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The physiological and ergogenic effects of exercise training with low carbohydrate availability: a review

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Due to the importance of glycogen for energy production, research has traditionally recommended sufficient carbohydrate (CHO) availability to maximise exercise performance. However, recent evidence has suggested that undertaking some training sessions with low CHO availability may bring about greater physiological adaptations. This strategy has commonly been termed as 'train low'. Although desirable adaptations in gene expression related to mitochondrial biogenesis and the activity of enzymes related to aerobic metabolism have been observed, research is conflicted towards the ergogenic impact this technique has on exercise performance. Additionally, this strategy may produce maladaptations such as reduced training intensity, immunosuppression, protein oxidation and reduced pyruvate dehydrogenase (PDH) activity. Therefore, if athletes are to adopt this strategy, it is suggested that they periodise 'train low' to solely low-intensity sessions which won't be impaired by a drop in work rate, but otherwise maintaining sufficient daily CHO intake. Also, athletes could negate potential maladaptations by using caffeine and/or CHO mouth rinse to maintain exercise intensity, and increasing protein ingestion to counteract increased protein oxidation. Future research should directly compare the effect of 'train low' between use in all sessions and solely low-intensity sessions within one comprehensive study to better understand the mechanisms behind the apparent superiority of the latter strategy.

Key words: glycogen; sports nutrition; exercise performance; mitochondrial biogenesis

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Introduction

As exercise intensity increases, carbohydrates (CHO) increasingly become the primary fuel source for ATP production (van Loon *et al.*, 2001). Consequently, glycogen depletion is strongly correlated with the onset of fatigue and thus, deteriorated performance (Ørtenblad *et al.*, 2013), which highlights the importance of this substrate for optimal exercise performance. As a result, it has traditionally been recommended that athletes maintain high CHO availability before, during and after bouts of exercise during training and competition, in order to sufficiently meet the demands of exercise volume and intensity (Bartlett *et al.*, 2015).

However, recent research has reported that training with low CHO availability may stimulate the mechanisms that bring about muscle adaptations more efficiently (Drust and Morton, 2009; Philp *et al.*, 2012). As such, this method, commonly referred to as 'train low', has gathered significant interest in recent years from researchers and athletes alike (Burke, 2007; Jeukendrup, 2017). However, it has also sparked an extensive debate as to the optimal strategy to bring about these benefits, with consensus yet to be made. Thus, rationalising an up-to-date, comprehensive review of this area to determine the current

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understanding, propose unexplored areas requiring future investigation and clarify the current recommendations for athletes.

Evidence of improved physiological adaptations such as heightened cell signalling and gene expression related to mitochondrial biogenesis, activity of enzymes related to aerobic metabolism and lipolysis has come to light in train low investigations, suggesting suitability for athletes. However, its effect on performance is debated, with some studies showing ergogenic effects (Hansen *et al.*, 2005; Marquet *et al.*, 2016a, 2016b), and others finding no such benefits (Yeo *et al.*, 2008; Morton *et al.*, 2009; Hulston *et al.*, 2010). The uncertainty regarding the ergogenic effects of this technique may be owed to the potential for maladaptations, as reduced training intensity (Yeo *et al.*, 2008; Hulston *et al.*, 2010), attenuated glycogen utilisation (Peters *et al.*, 2001; Stellingwerff *et al.*, 2006), heightened protein oxidation (Howarth *et al.*, 2010) and worsened immunity (Gleeson *et al.*, 2001) have all been reported.

The muscle glycogen content required to deliver optimal physiological adaptations from low CHO training generally ranged from 100-300 mmol/kg dry weight (dw) before commencing exercise in previous train low experiments that measured glycogen levels, according to Impey *et al.* (2018). This review deemed that starting training with ~ < 350 mmol/kg dw muscle glycogen concentration constituted low CHO availability, though some investigations did not quantify pre- and/or post-exercise glycogen concentration. Methods to instigate low CHO availability include a low CHO diet (Gleeson *et al.*, 2004), training twice daily with no feeding between sessions (Hansen et al., 2005; Yeo *et al.*, 2008; Cochran *et al.*, 2010; Hulston *et al.*, 2010), no CHO during recovery (Pilegaard *et al.*, 2005) and training after an overnight fast (Wojtaszewski *et al.*, 2003; Bartlett *et al.*, 2013; Psilander et al., 2013; Lane *et al.*, 2015; Marquet *et al.*, 2016a, 2016b).

This strategy has been argued to require a 'periodised' training schedule (Jeukendrup, 2017), in which selected training sessions are undertaken with low CHO availability, with a subsequent increase in CHO in order to support performance in high-intensity sessions, as well as during competition (Baar and McGee, 2008). Hence, also being termed as 'train low, compete high' (Hansen *et al.*, 2005). This is an important consideration for elite athletes who rely on physical strength, agility and endurance for optimal performance in competitive activity, which could be influenced by inadequately planned nutritional adjustments (Laquale, 2009). This is in contrast to recreational performers, who perform at an inferior level of intensity and therefore do not require specific sports nutrition (Laquale, 2009).
Due to the lack of consensus regarding the ergogenic benefits of 'train low' and how best to implement it, it is necessary to elucidate the recommendations for athletes in light of the current research. Therefore, this review aimed to determine whether training with low CHO availability brings about physiological adaptations and improvements in endurance performance.

Physiological Adaptations
Recent research shows that CHO is not only utilised as a source of fuel for cell energy production, but also as a key regulator of several intracellular signalling processes, thus, suggesting that CHO can exert a significant influence on the physiological adaptations experienced from exercise (Baar and McGee, 2008). Following this discovery, researchers have attempted to manipulate CHO feeding to elucidate the optimal level of CHO availability to bring about desired adaptations. Subsequently, research has examined the effect of the 'train low' strategy on physiological adaptations such as cell signalling, gene expression, enzyme activity and fat oxidation.

Training with low CHO availability can enhance muscle cell signalling pathways, as it has been shown to initiate increased activity of 5' AMP-activated protein kinase (AMPK), compared to higher levels of CHO availability (Wojtaszewski *et al.*, 2003; Cochran *et al.*, 2010; Yeo *et al.*, 2010; Bartlett et al., 2013; Lane *et al.*, 2015).

AMPK is known as the master regulator of metabolism, and can be activated by a number of conditions, including calorie restriction, and most notably, insufficient glycogen availability (Cantó and Auwerx, 2009). Activation of this particular signalling pathway is generally accepted to initiate greater expression of the gene peroxisome proliferator-activated receptor-γ coactivator 1-α (PGC-1α) (Thirupathi and De Souza, 2017), which is the master regulator of mitochondrial biogenesis. Thus, activation of the AMPK-PGC-1α pathway may be vital for physiological adaptations to aid endurance performance (Cantó and Auwerx, 2009).

Correspondingly, research finds that 'train low' also increases PGC-1α gene expression in comparison to higher levels of CHO availability (Bartlett *et al.*, 2013; Psilander *et al.*, 2013), although Lane *et al.* (2015) observed similar increases to controls. This heightened PGC-1α expression may have caused its translocation into the nucleus, to transcribe key transcription factors such as nuclear respiratory factor (NRF) 1 and NRF2, consequently activating the mitochondrial protein known as mitochondrial transcription factor A (TFAM) (Wu *et al.*, 1999; Liang and Ward, 2006; Safdar *et al.*, 2011). TFAM is capable of transcribing and replicating mitochondrial DNA to bring about mitochondrial biogenesis (Picca and Lezza, 2015), thus leading to increases in mitochondrial mass and therefore the ability of cells to undergo aerobic mitochondrial respiration (Lin *et al.*, 2005). This may exert an ergogenic effect on performance as greater aerobic capacity is correlated with greater endurance capacity during exercise. This heightened PGC-1α gene expression is most likely a result of the corresponding increase in AMPK activity after 'train low' also observed by both Bartlett *et al.* (2013) and Lane *et al.* (2015), as AMPK tightly regulates the expression of PGC-1α (Thirupathi and De Souza, 2017).

'Train low' also influences the activation of enzymes involved with oxidative metabolism, as significant increases in citrate synthase (CS) activity have been observed compared to controls in 'train low' trials (Hansen *et al.*, 2005; Yeo *et al.*, 2008), even though Gejl *et al.* (2014) only found a similar increase to controls. Though, this may be explained by CHO availability being too high to effect cell signalling in Gejl *et al.* (2014), at 431 mmol/kg/dw post-exercise in the 'train low' group. CS catalyses the conversion of acetyl-coenzyme A and oxaloacetate into citrate and coenzyme A, which is the essential initial stage of the tricarboxylic acid (TCA) cycle (Akram, 2014). The TCA cycle is a key biochemical pathway involved in aerobic metabolism, therefore, increases in CS activity may upregulate the TCA cycle activity, and consequently heighten aerobic energy contribution, which is directly reflective of greater endurance capacity.

Additionally, improvements in cytochrome c oxidase subunit IV (COX IV) enzyme activity have been detected after 'train low' interventions (Yeo *et al.*, 2008; Bartlett *et al.*, 2013), although Cochran *et al.* (2010) observed only similar increases in COX IV to controls. COX IV is the conclusive enzyme in the electron transport chain, where it transfers electrons into molecular oxygen (O2), and further reduces this to water within the inter-membrane space of the mitochondria, which facilitates the reactions which bring about subsequent ATP resynthesis (Hüttemann et al., 2012). Thus, heightened COX IV activity can augment the rate of mitochondrial oxidative phosphorylation, again indicative of heightened endurance potential during exercise (Levy and Deutschman, 2007). The increased activity of CS and COX IV is most likely a result of the initiation of the AMPK pathway, and thus, activation of PGC-1α after 'train low', as PGC-1α is a major regulator of both of these enzymes (Benton et al., 2008).

Also, increased β-hydroxyacyl-CoA dehydrogenase (β-HAD) activity has been reported in 'train low' studies (Hansen *et al.*, 2005; Yeo *et al.*, 2008; Cochran *et al.*, 2010; Hulston *et al.*, 2010), but with only Gejl et al. (2014) finding a similar increase in β-HAD activity to the control trial. β-HAD is a vital enzyme in fatty acid metabolism, as it catalyses the penultimate stage of fatty acid β-oxidation, which is the oxidation of

straight-chain 3-hydroxyacyl-CoA (Jain *et al.*, 2012). Hence, an increase in the activity of this enzyme may explain the corresponding significant increases in lipid oxidation after 'train low' observed by Yeo *et al.* (2008) and Hulston *et al.* (2010). This adaptation reduces glycogen dependence, conserving substrates for later application, which has been suggested to prolong the duration of endurance performance (Fan *et al.*, 2017). This could also suggest that these studies instigated a fat-adapted state, in which fat oxidation is chronically increased, even when higher CHO levels are returned (Burke *et al.*, 2017). However, research is equivocal regarding fat oxidation, as Marquet et al. (2016a, 2016b) found no difference in lipolysis between glycogen availability levels. This may be due to differences in study design, as Marquet et al. (2016a, 2016b) utilised training after an overnight fast, alongside a periodised, CHO-matched training schedule, with only low-intensity sessions undertaken in a low CHO state, which may have prevented chronic fat-adaptation.

Ergogenic Effects

Despite the numerous physiological adaptations observed, studies by Yeo *et al.* (2008) and Hulston *et al.* (2010) found no significant differences in the improvement in performance of cycling time trials performed with fully replenished CHO between train low groups and control groups of well-trained cyclists. This may be a result of 'train low' being utilised for high-intensity training sessions, in which glycogen depletion is a limiting factor of performance (van Loon *et al.*, 2001), hence the corresponding decline in training intensity which was also observed in the low CHO groups in both of these studies. Therefore, this may actually display some efficacy of 'train low' to still improve performance levels from baseline despite a greater disruption to high-intensity training than controls.

Similarly, recreationally active males also saw no significant differences in the improvement in performance of high-intensity exercise with fully replenished CHO between 'train low' and controls in a study by Morton *et al.* (2009), despite also observing improved aerobic enzyme activity. The lack of an ergogenic effect observed by Morton et al. (2009) may be explained by the possibility that the performance of the high-intensity exercise test adopted to determine ergogenic improvement is not aided by the aerobic nature of the adaptations exerted via the 'train low' strategy. Also, similarly to the protocol of Yeo *et al.* (2008) and Hulston *et al.* (2010), 'train low' was utilised for high-intensity training sessions (90% Vo_2 max) (Bacon et al., 2013) in which glycogen depletion is a limiting factor (van Loon *et al.*, 2001), and thus, may have also deteriorated training intensity. However, caution must be taken when generalising the results of recreational performers, as the CHO requirements of elite athletes far exceeds those of a recreational performer (Tarnopolsky *et al.*, 2005; Laquale, 2009).

Nonetheless, in untrained individuals, 'train low' improved endurance performance of a single-leg cycle ergometer-knee extensor exercise task to exhaustion after CHO was fully replenished (Hansen *et al.*, 2005). Despite this, the untrained sample and the simple nature of the exercise may inhibit the extrapolation of the data to trained athletes, and practical sporting contexts, respectively. In answer to this, more recent research revealed that a periodised, 'train low' intervention improved performance of both supramaximal cycling and 10km running time with replenished CHO availability in trained triathletes (Marquet *et al.*, 2016a). The same research group also observed that the same protocol improved the performance of both submaximal and time-trial cycling with replenished CHO availability in trained cyclists (Marquet *et al.*, 2016b), despite no increases in fatty acid oxidation observed in either study. However, no further physiological measures were observed in these investigations, alongside no measurement of pre- or post-exercise muscle glycogen content during 'train' low sessions, thus necessitating some speculation as to the mechanisms behind these ergogenic effects, and limiting the potential for reproduction of the study protocol.

The contrast between the results of Marquet et al. (2016a, 2016b) and those of Hulston *et al.*

(2010), Yeo *et al*. (2008) and Morton et al. (2009) may be due to the periodised training schedule by Marquet *et al*. (2016a, 2016b), which only utilised 'train low' during low-intensity sessions in which training intensity is not deteriorated by CHO deficiency. It may also be due to the same total volume of CHO being consumed between experimental groups (6 g/kg body mass/day), just consumed at different times. This could explain why participants in Marquet *et al*. (2016a, 2016b) did not become fat-adapted via increased lipolysis. Chronic fat-adaptation in Yeo *et al*. (2008), Morton et al. (2009) and Hulston *et al*. (2010) could have had a deleterious effect on their ability to utilise CHO when replenished, as fat-adaptation attenuates pyruvate dehydrogenase (PDH) activity via pyruvate dehydrogenase kinase-4 (PDK4) upregulation. In addition, the efficiency of ATP production could be impaired, as the metabolism of fats is less economic than CHO, consequently inflating the O2 cost of ATP resynthesis at a given work rate, driving attainment of VO2max and premature fatigue (Stellingwerff et al., 2006; Burke *et al*., 2017). Hence, this could explain why performance improvements were no different to controls in Yeo *et al*. (2008), Morton *et al*. (2009) and Hulston *et al*. (2010), despite the observation of valuable physiological adaptations in the 'train low' groups.

Limitations of 'Train Low'

'Train low' can reduce exercise intensity due to substrate deficiency (Havemann *et al*., 2006) and reduce muscle contraction as a result of compromised calcium regulation (Gejl *et al*., 2014). Consequently, this may not induce a high enough overload to see various adaptations occur, and also significantly worsen technical and tactical training performance (Baar and McGee, 2008). This may explain the decline in training intensity seen in the 'train low' studies of Yeo *et al*. (2008) and Hulston *et al*. (2010). However, periodisation of 'train low' to solely low-intensity sessions (Jeukendrup, 2017), CHO mouth rinse (Kasper *et al*., 2016) and caffeine supplementation (Silva-Cavalcante *et al*., 2013) have been recommended to counteract this performance deficit.

Training in this CHO-restricted state also increases cortisol production and reduces antibody production and lymphocyte proliferation, thus potentially leading to a transient period of immunosuppression and consequent heightened infection risk (Gleeson *et al*., 2001). An illness may enforce an involuntary reduction in training frequency, and each week of unfinished planned training is understood to decrease the probability of success by 26% in elite athletes (Raysmith and Drew, 2016). Therefore, excessive use of this strategy may best be avoided in order to protect the immunity of athletes. Nevertheless, these effects could be neutralised via supplementation with 400 to 2,000 IU/day vitamin D, which has been demonstrated to protect against the occurrence of respiratory tract infections (Charan *et al*., 2012; Rondanelli *et al*., 2018), as well as 80 to 207 mg/day zinc, which has been proven to limit the duration of respiratory tract infections following their onset (Hemilä, 2017; Rondanelli *et al*., 2018).
Exercising with low CHO availability increases protein metabolism, meaning prolonged exercise in this state could cause a reduction in skeletal muscle mass (Howarth *et al*., 2010). As maximal muscle force output is heavily reliant upon skeletal muscle mass, this process of sarcopenia may deteriorate exercise performance (Reid and Fielding, 2012). Though, this effect could be offset by merely increasing protein intake to stimulate greater muscle protein synthesis following exercise (Burke *et al*., 2011; Taylor *et al*., 2013).

As previously discussed, 'train low' may lead to reduced levels of the enzyme PDH when performing with replenished CHO (Stellingwerff *et al*., 2006). This is likely due to a corresponding increase in pyruvate dehydrogenase kinase-4 (PDK4) activity, as observed after training with low CHO availability (Peters *et al*., 2001; Pilegaard et al., 2005; Bartlett *et al*., 2013; Lane *et al*., 2015), because activation of this enzyme is widely understood to blunt PDH activity. PDH catalyses the conversion of pyruvate into acetyl-coenzyme A, which is the crucial antecedent of the TCA cycle known as the link (reaction. Therefore, this PDH downregulation

may attenuate the use of glycogen for aerobic ATP production, thus necessitating a greater reliance on either less efficient fat oxidation or metabolite-producing glycolytic metabolism, both of which can be detrimental to endurance performance (Jeong *et al.*, 2012). However, this PDH attenuation could be counteracted by simply ensuring sufficient daily CHO consumption (Stellingwerff *et al.*, 2006), but periodising intake throughout the day to avoid ingestion near to 'train low' sessions, as in Marquet *et al.* (2016a, 2016b).

Studies have generally failed to blind subjects of intervention group appointment, potentially enabling the emergence of placebo effects (Impey *et al.*, 2018). For instance, participants may have had pre-conceived biases about the intervention, potentially impacting their effort levels, hence the reduction in training intensity in many 'train low' investigations (Halson and Martin, 2013). Though, blinding of CHO intake may prove challenging, particularly during investigations utilising a pre- or post-exercise fasting period.

The physiological adaptations incurred in the previously discussed studies may be a result of a potential calorie restriction, rather than CHO restriction (Impey *et al.*, 2018). It is widely accepted that a negative energy balance increases levels of AMP and ADP, which can also elicit enhanced AMPK expression (Hardie and Sakamoto, 2006). Therefore this could have provided the mechanism behind the enhanced PGC-1α activity and subsequent desirable adaptations. Alternatively, any potential calorie restriction may have also reduced body mass, which has been argued to benefit endurance performance via an improved efficiency of movement and enhanced thermoregulation, and thus may explain any performance gains (O'Connor and Slater, 2011). However, until specific studies are undertaken to confirm this mechanism, generalisations must be taken with caution.

As well as peripheral adaptations such as mitochondrial biogenesis, optimal endurance performance also requires central adaptations such as heart hypertrophy, in order to augment cardiac output and thus muscle O2 supply, substrate delivery and metabolite clearance (Sharma *et al.*, 2000). In order to bring about this adaptation, one must exercise at an intensity which elevates heart rate and blood pressure for prolonged periods, thus increasing the physical demand on the heart (Fagard, 2003). As 'train low' restricts the ability to exercise at high intensities (Yeo *et al.*, 2008; Hulston *et al.*, 2010), it could be argued that the required heart rate isn't reached, or is not sustained for long enough to produce cardiac hypertrophy (Impey *et al.*, 2018). Therefore, to avoid preventing other key adaptations, athletes should periodise 'train low' to solely low-intensity sessions and/or employ this training method in moderation.

Conclusion

Regarding the physiological adaptations incurred following exercise (e.g. improved cell signalling, greater gene expression and greater activation of oxidative enzymes), it is evidently advantageous for athletes to train with low CHO availability. However, it is still contended whether this translates to improvements in endurance performance. It appears that studies which employ 'train low' for higher-intensity sessions or without matched total CHO feeding, observe worsened training intensity, as well as greater fat-adaptation, potentially impairing glycogen utilisation (Peters *et al.*, 2001) and exercise efficiency (Burke *et al.*, 2017). This could explain the corresponding absence of performance improvements greater than those of controls, despite observing other useful adaptations (Yeo *et al.*, 2008; Morton et al., 2009; Hulston *et al.*, 2010). Whereas studies which periodised 'train low' to only low-intensity sessions and employed matched total CHO feeding, observed no such decline in training intensity, alongside no increase in fat oxidation, and significant performance enhancements compared to controls (Marquet *et al.*, 2016a, 2016b). This may imply that if athletes and/or coaches are to utilise the 'train low' strategy, they should do so only in low-intensity sessions, alongside sufficient daily CHO intake.

Athletes should also avoid training too intensely and frequently with low CHO availability, in order to avoid the risk of the previously-mentioned potential maladaptations (e.g. immunosuppression and disproportionate protein metabolism) (Gleeson *et al.*, 2001; Howarth *et al.*, 2010), and to ensure that central adaptations are not compromised (Impey *et al.*, 2018). Lastly, athletes should also implement nutritional strategies during 'train low' by ingesting caffeine (Silva-Cavalcante *et al.*, 2013) and/or utilising CHO mouth rinse (Kasper *et al.*, 2016) to offset decreases in exercise intensity, consuming added protein to counteract heightened protein oxidation (Burke *et al.*, 2011; Taylor *et al.*, 2013) and supplementing with vitamin D and/or zinc to negate immunosuppression (Rondanelli *et al.*, 2018).

Although suggestions can be made to prescribe the best nutritional practice for athletes, a lack of concrete evidence for both the ergogenic efficacy, and optimal method of delivery of 'train low' remains evident, necessitating some speculation. Thus, this research field still requires further investigation before conclusive athlete guidelines can be distributed. Future research should directly compare the physiological and ergogenic effects of training between an intervention utilising 'train low' for higher-intensity sessions, and a periodised intervention only utilising 'train low' for low-intensity sessions within one comprehensive study, to better determine the mechanisms behind greater ergogenic improvements observed in periodised interventions. Also, future investigations should replicate the periodised, CHO-matched protocol of Marquet et al. (2016a, 2016b), but supplement this with further physiological measures such as cell signalling, gene expression and enzyme activity to better determine the mechanism that contributed to the ergogenic effects. Lastly, further studies are required to better understand how best to periodise 'train low' across all training cycles, including broader mesocycles such as pre-season and the competitive season.

References

Akram, M. (2014). Citric acid cycle and role of its intermediates in metabolism. *Cell Biochemistry and Biophysics*, 68(3), pp. 475-478.

Baar, K. and McGee, S. (2008). Optimizing training adaptations by manipulating glycogen. *European Journal of Sport Science*, 8(2), pp. 97-106.

Bacon, A. P., Carter, R. E., Ogle, E. A. and Joyner, M. J. (2013). VO 2 max trainability and high intensity interval training in humans: a meta-analysis. *PloS one*, 8(9), pp. e73182.

Bartlett, J. D., Hawley, J. A. and Morton, J. P. (2015). Carbohydrate availability and exercise training adaptation: too much of a good thing? *European Journal of Sport Science*, 15(1), pp. 3-12.

Bartlett, J. D., Louhelainen, J., Iqbal, Z., Cochran, A. J., Gibala, M. J., Gregson, W., Close, G. L., Drust, B. and Morton, J. P. (2013). Reduced carbohydrate availability enhances exercise-induced p53 signaling in human skeletal muscle: Implications for mitochondrial biogenesis. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, 304(6), pp. R450-R458.

Benton, C. R., Nickerson, J. G., Lally, J., Han, X. X., Holloway, G. P., Glatz, J. F. ... and Bonen, A. (2008). Modest PGC-1α overexpression in muscle in vivo is sufficient to increase insulin sensitivity and palmitate oxidation in subsarcolemmal, not intermyofibrillar, mitochondria. *Journal of Biological Chemistry*, 283(7), pp. 4228-4240.

Burke, L. M. (2007). New issues in training and nutrition: Train low, compete high? *Current Sports Medicine Reports*, 6(3), pp. 137-138.

Burke, L. M., Hawley, J. A., Wong, S. H. and Jeukendrup, A. E. (2011). Carbohydrates for training and competition. *Journal of Sports Sciences*, 29(sup1), pp. S17-S27

Burke, L. M., Ross, M. L., Garvican-Lewis, L. A., Welvaert, M., Heikura, I. A., Forbes, S. G. ... and Hawley, J. A. (2017). Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *The Journal of Physiology*, 595(9), pp. 2785-2807.

Cantó, C. and Auwerx, J. (2009). PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Current Opinion in Lipidology*, 20(2), pp. 98.

Charan, J., Goyal, J. P., Saxena, D. and Yadav, P. (2012). Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis. *Journal of Pharmacology & Pharmacotherapeutics*, 3(4), pp. 300-303.

Cochran, A. J., Little, J. P., Tarnopolsky, M. A. and Gibala, M. J. (2010) Carbohydrate feeding during recovery alters the skeletal muscle metabolic response to repeated sessions of high-intensity interval exercise in humans. *Journal of Applied Physiology*, 108(3) pp. 628–36.

Drust, B. and Morton, J. (2009). Promoting endurance training adaptations with nutritional interventions: the potential benefits of 'low carbohydrate' training. Kinesiology: *International Journal of Fundamental and Applied Kinesiology*, 41(1), pp. 19-24.

Fagard, R. (2003). Athlete's heart. Heart, 89(12), pp. 1455-1461.

Fan, W., Waizenegger, W., Lin, C. S., Sorrentino, V., He, M. X., Wall, C. E., Li, H., Liddle, C., Ruth, T. Y., Atkins, A. R. and Auwerx, J. (2017). PPARδ promotes running endurance by preserving glucose. *Cell Metabolism*, 25(5), pp. 1186-1193.

Gejl, K., Hvid, L. G., Frandsen, U., Jensen, K., Sahlin, K. and Ortenblad, N. (2014). Muscle glycogen content modifies SR Ca2+ release rate in elite endurance athletes. *Medicine & Science in Sports & Exercise*, 46(3), pp. 496-505.

Gleeson, M., Lancaster, G. I. and Bishop, N. C. (2001). Nutritional strategies to minimise exercise-induced immunosuppression in athletes. *Canadian Journal of Applied Physiology*, 26(S1), pp. S23-S35.

Gleeson, M., Nieman, D. C. and Pedersen, B. K. (2004). Exercise, nutrition and immune function. *Journal of Sports Sciences*, 22(1), pp. 115–125.

Halson, S. L. and Martin, D. T. (2013). Lying to win—placebos and sport science. International *Journal of Sports Physiology and Performance*, 8(6), pp. 597-599.

Hansen, A. K., Fischer, C. P., Plomgaard, P., Andersen, J. L., Saltin, B. and Pedersen, B. K. (2005). Skeletal muscle adaptation: training twice every second day vs. training once daily. *Journal of Applied Physiology*, 98(1), pp. 93-99.

Hardie, D. G. and Sakamoto, K. (2006). AMPK: A key sensor of fuel and energy status in skeletal muscle. *Physiology*, 21(1), pp. 48-60.

Havemann, L., West, S. J., Goedecke, J. H., Macdonald, I. A., St Clair Gibson, A., Noakes, T. D. and Lambert, E. V. (2006). Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance. *Journal of Applied Physiology*, 100(1), pp. 194-202.

Hemilä, H. (2017). *Zinc lozenges and the common cold: a meta-analysis comparing zinc acetate and zinc gluconate, and the role of zinc dosage*. JRSM Open, 8(5), pp. 2054270417694291.

Howarth, K. R., Phillips, S. M., MacDonald, M. J., Richards, D., Moreau, N. A. and Gibala, M. J. (2010). Effect of glycogen availability on human skeletal muscle protein turnover during exercise and recovery. *Journal of Applied Physiology*, 109(2), pp. 431-438.

Hulston, C. J., Venables, M. C., Mann, C. H., Martin, C., Philp, A., Baar, K. and Jeukendrup, A. E. (2010). Training with low muscle glycogen

cyclists. *Medicine & Science in Sports & Exercise*, 42(11), pp. 2046-2055.

Hüttemann, M., Lee, I., Grossman, L. I., Doan, J. W. and Sanderson, T. H. (2012). Phosphorylation of mammalian cytochrome c and cytochrome c oxidase in the regulation of cell destiny: respiration, apoptosis, and human disease. *Advances in Experimental Medicine and Biology*, 748, pp. 237–264.

Impey, S. G., Hearris, M. A., Hammond, K. M., Bartlett, J. D., Louis, J., Close, G. L. and Morton, J. P. (2018). Fuel for the work required: a theoretical framework for carbohydrate periodization and the glycogen threshold hypothesis. *Sports Medicine*, 48(5), pp. 1031-1048.

Jain, V., Chen, M. and Menon, R. K. (2012). Disorders of carbohydrate metabolism. In Gleason, C. A., Devaskar, S. U. (eds.), *Avery's Diseases of the Newborn*, 9th Edition (pp. 1320-1329). Philadelphia, USA: Elsevier Health Sciences.

Jeong, J. Y., Jeoung, N. H., Park, K. G. and Lee, I. K. (2012). Transcriptional regulation of pyruvate dehydrogenase kinase. *Diabetes & Metabolism Journal*, 36(5), pp. 328-335.

Jeukendrup, A. E. (2017). Periodized nutrition for athletes. Sports Medicine, 47(1), pp. 51-63.
Kasper, A. M., Cocking, S., Cockayne, M., Barnard, M., Tench, J., Parker, L. ... and Morton, J. P. (2016). Carbohydrate mouth rinse and caffeine improves high-intensity interval running capacity when carbohydrate restricted. *European Journal of Sport Science*, 16(5), pp. 560-568.

Lane, S. C., Camera, D. M., Lassiter, D. G., Areta, J. L., Bird, S. R., Yeo, W. K. ... and Hawley, J. A. (2015). Effects of sleeping with reduced carbohydrate availability on acute training responses. *Journal of Applied Physiology*, 119(6), pp. 643-655.

Laquale, K. (2009). Nutritional needs of the recreational athlete. *International Journal of Athletic Therapy and Training*, 14(1), pp. 12-15.

Levy, R. J. and Deutschman, C. S. (2007). Cytochrome c oxidase dysfunction in sepsis. *Critical Care Medicine*, 35(9), pp. S468-S475.

Liang, H. and Ward, W. F. (2006). PGC-1α: a key regulator of energy metabolism. Advances in Physiology Education, 30(4), pp. 145-151.

Lin, J., Handschin, C. and Spiegelman, B. M. (2005). Metabolic control through the PGC-1 family of transcription coactivators. *Cell* Metabolism, 1(6), pp. 361-370.

Marquet, L. A., Brisswalter, J., Louis, J., Tiollier, E., Burke, L., Hawley, J. and Hausswirth, C. (2016a). Enhanced endurance performance by periodization of CHO intake: "Sleep low" strategy. *Medicine & Science in Sports & Exercise*, 48(4), pp. 663-672.

Marquet, L. A., Hausswirth, C., Molle, O., Hawley, J., Burke, L., Tiollier, E. and Brisswalter, J. (2016b). Periodization of carbohydrate intake: Short-term effect on performance. *Nutrients*, 8(12), pp. 755.

Morton, J. P., Croft, L., Bartlett, J. D., MacLaren, D. P., Reilly, T., Evans, L., McArdle, A. and Drust, B. (2009). Reduced carbohydrate availability does not modulate training-induced heat shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle. *Journal of Applied Physiology*, 106(5), pp. 1513-1521.

O'Connor, H. and Slater G. (2011) Losing, gaining and making weight for athletes. In Lanham-New, S., Steer, S., Sherriffs, M., Collins, A. (eds.), *Sport and Exercise Nutrition* (pp. 210–232). West Sussex, UK: Wiley-Blackwell.

Ørtenblad, N., Westerblad, H. and Nielsen, J. (2013). Muscle glycogen stores and fatigue. *The Journal of Physiology*, 591(18), pp. 4405-4413.

Peters, S. J., Harris, R. A., Wu, P., Pehleman, T. L., Heigenhauser, G. J. and Spriet, L. L. (2001). Human skeletal muscle PDH kinase activity and isoform expression during a 3-day high-fat/low-carbohydrate diet. *American Journal*

of Physiology-Endocrinology and Metabolism, 281(6), pp. E1151-E1158.

Philp, A., Hargreaves, M. and Baar, K. (2012). More than a store: Regulatory roles for glycogen in skeletal muscle adaptation to exercise. *American Journal of Physiology-Endocrinology and Metabolism*, 302(11), pp. E1343-E1351.

Picca, A. and Lezza, A. M. S. (2015). Regulation of mitochondrial biogenesis through TFAM-mitochondrial DNA interactions: useful insights from aging and calorie restriction studies. *Mitochondrion*, 25, pp. 67-75.

Pilegaard, H., Osada, T., Andersen, L. T., Helge, J. W., Saltin, B. and Neufer, P. D. (2005). Substrate availability and transcriptional regulation of metabolic genes in human skeletal muscle during recovery from exercise. *Metabolism*, 54(8), pp. 1048-1055.

Psilander, N., Frank, P., Flockhart, M. and Sahlin, K. (2013). Exercise with low glycogen increases PGC-1α gene expression in human skeletal muscle. *European Journal of Applied Physiology*, 113(4), pp. 951-963.

Raysmith, B. P. and Drew, M. K. (2016). Performance success or failure is influenced by weeks lost to injury and illness in elite Australian track and field athletes: a 5-year prospective study. *Journal of Science and Medicine in Sport*, 19(10), pp. 778-783.

Reid, K. F. and Fielding, R. A. (2012). Skeletal muscle power: a critical determinant of physical functioning in older adults. *Exercise and Sport Sciences Reviews*, 40(1), pp. 4-12.

Rondanelli, M., Miccono, A., Lamburghini, S., Avanzato, I., Riva, A., Allegrini, P. ... and Perna, S. (2018). Self-care for common colds: the pivotal role of vitamin D, vitamin C, zinc, and echinacea in three main immune interactive clusters (physical barriers, innate and adaptive immunity) involved during an episode of common colds—practical advice on dosages and on the time to take these nutrients/botanicals in order to prevent or treat common colds. Evidence-Based *Complementary and Alternative Medicine*, 2018, pp. 5813095.

Safdar, A., Little, J. P., Stokl, A. J., Hettinga, B. P., Akhtar, M. and Tarnopolsky, M. A. (2011). Exercise increases mitochondrial PGC-1α content and promotes nuclear-mitochondrial cross-talk to coordinate mitochondrial biogenesis. *Journal of Biological Chemistry*, 286(12), pp. 10605-10617.

Sharma, S., Elliott, P. M., Whyte, G., Mahon, N., Virdee, M. S., Mist, B. and McKenna, W. J. (2000). Utility of metabolic exercise testing in distinguishing hypertrophic cardiomyopathy from physiologic left ventricular hypertrophy in athletes. *Journal of the American College of Cardiology*, 36(3), pp. 864-870.

Silva-Cavalcante, M. D., Correia-Oliveira, C. R., Santos, R. A., Lopes-Silva, J. P., Lima, H. M., Bertuzzi, R. ... and Lima-Silva, A. E. (2013). Caffeine increases anaerobic work and restores cycling performance following a protocol designed to lower endogenous carbohydrate availability. *PLoS One*, 8(8), pp. e72025.

Stellingwerff, T., Spriet, L. L., Watt, M. J., Kimber, N. E., Hargreaves, M., Hawley, J. A. and Burke, L. M. (2006). Decreased PDH activation and glycogenolysis during exercise following fat adaptation with carbohydrate restoration. *American Journal of Physiology - Endocrinology and Metabolism*, 290(2), pp. E380-E388.

Tarnopolsky, M. A., Gibala, M., Jeukendrup, A. E. and Phillips, S. M. (2005). Nutritional needs of elite endurance athletes. Part I: Carbohydrate and fluid requirements. *European Journal of Sport Science*, 5(1), pp. 3-14.

Taylor, C., Bartlett, J. D., van de Graaf, C. S., Louhelainen, J., Coyne, V., Iqbal, Z. … and Morton, J. P. (2013). Protein ingestion does not impair exercise-induced AMPK signalling when in a glycogen-depleted state: Implications for train-low compete-high. *European Journal of Applied Physiology*, 113(6), pp. 1457–1468.

Thirupathi, A. and De Souza, C. T. (2017). Multi-regulatory network of ROS: the interconnection of ROS, PGC-1 alpha, and AMPK-SIRT1 during exercise. *Journal of Physiology and Biochemistry*, 73(4), pp. 487-494.

van Loon, L. J., Greenhaff, P. L., Constantin-Teodosiu, D., Saris, W. H. and Wagenmakers, A. J. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of Physiology*, 536(1), pp. 295-304.

Wojtaszewski, J. F., MacDonald, C., Nielsen, J. N., Hellsten, Y., Hardie, D. G., Kemp, B. E., Kiens, B. and Richter, E. A. (2003). Regulation of 5′ AMP-activated protein kinase activity and substrate utilization in exercising human skeletal muscle. *American Journal of Physiology-Endocrinology and Metabolism*, 284(4), pp. E813-E822.

Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelmant, G., Mootha, V. ... and Spiegelman, B. M. (1999). Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell*, 98(1), pp. 115-124.

Yeo, W. K., McGee, S. L., Carey, A. L., Paton, C. D., Garnham, A. P., Hargreaves, M. and Hawley, J. A. (2010). Acute signalling responses to intense endurance training commenced with low or normal muscle glycogen. *Experimental Physiology*, 95(2), pp. 351-358.

Yeo, W. K., Paton, C. D., Garnham, A. P., Burke, L. M., Carey, A. L. and Hawley, J. A. (2008). Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens. *Journal of Applied Physiology*, 105(5), pp. 1462-1470.