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SYMPOSIUM REVIEW

Impact of pre-exercise feeding status on metabolic adaptations to endurance-type exercise training

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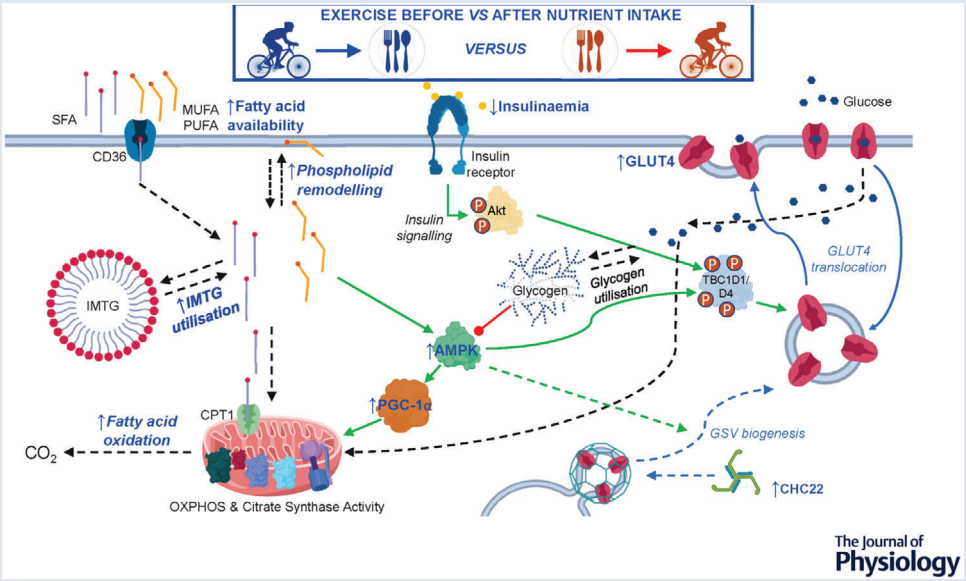
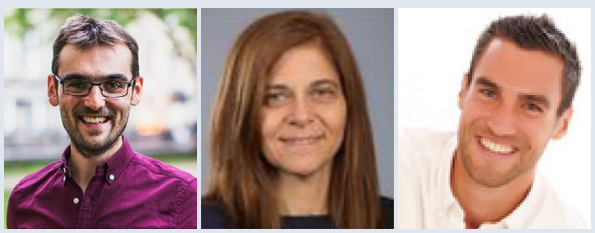


Nutrition and exercise metabolism are vibrant physiological fields, yet at times it feels as if greater progress could be made by better integrating these disciplines. Exercise is advocated for improving metabolic health, in part by increasing peripheral insulin sensitivity and glycaemic control. However, when a modest-to-high carbohydrate load is consumed before and/or during each exercise bout within a training programme, increases in oral glucose insulin sensitivity can be blunted in both men of a healthy weight and those with overweight/obesity. Exercise training-induced adaptation in the energy sensing AMP-activated protein kinase (AMPK) and



Rob Edinburgh recently completed a PhD at the University of Bath, having also gained an undergraduate degree in Sport and Exercise Science. His research to date has focused on the effects of nutrient–exercise timings on substrate utilisation during and after exercise, metabolism, and health-related adaptations to exercise. He is passionate about ways we can make exercise more efficacious for health. **Francoise Koumanov** is a Lecturer (Assistant Professor) at the University of Bath. Her research focuses on molecular signalling processes of glucose and insulin in physiological and pathophysiological states and aims to elucidate the roles of exercise and GLUT4 trafficking in the pathophysiology of insulin resistance. **Javier Gonzalez** is Reader (Associate Professor) at the University of Bath. His research focuses on interactions between nutrition and exercise in health and disease and explores the role of carbohydrate availability in the regulation of energy balance, metabolic health and sports performance.

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the insulin-sensitive glucose transporter GLUT4 protein levels are sensitive to pre-exercise feeding status in both healthy individuals and individuals classified as overweight or obese. Increased lipid oxidation may, in part, explain the enhanced adaptive responses to exercise training performed before (i.e. fasted-state exercise) *versus* after nutrient ingestion. Evidence in individuals with type 2 diabetes currently shows no effect of altering nutrient–exercise timing for measured markers of metabolic health, or greater reductions in glycated haemoglobin (HbA1c) concentrations with exercise performed after *versus* before nutrient provision. Since the metabolic inflexibility associated with type 2 diabetes diminishes differences in lipid oxidation between the fasted and fed states, it is plausible that pre-exercise feeding status does not alter adaptations to exercise when metabolic flexibility is already compromised. Current evidence suggests restricting carbohydrate intake before and during exercise can enhance some health benefits of exercise, but in order to establish clinical guidelines, further research is needed with hard outcomes and different populations.

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Abstract figure legend Candidate mechanisms linking nutrient–exercise timing to insulin sensitivity. Exercise performed in an overnight-fasted state (before nutrient intake), increases fatty acid availability for skeletal muscle, and also increases intramuscular triglyceride utilisation. An increase in skeletal muscle lipid turnover may result in phospholipid remodelling, with a relative reduction in saturated fatty acids within skeletal muscle phospholipids. Increased fatty acid availability can also increase AMPK activity and this increase PGC-1α levels. Exercise before *versus* after nutrient intake can also result in an increase in skeletal muscle GLUT4 and CHC22 levels, which are involved in insulin-stimulated glucose transport. Akt, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; CD36, fatty acid translocase; CHC22, clathrin heavy chain 22; CPT1, carnitine palmitoyltransferase 1; GLUT4, glucose transporter 4; GSV, GLUT4 storage vesicle; IMTG, intramuscular triglyceride; MUFA, monounsaturated fatty acid; OXPHOS, oxidative phosphorylation proteins; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1-α; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TBC1D1/D4, TBC1 domain family member 1/family member 4 (AS160).

Introduction

Nutritional intake cannot be fully understood without accounting for physical activity status, and similarly, knowledge of exercise metabolism requires consideration of nutrient status. From an evolutionary perspective, it might be considered that humans would be well-adapted to eat after exercise (*versus* before exercise) due to the need to perform physical activity in order to hunt or forage (Lieberman, 2015). However, it is also possible that humans could have evolved under conditions in which they ate carbohydrates whilst hunting and/or needed to run shortly after eating to avoid predation. Therefore, it is impossible to know with certainty under which conditions humans evolved under with respect to nutrient timing and physical activity. Nevertheless, it seems that nutrient timing does play an important role in regulating the acute and chronic effects of exercise. The role of physical activity (Bird & Hawley, 2017; Sylow & Richter, 2019) and nutrient–exercise timing (Haxhi *et al*. 2013; Wallis & Gonzalez, 2019; Aqeel *et al*. 2020; Mancilla *et al*. 2020) for improving aspects of metabolic health have been reviewed before. However, for nutrient–exercise timing, these reviews focused almost exclusively on acute metabolic responses to exercise. These reviews also called for further investigation into the role of exercise–nutrient interactions during training studies in populations at risk of, or diagnosed with, metabolic diseases. Several key studies have now been published, providing novel evidence on the role of nutrient–exercise timing in mediating health-related exercise adaptations in humans. The aims of this review are therefore to highlight (1) the latest research on nutrient–exercise timing and adaptations to exercise with implications for metabolic health and (2) candidate mechanisms underpinning adaptations to nutrient–exercise interactions.

Responses to endurance exercise training

Skeletal muscle, adipose tissue and the liver play roles in regulating the postprandial storage and oxidation of dietary carbohydrates (Kelley *et al*. 1988; Randle, 1998). A reduced peripheral insulin sensitivity to glucose uptake underpins many of the defects associated with metabolic diseases, and helps to account for an association between obesity and type 2 diabetes (Reaven, 1988). Although exercise training results in adaptations across

many physiological systems, the main focus of this review will be on skeletal muscle, due to the key role of this tissue in regulating postprandial glycaemia (DeFronzo *et al.* 1985; Baron *et al.* 1988). In addition, the role of nutrient–exercise timing for adaptations in adipose tissue and the liver during exercise training remains to be studied, but relevant acute studies will be highlighted.

Skeletal muscle responses to exercise which underlie improvements in blood glucose control can be attributed to two main effects (Sylow & Richter, 2019). First, there is an increase in skeletal muscle glucose uptake to help satisfy the higher energy demands of the activity. Each bout of exercise performed activates AMP-activated protein kinase (AMPK), Akt substrate of 160 kDa (AS160/TBC1D4) (Treebak *et al.* 2009; Herzig & Shaw, 2018), Rac1 (Gliemann *et al.* 2017) and Ca^{2+}/calmodulin-dependent protein kinase II (Witczak *et al.* 2010) pathways within skeletal muscle, which induces the translocation of the glucose transporter type 4 (GLUT4) protein to the plasma membrane and the *t*-tubules to facilitate increased transmembrane glucose transport (Hawley & Lessard, 2008). Together with increased microvascular perfusion, these mechanisms contribute to increased peripheral insulin sensitivity and skeletal muscle glucose uptake in the acute post-exercise period (Sjøberg *et al.* 2017; Hingst *et al.* 2018). However, it should be noted that while it was, until recently, accepted that AMPK is central for increasing glucose uptake during exercise via GLUT4 translocation, emerging animal studies report that this might not be the case and that it regulates glucose uptake and enhances insulin sensitivity only post-exercise (Kjøbsted *et al.* 2019; Hingst *et al.* 2020). Secondly, regular exercise training increases the time spent in this acute phase, but also induces longer-term adaptations across multiple tissues that can enhance blood glucose control (Sylow & Richter, 2019). The skeletal muscle responses to exercise training include a greater capacity to store glycogen and increased GLUT4 protein content and mitochondrial content and function (McGee & Hargreaves, 2006; Hawley *et al.* 2014; Marcelo *et al.* 2020). These adaptations are thought to largely result from the repeated stimuli from each bout of exercise, which then culminate in a change in phenotype. For example, acute metabolic responses to exercise produce a transient (<24 h) burst in the expression of transcriptional proteins such as peroxisome proliferator activated receptor γ coactivator 1-α (PGC1α) (Pilegaard *et al.* 2003; Perry *et al.* 2010). These mRNA bursts may be followed by an increase in protein levels after ∼24 h (Perry *et al.* 2010), which coincides with increases in skeletal muscle mitochondrial protein synthesis (Di Donato *et al.* 2014). With subsequent bouts of matched-intensity exercise, the magnitude of the mRNA bursts can be diminished, but the PGC1α protein accumulates in skeletal muscle, followed by an increase in the mitochondrial protein content (Perry *et al.* 2010). However, despite exercise being widely advocated as a means to improve aspects of metabolic health, many people fail to achieve physical activity recommendations, partly due to a perceived lack of time (Reichert *et al.* 2007; Korkiakangas *et al.* 2009). Therefore, strategies to maximise benefits of any exercise that is performed could be important. There is also evidence of inter-individual variability to exercise training for postprandial insulinaemic responses (de Lannoy *et al.* 2017). This may be accounted for, at least in part, by the feeding status under which exercise is performed.

Nutrient–exercise timing (acute studies)

The acute responses to nutrient–exercise timing have been reviewed for substrate utilisation (Vieira *et al.* 2016), glycaemia and lipidaemia following a single pre- or post-exercise meal (Haxhi *et al.* 2013), and for recovery from exercise (Arent *et al.* 2020). However, the primary focus of this review will be on adaptations to exercise training interventions. Acute responses will, however, be discussed briefly to provide context. Pre-exercise carbohydrate consumption decreases whole-body lipid oxidation in untrained and trained individuals, an effect mostly seen at moderate (40–60% $\dot{V}_{O_2max}$) exercise intensities (Bergman & Brooks, 1999; Vieira *et al.* 2016). Nutrient–exercise timing also alters the metabolic responses to the exercise session. For people with or without type 2 diabetes, the glycaemic response to a single meal can be attenuated to a greater extent with post- *versus* pre-meal exercise, partly through a greater oxidation of the ingested carbohydrates (Poirier *et al.* 2000, 2001). As such, it has been proposed that moderate-intensity physical activity could be undertaken between 30 and 120 min after carbohydrate-rich meals are consumed, to lower blood glucose excursions from that particular meal (Haxhi *et al.* 2013; Chacko, 2017). However, caution should be taken when extrapolating acute glycaemic responses to longer-term changes in blood glucose control. For example, if exercise is performed three times a week and when three meals are consumed per day, any glucose lowering effect of post- *versus* pre-meal exercise would apply to only 3 out of 15 meals (20%) consumed for that week.

Nutrient–exercise timing (training studies)

Whilst post- *versus* pre-meal exercise may offer additional benefits for blood glucose control at a single meal, regularly exercising before *versus* after nutrient (particularly carbohydrate) consumption may be a greater stimulus for more enduring adaptations relating to oral glucose insulin sensitivity. In a seminal paper

investigating nutrient–exercise timing for metabolic health, physically active men consumed a hyper-caloric (+ ∼30% kcal), high-fat diet (50% of kcal) and completed exercise for 300 min per week over 6 weeks (Van Proeyen *et al*. 2010). They consumed a carbohydrate-rich breakfast 90 min before any exercise and 1 g kg^{-1} of carbohydrate during exercise, or exercised in an overnight fasted state consuming the breakfast which they missed in the morning, and maltodextrin omitted during exercise, mid-afternoon. Relative to the control group, the glycaemic response to a 75 g oral glucose tolerance test (OGTT) decreased if exercise was performed in the fasted state, but not when carbohydrate was consumed before and during exercise training. The Matsuda insulin sensitivity index also increased only if the exercise was performed in the fasted state. However, feeding *versus* fasting can exert different physiological responses in lean people compared to individuals classified as overweight or obese, or with type 2 diabetes. For example, regularly extending the morning fast until noon upregulates the expression of genes involved in lipid turnover in the adipose tissue compared to the consumption of breakfast in lean humans, but not in individuals with obesity (Gonzalez *et al*. 2018). Also, the blunting of exercise adaptations resulting from carbohydrate consumption before and during exercise may not translate to the case in which carbohydrates are only consumed before exercise. The suppression of fatty acid availability in the latter scenario is less pronounced and there is even evidence that consuming carbohydrates before exercise can augment increases in skeletal muscle AMPK activity (Edinburgh *et al*. 2018).

A fully supervised exercise training intervention was recently completed in individuals classified as overweight or obese, but without type 2 diabetes (Edinburgh *et al*. 2020), with the aim of establishing whether the proof-of-principle in lean individuals extends to people with increased risk of metabolic disease. Moderate-intensity cycling exercise was performed before or after a carbohydrate-rich breakfast for 6 weeks. In the breakfast before exercise group, breakfast was consumed 2 h prior to exercise and in the exercise before breakfast group, breakfast was consumed at least 2 h post-exercise. Increases in oral glucose insulin sensitivity were reported with exercise before *versus* after breakfast, which was driven by reductions in postprandial insulinaemia. These measures were derived from a pre- and post-intervention OGTT with the follow-up OGTT completed between 48 and 72 h after the last exercise training session to reduce any residual effects of the last exercise bout performed on these measurements. The carbohydrate dose consumed was lower (∼120 g) than in a study in healthy men (Van Proeyen *et al*. 2010) and no carbohydrate was consumed during exercise. The exercise training also adhered to current physical activity guidelines, by the completion of moderate intensity cycling, three times a week, for 50 min per session. That study demonstrated that it is possible for people classified as overweight or obese to start exercise training but not improve postprandial glycaemic control or oral glucose insulin sensitivity if a carbohydrate-rich breakfast is consumed before exercise sessions. These responses to exercise before *versus* after breakfast occurred despite no differences between groups for the change in whole-body aerobic capacity, skeletal muscle citrate synthase activity, body mass, average daily self-reported energy intake and/or average daily physical activity energy expenditure. In that study, net skeletal muscle glycogen utilisation and acute skeletal muscle mRNA responses were largely unaffected by the same exercise performed before *versus* after breakfast. This is important because muscle glycogen availability can regulate adaptations to training (Burke & Hawley, 2018). Lower muscle glycogen concentrations are therefore unlikely to have driven the training responses observed in that particular study. Other studies also report changes in aerobic fitness (Van Proeyen *et al*. 2010, 2011; Gillen *et al*. 2013; Verboven *et al*. 2020) and body mass (Gillen *et al*. 2013; Schoenfeld *et al*. 2014; Escalante & Barakat, 2020) in response to exercise training to be independent of the pre-exercise feeding status.

Two exercise training studies have also recently been published with altered nutrient–exercise timing in individuals with type 2 diabetes. In one study, 30 patients experienced similar decreases in glycated haemoglobin (HbA1c) and serum insulin concentrations after high-intensity cycling (30 min) and strength training, performed three times a week for 8 weeks either before or after breakfast (Brinkmann *et al*. 2019). In a similar study, 25 patients completed a 12 week exercise intervention, with exercise (25 min walking and 20 min cycling per session) performed three times a week before or after breakfast (Verboven *et al*. 2020). In that study the breakfast was standardised within participant and self-selected, containing 375 ± 72 kcal (60 ± 4% carbohydrate (CHO)) for the exercise after breakfast group, which was consumed 1 h before exercise, and 479 ± 73 kcal (58 ± 4% CHO) for the exercise before breakfast group, which was consumed within 1 h of exercise completion. HbA1c concentrations decreased after exercise training, but there was a greater reduction when exercise was performed after *versus* before breakfast. One explanation for the differences between that study and research which used OGTTs (Van Proeyen *et al*. 2010; Edinburgh *et al*. 2020) is that HbA1c concentrations do not reflect glycaemic control *per se*, but rather average blood glucose concentrations over time. Whilst the study in individuals with type 2 diabetes has good ecological validity as breakfast and food intake was self-selected, it is possible that the HbA1c results were not reflective of glycaemic control alone, but

also the glycaemic load of the diet chosen by the patients throughout the training. Nonetheless, the OGTT also has limitations for assessing peripheral insulin sensitivity (Muniyappa *et al.* 2008) and there is now a need for a training study to alter nutrient–exercise timing and use a hyperinsulinaemic–euglycaemic clamp to measure peripheral insulin sensitivity. Other differences between studies include the exercise mode, the length of training and population studied (Table 1). For the latter point, if increased lipid oxidation alters adaptation to exercise with altered nutrient–exercise timing (discussed subsequently), a lack of effect of exercise before breakfast on markers of metabolic health in people with type 2 diabetes could be related to metabolic inflexibility, which blunts metabolic differences between fasting and feeding (Kelley & Simoneau, 1994; Kelley *et al.* 1999; Goodpaster & Sparks, 2017). In addition, in rodents, exercise-induced remodelling of skeletal muscle can be blunted by chronic hyperglycaemia, a common characteristic of diseases such as type 2 diabetes (MacDonald *et al.* 2020).

Candidate mechanisms for enhanced adaptations from exercise in a fasted state

Increased lipid oxidation. It is well established that exercising in an overnight-fasted state increases whole-body lipid oxidation, and decreases carbohydrate oxidation, compared to exercise in the fed state (Vieira *et al.* 2016). In men classified as overweight or obese, there are no clear differences in muscle glycogen utilisation during a single bout of moderate-intensity exercise performed before *versus* after a mixed-macronutrient breakfast, but net intramuscular triglyceride (IMTG) utilisation is blunted and plasma non-esterified fatty acid (NEFA) and glycerol concentrations are lower (likely due to adipose tissue NEFA re-esterification; Enevoldsen *et al.* 2004) during exercise after breakfast (Edinburgh *et al.* 2020). Fatty acids are ligands for peroxisome proliferated activator receptors (PPARs) which can mediate the expression of lipid metabolism proteins (Ehrenborg & Krook, 2009). For example, PPAR-δ helps regulate *CPT1* expression (Dressel *et al.* 2003) and fatty acid oxidation enzymes (Luquet *et al.* 2003). In rodents, increases in PPAR-δ activity can be augmented by exercising with a low carbohydrate availability (Philp *et al.* 2013). Together with increased AMPK activation (as discussed subsequently) this may augment some adaptations (Handschin & Spiegelman, 2006). In support of a possible role for increased lipid flux in mediating metabolic adaptations to exercise training, a positive correlation between changes in oral glucose insulin sensitivity and cumulative whole-body lipid utilisation during exercise training has been reported (Edinburgh *et al.* 2020). This highlights the possibility that for improving key aspects of metabolic health, exercise training may be more effective when the activity stimulates an increased utilisation of endogenous lipid stores, a concept which has recently been highlighted (Gemmink *et al.* 2020). In support of this theory is evidence that in individuals classified as overweight or obese, altering nutrient–exercise timing does not alter changes in postprandial glycaemia or markers of insulin sensitivity when the training is high-intensity (Gillen *et al.* 2013). Differences in lipid utilisation between exercise in the fasted *versus* fed state are less apparent at high exercise intensities, where carbohydrate is the predominant fuel source, irrespective of prior feeding status (Bergman & Brooks, 1999). The greater utilisation of IMTG with fasted *versus* fed exercise may be especially important as this is associated with increases in peripheral insulin sensitivity, potentially by alleviating any accumulation of the lipid metabolites (e.g. fatty acyl-CoA, ceramide or diacylglycerol) that can interfere with insulin signalling (Hulver & Dohm, 2004). Moreover, IMTG degradation during a subsequent bout of exercise can also be enhanced by prior exercise training in the fasted state but not in the fed state (Van Proeyen *et al.* 2011). Despite this, that the lipid store being utilised (i.e. IMTG *versus* adipose tissue-derived fatty acids) may explain the effect of nutrient–exercise timing for metabolic adaptations to exercise remains to be fully elucidated.

AMP-activated protein kinase. Of the molecular mechanisms that drive exercise adaptations, a protein with a key role is AMPK, an enzyme sensitive to the cellular energy status (Mounier *et al.* 2015). AMPK is a heterotrimeric protein complex consisting of α, β and γ subunits. Increases in the kinase activity of AMPK results in the phosphorylation of downstream targets (e.g. TBC1D1, TBC1D4 and acetyl coenzyme A carboxylase (ACC)) and nuclear transcription factors which regulate the expression of genes involved in glucose uptake, mitochondrial biogenesis and autophagy (Herzig & Shaw, 2018). In response to exercise, AMPK can be activated by an increased AMP:ATP ratio via the γ-subunit, and reductions in glycogen concentrations via the β-subunit (Pilegaard *et al.* 2002; Frøsig *et al.* 2004; McBride *et al.* 2009; Yeo *et al.* 2010). Recent *in vitro* research has demonstrated that long-chain fatty acyl-CoA esters can activate the β-subunit of AMPK and promote increased lipid oxidation (Pinkosky *et al.* 2020), a finding that is discussed elsewhere (Hardie, 2020). This supports earlier observations that skeletal muscle AMPK can be activated by increased fatty acid availability, independent of muscle glycogen or AMP concentrations (Watt *et al.* 2006). AMPK activation may contribute to regulating fuel metabolism during and immediately post-exercise, and this is partly mediated by increasing skeletal muscle glucose uptake via regulation of TBC1D1 and TBC1D4

Table 1. Overview of exercise training studies examining metabolic responses with manipulation of nutrient timing

First author (year)	Nutritional intervention	Exercise mode	Exercise intensity	Exercise frequency	Participants	Fasting glucose	Fasting insulin	Postprandial glucose	Postprandial insulin	Muscle adaptation
De Bock (2005)	CHO before and during exercise, *vs* after exercise	Cycling	75% $\dot{V}_{O_2peak}$	3 days/week, 6 weeks	Lean (M)	=	=	N/A	N/A (↑* exercise-induced reduction)	=: HKII, GLUT4, FAT/CD36, UCP3, IMCL utilisation ↑†: FABPm
Van Proeyen (2010)	CHO before and during exercise, *vs*. after exercise	Cycling	70–75% $\dot{V}_{O_2peak}$	4 days/week, 6 weeks	Lean (M), high-fat diet	=	=	↓†	↓†	↑*: COX ↑†: glycogen, GLUT4, p-AMPK, FAT/CD36, CPT1 =: CS, β-HAD
Van Proeyen (2011)	CHO before and during exercise, *vs*. after exercise	Cycling	~70% $\dot{V}_{O_2peak}$	4 days/week, 6 weeks	Lean (M), high-CHO diet	=	N/A	N/A	N/A	↑*: CS ↑†: β-HAD, type IIa IMCL utilisation
Gillen (2013)	Breakfast before exercise, *vs*. after exercise	Cycling intervals	~90% HR_{max}	3 days/week, 6 weeks	OW/OB (F)	=	=	=	=	=: CS, β-HAD, GLUT4
Brinkmann (2019)	Breakfast before exercise, *vs*. 12 h fast	Resistance/ cycling	~70-80% HR_{peak}	3 days/week, 8 weeks	T2D (M&F)	=	=	N/A	N/A	N/A
Verboven (2020)	Breakfast before exercise, *vs*. after exercise	Walking/ cycling	65% $\dot{V}_{O_2peak}$	3 days/week, 12 weeks	T2D (M)	=	=	N/A	N/A	=: mRNA
Edinburgh (2020)	CHO before exercise, *vs*. after exercise	Cycling	50–55% W_{peak}	3 days/week, 6 weeks	OW/OB (M)	=	=	=	↓†	↑*: AMPK, CHC22 ↑†: GLUT4 =: Akt, AS160, CPT1, FAT/CD36, OXPHOS, CS

*Difference between fasted-exercise and fed-exercise group ($P < 0.05$); †difference between fasted-exercise and control group ($P < 0.05$). Akt, protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; AS160, Akt substrate of 160 kDa (TBC1D4); β-HAD, 3-hydroxyacyl-CoA dehydrogenase; CHC22, clathrin heavy chain 22; CHO, carbohydrate; CPT1, carnitine palmitoyltransferase 1; COX, cytochrome *c* oxidase; CS, citrate synthase; F, females; FAT/CD36, fatty acid translocase; FABPm, fatty acid binding protein muscle; GLUT4, glucose transporter 4; HKII, hexokinase II; HR_{max}, maximal heart rate; HR_{peak}, peak heart rate, IMCL, intramuscular lipid; M, males; N/A, Not applicable/measured; OB, obese; OW, overweight; OXPHOS, oxidative phosphorylation proteins; p-AMPK, phosphorylated AMPK; T2D, type 2 diabetes; UCP3, uncoupling protein 3; $\dot{V}_{O_2peak}$, peak aerobic capacity; W_{peak}, peak power; =, no change.

activity (Szekeres *et al*. 2012; Richter & Hargreaves, 2013; Chadt *et al*. 2015; Stöckli *et al*. 2015) and lipid oxidation via the inhibition of ACC (Hardie, 1989; O'Neill *et al*. 2014). It has recently been reported that TBC1D1 and TBC1D4 activity can regulate long chain fatty acid uptake in skeletal muscle via the SLC27A4/FATP4 pathway, which is an additional mechanism by which exercise and nutrient status via AMPK may regulate lipid uptake and metabolism (Benninghoff *et al*. 2020). Skeletal muscle AMPK activation also stimulates enduring adaptations to exercise including increases in the GLUT4 protein levels and mitochondrial biogenesis via transcription factors including PGC1α (Wojtaszewski *et al*. 2000; Ojuka *et al*. 2002). Enhanced AMPK activation in response to exercise commenced with reduced muscle glycogen concentrations (35–45% of basal) may explain increased mitochondrial adaptations compared to the same exercise started with higher muscle glycogen concentrations (Morton *et al*. 2009; Hulston *et al*. 2010; Bartlett *et al*. 2013; Andrade-Souza et al., 2020). This may be important as obesity and peripheral insulin resistance have been associated with a lower mitochondrial oxidative capacity (Kelley *et al*. 1999; Simoneau *et al*. 1999). However, these studies used high exercise volumes and often asked participants to exercise multiple times over 24 h, which may not be sustainable for people exercising primarily for health. Nonetheless, in physically active healthy men, altering breakfast timing in relation to exercise (i.e. fasted exercise) can increase the content/expression of lipid metabolism proteins in skeletal muscle such as cluster of differentiation 36 (CD36) or carnitine palmitoyltransferase (CPT-1) over 6–8 weeks (De Bock *et al*. 2008; Nybo *et al*. 2009; Van Proeyen *et al*. 2011). In people classified as overweight or obese, the protein content of AMPK in skeletal muscle also increases by ∼3-fold with exercise before breakfast, whereas exercise after breakfast did not increase AMPK levels (Edinburgh *et al*. 2020). In that study, this AMPK response did not translate into changes in proteins involved in fatty acid transport or fatty acid utilisation (CD36 or CPT-1) or markers of mitochondrial oxidative capacity (OXPHOS complex protein levels or citrate synthase activity), but there was a 2-fold increase in the protein levels of GLUT4 in skeletal muscle and proteins in the GLUT4 biosynthetic pathway (i.e. CHC22) were also increased with exercise training before *versus* after breakfast (Abstract Figure). This AMPK and GLUT4 response to pre-meal *versus* post-meal exercise supports results reported in healthy men (Van Proeyen *et al*. 2010). As prior feeding status did not alter muscle glycogen utilisation during exercise in that study, increases in GLUT4 levels and oral glucose insulin sensitivity that were reported with exercise before *versus* after breakfast (Edinburgh *et al*. 2020) could be due to increased fatty acid flux driving skeletal muscle AMPK activation (Pinkosky *et al*. 2020) (Abstract Figure).

Phospholipid remodelling. There is also some suggestion of increased skeletal muscle phospholipid remodelling in people with obesity after exercise training performed before *versus* after breakfast (Edinburgh *et al*. 2020). An increased phospholipid saturated fatty acid (SFA) content and a decreased polyunsaturated fatty acid (PUFA) content has been associated with obesity and peripheral insulin resistance (Harayama & Riezman, 2018). Increasing the PUFA content increases membrane fluidity, insulin receptor number and insulin action and this is reversed if the saturated fatty acid content of the membranes is increased (Ginsberg *et al*. 1982; Field *et al*. 1988; Yorek *et al*. 1989). Increased PGC1α activity may regulate changes to the phospholipid content of exercise-trained skeletal muscle (Senoo *et al*. 2015). As exercising with a low carbohydrate availability increasingly activates proteins upstream of PGC1α such as AMPK, an increased PUFA: SFA ratio in skeletal muscle phospholipids (via AMPK) could also help to link nutrient–exercise timing to oral glucose insulin sensitivity (Abstract Figure). In support of this, reductions in the SFA content of skeletal muscle phospholipids has been shown to correlate with changes in postprandial insulinaemia after exercise (Edinburgh *et al*. 2020).

Future research

In men classified as overweight, feeding *versus* fasting before a single bout of exercise leads to a decreased expression of several genes involved in lipid metabolism, insulin signalling and glucose uptake in adipose tissue (Chen *et al*. 2017). Whether these acute responses to altered nutrient–exercise timing in adipose tissue are important for chronic metabolic health needs to be investigated with intervention studies. Glucose ingestion during exercise can also abolish reductions in hepatic glycogen content seen with a single bout of exercise (Gonzalez *et al*. 2015), which could blunt longer-term hepatic adaptations to exercise via AMPK activity. A single bout of exercise in the fasted state also leads to increased intrahepatic lipid content compared to exercise performed with glucose consumption (Bilet *et al*. 2015). However, it is unclear how acute changes in the hepatic lipid content reflect longer term responses, as exercise training lowers the hepatic lipid content (Brouwers *et al*. 2016). More work is required to establish a role of nutrient–exercise timing on adipose tissue and the liver. The timing of the first post-exercise meal may also be important for adaptations in response to altered nutrient–exercise timing. No clear differences were observed in the mRNA expression of many genes implicated in metabolic adaptations to exercise (e.g. *AMPK*, *PGC1α* and *GLUT4*) in overweight or obese individuals after a single bout of exercise before *versus* after breakfast, when the breakfast was consumed

immediately post-exercise (Edinburgh *et al*. 2020). Other studies that have provided the breakfast within 60 min of exercise completion in exercise before breakfast groups also observe no differences in protein levels of GLUT4 (Gillen *et al*. 2013) or the mRNA expression of genes involved in exercise adaptation (Verboven *et al*. 2020) with exercise before *versus* after breakfast. However, if the time window between exercise completion and breakfast consumption is extended (>2 h), augmented adaptations in skeletal muscle are reported with exercise before *versus* after breakfast (Van Proeyen *et al*. 2010; Edinburgh *et al*. 2020). This is in line with research showing that extended periods of fasting may have benefits for metabolic health (Parr *et al*. 2020). It is also possible that a lower insulinaemia in the immediate post-exercise period could contribute to these discordant results between studies. The studies in this review also investigated high-carbohydrate provision and recruited only men. As carbohydrate is the macronutrient which most potently regulates fat oxidation rates and muscle glycogen availability (Acheson *et al*. 2011), it is likely that low-carbohydrate pre-exercise meals produce outcomes more similar to the overnight fasted state than the carbohydrate-fed state, yet this would need confirming with empirical data. Omitting *versus* consuming nutrients prior to exercise increases lipid utilisation in both sexes, so it is plausible that the results discussed apply to women, but the magnitude of the response could differ (Wallis *et al*. 2006) and warrants investigation. Further research is also needed to better establish the potential effects of nutrient timing on other aspects of glucose metabolism and insulin availability, such as gastrointestinal absorption rate and hepatic insulin extraction. Current evidence would suggest that consuming a carbohydrate-rich meal prior to a single exercise bout increases exogenous glucose appearance rates post-exercise (reflecting a more rapid digestion and absorption of ingested glucose) (Edinburgh *et al*. 2018) and the lower insulinaemia seen with chronic exercise training in an overnight fasted state is most likely due to lower insulin secretion rates rather than increase hepatic insulin extraction, based on C-peptide data (Edinburgh *et al*. 2020). Finally, as compliance is a determinant of training effectiveness, future studies could investigate nutrient–exercise interactions in conditions where compliance to an exercise intervention could differ.

Summary

Exercising has many health benefits, such as increasing aerobic fitness independent of nutrient–exercise timing. However, recent research has shown that increases in oral glucose insulin sensitivity can depend on the nutrient-status in which exercise is performed. Specifically, exercise training after an extended, overnight-fast augments skeletal muscle adaptations such as GLUT4 and AMPK protein levels, and reductions in postprandial insulinaemia, even when energy balance is unaffected. This may occur to the extent to which it is possible for people with obesity to start exercise training, but achieve no clear benefits for oral glucose insulin sensitivity when carbohydrate-rich meals are consumed before exercise. The mechanisms underlying the adaptive response to exercise in a fasted state *versus* fed state are likely to include increased lipid oxidation, and greater remodelling of skeletal muscle relating to glucose uptake and metabolism, such as glucose transporters, energy sensing proteins and membrane phospholipid composition. Further research is needed with hard outcomes (e.g. fasting or 2 h glucose concentrations, HbA1c, LDL-cholesterol concentrations) and in different populations (e.g. females and people with cardiovascular disease or type 2 diabetes) in order to establish clinical guidelines. Nevertheless, the current evidence indicates that performing some exercise sessions in an overnight fasted state (i.e. before consumption of carbohydrate) may provide additional metabolic health benefits to exercise performed after high-carbohydrate meals.

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Additional information

Competing interests

None of the authors declare any conflicts of interest in relation to this work.

Author contributions

All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Keywords

energy balance, exercise, fasting, metabolic disease