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_2max) 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 milleu, 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_2max), 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_2peak) 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_{diff}), stable isotope and radioisotope tracers, and ^{13}C magnetic resonance spectroscopy (MRS). AV_{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

294 increases from ~60 mL/min at rest to 135 mL/min during exercise (34, 76,
295 120), therefore liver metabolic rate and glucose utilization will increase which
296 may augment liver glycogen utilization. Liver glycogen that is hydrolysed and
297 oxidized as glucose within the liver would not be detected by indirect methods
298 such as AV_{diff} or stable/radio-isotope techniques. Since ^{13}C MRS allows for a
299 direct assessment of liver glycogen content (46), it can be used to assess net
300 liver glycogenolysis in humans during exercise. However, ^{13}C MRS alone
301 cannot be used to determine turnover, therefore since all methods have
302 (different) limitations, combining methods would be a suitable strategy to best
303 understand liver glycogen metabolism. In order to gain insight into liver
304 glycogenolysis during exercise we performed a review of the literature
305 (PubMed, March 2016) including the search terms 'glycogenolysis',
306 'gluconeogenesis', 'glucose', 'glycogen' 'liver' and 'hepatic'. Studies were
307 limited to healthy humans only, studied during exercise in a fasted state
308 (**Table 1**). The vast majority of studies have been performed on adult males
309 during cycling-based exercise, with five studies reporting data from females
310 (38, 51, 84, 89, 90) and only one study using treadmill-based exercise (84).
311 Where studies had estimated rates of gluconeogenesis and endogenous
312 glucose production, the difference between the two was assumed to be net
313 liver glycogenolysis. In order to adequately assess the relationship between
314 exercise intensity, training status and liver glycogenolysis, only studies that
315 reported sufficient information to derive absolute (W) and relative (%
316 VO_2peak) exercise intensities during cycling were included in linear
317 regression analyses. Despite differences in methodologies and their inherent

318 assumptions, combining data across studies provides a remarkably consistent
319 picture regarding net liver glycogenolysis during exercise (**Figure 3)**.
320
321 In untrained individuals, rates of liver glycogenolysis markedly increase in the
322 transition from low to high intensity exercise, when expressed as either
323 absolute (**Figure 3A**) or relative (**Figure 3B**) intensities. The acceleration of
324 liver glycogenolysis with increasing exercise intensity is dampened in
325 endurance-trained athletes (**Figure 3C and 2D)** when compared to healthy,
326 untrained controls. This attenuation of liver glycogenolysis at higher exercise
327 intensities in trained athletes appears robust, since the difference in the
328 gradient of the line between trained vs untrained remains (and is in fact
329 augmented) if only studies that are of comparable exercise intensities are
330 included (data not shown). The attenuation of liver glycogenolysis in
331 endurance-trained athletes is likely to have implications for endurance
332 performance/capacity. For example, trained cyclists sustain ~82% VO_2peak
333 (~300 W) during 120 min time trials (103). The rate of liver glycogenolysis at
334 this relative intensity would be 6.9 vs 5.3 mg/kg/min in untrained vs trained
335 cyclists, respectively (Figures 2C vs 2D, respectively). When assuming liver
336 volume to be ~1.8 L (40), liver glycogen content would reach critically low
337 levels (>70% depletion) by 118 min of exercise at 80% VO_2peak in untrained
338 individuals, leading to an inability to maintain blood glucose homeostasis
339 and/or premature fatigue. Endurance-trained athletes, having a similar starting
340 liver glycogen concentration after overnight fasting (**Figure 2**), would not
341 reach a critically low liver glycogen content until 153 min of exercise,
342 performed at 80% VO_2peak, due to the lower rate of liver glycogenolysis.

343 Consistent with this reasoning, inhibition of adipose tissue lipolysis during
344 exercise by nicotinic acid impairs prolonged (120 min) cycling time trial
345 performance/capacity (~2.4%) and the decline in power output coincides with
346 a decline in plasma glucose concentrations occurring between 80 and 120
347 min (103). Inhibition of adipose tissue lipolysis accelerates plasma glucose
348 utilization exercise intensities above (49, 80), but not below ~60% VO_2max
349 (114, 116). Therefore considering most race-pace intensities are >80%
350 VO_2max, these findings are consistent with the idea that enhanced liver
351 glycogen depletion through reducing NEFA and glycerol availability (98),
352 leads to a decline in plasma glucose concentrations and impaired
353 performance/capacity in the absence of carbohydrate ingestion.
354
355 The mechanisms by which endurance-type exercise training influences liver
356 glycogen utilization during exercise are most likely due to changes in the
357 hormonal response to exercise. An acute bout of prolonged exercise results in
358 a rise in plasma glucagon, epinephrine and norepinephrine, and a reduction in
359 plasma insulin concentrations (23). Endurance type exercise training blunts
360 the rise in glucagon (22), norepinephrine and epinephrine (23) and lessens
361 the decline in plasma insulin during moderate intensity exercise (60%
362 VO_2peak) (23). During maximal exercise however, endurance trained athletes
363 display a greater rise in epinephrine, norepinephrine and glucagon
364 concentrations compared to untrained controls (61). This suggests that a
365 blunted hormonal response to exercise with endurance-type exercise training
366 is only observed when exercise is performed at the same absolute intensity
367 and/or a moderate intensity. Since liver glycogen metabolism has only been

368 studied at exercise intensities <80%VO_2max (Table 1), it is unknown whether
369 the exaggerated hormonal response seen in endurance-trained athletes alters
370 liver glycogen utilization during maximal exercise.
371
372 We propose that endurance-type exercise training reduces both liver and
373 muscle glycogen use during exercise at equivalent absolute, as well as
374 relative workloads, which may contribute to improved endurance
375 performance/capacity. Whilst liver glycogen concentrations do not seem to
376 differ between endurance-trained and untrained individuals, endurance-
377 trained athletes utilize less liver glycogen during moderate-to-high intensity
378 exercise (60-80 %VO_2max). The lower rate of liver glycogenolysis in the
379 endurance-trained state likely contributes to the greater endurance
380 performance/capacity by facilitating the maintenance of (high) carbohydrate
381 oxidation rates and blood glucose homeostasis during the latter stages of
382 exercise.
383
384 **Nutrition and liver glycogen metabolism during exercise**
385 From a quantitative perspective, carbohydrates form the most important fuel
386 source during prolonged moderate-to-high intensity (>60% VO_2max)
387 endurance-type exercise. Consequently, in the absence of exogenous
388 carbohydrate delivery, endogenous liver and muscle glycogen stores are
389 lowered by 40-60% within 90 min of exercise at 70% VO_2peak (18, 97).
390 Carbohydrate ingestion during prolonged exercise improves
391 performance/capacity (118). Mechanisms suggested to explain the
392 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,

418 59). No research to date has established whether liver glycogenolysis occurs
419 during exercise with carbohydrate ingestion at rates similar to those
420 recommended for performance/capacity in prolonged endurance-type
421 exercise (0.5-1.5 g/min). Whilst there are no detectable net changes in liver
422 glycogen concentration when large amounts of carbohydrates are ingested,
423 the ingested carbohydrates could either be stored as *de novo* glycogen and/or
424 directly released into the systemic circulation as glucose or lactate.
425
426 **Post-exercise liver glycogen synthesis**
427 The impact of endogenous glycogen stores on endurance
428 performance/capacity makes rapid post-exercise glycogen repletion a priority
429 when performance/capacity needs to be restored within a limited time-frame
430 (e.g. within 24 h). Such rapid repletion of endogenous glycogen stores is
431 important during multi-day tournaments and stage-races. Post-exercise
432 muscle glycogen repletion rates can be accelerated with ample carbohydrate
433 ingestion (1.2 g/kg BM/h) (8, 15, 115). It is also becoming increasingly
434 apparent that glucose-fructose mixtures are unlikely to further augment post-
435 exercise muscle glycogen repletion over glucose (polymers) alone (40, 106,
436 122). However, when ingesting such large amounts of carbohydrates (>1.2
437 g/kg BM/h) during the early stages of post-exercise recovery, the ingestion of
438 specific combinations and types of carbohydrates (glucose plus fructose
439 and/or sucrose) seem to be better tolerated than the ingestion of glucose
440 (polymers) only (40).
441

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_2max 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

492 g/cm^3 (123) and a wet-to-dry mass ratio of 4.28 (53)], respectively (18). The
493 restoration of these glycogen concentrations at exhaustion back to baseline
494 would require 11 vs 9 h for the liver vs muscle. This is in contrast to data from
495 rodents, which suggest that post-exercise liver glycogen restoration is more
496 rapid than muscle (24).
497
498 Current evidence suggests that glucose-fructose mixtures further enhance
499 post-exercise liver glycogen repletion rates over glucose (polymer) ingestion
500 only, whilst also reducing gastrointestinal discomfort. Co-ingestion of other
501 macronutrients with carbohydrate may modulate post-exercise liver glycogen
502 repletion but more work will be required to understand the impact of nutrition
503 on liver glycogen metabolism both during, as well as after exercise.
504
505 **Conclusions**
506 Liver glycogen is both an important substrate store and also represents a
507 strong signal facilitating appropriate fuel selection to support prolonged
508 endurance-type exercise. Changes in liver glycogen metabolism following
509 endurance-type exercise training include a reduction in net glycogenolysis
510 during moderate-to-high intensity exercise in the fasted state, at the same
511 absolute as well as the same relative workload, without an upregulation of
512 basal liver glycogen content. Nonetheless, this adaptation can be of sufficient
513 magnitude to explain the ergogenic effects of exercise training. In the absence
514 of carbohydrate ingestion, liver glycogen stores are substantially depleted
515 within 90 min of moderate-to-high intensity exercise. Ingesting carbohydrate in
516 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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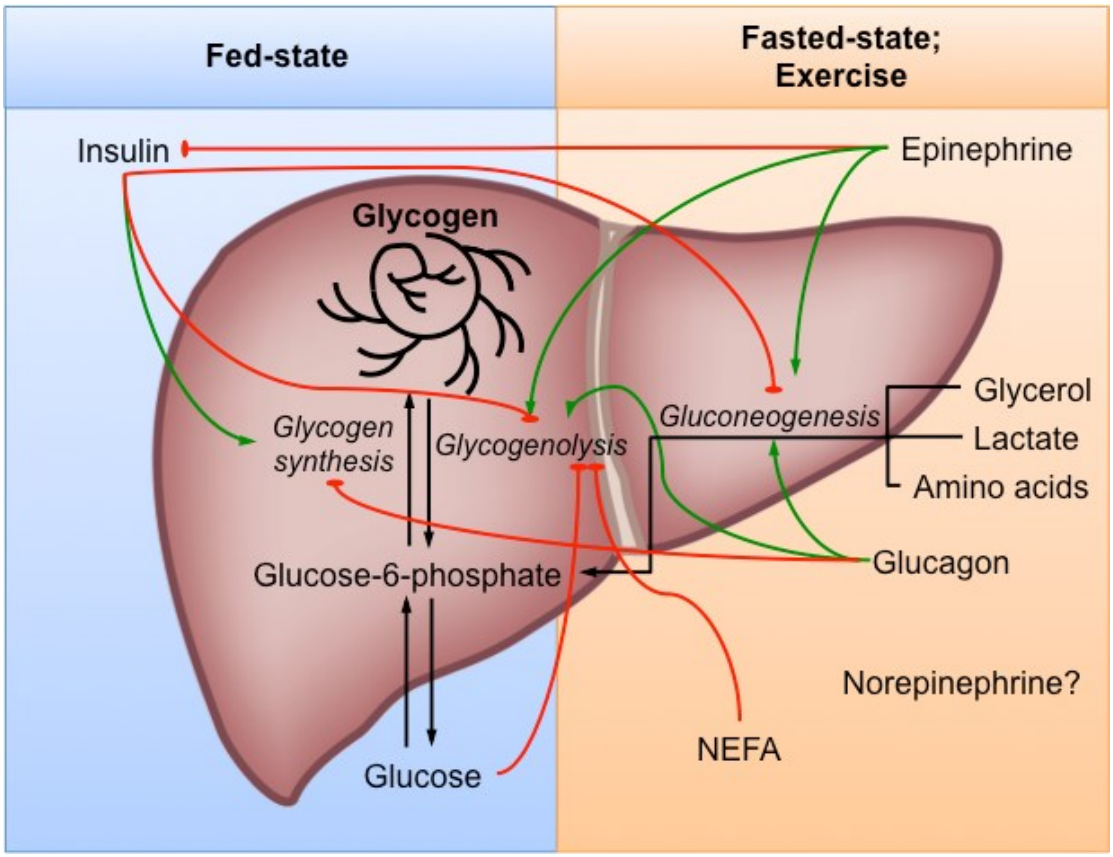

Figure 1. Regulation of liver glycogen metabolism under conditions of fasting, feeding and exercise. Glycogen synthesis is stimulated by insulin and inhibited by glucagon (and indirectly by epinephrine through insulin inhibition) (86). Glycogenolysis is stimulated by glucagon and epinephrine, and inhibited 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.

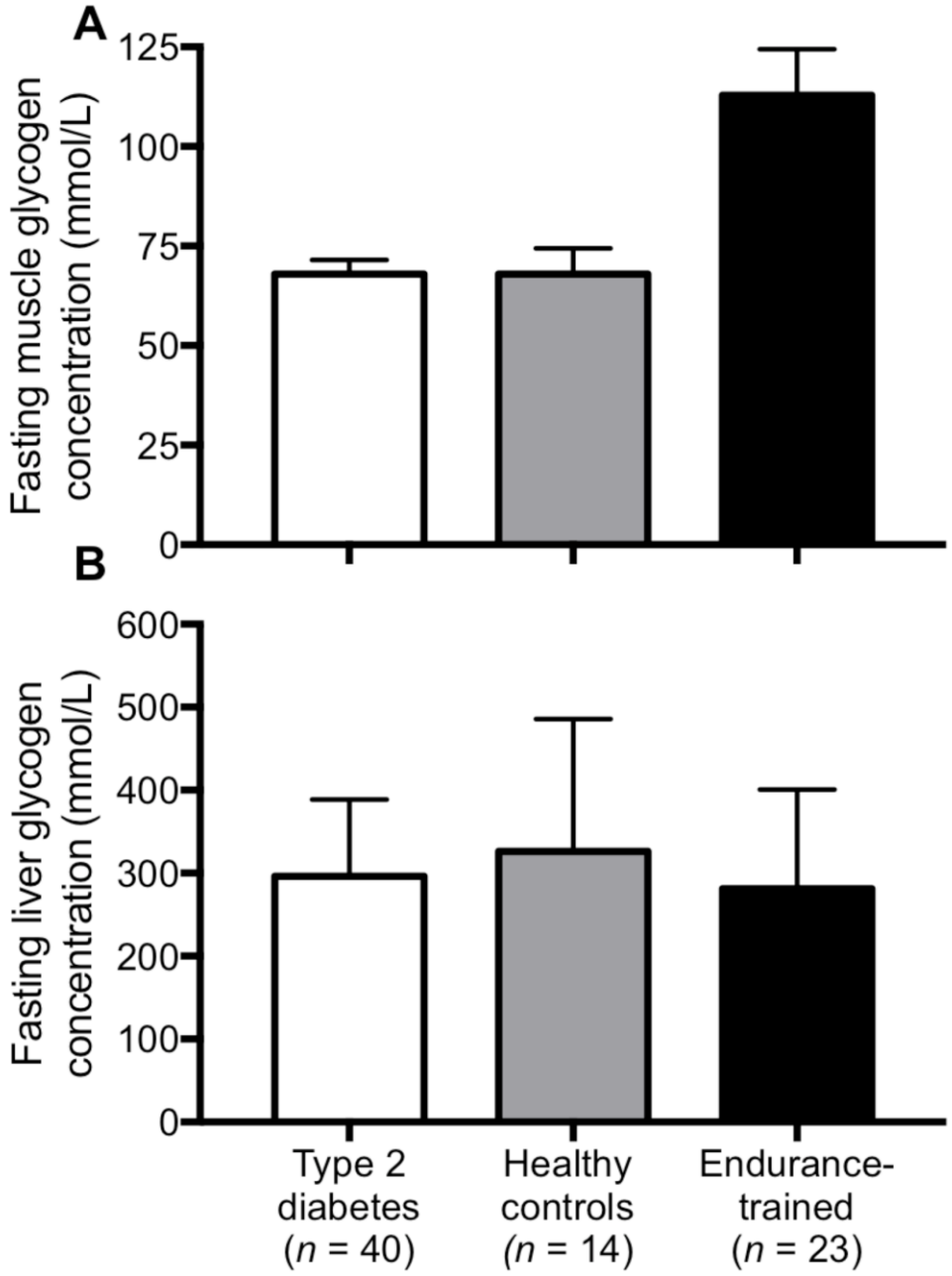

Figure 2. Liver (**A**) and muscle (**B**) glycogen concentrations in humans after an overnight fast in the resting state. Data are means ± 95%CI. Data extracted from Macauley et al. (67), Gonzalez et al. (44) and Stevenson et al. (97). For comparison to biopsy literature, muscle glycogen concentrations equate to 262 ± 19, 262 ± 35 and 434 ± 39 mmol/kg DM [assuming a muscle density of 1.112 g/cm^3 (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/cm^3 (94) and a wet-to-dry mass ratio of 2.4 (77)] in type 2 diabetes, healthy controls and endurance-trained, respectively.

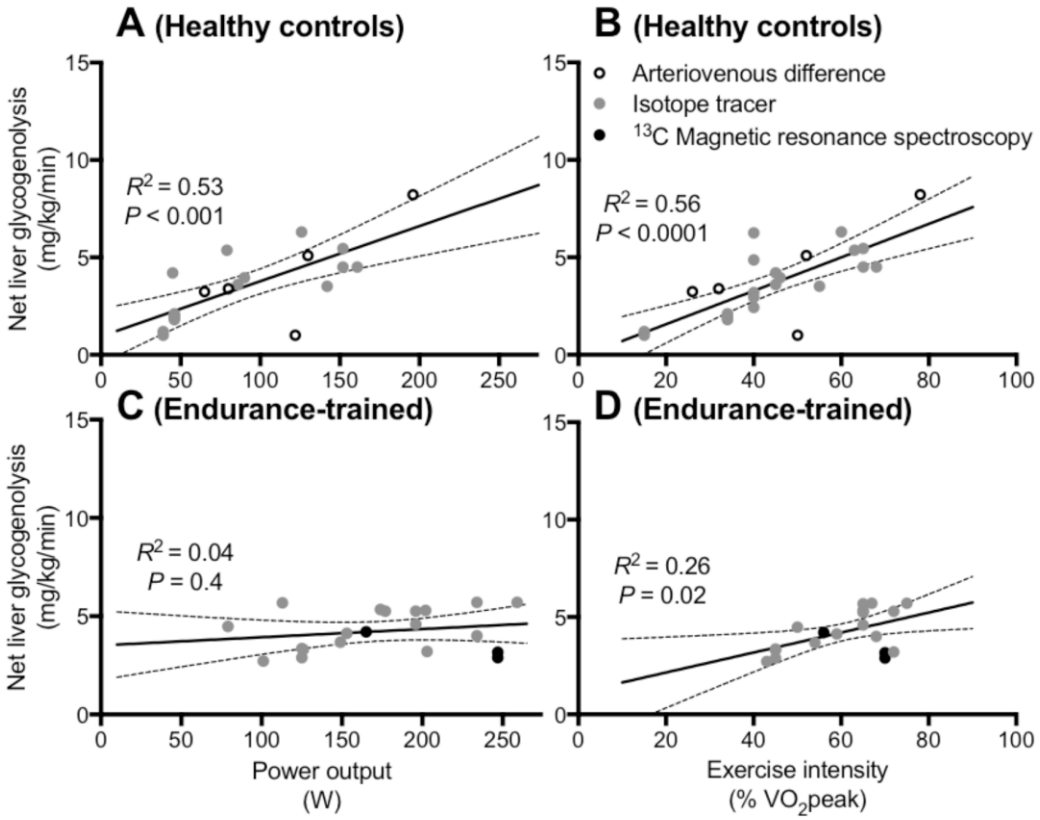

Figure 3. Net liver glycogenolysis rate as a function of absolute (**A and C**) and relative (**B and D**) exercise intensity in healthy untrained controls (**A and B**) and endurance-trained (**C and D**) humans. See Table 1 for details of studies. Dashed lines represent 95% CI.

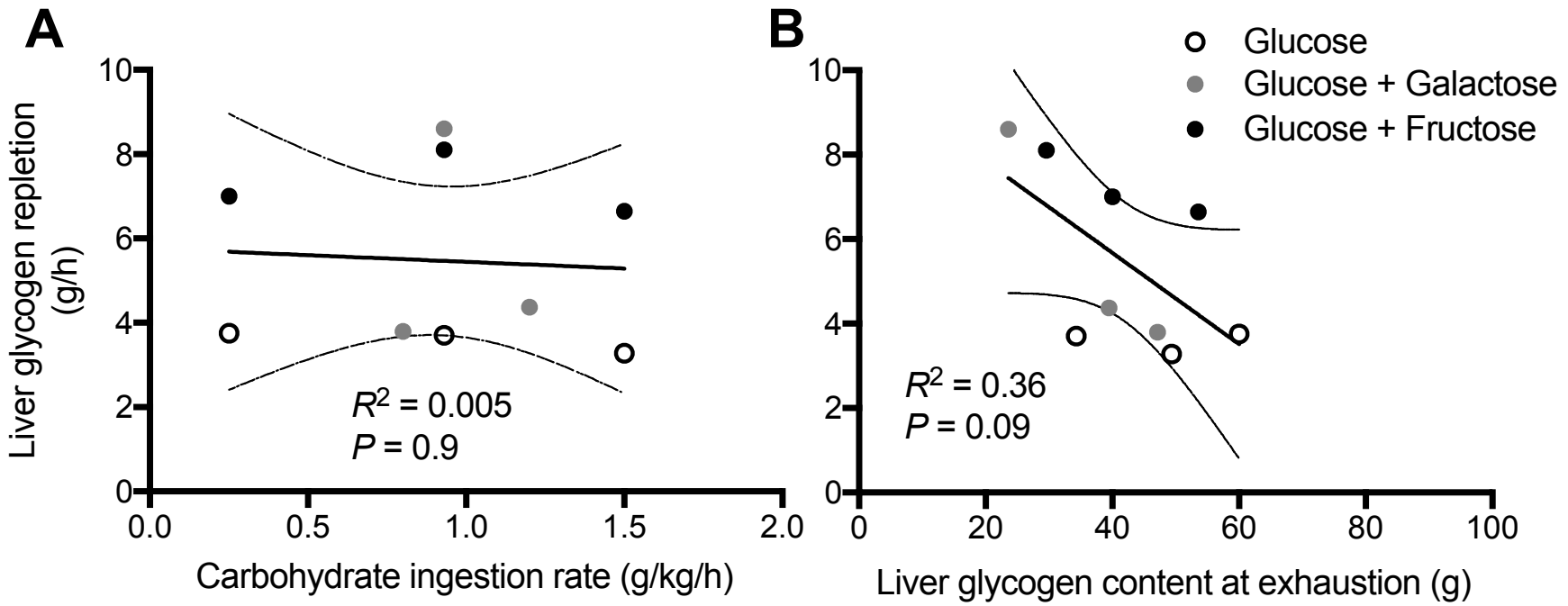

Figure 4. Post-exercise liver glycogen repletion rates during short-term recovery (4-6 hours) with varying types of carbohydrate ingestion plotted against carbohydrate ingestion rate (A) or liver glycogen content post-exercise (B). Data were extracted from references (18), (29) and (40). Where values 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.

Article	*n*	Participants	Exercise mode	Exercise Duration (min)	Exercise intensity (% VO_{2peak}) [W]	Net liver glycogenolysis (mg/kg/min)	Method
Wahren et al. 1971 (120)	10	Untrained (M)	Cycling	40	26 [65]	3.24	Splanchnic arteriovenous difference (total precursors)
	9	Untrained (M)	Cycling	40	52 [130]	5.09	
	6	Untrained (M)			78 [196]	8.22	
Ahlborg et al. 1974 (2)	6	Untrained (M)	Cycling	40	32 [80]	3.40	Splanchnic arteriovenous difference (total precursors)
Sestoft et al. 1977 (93)	5	Untrained (M)	Cycling	35	50 [122]	0.95	Splanchnic arteriovenous difference (total precursors)

Stanley et al. 1988 (95)	7	Trained (M)	Cycling	50	43 [101]	2.72	Isotope tracers ([^{13}C]- & [^{14}C]-lactate incorporation into glucose)
Coggan et al. 1995 (23)	6	Untrained (M)	Cycling	120	60 [126]	6.31	Isotope tracers ([^{13}C]-bicarbonate incorporation into [^{13}C]-glucose)
	6	Trained (M)	Cycling	120	45 [126]	3.30	Isotope tracers ([^{13}C]-bicarbonate incorporation into [^{13}C]-glucose)
Friedlander et al. 1997 (37)	19	Untrained (M)	Cycling	60	46 [90] 65 [152]	3.95 4.50	Isotope tracers ([^{13}C]-glucose recycling rate)
	19	Trained (M)	Cycling	60	59 [153] 65 [177]	4.13 5.25	Isotope tracers ([^{13}C]-glucose recycling rate)

Lavoie et al. 1997 (65)	5	Untrained (M)	Cycling	120	40 [NR]	4.87	Isotope tracers (2,3,4,6,6-^{2}H]-glucose and L-[1,2,3-^{13}C]-alanine incorporation into glucose)
Friedlander et al. 1998 (38)	17	Untrained (F)	Cycling	60	45 [45] 63 [79]	4.21 5.37	Isotope tracers ([^{13}C]-glucose recycling rate)
	17	Trained (F)	Cycling	60	50 [79] 65 [113]	4.48 5.68	Isotope tracers ([^{13}C]-glucose recycling rate)
Bergman et al. 2000 (11)	9	Untrained (M)	Cycling	60	45 [86] 65 [152]	3.60 5.46	Isotope tracers (6,6-$^{2}H_2$]-glucose and L-[3-^{13}C]-lactate into glucose)
	9	Trained (M)	Cycling	60	54 [149] 65	3.68 5.35	Isotope tracers (6,6-$^{2}H_2$]-glucose and L-[3-^{13}C]-lactate into glucose)

					[174]		
Casey et al. 2000 (18)	6	Trained (M)	Cycling	83	70 [NR]	12.03	^{13}C magnetic resonance spectroscopy at natural abundance
Trimmer et al. 2001 (104)	8	Trained (M)	Cycling	90	45 [125] 65 [196]	3.36 5.26	Isotope tracers (6,6-$^{2}H_2$]-glucose and 2-[^{13}C]-glycerol)
Trimmer et al. 2002 (105)	8	Trained (M)	Cycling	90	45 [125] 65 [196]	2.90 4.60	Isotope tracers (6,6-$^{2}H_2$]-glucose and 2-[^{13}C]-glycerol with mass isotopomer distribution analysis)
Roef et al. 2002 (90)	6	Untrained (F/M)	Cycling	90	15 [39]	1.21	Isotope tracers (6,6-$^{2}H_2$]-glucose and $^{2}H_2O$ by incorporation of ^{2}H from pyruvate into glucose at C-6)
Roef et al. 2003 (89)	7	Untrained (F/M)	Cycling	240	34 [46]	2.11	Isotope tracers (6,6-$^{2}H_2$]-glucose and $^{2}H_2O$ by incorporation of ^{2}H from pyruvate into glucose at C-6)

Petersen et al. 2004 (84)	6	Untrained (F/M)	Running	50	35 [-] 70 [-]	1.54 2.89	^{13}C magnetic resonance spectroscopy at natural abundance
Stevenson et al. 2009 (97)	9	Trained (M; high-glycemic index diet)	Cycling	90	70 [247]	3.17	^{13}C magnetic resonance spectroscopy at natural abundance
	9	Trained (M; low-glycemic index diet)	Cycling	90	70 [247]	2.90	^{13}C magnetic resonance spectroscopy at natural abundance
Huidekoper et al. 2013 (51)	4	Untrained (F/M)	Cycling	90	55 [142]	3.51	Isotope tracers (6,6-$^{2}H_2$]-glucose and $^{2}H_2O$ by incorporation of ^{2}H into glucose at C-5)
Emhoff et al. 2013 (33)	6	Untrained (M)	Cycling	60	68 [161]	4.50	Isotope tracers (6,6-$^{2}H_2$]-glucose and $_{L}$-[3-^{13}C]-lactate into

							glucose)
	6	Trained (M)	Cycling	60	75 [159] 67 [234] 68 [234]	5.70 5.70 4.00	Isotope tracers ($6,6\text{-}^{2}H_2$]-glucose and $_{L}$-[3-^{13}C]-lactate into glucose)
Gonzalez et al. 2015 (44)	14	Trained (M)	Cycling	180	56 [165]	4.20	^{13}C magnetic resonance spectroscopy at natural abundance
Webster et al. 2016 (124)	7	Trained (M)	Cycling	120	72 [202]	5.30	Isotope tracers ($6,6\text{-}^{2}H_2$]-glucose and $^{2}H_2O$ by incorporation of ^{2}H into glucose at C-1,3,4,5,6 and 6)
	7	Trained (M; low habitual carbohydrate intake)	Cycling	120	72 [203]	3.20	Isotope tracers ($6,6\text{-}^{2}H_2$]-glucose and $^{2}H_2O$ by incorporation of ^{2}H into glucose at C-1,3,4,5,6 and 6)

F, females; M, males; NR, not reported.