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Post-Exercise Muscle Glycogen Resynthesis in Humans

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Abstract

Since the pioneering studies conducted in the 1960s in which glycogen status was investigated utilizing the muscle biopsy technique, sports scientists have developed a sophisticated appreciation of the role of glycogen in cellular adaptation and exercise performance, as well as sites of storage of this important metabolic fuel. While sports nutrition guidelines have evolved during the past decade to incorporate sport-specific and periodized manipulation of carbohydrate (CHO) availability, athletes attempt to maximise muscle glycogen synthesis between important workouts or competitive events so that fuel stores closely match to the demands of the prescribed exercise. Therefore, it is important to understand the factors that enhance or impair this biphasic process. In the early post-exercise period (0-4 h), glycogen depletion provides a strong drive for its own resynthesis, with the provision of carbohydrate (CHO; ~ 1 g/kg body mass [BM]) optimizing this process. During the later phase of recovery (4-24 h), CHO intake should meet the anticipated fuel needs of the training/competition, with the type, form and pattern of intake being less important than total intake. Dietary strategies that can enhance glycogen synthesis from sub-optimal amounts of CHO or energy intake are of practical interest to many athletes; in this scenario, the co-ingestion of protein with CHO can assist glycogen storage. Future research should identify other factors that enhance the rate of synthesis of glycogen storage in a limited time-frame, improve glycogen storage from a limited CHO intake or increase muscle glycogen supercompensation.

Keywords: refueling, CHO intake, CHO loading, glycogen synthase

Introduction

Seminal work in the 1960s, using the percutaneous needle biopsy technique to excise small samples of human skeletal muscle, made it possible to conduct invasive studies of metabolism and determine the impact of training, diet and other manipulations on selected biochemical, metabolic, histological and contractile characteristics (for review see 41). Several studies identified muscle glycogen as a major determinant of endurance exercise capacity (10, 12, 80) and an inability to continue exercise when the glycogen stores were restricted (43). Furthermore, several days of diet-exercise manipulation resulted in 'super-compensated' muscle glycogen levels that, in turn, translated into significant improvements in performance of a 'real-life' endurance event (54). Since then, our knowledge about muscle glycogen has expanded to include roles such as fuel sensor, regulator of intracellular signaling pathways promoting exercise training adaptation and mediator of the osmotic characteristics of the muscle cell (38, 39, 50, 61, 81).

Current sport nutrition guidelines recognize that glycogen availability can be strategically manipulated to promote outcomes ranging from enhanced training adaptation through to optimal performance. Indeed, the reader is directed to recent reviews regarding strategies to enhance the cellular response to an exercise stimulus through training with low carbohydrate availability (6, 38). The aim of the current mini-review, however, is to revisit scenarios in which a performance benefit is associated with matching muscle glycogen stores to the fuel requirements of training or competition. We highlight recent advances in our understanding of the optimal nutritional strategies to promote rapid and effective restoration of this important muscle substrate and describe some of the molecular signals by which glucose transport is increased in the exercised muscle after strenuous exercise. The reader is also referred to previous comprehensive reviews on these topics (13, 50, 52).

General Background

Competitive endurance athletes undertake a prodigious volume of training with a substantial amount of exercise performed at intensities that are close to or faster than race pace (115). As such, preparation for and competition in endurance exercise events lasting up to 3 h is dependent on carbohydrate (CHO)-based fuels (muscle and liver glycogen, blood glucose and blood muscle and liver lactate) to sustain high rates of muscle energy production (16, 57, 75, 106). However, the body's reserves of CHO are not as plentiful as those of lipids or proteins, so an important goal of the athlete's daily diet is to provide the trained musculature with the substrates necessary to fuel the training program that supports optimal adaptation and recovery.

Rates of post-exercise glycogen synthesis have been investigated using a variety of exercise protocols and dietary regimens. Depletion of muscle glycogen provides a strong drive for its own resynthesis (116). Indeed, even in the absence of post-exercise CHO intake, glycogen synthesis occurs at rates of 1–2 mmol/kg wet weight (w.w.) of muscle/h through gluconeogenesis (63), or, particularly in the case of high-intensity exercise, lactate (44). However, post-exercise CHO ingestion is the most important determinant of muscle (and liver) glycogen synthesis, with the highest rates of resynthesis (typically within the range of 5–10 mmol/kg w.w./h) observed when large amounts of CHO are consumed soon after the completion of the exercise bout, and then continued throughout recovery. Several factors contribute to the enhanced synthesis rates during the first two hours after exercise: these include activation of glycogen synthase by glycogen depletion (83), as well as exercise-induced increases in insulin sensitivity (87) and permeability of the muscle cell membrane to glucose. Nevertheless, with a mean glycogen storage rate of 5–6 mmol/kg w.w./h, 20–24 h of recovery are normally required for normalization of muscle glycogen levels following extreme exercise depletion (30). This scenario provides a challenge to athletes who undertake multiple sessions of training in a 24 h period (e.g. swimmers, rowers or distance runners) or competition (e.g. tournament tennis, cycling tour) with less than 12-15 h recovery from the first session, after which muscle glycogen content is likely to be reduced by at least 50% (102).

Carbohydrates, Glucose Transport and Glycogen Storage in Human Skeletal Muscle

Glucose, fructose and galactose are the primary monosaccharides in the human diet having an energy value of 15.7 kJ/g and producing ~38 mol of ATP/mol monosaccharide. The most important monosaccharide for muscle metabolism is glucose, which is phosphorylated to glucose 6-phosphate by the enzyme hexokinase and either directed towards glycolysis or glycogen synthesis. Glycogen synthase catalyzes the incorporation of UDP-glucose through α-1-4-glycosidic linkages into the expanding glycogen polymer, with branching enzyme catalyzing formation of α-1,6-branchpoints (31). The many branching points formed by the α-1,6 bonds (approximately every 8-12 glucose units) on the glycogen molecule provide multiple sites for the addition of glucose residues during glycogen synthesis (glycogenesis), or glycogen breakdown during exercise (through glycogenolysis).

Until the discovery of the protein glycogenin as the mechanism for glycogen biogenesis (101), the source of the first glycogen molecule that acted as a primer in glycogen synthesis was not known. Glycogenin is located at the core of the glycogen molecules and is characterized by autocatalytic activity that enables it to transfer glucose residues from UDP-glucose to itself (3). Before glycogenin is able to synthesize a glycogen molecule, it must form a 1:1 complex with glycogen synthase (101). Glycogenin then initiates granule formation by the addition of 7-11 glucose residues to a single tyrosine residue on the protein, which serves as a substrate for glycogen synthase. The branching enzyme and glycogen synthase then act in concert to catalyze the formation of two distinct pools of glycogen: proglycogen (PG) and macro-glyocgen (MG) (59, 60). In the initial stages of glycogen formation, the PG granules grow by the addition of glucose residues forming the larger, mature MG. PG and MG contain the same amount of protein but differ in the number of glycogen units and also in their rates of degradation and synthesis (1, 3, 95). It appears that PG is more sensitive to dietary CHO and is synthesized more rapidly following exercise-induced glycogen depletion, reaching a plateau after 24 h (1). The synthesis of MG is a relatively slower process, persisting for 48 h post-exercise (1). The different rates of synthesis of the PG and MG granules explain, in part, the biphasic pattern of post-exercise glycogen storage (52), and demonstrate that the amount of glycogenin has a direct influence on how much glycogen the muscle cell can store. Factors that influence glycogenin concentrations are largely unexplored and required investigation.

In the period after glycogen-lowering exercise, glycogen synthesis is a key priority for the previously contracted muscles and glycogen synthase activity and glucose transport are increased dramatically to meet this obligatory requirement. Indeed, an enhanced metabolic action of insulin in skeletal muscle (glucose transport, glycogen synthase activity, glycogen synthesis) is observed after glycogen-depleting exercise (85) which can persist for up to 48 h (67). It is this enhanced insulin sensitivity in skeletal muscle that, in large part, contributes to the restoration and, depending on the degree of prior glycogen depletion, even a 'super-compensation' of muscle glycogen stores. While the molecular mechanisms involved in post-exercise increased insulin sensitivity are not fully understood (50), the magnitude of post-exercise glycogen depletion has been strongly linked to the enhanced metabolic action of insulin in this period (85).

Glycogen stores in human muscle (and liver) vary and are largely determined by the training status of the individual and their habitual CHO intake (42). The resting muscle glycogen content of an untrained person consuming a mixed diet is ~80-85 mmol/kg of muscle wet weight (w.w.) and somewhat higher at ~120 mmol/kg w.w. for individuals undertaking regular endurance type exercise training (12). After exhaustive glycogen-depleting exercise and with 36-48 h of a high (>8 g/kg BM) CHO diet, muscle glycogen content can be super-compensated (11), reaching 200 mmol/kg w.w. (97). Because 1 g of glycogen is stored in muscle with 3-5 g of water (76, 98), an athlete's BM typically increases 1-2% after several days of 'CHO-loading' (12). Whereas skeletal muscle glycogen stores provide between 300-700 g of glycogen (depending on the active musculature), a smaller amount of glycogen is stored in the liver, providing ~100-120 g glycogen in an average 75 kg male. Despite the relative small amounts of glycogen stored in the liver, it is the only endogenous source of glucose that directly regulates blood glucose homeostasis. Indeed, in the absence of exogenous CHO ingestion, hypoglycemia will occur when liver glycogen stores become depleted. However, when CHO is ingested during exercise liver glycogen is typically maintained (17, 34). Few studies have determined the impact of CHO ingestion on post-exercise repletion of liver glycogen (33) and brain glycogen (66) and these are beyond the scope of the present review.

Recently, the role and regulation of muscle glycogen have been specified to be dependent on its subcellular localization (74). Using transmission electron microscopy, studies undertaken in the 1970s and 1980s revealed both fiber type differences and a localization-dependent utilization of glycogen during exercise. A quantitative approach (64) has identified three distinct subcellular locations of glycogen: 1) intermyofibrillar glycogen, in which glycogen particles are located between the myofibrils next to sarcoplasmic reticulum and mitochondria; 2) intramyofibrillar glycogen, where glycogen particles are located within the myofibrils between the contractile filaments and 3) subsarcolemmal glycogen whereby glycogen particles are located from the outermost myofibril to the surface membrane. The implications of these distinct pools of glycogen for glycogen resynthesis, muscle function, and fatigue resistance are of key interest but require further investigation before practical recommendations can be made to exploit this knowledge. The remainder of this review will focus on factors that influence muscle glycogen synthesis and strategies that can be used by athletes to enhance muscle glycogen storage, with particular relevance to scenarios in which conditions for glycogen storage are sub-optimal; brief time periods between exercise sessions and/or the inability to consume adequate CHO intake.

Dietary Carbohydrate Intake and Muscle Glycogen Synthesis

Under most conditions, dietary CHO represents the main substrate for muscle glycogen synthesis with factors such as the quantity, timing, and type of CHO intake markedly influencing the rate of muscle glycogen storage.

Amount of carbohydrate intake

Synthesising data from a range of studies that have monitored glycogen storage over 24 h following exercise-induced depletion, including two dose-response studies (19, 28), a ‘glycogen storage threshold’ appears to occur at a daily CHO intake of ~7-10 g/kg body mass (BM) (24). Specific attention has been focussed on the early (0-4 h) phase of recovery because of the slightly higher muscle glycogen synthesis rates during this time, as well as the practical issues of the multi-day exercise programs undertaken by athletes. Initial guidelines recommended that athletes consume 50 g (~1 g/kg BM) of CHO every 2 h during the early period of recovery, based on observations of similar rates of post-exercise glycogen storage following CHO intakes of 0.7 and 1.4 g/kg BM (15), or 1.5 g and 3.0 g/kg BM (48) at such intervals. However, more recent work (33, 82, 109, 111) has reported 30-50% higher rates of glycogen synthesis (10–11 mmol kg ww/kg/h) over the first 4 h of recovery with larger CHO intakes (e.g. >1 g/kg/h), at least when CHO is consumed as repeated small feedings. Thus, when immediate post-exercise refuelling is a priority, current guidelines promote larger intakes of CHO in patterns of frequent consumption.

Timing of carbohydrate intake

The popular concept of a 'window of opportunity' for post-exercise refuelling was created by a well-publicized study (47) which reported that immediate intake of CHO after prolonged exercise resulted in higher rates of glycogen storage (7.7 mmol/kg ww/h) during the first 2 h of recovery, than when this same feeding was delayed after 2 h (~4.4 mmol/kg ww/h). Although these data show more effective glycogen synthesis during early post-exercise recovery, the key finding of that study was that glycogen synthesis rates remained very low until CHO feeding was initiated. Thus, immediate provision of CHO to the muscle cell should be seen as a strategy to initiate effective refuelling rather than to simply take advantage of a period of moderately enhanced glycogen synthesis. This has significance when there is only 4-8 h of recovery between exercise sessions, but a longer (>8 h) recovery time (78) may compensate for a delay in the initial feeding. Indeed, the negative feedback loop from glycogen concentrations on its own synthesis (116) may contribute to the equalization of muscle glycogen content over time.

The frequency of intake of the recommended amounts of CHO (e.g. large meals versus a series of snacks) does not affect glycogen storage in longer-term recovery, despite marked differences in blood glucose and insulin responses (21, 28). This is in apparent conflict to the observations of higher rates of muscle glycogen synthesis during the first 4–6 h of recovery when large amounts of CHO are fed at 15- to 30-min intervals (51, 109, 111). One theory to explain this 'paradox' is that the maintenance of blood glucose and insulin profiles is most important during the first hours of recovery and perhaps when total CHO intake is sub-optimal. However, during longer periods of recovery, or when total CHO intake is above this 'threshold,' manipulations of plasma substrates and hormones within physiological ranges do not confer any additional benefit.

Type of carbohydrate intake

Early studies of single nutrient feedings showed glucose and sucrose to be more effective than fructose in restoring muscle glycogen after exercise (15). This confirmed the hypothesis that glycogen synthesis is more effective with dietary CHO sources that elicit higher blood glucose and insulin responses. However, the results of the first studies of food-derived CHO were inconsistent (28, 88), due to the misuse of the structural classification of 'simple' or 'complex' to predict the glycaemic impact of CHO-rich foods. The subsequent use of published glycaemic index (GI) stores to construct post-exercise diets found that glycogen storage was increased during 24 hours of recovery with a CHO-rich meals based on high-GI foods compared with an identical amount of CHO eaten in the form of low-GI foods (22). However, the magnitude of increase in glycogen storage (~30%) was substantially greater than the difference in 24-h blood glucose and insulin profiles, particularly because the immediate post-exercise meal produced a large glycemic and insulinemic response, independent of the GI of the CHO consumed. Other studies have confirmed greater gut glucose release and greater hepatic glucose output in response to meals immediately post exercise, favouring an increase in muscle glucose uptake and glycogen storage (91). The malabsorption of some very low GI CHO-rich foods was postulated to account for less efficient glycogen storage by reducing the effective amount of CHO consumed; this is supported by observations of lower post-exercise glycogen storage from a poorly digestible high amylose starch mixture compared with intake of glucose, maltodextrins and a high amylopectin starch (53). Finally, a drink containing a special glucose polymer of high molecular weight and low osmolarity was found to enhance glycogen synthesis in the first 2 h of recovery, although this effect disappeared thereafter (82). This benefit was attributed to a faster rate of gastric emptying (58) and may point to the benefits of foods that are rapidly digested and emptied when more rapid glycogen restoration is needed. Nevertheless, in other studies, solid and liquid forms of CHO-rich foods have been found to be equally effective in providing substrate for muscle glycogen synthesis over 2-24 h (55, 84). Indeed, direct comparison to intravenous administration of matched concentrations of glucose in one investigation showed that gastric emptying of foods/drinks was not the rate-limiting process for glycogen synthesis. A separate study, which found that intravenous delivery of supra-physiological concentrations of glucose and insulin can increase rates of post-exercise glycogen synthesis over 8 h to levels achieved by glycogen super-compensation protocols (37), is largely of theoretical interest only since its use contravenes anti-doping rules in sport.

Effect of other dietary factors on glycogen synthesis

Although dietary CHO intake has the most robust effect on muscle glycogen synthesis, rates of glycogen storage may be manipulated by other nutrients or nutrition-related factors. Outcomes of this knowledge can be used to increase glycogen storage by employing strategies to increase muscle glycogen synthesis rates when conditions are sub-optimal (e.g. when total carbohydrate intake is below targets set for maximal synthesis rates or when the refuelling period is limited) or by avoiding factors that can interfere with optimal muscle glycogen synthesis.

Energy intake/energy availability

There is increasing awareness that sub-optimal intake of energy in relation to exercise energy expenditure (termed Relative Energy Deficiency in Sport – RED-S) results in an impairment of energy-requiring activities involved in body maintenance and health such as protein synthesis, bone turnover or hormone pulsatility (69). It is intuitive that glycogen storage could be decreased in the face of inadequate energy intake, either by a down-regulation of the energetics of glycogen synthesis or the reduced availability of glucose for storage due to demands for immediate oxidation. Indeed, there is evidence that the relationship between dietary CHO and glycogen storage is underpinned by total energy intake. For example, glycogen super-compensation protocols were reported to be less effective in female than male athletes (103), but this finding was later reinterpreted as an outcome of the relatively lower energy intake in the female cohort (104). In the latter study, female subjects showed a substantial enhancement of muscle glycogen storage associated with increased dietary CHO intake only after total energy intake was also increased (104). It should be noted that these studies involved a 4-day glycogen loading protocol and did not collect data that would explain the mechanism of energy-related glycogen storage changes. Therefore we are left to speculate whether this is an acute issue related to alternate fates for exogenous CHO when energy intake is sub-optimal and/or a more chronic suppression of glycogen synthesis in the face of low energy availability.

Co-ingestion of other macronutrients

The co-ingestion of other macronutrients, either present in CHO-rich foods or consumed at the same meal, may directly influence muscle glycogen restoration independent of their effect on energy intake. Factors that may directly or indirectly affect glycogen storage include the provision of gluconeogenic substrates, as well as effects on digestion, insulin secretion or the satiety of meals. Protein has received most attention, since an insulinotropic amino acid and/or protein mixture can augment postprandial insulin release and stimulate both glucose uptake and glycogen synthase activity in skeletal muscle tissue (26, 113), thus further accelerating muscle glycogen synthesis. Indeed there is evidence that this occurs when amino acids and/or protein are co-ingested with CHO below the threshold for glycogen storage (e.g. 0.5–0.8 g CHO/kg/h) (9, 45, 46, 111, 112, 117). However, as discussed by Betts and Williams (13), when CHO intake is adequate (e.g. >1 g/kg/h), the co-ingestion of protein has no further effect on glycogen synthesis (8, 51, 109). Protein intakes of around 0.3-0.4 g/kg appear to maximize this effect (13); this is also considered the optimal amount to promote muscle protein synthesis goals (68). The effects of co-ingesting fat with CHO-rich meals on post-exercise glycogen storage have not been systematically investigated. In the only available study involving endurance sport, the addition of fat and protein (0.4 g/kg and 0.3 g/kg BM per meal, respectively) to a diet containing adequate CHO to achieve maximal glycogen storage over 24 h of refueling failed to increase rates of glycogen synthesis despite markedly different responses in blood glucose and free fatty acid concentrations (19).

The consumption of large amounts of alcohol is of interest since this practice often occurs in the post-competition period, particularly in team sports. Separate studies of 8 h and 24 h recovery from glycogen-depleting exercise in well-trained cyclists who consumed ~120 g alcohol (equal to twelve standard drinks) have been undertaken (20). Muscle glycogen storage was reduced during both recovery periods when alcohol displaced an energy-matched amount of

CHO from a standard recovery diet. Evidence for a direct effect of elevated blood alcohol concentrations on muscle glycogen synthesis was unclear, but it appeared that if an immediate impairment of glycogen synthesis existed, it might be compensated by adequate CHO intake and longer recovery time (20).

Other dietary agents that promote glycogen storage

A range of other dietary substances has been studied in relation to their potential to accelerate the rates of muscle glycogen storage or increase glycogen storage from a given amount of CHO, through mechanisms including increased muscle glucose uptake and insulin sensitivity as well as an enhancement of cellular signalling events. With regard to the latter issue, short-term supplementation with creatine monohydrate to increase muscle total creatine content has been shown to upregulate the mRNA content of select genes and proteins involved in a range of cellular activities including glycogen synthesis, with the suggested mechanism being a change in cellular osmolarity (93). **Table 1** summarises studies of glycogen storage in relation to exercise which prior or simultaneous creatine supplementation has been undertaken and includes investigations in which an increase in glycogen storage has been observed in muscle that has been creatine-loaded (32, 71, 77, 90, 100). Although it is not a universal finding, Sewell and colleagues (94) postulated that the glycogen depleting or 'muscle sensitising' effect of exercise is needed to achieve the stimulatory effect of creatine loading on post-exercise glycogen loading. Recently, Roberts et al. (88) reported a greater increase in post-exercise muscle glycogen storage following creatine (20 g/d) supplementation in addition to a high CHO diet. The greater post-exercise increase in muscle glycogen became evident as early as 24 h after exercise and was maintained following 6 days of post-exercise recovery on a CHO-rich diet. Although the mechanism(s) underlying this observation remains to be elucidated, it seems evident that creatine supplementation can further augment muscle glycogen storage. However, it remains to be established whether this effect occurs in highly-trained athletes. Furthermore, the practical implications of any benefits of creatine use to refuelling in endurance athletes should be weighed against the 1-2% gain in body mass that is associated with creatine loading.

Here it should also be noted that changes in muscle water content secondary to the whole body fluid changes experienced by athletes (i.e. hyperhydration and, more commonly, dehydration) could also alter glycogen synthesis due to changes in cell osmolarity and cell volume. This has not been systematically addressed, although an early study investigated the effect of dehydration on glycogen synthesis, based on the hypothesis that the binding of water to glycogen might make cellular hydration a permissive factor in muscle glycogen storage (72). This study found that dehydration equivalent to loss of ~5% BM or 8% body water did not interfere with glycogen storage during 15 h following cycling exercise, although muscle water content was lower than in the trial involving euhydrated recovery. Further investigation is warranted (72).

Other dietary constituents with purported effects on insulin sensitivity and glucose tolerance have been investigated in relation to muscle glycogen storage in various trained and untrained human populations. Studies have shown varying effects of caffeine use on muscle glycogen storage in trained individuals. In one investigation, intake of caffeine (8 mg/kg) with CHO (1 g/kg/h) resulted in substantially higher rates of muscle glycogen storage over 4 h of recovery (79). However, another study (7) found no difference in muscle glycogen synthesis when an hourly caffeine intake of 1.7 mg/kg/h was added to large CHO feedings (1.2 g/kg/h) for a post-exercise recovery period of 6 h. There is no apparent explanation for the discrepancy in these findings and the practicality of using caffeine as a post-exercise refuelling aid must also be questioned in view of its interruption to sleep patterns.

Isolated studies, (**Table 1**), have reported enhancement of muscle glycogen storage following the use of the insulin mimetic fenugreek (containing the unique amino acid 4-hydroxy-leucine, conjugated linoleic acid (CLA), and hydoxycitric acid (HCA) (found in Garcinia Cambogia fruit). However, these findings have not been replicated. For example, although muscle glycogen synthesis during 4 h of recovery was found to be enhanced when an extract isolated from fenugreek was added to a high dose of dextrose (92), a subsequent investigation from the same group failed to find any refuelling advantages after 4 or 15 h of post-exercise recovery when this product was consumed in combination with CHO (99). Therefore it would be premature to consider these ingredients as an aid to accelerate muscle glycogen recovery for competitive athletes.

Non-Dietary Issues: Effects on Glycogen Storage

The effects of muscle damage from the prior exercise bout needs to be considered in the context of refuelling. In particular, rates of glycogen synthesis are impaired after muscle-damaging eccentric contractions and/or impact injuries, due to reductions in GLUT 4 translocation (5) as well as reduced glucose uptake (4). Early laboratory-based work from Costill and colleagues reported that isolated eccentric exercise (29) or exhaustive running (14) was associated with reduced rates of muscle glycogen restoration during 24 and 72 h of post-exercise recovery, with a time course suggesting that this phenomenon did not occur in the early phase (0-6 h) of recovery but was associated with later recovery (114). Although these findings are generally attributed to damage to muscle fibres and local inflammation, glycogen synthesis in damaged muscles might be partially overcome by increased amounts of CHO intake during the first 24 h after exercise (29). Of course, few studies have followed the time-course of muscle glycogen recovery after real-life sporting activities. Several investigations of recovery from competitive soccer have reported a delay in glycogen restoration following football matches (36, 49, 56) such that it remained below resting levels after 24 h of recovery in both Type 1 and Type II fibres and after as much as 48 h of recovery in Type II fibres, despite relative high CHO intakes (36). Although these findings are generally attributed to the eccentric component of the movement patterns in soccer (sudden changes in direction and speed) and direct contact between players, an intervention within one study also found rates of glycogen storage below rates normally associated with recovery from cycling exercise when simulated soccer activities of different duration were undertaken with the removal of the body contact and a reduction in eccentric movements (36). Therefore, further observations of muscle glycogen recovery following competitive sports events is warranted, including the investigation of mechanisms that could explain attenuated muscle synthesis rates.

Since athletes frequently undertake specialised activities after competition or key training sessions to promote various aspects of recovery, it is of interest to consider how such practices might interact with glycogen storage goals. For example, therapies that alter local muscle temperature to alleviate symptoms of exercise-induced muscle damage appear to have some effect on factors that are important in muscle glycogen synthesis, although the overall effect is unclear. In one study, intermittent application of ice reduced net glycogen storage over 4 h of recovery compared to a control leg (108), while in a companion study by the same laboratory, the application of heat was associated with greater refuelling (100). Alterations in blood flow to the muscle secondary to temperature changes were presumed to play a role in these findings, although a reduction in muscle enzyme activities was also suspected to be a factor in explaining the outcomes of ice therapy. However, another study of cold-water immersion following exercise failed to find evidence of impaired glycogen storage during the recovery period (35). Therefore, the benefits of post-exercise application of cold or heat on muscle glycogen repletion following exercise remains to be addressed in future research.

Glycogen supercompensation

Strategies to achieve glycogen super-compensation have slowly evolved since the first description of this phenomenon in the pioneering studies of Bergstrom and co-workers (2, 10-12, 43). These researchers (using themselves as subjects), showed that several days of a low-CHO diet followed by a similar period of high CHO intake resulted in a localized doubling of muscle glycogen concentrations in muscle that had been previously depleted of glycogen through exercise. From this finding, emanated the ‘classical’ 7-day model of CHO loading, involving a 3–4 day ‘depletion’ phase of hard training and low CHO intake, finishing with a 3–4 day ‘loading’ phase of high CHO eating and exercise taper. A subsequent field study (54) and documented implementation by successful athletes illustrated its benefits to performance of distance running and cemented CHO loading into the practice and language of sports nutrition for endurance sports (18). Surprisingly, there have been few refinements of this potentially valuable technique, despite the fact that it was derived from observations on active but essentially untrained individuals. These increments in knowledge are illustrated in **Figure 1**

A decade later, Sherman and colleagues showed that well-trained runners were able to supercompensate muscle glycogen stores with 3 d of taper and a high CHO intake, regardless of whether this was preceded by a depletion phase or a more typical diet and training preparation (97). This ‘modified’ and more practical CHO loading protocol avoids the fatigue and complexity of extreme diet and training requirements associated with the previous depletion phase. A more recent update on the time course of glycogen storage found that it increased significantly from ~90 mmol to ~180 mmol/kg ww with 24 h of rest and high CHO intake, and thereafter remained stable despite another 2 days of the same conditions (25). Although the authors concluded that this was an ‘improved 1-day CHO loading protocol’ (25), the true loading phase from the last training session was ~36 h. In essence, the study provides a midpoint to the glycogen storage observations of Sherman and colleagues (25) and suggests that supercompensation is probably achieved within 36–48 hours of the last exercise session, at least when the athlete rests and consumes adequate CHO intake. Of course, it is not always desirable for athletes to achieve total inactivity in the days prior to competition, since even in a taper some stimulus is required to maintain previously acquired training adaptations (70).

An athlete’s ability to repeat glycogen supercompensation protocols has also been examined. Well-trained cyclists who undertook two consecutive periods of exercise depletion, followed by 48 hours of high CHO intake (12 g/kg/d) and rest, were found to elevate their glycogen stores above resting levels on the first occasion but not the next (62). Further studies are needed to confirm this finding and determine why glycogen storage is attenuated with repeated CHO loading.

Implications for athlete practice

Current sports nutrition guidelines no longer promote a universal message of ‘high CHO intakes at all time’ or the need to maximize muscle glycogen storage. Indeed CHO requirements may be low on days or for athletes where a light/moderate training load has only a modest requirement for glycogen utilization or replacement (23). Intakes may be similarly low when there is a deliberate decision to undertake exercise with low glycogen stores to induce a greater skeletal muscle adaptive response (6), and there may even be benefits from deliberately withholding CHO after a high quality training session to minimise glycogen restoration and extend the period during which adaptive responses are elevated (65). Nevertheless, there are numerous real-life scenarios in which athletes want to optimise muscle glycogen storage, either by accelerating the rates of glycogen synthesis, by promoting greater storage from a given amount of dietary CHO, or by increasing the total muscle glycogen pool. These include super-compensating muscle glycogen stores prior to an endurance/ultra-endurance event (e.g. preparation for a marathon), normalising muscle glycogen for shorter games/events within the weekly training microcycle (e.g. weekly or bi-weekly soccer game), rapidly restoring muscle glycogen between two events or key training sessions held less than 8 h apart (two matches within a tennis tournament or a swimmer's twice daily workouts), and maximising muscle glycogen storage from a diet in which energy intake is restricted (athlete on a weight loss program, restrained eater or an athlete in a weight-making sport). Current sports nutrition guidelines for muscle glycogen storage, summarized in **Table 2**, provide recommendations for both short-term (e.g. 0-6 hours post glycogen-depleting exercise) and longer-term (12-48 h) refuelling (23, 105). While these strategies provide useful practices for many athletes, they are biased towards conditions in which the athlete is able to consume large/optimal amounts of carbohydrate. A range of questions that can extend our current knowledge on muscle glycogen synthesis in more practical ways is provided in **Table 3**.

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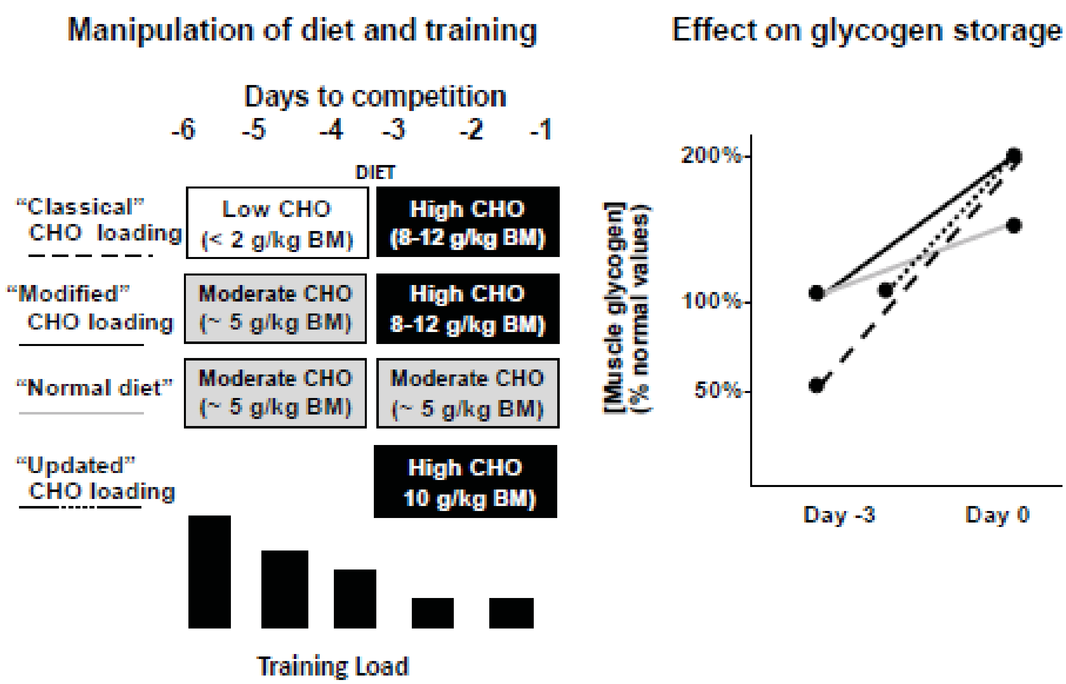
Figure Legends

Figure 1. Evolution of knowledge regarding protocols for carbohydrate (CHO) loading, as illustrated by diet and training manipulations in the 7 day prior to an endurance event. The "Classical" loading protocol for glycogen supercompensation was developed by Bergstrom et al. (10) in untrained active individuals and confirmed in well-trained individuals by Sherman and colleagues (97). A "modified" protocol of high CHO intake and exercise taper, deleting the depletion phase, was found to be similarly successful in athletes in the latter study (96). More recent work suggests that the super-compensation occurs in 24-48 h of taper and high CHO intake in well-trained individuals (25).

Figure 1



Manipulation of diet and training
Effect on glycogen storage
Days to competition
-6 -5 -4 -3 -2 -1
DIET
"Classical" CHO loading
Low CHO (< 2 g/kg BM)
High CHO (8-12 g/kg BM)
"Modified" CHO loading
Moderate CHO (~ 5 g/kg BM)
High CHO 8-12 g/kg BM)
"Normal diet"
Moderate CHO (~ 5 g/kg BM)
Moderate CHO (~ 5 g/kg BM)
"Updated" CHO loading
High CHO 10 g/kg BM)
Training Load
[Muscle glycogen] (% normal values)
200%
100%
50%
Day -3
Day 0

756

757

Table 1. Summary of studies of other dietary constituents that may increase post-exercise muscle glycogen storage

Study	Subject population	Exercise protocol	Supplementation and Recovery feeding protocol	Enhancement of glycogen storage
Caffeine (Caf) – acute supplementation				
Pedersen et al. 2008 (79)	Well trained cyclists (n = 7M)	0-4 h recovery after Severe glycogen severely depleted by intermittent high-intensity cycling bout to fatigue + low CHO diet + 2nd session of steady state exercise to fatigue	Post exercise: 8 mg/kg caffeine + 1 g/kg/h CHO CHO consumed in hourly feedings, while CHO+Caf consumed in two feedings, 2 h apart	Yes Rate of glycogen storage: 13.7 ± 4.4 vs. 9.0 ± 1.8 mmol/kg ww/h (*P* < 0.05) for CHO+Caf vs CHO, with differences occurring due to continued elevation of rates after 1 h. Attributed to higher glucose and insulin concentrations with CHO+Caf trial. Note that glycogen storage rates with CHO+Caf are highest recorded in literature with dietary intakes.
Beelen et al 2012 (7)	Trained cyclists (n = 14 M)	0-6 h recovery after glycogen depleted by intermittent high-intensity cycling bout to fatigue	Post-exercise: 1.7 mg/kg/h caffeine + 1.2 g/kg/h CHO Caf and CHO consumed in snacks every 30 min	No Rate of glycogen storage: 7.1 ± 1 vs. 7.1 ± 1 mmol/kg ww/h (NS) for CHO+Caf vs CHO (Not Significant). Tracer determined rates of exogenous glucose appearance

				showed no difference in absorption of drink CHO.
Creatine (Cr) supplementation – rapid loading or chronic supplementation				
Robinson et al., 1999 (90)	Healthy young subjects (n = 14 M)	Cycling to fatigue (one-legged protocol)	20 g/d Cr + high CHO diet for 5 days after exercise trial	Yes Glycogen was increased above non-exercised concentrations in the exercised limb to a greater degree in the CHO + Cr group (P =0.06) over CHO only
Nelson et al., 2001 (71)	Physically active but untrained young subjects (n = 12 M)	Cycling to fatigue	20 g/d Cr for 5 days prior to exercise trial + 3 d high CHO diet afterwards	Yes Compared with a previous trial involving glycogen depletion + CHO loading, prior Cr loading was associated with ~10% increase in glycogen stores. Noted that prior Cr loading increased efficiency of glycogen storage but not necessarily threshold of glycogen stores.
Op t Eijnde et al., 2001 (77)	Healthy young subjects (n = 13 M, 9 F)	Leg immobilization for 2 weeks followed by 10 w resistance training	20 g/d for 2 weeks of immobilization, 15g/d for first 3 weeks of	Yes, for a period Muscle glycogen levels were higher in the creatine group after 3 weeks of

			rehabilitation, 5g/day for following 7 weeks	rehabilitation (P<0.05) but not after 10 weeks.
Derave et al., 2003 (32)	Healthy young subjects (n = 26 M, 7F)	Leg immobilization for 2 weeks followed by 6 w resistance training	15 g/d Cr during immobilization, 2.5 g/d Cr during training	Yes Creatine supplementation increased muscle glycogen and GLUT-4 protein contents.
Safdar et al., 2008 (93)	Collegiate track and field athletes (n = 12 M)	60 min running exercise and a 100 m sprint running exercise	12 g/day Cr for 15 days	Yes Cr supplementation significantly upregulated (P<0.05) the mRNA and protein content of various proteins involved in the regulation of glycogen synthesis.
Roberts et al., 2016 (89)	Recreationally active males (n = 14 M)	Cycling to fatigue @ 70% VO_2peak	20 g/day Cr + high CHO diet for 6 d after exercise trial	Yes Cr supplementation significantly augmented the post-exercise increase in muscle glycogen content, with differences most apparent during the first 24 h of post-exercise recovery.

Fenugreek – acute supplementation				
Ruby et al. 2005 (92)	Trained cyclists (n = 6 M)	0-4 h recovery after glycogen depletion by 90 min intermittent high intensity cycling bout	Post-exercise: 0.9 g/kg/h CHO + fenugreek extract providing 4 mg/kg 4-hydroxy-leucine CHO consumed in 2 feedings at 15 min and 2 h	Yes Rate of glycogen storage: 10.6 ± 3.3 vs. 6.5 ± 2.6 mmol/kg ww/h for CHO+Fenugreek vs CHO ($p < 0.05$). Underlying mechanism unclear since no differences in blood glucose or insulin concentrations between trials were observed.
Slivka et al. 2008 (99)	Trained cyclists (n = 8 M)	0-4 h and 4-15 h recovery after glycogen depletion by 5 h cycle @ 50% Peak Power Output	Post-exercise: 0.9 g/kg/h CHO + fenugreek extract providing 4 mg/kg 4-hydroxy-leucine CHO consumed in 2 feedings at 15 min and 2 h Further feeding of CHO-rich meals + fenugreek with 2 mg/kg 4-hydroxy-leucine	No No difference in muscle glycogen synthesis at 4 h or 15 h with CHO+Fenugreek vs CHO trials. (Subsequent performance of 40 km TT also unaffected by Fenugreek). Rationale for contradiction of findings of earlier study unclear although differences in glycogen-depleting exercise was noted.

Hydroxycitrate (HCA) - acute supplementation				
Cheng et al. 2012 (27)	12 healthy males Glycogen depletion by 1 h cycling@ 75% VO_2max	0-3 h	Post-exercise: 0.66 g/kg/h CHO + 500 mg HCA Consumed as single meal at 0 h	Yes Rates of muscle glycogen higher post-exercise and post-recovery in CHO+HCA vs CHO ((~ 9 vs 4.1 mmol/kg ww/h). Reduction in GLUT4 protein expression and increase in FAT-CD36 mRNA at 3h in CHO-CLA trial. Blood insulin concentrations lower in CHO+HCA despite similar glucose concentrations. Authors suggested increased glycogen storage due to enhanced lipid metabolism and increase insulin sensitivity.
Conjugated Linoleic Acid (CLA) - chronic supplementation				
Tsao et al. 2015 (107)	12 healthy males	0-3 h recovery after glycogen depletion by 1 h cycling@ 75% VO_2max	Prior supplementation: 8 w @ 3.8 g/d CLA Post-exercise: 0.66 g/kg/h CHO Consumed as single meal at 0 h	Yes Muscle glycogen higher post-exercise and post-recovery in CLA trial than control with elevated rates of storage (~ 5.8 vs 3.3 mmol/kg ww/h). Increased in

				GLUT4 protein expression at 0 and 3 h in CLA trial.

Table 2. Guidelines for promoting post-exercise glycogen storage by athletes (23, 24, 105)

Time period/scenario	Evidence-based guidelines
Optimal storage of glycogen following or between glycogen-limited workouts/events (early phase 0-6 h)	• When the period between exercise sessions is < 8 h, the athlete should consume carbohydrate as soon as practical after the first workout to maximise the effective recovery time • Early post-exercise recovery (0-4 h) may be enhanced by a higher rate of carbohydrate intake (~1 g/kg BM/h), especially when consumed in frequent small feedings • Carbohydrate-rich foods with a moderate-high glycemic index (GI) provide a readily available source of substrate for glycogen synthesis. This may be important in situations where maximum glycogen storage is required in the hours after an exercise bout. Foods with a low GI appear to be less effective in promoting glycogen storage. However, this may be partly due to poor digestibility that overestimates actual carbohydrate intake and may be compensated by additional intake of

	these foods, or the addition of foods with a high GI to meals and snacks. • Adequate energy availability is required to optimise glycogen storage from a given amount of CHO. • The selection of CHO-rich foods and drinks, or the combination of these in meals and snacks should be integrated with the athlete's other nutritional goals related to recovery (e.g. rehydration, muscle protein synthesis) • Athletes should follow sensible practices regarding alcohol intake at all times, but particularly in the recovery period after exercise. Excessive intake of alcohol after exercise may directly inhibit glycogen storage during the period of elevated blood alcohol concentration. However, the most important effects of alcohol intake on refuelling (and other recovery issues) is through a reduced ability, or interest, to implement sports nutrition goals and sensible lifestyle choices
Optimal glycogen storage over 24 h to meet fuel requirements of upcoming events or workouts	• Targets for daily carbohydrate intake are usefully based on body mass (or proxy for the volume of active muscle) and exercise load.

where it is important to perform well and/or with high intensity.	Guidelines can be suggested but need to be fine-tuned according to the athlete's overall dietary goals and feedback from training. ◦ Moderate exercise load: 5-7 g/kg/24 h ◦ Heavy exercise load: 6-10 g/kg/24 h ◦ Extreme exercise load: 8-12 g/kg/24 h • During longer recovery periods (6 h+) when the athlete can consume adequate energy and carbohydrate, the types, pattern and timing of carbohydrate-rich meals and snacks can be chosen according to what is practical and enjoyable. In these circumstances, it doesn't seem to matter whether CHO is consumed as meals or frequent snacks, or in liquid or solid form as long as sufficient CHO is consumed • The selection of CHO-rich foods and drinks, or the combination of these in meals and snacks should be integrated with the athlete's other nutritional goals related to general health and performance (e.g. nutrient density, energy requirements) as well as ongoing recovery goals
Enhanced glycogen storage when the athlete is unable to consume adequate energy or CHO to	• The addition of protein to CHO-rich meals and snacks may promote glycogen storage when carbohydrate intake is sub-optimal especially

optimise glycogen storage (e.g. poor appetite, restrained eater, low energy availability)	during the first hours of recovery. An intake of ~20-25 g of high quality protein appears to optimize this effect while also meeting goals for post-exercise muscle protein synthesis
Glycogen supercompensation prior to endurance events of > 90 min of sustained or intermittent high-intensity exercise	• In the absence of muscle damage, a CHO intake of 8-12 g/kg/ 24 h for 36-48 h in combination with exercise taper can supercompensate muscle glycogen concentrations

Table 3. High priority areas for further research on post-exercise glycogen storage by athletes

- Can dietary strategies alter the restoration of the glycogen stores in various cellular locations and which is more important for performance outcomes?
- What is the role of glycogenin as a permissive or limiting factor for glycogen storage and can it be manipulated?
- Can various dietary strategies enhance muscle glycogen storage from sub-optimal amounts of CHO intake by manipulating more favourable blood glucose and insulin concentrations?
    - Manipulation of pattern of intake of meals and snacks
    - Choice of CHO-rich foods with high glycemic and insulinemic responses
- Can dietary compounds with insulin mimetic activity enhance muscle glycogen storage?
- Can caffeine increase muscle glycogen storage when consumed in modest amounts that are consistent with other health or recovery goals (e.g. lack of interference with sleep)?
    - What is the mechanism of action of any positive effect?
- Can prior or concurrent supplementation with creatine enhance muscle glycogen concentration in well-trained athletes?
    - What is the mechanism of action of any positive effect?
    - Under what conditions does the effect of enhanced muscle fuel stores overcome the weight gain associated with creatine loading?
- Is the positive effect of any such dietary components/manipulations to enhance glycogen storage achieved by increasing glycogen synthesis from a given amount of dietary CHO, increasing the rate of muscle glycogen storage over a given time and/or increasing total muscle glycogen storage capacity or level of supercompensation?

- Does reduced glycogen storage during energy restriction/low energy availability reflect down-regulation of glycogen storage and/or lack of substrate?
- What is the mechanism of the failure to repeat glycogen supercompensation in close succession and can it be overcome?
- What is the mechanism of delayed resynthesis of glycogen following some sporting activities and can it be overcome?
- Do other recovery activities that affect muscle blood flow or temperature enhance or impair muscle glycogen storage?
- How can the impairment of glycogen storage by muscle damage be attenuated?
- Are there special issues for different athlete populations – for example, athletes with disabilities, adolescent and masters athletes?