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REVIEW ARTICLE

Impact of Endurance Exercise Training in the Fasted State on Muscle Biochemistry and Metabolism in Healthy Subjects: Can These Effects be of Particular Clinical Benefit to Type 2 Diabetes Mellitus and Insulin-Resistant Patients?

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Abstract Exercise training intervention is a cornerstone in the care of type 2 diabetes mellitus (T2DM) and insulin resistance (IR), and it is pursued in order to optimize exercise interventions for these patients. In this regard, the nutritional state of patients during exercise (being in the fed or fasted state) can be of particular interest. The aim of the present review is to describe the impact of endurance exercise (training) in the fasted versus fed state on parameters of muscle biochemistry and metabolism linked to glycemic control or insulin sensitivity in healthy subjects. From these data it can then be deduced whether exercise training in the fasted state may be relevant to patients with T2DM or IR. In healthy subjects, acute endurance exercise in the fasted state is accompanied by lower blood insulin and elevated blood free fatty acid concentrations, stable blood glucose concentrations (in the first 60–90 min), superior intramyocellular triacylglycerol oxidation and whole-body lipolysis, and muscle glycogen preservation. Long-term exercise training in the fasted state in healthy subjects is associated with greater improvements in insulin sensitivity, basal muscle fat uptake capacity, and oxidation. Therefore, promising results of exercise (training) in the fasted state have been found in healthy subjects on parameters of muscle biochemistry and metabolism linked to insulin sensitivity and glycemic control. Whether exercise training intervention in which exercise sessions are organized in the fasted state may be more effective in improving insulin sensitivity or glycemic control in T2DM patients and insulin-resistant individuals warrants investigation.

Key Points

Exercise training intervention is a cornerstone in the care of type 2 diabetes mellitus (T2DM) and insulin resistance, and the optimization of exercise interventions for these patients should be pursued by, for example, manipulating the nutritional state during exercise (training).

In healthy subjects, acute endurance exercise and exercise training in the fasted state are accompanied by significantly greater beneficial adaptations that lead to improvements in insulin sensitivity.

Whether exercise training intervention in which exercise sessions are organized in the fasted state may be more effective in improving insulin sensitivity or glycemic control in T2DM patients and insulin-resistant individuals warrants further investigation.

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1 Introduction

Structured exercise intervention is a cornerstone in the care of type 2 diabetes mellitus (T2DM) patients as it leads to an ~0.7 % reduction in blood glycated hemoglobin (HbA_{1c}) content, even when these patients already take

blood glucose-lowering medication [1]. Considering the significant relationship between blood HbA_{1c} content and the risk of cardiovascular complications and premature death, such a decline in blood HbA_{1c} content is clinically relevant [2, 3]. In addition to improvements in glycemic control, exercise training interventions lead to improvements in exercise capacity, decrements in adipose tissue mass, blood inflammatory markers (in the basal state, although exercise is known to affect blood inflammatory markers for up to 1–2 days) and blood pressure, augmentations in lean tissue mass and quality of life, and improvements in the blood lipid profile [4–7].

Various official international recommendations therefore promote exercise intervention in the care of T2DM [8–11]: patients with T2DM should exercise 3–5 days/week, at 40–70 % of peak oxygen uptake ($\dot{V}O_{2peak}$), achieving a minimum exercise duration of 150 min/week, in combination with resistance exercises (5–10 exercises/session, three series/exercise, 10–15 repetitions/series).

Even for the prevention of T2DM, for example in patients experiencing insulin resistance (IR) or glucose intolerance, the implementation of exercise intervention has been shown to be very potent: significant reductions in T2DM incidence can be achieved by increasing physical activity in this particular population [12]. Exercise recommendations for T2DM patients are therefore also often promoted in patients with IR.

In recent decades, further augmentation of the clinical benefits of exercise intervention in T2DM or IR have been pursued [13]. It has been shown that greater improvements in glycemic control are achieved in T2DM or insulin-resistant patients when prolonging the exercise intervention [13], increasing the volume of resistance exercises on top of endurance exercises [14], and exercising more frequently [14]. In addition, evidence in support of the potential clinical benefits of high-intensity interval training (HIIT) in patients with T2DM and IR is mounting [15]. Multiple studies have shown an equal, or sometimes even greater, clinical effectiveness of HIIT as opposed to continuous moderate-intensity endurance exercise training in these populations. However, most studies examined a small number of participants with a relatively short follow-up duration, thus warranting further study [15]. Despite the promising improvements in the clinical effectiveness of exercise interventions in T2DM or insulin-resistant patients from such adaptations in exercise prescription, room for further improvement remains. In particular, increased efficiency of exercise training (meaning greater clinical benefits with equal effort by the patient) should be aimed for. In this regard, optimization of the nutritional state during exercise in T2DM or insulin-resistant patients remains to be achieved.

The impact of exercise training in the fasted versus fed state has received considerable interest and is currently a topic of intense debate [16]. This interest stems from the observation of advantageous metabolic adaptations that particularly occur when exercising in the fasted state (see Sect. 3.1). Such acute metabolic adaptations during exercise in the fasted state could also be instrumental in gaining greater improvement in glycemic control and insulin sensitivity, especially when such exercise sessions are repeated during a long-term training intervention.

During the last 5 years many studies on the impact of exercise training in the fasted state have been published, but an examination of the potential clinical benefits of this type of exercise training specifically relating to the optimization of insulin sensitivity and glycemic control is absent. The aim of the present review is to provide an in-depth description of the impact of exercise (training) in the fasted versus fed state in healthy individuals on the parameters of muscle biochemistry and metabolism that are instrumental in greater improvements in insulin sensitivity/glycemic control. This information will contribute greater insight into the impact of exercise (training) in the fasted state on insulin sensitivity and glycemic control, and aid in the generation of an appropriate hypothesis that can be examined in T2DM or insulin-resistant patients.

2 Methodology

2.1 Literature Search

Two reviewers (DH and DDS) independently performed searches in PubMed and Web of Science for articles published up to January 2016. To find as many relevant articles as possible, the following keywords were used individually and in combination: ‘exercise’, ‘fasted’, ‘fasting’, ‘aerobic exercise’, ‘endurance exercise’, ‘insulin sensitivity’ (see Table 1). The literature search was limited to human studies and English-language articles. In addition, the reference lists of included manuscripts were checked, as well as related citations on PubMed and citations from other journals via Google Scholar. In this way, the risk of missing relevant publications was minimized.

2.2 Selection Criteria

Individual studies in which healthy adults (≥18 years, free from any disease, including overweight/obesity and T2DM/IR) were studied and endurance exercise (training) in the fasted state was compared directly with exercise (training) in the fed state (with a crossover design or in a randomized clinical trial) were selected. There were no

Table 1 Search keywords and number of hits (*n*)

Search keywords	*n*
Exercise AND fasted	437
Exercise AND fasting	3842
Endurance exercise AND fasted	82
Endurance exercise AND fasting	246
Aerobic exercise AND fasted	437
Aerobic exercise AND fasting	3842
Aerobic exercise AND fasting AND insulin sensitivity	1183
Aerobic exercise AND fasted AND insulin sensitivity	72

Search was limited to English papers and human studies

restrictions in age above 18 years, sex, and physical activity level. Studies that included resistance exercises were excluded. The fed state was defined as having consumed a meal before exercise (studies examining the impact of ingestion of a carbohydrate-containing drink only were not included) while the fasted state was defined as food intake cessation after a single overnight fast (>10 h). In studies examining the acute impact of exercise in a different nutritional state, the same nutritional state had to be maintained during the entire observed exercise bout (subjects should remain in the fasted state during exercise and/or subjects in the fed state should not consume nutrients during exercise, although the consumption of water was allowed). However, in studies examining the impact of exercise training in the fasted versus fed state, this was not always the case. Studies examining Ramadan fasting (in which cessation of food intake is observed during the daytime) were not included due to a very different endocrinologic impact. At least one of following outcome parameters/items should have been assessed in these studies: blood insulin, glucose, free fatty acid or HbA_{1c} concentration, (muscle) fat or carbohydrate oxidation rates, lipid mobilization (lipolysis) rates, insulin sensitivity (as assessed by Homeostasis Model Assessment for Insulin Resistance [HOMA-IR] index, oral glucose tolerance test, or euglycemic hyperinsulinemic clamp), blood hormones ([nor]epinephrine, glucagon, cortisol, C-peptide, growth hormone), and/or other muscle biochemistry parameters that affect glycemic control or insulin sensitivity (such as glucose transporter type 4 [GLUT-4] protein expression, muscle intramyocellular triacylglycerol [IMTG], glycogen content).

Initially, 3842 initial hits were found. After applying inclusion and exclusion criteria and checking the titles and/or abstracts, 48 articles were retained. Next, the full text of these studies was checked carefully and additional relevant articles found in the references lists of these publications were searched for via PubMed, Web of Science, and Google Scholar. Eventually, 27 papers were included in the present review. Nineteen studies examined the acute impact of endurance exercise in the fasted versus fed state, while eight studies examined the impact of endurance exercise training (see Tables 2, 3).

2.3 Data Extraction

The extracted data from each included study are presented in Tables 2 and 3 in order of publication date. Two topics are described in chronological order throughout the present review: (1) the impact of acute endurance exercise in the fasted versus fed state on glycemic control and insulin sensitivity, muscle biochemistry, and metabolism in healthy individuals; and (2) the impact of endurance exercise training in the fasted versus fed state on glycemic control and insulin sensitivity, muscle biochemistry, and metabolism in healthy individuals. Meta-analytical calculations or comparisons were not conducted due to the great diversity of outcome measurements and methods within the different studies.

3 Findings and Discussion

3.1 Impact of Acute Endurance Exercise on Muscle Biochemistry and Metabolism Related to Glycemic Control and Insulin Sensitivity

The first studies that examined the impact of endurance exercise in the fasted versus fed state on muscle biochemistry and metabolism related to glycemic control and insulin sensitivity were published in the mid-1980s. In these studies, exercise in the fed state was initiated within 15 [27], 30 [23], 45 [19], 60 [17, 28, 45], 90 [22, 29, 33], 120 [18, 20, 21], 180 [24, 43, 44], or 240 min [25, 31] after meal consumption.

According to some studies [17–19], when endurance exercise is performed in the fasted state in healthy subjects, lower blood glucose levels are present during exercise, as opposed to an exercise bout after a meal, although the majority of studies reported elevated or stable blood glucose concentrations when endurance exercise is performed in the fasted state [20–25]. A lowered blood glucose concentration during exercise in the fed state may seem counterintuitive, but is believed to be related to the hyperinsulinemic state that typically occurs in the postprandial phase preceding the exercise bout [22]. When exercise is initiated in that particular hyperinsulinemic condition, while insulin sensitivity acutely increases together with muscle glucose uptake after initiation of exercise, significant and rapid decrements in blood glucose concentrations can be expected. This phenomenon particularly occurs in the first part (first ±30 min) of the exercise bout.

Table 2 Impact of acute endurance exercise in the fasted versus fed state

Study (year)	Subjects (*n*)	Exercise bout characteristics	Outcome parameters	Findings during exercise
Coyle et al. [25] (1985)	7 male cyclists	105 min cycling at 70 % $\dot{V}O_{2peak}$	Blood glucose, insulin, and FFA concentration	Elevated blood glucose, insulin, and FFA concentration during exercise in the fasted state
			RQ	Lowered RQ during exercise in the fasted state
			Muscle glycogen concentration	Smaller muscle glycogen concentration reduction during exercise in the fasted state
Dohm et al. [32] (1986)	9 male runners	90 min treadmill running at 70 % $\dot{V}O_{2peak}$	Blood glucose and FFA concentration	Blood glucose concentration not different between different exercise conditions. Elevated blood FFA concentration during exercise in the fasted state
			RQ	Lower RQ during exercise in the fasted state
Loy et al. [31] (1986)	10 male cyclists	Cycling to exhaustion at 79 % $\dot{V}O_{2peak}$ Cycling to exhaustion at 86 % $\dot{V}O_{2peak}$	Blood glucose, insulin, FFA, (nor)epinephrine concentration	Blood glucose, norepinephrine, and insulin concentration elevated, and blood FFA concentration lowered during high-intensity exercise in the fasted state, but not during moderate-intensity exercise in the fasted state
			Muscle glycogen concentration	Changes in muscle glycogen concentration not affected by nutritional state during exercise
			RQ	RQ not affect by nutritional state during exercise
Nieman et al. [43] (1987)	9 male marathon runners	90 min treadmill running at 70 % $\dot{V}O_{2peak}$	RQ	Lowered RQ during exercise in the fasted state
			Blood glucose, insulin, FFA, (nor)epinephrine, glucagon concentration	Greater blood insulin and norepinephrine concentration during exercise in the fasted state, no differences in other parameters between exercise conditions
			Muscle glycogen concentration	No differences in muscle glycogen concentration between exercise conditions
Schneiter et al. [33] (1995)	9 healthy males/ females	45 min on treadmill (5 km/h, slope 10 %)	Fat and glucose oxidation	Lower glucose oxidation and elevated fat oxidation during exercise in the fasted state
			Muscle glycogen turnover	Greater muscle glycogen turnover during exercise in the fasted state leading to glycogen sparing effect
Paul et al. [22] (1996)	12 healthy males/ females	90 min cycling at 60 % $\dot{V}O_{2peak}$	Fat and carbohydrate oxidation	Lowered carbohydrate oxidation and elevated fat oxidation during exercise in the fasted state when compared with exercise after corn, oat, and wheat consumption
			Blood glucose, FFA, and insulin levels	Elevated blood glucose/FFA level during exercise in the fasted state when compared with exercise after corn, oat, and wheat consumption
Horowitz et al. [17] (1997)	6 healthy males	45 % $\dot{V}O_{2peak}$ for 60 min	Blood glucose, insulin, FFA concentration	Blood glucose and insulin levels decrease in the fed but not in the fasted state, plasma FFA concentrations are elevated in the fasted vs. fed state
			Lipolysis and fat oxidation	Lipolysis and fat oxidation are greater in the fasted vs. fed state
			Glucose kinetics and glucose oxidation	Glucose rate of appearance and disappearance smaller in the fasted vs. fed state, muscle glycogen use smaller in the fasted vs. fed state
Whitley et al. [31] (1998)	8 male cyclists	90 min of cycling at 70 % $\dot{V}O_{2peak}$	Blood glucose, insulin, FFA, (nor)epinephrine, cortisol, growth hormone concentration	Lowered blood FFA and epinephrine concentration and elevated insulin concentrations during exercise in the fasted state, as opposed to exercise after high-carbohydrate meal
			Fat and carbohydrate oxidation	Elevated blood growth hormone concentration during exercise in the fasted state, as opposed to exercise after high-fat meal

Table 2 continued

Study (year)	Subjects (*n*)	Exercise bout characteristics	Outcome parameters	Findings during exercise
DeMarco et al. [23] (1999)	10 trained cyclists	120 min cycling at 70 % $\dot{V}O_{2peak}$	Blood glucose and insulin concentration	Greater blood glucose concentration and lower blood insulin concentration during exercise in the fasted state, as opposed to exercise after a high or low glycemic index meal
			RQ	Lowered RQ during exercise in the fasted state, but only when compared to exercise after a meal with a high glycemic index
Bergman and Brooks [44] (1999)	14 healthy males	Cycling at 20–40 % $\dot{V}O_{2peak}$ for 120 min, followed by cycling at 60 % $\dot{V}O_{2peak}$ for 90 min, followed by cycling at 80 % $\dot{V}O_{2peak}$ for 30 min	RQ	Lower RQ during exercise in the fasted state
Wu et al. [24] (2003)	9 male runners	60 min treadmill running at 65 % $\dot{V}O_{2peak}$	Blood glucose, insulin, FFA concentration	Elevated blood glucose and FFA concentration during exercise in the fasted state when compared with exercise after a high or low glycemic index meal
			Fat and carbohydrate oxidation	Elevated fat oxidation and lowered carbohydrate oxidation during exercise in the fasted state, especially when compared with exercise after a meal with high glycemic index
Enevoldsen et al. [28] (2004)	7 healthy males	55 % $\dot{V}O_{2peak}$ for 60 min	Blood glucose, insulin, FFA, glycerol concentration	Blood insulin concentration lower in the fasted vs. fed state, blood FFA and glycerol concentration higher in the fasted vs. fed state, comparable blood glucose levels between trials
			Lipolysis and fat oxidation	Lipolysis was comparable between trials, fat oxidation greater in the fasted vs. fed state
			Blood catecholamine concentration	Comparable changes between trials
De Bock et al. [18] (2005)	9 healthy males	75 % $\dot{V}O_{2peak}$ for 120 min	Muscle IMTG concentration	Greater reduction in type 1 muscle fibers as result of exercise training in the fasted state
			Muscle glycogen concentration	Comparable reduction between different conditions
			Muscle AMPK activity	Comparable increase between different conditions
			Blood glucose and FFA concentration	Lower blood glucose and higher blood FFA concentration during exercise in the fasted state
			Blood insulin, (nor)epinephrine, leptin, cortisol concentration	Lower blood insulin and leptin concentration during exercise in the fasted state, greater blood epinephrine concentration during exercise in the fasted state
			Fat and carbohydrate oxidation	Greater fat and lower carbohydrate oxidation during exercise in the fasted state
Bennard and Doucet [19] (2006)	8 healthy males	Treadmill running at 50 % $\dot{V}O_{2peak}$ for 40 min	Whole-body fat oxidation	Greater whole-body fat oxidation during exercise in the fasted state when compared with exercise after a meal with high or low glycemic index
Scott et al. [21] (2012)	10 healthy males	60 min treadmill running at 65 % $\dot{V}O_{2peak}$	Blood glucose, cortisol, leptin, and ghrelin concentration	Greater blood glucose levels during exercise in the fasted state. No difference between exercise trials in blood cortisol, leptin, and ghrelin levels

Table 2 continued

Study (year)	Subjects (*n*)	Exercise bout characteristics	Outcome parameters	Findings during exercise
Lane et al. [20] (2013)	12 competitive male cyclists	60 min cycling time trial	Blood glucose levels	Greater blood glucose levels during exercise in the fasted state
Kang et al. [45] (2013)	20 healthy males/females	30 min cycling exercise, increasing from 50 up to 70 % $\dot{V}O_{2peak}$ every 10 min	Fat and carbohydrate oxidation	Elevated fat and lowered carbohydrate oxidation during exercise in the fasted state
Schwalm et al. [29] (2015)	23 trained male athletes	120 min cycling at 55 % $\dot{V}O_{2peak}$ 120 min cycling at 70 % $\dot{V}O_{2peak}$	Blood insulin concentration Muscle mTOR and AMPK phoshorylation	Lower blood insulin concentration during exercise in the fasted state Lower mTOR phosphorylation during exercise in the fasted state, AMPK phosphorylation not affected by different nutritional state
de Lima et al. [27] (2015)	10 healthy participants	36 min treadmill running at 65 % $\dot{V}O_{2peak}$	Blood glucose concentration	Reduction in blood glucose concentration during exercise in the fed state, no reduction in blood glucose concentration during exercise in the fasted state

AMPK adenosine monophosphate-activated protein kinase, *FFA* free fatty acid, *IMTG* intramyocellular triacylglycerol, *mTOR* mammalian target of rapamycin, *RQ* respiratory quotient, $\dot{V}O_{2peak}$ peak oxygen uptake

This also implies that when exercise is performed later after consumption of a meal, smaller decrements in blood glucose during exercise can be anticipated. In agreement with this reasoning, Montain et al. [26] discovered that when endurance exercise is performed within 2 h after meal consumption, reductions in blood glucose concentrations are significantly greater (especially in the first 30 min of exercise) than with exercise initiated 6–12 h after meal consumption.

In addition, and in contrast to the current widely held belief, the risk of hypoglycemia during exercise in the fasted state is not greater in healthy individuals because blood glucose levels remain stable throughout such an exercise bout, at least in first hour [17–19, 27]. However, decrements in blood glucose concentrations during exercise in the fasted state have been observed between 60 and 120 min after endurance exercise [38], although most T2DM or insulin-resistant patients do not exercise for such long periods, particularly when initiating exercise interventions. The stabilization of blood glucose levels during endurance exercise (thus in later stages during exercise) in the fasted state is likely as a result of lower (pre-exercise) blood insulin levels, which will lead to smaller hepatic and muscle glucose uptake [17–19, 28, 29], and higher blood epinephrine levels, which will lead to elevated hepatic glucose production [18]. As a result, compensatory hormonal changes during exercise training in the fasted state prevent the development of hypoglycemia. It thus follows that exercise training in the fasted state during the first 60 min is medically safe from an endocrine perspective, at least in healthy individuals.

However, the impact of exercise in the fasted versus fed state on glucose and insulin metabolism during exercise is a very complex affair. For example, the application of different relative exercise intensities affects blood glucose and insulin concentrations significantly [30]. In trained cyclists it was found that blood glucose and insulin concentrations were elevated during high-intensity exercise (at 86 % of $\dot{V}O_{2peak}$, which was above the anaerobic threshold) in the fasted state (as opposed to exercise in the fed state). In contrast, during moderate-intensity endurance exercise (at 79 % of $\dot{V}O_{2peak}$, which was below the anaerobic threshold), blood glucose and insulin concentrations were comparable in the fasted versus fed state. Changes in the blood glucose level during exercise thus depend, at least in part, on exercise intensity. It is well-known that high-intensity exercise is associated with a greater epinephrine and growth hormone synthesis/release, which will stimulate hepatic glucose output [46]. Due to greater glucose production, as opposed to glucose utilization (in skeletal muscle cells), increments in blood glucose concentrations may occur during high-intensity exercise (above the anaerobic threshold) [46].

Table 3 Impact of exercise training in the fasted versus fed state

Study (year)	Subjects	Comparison	Outcome parameters	Findings after exercise intervention
De Bock et al. [35] (2008)	20 healthy males	Fasted vs. fed endurance exercise training at 75 % $\dot{V}O_{2peak}$ for 6 weeks when following normal diet	Basal muscle protein GLUT-4, hexokinase-2, FABP, CD36, UCP-3 concentration	Greater increase in muscle FABP and CD36 protein concentration after exercise training in the fasted state, equal changes between groups for other parameters
			Basal muscle SDH activity	Equal changes in type 1 muscle fiber between groups, greater increase in type 2 muscle fibers as result of exercise training in the fasted state
			Basal muscle glycogen and IMTG concentration	Greater increase in muscle glycogen concentration in the fed training group, no changes in muscle IMTG concentration between groups
			Changes during acute exercise in above-mentioned muscle parameters	Greater muscle glycogen breakdown in the fed training group, equal increase in muscle IMTG breakdown between groups
			Fat and carbohydrate oxidation during acute exercise	No changes
			Basal blood insulin, (nor)epinephrine and glucose concentration	No changes
			Changes during acute exercise in above-mentioned blood parameters	No changes
Van Proeyen et al. [36] (2010)	27 healthy males	Fasted vs. fed exercise training for 6 weeks at 70–75 % $\dot{V}O_{2peak}$ when following a high-fat diet	Oral glucose tolerance	Greater reductions in postprandial blood glucose levels after exercise training in the fasted state
			Insulin sensitivity (by Matsuda ISI)	Greater improvement as result of exercise training in the fasted state
			Basal muscle GLUT-4, AMPKα protein concentration	Greater increase in muscle GLUT-4 and AMPKα protein concentration after exercise training in the fasted state
			Basal muscle glycogen and lipid concentration	Greater increase in muscle glycogen concentration after exercise training in the fasted state, equal changes in muscle fat concentration between different training programs
			Basal muscle FAT/CD36 and CPT-1 mRNA concentration	Greater increase in muscle FAT/CD36 and CPT-1 mRNA concentration after exercise training in the fasted state
			Basal muscle CS, β-HAD, and COX activity	Equal changes between different training programs
			Basal blood FFA concentration	Equal reductions between different training programs
Stannard et al. [37] (2010)	14 healthy males/ females	Fasted vs. fed exercise training for 4 weeks at 65 % $\dot{V}O_{2peak}$ when following normal diet	Fat oxidation during submaximal exercise	Equal decrease between different training programs
			Basal muscle glycogen concentration	Greater increase as result of fasted exercise training
			Basal muscle CS and β-HAD activity	Equal increase in muscle CS activity between different training interventions, no changes in muscle β-HAD activity

Table 3 continued

Study (year)	Subjects	Comparison	Outcome parameters	Findings after exercise intervention
Van Proeyen et al. [38] (2011)	20 healthy males	Fasted vs. fed exercise training for 6 weeks at 70 % $\dot{V}O_{2peak}$ when following normal diet	Fat oxidation capacity during submaximal exercise	Equal improvement between different training programs
			Basal muscle IMTG concentration	No changes
			Muscle IMTG concentration before and after acute endurance exercise	Greater reduction in type 1 and 2a muscle fiber IMTG concentration during acute endurance exercise as result of exercise training in the fasted state
			Basal muscle glycogen concentration	Increase in basal muscle glycogen concentration as result of exercise training in the fasted state, no changes as result of exercise training in the fed state
			Muscle glycogen concentration before and after acute endurance exercise	Equal reduction in muscle glycogen concentration during endurance exercise between different training interventions
			Basal muscle GLUT-4 and AMPKα activity	Equal increases between different training programs
			Basal muscle CS and β-HAD activity	Increase in muscle CS and β-HAD activity as result of fasted exercise training but not as result of fed exercise training
			Basal blood glucose concentration	No changes
			Blood glucose concentration during acute endurance exercise	Equal reduction at 60 min of acute endurance exercise between different training programs
			Basal blood FFA concentration	No changes
Van Proeyen et al. [39] (2011)	28 healthy males	Fasted vs. fed exercise training for 6 weeks at 70–75 % $\dot{V}O_{2peak}$ when following a high-fat diet	Basal muscle IMTG concentration	Equal increase between different training programs
			Muscle IMTG breakdown during acute endurance exercise	Equal greater level of breakdown in muscle IMTG concentration between different training programs
			Basal muscle glycogen concentration	Equal increase in between different training programs
			Muscle glycogen concentration before and after acute endurance exercise	No changes
			Basal muscle AMPKα protein concentration	Elevated after exercise training in the fasted state, but not after exercise training in the fed state
			Muscle AMPKα protein concentration changes during acute endurance exercise	Elevated during acute exercise after exercise training in the fasted condition, but not after exercise training in the fed condition
			Basal blood FFA concentration	Equally reduced between different training programs
			Blood FFA changes during acute endurance exercise	Greater reduction in blood FFA concentration during acute endurance exercise as result of exercise training in the fasted state
Van Proeyen et al. [40] (2011)	20 healthy males	Fasted vs. fed exercise training for 6 weeks at 75 % $\dot{V}O_{2peak}$ when following normal diet	Basal muscle AMPKα phosphorylation	Equal increase between different training programs
			Muscle AMPKα phosphorylation changes during acute endurance exercise	Equal decrease between different training programs
			Basal muscle glycogen concentration	Increased after exercise training in the fed state, but not after exercise training in the fasted state
			Changes in muscle glycogen concentration during acute endurance exercise	Equally increased post-exercise muscle glycogen concentration between different training programs

Table 3 continued

Study (year)	Subjects	Comparison	Outcome parameters	Findings after exercise intervention
Schoenfeld et al. [41] (2014)	20 healthy females	Fasted vs. fed exercise training for 4 weeks at 70 % of maximal heart rate when following a hypocaloric diet	Fat mass, fat-free mass, waist circumference	Equal reductions between groups

β-HAD β-hydroxyacyl coenzyme A dehydrogenase, *AMPK* adenosine monophosphate-activated protein kinase, *COX* cyclo-oxygenase, *CPT-1* carnitine palmitoyltransferase I, *CS* citrate synthase, *FABP* fatty acid binding protein, *FAT/CD36* fatty acid translocase CD36, *FFA* free fatty acid, *GLUT-4* glucose transporter type 4, *IMTG* intramuscular triacylglycerol, *ISI* insulin sensitivity index, *mRNA* messenger RNA, *SDH* succinate dehydrogenase, *UCP-3* mitochondrial uncoupling protein 3, $\dot{V}O_{2peak}$ peak oxygen uptake

In addition, the macronutrient composition or glycemic index of the meals that precede endurance exercise in the fed state may significantly affect blood glucose and/or insulin concentrations, and thus change the outcome as opposed to exercise in the fasted state [19, 22–24, 31]. In contrast, the glycemic index of the last meal before acute exercise does not seem to affect blood glucose and free fatty acid or glycerol concentrations during exercise [23, 24]. Discrepancies in results between studies were, however, found for (changes in) blood insulin concentrations and fat or carbohydrate oxidation rates when a meal with a high or low glycemic index was consumed before exercise [19, 23, 24]. Some studies observed the largest differences in oxidation rate between exercise in the fasted state versus exercise after consumption of high glycemic index meal, while other studies were unable to detect differences between meals of low or high glycemic indexes on these oxidation rates (even though fat oxidation was elevated during exercise in the fasted state). Due to differences in exercise protocols and nutritional manipulations between these studies, it remains difficult to explain the discrepancies in results. However, it is felt that this aspect is important in order to obtain a full understanding of the (beneficial) impact of endurance exercise in the fasted state. Further study in this field is thus encouraged.

Two studies examined the clinical benefits of exercise in the fasted state as opposed to exercise after meals with a different macronutrient composition and/or fiber content [22, 31]. In one study [22] it was found that such a different macronutrient composition and fiber content of the last meal preceding endurance exercise could affect blood glucose concentrations during exercise, but not blood insulin and free fatty acid concentrations or fat oxidation rate. Another study reported a significant impact of a different macronutrient composition of the last meal preceding exercise (high-fat or high-carbohydrate meal) in comparison with exercise in the fasted state [31]. When compared with exercise after a high-fat meal, exercise in the fasted state was associated with elevated blood growth hormone concentrations. On the other hand, when compared with exercise after a high-carbohydrate meal, exercise in the fasted state was associated with lowered blood epinephrine and insulin concentrations and elevated blood free fatty acid concentrations. These data highlight an important issue: the acute clinical benefits of exercise in the fasted state, as opposed to exercise in the fed state, are highly dependent on the type of meal (macronutrient composition) that was consumed in the fed state. Unfortunately, too few studies have been published in this domain ($n = 2$) and, as far as the authors are aware, muscle biochemistry was not examined in these studies. It thus follows that more research is needed in this particular field.

Although it has become apparent that the macronutrient composition and/or glycemic index of the last meal preceding the exercise bout in the fed state may affect blood glucose and insulin concentration or fat/carbohydrate oxidation during endurance exercise, a carbohydrate-rich meal with a higher glycemic index was provided to the subjects in the 'exercising in the fed' condition in most studies. This may explain why similar results were reported in the majority of the studies.

A typical, often noted characteristic of endurance exercise in the fasted state is the significantly elevated fat oxidation and lipolysis rate (often accompanied by elevated blood free fatty acid concentrations) [17–19, 22, 24, 25, 28, 32]. Elevated blood free fatty acid concentrations during endurance exercise in the fasted state stems from enhanced lipolysis. The enhancement in lipolysis during such exercise is very likely due to lower blood insulin concentrations (which thus leads to a smaller inhibitory effect on lipolysis) and equal or elevated blood epinephrine concentrations (which thus leads to preservation or stimulation of lipolysis) during exercise [17–19, 28]. These metabolic adaptations that typically occur during endurance exercise in the fasted state are often thought to result in greater adipose tissue mass loss: this could thus be an argument for (endurance) athletes and obesity patients to exercise in the fasted state. The validity of this hypothesis is examined in Sect. 3.2.

In addition, and less speculative, these circulatory adaptations in glucose and free fatty acid levels during endurance exercise in the fasted state are associated with changes in muscle substrate selection preference. Greater IMTG oxidation and breakdown emerges when exercising in the fasted state, especially in type 1 muscle fibers [17]. The greater IMTG breakdown for muscle adenosine triphosphate (ATP) resynthesis can explain the muscle glycogen sparing that is often observed in endurance exercise in the fasted state [17, 25, 34]. However, the greater muscle glycogen sparing effect during endurance exercise in the fasted state was not explained by basal (pre-exercise) differences in muscle glycogen concentration in all studies: even after a 10-h fast, the muscle glycogen concentration was not lowered before exercise in some studies [18]. It could be speculated that greater IMTG breakdown during endurance exercise in the fasted state is probably due to greater activation in hormone-sensitive lipase (HSL) within the muscle cell, especially in the presence of elevated blood epinephrine/insulin ratios [18]. However, the latter hypothesis remains to be confirmed.

The facilitation of IMTG oxidation or breakdown during endurance exercise in the fasted state could be clinically relevant to patients with T2DM or IR. Elevations in IMTG concentrations in the presence of a lowered or disturbed IMTG oxidation capacity are believed to, at least in part, lead to the development of IR [34]. It thus follows that acute endurance exercise in the fasted state leads to promising metabolic adaptations that are relevant to T2DM or insulin-resistant patients (i.e., greater stimulation of insulin sensitivity).

One study examined the impact of a different nutritional status during exercise on muscle signaling for mitochondrial biogenesis (by examination of adenosine monophosphate-activated protein kinase alpha [AMPKα] phosphorylation) and myofibrillar biogenesis (by examination of mammalian target of rapamycin [mTOR] phosphorylation) [29]. Stimulated muscle AMPKα phosphorylation may be important for greater mitochondrial biogenesis and thus greater improvements in muscle fat oxidation capacity and prevention of IMTG accumulation. Greater muscle mTOR phosphorylation may, on the other hand, be important for greater myofibrillar biogenesis, muscle hypertrophy, and thus greater muscle glucose uptake. It has been discovered that endurance exercise in the fasted state was associated with lowered mTOR phosphorylation, while muscle AMPKα phosphorylation was not affected by a different nutritional state [29]. However, it remains speculative as to whether a different muscle signaling for myofibrillar biogenesis during endurance exercise relates to different muscular adaptations, and changes in insulin sensitivity or glycemic control, during long-term exercise intervention under different nutritional states. This hypothesis is explored in Sect. 3.2.

Some methodological limitations were discovered in the published studies that should be taken into account. It remains difficult to generalize these promising findings to the general healthy population: the impact of high-intensity (>75 % of $\dot{V}O_{2peak}$) endurance exercise in the fasted state is not studied frequently, mostly males were examined (often with a good endurance exercise tolerance), and relatively young subjects were most often evaluated. These study limitations should thus be taken into account throughout this review. Studies that examined the impact of fasted endurance exercise at high intensities remain to be initiated, and more females, older individuals (>50 years), and/or less physically fit individuals should be selected as well. As a result, we should take care regarding the opinion that exercise training in the fasted state should be implemented more often in healthy individuals in order to affect glucose metabolism or insulin sensitivity to a greater extent. Considering the significantly different phenotype between the examined subjects and T2DM or insulin-resistant individuals (older, greater fat mass, lower exercise tolerance, etc.), even greater caution is warranted when generalizing these findings to these patient groups.

In summary, endurance exercise in the fasted state in healthy subjects is generally assumed to be associated with

lower blood insulin concentrations, stable blood glucose concentrations (in the first 60 min), elevated blood free fatty acid concentrations and fat oxidation, greater IMTG oxidation and muscle glycogen sparing, and lowered muscle mTOR phosphorylation. In particular, the higher level of IMTG oxidation during exercise in the fasted state may predict greater improvements in glycemic control and/or insulin sensitivity. To optimize the medical safety of exercise in the fasted state in T2DM and insulin-resistant patients (i.e., prevent hypoglycemia), it may be advisable to limit the exercise session duration to up to 60–90 min.

In the next section, we review the impact of exercise training in the fasted versus fed state on these parameters to examine whether these predictions hold true during long-term exercise intervention.

3.2 Impact of Endurance Exercise Training on Glycemic Control and Insulin Sensitivity, Muscle Biochemistry, and Metabolism

The first studies that examined the impact of endurance exercise training in the fasted versus fed state on parameters related to insulin sensitivity or glycemic control in healthy individuals were published in the late 2000s. The impact of exercise training in the fasted versus fed state has been examined frequently in healthy subjects, especially in the last 5 years, and a detailed examination of its impact on parameters of metabolism and muscle biochemistry related to insulin sensitivity and glycemic control has often been achieved [35–41].

To review the observed impact of endurance exercise training intervention in the fasted versus fed state, we first discuss changes in parameters in basal/resting state, followed by changes in various parameters during acute submaximal endurance exercise.

The available data indicate that few consistent changes in fasting blood parameters can be expected when an endurance exercise training intervention in the fasted state is implemented in healthy individuals. Some studies did not observe changes in fasted blood free fatty acid, glucose, or insulin concentrations as result of exercise training in the fasted or fed state [35, 38] while other studies reported equal changes in these parameters between different training interventions [36, 39]. However, under more dynamic circumstances, such as during an oral glucose tolerance test, endurance exercise training in the fasted versus fed state seems to have a different impact. Van Proeyen et al. [36] recently discovered that exercise training in the fasted state led to a significantly greater improvement in oral glucose tolerance and insulin sensitivity (according to the Matsuda insulin sensitivity index [ISI]), as opposed to exercise training in the fed state. It is speculated that a greater improvement in insulin sensitivity and oral glucose tolerance as result of an endurance exercise training intervention in the fasted state is attributed to greater augmentation in (fasted) muscle GLUT-4 protein content [36]. The latter adaptation will lead to enhanced muscle glucose uptake, especially in situations where hyperglycemia is provoked (such as during an oral glucose load). However, it has to be mentioned that the subjects examined in this study were on a high-fat diet. It remains to be determined whether similar effects of endurance exercise training in the fasted state also occur when healthy subjects follow a low-fat diet, or any other diet (such as a high-carbohydrate diet). For example, in another study where subjects were on a diet with significantly lower fat intake during a 6-week exercise training intervention in the fasted state, no such additional beneficial effects of exercise training in the fasted state on basal muscle GLUT-4 protein concentration were observed [35, 38]. It can thus be hypothesized that the surplus effects of exercise training in the fasted versus fed state are also dependent on the macronutrient composition of the diet that is followed by the subjects in the fed group. Unfortunately, the interactions between the type of diet and impact of (fasted) exercise training on metabolism, glycemic control and insulin sensitivity, and muscle biochemistry are far from established and deserve further investigation.

Regardless of the impact of the type of diet that is followed by the individuals, endurance exercise training in the fasted state markedly improves basal muscle fat transport and oxidation capacity, as evidenced by significantly greater increments in basal muscle fatty acid translocase CD36 (FAT/CD36) protein and fatty acid binding protein (FABP) content, and in this respect is superior to exercise training in the fed state [35, 36]. However, a greater muscle fat uptake capacity is not associated with greater elevations in IMTG content as result of endurance exercise training in the fasted state [35, 36, 38, 39]. In the presence of elevations in muscle fat uptake capacity, but without concomitant elevations in IMTG content, it may be speculated that the basal muscle fat oxidation capacity is enhanced with a significantly greater magnitude when following an endurance exercise training intervention in the fasted state. This will probably lead to a better protection against IR from IMTG accumulation [34]. However, the impact of endurance exercise training in the fasted state on muscle β-hydroxyacyl coenzyme A dehydrogenase (β-HAD) activity/content (which is a key enzyme involved in β-oxidation or oxidation of fatty acids in the muscle cell) remains uncertain [36, 38]. Therefore, whether endurance exercise training in the fasted state actually leads to greater muscle fat oxidation capacity should be studied in greater detail. This would confirm the above-mentioned beneficial physiological cascades that emerge from endurance exercise training in the fasted state.

Changes in basal muscle glycogen content after endurance exercise training in the fasted versus fed state remain equivocal: some studies observed greater increases in basal muscle glycogen content as a result of endurance exercise training in the fasted state [36–38], while others observed greater increases in basal muscle glycogen content after endurance exercise training in the fed state [35, 40], and still others observed equal changes in basal muscle glycogen content between different exercise interventions [39]. Similar equivocal results were found for changes in basal muscle AMPKα activity or phosphorylation [36, 38–40]. An explanation for the discrepancy in results between studies remains to be provided, although it can be hypothesized that differences in the type of diet between studies may be instrumental in these inconsistent findings. Subject characteristics and endurance exercise training modalities were comparable between studies.

A recent trial examined the impact of 4 weeks of exercise training in the fed or fasted state on body composition, when following a diet, in healthy normal-weight females [41]. Reductions in fat mass in particular were instrumental in improving glycemic control and thus are relevant to T2DM and insulin-resistant patients. Changes in fat and fat-free mass were comparable between groups. Similar findings have been reported in obese females participating in interval endurance exercise training interventions in the fed or fasted state [42]. On the other hand, when young healthy volunteers followed a high-fat diet for 6 weeks, increments in body weight and subcutaneous skinfold thickness were observed, which was not entirely prevented when exercising in the fed state [36]. Conversely, exercise training in the fasted state was associated with a prevention of increments in body weight and skinfold thickness. These data thus seem to indicate that exercise training in the fasted state may be instrumental in preventing adipose tissue mass gain in previously lean individuals, although more studies remain warranted to further explore this finding. Even though muscle mTOR phosphorylation during a single exercise bout seems to be affected by the nutritional state (see above), long-term changes in muscle mass seem to be unaffected by the nutritional state during exercise. In addition, even though acute endurance exercise in the fasted state is associated with greater lipolysis, this does not seem to translate into greater fat mass loss during long-term exercise interventions.

Some studies evaluated the impact of endurance exercise training intervention in the fasted versus fed state on the physiological/metabolic response to acute submaximal endurance exercise. Despite the promising effects of endurance exercise training in the fasted state on basal muscle fat uptake and oxidation capacity, these beneficial effects do not automatically translate into greater whole-body fat oxidation or greater IMTG breakdown during submaximal endurance exercise [35, 37–39]. These data thus indicate that changes in muscle fat oxidation and uptake capacity in basal state as a result of endurance exercise interventions under different nutritional circumstances do not predict similar changes during acute endurance exercise. However, it may be questioned whether the submaximal exercise tests were of an appropriate methodology to be able to detect such relationships: the workload of the exercise test should have been adapted in accordance to changes in workload capacity or exercise tolerance of the individuals. It may be hypothesized that increments in exercise tolerance are associated with shifts in workloads in which maximal fat oxidation is achieved. This was not corrected for in these studies. Therefore, relative exercise intensities were significantly different between subjects at the follow-up/second submaximal exercise test. The latter significantly affects the observed muscle and whole-body fat oxidation during such an exercise test. In addition, it remains to be determined whether beneficial adaptations preferentially in the basal state, or preferentially during endurance exercise testing, are predictive of improvements in glycemic control and insulin sensitivity in healthy individuals.

These data should be evaluated in light of the study limitations: mainly young males with a normal or elevated endurance exercise capacity were examined and, in addition, the implemented exercise training interventions were of a relatively short duration (<7 weeks) and of moderate intensity only. It thus follows that the generalization of these findings towards females, older, and/or less fit individuals is not appropriate. Furthermore, the impact of long-term endurance exercise training (>12 weeks) interventions in the fasted state at a high (>80 % $\dot{V}O_{2peak}$) or low (<65 % $\dot{V}O_{2peak}$) intensity remains unknown. Patients with T2DM or IR typically have a significantly different phenotype, which further complicates the generalization of these results to these patients. In addition, exercise training interventions for these patients are typically of a longer duration (at least 12 weeks) and often combined with a hypocaloric diet [8–11].

In conclusion, as result of exercise training in the fasted state, it is generally assumed that such intervention is associated with greater improvements in basal fat oxidation capacity (but not during exercise) and greater improvements in insulin sensitivity. These adaptations may thus be instrumental in greater improvements in glycemic control.

4 Conclusion

In healthy subjects, acute endurance exercise in the fasted state is accompanied by lower blood insulin and elevated blood free fatty acid concentrations, stable blood glucose concentrations (at least in the first 60–90 min), superior

IMTG oxidation and whole-body lipolysis, and muscle glycogen preservation. Long-term endurance exercise training in the fasted state is accompanied by greater improvements in insulin sensitivity and basal muscle fat oxidation capacity (but not during exercise). Due to these promising results, the impact of endurance exercise training in the fasted state should be studied in T2DM and insulin-resistant patients.

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References

1. Umpierre D, Ribeiro PAB, Kramer CK, et al. Physical activity advice only or structured exercise training and association with HbA1c levels in type 2 diabetes: a systematic review and meta-analysis. JAMA. 2011;305:1790–9.
2. UK Prospective Diabetes Study Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risks of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998;352:837–53.
3. Khaw K, Wareham N, Luben R, et al. Glycated haemoglobin, diabetes and mortality in men in Norfolk cohort of European Prospective Investigation of Cancer and Nutrition (EPICNorfolk). BMJ. 2001;322:15–8.
4. Sigal RJ, Kenny GP, Boule NG, et al. Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes: a randomized trial. Ann Intern Med. 2007;147:357–69.
5. Dunstan DW, Mori TA, Puddey IB, et al. The independent and combined effects of aerobic exercise and dietary fish intake on serum lipids and glycaemic control in NIDDM. Diabetes Care. 1997;20:913–21.
6. Praet SF, van Rooij ESJ, Wijtvliet A, et al. Brisk walking compared with an individual medical fitness programme for patients with type 2 diabetes: a randomised controlled trial. Diabetologia. 2008;51:736–46.
7. Boule NG, Kenny GP, Haddad E, et al. Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in type 2 diabetes mellitus. Diabetologia. 2003;46:1071–81.
8. Vanhees L, Geladas N, Hansen D, et al. Importance of characteristics and modalities of physical activity and exercise in the management of cardiovascular health in individuals with cardiovascular risk factors: recommendations from the European Association of Cardiovascular Prevention and Rehabilitation. Eur J Prev Cardiol. 2012;19:1005–33.
9. Colberg SR, Albright AL, Blissmer BJ, et al. Exercise and type 2 diabetes. American College of Sports Medicine and the American Diabetes Association: joint position statement. Med Sci Sports Exerc. 2010;42:2282–303.
10. American Diabetes Association. Standards of medical care in diabetes—2012. Diabetes Care. 2012;35:S11–63.
11. Hansen D, Peeters S, Zwaenepoel B, et al. Exercise assessment and prescription in patients with type 2 diabetes in the private and home care setting: clinical recommendations from AXXON (Belgian Physical Therapy Association). Phys Ther. 2013;93:597–610.
12. Laaksonen DE, Lindström J, Lakka TA, et al. Finnish diabetes prevention study. physical activity in the prevention of type 2 diabetes: the Finnish Diabetes Prevention Study. Diabetes. 2005;54:158–65.
13. Hansen D, Dendale P, van Loon LJC, et al. The effects of training modalities on clinical benefits of exercise intervention in cardiovascular disease risk patients or type 2 diabetes mellitus. Sports Med. 2010;40:921–40.
14. Umpierre D, Ribeiro PA, Schaan BD, et al. Volume of supervised exercise training impacts glycaemic control in patients with type 2 diabetes: a systematic review with meta-regression analysis. Diabetologia. 2013;56:242–51.
15. Francois ME, Little JP. Effectiveness and safety of high-intensity interval training in patients with type 2 diabetes. Diabetes Spectr. 2015;28:39–44.
16. Haxhi J, Scotto di Palumbo A, Sacchetti M. Exercising for metabolic control: is timing important? Ann Nutr Metab. 2012;62:14–25.
17. Horowitz JF, Mora-Rodriguez R, Byerley LO, et al. Lipolytic suppression following carbohydrate ingestion limits fat oxidation during exercise. Am J Physiol Endocrinol Metab. 1997;273:E768–75.
18. De Bock K, Richter EA, Russell AP, et al. Exercise in the fasted state facilitates fibre type-specific intramyocellular lipid breakdown and stimulates glycogen resynthesis in humans. J Physiol. 2005;564:649–60.
19. Bennard P, Doucet E. Acute effects of exercise timing and breakfast meal glycemic index on exercise-induced fat oxidation. Appl Physiol Nutr Metab. 2006;31:502–11.
20. Lane SC, Bird SR, Burke LM, et al. Effect of a carbohydrate mouth rinse on simulated cycling time-trial performance commenced in a fed or fasted state. Appl Physiol Nutr Metab. 2013;38:134–9.
21. Scott JPR, Sale C, Greeves JP, et al. Effect of fasting versus feeding on the bone metabolic response to running. Bone. 2012;51:990–9.
22. Paul GL, Rokusek JT, Dykstra GL, et al. Oat, wheat or corn cereal ingestion before exercise alters metabolism in humans. J Nutr. 1996;126:1372–81.
23. DeMarco HM, Sucher KP, Cisar CJ, et al. Pre-exercise carbohydrate meals: application of glycemic index. Med Sci Sports Exerc. 1999;31:164–70.
24. Wu CL, Nicholas C, Williams C, et al. The influence of high-carbohydrate meals with different glycaemic indices on substrate utilisation during subsequent exercise. Br J Nutr. 2003;90:1049–56.
25. Coyle EF, Coggan AR, Hemmert MK, et al. Substrate usage during prolonged exercise following a preexercise meal. J Appl Physiol. 1985;59:429–33.
26. Montain SJ, Hopper MK, Coggan AR, Coyle EF. Exercise metabolism at different time intervals after a meal. J Appl Physiol. 1991;70(2):882–8.
27. de Lima FD, Correia AL, Teixeira Dda S, et al. Acute metabolic response to fasted and postprandial exercise. Int J Gen Med. 2015;8:255–60.
28. Enevoldsen LH, Simonsen L, Macdonald IA, et al. The combined effects of exercise and food intake on adipose tissue and splanchnic metabolism. J Physiol. 2004;561:871–82.
29. Schwalm C, Jamart C, Benoit N, et al. Activation of autophagy in human skeletal muscle is dependent on exercise intensity and AMPK activation. FASEB J. 2015;29:3515–26.
30. Loy SF, Conlee RK, Winder WW, et al. Effects of 24-hour fast on cycling endurance time at two different intensities. J Appl Physiol. 1986;61:654–9.
31. Whitley HA, Humphreys SM, Campbell IT, et al. Metabolic and performance responses during endurance exercise after high-fat and high-carbohydrate meals. J Appl Physiol. 1998;85:418–24.

32. Dohm GL, Beeker RT, Israel RG, et al. Metabolic responses to exercise after fasting. J Appl Physiol. 1986;61:1363–8.
33. Schneiter P, Di Vetta V, Jequier E, et al. Effect of physical exercise on glycogen turnover and net substrate utilization according to nutritional state. Am J Physiol Endocrinol Metab. 1995;32:E1031–6.
34. Dumas JF, Simard G, Flamment M, et al. Is skeletal muscle mitochondrial dysfunction a cause or an indirect consequence of insulin resistance in humans? Diabetes Metab. 2009;35:159–67.
35. De Bock K, Derave W, Eijnde BO, et al. Effect of training in the fasted state on metabolic responses during exercise with carbohydrate intake. J Appl Physiol. 2008;104:1045–55.
36. Van Proeyen K, Szlufcik K, Nielens H, et al. Training in the fasted state improves glucose tolerance during fat-rich diet. J Physiol. 2010;588:4289–302.
37. Stannard SR, Buckley AJ, Edge JA, et al. Adaptations to skeletal muscle with endurance exercise training in the acutely fed versus overnight-fasted state. J Sci Med Sport. 2010;13:465–9.
38. Van Proeyen K, Szlufcik K, Nielens H, et al. High-fat diet overrules the effects of training on fiber-specific intramyocellular lipid utilization during exercise. J Appl Physiol. 2011;111:108–16.
39. Van Proeyen K, Szlufcik K, Nielens H, et al. Beneficial metabolic adaptations due to endurance exercise training in the fasted state. J Appl Physiol. 2011;110:236–45.
40. Van Proeyen K, De Bock K, Hespel P. Training in the fasted state facilitates re-activation of eEF2 activity during recovery from endurance exercise. Eur J Appl Physiol. 2011;111:1297–305.
41. Schoenfeld BJ, Aragon AA, Wilborn CD, et al. Body composition changes associated with fasted versus non-fasted aerobic exercise. J Int Soc Sports Nutr. 2014;11:54.
42. Gillen JB, Percival ME, Ludzki A, et al. Interval training in the fed or fasted state improves body composition and muscle oxidative capacity in overweight women. Obesity. 2013;21:2249–55.
43. Nieman DC, Carlson KA, Brandstater ME, et al. Running endurance in 27-h-fasted humans. J Appl Physiol. 1987;63:2502–9.
44. Bergman BC, Brooks GA. Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. J Appl Physiol. 1999;86:479–87.
45. Kang J, Raines E, Rosenberg J, et al. Metabolic responses during postprandial exercise. Res Sports Med. 2013;21:240–52.
46. Peake JM, Tan SJ, Markworth JF, et al. Metabolic and hormonal responses to isoenergetic high-intensity interval exercise and continuous moderate-intensity exercise. Am J Physiol Endocrinol Metab. 2014;307:E539–52.