LJMU Research Online

Close, GL, Hamilton, L, Philp, A, Burke, L and Morton, JP

 New Strategies in Sport Nutrition to Increase Exercise Performance.

http://researchonline.ljmu.ac.uk/2977/

Article

New strategies in sport nutrition to increase exercise performance

Close, GL[1], Hamilton, L[2], Philp, A[3], Burke, L[4] and Morton, JP[1]

1. Research Institute for Sport and Exercise Science (RISES), Liverpool John Moores University, Liverpool, United Kingdom.

2. Health and Exercise Sciences Research Group, University of Stirling, Stirling, United Kingdom

3. School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Birmingham, United Kingdom.

4. Sports Nutrition, Australian Institute of Sport, Canberra, ACT, Australia

Corresponding Author:

Dr. Graeme L. Close

Research Institute for Sport and Exercise Science,

Liverpool John Moores University

Tom Reilly Building,

Byrom Street,

Liverpool,

L3 3AF

g.l.close@ljmu.ac.uk

ABSTRACT

Despite over 50 years of research, the field of sports nutrition continues to grow at a rapid rate. Whilst the traditional research focus was one that centred on strategies to maximize competition performance, emerging data in the last decade has demonstrated how both macronutrient and micronutrient availability can play a prominent role in regulating those cell signalling pathways that modulate skeletal muscle adaptations to endurance and resistance training. Nonetheless, in the context of exercise performance, it is clear that carbohydrate (but not fat) still remains king and that carefully chosen ergogenic aids (e.g. caffeine, creatine, sodium bicarbonate, beta-alanine, nitrates) can all promote performance in the correct exercise setting. In relation to exercise training, however, it is now thought that strategic periods of reduced carbohydrate and elevated dietary protein intake may enhance training adaptations whereas high carbohydrate availability and antioxidant supplementation may actually attenuate training adaptation. Emerging evidence also suggests that vitamin D may play a regulatory role in muscle regeneration and subsequent hypertrophy following damaging forms of exercise. Finally, novel compounds (albeit largely examined in rodent models) such as epicatechins, nicotinamide riboside, resveratrol, β-hydroxy β-methylbutyrate, phosphatidic acid and ursolic acid may also promote or attenuate skeletal muscle adaptations to endurance and strength training. When taken together, it is clear that sports nutrition is very much at the heart of the Olympic motto, *Citius, Altius, Fortius (faster, higher, stronger).*

1. Introduction into the growing role of sport nutrition

In keeping with the Olympic motto "*Citius, Altius, Fortius*", the traditional research focus in the field of sports nutrition has been one that has largely centred on those strategies that may improve performance on competition day. In this way, over 50 years of research has investigated strategies to prepare for competition (e.g. pre-exercise fuelling), promote performance during competition (e.g. fluid intake and carbohydrate feeding) and recover from competition (e.g. carbohydrate and protein feeding to promote muscle recovery). Additionally, many investigators have researched those ergogenic aids that may improve exercise performance and/or fatigue through modulating either central or peripheral aspects of fatigue. When taken together, it is clear that those competition nutrition strategies that focus on sufficient macronutrient intake and ergogenic aids to promote energy availability and delay the biochemical determinants of fatigue are now largely based on sound scientific evidence.

However, in the last decade of research, accumulating data have now demonstrated a potent role of both macro- and micro-nutrient availability in regulating those exercise-induced cell-signalling pathways that are thought to regulate skeletal muscle adaptations to exercise training. As such, both researchers and practitioners alike are now beginning to treat "competition nutrition" and "training nutrition" as two separate entities, the former having an obvious performance focus but the latter having an adaptive focus. For example, in the case of endurance exercise, emerging data suggest that deliberate periods of *reduced* carbohydrate availability (and potentially, high fat availability) can enhance those adaptations fundamental to endurance performance including mitochondrial biogenesis, increased lipid oxidation and increased fatigue resistance. Similarly, many novel compounds are also emerging (albeit from rodent studies) that can also regulate those signalling pathways inherent to endurance training adaptations. In the context of resistance exercise, it is also now well known that increased dietary protein is a necessary nutrient to promote muscle growth by providing those necessary amino acids to both activate and promote muscle protein synthesis.

In the present paper, we review the current developments in sports nutrition by providing a narrative that simultaneously discusses traditional and novel strategies that serve to enhance exercise performance and training adaptations. We begin by providing an overview of the molecular regulation of skeletal muscle adaptations to endurance and resistance

training and discuss where appropriate, the role of carbohydrate, fat and protein in modulating performance and training adaptations. We then proceed to review our latest thinking on evidence-based supplements that can promote performance. Finally, we close by outlining a variety of potential novel compounds that may also regulate training adaptations.

2. Nutrition gene interactions

Multiple molecular pathways are activated by exercise and these pathways have been shown to contribute to the adaptive remodelling of skeletal muscle [1, 2]. These same pathways are shared with a number of the nutrient sensing mechanisms [3, 4]. This cross-talk between nutrient sensitive and exercise sensitive pathways opens up the possibility that nutrient provision strategies could influence not only acute exercise performance through fuelling the activity, but also the magnitude of the adaptive response following a period of structured exercise training. In the following sections we will discuss some of these nutrient-gene interactions and the potential for exploiting these interactions through strategically delivered or withheld nutrition to enhance the adaptive stimulus and ultimately to improve exercise and athletic performance.

2.1 The molecular regulation of endurance training adaptation. Endurance training is typically defined by rhythmically performing relatively low intensity contractions for a relatively long period of time [5], however recent studies show that short duration high intensity interval training may in fact result in similar adaptations to typical endurance exercise [6]. Structured endurance training leads to improvements in fatigue resistance partly by altering the phenotype of the skeletal muscle performing the work [7]. At the level of the muscle a period of endurance training leads to improvements in blood flow, mitochondrial content and an improved ability to extract and utilise oxygen during exercise [7]. These adaptive processes are driven by transcriptional changes in response to each bout of exercise [2, 8]. The transcriptional changes accumulate over time such that the protein expression profile of muscle is changed resulting in an altered phenotype [2, 8]. The question then remains as to how contracting skeletal muscle senses the work and conveys that signal to a gene expression change, ultimately resulting in improved fatigue resistance.

The answer may lie partly in the metabolite changes in skeletal muscle during contraction. Of primary importance for this review are the changes in intramuscular AMP levels, glycogen stores and fatty acid flux (**Figure 1**). AMP is a product of the adenylate kinase reaction which works to maintain the ATP:ADP ratio by converting two ADP molecules to one ATP and one AMP molecule [9]. The exercise dependent increase in skeletal muscle AMP [10] is thought to not only allosterically activate glycolysis [11] but also activate the evolutionary conserved fuel gauge protein AMP-activated protein kinase (AMPK) [12]. When the AMP:ATP ratio increases the probability of AMPK being in the AMP bound state increases. This allosterically activates AMPK [13] and makes AMPK a more efficient substrate for its upstream kinase LKB1, phosphorylation by which leads to full activation of the kinase [14]. Activated AMPK phosphorylates and inhibits its downstream substrate Acetyl-CoA carboxylase (ACC) [12]. The inhibition of ACC by AMPK leads to a reduction in the levels of manonyl CoA, which allows for the activation of the mitochondrial fatty acid transporter Carnitine palmitoyltransferase I (CPT1) allowing for a greater flux of fatty acids into the mitochondria for oxidation [15]. In addition to this event exercise induced AMPK is also thought to phosphorylate and inhibit the Rab-GAP proteins TBC1D1/TBC1D4 [16-18]. The reduced activity of these Rab-GAPs allows for more of the Rabs that control glucose transporter 4 (GLUT4) translocation to be in the GTP bound state which increases the translocation of GLUT4 to the plasma membrane increasing glucose uptake and oxidation [19]. In addition to being a direct AMP:ATP ratio sensor, AMPK also has the capacity to be modulated by the glycogen storage status of a cell [20]. The β-subunit of AMPK binds to specific branch points on glycogen inhibiting the enzyme and preventing it from being phosphorylated by upstream kinases [20]. These data suggest that when glycogen is depleted AMPK is more active. Indeed substantial experimental evidence in humans supports this. For example, when exercise is commenced in the glycogen depleted state the phosphorylation of AMPK is greater in the post exercise recovery when muscle glycogen is low [21-23]. Interestingly, in rodent studies when exercise occurs in the low glycogen state AMPK nuclear content is increased [24]. Partly through these mechanisms the exercise dependent increases in AMP:ATP ratio and depletion of glycogen lead to a co-ordinated metabolic response, partly through AMPK activation, which increases substrate uptake and oxidation to fuel the activity (**Figure 1**).

AMPK also plays a key role in controlling running capacity and certain adaptive processes in response to endurance exercise training [25, 26]. In a feed forward fashion AMPK can

translocate to the nucleus [24, 27] where it regulates Histone de-aceytlases, transcription factors and transcriptional co-activators such as histone deacetylase 5 (HDAC5), myocyte enhancing factor 2 (MEF2) and peroxisome proliferator activated receptor-γ co-activator-1α (PGC-1α) respectively (**Figure 1**) [28-30]. The AMPK dependent regulation of HDAC5 leads to the nuclear export of this de-acetylase [30]. HDAC5 functions to acetylate histones, causing the chromatin to be wound tighter restricting the access of transcription factors to promoter regions [31]. The nuclear export of HDAC5 therefore leads to a relaxation of the chromatin and in an unknown manner leads to increases in transcription of GLUT4 [30]. The AMPK dependent phosphorylation of PGC-1α additionally is associated with an increase in the expression of mitochondrial genes [28]. As we mentioned PGC-1α acts as a transcriptional co-activator where it moderates the activity of multiple transcription factors including the PPAR's (PPAR-γ, PPAR-α and PPAR-δ), mitochondrial transcription factor A (TFAM), nuclear respiratory factors 1/2 (NRF1/2) and estrogen related receptor-α (ERR-α) (**Figure 1**) [32]. The PPAR's are also of interest with regard to nutritional interventions as they are sensitive to changes in fatty acid levels in that increased free fatty acid (FFA) availability leads to increases in the activity of these transcription factors which control the expression of FFA metabolism genes [33].

2.2. Is carbohydrate still king?

The principle of ensuring adequate carbohydrate (CHO) availability to promote exercise performance is the foundation of which contemporary sports nutrition practices have typically been built upon. Indeed, the importance of muscle glycogen as a determinant of exercise capacity was first recognized as early as the late 1960s with the introduction of the muscle biopsy technique into exercise physiology research [34]. Since this landmark study, a wealth of studies conducted over the next 40 years unequivocally confirmed that high pre-exercise muscle glycogen stores (i.e. >500 mmol.kg^{-1} dw) can improve endurance and team sport performance in those instances where exercise duration is >60-90 minutes [35]. As such, elite athletes are now advised to consume at least 6-12 g/kg body mass of CHO in the 24-36 h prior to competition so as to adequately "CHO load" for competition day [36].

In addition to high endogenous pre-exercise muscle glycogen stores, it is widely accepted that exogenous CHO feeding during exercise also improves physical, cognitive and technical elements of performance [37]. Whereas it was generally accepted that exogenous CHO oxidation rates were thought to be limited at approximately 1 g/min due to saturation of

intestinal glucose transporters, it is now known that exogenous CHO oxidation rates can increase to 1.8 g/min with the addition of sucrose or fructose to the CHO blend [38]. When taken together, it is currently thought that CHO feeding during exercise may therefore augment exercise performance via multiple mechanisms consisting of muscle glycogen sparing [39], liver glycogen sparing [40] and maintenance of plasma glucose and CHO oxidation rates [41]. It is noteworthy, however, that exogenous CHO feeding during exercise also improves performance when exercise duration is <60 minutes [42], an effect that is not apparent when glucose is directly infused to the bloodstream during exercise [43]. Such data suggest that CHO feeding may also improve exercise performance via non-metabolic effects but through direct effects on the central nervous system [44]. To this end, the last decade of research has resulted in a growing body of literature demonstrating that simply "rinsing" CHO in the oral cavity (for 10-second periods every 5-10 minutes during exercise) is also ergogenic to performance [45], an effect that is independent of sweetness [46] and that is especially apparent in the absence of a pre-exercise CHO meal [47] and low pre-exercise muscle glycogen [48].

The conventional approach to CHO fuelling during exercise is to consume 6-8% CHO beverages, although relying solely on this approach does not allow for flexibility in terms of individual variations in body mass or actual fluid requirements given variations in ambient conditions [49]. As such, many athletes rely on a CHO fuelling approach that is based on a combination of solids (e.g. bars), semi-solids (e.g. gels) and fluids (e.g. sports drinks) so as to collectively meet their personalized exogenous CHO targets, typically in the region of 30-90 g/h depending on exercise duration [38]. Nevertheless, although there is little difference in exogenous CHO oxidation rates (albeit in fluid matched conditions) between the aforementioned sources [50, 51], it is noteworthy that many athletes experience gastrointestinal discomfort when attempting to hit these targets, possibly related to extreme differences in osmolality between commercially available CHO gels [52] as well as the presence of fibre, fat and protein in energy bars [53]. As such, it is now advised that athletes should clearly practice their approach to in-competition fuelling during those training sessions of similar intensity and duration as competition [54].

Although CHO guidelines for competition are now generally accepted, considerable controversy exists as to the optimal targets of endogenous and exogenous CHO availability for which to adhere to during both moderate and intensive training periods. Indeed, whilst

both low endogenous [55] and exogenous [56] CHO availability can undoubtedly impair training intensity, accumulating data have now demonstrated a potent effect of reduced carbohydrate availability in modulating those acute exercise-induced increase in cell signalling and gene expression responses that regulate endurance training adaptations [57, 58]. In this regard, we and others have collectively observed that reducing endogenous and/or exogenous CHO availability during short-term (e.g. 3-10 week) endurance training increases mitochondrial enzyme activity and protein content [55, 59, 60] increases both whole body [55] and intramuscular lipid oxidation [61] and in some instances, improves exercise capacity [62, 63]. These data have therefore led to the innovative *"train-low (or smart), compete-high"* model surmising that athletes deliberately complete a portion of their training programme with reduced CHO availability so as to augment training adaptation but yet always ensure high CHO availability prior to and during competition in an attempt to promote maximal performance [64]. The augmented training response observed with training-low strategies are currently thought to be regulated via the enhanced activation of upstream cell signalling kinases including both AMPK [23] and p38MAPK [65] that ultimately converge on the downstream regulation of key transcription factors and co-activators such as PGC-1α [66], p53 [21] and PPARδ [24] (**Figure 1**). In this way, training with low CHO availability thereby leads to a co-ordinated up-regulation of both the nuclear and mitochondrial genomes.

Despite the theoretical rationale for the training-low paradigm, potential pitfalls to long-term training with low CHO availability include perturbations to immune function [67] impaired training intensity [55], reduced ability to oxidise exogenous CHO during competition [68] and increased muscle protein oxidation [69]. As such, many challenges remain as to how best to periodise train-low into an elite athlete's training programme without increasing the risk of the aforementioned maladaptive responses. At present, research studies examining the efficacy of train-low strategies have largely adopted fasted training protocols [60], protein only sessions [70], training twice per day models [62] reducing CHO intake in the post-exercise period [71] and most recently, both sleeping and training (i.e. sleep low-train low models) on the subsequent morning with reduced CHO intake [21, 72]. Ultimately, the simplest advice at present may be to adopt the practical concept of *"fuelling for the work required"* in that completing pre-determined training workloads that can be readily performed with reduced muscle glycogen and without exogenous CHO feeding may represent a strategic approach for which to implement day-to-

day nutrient-exercise periodization protocols. Alternatively, when the goals of the training session are to complete the highest workload possible, then adequate CHO should be provided in the 24 h period prior to and during the specific training session. Despite many unanswered questions to the precise molecular mechanisms underpinning the enhanced training adaptations associated with training-low as well as optimal practical application models, it is now readily apparent 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.
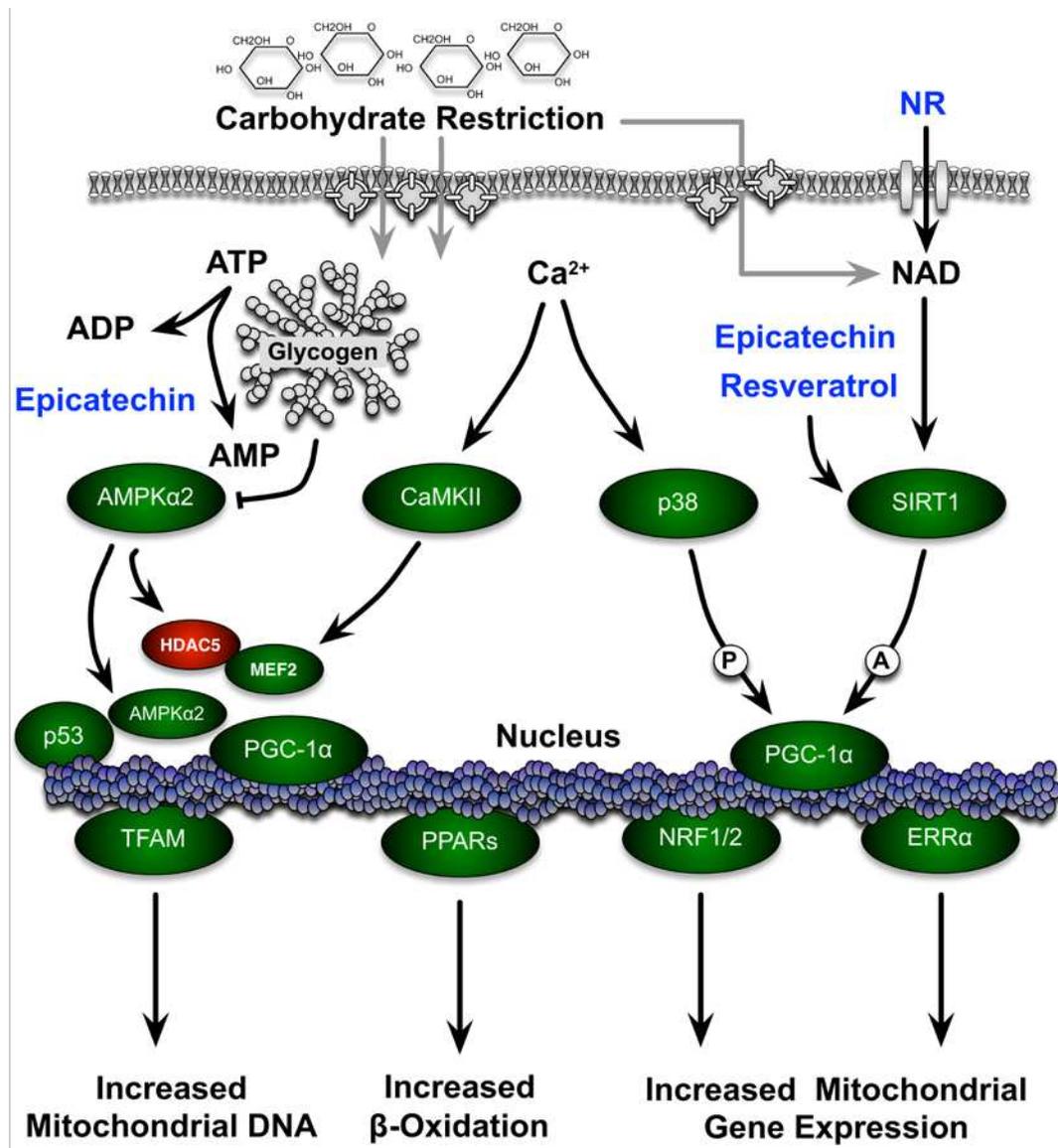

Figure 1. Overview of the molecular signalling pathways activated in skeletal muscle in response to endurance exercise. Contraction results in alterations in AMP, Ca2+ and NAD which activate numerous cellular energy sensing proteins (AMPK, CaMKII, p38 MAPK, SIRT1). These signalling proteins converge on the transcriptional co-activator PGC-1α

which leads to increases in mitochondrial biogenesis through the activation of numerous nuclear transcription factors (TFAM, PPARs, NRF1/2, ERRα). Carbohydrate restriction and/or glycogen depletion during exercise leads to further enhances in mitochondrial biogenesis through increased activity of AMPK, p38 and SIRT1. In addition, the small compounds epicatechins, nicotinamide riboside (NR) and resveratrol have all been suggested to enhance endurance-training responses in skeletal muscle through a number of signalling pathways (blue).

2.3 Adaptation to high fat diets and exercise performance.

Endurance athletes develop a high capacity to fuel exercise via fat oxidation as an adaptation to their training. However, there have been cycles of interest in strategies that can further up-regulate the contribution of fat as a substrate for exercise; specifically, the chronic consumption of a low-carbohydrate, high-fat (LCHF) diet. Models have included moderate (<20% of energy) to extreme (< 50 g/d) carbohydrate restriction, with fat increasing to ~65% or ~80% of energy respectively [73]. Such regimens, which should not be confused with other popular high-protein, CHO-reduced diets such as the Paleo Diet, have claimed benefits to sports performance via an enhanced exercise utilisation of the relatively large body fat stores as well as other effects from chronic adaptation to high levels of circulating ketones ("keto-adaptation") [74].

Original interest in LCHF for sports performance stemmed from an 1983 study which measured exercise capacity in 5 well-trained cyclists before and after 4 weeks of a ketogenic LCHF diet [75]. Despite conditions that should have favoured a benefit to endurance (additional 4 weeks of training, overnight fasting and water only during cycling, very moderate intensity ~60% VO_{2max} workloads), there was no mean improvement in time to exhaustion over the baseline values completed with a high carbohydrate diet. Moreover, the results were skewed by a large increase in endurance in one subject, and the authors also noted that the enhanced utilisation of fat and sparing of carbohydrate at moderate intensity was "a limitation of the intensity of exercise that can be performed" and a "throttling of function near VO_{2max}" [75].

During the period from 1995-2005, researchers from a number of laboratories examined the

effect of non-ketogenic LCHF diets on exercise/sports performance [73]. Results confirmed the lack of a clear effect on performance despite marked changes in ability to utilise fat, but identified some scenarios, such as submaximal exercise carried out with depleted muscle glycogen stores, in which some benefits might be observed. The finding that shifts in substrate utilisation during exercise occurred in as little as 5 days of exposure to the LCHF paved the way for a further series of studies in which athletes first undertook such "fat adaptation", then restored carbohydrate availability just before and during an exercise bout with the intention of promoting performance via optimized contributions of both fat and carbohydrate pathways [73]. Here, it should be noted that a shift in measurements of respiratory exchange ratio during exercise, which are often used to mark shifts in substrate utilization, can reflect the prevailing availability of substrate rather than a true adaptation in the muscle. However, several studies confirmed that exposure to the LCHF diet achieved a robust alteration to regulatory factors in fat utilisation; changes include an increase in muscle triglyceride stores, increased activity of hormone sensitive lipase [HSL] which mobilizes triglycerides in muscle and adipose tissue, and increases in key fat transport molecules such as fatty acid translocase [FAT-CD36] and Carnitine Palmitoyl Transferase (CPT) [76]. Furthermore, the increases in fat utilisation during exercise persisted in the face of abundant carbohydrate supplies. However, again, these investigations failed to find evidence of universal performance benefits, but uncovered mechanisms to explain metabolic changes in the muscle as well as information on scenarios in which fat adaptation might be useful/benign and those in which it would, in fact, impair sports performance [73].

A landmark study investigated the effect of fat adaptation and carbohydrate restoration on a real-life simulation of sports performance which involved the completion of a 100 km cycling time trial during which subjects were required to complete "sprints" at intensities of >90% peak power output [77]. Although the overall result was a (non-significant) benefit of ~ 3 min in the control trial, the striking outcome was the observation that the cyclist's ability to exercise at higher intensities was impaired following the fat-adaptation strategy. A separate investigation completed around the same time provided the unifying mechanistic explanation of all the previous literature: chronic intake of the LCHF diet specifically impairs rather than spares glycogen utilisation during exercise by reducing glycogenolysis and reducing the active form of pyruvate dehydrogenase (PDHa) to down-regulate the entry of carbohydrate into the citric acid cycle [78]. This finding elicited the opinion that the LCHF

had little role to play in the preparation of competitive athletes since it would likely impair their capacity for the high-intensity exercise that is a pre-requisite for success in the majority of conventional sports [79].

A renewed and fervent interest in the ketogenic version of the LCHF has recently emerged, supported by peer-reviewed summaries of theoretical claims for health and sports performance [74], but largely promoted by the phenomenon of social media. While this merits further examination, it is difficult to make different conclusions in the absence of new data. However, it has been noted that a further frustration around this topic is a relentless misrepresentation of the current sports nutrition guidelines by proponents of the LCHF movement [73]. Rather than promoting "high carbohydrate diets for all athletes", both the guidelines and the practices of contemporary sports nutritionists have moved away from such a universal message. Instead they promote an individualized and periodized approach to avoid unnecessary and excessive intake of carbohydrate *per se*, to optimise training outcomes via modification of the timing, amount and type of carbohydrate-rich foods and drinks to balance periods of low and high carbohydrate availability and to adopt well-practiced competition strategies that provide appropriate carbohydrate availability according to the needs and opportunities provided by the event and individual experience [73] [see section 2.2]. Further research is needed to continue to evolve these models, including examination of scenarios in which the LCHF diet may be beneficial or at least not detrimental to sports performance.

2.4 The molecular regulation of resistance training adaptation. Resistance training is typically defined as performing relatively high intensity contractions against an external resistance for a relatively short period of time [5]. Resistance exercise is usually performed utilising weights and is used as an adjunct to almost all sports with some sports such as Olympic weight lifting or para-power lifting performing the same or variations of the lifts in training as in the sport. In other sports such as rugby, weight lifting is used to add/maintain functional weight to the athlete and improve power on sport specific tasks is critical. An appropriately structured resistance training program will lead to improved strength partly through improvements in muscle mass [80]. An individual's strength is highly, but not wholly dependent upon muscle mass [81, 82]. Resistance exercise builds muscle mass by increasing the remodelling of muscle proteins and by sensitising the skeletal muscle protein synthesis machinery to subsequent meals [83]. As with endurance exercise, nutrition can play a critical

role in augmenting the adaptive stimulus that comes from resistance exercise [84]. Whilst endurance exercise adaptations are believed to be driven primarily by transcriptional responses [2, 8], the muscle growth adaptation to resistance exercise is driven primarily by changes in translation, in particular by increases in mRNA activity (protein produced per unit of mRNA) [85-87]. The multi-protein complex mechanistic Target of Rapamycin Complex 1 (mTORC1) is a key controller of protein synthesis through the control that it exerts on mRNA activity via increasing protein translation initiation [88] (**Figure 2**).

The mechanically sensitive pathways that respond to loading across the muscle to increase MPS converge with those that respond to increases in intracellular amino acid concentrations and insulin at mTORC1 [88] (**Figure 2**). mTORC1 is well defined as essential to loading induced muscle growth in rodents [89] and stimulus induced increases in MPS in humans [90, 91]. Activation of mTORC1 is required for increases in protein synthesis with amino acids [90] and resistance exercise [91]. When mTORC1 activity [89] or the activity of its down stream target p70S6K1 [92, 93] are impaired then muscle mass is impinged. It therefore is logical to assume that increases in muscle protein synthesis could be enhanced by enhancing the activation of mTORC1. This can be achieved nutritionally in several ways, 1) with high quality protein or essential amino acids and 2) with carbohydrate driven increases in insulin.

The activity of the catalytic component of mTORC1, mTOR, against its substrates is highly dependent upon the formation of the mTOR complex. This complex consists of a number of different proteins, mTOR, GbetaL, raptor, GTP bound Rheb in addition to an apparent required association with lysosomal membranes [94]. Furthermore, mTORC1 has a number of repressors, which must be dissociated from the complex to allow it to become active, such as PRAS40 and DEPTOR [94]. Finally the amino acid sensitive lipid kinase hVPS34 also plays a key role in mTORC1 activation [95] as does the MAPK family member MAP4K3 [96] (**Figure 2**). Cell based work has shown that this amino acid sensing system is incredibly sensitive with an ~7% increase in intracellular leucine leading to 50% maximal activation of mTORC1 [97]. Furthermore, only a fraction of maximal mTORC1 activity (~30%) is required to fully saturate muscle protein synthesis [98, 99]. The amino acid sensing by mTORC1 also seems to be driven not by extracellular, but instead by intracellular amino acid concentrations [100]. The mechanisms by which this occurs are incredibly complex and not yet fully defined. However, some key events seem to relate to the GTP loading status of a

number of GTPases. Through an unknown mechanism the GTPase activity of the RagGTPases (RagA/B and RagC/D) is sensitive to the intracellular amino acid content [101]. When amino acids are above a certain threshold these Rags dimerise with the correct GTP loading status in such a way as to allow mTORC1 complex assembly [101]. As we mentioned the amino acid induced activation of mTORC1 is distinct from the mechanical activation of mTORC1, which again is not yet fully defined, but is thought to be dependent upon the secondary messenger phosphatidic acid (PA) derived from diacylglycerol kinase (DGK) [102]. Because the mechanically sensitive pathways and the resistance exercise pathways are distinct, consuming essential amino acids following resistance exercise significantly activates mTORC1 above resistance exercise alone [103]. We have known for several decades that consuming essential amino acids in close proximity to resistance exercise can enhance the protein synthetic response in the skeletal muscle [104] and we now know that ~20g of high quality protein in the fasted [105] or fed [106] state is sufficient to saturate the protein synthetic response following resistance exercise in young men. Furthermore supplementation of protein during a program of resistance training is well proven to enhance lean mass/muscle gains [84].

The key trigger for the protein feeding induced increases in MPS seems to be the leucine content of the digested protein [107] and possibly the leucine metabolite HMB (β-Hydroxy β-methylbutyrate) [108]. Both of which activate mTORC1 in human skeletal muscle when consumed [108]. It therefore seems that amino-acids and resistance exercise enhance MPS via a dual effect on increasing mTORC1 activity (**Figure 2**). However, not all stimuli that activate mTORC1 lead to improved protein synthesis. As we mentioned earlier, carbohydrate driven increases in insulin may increase mTORC1 activity. However, when insulin is infused to supra-physiological levels mTORC1 is potently activated without a concomitant increase in muscle protein synthesis [99]. So while the data are clear that mTORC1 is required for load-induced growth [89], resistance exercise [91] and feeding [90], it is still very unclear if manipulating mTORC1 above what occurs physiologically will lead to enhanced muscle growth.

2.5. New/Underexplored areas in protein nutrition?

A key question in the sports nutrition field has always been, "how much protein do I need to maximise muscle growth?" Several protein dose response studies have demonstrated that ~20g of high quality protein (egg white protein or whey protein) following resistance

exercise with a small amount of muscle mass (single leg training) is sufficient to maximally stimulate MPS in the trained leg [105, 106]. These studies have gone a long way to optimising post exercise nutrition. However, no study has assessed if increasing the amount of muscle mass worked, or if the size of the individual, plays any role in the protein requirements post exercise. Recently however, work from Stu Phillips laboratory has retrospectively analysed a series of studies on muscle protein synthesis (MPS) in an attempt to identify if maximum rates are dependent upon body mass. This retrospective analysis has suggested that the optimal dose of protein post exercise in healthy young men may be best quantified in a g/kg basis or even a g/kg lean mass basis with ~0.25 g/kg body mass and 0.25 g/kg lean mass appearing to elicit the maximum rates of MPS [109].

In addition to the benefits of consuming high quality proteins on muscle mass and exercise recovery, foods rich in high quality protein also tend to be rich in other nutrients [110]. These other nutrients can potentially have benefits beyond the protein content [110]. In particular dairy protein sources, due to the high calcium content has been lauded for this reason [111]. Additionally, a number of studies have highlighted the benefits of milk-based protein, particularly whey, over plant based proteins for stimulating muscle protein synthesis [112]. This finding is thought to be due to the higher leucine content of whey. However, an area that has been highly speculated about, but very underexplored is the possibility of digested peptides from ingested proteins having beneficial, biological activities [113]. Milk based proteins in particular can be digested via gastrointestinal peptidases to tryptophan containing peptides, which in cell and biochemical based assays can have biological activities which may positively impact human physiology from blood pressure control to satiation [114]. Finally, protein-containing foods also contain fat and the role that the fat fraction plays in regulating the feeding response to the protein is very underexplored. For instance, whole milk consumed after resistance exercise may be more effective at stimulating amino acid incorporation into skeletal muscle than fat free milk [115]. These points demonstrate that, for the field of protein nutrition, there is still much to do to optimise the source and quantity of protein to support human health and performance.
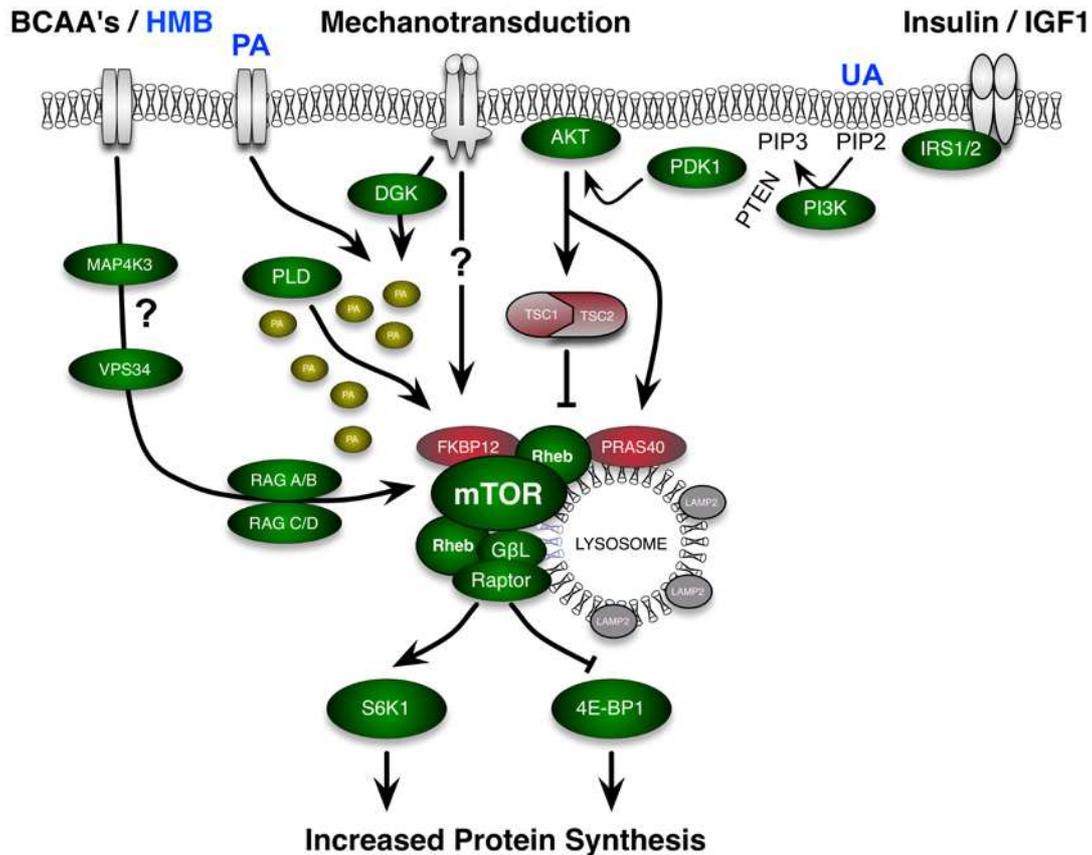
Figure 2. Overview of the molecular signalling pathways activated in skeletal muscle in response to resistance exercise. Resistance exercise increases protein synthesis in skeletal muscle via 3 distinct processes that converge on the protein kinase mTOR. Insulin/IGF1 is thought to activate mTOR through AKT mediated phosphorylation of TSC1/2 and PRAS40. In parallel, mechanical loading of skeletal muscle activates mTOR through the generation of phosphatidic acid in addition to unknown mechanisms. Finally, mTOR is activated through an amino acid pathway via the activation of VPS34, MAP4K3 and the Rag A-D proteins. The small compounds HMB, PA and ursolic acid (UA) have all been suggested to enhance resistance-training responses in skeletal muscle through a number of signalling pathways (blue).

3. Traditional Sports Supplements – the ones that still work in 2015

Although supplements are still an integral part of an elite athletes daily routine [116, 117] there is a growing shift in priorities with many athletes now adopting a "food first" approach. Given the risk of supplement contamination and the potential for failed drug tests [118] supplements are now often only given when there is a clear rationale for their use combined with the availability of independently drug-tested products. It is beyond the scope

of this review to cover all supplements therefore the most popular ones are categorized according to the level of evidence supporting their benefit and summarized in Table 1. Supplements have been divided into those claimed to increase endurance performance, strength/size adaptions or boost general health. There is however considerable evidence behind the effectiveness of caffeine, creatine, nitrates, beta alanine, antioxidants and vitamin D and therefore these have been given special consideration.

Table 1. Summary of some of the most common supplements grouped as Green – Strong evidence of a performance effect, Amber – moderate or emerging evidence or Red – Lack of evidence, high risk of contamination and/or currently prohibited by WADA based upon authors interpretation of the existing published literature.

	Green	Amber	Red
Endurance	Caffeine Carbohydrate Gels/drinks Beta-alanine Beetroot Juice Sodium Bicarb/Citrate Antioxidants	Taurine Cherry Active L-Carnitine	Ephedrine Methylhaxanemine Herbal Supplements Citruline Malate L-Arginine Synephrine
Strength/Size	Creatine Protein	Leucine BCAAs	ZMA Anything 'Anabolic' Testosterone Boosters Herbal Supplements Colostrum
Health	Probiotics Electrolytes Vitamin D	Vitamin C Multi Vitamin Glucosamine Quercetin Glutamine Fish Oil Collagen	Magnesium Herbal Supplements

Caffeine, tri-methyl xanthine, is a naturally occurring compound in plant foods which has been used for many centuries across many cultures to promote well-being and work capacity. Today, about 90 percent of adults regularly consume commonly available dietary sources such as coffee, tea and cola or energy drinks for a mixture of social, cultural and lifestyle enhancement reasons [119]. Meanwhile, athletes have more than 500 peer-reviewed publications and entire textbooks [119] to guide their more specific use of caffeinated sports products and functional foods (including gum, gels, confectionery and

drinks) to achieve sporting goals [120]. The most relevant of caffeine's many pharmacological and physiological effects as an adenosine receptor antagonist and modifier of muscle contractility is the reduced perception of effort, fatigue or pain associated with exercise [120]. Recent changes to knowledge and practice around caffeine and sports performance include recognition that benefits apply across a large range of sports (high-intensity events of 1-60 min, endurance/ultra-endurance events and team/racket/combat sports with intermittent efforts) [121]. Caffeine may also enhance training outcomes, especially when used to allow the athlete to train harder during key sessions that are deliberately fatiguing [122] (for example when training in a low carbohydrate state as discussed in section 2.2). A range of protocols involving small-moderate doses of caffeine (3 mg/kg) before and during the event, according to practical considerations and athlete experience, may be used [119, 121]. Following its 2004 removal from the WADA Prohibited List, caffeine can be considered safe, effective and legal when used according to established and practiced protocols. Athletes should avoid risky and unnecessary practices such as taking unnecessarily high doses and/or mixing caffeine with other stimulants. Furthermore, evolving confirmation of genetic and other sources of individual variability in caffeine metabolism provides support for the well-known observations that some athletes are low-responders [123] to caffeine or suffer side effects.

Creatine is an amino acid derived metabolite found predominantly in skeletal muscle from both endogenous synthesis and dietary intake (meat). Since 1992, when the first scientific publication regarding creatine supplementation [124] aligned with testimonials regarding its use by successful athletes from the Barcelona Olympic Games, creatine sales and science have both flourished. Muscle creatine stores are elevated by ~ 20% to a threshold level by oral creatine supplementation, either by loading protocols (5 d @ 20 g/d in split doses) or by longer periods (~4 weeks) of a maintenance dose (~3 g/d) [125]. Despite the marketing of exotic creatine compounds, creatine monohydrate remains an effective form of supplemental creatine, with muscle uptake being optimized by its co-ingestion with carbohydrate [125]. By increasing the muscle phosphocreatine reservoir, creatine supplementation can enhance the rapid regeneration of ATP during brief high-intensity exercise bouts, particularly when they are repeated with short recovery intervals. This can acutely benefit the performance of sports involving such work patterns (e.g. team sports), as well as chronically enhance the athlete' capacity to undertake training sessions of this

nature (e.g. resistance or interval training) [125]. Although creatine loading is most associated with sports involving increased muscle mass, strength, power or intermittent activity, other less well-explored actions on cellular osmolarity (e.g. increases in gene expression and glycogen storage) may extend its value to other sports or exercise scenarios [126]. Creatine supplementation may have important clinical roles and benefits to ageing populations [127]. Despite publicity around safety aspects, careful studies of established creatine supplementation protocols have not found evidence of increased health risks4 and have reported reduced rather than increased prevalence of muscle damage or impaired thermoregulation associated with exercise [128].

Beetroot juice (BJ) has become a recent addition to the athlete's armoury of evidence-based supplements, with commercial preparations providing a safe, cost-effective and reliable source of inorganic nitrate in countries where the use of sodium nitrate is not permitted [129]. Both acute (2.5 h pre-exercise) and chronic (6 d) of intake of ~ 8 mmol of dietary nitrate has been shown to increase plasma nitrate concentrations and increase the capacity of the nitrate-nitrite-NO pathway to produce NO2. In addition to a number of health and benefits in clinical populations, enhanced NO availability appears to be responsible for improved exercise capacity via mechanisms including an increase in oxygen economy and direct effects on muscle contractility [130]. However, the situations in which this results in benefits to sports performance are presently unclear, particularly in the case of elite athletes who seem to be less responsive to nitrate supplementation protocols [131, 132]. Reasons for this observation include a greater dietary nitrate intake and/or greater capacity for arginine-derived production of NO in highly trained individuals, as well as better genetic and training-supported characteristics in the muscle, which enhance oxygen delivery and buffer metabolic acidosis, thus reducing the conditions under which the nitrate-nitrite-NO pathway may be important [130]. Nevertheless, there seems good evidence that nitrate/BJ supplementation can enhance sports performance in moderate caliber athletes and individuals among elite athlete populations [130], with emphasis on adequate supplementation protocols and sports or scenarios which rely on the recruitment of the less-oxidative type II muscle fibres, and local or environmental conditions of hypoxia or acidosis (e.g. exercise at high altitude, sports involving high-intensity exercise or involvement of a small muscle mass) [131, 132].

Sodium bicarbonate. Performance of sports requiring high rates of energy production from anaerobic glycolysis is often limited by excessive accumulation of hydrogen ions (H+) in the muscle. This includes sustained high-intensity events lasting 1-7 minutes, but also sports involving repeated sprints (e.g team sports) or longer (30-60 min) sustained efforts just below the "lactate threshold" in which there are surges in pace. Supplements that enhance intracellular and/or extracellular buffering capacity may benefit these sports by managing the progressive increase in intracellular acidosis.

The extracellular anion bicarbonate largely manages pH and electrolyte gradients between intra and extracellular environments. Acute ingestion of dietary bicarbonate, if it achieves a meaningful albeit temporary increase in blood bicarbonate concentrations and pH, can enhance extracellular disposal of the H+ efflux from the working muscle. Typical protocols involve intake of 300 mg/kg BM of bicarbonate, 1-2 hours prior to the targeted exercise [133]. Powered sodium bicarbonate is a cheap and widely available household product, but alternative forms include pharmaceutical urinary alkalizers used for discomfort relief with urinary tract infections [133].

A comprehensive meta-analysis of the lengthy history of bicarbonate supplementation and sports performance found a moderate (1.7 ± 2.0%, 90%CL) enhancement of a single 1-minute sprint in male athletes with the typical protocol [134]. This increases by ~0.5% (±0.5%) with a larger (+0.1 mg/kg) dose or five extra sprint bouts, and reduces by a similar magnitude with each 10-fold increase in test duration (e.g. 1-10 minutes) or females. Gastrointestinal disturbances often occur with bicarbonate use, but can be managed by consuming the dose in a split protocol with the intake of a small meal/snack [135].

Beta-alanine. More recently, muscle concentrations of the dipeptide carnosine have been shown to respond to supplementation with β-alanine, the rate-limiting precursor for carnosine synthesis. The optimal loading protocol is unknown but intakes of 3-6 g/d for 4-12 weeks increase this intracellular buffer by 50-85% [136]. To date, no threshold for carnosine concentrations has been found, but a daily intake of ~ 1.2 g appears to maintain muscle carnosine elevations and a return to baseline may require 6-20 weeks of supplement withdrawal [137]. Dietary beta-alanine includes meat from animals that are highly

anaerobic (e.g. breast meat from poultry) or live in hypoxic environments (e.g. whale). Supplementation with doses > 800 mg of purified b-alanine is associated with symptoms of paresthesia (skin tingling), but this sometimes unpleasant side-effect can be managed with slow-release tablets or spreading doses over the day [136]. Theoretically this chronically applied supplement can enhance the performance of a single competition, but also support the training process. Indeed, review of the accumulating literature shows some support for the benefits of beta-alanine supplementation on high-intensity exercise and a potential additive effect when combined with bicarbonate supplementation. However, further work is needed to define specific applications including targeted sporting events, benefits to elite vs recreational athletes, and whether other muscle carnosine actions such as Ca handling are also responsible [138].

Vitamin D. Over the past decade interest in Vitamin D has exploded. The reason for this increase in interest is partly due to the re-emergence of the debilitating bone disease rickets, but also due to a better understanding of the many biological roles of this 'prohormone'. It is now known that many tissues in the human body express the vitamin D receptor suggesting that Vitamin D (or more specifically active vitamin D metabolites) play a fundamental physiological role that have previously been somewhat ignored [reviewed by 139]. It is now clear that both innate and acquired immune function, cardiovascular health and even muscle growth and repair may be regulated by vitamin D. This emerging knowledge, combined with a plethora of research suggesting that many athletes are vitamin D deficient [140], largely due to a sun-shy lifestyle and poor dietary sources of vitamin D, even those living in sunny climates [141] has resulted in vitamin D being one of the most widely supplemented vitamins in sports nutrition.

It must be stressed that direct evidence indicating a performance enhancing effect of vitamin D in athletic populations is at best equivocal [142]. Whilst some studies have shown improved markers of muscle function the majority have failed to show any meaningful effects. Perhaps the main reason for the discrepancies lies with the baseline vitamin D concentration. Classification of vitamin D deficiency is complex and subject to significant debate. Currently the US Institute of Medicine state that >50nmol/L is sufficient although many leading researchers seriously question this as being too conservative [143, 144]. In our laboratory, we have reported detrimental effects on muscle function when concentrations are below 30nmol/L [140] with no performance enhancing effects of vitamin D supplements

if the starting concentration is around 50nmol/L [145]. In terms of a supplemental dose, the EFSA have recently stated that 4000iU is the maximum dose that should be used which we have also shown to be an effective dose in correcting deficiencies [146]. Recent work from our group [147] has also demonstrated that vitamin D deficiencies may impair muscle regeneration and perhaps more importantly this effect may occur even when vitamin D concentrations are around 50nmol/L. It is clear that much research is required on vitamin D and athletic performance with future studies likely to assess if different biological functions require differing vitamin D concentrations. Perhaps at present the best advice is to check athletes to ensure deficiencies are identified and corrected although we would add a word of caution that emerging work form our group is beginning to suggest that high dose supplementation, in non-deficient individuals, could have negative consequences related to the function of the vitamin D endocrine system.

Antioxidants It is now well recognized that exercise-induced disruptions in skeletal muscle homeostasis regulate exercise adaptations with repeated activation [148, 149] (**Figure 1**) This is best exemplified by the notion that decreasing reactive oxygen species (ROS) generation with nutritional antioxidants, attenuates exercise-induced redox signalling, and thereby blunts exercise adaptation [150, 151]. Over the last decade research has therefore somewhat switched focus from looking at exercise-induced ROS generation as being damaging at all times with nutrition focused on prevent any increase in ROS production [152] to now appreciating this generation as being an essential signaling process in skeletal muscle adaptation [153, 154]. In the sport and exercise world, there is still a great deal of confusion when it come to exercise-induced ROS generation and as a consequence the advice often offered to athletic populations is at best misguided and at worst, detrimental to performance and even long term health [155]. Confusion generally stems from three major important issues:

1. **Inappropriate methodological techniques**. ROS are facile species and are inherently difficult to measure directly [156]. Unfortunately, many of the assays in the sports science literature merely serve to create confusion as they are fundamentally ill suited to deciphering whether a compound with antioxidant activity is actually acting as an antioxidant. For example, the vast majority of sport science research has used blood markers of "total antioxidant status" an inappropriate assay to assay

antioxidant status in vivo [157] or "TBARS" a purported marker of lipid peroxidation that is generated by several non-redox regulated means and is prone to methodological artefact to the extent that it is no longer recommended for use in the parent discipline [158]. It is therefore not surprising that inapposite conclusions are drawn when a non-specific antioxidant does or does not affect some non-specific blood borne markers. To really advance this field of research sport science needs to collaborate with redox biologists and combine the skills of both sets of scientists.

2. **Antioxidants are heterogeneous** [159] they work in distinct ways and importantly they do not solely regulate ROS. This is important because just because one is using an antioxidant it is not to say that it is acting as an antioxidant, which is especially important for nutritional antioxidants. To eliminate confusion we recommend that findings from one antioxidant or combination thereof are not automatically extrapolated to others [148].

3. **Context:** perhaps most importantly when formulating recommendations context is everything. For example, NAC blunts training adaptations [160] but enhances acute performance [161, 162]. Thus when adaptation is inconsequential (e.g., competitive events) short-term NAC supplementation may be beneficial although it should be stressed that effective doses may result in gastro-intestinal discomfort [161]. At present it may be best to consider exercise as 2 distinct outcomes, one being training adaptions and one being athletic performance and judge the need for supplementation based on the exact context of the required stimuli. There could also be a case for some polyphenolic compounds to be supplemented post-exercise, such as tart cherry juice [163] to attenuate muscle soreness although if this is through a direct scavenging effect remains unclear (see point 2 above and reference [148] for criteria that need to fulfilled to satisfy a scavenging affect).

We recommend that more work is done to decipher whether nutritional antioxidants are in fact working in an antioxidant fashion and at present supplementation to athletes should be undertaken with caution. A final note is that to date there have been no data to suggest that eating high quality fruit and vegetables attenuates adaptations to exercise so it may be best to advise athletes to consume a high quality diet and avoid mega dose micronutrient supplementation, it really could be that simple [164].

4. Novel compounds

4.1 Supporting endurance training adaptation

(-)-Epicatechins. Consumption of dark chocolate has been reported to have multiple health benefits in humans [165]. The active ingredient of dark chocolate that appears to induce this metabolic remodeling is the cocoa-derived (-)-epicatechin. Noguiera et al. (2011) were the first to report that fifteen days (-)-epicatechin supplementation increased skeletal muscle fatigue resistance, mitochondrial volume and angiogenesis in mice compared to activity-matched controls [166]. Importantly, (-)-epicatechin supplementation was not as potent as endurance exercise in remodeling skeletal muscle, however (-)-epicatechin supplementation in combination with exercise training had a synergistic effect. As such these results indicate that (-)-epicatechin supplementation may be a nutritional approach to enhance skeletal muscle adaptation to endurance training (**Figure 1**). The first translation of these rodent studies into human investigation was recently performed by Gutierrez-Salmean and colleagues (2014), who investigated the effects of epicatechin supplementation on post-prandial fat metabolism in normal and overweight adults [167]. Following supplementation of (-)-epicatechin (1 mg/kg), participants displayed a lower RER, indicative of increased lipid oxidation. In addition, lower plasma glucose concentrations were observed following the supplementation [167]. From the available data, it would appear that cocoa-derived -(-)epicatechin is a promising ergogenic aid for increasing mitochondrial biogenesis and lipid oxidation. However, it is currently unknown whether (-)-epicatechin supplementation can promote mitochondrial biogenesis and enhance endurance-training adaptation in human skeletal muscle.

Nicotinamide riboside (NR). Vitamin B3 (niacin) is a naturally occurring substance found in meat, poultry, fish, eggs, and green vegetables [168]. Niacin is a combination of Nicotinic acid (NA) and nicotinamide (NAM), whereas Nicotinamide Riboside (NR) is a pyridine-nucleoside form of niacin containing an associated ribose bond in addition to nicotinamide [168]. NR has garnered recent attention, as it is a direct precursor for NAD^+ synthesis in skeletal muscle through the Nicotinamide Riboside Kinase 1/2 (NRK1/2) pathway [169]. As a dietary derived NAD^+ donor in skeletal muscle, NR is thought to impact skeletal muscle mitochondrial function through the NAD^+/SIRT1/PGC-1α signaling cascade [170] (**Figure 1**). Recently, Canto et al (2012) who showed that NR supplementation in C2C12 myotubes increased NAD^+ content, whilst NR feeding to mice (400 mg/kg/day) resulted in modest

increases in skeletal muscle NAD$^+$ (~5%) following 1-week supplementation [171]. The authors proposed that the metabolic action of NR supplementation was mediated through SIRT1, as the adaptive response of C2C12 myotubes to NR supplementation was lost following SIRT1 siRNA mediated knockdown. Interestingly, NR supplementation protected mice from the deleterious effects of 8 weeks high fat feeding, principally through an increase in energy expenditure and a reduction in cholesterol levels [171]. In parallel to metabolic adaptation, endurance capacity also increased by ~25% in the NR supplemented mice, coupled to an increase in mitochondrial to nuclear DNA ratios (a marker of mitochondrial mass) and increased mitochondrial protein content. Thus NR supplementation appears capable of altering skeletal muscle NAD$^+$ content which in turn increases skeletal muscle mitochondrial biogenesis through a SIRT1-dependent process [171] (**Figure 1**). To date, no studies have examined the effect of NR supplementation on mitochondrial adaptation in human skeletal muscle.

Resveratrol. Resveratrol, a stilbenoid polyphenol, belongs to the phenylpropanoid family commonly found in red wine [172]. As the prototypical SIRT1 activator, numerous reports have identified resveratrol as a potent activator of mitochondrial biogenesis in skeletal muscle (**Figure 1)**, in addition to protecting skeletal muscle from the deleterious effects of high fat feeding in mice [173]. Further, Resveratrol has been shown to promote fat oxidation and enhance endurance performance in mice [174]. Translational studies in obese male volunteers have suggested that 30 days resveratrol supplementation (150mg/day resVida) can reduce intrahepatic lipid content, circulating glucose, triglycerides, alanine-aminotransferase, and inflammation markers in addition to improving estimates of insulin sensitivity [175]. In parallel resveratrol supplementation increased skeletal muscle citrate synthase activity without a change in mitochondrial content, and improved muscle mitochondrial respiration in response to a fatty acid-derived substrate [175]. As such, there is growing support that resveratrol may be a beneficial approach to remodel skeletal muscle in humans. Whilst the data from Timmers et al (2011) was encouraging, recently Scribbans and colleagues (2014) reported that resveratrol supplementation during exercise training in healthy individuals can result in a maladaptive response in exercise-stimulated gene expression [176]. In agreement with this observation, Gliemann et al. (2013) showed that resveratrol supplementation in combination with high-intensity training in older men not only blunted the increase in maximal oxygen uptake observed in the placebo group, but also eradicated the effects of the exercise to reduce low-density lipoprotein, total cholesterol and triglyceride concentrations in the blood [177]. Using a similar protocol, Olesen et al.

(2014) recently showed that resveratrol supplementation also blunted training-induced decreases in protein carbonylation and tumour necrosis factor α (TNFα) mRNA within older individuals' skeletal muscle [178]. Thus, there are clear discrepancies between cell, rodent and human studies investigating resveratrol supplementation and it is currently unclear as to why resveratrol supplementation may display a negative effect on whole body/skeletal muscle adaptation when combined with endurance-exercise training in healthy individuals. Certainly the human research to date suggests that resveratrol does not have the metabolic benefits *in vivo* as previously proposed in cell and rodent studies. Clearly further research into the overlapping effects of exercise and resveratrol in humans is warranted.

4.2 Novel compounds supporting resistance training adaptations

β-hydroxy β-methylbutyrate (HMB). As discussed in previous sections, the BCAA leucine is a potent regulator of protein balance in skeletal muscle [179]. As such, there has been considerable interest in the metabolism of leucine in skeletal muscle, and the design of nutritional approaches to maximize this signaling cascade in the context of resistance training adaptation [179]. One of the key leucine intermediates appears to be the derivative β-hydroxy β-methylbutyrate (HMB), which like leucine appears to have potent anabolic properties in skeletal muscle [179] (**Figure 2**). Supplementation of HMB (3g/day) has previously been shown to enhance gains in fat-free mass following 6 weeks of resistance training [180]. This adaptive response appeared to be mediated by prevention in exercise-induced proteolysis (as assessed via urine 3-methylhistidine appearance), muscle damage and resulted in larger gains in muscle function associated with resistance training [180]. To examine the synergy between leucine and HMB-mediated increases in Myofibrillar Protein Synthesis (MPS), Wilkinson et al. (2013) directly compared the effects of leucine and HMB on [108]. Interestingly, the authors demonstrated that oral consumption of HMB (3.42 g free-acid (FA-HMB) providing 2.42 g of pure HMB) exhibited rapid bioavailability in plasma and muscle and, similarly to 3.42 g Leucine (Leu), stimulated muscle protein synthesis (MPS; HMB +70% vs. Leu +110%). HMB consumption also attenuated muscle protein breakdown (MPB; -57%) in an insulin-independent manner [108]. Further, HMB supplementation increased mTORC1 activity (as assessed via the phosphorylation of mTORC1 substrates S6K1^{Thr389} and 4E-BP1$^{Ser65/Thr70}$ phosphorylation) in a similar manner to Leu, however mTORC1 activation was more pronounced in the Leu group compared to HMB [108] suggesting that the mechanism of Leu action may function in additional processes compared to HMB (**Figure**

2). Further long-term training studies are clearly warranted to assess the efficacy of HMB supplementation for use in human resistance-exercise training studies.

Phosphatidic acid (PA). The diacyl-glycerophospholipid, Phosphatidic acid (PA) is a precursor for the synthesis of numerous lipids [181]. As such, PA plays a fundamental role in the regulation of cellular metabolism [181]. As discussed in previous sections, in addition to its metabolic role, PA has also emerged as a key signaling intermediate in skeletal muscle following the observation that PA can activate mTORC1 and by extension increase protein synthesis *in vitro* [182] (**Figure 2**). Whilst the interaction between PA and mTORC1 has been well established in cell and rodent models, the ability of PA to activate mTORC1 in human skeletal muscle is less clear. Recently, Hoffman et al. (2012) examined whether the oral ingestion (750mg/day) of a commercially available PA supplement (Mediator^{TM}: Chemi Nutra, USA) could enhance adaptation to an 8-week resistance-training program [183]. Following the training period, the authors reported a 12.7% increase in squat strength and a 2.6% increase in LBM in the PA group, compared to a 9.3% improvement in squat strength and a 0.1% change in LBM in the placebo group. In a subsequent study from the same group, Joy et al. (2014) reported that in contrast to their previous study [183], PA supplementation (750mg/day) during 8 weeks resistance training lead to significant increases in lean body mass (+2.4 kg), skeletal muscle cross sectional area (+1.0 cm), and leg press strength (+51.9 kg) when compared to placebo [184]. Finally, Mobley et al (2015) recently examined the effect of PA, whey protein concentrate (WPC) and a combination of PA+WPC administration on acute signaling responses in rat skeletal muscle [185]. Interestingly, WPC ingestion was the only intervention that significantly increased MPS 3h post administration, whilst PA actually lead to an ~50% reduction in the WPC-mediated MPS response. Thus, based on the current data available, it would appear that PA supplementation (750mg/day) might enhance resistance training mediated increases in mass and function (**Figure 2**). However, the fact that PA has also been reported to have no effect on resistance training [183], or even have a negative effect on WPC-stimulated protein synthesis would suggest that further, well controlled human studies are required to define the role of PA supplementation in skeletal muscle adaptation to resistance training in humans.

Ursolic acid (UA). Ursolic acid (UA) is a natural, water-insoluble, pentacyclic triterpenoid carboxylic acid found widely in leaf extracts including rosemary plant and holy basil [186]. Interest in the efficacy of UA as a nutritional aid to augment muscle mass has sparked following the observations from Kunkel et al (2011) that UA supplementation in mice (200

mg / kg via i.p. injection twice daily for 7 days) reduced muscle atrophy following denervation, whilst 5 weeks of supplemented diet (0.27% UA) induced hypertrophy [187]. UA appeared to mediate its effects through enhancement of insulin/IGF-I signaling and a reduction in the expression of the atrophy-associated genes MuRF1 and MAFbx [187] (**Figure 2**). Ogasawara et al (2013) recently reported that UA administration enhanced S6K1^{Thr389} phosphorylation 6 hours following a single bout of resistance exercise in rats [188], indicating that UA might also enhance mTORC1 activity in skeletal muscle. Whether UA administration promotes skeletal muscle hypertrophy in human skeletal muscle is less clear. Bang et al. (2014) recently reported that supplementation of UA (1350mg/day) reduced body fat percentage, increased maximal leg strength and IGF-1 activation following 8 weeks resistance training in healthy male participants [189]. However, a recent study failed to translate the findings from Ogasawara et al (2013) into humans, with the observation that UA ingestion (3000mg) had no effect on Akt^{Thr308}, IGF-1^{Try1131}, S6K^{Thr389} or mTORC1^{Ser2448} phosphorylation following a single bout of resistance exercise [190]. Therefore, it is presently unclear whether UA supplementation in humans can reproduce the anti-atrophy or pro-hypertrophic data reported previously [187]. However, given the potent effect of UA in rodent skeletal muscle, future translational research in humans is clearly warranted.

5. Summary and future directions

It is clear that sport nutrition is rapidly evolving and we are now entering a new era, one that could be best described as "targeted nutritional periodization". In terms of nutrition and the elite athlete it is essential that the purpose of the exercise session is clearly defined in the days and hours prior to the session in order to maximize performance or adaptation. It is also essential that coaches and athletes appreciate that the nutritional strategies to enhance *performance* or *adaptation* are quite different and at times are not always compatible. This is perhaps best demonstrated by the growing literature that carbohydrate restriction may enhance mitochondrial biogenesis and potentially long term adaption although it may also impair performance in a given training session. This growing need for "*targeted nutritional periodization*" requires that sports teams work with sport nutritionists / dieticians who have a solid understanding of exercise biochemistry to allow such strategies to be correctly implemented and is also beginning to highlight the need for sporting teams to employ full time nutrition support.

Despite the growing research in sport nutrition this review has highlighted that there

remains many unanswered questions, which must be addressed to further improve athletic performance. These questions include, but are not limited to 1) What is the best way to implement the train low (carbohydrate) strategy, 2) are there scenarios where fat adaption may enhance performance and if so how long does it take to fat adapt, 3) will the novel compounds that are emerging in rodent models translate to human performance, 4) given that the vast majority of research is on non elite performers, how much of this review directly translates to the elite athlete? These questions, amongst many others, will no doubt be addressed in the coming years ultimately helping athletes to continually be "*Citius, Altius, Fortius*".

Acknowledgments

References

[1]   Baar, K. The signaling underlying FITness. *Appl Physiol Nutr Metab* **34:**411-419; 2009.
[2]   Egan, B.; Zierath, J. R. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab* **17:**162-184; 2013.
[3]   Weigl, L. G. Lost in translation: regulation of skeletal muscle protein synthesis. *Curr Opin Pharmacol* **12:**377-382; 2012.
[4]   Hardie, D. G. Sensing of energy and nutrients by AMP-activated protein kinase. *Am J Clin Nutr* **93:**891S-896; 2011.
[5]   Booth, F. W.; Thomason, D. B. Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. *Physiol Rev* **71:**541-585; 1991.
[6]   Gibala, M. J.; Little, J. P.; van Essen, M.; Wilkin, G. P.; Burgomaster, K. A.; Safdar, A.; Raha, S.; Tarnopolsky, M. A. Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *J Physiol* **575:**901-911; 2006.
[7]   Rivera-Brown, A. M.; Frontera, W. R. Principles of exercise physiology: responses to acute exercise and long-term adaptations to training. *PM R* **4:**797-804; 2012.
[8]   Perry, C. G.; Lally, J.; Holloway, G. P.; Heigenhauser, G. J.; Bonen, A.; Spriet, L. L. Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J Physiol* **588:**4795-4810; 2010.

[9]   Tamura, T.; Shiraki, H.; Nakagawa, H. Purification and characterization of adenylate kinase isozymes from rat muscle and liver. *Biochim Biophys Acta* **612:**56-66; 1980.

[10]   Meyer, R. A.; Terjung, R. L. AMP deamination and IMP reamination in working skeletal muscle. *Am J Physiol* **239:**C32-38; 1980.

[11]   Trigun, S. K.; Singh, S. N. Changes in the modulations of kinetics and allosteric properties of muscle phosphofructokinase of young and old rats. *Arch Gerontol Geriatr* **7:**239-247; 1988.

[12]   Winder, W. W.; Hardie, D. G. Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am J Physiol* **270:**E299-304; 1996.

[13]   Gowans, G. J.; Hawley, S. A.; Ross, F. A.; Hardie, D. G. AMP is a true physiological regulator of AMP-activated protein kinase by both allosteric activation and enhancing net phosphorylation. *Cell Metab* **18:**556-566; 2013.

[14]   Sakamoto, K.; Goransson, O.; Hardie, D. G.; Alessi, D. R. Activity of LKB1 and AMPK-related kinases in skeletal muscle: effects of contraction, phenformin, and AICAR. *Am J Physiol Endocrinol Metab* **287:**E310-317; 2004.

[15]   Alam, N.; Saggerson, E. D. Malonyl-CoA and the regulation of fatty acid oxidation in soleus muscle. *Biochem J* **334 (Pt 1):**233-241; 1998.

[16]   Pehmoller, C.; Treebak, J. T.; Birk, J. B.; Chen, S.; Mackintosh, C.; Hardie, D. G.; Richter, E. A.; Wojtaszewski, J. F. Genetic disruption of AMPK signaling abolishes both contraction- and insulin-stimulated TBC1D1 phosphorylation and 14-3-3 binding in mouse skeletal muscle. *Am J Physiol Endocrinol Metab* **297:**E665-675; 2009.

[17]   Howlett, K. F.; Mathews, A.; Garnham, A.; Sakamoto, K. The effect of exercise and insulin on AS160 phosphorylation and 14-3-3 binding capacity in human skeletal muscle. *Am J Physiol Endocrinol Metab* **294:**E401-407; 2008.

[18]   Howlett, K. F.; Sakamoto, K.; Garnham, A.; Cameron-Smith, D.; Hargreaves, M. Resistance exercise and insulin regulate AS160 and interaction with 14-3-3 in human skeletal muscle. *Diabetes* **56:**1608-1614; 2007.

[19]   Sakamoto, K.; Holman, G. D. Emerging role for AS160/TBC1D4 and TBC1D1 in the regulation of GLUT4 traffic. *Am J Physiol Endocrinol Metab* **295:**E29-37; 2008.

[20]   McBride, A.; Ghilagaber, S.; Nikolaev, A.; Hardie, D. G. The glycogen-binding domain on the AMPK beta subunit allows the kinase to act as a glycogen sensor. *Cell Metab* **9:**23-34; 2009.

[21]   Bartlett, J. D.; Louhelainen, J.; Iqbal, Z.; Cochran, A. J.; Gibala, M. J.; Gregson, W.; Close, G. L.; Drust, B.; Morton, J. P. Reduced carbohydrate availability enhances exercise-induced p53 signaling in human skeletal muscle: implications for mitochondrial biogenesis. *Am J Physiol Regul Integr Comp Physiol* **304:**R450-458; 2013.

[22]   Wojtaszewski, J. F.; MacDonald, C.; Nielsen, J. N.; Hellsten, Y.; Hardie, D. G.; Kemp, B. E.; Kiens, B.; Richter, E. A. Regulation of 5'AMP-activated protein kinase activity and substrate utilization in exercising human skeletal muscle. *Am J Physiol Endocrinol Metab* **284:**E813-822; 2003.

[23]   Yeo, W. K.; McGee, S. L.; Carey, A. L.; Paton, C. D.; Garnham, A. P.; Hargreaves, M.; Hawley, J. A. Acute signalling responses to intense endurance training commenced with low or normal muscle glycogen. *Exp Physiol* **95:**351-358; 2010.

[24]   Philp, A.; MacKenzie, M. G.; Belew, M. Y.; Towler, M. C.; Corstorphine, A.; Papalamprou, A.; Hardie, D. G.; Baar, K. Glycogen content regulates peroxisome proliferator activated receptor- partial differential (PPAR- partial differential) activity in rat skeletal muscle. *PLoS One* **8:**e77200; 2013.

[25]   Tanner, C. B.; Madsen, S. R.; Hallowell, D. M.; Goring, D. M.; Moore, T. M.; Hardman, S. E.; Heninger, M. R.; Atwood, D. R.; Thomson, D. M. Mitochondrial and performance adaptations to exercise training in mice lacking skeletal muscle LKB1. *Am J Physiol Endocrinol Metab* **305:**E1018-1029; 2013.

[26]   Fentz, J.; Kjobsted, R.; Kristensen, C. M.; Hingst, J. R.; Birk, J. B.; Gudiksen, A.; Foretz, M.; Schjerling, P.; Viollet, B.; Pilegaard, H.; Wojtaszewski, J. F. AMPKalpha is essential for acute exercise-induced gene responses but not for exercise training-induced adaptations in mouse skeletal muscle. *Am J Physiol Endocrinol Metab***:**ajpendo 00157 02015; 2015.

[27]   McGee, S. L.; Howlett, K. F.; Starkie, R. L.; Cameron-Smith, D.; Kemp, B. E.; Hargreaves, M. Exercise increases nuclear AMPK alpha2 in human skeletal muscle. *Diabetes* **52:**926-928; 2003.

[28]   Jager, S.; Handschin, C.; St-Pierre, J.; Spiegelman, B. M. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A* **104:**12017-12022; 2007.

[29]   McGee, S. L.; Sparling, D.; Olson, A. L.; Hargreaves, M. Exercise increases MEF2- and GEF DNA-binding activity in human skeletal muscle. *FASEB J* **20:**348-349; 2006.

[30]   McGee, S. L.; van Denderen, B. J.; Howlett, K. F.; Mollica, J.; Schertzer, J. D.; Kemp, B. E.; Hargreaves, M. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes* **57:**860-867; 2008.

[31]   McGee, S. L.; Hargreaves, M. Histone modifications and exercise adaptations. *J Appl Physiol (1985)* **110:**258-263; 2011.

[32]   Gilde, A. J.; Van Bilsen, M. Peroxisome proliferator-activated receptors (PPARS): regulators of gene expression in heart and skeletal muscle. *Acta Physiol Scand* **178:**425-434; 2003.

[33]   Muoio, D. M.; Koves, T. R. Skeletal muscle adaptation to fatty acid depends on coordinated actions of the PPARs and PGC1 alpha: implications for metabolic disease. *Appl Physiol Nutr Metab* **32:**874-883; 2007.

[34]   Bergstrom, J.; Hermansen, L.; Hultman, E.; Saltin, B. Diet, muscle glycogen and physical performance. *Acta Physiol Scand* **71:**140-150; 1967.

[35]   Hawley, J. A.; Schabort, E. J.; Noakes, T. D.; Dennis, S. C. Carbohydrate-loading and exercise performance. An update. *Sports Med* **24:**73-81; 1997.

[36]   Burke, L. M.; Hawley, J. A.; Wong, S. H.; Jeukendrup, A. E. Carbohydrates for training and competition. *J Sports Sci* **29 Suppl 1:**S17-27; 2011.

[37]   Stellingwerff, T.; Cox, G. R. Systematic review: Carbohydrate supplementation on exercise performance or capacity of varying durations. *Appl Physiol Nutr Metab* **39:**998-1011; 2014.

[38]   Jeukendrup, A. A step towards personalized sports nutrition: carbohydrate intake during exercise. *Sports Med* **44 Suppl 1:**S25-33; 2014.

[39]   Stellingwerff, T.; Boon, H.; Gijsen, A. P.; Stegen, J. H.; Kuipers, H.; van Loon, L. J. Carbohydrate supplementation during prolonged cycling exercise spares muscle glycogen but does not affect intramyocellular lipid use. *Pflugers Arch* **454:**635-647; 2007.

[40]   Gonzalez, J. T.; Fuchs, C. J.; Smith, F. E.; Thelwall, P. E.; Taylor, R.; Stevenson, E. J.; Trenell, M. I.; Cermak, N. M.; van Loon, L. J. Ingestion of Glucose or Sucrose Prevents Liver but not Muscle Glycogen Depletion During Prolonged Endurance-type Exercise in Trained Cyclists. *Am J Physiol Endocrinol Metab***:**ajpendo 00376 02015; 2015.
[41]   Coyle, E. F.; Coggan, A. R.; Hemmert, M. K.; Ivy, J. L. Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* **61:**165-172; 1986.
[42]   Jeukendrup, A.; Brouns, F.; Wagenmakers, A. J.; Saris, W. H. Carbohydrate-electrolyte feedings improve 1 h time trial cycling performance. *Int J Sports Med* **18:**125-129; 1997.
[43]   Carter, J. M.; Jeukendrup, A. E.; Mann, C. H.; Jones, D. A. The effect of glucose infusion on glucose kinetics during a 1-h time trial. *Med Sci Sports Exerc* **36:**1543-1550; 2004.
[44]   Carter, J. M.; Jeukendrup, A. E.; Jones, D. A. The effect of carbohydrate mouth rinse on 1-h cycle time trial performance. *Med Sci Sports Exerc* **36:**2107-2111; 2004.
[45]   Burke, L. M.; Maughan, R. J. The Governor has a sweet tooth - mouth sensing of nutrients to enhance sports performance. *Eur J Sport Sci* **15:**29-40; 2015.
[46]   Chambers, E. S.; Bridge, M. W.; Jones, D. A. Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. *J Physiol* **587:**1779-1794; 2009.
[47]   Lane, S. C.; Bird, S. R.; Burke, L. M.; Hawley, J. A. Effect of a carbohydrate mouth rinse on simulated cycling time-trial performance commenced in a fed or fasted state. *Appl Physiol Nutr Metab* **38:**134-139; 2013.
[48]   Kasper, A. M.; Cocking, S.; Cockayne, M.; Barnard, M.; Tench, J.; Parker, L.; McAndrew, J.; Langan-Evans, C.; Close, G. L.; Morton, J. P. Carbohydrate mouth rinse and caffeine improves high-intensity interval running capacity when carbohydrate restricted. *Eur J Sport Sci***:**1-9; 2015.
[49]   Lee, M. J.; Hammond, K. M.; Vasdev, A.; Poole, K. L.; Impey, S. G.; Close, G. L.; Morton, J. P. Self-selecting fluid intake while maintaining high carbohydrate availability does not impair half-marathon performance. *Int J Sports Med* **35:**1216-1222; 2014.
[50]   Pfeiffer, B.; Stellingwerff, T.; Zaltas, E.; Jeukendrup, A. E. CHO oxidation from a CHO gel compared with a drink during exercise. *Med Sci Sports Exerc* **42:**2038-2045; 2010.
[51]   Pfeiffer, B.; Stellingwerff, T.; Zaltas, E.; Jeukendrup, A. E. Oxidation of solid versus liquid CHO sources during exercise. *Med Sci Sports Exerc* **42:**2030-2037; 2010.
[52]   Zhang, X.; O'Kennedy, N.; Morton, J. P. Extreme Variation of Nutritional Composition and Osmolality of Commercially Available Carbohydrate Energy Gels. *Int J Sport Nutr Exerc Metab* **25:**504-509; 2015.
[53]   Pfeiffer, B.; Stellingwerff, T.; Hodgson, A. B.; Randell, R.; Pottgen, K.; Res, P.; Jeukendrup, A. E. Nutritional intake and gastrointestinal problems during competitive endurance events. *Med Sci Sports Exerc* **44:**344-351; 2012.
[54]   de Oliveira, E. P.; Burini, R. C.; Jeukendrup, A. Gastrointestinal complaints during exercise: prevalence, etiology, and nutritional recommendations. *Sports Med* **44 Suppl 1:**S79-85; 2014.

[55]   Yeo, W. K.; Paton, C. D.; Garnham, A. P.; Burke, L. M.; Carey, A. L.; Hawley, J. A. Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens. *J Appl Physiol* **105:**1462-1470; 2008.

[56]   Widrick, J. J.; Costill, D. L.; Fink, W. J.; Hickey, M. S.; McConell, G. K.; Tanaka, H. Carbohydrate feedings and exercise performance: effect of initial muscle glycogen concentration. *J Appl Physiol (1985)* **74:**2998-3005; 1993.

[57]   Hawley, J. A.; Morton, J. P. Ramping up the signal: promoting endurance training adaptation in skeletal muscle by nutritional manipulation. *Clin Exp Pharmacol Physiol* **41:**608-613; 2014.

[58]   Bartlett, J. D.; Hawley, J. A.; Morton, J. P. Carbohydrate availability and exercise training adaptation: too much of a good thing? *Eur J Sport Sci* **15:**3-12; 2015.

[59]   Morton, J. P.; Croft, L.; Bartlett, J. D.; Maclaren, D. P.; Reilly, T.; Evans, L.; McArdle, A.; Drust, B. Reduced carbohydrate availability does not modulate training-induced heat shock protein adaptations but does upregulate oxidative enzyme activity in human skeletal muscle. *J Appl Physiol* **106:**1513-1521; 2009.

[60]   Van Proeyen, K.; Szlufcik, K.; Nielens, H.; Ramaekers, M.; Hespel, P. Beneficial metabolic adaptations due to endurance exercise training in the fasted state. *J Appl Physiol (1985)* **110:**236-245; 2011.

[61]   Hulston, C. J.; Venables, M. C.; Mann, C. H.; Martin, C.; Philp, A.; Baar, K.; Jeukendrup, A. E. Training with low muscle glycogen enhances fat metabolism in well-trained cyclists. *Med Sci Sports Exerc* **42:**2046-2055; 2010.

[62]   Hansen, A. K.; Fischer, C. P.; Plomgaard, P.; Andersen, J. L.; Saltin, B.; Pedersen, B. K. Skeletal muscle adaptation: training twice every second day vs. training once daily. *J Appl Physiol (1985)* **98:**93-99; 2005.

[63]   Cochran, A. J.; Myslik, F.; MacInnis, M. J.; Percival, M. E.; Bishop, D.; Tarnopolsky, M. A.; Gibala, M. J. Manipulating Carbohydrate Availability Between Twice-Daily Sessions of High-Intensity Interval Training Over 2 Weeks Improves Time-Trial Performance. *Int J Sport Nutr Exerc Metab* **25:**463-470; 2015.

[64]   Burke, L. M. Fueling strategies to optimize performance: training high or training low? *Scand J Med Sci Sports* **20 Suppl 2:**48-58; 2010.

[65]   Cochran, A. J.; Little, J. P.; Tarnopolsky, M. A.; Gibala, M. J. Carbohydrate feeding during recovery alters the skeletal muscle metabolic response to repeated sessions of high-intensity interval exercise in humans. *J Appl Physiol* **108:**628-636; 2010.

[66]   Psilander, N.; Frank, P.; Flockhart, M.; Sahlin, K. Exercise with low glycogen increases PGC-1alpha gene expression in human skeletal muscle. *Eur J Appl Physiol* **113:**951-963; 2013.

[67]   Nieman, D. C. Marathon training and immune function. *Sports Med* **37:**412-415; 2007.

[68]   Cox, G. R.; Clark, S. A.; Cox, A. J.; Halson, S. L.; Hargreaves, M.; Hawley, J. A.; Jeacocke, N.; Snow, R. J.; Yeo, W. K.; Burke, L. M. Daily training with high carbohydrate availability increases exogenous carbohydrate oxidation during endurance cycling. *J Appl Physiol (1985)* **109:**126-134; 2010.

[69]   Howarth, K. R.; Moreau, N. A.; Phillips, S. M.; Gibala, M. J. Coingestion of protein with carbohydrate during recovery from endurance exercise stimulates skeletal muscle protein synthesis in humans. *J Appl Physiol* **106:**1394-1402; 2009.

[70]   Impey, S. G.; Smith, D.; Robinson, A. L.; Owens, D. J.; Bartlett, J. D.; Smith, K.; Limb, M.; Tang, J.; Fraser, W. D.; Close, G. L.; Morton, J. P. Leucine-enriched protein feeding does not impair exercise-induced free fatty acid availability and lipid oxidation: beneficial implications for training in carbohydrate-restricted states. *Amino Acids* **47:**407-416; 2015.

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

[72]   Lane, S. C.; Camera, D. M.; Lassiter, D. G.; Areta, J. L.; Bird, S. R.; Yeo, W. K.; Jeacocke, N. A.; Krook, A.; Zierath, J. R.; Burke, L. M.; Hawley, J. A. Effects of sleeping with reduced carbohydrate availability on acute training responses. *J Appl Physiol (1985)* **119:**643-655; 2015.

[73]   Burke, L. Re-Examining High-Fat Diets for Sports Performance: Did We Call the 'Nail in the Coffin' Too Soon? . *Sports Med.* **Nov 9:**epub ahead of print; 2015.

[74]   Volek, J. S.; Noakes, T.; Phinney, S. D. Rethinking fat as a fuel for endurance exercise. *Eur J Sport Sci* **15:**13-20; 2015.

[75]   Phinney, S. D.; Bistrian, B. R.; Evans, W. J.; Gervino, E.; Blackburn, G. L. The human metabolic response to chronic ketosis without caloric restriction: preservation of submaximal exercise capability with reduced carbohydrate oxidation. *Metabolism* **32:**769-776; 1983.

[76]   Yeo, W. K.; Carey, A. L.; Burke, L.; Spriet, L. L.; Hawley, J. A. Fat adaptation in well-trained athletes: effects on cell metabolism. *Appl Physiol Nutr Metab* **36:**12-22; 2011.

[77]   Havemann, L.; West, S. J.; Goedecke, J. H.; Macdonald, I. A.; St Clair Gibson, A.; Noakes, T. D.; Lambert, E. V. Fat adaptation followed by carbohydrate loading compromises high-intensity sprint performance. *J Appl Physiol (1985)* **100:**194-202; 2006.

[78]   Stellingwerff, T.; Spriet, L. L.; Watt, M. J.; Kimber, N. E.; Hargreaves, M.; Hawley, J. A.; Burke, L. M. Decreased PDH activation and glycogenolysis during exercise following fat adaptation with carbohydrate restoration. *Am J Physiol Endocrinol Metab* **290:**E380-388; 2006.

[79]   Burke, L. M.; Kiens, B. "Fat adaptation" for athletic performance: the nail in the coffin? *J Appl Physiol (1985)* **100:**7-8; 2006.

[80]   Faigenbaum, A. D.; Lloyd, R. S.; MacDonald, J.; Myer, G. D. Citius, Altius, Fortius: beneficial effects of resistance training for young athletes. *Br J Sports Med*; 2015.

[81]   Moritani, T.; deVries, H. A. Neural factors versus hypertrophy in the time course of muscle strength gain. *Am J Phys Med* **58:**115-130; 1979.

[82]   Seynnes, O. R.; de Boer, M.; Narici, M. V. Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training. *J Appl Physiol (1985)* **102:**368-373; 2007.

[83]   Churchward-Venne, T. A.; Burd, N. A.; Phillips, S. M. Nutritional regulation of muscle protein synthesis with resistance exercise: strategies to enhance anabolism. *Nutr Metab (Lond)* **9:**40; 2012.

[84]   Cermak, N. M.; Res, P. T.; de Groot, L. C.; Saris, W. H.; van Loon, L. J. Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis. *Am J Clin Nutr* **96:**1454-1464; 2012.

[85]   Baar, K.; Esser, K. Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise. *Am J Physiol* **276:**C120-127; 1999.

[86]   Chen, Y. W.; Nader, G. A.; Baar, K. R.; Fedele, M. J.; Hoffman, E. P.; Esser, K. A. Response of rat muscle to acute resistance exercise defined by transcriptional and translational profiling. *J Physiol* **545:**27-41; 2002.

[87]   Chesley, A.; MacDougall, J. D.; Tarnopolsky, M. A.; Atkinson, S. A.; Smith, K. Changes in human muscle protein synthesis after resistance exercise. *J Appl Physiol (1985)* **73:**1383-1388; 1992.

[88]   McCarthy, J. J.; Esser, K. A. Anabolic and catabolic pathways regulating skeletal muscle mass. *Curr Opin Clin Nutr Metab Care* **13:**230-235; 2010.

[89]   Bodine, S. C.; Stitt, T. N.; Gonzalez, M.; Kline, W. O.; Stover, G. L.; Bauerlein, R.; Zlotchenko, E.; Scrimgeour, A.; Lawrence, J. C.; Glass, D. J.; Yancopoulos, G. D. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. *Nat Cell Biol* **3:**1014-1019; 2001.

[90]   Dickinson, J. M.; Fry, C. S.; Drummond, M. J.; Gundermann, D. M.; Walker, D. K.; Glynn, E. L.; Timmerman, K. L.; Dhanani, S.; Volpi, E.; Rasmussen, B. B. Mammalian target of rapamycin complex 1 activation is required for the stimulation of human skeletal muscle protein synthesis by essential amino acids. *J Nutr* **141:**856-862; 2011.

[91]   Drummond, M. J.; Fry, C. S.; Glynn, E. L.; Dreyer, H. C.; Dhanani, S.; Timmerman, K. L.; Volpi, E.; Rasmussen, B. B. Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis. *J Physiol* **587:**1535-1546; 2009.

[92]   Mieulet, V.; Roceri, M.; Espeillac, C.; Sotiropoulos, A.; Ohanna, M.; Oorschot, V.; Klumperman, J.; Sandri, M.; Pende, M. S6 kinase inactivation impairs growth and translational target phosphorylation in muscle cells maintaining proper regulation of protein turnover. *Am J Physiol Cell Physiol* **293:**C712-722; 2007.

[93]   Ohanna, M.; Sobering, A. K.; Lapointe, T.; Lorenzo, L.; Praud, C.; Petroulakis, E.; Sonenberg, N.; Kelly, P. A.; Sotiropoulos, A.; Pende, M. Atrophy of S6K1(-/-) skeletal muscle cells reveals distinct mTOR effectors for cell cycle and size control. *Nat Cell Biol* **7:**286-294; 2005.

[94]   Zheng, X.; Liang, Y.; He, Q.; Yao, R.; Bao, W.; Bao, L.; Wang, Y.; Wang, Z. Current models of mammalian target of rapamycin complex 1 (mTORC1) activation by growth factors and amino acids. *Int J Mol Sci* **15:**20753-20769; 2014.

[95]   Mackenzie, M. G.; Hamilton, D. L.; Murray, J. T.; Baar, K. mVps34 is activated by an acute bout of resistance exercise. *Biochem Soc Trans* **35:**1314-1316; 2007.

[96]   Dodd, K. M.; Tee, A. R. Leucine and mTORC1: a complex relationship. *Am J Physiol Endocrinol Metab* **302:**E1329-1342; 2012.

[97]   Christie, G. R.; Hajduch, E.; Hundal, H. S.; Proud, C. G.; Taylor, P. M. Intracellular sensing of amino acids in Xenopus laevis oocytes stimulates p70 S6 kinase in a target of rapamycin-dependent manner. *J Biol Chem* **277:**9952-9957; 2002.

[98]   Crozier, S. J.; Kimball, S. R.; Emmert, S. W.; Anthony, J. C.; Jefferson, L. S. Oral leucine administration stimulates protein synthesis in rat skeletal muscle. *J Nutr* **135:**376-382; 2005.

[99]   Greenhaff, P. L.; Karagounis, L. G.; Peirce, N.; Simpson, E. J.; Hazell, M.; Layfield, R.; Wackerhage, H.; Smith, K.; Atherton, P.; Selby, A.; Rennie, M. J. Disassociation between the effects of amino acids and insulin on signaling, ubiquitin ligases, and protein turnover in human muscle. *Am J Physiol Endocrinol Metab* **295:**E595-604; 2008.

[100]   Beugnet, A.; Tee, A. R.; Taylor, P. M.; Proud, C. G. Regulation of targets of mTOR (mammalian target of rapamycin) signalling by intracellular amino acid availability. *Biochem J* **372:**555-566; 2003.

[101]   Bar-Peled, L.; Sabatini, D. M. Regulation of mTORC1 by amino acids. *Trends Cell Biol* **24:**400-406; 2014.

[102]   You, J. S.; Lincoln, H. C.; Kim, C. R.; Frey, J. W.; Goodman, C. A.; Zhong, X. P.; Hornberger, T. A. The role of diacylglycerol kinase zeta and phosphatidic acid in the mechanical activation of mammalian target of rapamycin (mTOR) signaling and skeletal muscle hypertrophy. *J Biol Chem* **289:**1551-1563; 2014.

[103]   Apro, W.; Moberg, M.; Hamilton, D. L.; Ekblom, B.; Rooyackers, O.; Holmberg, H. C.; Blomstrand, E. Leucine does not affect mechanistic target of rapamycin complex 1 assembly but is required for maximal ribosomal protein s6 kinase 1 activity in human skeletal muscle following resistance exercise. *FASEB J*; 2015.

[104]   Tipton, K. D.; Ferrando, A. A.; Phillips, S. M.; Doyle, D., Jr.; Wolfe, R. R. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am J Physiol* **276:**E628-634; 1999.

[105]   Moore, D. R.; Robinson, M. J.; Fry, J. L.; Tang, J. E.; Glover, E. I.; Wilkinson, S. B.; Prior, T.; Tarnopolsky, M. A.; Phillips, S. M. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *Am J Clin Nutr* **89:**161-168; 2009.

[106]   Witard, O. C.; Jackman, S. R.; Breen, L.; Smith, K.; Selby, A.; Tipton, K. D. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *Am J Clin Nutr* **99:**86-95; 2014.

[107]   Breen, L.; Churchward-Venne, T. A. Leucine: a nutrient 'trigger' for muscle anabolism, but what more? *J Physiol* **590:**2065-2066; 2012.

[108]   Wilkinson, D. J.; Hossain, T.; Hill, D. S.; Phillips, B. E.; Crossland, H.; Williams, J.; Loughna, P.; Churchward-Venne, T. A.; Breen, L.; Phillips, S. M.; Etheridge, T.; Rathmacher, J. A.; Smith, K.; Szewczyk, N. J.; Atherton, P. J. Effects of leucine and its metabolite beta-hydroxy-beta-methylbutyrate on human skeletal muscle protein metabolism. *J Physiol* **591:**2911-2923; 2013.

[109]   Moore, D. R.; Churchward-Venne, T. A.; Witard, O.; Breen, L.; Burd, N. A.; Tipton, K. D.; Phillips, S. M. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *J Gerontol A Biol Sci Med Sci* **70:**57-62; 2015.

[110]   Phillips, S. M.; Fulgoni, V. L., 3rd; Heaney, R. P.; Nicklas, T. A.; Slavin, J. L.; Weaver, C. M. Commonly consumed protein foods contribute to nutrient intake, diet quality, and nutrient adequacy. *Am J Clin Nutr*; 2015.

[111]   Phillips, S. M.; Zemel, M. B. Effect of protein, dairy components and energy balance in optimizing body composition. *Nestle Nutr Inst Workshop Ser* **69:**97-108; discussion 108-113; 2011.

[112]   Devries, M. C.; Phillips, S. M. Supplemental protein in support of muscle mass and health: advantage whey. *J Food Sci* **80 Suppl 1:**A8-A15; 2015.

[113]   Nongonierma, A. B.; FitzGerald, R. J. Bioactive properties of milk proteins in humans: A review. *Peptides* **73:**20-34; 2015.

[114]   Nongonierma, A. B.; FitzGerald, R. J. Milk proteins as a source of tryptophan-containing bioactive peptides. *Food Funct* **6:**2115-2127; 2015.

[115]   Elliot, T. A.; Cree, M. G.; Sanford, A. P.; Wolfe, R. R.; Tipton, K. D. Milk ingestion stimulates net muscle protein synthesis following resistance exercise. *Med Sci Sports Exerc* **38:**667-674; 2006.

[116]   Knapik, J. J.; Steelman, R. A.; Hoedebecke, S. S.; Austin, K. G.; Farina, E. K.; Lieberman, H. R. Prevalence of Dietary Supplement Use by Athletes: Systematic Review and Meta-Analysis. *Sports Med*; 2015.

[117]   Knapik, J. J.; Steelman, R. A.; Hoedebecke, S. S.; Farina, E. K.; Austin, K. G.; Lieberman, H. R. A systematic review and meta-analysis on the prevalence of dietary supplement use by military personnel. *BMC Complement Altern Med* **14:**143; 2014.

[118]   Outram, S.; Stewart, B. Doping through supplement use: a review of the available empirical data. *Int J Sport Nutr Exerc Metab* **25:**54-59; 2015.

[119]   Burke, L.; Desbrow, B.; Spriet, L. *Caffeine for sports performance.* Champaign Illinois: Human Kinetics; 2013.

[120]   Spriet, L. L. Exercise and sport performance with low doses of caffeine. *Sports Med* **44 Suppl 2:**S175-184; 2014.

[121]   Burke, L. M. Caffeine and sports performance. *Appl Physiol Nutr Metab* **33:**1319-1334; 2008.

[122]   Lane, S. C.; Areta, J. L.; Bird, S. R.; Coffey, V. G.; Burke, L. M.; Desbrow, B.; Karagounis, L. G.; Hawley, J. A. Caffeine ingestion and cycling power output in a low or normal muscle glycogen state. *Med Sci Sports Exerc* **45:**1577-1584; 2013.

[123]   Womack, C. J.; Saunders, M. J.; Bechtel, M. K.; Bolton, D. J.; Martin, M.; Luden, N. D.; Dunham, W.; Hancock, M. The influence of a CYP1A2 polymorphism on the ergogenic effects of caffeine. *J Int Soc Sports Nutr* **9:**7; 2012.

[124]   Harris, R. C.; Soderlund, K.; Hultman, E. Elevation of creatine in resting and exercised muscle of normal subjects by creatine supplementation. *Clin Sci (Lond)* **83:**367-374; 1992.

[125]   Buford, T. W.; Kreider, R. B.; Stout, J. R.; Greenwood, M.; Campbell, B.; Spano, M.; Ziegenfuss, T.; Lopez, H.; Landis, J.; Antonio, J. International Society of Sports Nutrition position stand: creatine supplementation and exercise. *J Int Soc Sports Nutr* **4:**6; 2007.

[126]   Safdar, A.; Yardley, N. J.; Snow, R.; Melov, S.; Tarnopolsky, M. A. Global and targeted gene expression and protein content in skeletal muscle of young men following short-term creatine monohydrate supplementation. *Physiol Genomics* **32:**219-228; 2008.

[127]   Gualano, B.; Roschel, H.; Lancha-Jr, A. H.; Brightbill, C. E.; Rawson, E. S. In sickness and in health: the widespread application of creatine supplementation. *Amino Acids* **43:**519-529; 2012.

[128]   Dalbo, V. J.; Roberts, M. D.; Stout, J. R.; Kerksick, C. M. Putting to rest the myth of creatine supplementation leading to muscle cramps and dehydration. *Br J Sports Med* **42:**567-573; 2008.

[129]   Burke, L. M. To beet or not to beet? *J Appl Physiol (1985)* **115:**311-312; 2013.

[130]   Jones, A. M. Dietary nitrate supplementation and exercise performance. *Sports Med* **44 Suppl 1:**S35-45; 2014.

[131]   Jonvik, K. L.; Nyakayiru, J.; van Loon, L. J.; Verdijk, L. B. Can elite athletes benefit from dietary nitrate supplementation? *J Appl Physiol (1985)* **119:**759-761; 2015.

[132]   Hultstrom, M.; Amorim de Paula, C.; Antonio Peliky Fontes, M.; Porcelli, S.; Bellistri, G.; Pugliese, L.; Rasica, L.; Marzorati, M.; Pavei, G.; Ferguson, S. K.; Holdsworth, C. T.; Musch, T. I.; Poole, D. C.; Bourdillon, N.; Hoon, M. W.; Burke, L. M.; Michielli, D. W.; Faiss, R.; Millet, G. P.; Corona, B. T.; Green, M. S.; da Silveira, A. L.; Sindler, A. L.; Casey, D. P.; Johnson, B. D.; Wheatley, C. M.; Kunces, L. J.; Bescos, R.; Cody, L. C.; Martens, C. R.; Justice, J. N.; Ballak, S. B.; Ballak, D. B.; Wanner, S. P.; Rehman, S. Commentaries on Viewpoint: Can elite athletes benefit from dietary nitrate supplementation? *J Appl Physiol (1985)* **119:**762-769; 2015.

[133]   Burke, L. Practical considerations for bicarbonate loading and sports performance. In: Tipton, K.; van Loon, L., eds. *Nutritional strategy to modulate training efficiency*; 2013: 15-12.

[134]   Carr, A. J.; Hopkins, W. G.; Gore, C. J. Effects of acute alkalosis and acidosis on performance: a meta-analysis. *Sports Med* **41:**801-814; 2011.

[135]   Carr, A. J.; Slater, G. J.; Gore, C. J.; Dawson, B.; Burke, L. M. Effect of sodium bicarbonate on [HCO3-], pH, and gastrointestinal symptoms. *Int J Sport Nutr Exerc Metab* **21:**189-194; 2011.

[136]   Stellingwerff, T.; Decombaz, J.; Harris, R. C.; Boesch, C. Optimizing human in vivo dosing and delivery of beta-alanine supplements for muscle carnosine synthesis. *Amino Acids*; 2012.

[137]   Stegen, S.; Bex, T.; Vervaet, C.; Vanhee, L.; Achten, E.; Derave, W. beta-Alanine dose for maintaining moderately elevated muscle carnosine levels. *Med Sci Sports Exerc* **46:**1426-1432; 2014.

[138]   Blancquaert, L.; Everaert, I.; Derave, W. Beta-alanine supplementation, muscle carnosine and exercise performance. *Curr Opin Clin Nutr Metab Care* **18:**63-70; 2015.

[139]   Demay, M. B. Mechanism of vitamin D receptor action. *Annals of the New York Academy of Sciences* **1068:**204-213; 2006.

[140]   Close, G. L.; Russell, J.; Cobley, J. N.; Owens, D. J.; Wilson, G.; Gregson, W.; Fraser, W. D.; Morton, J. P. Assessment of vitamin D concentration in non-supplemented professional athletes and healthy adults during the winter months in the UK: implications for skeletal muscle function. *J Sports Sci* **31:**344-353; 2013.

[141]   Hamilton, S. A.; McNeil, R.; Hollis, B. W.; Davis, D. J.; Winkler, J.; Cook, C.; Warner, G.; Bivens, B.; McShane, P.; Wagner, C. L. Profound Vitamin D Deficiency in a Diverse Group of Women during Pregnancy Living in a Sun-Rich Environment at Latitude 32 degrees N. *Int J Endocrinol* **2010:**917428; 2010.

[142]   Owens, D. J.; Fraser, W. D.; Close, G. L. Vitamin D and the athlete: emerging insights. *Eur J Sport Sci* **15:**73-84; 2015.

[143]   Vieth, R.; Bischoff-Ferrari, H.; Boucher, B. J.; Dawson-Hughes, B.; Garland, C. F.; Heaney, R. P.; Holick, M. F.; Hollis, B. W.; Lamberg-Allardt, C.; McGrath, J. J.; Norman, A. W.; Scragg, R.; Whiting, S. J.; Willett, W. C.; Zittermann, A. The urgent need to recommend an intake of vitamin D that is effective. *Am J Clin Nutr* **85:**649-650; 2007.

[144]   Zittermann, A. Vitamin D in preventive medicine: are we ignoring the evidence? *British Journal of Nutrition* **89:**552-572; 2003.

[145]   Owens, D. J.; Webber, D.; Impey, S. G.; Tang, J.; Donovan, T. F.; Fraser, W. D.; Morton, J. P.; Close, G. L. Vitamin D supplementation does not improve human skeletal muscle contractile properties in insufficient young males. *Eur J Appl Physiol* **114:**1309-1320; 2014.

[146]   Agostoni, C.; Bresson, J.; Fairweather-Tait, S.; Flynn, A.; Golly, I.; Korhonen, H.; Lagiou, P.; Lovik, M.; Marchelli, R.; Martin, A.; Moseley, B.; Neuhauser-Berthold, M.; Przyrembel, H.; Salminen, S.; Sanz, Y.; Strain, S.; Strobel, S.; Tetens, I.; Tome, D.; van Loveren, H.; Verhagen, H. Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. *European Food Safety Authority (EFSA) Journal* **10:**2813; 2012.

[147]   Owens, D. J.; Sharples, A. P.; Polydorou, I.; Alwan, N.; Donovan, T. F.; Tang, J.; Cooper, R. G.; Fraser, W. D.; Morton, J. P.; Stewart, C.; Close, G. L. A Systems Based Investigation into Vitamin D and Skeletal Muscle Repair, Regeneration and Hypertrophy. *Am J Physiol Endocrinol Metab***:**ajpendo 00375 02015; 2015.

[148]   Cobley, J. N.; McHardy, H.; Morton, J. P.; Nikolaidis, M. G.; Close, G. L. Influence of vitamin C and vitamin E on redox signaling: Implications for exercise adaptations. *Free Radic Biol Med* **84:**65-76; 2015.

[149]   Cobley, J. N.; Margaritelis, N. V.; Morton, J. P.; Close, G. L.; Nikolaidis, M. G.; Malone, J. K. The basic chemistry of exercise-induced DNA oxidation: oxidative damage, redox signaling, and their interplay. *Front Physiol* **6:**182; 2015.

[150]   Paulsen, G.; Hamarsland, H.; Cumming, K. T.; Johansen, R. E.; Hulmi, J. J.; Borsheim, E.; Wiig, H.; Garthe, I.; Raastad, T. Vitamin C and E supplementation alters protein signalling after a strength training session, but not muscle growth during 10 weeks of training. *J Physiol* **592:**5391-5408; 2014.

[151]   Morrison, D.; Hughes, J.; Della Gatta, P. A.; Mason, S.; Lamon, S.; Russell, A. P.; Wadley, G. D. Vitamin C and E supplementation prevents some of the cellular adaptations to endurance-training in humans. *Free Radic Biol Med* **89:**852-862; 2015.

[152]   Thompson, D.; Williams, C.; McGregor, S. J.; Nicholas, C. W.; McArdle, F.; Jackson, M. J.; Powell, J. R. Prolonged vitamin C supplementation and recovery from demanding exercise. *Int J Sport Nutr Exerc Metab* **11:**466-481; 2001.

[153]   Gomez-Cabrera, M. C.; Vina, J.; Ji, L. L. Interplay of oxidants and antioxidants during exercise: implications for muscle health. *Phys Sportsmed* **37:**116-123; 2009.

[154]   Gomez-Cabrera, M. C.; Borras, C.; Pallardo, F. V.; Sastre, J.; Ji, L. L.; Vina, J. Decreasing xanthine oxidase-mediated oxidative stress prevents useful cellular adaptations to exercise in rats. *J Physiol* **567:**113-120; 2005.

[155]   Ristow, M.; Zarse, K.; Oberbach, A.; Kloting, N.; Birringer, M.; Kiehntopf, M.; Stumvoll, M.; Kahn, C. R.; Bluher, M. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A* **106:**8665-8670; 2009.

[156]   Halliwell, B.; Whiteman, M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *Br J Pharmacol* **142:**231-255; 2004.

[157]   Sies, H. Total antioxidant capacity: appraisal of a concept. *J Nutr* **137:**1493-1495; 2007.

[158]   Forman, H. J.; Augusto, O.; Brigelius-Flohe, R.; Dennery, P. A.; Kalyanaraman, B.; Ischiropoulos, H.; Mann, G. E.; Radi, R.; Roberts, L. J., 2nd; Vina,

J.; Davies, K. J. Even free radicals should follow some rules: a guide to free radical research terminology and methodology. *Free Radic Biol Med* **78:**233-235; 2015.

[159]   Murphy, M. P.; Holmgren, A.; Larsson, N. G.; Halliwell, B.; Chang, C. J.; Kalyanaraman, B.; Rhee, S. G.; Thornalley, P. J.; Partridge, L.; Gems, D.; Nystrom, T.; Belousov, V.; Schumacker, P. T.; Winterbourn, C. C. Unraveling the biological roles of reactive oxygen species. *Cell Metab* **13:**361-366; 2011.

[160]   Petersen, A. C.; McKenna, M. J.; Medved, I.; Murphy, K. T.; Brown, M. J.; Della Gatta, P.; Cameron-Smith, D. Infusion with the antioxidant N-acetylcysteine attenuates early adaptive responses to exercise in human skeletal muscle. *Acta Physiol (Oxf)* **204:**382-392; 2012.

[161]   Cobley, J.; McGlory, C.; Morton, J.; Close, G. L. N-Acetylcysteine's attenuation of fatigue after repeated bouts of intermittent exercise: practical implications for tournament situations *Int J Sport Nutr Exerc Metab* **21:**451-461; 2011.

[162]   Reid, M. B. Free radicals and muscle fatigue: Of ROS, canaries, and the IOC. *Free Radic Biol Med* **44:**169-179; 2008.

[163]   Howatson, G.; McHugh, M. P.; Hill, J. A.; Brouner, J.; Jewell, A. P.; van Someren, K. A.; Shave, R. E.; Howatson, S. A. Influence of tart cherry juice on indices of recovery following marathon running. *Scand J Med Sci Sports* **20:**843-852; 2010.

[164]   Close, G. L.; Jackson, M. J. Antioxidants and exercise: a tale of the complexities of relating signalling processes to physiological function? *J Physiol* **592:**1721-1722; 2014.

[165]   Buijsse, B.; Weikert, C.; Drogan, D.; Bergmann, M.; Boeing, H. Chocolate consumption in relation to blood pressure and risk of cardiovascular disease in German adults. *Eur Heart J* **31:**1616-1623; 2010.

[166]   Nogueira, L.; Ramirez-Sanchez, I.; Perkins, G. A.; Murphy, A.; Taub, P. R.; Ceballos, G.; Villarreal, F. J.; Hogan, M. C.; Malek, M. H. (-)-Epicatechin enhances fatigue resistance and oxidative capacity in mouse muscle. *J Physiol* **589:**4615-4631; 2011.

[167]   Gutierrez-Salmean, G.; Ortiz-Vilchis, P.; Vacaseydel, C. M.; Rubio-Gayosso, I.; Meaney, E.; Villarreal, F.; Ramirez-Sanchez, I.; Ceballos, G. Acute effects of an oral supplement of (-)-epicatechin on postprandial fat and carbohydrate metabolism in normal and overweight subjects. *Food Funct* **5:**521-527; 2014.

[168]   MacKay, D.; Hathcock, J.; Guarneri, E. Niacin: chemical forms, bioavailability, and health effects. *Nutr Rev* **70:**357-366; 2012.

[169]   Bieganowski, P.; Brenner, C. Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a Preiss-Handler independent route to NAD+ in fungi and humans. *Cell* **117:**495-502; 2004.

[170]   Canto, C.; Menzies, K. J.; Auwerx, J. NAD(+) Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus. *Cell Metab* **22:**31-53; 2015.

[171]   Canto, C.; Houtkooper, R. H.; Pirinen, E.; Youn, D. Y.; Oosterveer, M. H.; Cen, Y.; Fernandez-Marcos, P. J.; Yamamoto, H.; Andreux, P. A.; Cettour-Rose, P.; Gademann, K.; Rinsch, C.; Schoonjans, K.; Sauve, A. A.; Auwerx, J. The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. *Cell Metab* **15:**838-847; 2012.

[172]   Pervaiz, S. Resveratrol: from grapevines to mammalian biology. *FASEB J* **17:**1975-1985; 2003.

[173]   de Ligt, M.; Timmers, S.; Schrauwen, P. Resveratrol and obesity: Can resveratrol relieve metabolic disturbances? *Biochim Biophys Acta* **1852:**1137-1144; 2015.

[174]   Murase, T.; Haramizu, S.; Ota, N.; Hase, T. Suppression of the aging-associated decline in physical performance by a combination of resveratrol intake and habitual exercise in senescence-accelerated mice. *Biogerontology* **10:**423-434; 2009.

[175]   Timmers, S.; Konings, E.; Bilet, L.; Houtkooper, R. H.; van de Weijer, T.; Goossens, G. H.; Hoeks, J.; van der Krieken, S.; Ryu, D.; Kersten, S.; Moonen-Kornips, E.; Hesselink, M. K.; Kunz, I.; Schrauwen-Hinderling, V. B.; Blaak, E. E.; Auwerx, J.; Schrauwen, P. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* **14:**612-622; 2011.

[176]   Scribbans, T. D.; Ma, J. K.; Edgett, B. A.; Vorobej, K. A.; Mitchell, A. S.; Zelt, J. G.; Simpson, C. A.; Quadrilatero, J.; Gurd, B. J. Resveratrol supplementation does not augment performance adaptations or fibre-type-specific responses to high-intensity interval training in humans. *Appl Physiol Nutr Metab* **39:**1305-1313; 2014.

[177]   Gliemann, L.; Schmidt, J. F.; Olesen, J.; Bienso, R. S.; Peronard, S. L.; Grandjean, S. U.; Mortensen, S. P.; Nyberg, M.; Bangsbo, J.; Pilegaard, H.; Hellsten, Y. Resveratrol blunts the positive effects of exercise training on cardiovascular health in aged men. *J Physiol* **591:**5047-5059; 2013.

[178]   Olesen, J.; Gliemann, L.; Bienso, R.; Schmidt, J.; Hellsten, Y.; Pilegaard, H. Exercise training, but not resveratrol, improves metabolic and inflammatory status in skeletal muscle of aged men. *J Physiol* **592:**1873-1886; 2014.

[179]   Atherton, P. J.; Smith, K. Muscle protein synthesis in response to nutrition and exercise. *J Physiol* **590:**1049-1057; 2012.

[180]   Nissen, S.; Sharp, R.; Ray, M.; Rathmacher, J. A.; Rice, D.; Fuller, J. C., Jr.; Connelly, A. S.; Abumrad, N. Effect of leucine metabolite beta-hydroxy-beta-methylbutyrate on muscle metabolism during resistance-exercise training. *J Appl Physiol (1985)* **81:**2095-2104; 1996.

[181]   Athenstaedt, K.; Daum, G. Phosphatidic acid, a key intermediate in lipid metabolism. *Eur J Biochem* **266:**1-16; 1999.

[182]   Fang, Y.; Vilella-Bach, M.; Bachmann, R.; Flanigan, A.; Chen, J. Phosphatidic acid-mediated mitogenic activation of mTOR signaling. *Science* **294:**1942-1945; 2001.

[183]   Hoffman, J. R.; Stout, J. R.; Williams, D. R.; Wells, A. J.; Fragala, M. S.; Mangine, G. T.; Gonzalez, A. M.; Emerson, N. S.; McCormack, W. P.; Scanlon, T. C.; Purpura, M.; Jager, R. Efficacy of phosphatidic acid ingestion on lean body mass, muscle thickness and strength gains in resistance-trained men. *J Int Soc Sports Nutr* **9:**47; 2012.

[184]   Joy, J. M.; Gundermann, D. M.; Lowery, R. P.; Jager, R.; McCleary, S. A.; Purpura, M.; Roberts, M. D.; Wilson, S. M.; Hornberger, T. A.; Wilson, J. M. Phosphatidic acid enhances mTOR signaling and resistance exercise induced hypertrophy. *Nutr Metab (Lond)* **11:**29; 2014.

[185]   Mobley, C. B.; Hornberger, T. A.; Fox, C. D.; Healy, J. C.; Ferguson, B. S.; Lowery, R. P.; McNally, R. M.; Lockwood, C. M.; Stout, J. R.; Kavazis, A. N.; Wilson, J. M.; Roberts, M. D. Effects of oral phosphatidic acid feeding with or without whey

protein on muscle protein synthesis and anabolic signaling in rodent skeletal muscle. *J Int Soc Sports Nutr* **12:**32; 2015.

[186] Ikeda, Y.; Murakami, A.; Ohigashi, H. Ursolic acid: an anti- and pro-inflammatory triterpenoid. *Mol Nutr Food Res* **52:**26-42; 2008.

[187] Kunkel, S. D.; Suneja, M.; Ebert, S. M.; Bongers, K. S.; Fox, D. K.; Malmberg, S. E.; Alipour, F.; Shields, R. K.; Adams, C. M. mRNA expression signatures of human skeletal muscle atrophy identify a natural compound that increases muscle mass. *Cell Metab* **13:**627-638; 2011.

[188] Ogasawara, R.; Sato, K.; Higashida, K.; Nakazato, K.; Fujita, S. Ursolic acid stimulates mTORC1 signaling after resistance exercise in rat skeletal muscle. *Am J Physiol Endocrinol Metab* **305:**E760-765; 2013.

[189] Bang, H. S.; Seo, D. Y.; Chung, Y. M.; Oh, K. M.; Park, J. J.; Arturo, F.; Jeong, S. H.; Kim, N.; Han, J. Ursolic Acid-induced elevation of serum irisin augments muscle strength during resistance training in men. *Korean J Physiol Pharmacol* **18:**441-446; 2014.

[190] Church, D.; Schwartz, N.; Spillane, M.; McKinley, S.; Andre, T.; Willoughby, D. S. A comparison of the effects of ursolic acid and l-leucine supplementation on IGF-1 receptor and AKT-mTOR signaling in response to resistance exercise in trained men. *J Int Soc Sports Nutr* **11:**19; 2014.