Liver glycogen metabolism during and after prolonged endurance-type exercise

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Running head: Hepatic glycogen and endurance exercise

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Abstract

Carbohydrate and fat are the main substrates utilized during prolonged endurance-type exercise. The relative contribution of each is primarily determined by the intensity and duration of exercise, along with individual training and nutritional status. During moderate-to-high intensity exercise, carbohydrate represents the main substrate source. As endogenous carbohydrate stores (primarily in liver and muscle) are relatively small, endurance-type exercise performance/capacity is often limited by endogenous carbohydrate availability. Much exercise metabolism research to date has focused on muscle glycogen utilization with little attention to the contribution of liver glycogen. $^{13}$C magnetic resonance spectroscopy permits direct, non-invasive measurements of liver glycogen content and has increased understanding of the relevance of liver glycogen during exercise. In contrast to muscle, endurance-trained athletes do not exhibit elevated basal liver glycogen concentrations. However, there is evidence that liver glycogenolysis may be lower in endurance-trained athletes compared to untrained controls during moderate-to-high intensity exercise. Liver glycogen sparing in an endurance-trained state may therefore partly account for training-induced performance/capacity adaptations during prolonged (>90 min) exercise. Ingestion of carbohydrate at a relatively high rate (>1.5 g/min) can prevent liver glycogen depletion during moderate-intensity exercise, independent of the type of carbohydrate (e.g. glucose vs sucrose) ingested. To minimize gastrointestinal discomfort, it is recommended to ingest specific combinations or types of carbohydrates (glucose plus fructose and/or sucrose). By co-ingesting glucose with either galactose or fructose, post-exercise liver
glycogen repletion rates can be doubled. There are currently no guidelines for carbohydrate ingestion to maximize liver glycogen repletion.

Introduction

Carbohydrate and fat are the primary substrates utilized during prolonged, endurance-type exercise activities in humans (91, 111). The major determinants of fuel selection are the intensity and duration of exercise (19, 91, 111), in addition to training (37, 38, 112) and nutritional status (16, 45, 121). Endogenous carbohydrates are stored as glycogen, primarily in muscle and liver. In contrast to endogenous fat stores (>100,000 kcal; >400 MJ for a 75 kg individual with 15% body fat), glycogen stores are small (<3000 kcal; 13 MJ) and so may limit the capacity for exercise tasks of a moderate-to-high intensity (~50-90% VO$_2$max) lasting more than 45 min (3, 12, 18). The importance of muscle glycogen availability during prolonged exercise has received much attention over the last 50 years (13, 19). In contrast, the role of hepatic glycogen as a substrate source during exercise has been less well studied, largely due to the inaccessibility of tissue samples. This review provides an overview of liver glycogen metabolism during exercise, and the impact of nutritional strategies to modulate hepatic glycogen use and subsequent repletion.

Historical Perspective on Liver Glycogen

The role of carbohydrate-based fuels in manipulating the perception of effort during endurance-type exercise has been known for almost a century (63). The greater reliance on carbohydrate as a substrate source during exercise of
a moderate-to-high intensity was already demonstrated in the 1930’s (20).

The utilization and importance of muscle glycogen as a substrate source during exercise were demonstrated in the 1960’s following the re-introduction of the Bergstrom muscle biopsy technique (12, 13). Since then, there has been much focus on optimizing muscle glycogen availability in relation to human function. Presumably because of the methodological limitations when trying to assess liver glycogen content, only few data have been obtained on the use of liver glycogen during exercise.

Whilst suggestions that liver glycogen contributes to blood glucose homeostasis have been made since at least 1855 (14), it wasn’t until the 1960’s (9, 100) and 70’s (39, 77-79) that researchers were able to take advantage of the “one-second” liver biopsy technique described by Menghini (71) to report on liver glycogen utilization in vivo in humans. It was demonstrated that fasting rapidly depleted liver glycogen content (100), with near complete depletion within 48 h of fasting, or following a (very) low carbohydrate diet (79). Only when sufficient carbohydrate was included in the diet did net repletion of liver glycogen stores begin (79). This was quite a novel finding since the prevailing theory held that gluconeogenesis was the major pathway for liver glycogen synthesis, so would rapidly restore liver glycogen stores even during fasting or carbohydrate intake restriction (5, 48).

In humans, gluconeogenesis [from the major precursors: glycerol, glucogenic amino acids (e.g. alanine) and lactate] contributes ~55% of endogenous glucose production during the first 10 h of fasting (87). Prolonged fasting (64 h) increases the relative contribution of gluconeogenesis to ~96% of
endogenous glucose production, without drastically altering the absolute rate of gluconeogenesis (from ~7 μmol/kg/min to ~8.5 μmol/kg/min (87). The observation that some non-human species (rodents) can synthesize relatively large amounts of liver glycogen during fasting or carbohydrate intake restriction – presumably from gluconeogenesis – highlights the importance of studying liver glycogen physiology in vivo in humans (31, 42, 72, 75).

It wasn’t until the late 1980’s and early 1990’s that $^{13}$C magnetic resonance spectroscopy (MRS) was employed as a non-invasive human liver glycogen measurement tool (60, 92). This non-invasive method allows repeated measures of liver glycogen content to be made, without inducing the catecholamine response that sometimes is induced by biopsy procedures in unaccustomed individuals (102). A theoretical limitation of the method is that only $^{13}$C are detected (since nuclei of $^{12}$C do not posses the magnetic moment required to align with or against the magnetic field). Therefore, consumption of diets differing strongly in the $^{13}$C/$^{12}$C enrichment level of the various carbohydrates may influence the assessment of glycogen content and reduce the signal-to-noise ratio. Nevertheless, the differences in $^{13}$C abundance of C$_3$ and C$_4$ plants and therefore foods is relatively small [1.09 vs 1.10 %$^{13}$C for the C-1 position of glycosyl units in sugar beet vs sugar cane, respectively (43)] when compared to the large changes in liver glycogen concentrations with fasting, exercise and feeding (40, 44, 87). This large signal-to-noise ratio means that differences in carbon fixation between sources of carbohydrates can likely be neglected as a confounding factor in most study designs applying $^{13}$C MRS to assess (liver) glycogen content.
**Regulation of liver glycogen metabolism**

Liver glycogen metabolism is fundamental in the regulation of substrate selection. The most obvious role is in blood glucose homeostasis, with liver glycogen contributing ~45% to total endogenous glucose production during the initial periods of fasting (83, 92), thereby contributing heavily to the maintenance of euglycemia. In the postprandial state, the anatomical location of the liver allows for hepatic glycogen synthesis to buffer excess blood glucose being released into the periphery, attenuating post-prandial hyperglycemia. The vital physiological functions of liver glycogen require rapid metabolic regulation. It is not surprising that liver glycogenolysis and glycogen synthesis occur simultaneously (69, 82, 86), allowing rapid changes in glucose flux. Rates of liver glycogen turnover (glycogen cycling) in humans are not negligible. For example, it has been estimated that during net glycogen synthesis, glycogenolysis can occur at >57% of the rate of net synthesis (69). Similar to muscle glycogen, it has been suggested that a high liver glycogen concentration may directly stimulate liver glycogenolysis (87) and inhibit glycogen synthesis (35) thereby conforming to autoregulation.

The clear importance of liver glycogen metabolism for metabolic control is evidenced both by hypoglycemia during fasting, and by postprandial hyperglycemia in individuals with various disorders of liver glycogen metabolism (66). A complete absence of liver glycogen synthase (glycogen storage disease type 0; GSD-0) is associated with an almost complete inability to store liver glycogen, excess hepatic lipid accumulation, fasting
hypoglycemia and postprandial hyperglycemia (66). A deficiency of glucose-6-phosphatase (GSD-1a) is associated with excessive liver glycogen accumulation and also produces fasting hypoglycemia (26). Therefore, both an inability to adequately synthesize or hydrolyze liver glycogen is associated with numerous metabolic abnormalities.

In addition to assisting in the delivery and storage of glucose under fasting and postprandial conditions, liver glycogen may also assist with blood glucose homeostasis by modulating non-esterified fatty acid (NEFA) availability during periods of limited carbohydrate availability. In rodents, liver glycogen may partly regulate adipose tissue lipolysis during fasting whereby the increase in adipose tissue lipolysis correlates with the reduction in liver glycogen content (52). Overexpression of glycogen synthase 2 increases liver glycogen content and adipose tissue mass, while suppressing HSL phosphorylation in adipose tissue (52). Furthermore, knockdown of glycogen synthase 2 reduces liver glycogen and accelerates the loss of adipose tissue mass, which appears to be due to liver glycogen per se and not due to downstream metabolites in response to glycogenolysis (52). Interestingly, this regulation of adipose tissue lipolysis by liver glycogen is dependent on neural circuitry rather than hormonal milieu, since hepatic vagotomy suppresses the effect of liver glycogen depletion on adipose tissue lipolysis (52). This interaction between liver glycogen and adipose tissue would presumably assist in maintaining blood glucose homeostasis by allowing muscle and other organs access to NEFAs for oxidation and thereby allow for a reduction in blood glucose utilization. There is also evidence in humans of hepatic glycogen regulation by
fatty acid and glycerol delivery, whereby NEFAs and glycerol can potently suppress net hepatic glycogenolysis by ~84% (98). Moreover, this does not appear to be solely due to glycerol delivery as a gluconeogenic precursor, since glycerol delivery alone only suppressed hepatic glycogenolysis by ~46% (98). This demonstrates the intricate crosstalk between liver and adipose tissue to maintain adequate substrate availability during extreme conditions.

Hepatic glycogen regulation is also under the control of circulating insulin, glucagon, epinephrine and possibly norepinephrine concentrations (Figure 1). Hepatic glycogen synthesis rates are half-maximal at a portal vein insulin concentration of ~160 pmol/L and plateau above ~200 pmol/L (86). Nonetheless, even when hepatic glycogen synthase activity is maximal, other factors can further augment net hepatic glycogen synthesis rates. Hyperglycemia (10 mmol/L) augments net glycogen synthesis rates in the presence of hyperinsulinemia by suppressing glycogen phosphorylase activity (82). Suppression of glucagon secretion also results in higher hepatic glycogen synthase activity and thus elevates net hepatic glycogen synthesis rates by ~66% compared to fasting concentrations (86).

Liver glycogen can be synthesized via either a direct pathway (glucose → glucose-6-phosphate → glucose-1-phosphate → uridine diphosphate-glucose → glycogen), or via an indirect pathway through 3-carbon atom precursors and subsequent gluconeogenesis (64). In resting humans in the overnight fasted state, consumption of a substantive (824 kcal) mixed-macronutrient meal containing ~140 g (~1.82 g/kg BM) glucose, increases liver glycogen
content through both direct (46-68% contribution in early and late postprandial periods) and indirect pathways (101). The average rate of net glycogen repletion from pre-feeding until peak liver glycogen concentration (~5 h postprandial) was 20 mmol/L/h (~6 g/h) (101). When subsequent meals are ingested, the proportion of liver glycogen synthesis via the direct pathway increases to ~77% (68).

The catecholamine epinephrine may also be directly involved in liver glycogen regulation. In patients with skeletal muscle metabolic disorders such as McArdle's disease (glycogen phosphorylase deficiency), epinephrine concentrations and hepatic glucose output are both more than two-fold higher during exercise, compared to healthy controls, which compensates for impaired muscle glycogen metabolism (119). Epinephrine is a potent stimulator of hepatic glucose output both directly and indirectly (by reducing insulinemia). When infused at rates equivalent to that seen during moderate-to-high intensity exercise (60-80% VO\textsubscript{2}max), epinephrine increases endogenous glucose production 2.5-fold above basal (32). Interestingly, this increase is almost entirely accounted for by hepatic glycogenolysis, which rises 4-fold above basal, whereas gluconeogenesis does not contribute substantially until >60 min of epinephrine infusion (32). During short-duration (20 min), high intensity (78% VO\textsubscript{2}peak) exercise however, the role of catecholamines is less clear, as α- and β-adrenergic antagonists do not alter endogenous glucose appearance (50). Norepinephrine is ~30-fold less potent at stimulating endogenous glucose production than epinephrine and is likely to play little if any role in hepatic glycogen regulation (25, 39, 70). Sympathetic
hepatic neurons are also unlikely to play a major role in liver glycogenolysis during exercise, as liver transplant recipients (assumed to have no hepatic innervation) have similar exercise-induced endogenous glucose appearance rates compared to controls of kidney transplant recipients (62). This suggests that hepatic neurons may not play a major role in endogenous glucose appearance in healthy humans. Whether the balance between gluconeogenesis and glycogenolysis is regulated by innervation remains to be determined.

Training status, muscle and liver glycogen content

Both acute (13) and chronic (10) exercise drastically alter muscle glycogen availability. Supercompensation of muscle glycogen occurs after a single bout of exercise and is specific to the muscle that was recruited during exercise (13). Endurance-type exercise training leads to a chronic upregulation of muscle glycogen concentrations in the basal state, with availability increased by 20-66%, compared to concentrations observed in the untrained state (10, 44, 67, 97, 117). Insulin sensitivity may play a role in this effect since insulin resistance is strongly associated with impaired muscle glycogen storage (81), and thus individuals with type 2 diabetes (T2D) display little variation in muscle glycogen content with feeding throughout a day (67). Interestingly, this is despite no structural differences in fasting muscle glycogen contents between T2D and healthy, age- and bodyweight-matched controls (67). Therefore, insulin sensitivity may be more tightly coupled to muscle glycogen turnover rather than absolute muscle glycogen content.
Higher basal muscle glycogen availability, in combination with a reduced reliance on muscle glycogen as a substrate source during prolonged endurance-type exercise, may postpone the point at which muscle glycogen depletion contributes to fatigue. However, in the trained athlete, higher absolute and relative exercise intensities can be maintained for a prolonged period of time (28), making it still possible to reach a critically low level of muscle glycogen. Therefore greater muscle glycogen storage may, at least partly, be responsible for greater performance/capacity during prolonged endurance-type exercise.

Liver glycogen stores do not appear to differ following prolonged endurance-type exercise training nor with differing insulin sensitivity. Following the ingestion of mixed-macronutrient meals containing carbohydrate, there is no detectable difference in net liver glycogen synthesis in individuals with insulin resistance (81) or T2D (67). T2D patients, however, display a 50% higher contribution from indirect pathways, at the expense of direct pathways of liver glycogen synthesis (21). Moreover, by combining data from studies including both muscle and liver glycogen data in humans in the overnight fasted state (44, 67, 97), it is apparent that basal liver and muscle glycogen stores respond similarly to insulin resistance but differently to endurance training (Figure 2). Whilst the archetypal adaptation in muscle of a ~66% increase in fasting glycogen concentration is observed (Figure 2A), there is no difference in fasting liver glycogen concentrations across the spectrum of insulin sensitivity (Figure 2B). These findings are also supported by the lack of change in liver glycogen storage with acute exercise in the presence of
enhanced muscle glycogen storage (85). Future work should seek to establish 
whether endurance-type exercise training alters liver glycogen storage in the 
early postprandial period, which would have implications for endurance 
performance in competitive events when pre-event meals are consumed. It is 
also interesting to note that the liver has a ~5-fold higher glycogen 
concentration than in muscle in untrained individuals, and that the diameter of 
glycogen in liver is also ~7-fold larger than glycogen in muscle (1). Since liver 
glycogen content (in the overnight fasted state) does not appear to be 
elevated in endurance trained athletes when compared to healthy controls, 
this cannot contribute to the enhanced performance/capacity seen with 
endurance-type exercise training.

**Liver glycogen metabolism during exercise**

Liver glycogenolysis during exercise has been estimated using numerous 
methods. These include arteriovenous difference (AV$_{\text{diff}}$), stable isotope and 
radioisotope tracers, and $^{13}$C magnetic resonance spectroscopy (MRS). AV$_{\text{diff}}$ 
and stable-/radio-isotope tracers provide an indirect estimate of net 
glycogenolysis by subtracting estimated rates of gluconeogenesis (by 
gluconeogenic precursor tracer incorporation into glucose) from estimates of 
endogenous glucose production (by isotope tracer dilution). These methods 
are subject to inherent assumptions, some of which include estimating the 
fractional contribution of a certain precursor to total gluconeogenesis, the 
inability to account for other endogenous sources of glucose (73, 99), and the 
inability to account for liver glycogen that is either converted to lactate (95) or 
oxidized within the liver before entering the systemic circulation. Hepatic VO$_2$
increases from ~60 mL/min at rest to 135 mL/min during exercise (34, 76, 120), therefore liver metabolic rate and glucose utilization will increase which may augment liver glycogen utilization. Liver glycogen that is hydrolysed and oxidized as glucose within the liver would not be detected by indirect methods such as AV_diff or stable/radio-isotope techniques. Since $^{13}$C MRS allows for a direct assessment of liver glycogen content (46), it can be used to assess net liver glycogenolysis in humans during exercise. However, $^{13}$C MRS alone cannot be used to determine turnover, therefore since all methods have (different) limitations, combining methods would be a suitable strategy to best understand liver glycogen metabolism. In order to gain insight into liver glycogenolysis during exercise we performed a review of the literature (PubMed, March 2016) including the search terms ‘glycogenolysis’, ‘gluconeogenesis’, ‘glucose’, ‘glycogen’ ‘liver’ and ‘hepatic’. Studies were limited to healthy humans only, studied during exercise in a fasted state (Table 1). The vast majority of studies have been performed on adult males during cycling-based exercise, with five studies reporting data from females (38, 51, 84, 89, 90) and only one study using treadmill-based exercise (84). Where studies had estimated rates of gluconeogenesis and endogenous glucose production, the difference between the two was assumed to be net liver glycogenolysis. In order to adequately assess the relationship between exercise intensity, training status and liver glycogenolysis, only studies that reported sufficient information to derive absolute (W) and relative (% VO$_2$peak) exercise intensities during cycling were included in linear regression analyses. Despite differences in methodologies and their inherent
assumptions, combining data across studies provides a remarkably consistent picture regarding net liver glycogenolysis during exercise (Figure 3).

In untrained individuals, rates of liver glycogenolysis markedly increase in the transition from low to high intensity exercise, when expressed as either absolute (Figure 3A) or relative (Figure 3B) intensities. The acceleration of liver glycogenolysis with increasing exercise intensity is dampened in endurance-trained athletes (Figure 3C and 2D) when compared to healthy, untrained controls. This attenuation of liver glycogenolysis at higher exercise intensities in trained athletes appears robust, since the difference in the gradient of the line between trained vs untrained remains (and is in fact augmented) if only studies that are of comparable exercise intensities are included (data not shown). The attenuation of liver glycogenolysis in endurance-trained athletes is likely to have implications for endurance performance/capacity. For example, trained cyclists sustain ~82% VO2peak (~300 W) during 120 min time trials (103). The rate of liver glycogenolysis at this relative intensity would be 6.9 vs 5.3 mg/kg/min in untrained vs trained cyclists, respectively (Figures 2C vs 2D, respectively). When assuming liver volume to be ~1.8 L (40), liver glycogen content would reach critically low levels (>70% depletion) by 118 min of exercise at 80% VO2peak in untrained individuals, leading to an inability to maintain blood glucose homeostasis and/or premature fatigue. Endurance-trained athletes, having a similar starting liver glycogen concentration after overnight fasting (Figure 2), would not reach a critically low liver glycogen content until 153 min of exercise, performed at 80% VO2peak, due to the lower rate of liver glycogenolysis.
Consistent with this reasoning, inhibition of adipose tissue lipolysis during exercise by nicotinic acid impairs prolonged (120 min) cycling time trial performance/capacity (~2.4%) and the decline in power output coincides with a decline in plasma glucose concentrations occurring between 80 and 120 min (103). Inhibition of adipose tissue lipolysis accelerates plasma glucose utilization exercise intensities above (49, 80), but not below ~60% VO₂max (114, 116). Therefore considering most race-pace intensities are >80% VO₂max, these findings are consistent with the idea that enhanced liver glycogen depletion through reducing NEFA and glycerol availability (98), leads to a decline in plasma glucose concentrations and impaired performance/capacity in the absence of carbohydrate ingestion.

The mechanisms by which endurance-type exercise training influences liver glycogen utilization during exercise are most likely due to changes in the hormonal response to exercise. An acute bout of prolonged exercise results in a rise in plasma glucagon, epinephrine and norepinephrine, and a reduction in plasma insulin concentrations (23). Endurance type exercise training blunts the rise in glucagon (22), norepinephrine and epinephrine (23) and lessens the decline in plasma insulin during moderate intensity exercise (60% VO₂peak) (23). During maximal exercise however, endurance trained athletes display a greater rise in epinephrine, norepinephrine and glucagon concentrations compared to untrained controls (61). This suggests that a blunted hormonal response to exercise with endurance-type exercise training is only observed when exercise is performed at the same absolute intensity and/or a moderate intensity. Since liver glycogen metabolism has only been
studied at exercise intensities <80%VO$_2$max (Table 1), it is unknown whether
the exaggerated hormonal response seen in endurance-trained athletes alters
liver glycogen utilization during maximal exercise.

We propose that endurance-type exercise training reduces both liver and
muscle glycogen use during exercise at equivalent absolute, as well as
relative workloads, which may contribute to improved endurance
performance/capacity. Whilst liver glycogen concentrations do not seem to
differ between endurance-trained and untrained individuals, endurance-
trained athletes utilize less liver glycogen during moderate-to-high intensity
exercise (60-80 %VO$_2$max). The lower rate of liver glycogenolysis in the
endurance-trained state likely contributes to the greater endurance
performance/capacity by facilitating the maintenance of (high) carbohydrate
oxidation rates and blood glucose homeostasis during the latter stages of
exercise.

**Nutrition and liver glycogen metabolism during exercise**

From a quantitative perspective, carbohydrates form the most important fuel
source during prolonged moderate-to-high intensity (>60% VO$_2$max)
endurance-type exercise. Consequently, in the absence of exogenous
carbohydrate delivery, endogenous liver and muscle glycogen stores are
lowered by 40-60% within 90 min of exercise at 70% VO$_2$peak (18, 97).

Carbohydrate ingestion during prolonged exercise improves
performance/capacity (118). Mechanisms suggested to explain the
improvement in exercise tolerance include maintenance of euglycemia,
maintenance of (high) carbohydrate oxidation rates, and sparing of muscle
glycogen (19, 108). Muscle glycogen sparing has been demonstrated by
some (96, 109, 110), but not all studies (27, 36, 47, 58), which is likely
attributed to the timing of measurements performed (96), and the type of
exercise and/or muscle fiber type recruitment (110).

Studies using stable isotope or radioisotope tracers to assess hepatic glucose
output have demonstrated that moderate glucose ingestion (~0.6-0.8 g/min)
can suppress (17), and large amounts (~3 g/min) even abolish hepatic
glucose output during exercise (59). Based upon these findings, it has also
been suggested that carbohydrate ingestion during exercise inhibits liver
glycogenolysis and as such, attenuates the decline in liver glycogen content
(19). This was recently tested with the application of $^{13}$C magnetic resonance
spectroscopy to assess net changes in liver glycogen content during exercise
with or without carbohydrate ingestion (44). Whereas liver glycogen content
was reduced by 50% during 3 hours of cycling, exogenous carbohydrate
ingestion (1.7 g/min glucose or sucrose) fully prevented a net decline in liver
glycogen content (44). Therefore, when attempting to prevent or reduce liver
glycogen depletion during endurance-type exercise, it is advisable to
consume exogenous carbohydrate.

It remains unknown whether carbohydrate ingestion during exercise
influences liver glycogen turnover. Based on previous literature, a relatively
high rate of glucose ingestion (1.7 g/min) would suppress endogenous
glucose appearance by anywhere from 60% to complete suppression (17,
No research to date has established whether liver glycogenolysis occurs during exercise with carbohydrate ingestion at rates similar to those recommended for performance/capacity in prolonged endurance-type exercise (0.5-1.5 g/min). Whilst there are no detectable net changes in liver glycogen concentration when large amounts of carbohydrates are ingested, the ingested carbohydrates could either be stored as de novo glycogen and/or directly released into the systemic circulation as glucose or lactate.

**Post-exercise liver glycogen synthesis**

The impact of endogenous glycogen stores on endurance performance/capacity makes rapid post-exercise glycogen repletion a priority when performance/capacity needs to be restored within a limited time-frame (e.g. within 24 h). Such rapid repletion of endogenous glycogen stores is important during multi-day tournaments and stage-races. Post-exercise muscle glycogen repletion rates can be accelerated with ample carbohydrate ingestion (1.2 g/kg BM/h) (8, 15, 115). It is also becoming increasingly apparent that glucose-fructose mixtures are unlikely to further augment post-exercise muscle glycogen repletion over glucose (polymers) alone (40, 106, 122). However, when ingesting such large amounts of carbohydrates (>1.2 g/kg BM/h) during the early stages of post-exercise recovery, the ingestion of specific combinations and types of carbohydrates (glucose plus fructose and/or sucrose) seem to be better tolerated than the ingestion of glucose (polymers) only (40).
In contrast to the wealth of data pertaining to skeletal muscle, only a handful of studies have investigated the impact of carbohydrate ingestion on post-exercise liver glycogen repletion (18, 29, 30, 40, 74). When only glucose (polymers) are ingested, maximum liver glycogen repletion rates are ~13 mmol/L/h, which translates to ~4 g of liver glycogen per hour (18, 29, 40). Interestingly this appears to be independent of the amount of carbohydrate ingested within the range of 0.25-1.5 g/kg BM/h (18, 29, 30, 40). The reported liver glycogen repletion rates following post-exercise glucose (polymer) feeding tend to be substantially lower than the ~20 mmol/L/h (~6 g/h) liver glycogen repletion rates reported at rest following a mixed-macronutrient meal (101). It could be speculated that fat and protein co-ingestion with carbohydrate might further augment net liver glycogen synthesis by providing gluconeogenic precursors (from glycerol and some amino acids). Furthermore, the greater post-prandial insulin release following the ingestion of a mixed meal may augment net glucose uptake and storage in liver glycogen (4, 16, 113, 115).

Since fructose and galactose are preferentially metabolized by the liver at rest (7, 41, 78), co-ingestion of either fructose or galactose with glucose can further augment post-exercise liver glycogen repletion rates (18, 29, 40). The ingestion of fructose (including sucrose) (18, 29, 40) or galactose (29, 30) with glucose can nearly double liver glycogen repletion rates from ~13 to ~25 mmol/L/h (from ~ 4 to ~8 g/h), largely independent of the total amount of carbohydrate ingested (Figure 4A). The magnitude of liver glycogen depletion however, may also modulate liver glycogen repletion rates (Figure 4B) (35).
Co-ingesting fructose alongside glucose likely accelerates liver glycogen repletion due to faster intestinal absorption of glucose-fructose mixtures when compared to the ingestion of either glucose or fructose in isolation (54, 56, 57). Moreover, combined ingestion of glucose with fructose enhances fructose absorption (107) via mechanism(s) that remain to be elucidated. The greater intestinal absorption rate following combined ingestion of glucose plus fructose, making use of both apical membrane transport proteins (SGLT1 and GLUT5 (6, 88)) also accounts for the reduction in gastrointestinal discomfort when large amounts of carbohydrate are ingested (29, 55).

To directly compare liver and muscle glycogen repletion rates post-exercise, measurements of both muscle and liver glycogen concentration within the same individual are required. To date, this has only been performed in vivo in humans in two studies, following ingestion of either a low- (0.25/kg BM/h) (18) or a high-carbohydrate ingestion rate (1.5 g/kg BM/h) (40). When ample amounts of carbohydrate were ingested (1.5 g/kg BM/h) as a glucose-fructose mixture, glycogen repletion rates were shown to be substantially higher in liver than muscle, at least when expressed per unit volume: ~19 vs ~11 mmol/L/h in liver vs muscle, respectively (40). However, when expressed as time to complete restoration of glycogen stores, liver repletion may take considerably longer than muscle glycogen repletion. For example, cycling to exhaustion at 70% VO$_2$max can reduce liver and muscle glycogen concentrations from ~386 to ~170 mmol/L [-874 to ~385 mmol/kg DM assuming a liver density of 1.06 g/cm$^3$ (94) and a wet-to-dry mass ratio of 2.4 (77)] and from ~159 to ~62 mmol/L [-600 to ~240 mmol/kg DM assuming a muscle density of 1.112 g/cm$^3$]
g/cm³ (123) and a wet-to-dry mass ratio of 4.28 (53), respectively (18). The restoration of these glycogen concentrations at exhaustion back to baseline would require 11 vs 9 h for the liver vs muscle. This is in contrast to data from rodents, which suggest that post-exercise liver glycogen restoration is more rapid than muscle (24).

Current evidence suggests that glucose-fructose mixtures further enhance post-exercise liver glycogen repletion rates over glucose (polymer) ingestion only, whilst also reducing gastrointestinal discomfort. Co-ingestion of other macronutrients with carbohydrate may modulate post-exercise liver glycogen repletion but more work will be required to understand the impact of nutrition on liver glycogen metabolism both during, as well as after exercise.

Conclusions
Liver glycogen is both an important substrate store and also represents a strong signal facilitating appropriate fuel selection to support prolonged endurance-type exercise. Changes in liver glycogen metabolism following endurance-type exercise training include a reduction in net glycogenolysis during moderate-to-high intensity exercise in the fasted state, at the same absolute as well as the same relative workload, without an upregulation of basal liver glycogen content. Nonetheless, this adaptation can be of sufficient magnitude to explain the ergogenic effects of exercise training. In the absence of carbohydrate ingestion, liver glycogen stores are substantially depleted within 90 min of moderate-to-high intensity exercise. Ingesting carbohydrate in the form of either glucose or sucrose (glucose-fructose) lessens – and can
even fully prevent - the decline in liver glycogen content during endurance-type exercise, which is likely to be a key aspect in positively influencing exercise performance/capacity.

When rapid replenishment of liver glycogen stores is an aim, ingestion of glucose plus fructose allows more rapid liver glycogen repletion rates when compared to the ingestion of glucose only. There is currently a lack of evidence on the appropriate type and amount of ingested carbohydrate necessary to prevent liver glycogen depletion during exercise, or to maximize post-exercise liver glycogen repletion. Further work is warranted to assess the impact of co-ingesting other macronutrients on liver glycogen metabolism.

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References


Figure 1. Regulation of liver glycogen metabolism under conditions of fasting, feeding and exercise. Glycogen synthesis is stimulated by insulin and inhibited by glucagon (and indirectly by epinephrine through insulin inhibition) (86). Glycogenolysis is stimulated by glucagon and epinephrine, and inhibited by insulin, glucose and non-esterified fatty acids (NEFA) (32, 82, 98). Gluconeogenesis is stimulated by glucagon and epinephrine and inhibited by insulin (32). The role of norepinephrine in hepatic glycogen metabolism is likely to be minimal in humans (25, 39, 70). Green lines indicate stimulation, red lines indicate inhibition.
Figure 2. Liver (A) and muscle (B) glycogen concentrations in humans after an overnight fast in the resting state. Data are means ± 95%CI. Data extracted from Macauley et al. (67), Gonzalez et al. (44) and Stevenson et al. (97). For comparison to biopsy literature, muscle glycogen concentrations equate to 262 ± 19, 262 ± 35 and 434 ± 39 mmol/kg DM [assuming a muscle density of 1.112 g/cm³ (123) and a wet-to-dry mass ratio of 4.28 (53)] in type 2 diabetes, healthy controls and endurance-trained, respectively. Liver glycogen concentrations equate to 670 ± 70, 738 ± 111 and 636 ± 96 mmol/kg DM [assuming a liver density of 1.06 g/cm³ (94) and a wet-to-dry mass ratio of 2.4 (77)] in type 2 diabetes, healthy controls and endurance-trained, respectively.
Figure 3. Net liver glycogenolysis rate as a function of absolute (A and C) and relative (B and D) exercise intensity in healthy untrained controls (A and B) and endurance-trained (C and D) humans. See Table 1 for details of studies. Dashed lines represent 95% CI.
Figure 4. Post-exercise liver glycogen repletion rates during short-term recovery (4-6 hours) with varying types of carbohydrate ingestion plotted against carbohydrate ingestion rate (A) or liver glycogen content post-exercise (B). Data were extracted from references (18), (29) and (40). Where values were reported as mmol/L/h (30), liver volume was assumed to be 1.8 L to convert to g/h.
Table 1: Studies estimating liver glycogenolysis during endurance-type exercise in healthy humans.

<table>
<thead>
<tr>
<th>Article</th>
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<th>Participants</th>
<th>Exercise mode</th>
<th>Exercise Duration (min)</th>
<th>Exercise intensity (% VO_{peak})</th>
<th>Net liver glycogenolysis (mg/kg/min)</th>
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<td>2.72</td>
<td>Isotope tracers ([(^{13})C]- &amp; [(^{14})C]-lactate incorporation into glucose)</td>
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<td>Coggan et al. 1995</td>
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<td>Isotope tracers ([(^{13})C]-bicarbonate incorporation into [(^{13})C]-glucose)</td>
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<td>3.95</td>
<td>Isotope tracers ([(^{13})C]-glucose recycling rate)</td>
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<td>(2,3,4,6-2H)-glucose and L-[1,2,3-\textsuperscript{13}C]-alanine incorporation into glucose</td>
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<td>Friedlander et al. 1998</td>
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<td>60 min</td>
<td>45 min</td>
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<td>(\textsuperscript{13}C)-glucose recycling rate (\textsuperscript{13}C)-glucose recycling rate</td>
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<td>(6,6-\textsuperscript{2}H\textsubscript{2})-glucose and L-[\textsuperscript{3-13}C]-lactate into glucose</td>
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<td>Casey et al. 2000</td>
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<td>Huidekoper et al. 2013 (51)</td>
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<td>Time (min)</td>
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<td>Gonzalez et al. 2015</td>
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<td>Webster et al. 2016</td>
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<td>Isotope tracers $\text{(6,6-}$-$^2\text{H}_2$-$^\text{glucose and }^2\text{H}_2\text{O by incorporation of}$ $^2\text{H into glucose at C-1,3,4,5,6 and 6)}$</td>
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F, females; M, males; NR, not reported.