Liver glycogen metabolism during and after prolonged endurance-type exercise

Javier T. Gonzalez,1 Cas J. Fuchs,2 James A. Betts,1 and Luc J. C. van Loon2

1Department for Health, University of Bath, Bath, United Kingdom; and 2Department of Human Biology and Movement Sciences, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre+, Maastricht, The Netherlands

Submitted 17 June 2016; accepted in final form 12 July 2016

Gonzalez JT, Fuchs CJ, Betts JA, van Loon LJ. Liver glycogen metabolism during and after prolonged endurance-type exercise. Am J Physiol Endocrinol Metab 311: E543–E553, 2016. First published July 19, 2016; doi:10.1152/ajpendo.00232.2016.—Carbohydrate and fat are the main substrates utilized during prolonged endurance-type exercise. The relative contribution of each is determined primarily 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. Because 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 paid to the contribution of liver glycogen. 13C magnetic resonance spectroscopy permits direct, noninvasive 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 with untrained controls during moderate- to high-intensity exercise. Therefore, liver glycogen sparing in an endurance-trained state may account partly 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 coingesting glucose with either galactose or fructose, postexercise liver glycogen repletion rates can be doubled. There are currently no guidelines for carbohydrate ingestion to maximize liver glycogen 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 1930s (20). The utilization and importance of muscle glycogen as a substrate source during exercise were demonstrated in the 1960s following the reintroduction of the Bergstrom muscle biopsy technique (12, 13). Ever 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.

Although suggestions that liver glycogen contributes to blood glucose homeostasis have been made since at least 1855 (14), it was not until the 1960s (9, 100) and 1970s (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 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, and so it 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 to ~8.5 μmol·kg⁻¹·min⁻¹) (87). The observation that some nonhuman 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 1980s and early 1990s that 13C magnetic resonance spectroscopy (MRS) was employed as a noninvasive human liver glycogen measurement tool (60, 92). This noninvasive method allows repeated measurements 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 13C is detected (since nuclei of 12C do not possess the magnetic moment required to align with or against the magnetic field). Therefore, consumption of diets differing strongly in the 13C/12C enrichment level of the various carbohydrates may influence the assessment of glycogen content and reduce the signal-to-noise ratio. Nevertheless, the differences in 13C abundance of C₃ and C₄ plants are relatively small (1.09 vs. 1.10% 13C for the C-1 position of glycosyl units in sugar beet vs. sugar cane, respectively; see Ref. 43) compared with 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 13C 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 anatomic location of the liver allows for hepatic glycogen synthesis to buffer excess blood glucose being released into the periphery, attenuating postprandial 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) 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 is associated with excessive liver glycogen accumulation and also produces fasting hypoglycemia (26). Therefore, 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 nonesterified fatty acid (NEFA) availability during periods of limited carbohydrate availability. In rodents, liver glycogen may partially 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 hormone-sensitive lipase 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 due solely to glycerol delivery as a gluconeogenic precursor, since glycerol delivery alone suppressed hepatic glycogenolysis by only ~46% (98). This demonstrates the intricate cross-talk 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 norepi-
Epinephrine is a potent stimulator of hepatic glucose output during exercise compared with healthy controls, which compensates for impaired muscle glycogen metabolism (119). 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 twofold higher during exercise compared with 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% \(V_{\text{O2 max}}\)), epinephrine increases endogenous glucose production 2.5-fold above basal (32). Interestingly, this increase is accounted for almost entirely by hepatic glycogenolysis, which rises fourfold above basal, whereas gluconeogenesis does not contribute substantially until >60 min of epinephrine infusion (32). During short-duration (20 min), high-intensity (78% \(V_{\text{O2 peak}}\)) exercise, however, the role of catecholamines is less clear, as \(\alpha-\) and \(\beta\)-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 with controls of kidney transplant recipients (62). This suggests that hepatic neurons may not play a major role in endogenous glucose appearance in 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 with 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 typical day (67). Interestingly, this is true despite no structural differences in fasting muscle glycogen contents between T2D and healthy, age-matched, and body weight-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 be at least partly 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 (Fig. 2). Whereas the archetypal adaptation in muscle of an ~66% increase in fasting glycogen concentration is observed (Fig. 2A), there is no difference in fasting liver glycogen concentrations across the spectrum of insulin sensitivity (Fig. 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 an approximately fivefold higher glycogen concentration than in muscle in untrained individuals and that the diameter of glycogen in liver is also approximately sevenfold 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 compared with 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 (AVdiff), stable isotope and radioisotope tracers, and $^{13}$C magnetic resonance spectroscopy (MRS). AVdiff and stable/radioisotope 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 $\dot{V}_{\text{O}_2}$ increases from ~60 ml/min at rest to 135 ml/min during exercise (34, 76, 120), and therefore, liver metabolic rate and glucose utilization will increase, which may augment liver glycogen utilization. Liver glycogen that is hydroyzed and oxidized as glucose within the liver would not be detected by indirect methods such as AVdiff or stable/radioisotope 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, and therefore, since all methods have (different) limitations, combining methods would be a suitable strategy to best understand liver glycogen metabolism. To gain insight into liver glycogenolysis during exercise, we performed a review of the literature (PubMed, March 2016), including the search terms “glycogenolysis,” “gluconeogenesis,” “glycogen,” “liver,” “glycogen,” 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. 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 ($\%\dot{V}_{\text{O}_2,\text{peak}}$) 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 (Fig. 3).

In untrained individuals, rates of liver glycogenolysis markedly increase in the transition from low- to high-intensity exercise when expressed as either absolute (Fig. 3A) or relative (Fig. 3B) intensities. The acceleration of liver glycogenolysis with increasing exercise intensity is dampened in endurance-trained athletes (Fig. 3, C and D) compared with 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

---

**AJP-Endocrinol Metab • doi:10.1152/ajpendo.00232.2016 • www.ajpendo.org**
Table 1. Studies estimating liver glycogenolysis during endurance-type exercise in healthy humans

<table>
<thead>
<tr>
<th>Article</th>
<th>n</th>
<th>Participants</th>
<th>Exercise Mode</th>
<th>Exercise Duration, min</th>
<th>Exercise Intensity, %V\textsubscript{O2peak} (W)</th>
<th>Net Liver Glycogenolysis, mg·kg\textsuperscript{-1}·min\textsuperscript{-1}</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wahren et al. (120)</td>
<td>10</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>40</td>
<td>26 (65)</td>
<td>3.24</td>
<td>Splanchnic arteriovenous difference (total precursors)</td>
</tr>
<tr>
<td>Wahren et al. (120)</td>
<td>9</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>40</td>
<td>52 (130)</td>
<td>5.09</td>
<td></td>
</tr>
<tr>
<td>Wahren et al. (120)</td>
<td>6</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>40</td>
<td>78 (196)</td>
<td>8.22</td>
<td></td>
</tr>
<tr>
<td>Ahlborg et al. (2)</td>
<td>6</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>40</td>
<td>32 (80)</td>
<td>3.40</td>
<td>Splanchnic arteriovenous difference (total precursors)</td>
</tr>
<tr>
<td>Sestoft et al. (93)</td>
<td>5</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>35</td>
<td>50 (122)</td>
<td>0.95</td>
<td>Splanchnic arteriovenous difference (total precursors)</td>
</tr>
<tr>
<td>Stanley et al. 1988 (95)</td>
<td>7</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>50</td>
<td>43 (101)</td>
<td>2.72</td>
<td>Isotope tracers [\textsuperscript{13}C- and \textsuperscript{14}C-lactate incorporation into glucose]</td>
</tr>
<tr>
<td>Coggan et al. (23)</td>
<td>6</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>120</td>
<td>60 (126)</td>
<td>6.31</td>
<td>Isotope tracers [\textsuperscript{13}C-bicarbonate incorporation into \textsuperscript{13}C-glucose]</td>
</tr>
<tr>
<td>Coggan et al. (23)</td>
<td>6</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>120</td>
<td>45 (126)</td>
<td>3.30</td>
<td>Isotope tracers [\textsuperscript{13}C-bicarbonate incorporation into \textsuperscript{13}C-glucose]</td>
</tr>
<tr>
<td>Friedlander et al. (37)</td>
<td>19</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>60</td>
<td>46 (90)</td>
<td>3.95</td>
<td>Isotope tracers [\textsuperscript{13}C-glucose recycling rate]</td>
</tr>
<tr>
<td>Friedlander et al. (37)</td>
<td>19</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>60</td>
<td>59 (153)</td>
<td>4.13</td>
<td>Isotope tracers [\textsuperscript{13}C-glucose recycling rate]</td>
</tr>
<tr>
<td>Lavoie et al. (65)</td>
<td>5</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>120</td>
<td>40 (NR)</td>
<td>4.87</td>
<td>Isotope tracers [\textsuperscript{2,3,4,6,6-2H}glucose and \textsuperscript{L-[1,2,3-13C]}alanine incorporation into glucose]</td>
</tr>
<tr>
<td>Friedlander et al. (38)</td>
<td>17</td>
<td>Untrained (F)</td>
<td>Cycling</td>
<td>60</td>
<td>45 (45)</td>
<td>4.21</td>
<td>Isotope tracers [\textsuperscript{13}C-glucose recycling rate]</td>
</tr>
<tr>
<td>Friedlander et al. (38)</td>
<td>17</td>
<td>Trained (F)</td>
<td>Cycling</td>
<td>60</td>
<td>50 (79)</td>
<td>4.48</td>
<td>Isotope tracers [\textsuperscript{13}C-glucose recycling rate]</td>
</tr>
<tr>
<td>Bergman et al. 2000 (11)</td>
<td>9</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>60</td>
<td>45 (86)</td>
<td>3.60</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{L-(3-13C)}lactate into glucose]</td>
</tr>
<tr>
<td>Bergman et al. 2000 (11)</td>
<td>9</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>60</td>
<td>54 (149)</td>
<td>3.68</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{L-(3-13C)}lactate into glucose]</td>
</tr>
<tr>
<td>Casey et al. (18)</td>
<td>6</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>83</td>
<td>70 (NR)</td>
<td>12.03</td>
<td>\textsuperscript{13}C magnetic resonance spectroscopy at natural abundance</td>
</tr>
<tr>
<td>Trimmer et al. (104)</td>
<td>8</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>90</td>
<td>45 (125)</td>
<td>3.36</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{2-(13C)}glycerol]</td>
</tr>
<tr>
<td>Trimmer et al. (105)</td>
<td>8</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>90</td>
<td>45 (125)</td>
<td>5.26</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{2-(13C)}glycerol with mass isotopomer distribution analysis]</td>
</tr>
<tr>
<td>Roef et al. (90)</td>
<td>6</td>
<td>Untrained (F/M)</td>
<td>Cycling</td>
<td>90</td>
<td>15 (39)</td>
<td>1.21</td>
<td>\textsuperscript{13}C magnetic resonance spectroscopy at natural abundance</td>
</tr>
<tr>
<td>Roef et al. 2003 (89)</td>
<td>7</td>
<td>Untrained (F/M)</td>
<td>Cycling</td>
<td>240</td>
<td>34 (46)</td>
<td>2.11</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{2H2O} by incorporation of \textsuperscript{2H} from pyruvate into glucose at C-6]</td>
</tr>
<tr>
<td>Petersen et al. (84)</td>
<td>6</td>
<td>Untrained (F/M)</td>
<td>Running</td>
<td>50</td>
<td>35 (–)</td>
<td>1.54</td>
<td>\textsuperscript{13}C magnetic resonance spectroscopy at natural abundance</td>
</tr>
<tr>
<td>Stevenson et al. (97)</td>
<td>9</td>
<td>Trained (M; high-glycemic index diet)</td>
<td>Cycling</td>
<td>90</td>
<td>70 (247)</td>
<td>3.17</td>
<td>\textsuperscript{13}C magnetic resonance spectroscopy at natural abundance</td>
</tr>
<tr>
<td>Stevenson et al. (97)</td>
<td>9</td>
<td>Trained (M; low-glycemic index diet)</td>
<td>Cycling</td>
<td>90</td>
<td>70 (247)</td>
<td>2.90</td>
<td>\textsuperscript{13}C magnetic resonance spectroscopy at natural abundance</td>
</tr>
<tr>
<td>Huidekoper et al. (51)</td>
<td>4</td>
<td>Untrained (F/M)</td>
<td>Cycling</td>
<td>90</td>
<td>55 (142)</td>
<td>3.51</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{2H2O} by incorporation of \textsuperscript{2H} from pyruvate into glucose at C-6]</td>
</tr>
<tr>
<td>Emhoff et al. (33)</td>
<td>6</td>
<td>Untrained (M)</td>
<td>Cycling</td>
<td>60</td>
<td>68 (161)</td>
<td>4.50</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{L-[3-13C]}lactate into glucose]</td>
</tr>
<tr>
<td>Emhoff et al. (33)</td>
<td>6</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>60</td>
<td>75 (159)</td>
<td>5.70</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{L-[3-13C]}lactate into glucose]</td>
</tr>
<tr>
<td>Gonzalez et al. 2015 (44)</td>
<td>14</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>180</td>
<td>56 (165)</td>
<td>4.20</td>
<td>\textsuperscript{13}C magnetic resonance spectroscopy at natural abundance</td>
</tr>
<tr>
<td>Webster et al. (124)</td>
<td>7</td>
<td>Trained (M)</td>
<td>Cycling</td>
<td>120</td>
<td>72 (202)</td>
<td>5.30</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{2H2O} by incorporation of \textsuperscript{2H} from pyruvate into glucose at C-1,3,4,5,6 and 6]</td>
</tr>
<tr>
<td>Webster et al. (124)</td>
<td>7</td>
<td>Trained (M; low habitual carbohydrate intake)</td>
<td>Cycling</td>
<td>120</td>
<td>72 (203)</td>
<td>3.20</td>
<td>Isotope tracers [\textsuperscript{6,6-2H2}glucose and \textsuperscript{2H2O} by incorporation of \textsuperscript{2H} from pyruvate into glucose at C-1,3,4,5,6 and 6]</td>
</tr>
</tbody>
</table>

F, females; M, males; NR, not reported.
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% VO₂ peak) (23). During maximal exercise, however, endurance-trained athletes display a greater rise in epinephrine, norepinephrine, and glucagon concentrations compared with untrained controls (61). This suggests that a blunted hormonal response to exercise with endurance-type exercise training is observed only when exercise is performed at the same absolute intensity and/or a moderate intensity. Since liver glycogen metabolism has been studied only at exercise intensities <80% VO₂ 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. Whereas 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% VO₂ 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% \( \dot{V}O_2_{max} \)) 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% \( \dot{V}O_2_{peak} \) (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) can 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 h 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 carbohydrates.

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, 59). 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). Although there are no detectable net changes in liver glycogen concentration when large amounts of carbohydrates are ingested, the ingested carbohydrates could be either stored as de novo glycogen and/or directly released into the systemic circulation as glucose or lactate.

Postexercise Liver Glycogen Synthesis

The impact of endogenous glycogen stores on endurance performance/capacity makes rapid postexercise 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 multiday tournaments and stage races. Postexercise muscle glycogen repletion rates can be accelerated with ample carbohydrate ingestion (1.2 g·kg body mass\(^{-1}\)·h\(^{-1}\)) (8, 15, 115). It is also becoming increasingly apparent that glucose-fructose mixtures are unlikely to further augment postexercise muscle glycogen repletion over glucose (polymers) alone (40, 106, 122). However, when ingesting such large amounts of carbohydrates (>1.2 g·kg body mass\(^{-1}\)·h\(^{-1}\)) during the early stages of postexercise 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 postexercise liver glycogen repletion (18, 29, 30, 40, 74). When only glucose (polymers) are ingested, maximum liver glycogen repletion rates are ∼13 mmol·l\(^{-1}\)·h\(^{-1}\), which translates to ∼4 g liver glycogen/h (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 body mass\(^{-1}\)·h\(^{-1}\) (18, 29, 30, 40). The reported liver glycogen repletion rates following postexercise glucose (polymer) feeding tend to be substantially lower than the ∼20 mmol·l\(^{-1}\)·h\(^{-1}\) (∼6 g/h) liver glycogen repletion rates reported at rest following a mixed-macronutrient meal (101). It could be speculated that fat and protein coingestion with carbohydrate might further augment net liver glycogen synthesis by providing gluconeogenic precursors (from glycerol and some amino acids). Furthermore, the greater postprandial 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), coingestion of either fructose or galactose with glucose can further augment postexercise 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·1⁻¹·h⁻¹ (from ~4 to ~8 g/h), largely independent of the total amount of carbohydrate ingested (Fig. 4A). The magnitude of liver glycogen depletion, however, may also modulate liver glycogen repletion rates (Fig. 4B) (35). Co-ingesting fructose alongside glucose likely accelerates liver glycogen repletion due to faster intestinal absorption of glucose-fructose mixtures compared with the ingestion of either glucose or fructose in isolation (54, 56, 57). Moreover, combined ingestion of glucose with fructose enhances fructose absorption (107) via a mechanism(s) that remains to be elucidated. The greater intestinal absorption rate following combined ingestion of glucose plus fructose, making use of both apical membrane transport proteins(sodium-dependent glucose transporter 1 and glucose transporter 5; see Refs. 6 and 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 postexercise, measurements of both muscle and liver glycogen concentration within the same individual are required. To this date, this has only been performed in vivo in humans in two studies following ingestion of either a low-(0.25 g·kg body mass⁻¹·h⁻¹) (18) or a high-carbohydrate ingestion rate (1.5 g·kg body mass⁻¹·h⁻¹) (40). When ample amounts of carbohydrate were ingested (1.5 g·kg body mass⁻¹·h⁻¹) as a glucose-fructose mixture, glycogen repletion rates were shown to be substantially higher in liver than in muscle, at least when expressed per unit volume: ~19 vs. ~11 mmol·1⁻¹·h⁻¹ 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% VO₂max can reduce liver and muscle glycogen concentrations from ~386 to ~170 mmol/l (~874 to ~385 mmol/kg dry mass (DM), assuming a liver density of 1.06 g/cm³ (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/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 postexercise liver glycogen restoration is more rapid than muscle (24).

Current evidence suggests that glucose-fructose mixtures further enhance postexercise liver glycogen repletion rates over glucose (polymer) ingestion only while also reducing gastrointestinal discomfort. Coingestion of other macronutrients with carbohydrate may modulate postexercise 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 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 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) lessen 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 compared with 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 postexercise liver glycogen repletion. Further work is warranted to assess the impact of coingesting other macronutrients on liver glycogen metabolism.

DISCLOSURES

No sources of funding were used to prepare this article. The authors declare no conflicts of interest that directly relate to this paper.

AUTHOR CONTRIBUTIONS


REFERENCES

10. Bergman BC, Butterfield GE, Wolfe EE, Lopaschuk GD, Casazza GA, Horning MA, Brooks GA. Muscle net glucose uptake and glucose...


60. Krogh A, Lindhard J.
72. Lewis GM, Spencer-Peet J, Stewart KM.
73. Lavoie C, Ducros F, Bourque J, Langelier H, Chiasson JL.