing regular fasted training sessions (i.e. exercising before breakfast) decreases exercise-induced glycogen breakdown, increases lipid transport proteins and also augmented CS and β-HAD activity compared with the consumption of breakfast before training (Van Proeyen, Szlufcik, Nielens, Ramaekers, & Hespel, 2011). Nevertheless, despite enhanced skeletal muscle adaptations there were similar improvements in $\dot{V}O_{2max}$ and cycling time-trial performance when training in the fasted or fed state.

It is noteworthy that the exercise-induced increase in PGC-1α mRNA is not affected by CHO feeding before and during exercise (Cochran et al., 2010) suggesting that there are alternative signalling mechanisms at play. In this regard, the increased availability of both extra- and intra-muscular free fatty acids (FFAs) may act as signalling intermediates. Indeed, the enhanced catecholamine response when training low enhances adipose tissue lipolysis and intra-muscular triglyceride breakdown such that FFA availability is increased. In this way, increased FFA availability during exercise can modulate p38MAPK activity (Cochran et al., 2010; Zbinden-Foncea, van Loon, Raymackers, Francaux, & Deldicque, 2013) and the binding of the transcription factor PPARδ to target genes such as CPT1 is also increased during conditions of low glucose (Philp et al., 2013). As such, these data suggest that PPARδ signalling is important for the enhanced capacity to oxidise lipids following a period of train low.

Practical applications

'Train low' has now become a catchphrase in athletic circles as well as in the scientific literature though its practical application is not without limitations. Indeed, consistently performing repeated training sessions in the face of reduced CHO availability may lead to an inability to maintain the desired training intensity (Hulston et al., 2010; Yeo et al., 2008) and hence could potentially lead to a lower overall training impulse (i.e. volume × intensity). Furthermore, performing both long duration and/or high-intensity training sessions in a CHO restricted state may also increase the susceptibility to illness and infection given the role of CHO in offsetting exercise-induced immunosuppression (Gleeson, Nieman, & Pedersen, 2004). Exercising with low CHO availability (especially conditions of reduced muscle glycogen) also increases muscle protein breakdown (Howarth et al., 2010) and if performed chronically in the presence of reduced

CHO intake (e.g. a low CHO diet) could lead to a loss of skeletal muscle mass. Finally, exercising without regular exogenous CHO provision impairs the ability to subsequently oxidise exogenous CHO (Cox et al., 2010), an adaptation that may therefore negate competition performance. The above limitations may also become exacerbated when coaches and athletes incorrectly interpret the train-low terminology as 'training zero', that is training chronically on a zero or an extremely low CHO diet. Rather, we wish to stress that training low (or training smart which may be a more representative term) is simply intended to be a well-structured example of nutritional periodisation where CHO is deliberately withheld before, during and/or after carefully selected training sessions in an attempt to enhance training adaptation. With these caveats in mind, we propose the below practical recommendations to help strategically implement train low into an athlete's training programme:

1. Practical approaches to training-low include training in the fasted state (i.e. 6–10 h after the last meal); training twice per day (where the second session is thus performed with reduced glycogen stores); and/or restricting CHO intake in the recovery period post-exercise (see Table II). Although it is presently not known which of these approaches provides the most potent training stimulus, it is recommended that where training intensity and duration lend themselves to the training-low approach (i.e. training loads are not overly compromised), then athletes would benefit from incorporating elements of train-low into their training programme.

Table II. Overview of the practical strategies to 'train low' in order to increase oxidative adaptations to endurance type training

Exercise–diet strategy	Outcome	Supporting references
Chronically low CHO diet both from endogenous and exogenous CHO sources	Chronically low CHO diet resulting in mismatch between energy intake and energy requirements May impair absolute training intensities May reduce immune function or accentuate immunosuppression that occurs post-exercise	Gleeson et al. (2004)
Twice per day training (specifically withholding CHO intake in between two endurance training sessions performed in the same day)	Reduction of endogenous and exogenous CHO availability for the muscle during the second session May impair absolute training intensities May reduce immune function or accentuate immunosuppression that occurs post-exercise	Hansen et al. (2005), Hulston et al. (2010), Morton et al. (2009), Yeo et al. (2008)
Training after an overnight fast	Reduction in exogenous CHO availability for the muscle for the specific session Potential reduction in endogenous CHO availability if sub-optimal recovery has occurred following the previous days' training May reduce immune function or accentuate immunosuppression that occurs post-exercise	Van Proeyen et al. (2011)
Withholding CHO within the first few hours of recovery	Adequate CHO availability for the muscle for the specific session but amplifies the cell-signalling response in the targeted post-exercise recovery phase. Could achieve both a 'training harder' and 'training smarter' effect Could interfere with refuelling for subsequent training sessions if CHO intake is reduced as opposed to delayed May reduce immune function or accentuate immunosuppression that occurs post-exercise	Pilegaard et al. (2005)
'Sleep low/train low'	Evening training session to deplete endogenous CHO stores followed by overnight fast and subsequent training session the next morning. Represents a combination of all of the above strategies to provide a prolonged period of CHO restriction before (i.e. overnight), during and after morning training May impair absolute training intensities May reduce immune function or accentuate immunosuppression that occurs post-exercise	Bartlett et al. (2013)

Source: Adapted from Burke (2010).

2. In order to minimise any exercise-induced immunosuppression, training-low should be undertaken during sessions that are not dedicated to uncustomary training loads (i.e. supramaximal efforts, prolonged, intense workouts).
3. In an attempt to maintain training intensity during train-low sessions, athletes would benefit from pre-training caffeine ingestion (Lane, Areta et al., 2013) and/or CHO mouth rinse during exercise (Lane, Bird, Burke, & Hawley, 2013) as both approaches can partially offset the reduced training intensity that accompanies training with low endogenous and/or exogenous CHO availability.
4. Protein ingestion (e.g. 20–25 g) should be ingested before, during and/or immediately after exercise in order to attenuate muscle protein breakdown, and to promote protein synthesis. Increased protein availability before/during/after exercise does not attenuate the activation of the key signalling cascades associated with train-low (e.g. AMPK-PGC-1), suggesting that amino acid provision will not down-regulate the beneficial adaptations induced by training low (Taylor et al., 2013).
5. The practice of training low should also be undertaken alongside deliberate sessions of training high where the intended competition-fuelling schedule (glycogen loaded, pre-exercise meal and exogenous CHO provision during exercise) is simulated (Stellingwerff, 2012). These sessions are likely to be best undertaken when the intensity and duration of training simulate the physiological demands of competition.

Conclusions and future research directions

The concept of deliberately training in CHO-restricted states is now one of the most widely debated topics amongst athletes, coaches and sports scientists. However, although our understanding of this area has advanced considerably in recent years, many important questions remain unanswered that ultimately limit our ability to strategically implement the train-low paradigm. Most notable is the absence of definitive studies demonstrating the optimal approach to structure and periodise elements of train low into an athlete's overall training macro-cycle. For example, should train low be best practised as a training block dedicated solely to this purpose or alternatively should it be implemented simultaneously alongside periods of training high within each micro- and meso-cycle of training? Furthermore, is train low best left to steady-state type training sessions that are performed several hours after a high-intensity interval type session so that high glycogen has therefore been previously available to support high-training intensities? Alternatively, is it the actual completion of a high-intensity stimulus in the presence of reduced CHO availability that is required to really augment the signalling and adaptive response? Should train low sessions be focused on the requirement to achieve a set or fixed training load or should athletes aim to complete as much work as physically possible during CHO-restricted sessions? Moreover, can elite athletes benefit from train low just as much as sub-elite or recreationally active individuals? As alluded to earlier, it is also still not known whether the optimal approach to train low is to perform fasted exercise, glycogen-depleted exercise, post-exercise CHO restriction or indeed, a combination of all three? Perhaps there is a glycogen threshold that needs to be exceeded in order to activate the proposed regulatory signalling cascades? Most important of all, does train low really enhance real-world measures of exercise performance? These questions and many more are likely to keep researchers highly active in the coming years and will hopefully advance our understanding of CHO metabolism in sport and exercise. What has already become apparent, however, is that we can no longer think of CHO as a simple fuel source of which, depletion causes of fatigue. Rather, we must now add the term of 'training regulator' to its known functions.

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