Effects of aerobic exercise performed in fasted *v.* fed state on fat and carbohydrate metabolism in adults: a systematic review and meta-analysis

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Abstract

This study aimed to verify the effect of aerobic exercise performed in the fasted *v.* fed states on fat and carbohydrate metabolism in adults. Searches were conducted in March 2015, and updated in July 2016, using PubMed®, Scopus and Cochrane databases (terms: 'fasting', 'exercise', 'aerobic exercise', 'substrate', 'energy metabolism', 'fat', 'glucose', 'insulin' and 'adult') and references from selected studies. Trials that compared the metabolic effects of aerobic exercise (duration ≤120 min) performed in the fasted *v.* fed states in adults were accepted. The outcomes evaluated were fat oxidation during exercise and the plasma concentrations of insulin, glucose and NEFA before and immediately after exercise; two independent reviewers extracted the data (A. F. V. and L. C.). The results were presented as weighted mean differences between treatments, with 95 % CI. Of 10 405 articles identified, twenty-seven studies – with a total of 273 participants – were included. There was a significant increase in fat oxidation during exercise performed in the fasted, compared with fed, state (−3·08 g; 95 % CI −5·38, −0·79; I^2 39·1 %). The weighted mean difference of NEFA concentrations was not significantly different between states (0·00 mmol/l; 95 % CI −0·07, 0·08; I^2 72·7 %). However, the weighted mean differences of glucose (0·78 mmol/l; 95 % CI 0·43, 1·14; I^2 90·8 %) and insulin concentrations (104·5 pmol/l; 95 % CI 70·8, 138·2; I^2 94·5 %) were significantly higher for exercise performed in the fed state. We conclude that aerobic exercise performed in the fasted state induces higher fat oxidation than exercise performed in the fed state.

Key words: Fasting: Exercise: Energy metabolism: Reviews

Fasting is characterised by the absence of food and/or energy beverage intake for a period of time, which may last from several hours to a few weeks[1,2]. However, most people fast for 8–12 h daily – the 'overnight fasting' period[2]. During this period, NEFA, ketone bodies and glucose derived from liver glycogen and gluconeogenesis are the predominant energy sources[3].

During exercise, NEFA also make a considerable contribution to energy metabolism owing to the increased availability of these substrates in the plasma. This is caused by increased adrenaline levels and decreased insulin concentrations in the blood[4]. Fasting promotes low levels of insulin and hepatic glycogen[2]. Thus, when aerobic exercise is performed under these conditions, an increase in the utilisation of fat as an energy substrate is observed, when compared with exercise performed in the fed state[5,6]. The decrease in fat oxidation during exercise in the fed state can be mainly attributed to higher insulin concentrations caused by a meal, which may inhibit the breakdown of intramuscular TAG (IMTG) and reduce the availability of NEFA for oxidation[7,8].

Several studies have indicated that regular exercise promotes beneficial effects in terms of health and body composition[9–11], including an improvement in insulin sensitivity and maintenance and reduction of body weight and body fat. It has been suggested that exercise enhances fat oxidation and that this adaptation may be associated with improved insulin sensitivity[12]. Furthermore, higher fat oxidation capacity during exercise seems to be related to a decrease in the number of metabolic risk factors[13]. Venables & Jeukendrup[14] demonstrated that participating in a training programme for 4 weeks, with continuous aerobic exercise programmed for the maximum contribution of fat as the energy substrate during each session, can further increase fat oxidation. This higher oxidation was associated with improvements in insulin sensitivity in obese men. In healthy, young men, the maximal fat oxidation

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Abbreviation: IMTG; intramuscular TAG.

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during exercise was positively associated with insulin sensitivity and 24-h fat oxidation[15]. Studies have demonstrated that exercise performed in the fasted state can increase the rate of fat oxidation at rest from 9[16] to 24 h[17–19] after exercise when compared with the same exercise performed after a meal. This higher utilisation of fat as an energy source at rest may promote reduction in body fat.

On the basis of these data, aerobic exercise performed in the fasted state has been considered a strategy to increase fat oxidation during exercise and, chronically, to promote adaptations that may be beneficial to health. However, although most studies reported higher fat oxidation under these conditions compared with a carbohydrate-fed state, it is not clear whether the stimulation of lipolytic activity and/or decreased re-esterification of NEFA that occur during the fasted state[20] result in a significantly increased use of fat as an energy substrate during exercise. This systematic review with meta-analysis aimed to verify the effect of aerobic exercise performed during fasted *v.* fed states on fat and carbohydrate metabolism in adults.

Methods

Eligibility criteria

This review considered clinical trials (parallel and randomised cross-over designs) evaluating the effect of performing an aerobic exercise intervention of no >120 min duration (or data at 120 min for those interventions with longer durations) in a fasted state among adults aged 19–59 years. These interventions had to be compared with the same exercise performed in the fed state (prior consumption of meals containing at least 25 g of carbohydrates)[21]. Included studies evaluated the following outcome measures: fat oxidation during exercise, considered the primary end point; and serum concentrations of NEFA, glucose and insulin immediately before and after the exercise session; the absolute weighted mean differences of these concentrations were considered the secondary end points. Studies that evaluated these acute responses to aerobic exercise were included; trials that did not present acute outcome data were excluded. In the case of trials with several publications (or sub-studies), the study was included only once.

Search strategy

The electronic databases MEDLINE® (via PubMed®), Scopus and Cochrane were used. In addition, manual searches were conducted of references of studies identified for inclusion. The search was conducted in March 2015 and updated in July 2016. The terms 'fasting', 'exercise', 'aerobic exercise', 'substrate', 'energy metabolism', 'fat', 'glucose', 'insulin' and 'adult' as well as related entry terms were used. The searches were limited to articles published in English, Portuguese and Spanish languages. The search strategy used in the PubMed® database is available as the online Supplementary Material. Details of other strategies may be obtained upon request. This systematic review and meta-analysis was prepared and is presented in accordance with 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' guidelines[22,23].

Selection of studies

Selection of studies for review was performed independently and duplicated, without restriction on the date of publication. First, the titles and abstracts of all articles identified by the search strategy were evaluated for inclusion independently by two researchers (A. F. V. and L. C.), in duplicate form. Whenever the abstract did not provide sufficient information about inclusion and exclusion criteria, the full article was evaluated. Second, the same reviewers independently evaluated the full articles of those identified as appropriate from the abstract screening process, and made their selection according to eligibility criteria. Disagreements between reviewers were resolved by consensus, and in the case of continuing disagreement the evaluation was made by a third reviewer (R. R. C.). To avoid possible double counting of participants included in more than one report by the same authors/working groups, the periods of recruitment of participants and areas of recruitment were evaluated, and authors were contacted for clarification where necessary.

Data extraction

Data extraction was performed by two reviewers (A. F. V. and L. C.) independently concerning methodological characteristics, interventions and outcomes of the studies using a standardised form. As in the selection stage, disagreements were resolved by consensus or by a third reviewer (R. R. C.). The extracted data included average age, BMI, sex and training status of participants; exercise duration and intensity; time between dietary intake and the start of exercise; amount of carbohydrate consumed in the pre-exercise meal; and the end points analysed. If the required data were not found in the published report, the corresponding author was contacted to provide missing data and, in the absence of responses or data extraction alternatives, the study or missing end point was excluded from the review. Data presented only graphically, and for which more detail was not provided despite a request to the corresponding authors, were extracted using 'DigitizeIt' software. Where it was not possible to extract means or standard deviations from graphs at the required points, the variable was excluded from the analysis.

In this phase, studies that included diabetic participants, or those in which carbohydrates were provided during exercise as part of the study protocol, were excluded to avoid possible bias in the results. The primary end point we assessed was the total absolute average fat oxidation during exercise. Secondary end points were the weighted mean difference in insulin, glucose and NEFA concentrations. Weighted mean differences were calculated from values taken immediately before and during the last minute of exercise for studies lasting ≤120 min. For studies of longer duration, the time '120 min' was considered the last minute of exercise.

In studies where the total absolute average fat oxidation during exercise was not presented in the published article, a request was submitted to the authors, and if means for VO_2 and carbon dioxide production values were provided these were applied to the formula determined by Péronnet & Massicotte[24] in order to determine the fat oxidation rate. The units of measurements used in this review were grams for fat oxidation, mmol/l for concentrations of NEFA and glucose, and pmol/l for insulin

concentrations. Study data not presented in these units were converted. For instance, where fat oxidation was presented using an energy value (kJ/kcal), these averages were divided by 40·79 kJ (9·75 kcal) in order to obtain the value in grams[25]. If these data were not provided by authors, or if it was not possible to calculate the total oxidised absolute average during exercise, the variable or the study was excluded. Studies with two or more comparison groups with the same population were included with only one comparator, which was selected according to the time between dietary intake and exercise and/or the nutritional characteristics of meals consumed that most closely resembled the other studies being reviewed, in an effort to standardise results. For studies with two or more intervention groups, a single group was also included, selected according to characteristics similar to other studies.

Evaluation of risk of bias

The assessment of the methodological quality of included studies was performed according to criteria proposed by Cochrane[26]: appropriate use of randomisation sequences, allocation concealment, blinding of participants and/or therapists, blinding of assessors to outcomes, and description of losses and exclusions. When these processes had been described in the published document, it was considered that criteria had been met and these studies were classified as being at 'low risk' of bias and, in opposition, as 'high risk'. Studies that did not report these data were classified as 'unclear risk'. Descriptions of losses and exclusions were considered 'low risk' when the number of participants evaluated were presented in the legends of charts and graphs. Quality evaluation was performed independently by two reviewers (A. F. V. and L. C.).

Data analysis

Results are presented as weighted mean differences for absolute values between treatments with 95 % CI. The standard deviation of mean difference values not provided by studies was imputed according to the equation proposed by Higgins et al.[27]. Statistical heterogeneity of treatment effects between studies was evaluated by Cochran's Q test and I^2 inconsistency test; values above 50 % indicated high heterogeneity[28]. In case of low heterogeneity, the fixed effect model was used to pool study results for the outcomes. When significant heterogeneity was observed ($I^2 > 50\%$), the random effects model was applied. Meta-analyses comprised comparisons of fat oxidation during aerobic exercise performed in the fasted v. fed state and the changes in concentrations (expressed using weighted mean differences) of glucose, NEFA and insulin from immediately before exercise to the last minute of exercise (post-exercise). Values of $\alpha \leq 0.05$ were considered statistically significant.

For variables with high heterogeneity, sensitivity analyses were performed according to the following criteria: exercise time, exercise intensity, sex of participants, BMI of participants, training level of participants, pre-exercise values for each variable, time between dietary intake and the start of exercise, and amount of carbohydrate consumed in the pre-exercise meal.

Furthermore, publication bias was assessed using funnel plots for each outcome (of each trial's effect size against the standard error). Funnel plot asymmetry was evaluated using Begg and Egger tests[29], and a significant publication bias was considered if $P < 0.10$. The trim-and-fill computation was used to estimate the effect of publication bias on the interpretation of results.

All analyses were performed using Comprehensive Meta-Analysis version 2.0, except the risk of bias, which was performed using Review Manager version 5.3 (Cochrane Collaboration).

Results

Description of studies

Of the 10 405 studies identified from the database searches, twenty-three met our inclusion criteria. An additional four studies were included from a manual search of the reference lists of the included studies, bringing the total number of articles included to twenty-seven. Of these, three studies[30–32] were included twice because they had met eligibility criteria for two groups with different populations, in which each population had a different intervention group and control group: references 'Bergman & Brooks, 1999a' and 'Montain et al., 1991a' related to populations comprised of trained men, 'Bergman & Brooks, 1999b' and 'Montain et al., 1991b' related to populations comprised of untrained men, and 'Isacco et al., 2012a' and 'Isacco et al., 2012b' related to populations of women who did not and did use the contraceptive pill, respectively. Thus, thirty comparisons were used in this meta-analysis (Fig. 1). In total, 270 and 269 participants were included in the fasted and fed groups, respectively. The majority of studies (80 %) analysed men, whereas 13·3 % analysed women, and 6·6 % analysed both sexes. Most samples comprised physically active individuals (86·7 %), and exercise sessions lasted an average of 73 min. The meals were provided 30–240 min before the interventions and were composed of a maximum of 215 g of carbohydrates (Table 1).

In all, four studies were excluded from our analysis: one study was unaccessible[33], and the other three met all eligibility criteria, but were not used because of the unavailability of results[34], or the presentation of averages[19] and standard deviations[35] graphically, with no clarification received from authors and no possibility of data extraction using 'DigitizeIt' software.

From some of the studies included, it was necessary to exclude certain variables because absolute averages were not given – for example, the absolute average of fat oxidation during exercise[6,7,36–38]. Other variables were excluded as it was impossible to extract values for standard deviation of insulin[39–42], glucose[40,41,43] and NEFA[32,40] concentrations. These data were all requested from authors but were not provided. 'DigitizeIt' software was used to extract the average relating to fat oxidation from one study[44], relating to NEFA concentrations from fourteen studies[6,7,37,38,41–43,45–51], relating to glucose concentrations from sixteen studies[6,7,32,36–39,42,45–52] and relating to insulin concentrations from thirteen studies[6,7,37,38,43,45–52].

Risk of bias

Of the included studies, 80 % showed adequate generation of randomisation sequence, 6·6 % reported allocation
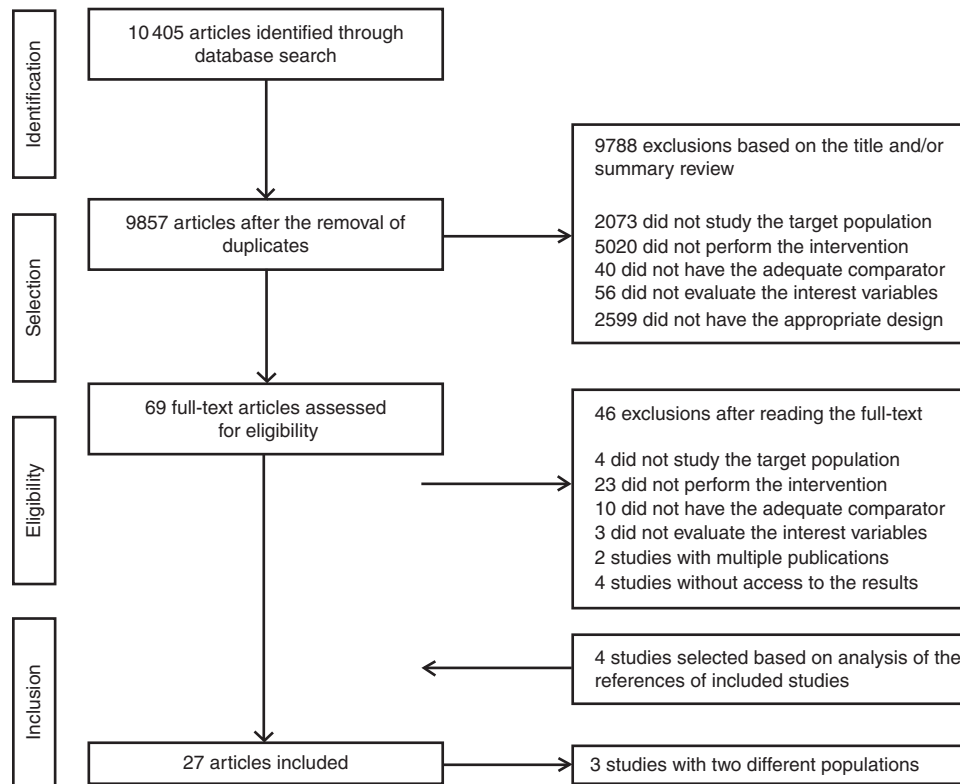
Fig. 1. Flow chart of the included studies.

concealment, 20 % had blinded participants and/or therapists, 6·6 % had blinded the assessors to the outcomes and 66·6 % described losses to follow-up and exclusions (Fig. 2 and 3).

Effects of interventions

Fat oxidation. Data on fat oxidation were available from eleven studies[30,31,39–41,43,44,47,50–52], with a total of 117 individuals evaluated (Fig. 4). Aerobic exercise performed in the fasted state was associated with a significant increase in fat oxidation during exercise when compared with the fed state (effect size: −3·53; 95 % CI −4·76, −2·30; I^2 39·1 %). Aerobic exercise performed in the fasted state led to an increase in fat oxidation of approximately 3·53 g, compared with execution of the same exercise after consumption of meals containing carbohydrates. However, the analysis of publication bias identified a significant bias ($P = 0·007$), and thus the adjusted value of the effect size, according to the Duval & Tweedie's trim and fill test, resulted in 3·08 g.

Given the influence of exercise intensity on fat oxidation, sensitivity analyses were performed to identify whether there was an effect difference when stratified by two different intensity ratings – VO_{2max} <70 % and VO_{2max} ≥70 %. Thus, even though the meta-analysis did not demonstrate significant heterogeneity ($P = 0·07$), sensitivity analyses were performed: <70 % VO_{2max} (3·45 g; 95 % CI 2·19, 4·71; $P < 0·001$; I^2 50 %) and ≥70 % VO_{2max} (5·38 g; 95 % CI −0·45, 11·21; $P = 0·07$; I^2 0 %). Aerobic exercise of low-to-moderate intensity performed in the fasted state induced a higher fat oxidation compared with a fed state. On the other hand, there was no significant difference between fasted and fed states in relation to fat oxidation during aerobic exercise of moderate-to-high intensity.

Sensitivity analyses were also performed for fat oxidation taking the following into account: exercise time (≤60 min: 3·35 g; 95 % CI 2·07, 4·62; $P < 0·001$; I^2 54 %; >60 min: 6·13 g; 95 % CI 1·37, 10·88; $P = 0·01$; I^2 9 %); sex of participants (male: 6·39 g; 95 % CI 3·84, 8·94; $P < 0·001$; I^2 0 %; female: 2·60 g; 95 % CI 1·19, 4·01; $P = 0·0003$; I^2 0 %); BMI of participants (<25 kg/m²: 2·79 g; 95 % CI 1·42, 4·17; $P < 0·001$; I^2 30 %); training level of participants (physically active: 3·74 g; 95 % CI 1·97, 5·52; $P < 0·001$; I^2 49 %; sedentary: 3·34 g; 95 % CI 1·62, 5·05; $P = 0·0001$; I^2 23 %); time between consumption of meal and the beginning of exercise (<100 min: 3·41 g; 95 % CI 1·68, 5·14; $P = 0·0001$; I^2 57 %; >100 min: 3·66 g; 95 % CI 1·91, 5·41; $P < 0·001$; I^2 37 %); and quantity of carbohydrate consumed in the pre-exercise meal (<100 g: 3·51 g; 95 % CI 1·84, 5·17; $P < 0·001$; I^2 34 %; ≥100 g: 3·56 g; 95 % CI 1·73, 5·39; $P = 0·0001$; I^2 53 %). Thereby, these results demonstrated no change in the pattern already presented, of higher fat oxidation when the exercise is performed in the fasted state, regardless of the adopted criteria for the sensitivity analyses.

NEFA. Data on NEFA concentrations were available from sixteen studies[6,7,31,37,38,41–43,45–52], with a total of 144 individuals evaluated (Fig. 5). All but one of these studies used the same sample populations for both interventions[37]. The weighted mean difference of NEFA before and after exercise

Table 1. Characteristics of included studies
(Mean values and standard deviations)*

Studies	Age (years)		Sex/n	Training status	Exercise duration (min)	Exercise intensity	Time between meal and exercise (min)	Amount of carbohydrate pre exercise meal (g)
	Mean	SD						
Aziz et al.[53]	27·3	7·2	Male/10	Physically active	60	65·0 % VO_{2max}	180 to 240	126·7
Bergman & Brooks[30]	25·1	4·8	Male/7	Physically active	90	60·0 % VO_{2peak}	180	119·6
Bergman & Brooks[30]	22·1	3·4	Male/7	Sedentary	120	40·0 % VO_{2peak}	180	119·6
Bouhlel et al.[54]	19·0	2·0	Male/9	Physically active	30	20·0, 30·0, 40·0, 50·0, 60·0 % W_{max}	Does not mention	Does not mention
Coyle et al.[45]	25·0	5·0	Male/7	Physically active	105	70·0 % VO_{2max}	240	141·8
Coyle et al.[7]	22·0	4·9	Male/6	Physically active	40	50·0 % VO_{2max}	60 and 10	96·6
Dohm et al.[46]	28·7	3·9	Male/9	Physically active	90 or until exhaustion (about 80)	70·0–75·0 % VO_{2max}	120 to 240	47·0
Farah & Gill[39]	28·1	10·7	Male/10	Sedentary	60	50·0 % VO_{2max}	30	56·5
Gonzalez et al.[47]	23·2	4·3	Male/12	Physically active	59	61·1 % VO_{2peak}	120	66·6
Guéye et al.[55]	22·5	1·7	Male/12	Physically active	60	75·0 % HR_{max}	Does not mention	Does not mention
Horowitz et al.[6]	26·5	9·3	Male/6	Physically active	60	44·0 % VO_{2peak}	60	60·0
Isacco et al.[31]	22·9	3·6	Female/10	Sedentary	45	65·0 % VO_{2max}	180	72·2
Isacco et al.[31]	21·2	1·9	Female/11	Sedentary	45	65·0 % VO_{2max}	180	73·4
Kirwan et al.[48]	22·0	2·4	Male/6	Physically active	Until exhaustion (120)	60·0 % VO_{2peak}	45	75·0
Kirwan et al.[49]	24·0	4·9	Female/6	Physically active	Until exhaustion (120)	60·0 % VO_{2peak}	45	75·0
Little et al.[36]	23·3	3·8	Male/7	Physically active	90 (45)	V_{max}	180	86·0
Little et al.[50]	22·8	3·2	Male/13	Physically active	105	V_{max}	120	Does not mention (1·5 g/kg)
Massicotte et al.[52]	24·8 (SD 6·9) (fast) 22·1 (SD 5·8) (fed)		Male/5	Physically active	120 (60)	52·0 % VO_{2max}	180	50·0
Maughan & Gleeson[40]	34·0	8·9	Male/5	Physically active	Until exhaustion (90)	70·0 % VO_{2max}	45	69·8
Montain et al.[32]	Does not mention		Male/9	Physically active	30	70·0 % VO_{2peak}	120	131·6
Montain et al.[32]	Does not mention		Male/8	Physically active	30	70·0 % VO_{2peak}	120	154·6
Paul et al.[41]	24·9	3·4	Mixed/12	Physically active	90	60·0 % VO_{2peak}	90	32·4
Ramos-Jiménez et al.[56]	22·5	3·7	Mixed/30	Physically active	8 to 15	98·0 % HR_{max}	70	Does not mention (60 % ETV meal)
Satabin et al.[42]	25·2	17·7	Male/9	Physically active	110	60·0 % VO_{2max}	60	100·0
Schabort et al.[37]	26·0	7·9	Male/7	Physically active	105	70·0 % VO_{2max}	180	100·0
Shin et al.[38]	23·3	2·5	Male/8	Physically active	60	50·0 % VO_{2max}	30	66·4
Whitley et al.[43]	21·0	10·8	Male/8	Physically active	90	70·0 % VO_{2max}	240	215·0
Willcutts et al.[44]	23·7	2·4	Female/8	Physically active	30 (23)	62·0 % VO_{2max}	90	109·3
Wu et al.[51]	26·8	3·3	Male/9	Physically active	60	65·0 % VO_{2max}	180	141·0
Ziogas & Thomas[57]	27·4	3·8	Male/7	Physically active	60	60·0 % VO_{2max}	180	111·5

VO_{2peak}, peak VO_2; W_{max}, maximum power; HR_{max}, maximum heart rate; V_{max}, maximum velocity; ETV, energy total value.
* Exercise duration: total time of exercise duration evaluated in the study (time post exercise extracted).
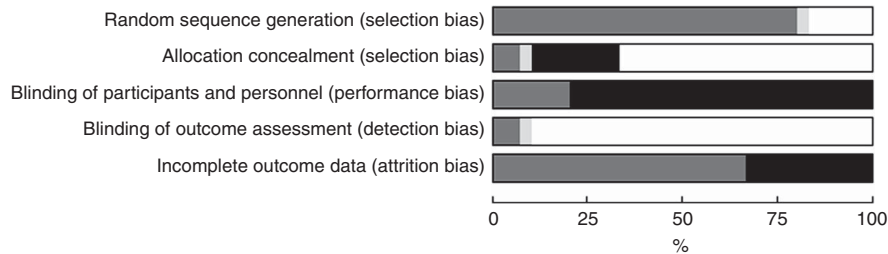
Fig. 2. Risk of bias in the included studies. ■, Low risk of bias; □, unclear risk of bias; ■, high risk of bias.

was not demonstrated to be significantly different between exercise performed in the fasted or fed states (effect size: 0·00; 95 % CI −0·07, 0·08; I^2 72·7 %). The analysis of publication bias for this outcome showed no significant bias ($P = 0.124$).

Owing to the high heterogeneity ($P < 0.001$) found in the analysis of this variable, sensitivity analyses were performed. Significant heterogeneity was found for most of the variables analysed: exercise time (≤60 min: I^2 93 %; $P < 0.001$; >60 min: I^2 83 %; $P < 0.001$); exercise intensity (<70 % VO$_{2max}$: I^2 92 %; $P < 0.001$; ≥70 % VO$_{2max}$: I^2 74 %; $P = 0.004$); sex of participants (male: I^2 90 %; $P < 0.001$; female: I^2 90 %; $P < 0.001$); BMI of participants (<25 kg/m^2: I^2 90 %; $P < 0.001$; >25 kg/m^2: I^2 0 %; $P = 0.76$); training level of participants (physically active: I^2 90 %; $P < 0.001$; sedentary: I^2 0 %; $P = 0.65$); pre-exercise values for fasting (<1 mmol/l: I^2 90 %; $P < 0.001$); time between consumption of meal and the beginning of exercise (<100 min: I^2 85 %; $P < 0.001$; >100 min: I^2 82 %; $P < 0.001$); and quantity of carbohydrate consumed in the pre-exercise meal (<100 g: I^2 87 %; $P < 0.001$; ≥100 g: I^2 90 %; $P < 0.001$). Sensitivity analyses for the criterion 'pre-exercise values in fasting >1 mmol/l' were not performed because only one study presented this characteristic. The criteria 'BMI > 25 kg/m^2' and 'sedentary' showed no significant heterogeneity, although sensitivity analyses were performed with only two studies for each. Owing to the maintenance of high heterogeneity and/or low number of studies, the data presented graphically (Fig. 5) refer to the general analysis (disregarding the sensitivity analysis).

Glucose. Data on glucose concentrations were available from twenty-two studies[6,7,31,32,36–39,42,45–57], with a total of 226 individuals evaluated (Fig. 6). All but one of these studies used the same sample populations for both interventions[37]. Significantly lower variation was reported for glucose concentrations from before to after exercise in the fasted *v.* fed states (effect size: 0·60; 95 % CI 0·25, 0·94; I^2 90·8 %). Nevertheless, the analysis of publication bias identified a significant bias ($P = 0.057$), and thus the adjusted value of the effect size, according to the Duval & Tweedie's trim-and-fill test, resulted in 0·78 mmol/l.

Because of the high heterogeneity ($P = 0.001$) found for this variable, sensitivity analyses were performed and, again, significant heterogeneity was found in most analyses. These variables were as follows: exercise time (≤60 min: I^2 95 %; $P < 0.001$; >60 min: I^2 99 %; $P < 0.001$); exercise intensity (<70 % VO$_{2max}$: I^2 98 %; $P < 0.001$; ≥70 % VO$_{2max}$: I^2 80 %;

$P < 0.001$); sex of participants (male: I^2 95 %; $P < 0.001$; female: I^2 99 %; $P < 0.001$); BMI of participants (<25 kg/m^2: I^2 99 %; $P < 0.001$; >25 kg/m^2: I^2 82 %; $P = 0.001$); training level of participants (physically active: I^2 97 %; $P < 0.001$; sedentary: I^2 16 %; $P = 0.30$); pre-exercise values in fasting (<5 mmol/l: I^2 95 %; $P < 0.001$; >5 mmol/l: I^2 99 %; $P < 0.001$); time between consumption of meal and the beginning of exercise (<100 min: I^2 98 %; $P < 0.001$; >100 min: I^2 69 %; $P < 0.001$); and quantity of carbohydrate consumed in the pre-exercise meal (<100 g: I^2 98 %; $P < 0.001$; ≥100 g: I^2 44 %; $P = 0.08$). Again, the criterion 'sedentary' showed no significant heterogeneity, although the sensitivity analysis was performed with only two studies. The criterion 'quantity of carbohydrate consumed in the pre-exercise meal ≥100 g' was analysed with eight interventions (n 66), and did not show significant heterogeneity. Therefore, in this case, the weighted mean difference of relative glucose concentrations did not appear to differ significantly when exercise was performed in a fasted *v.* fed state ($P = 0.91$). Because of the high heterogeneity and/or low number of studies, the data presented graphically (Fig. 6) refer to the general analysis (disregarding the sensitivity analysis). More detailed results of the sensitivity analysis performed for this variable relating to the criterion 'quantity of carbohydrate consumed in the pre-exercise meal ≥100 g' can be provided on request.

Insulin. Data on insulin concentrations were available from fifteen studies[6,7,31,32,37,38,43,45–52], with a total of 140 individuals evaluated (Fig. 7). Again, all but one of these studies used the same sample populations for both interventions[37]. Significantly lower variation was reported for insulin concentrations from before to after exercise in the fasted *v.* fed states (effect size: 104·5; 95 % CI 70·8, 138·2; I^2 92·5 %). However, the analysis of publication bias identified a significant bias ($P < 0.001$), and thus the adjusted value of the effect size, according to the Duval & Tweedie's trim and fill test, resulted in 104·5 pmol/l.

As with the other blood variables, high heterogeneity was found ($P < 0.001$) and sensitivity analyses were consequently performed. Once again, significant heterogeneity was found for most comparisons: exercise time (≤60 min: I^2 82 %; $P < 0.001$; >60 min: I^2 95 %; $P < 0.001$); exercise intensity (<70 % VO$_{2max}$: I^2 91 %; $P < 0.001$; ≥70 % VO$_{2max}$: I^2 89 %; $P < 0.001$); sex of participants (male: I^2 93 %; $P < 0.001$; female: I^2 96 %; $P < 0.001$); BMI of participants (<25 kg/m^2: I^2 90 %; $P < 0.001$; >25 kg/m^2: I^2 98 %; $P < 0.001$); training level of participants

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)
Aziz et al., 2010	?	?	−	?	+
Bergman & Brooks, 1999a	+		−		−
Bergman & Brooks, 1999b	+		−		−
Bouhlel et al., 2006			−		−
Coyle et al., 1985	+		−		+
Coyle et al., 1997	+		−		+
Dohm et al., 1986		−	−		+
Farah & Gill., 2013	+		−		+
Gonalez et al., 2013	+		−		+
Guéye et al., 2003		−	−		−
Horowitz et al., 1997			−		+
Isacco et al., 2012a	+		−		−
Isacco et al., 2012b	+		−		−
Kirwan et al., 2001a	+		+		+
Kirwan et al., 2001b	+		+		+
Little et al., 2009	+		+	+	−
Little et al., 2010	+		+	?	−
Massicotte et al., 1990	+		−		−
Maughan & Gleeson, 1988	+	+	−		−
Montain et al., 1991a	+	−	−		+
Montain et al., 1991b	+	−	−		+
Paul et al., 1996		−	+		+
Ramos-Jiménez et al., 2014	+		−		+
Satabin et al., 1987	+		−		+
Schabort et al., 1999	+		−		+
Shin et al., 2013	+	+	−		+
Whitley et al., 1998	+	−	−		+
Willcutts et al., 1988	+		−		−
Wu et al., 2003	+		−		+
Ziogas & Thomas, 1998	+	−	−		+

Fig. 3. Summary of risk of bias in the included studies.

(physically active: I^2 95 %; $P < 0.001$; sedentary: I^2 0 %; $P = 0.39$); pre-exercise values in the fed state (<200 pmol/l: I^2 92 %; $P < 0.001$; >200 pmol/l: I^2 83 %; $P < 0.001$); time between consumption of meal and the beginning of exercise (<100 min: I^2 95 %; $P < 0.001$; >100 min: I^2 91 %; $P < 0.001$); and quantity of carbohydrate consumed in the pre-exercise meal (<100 g: I^2 91 %; $P < 0.001$; ≥100 g: I^2 92 %; $P < 0.001$). Once again, the variable 'sedentary' showed no significant heterogeneity, although the sensitivity analysis was performed with only two interventions. Owing to the high heterogeneity and/or low number of studies, the data presented graphically (Fig. 7) refer to the general analysis (disregarding the sensitivity analysis).

Discussion

The major finding of this systematic review with meta-analysis is that performing aerobic exercise at low-to-moderate intensity in the fasted state induces a significant increase (3·08 g) in fat oxidation while exercise is being performed. No difference was seen in the variation of NEFA concentrations between exercise performed in the fasted v. fed states. However, greater variations in glucose and insulin concentrations were seen when exercise was performed in a fed state.

Carbohydrates and fats are the most important sources of fuel during rest and exercise[4]. In general, the lipolytic activity of adipose tissue is regulated by the balance between stimulating hormones such as catecholamines and those that inhibit the enzyme responsible for lipolysis (lipase sensitive hormone), especially insulin[58]. Because of higher muscular energy exigency and increased availability of NEFA[58], mediated by increased adrenergic stimulation[59], exercise alone can increase fat oxidation compared with rest[39].

Among the primary responses to fasting are the partial mobilisation of TAG reserves contained in the adipose tissue and the decreased re-esterification of NEFA. This leads to an increase in the concentration of circulating NEFA in plasma and, consequently, greater availability of this fuel source for the muscles[2,20]. These fundamental principles can explain the findings of the present study, suggesting that when exercise is performed in the fasted state, lipolytic activity is increased further because of the action of lipolysis-stimulating hormones and limited action of insulin. However, increased plasma concentrations of NEFA during exercise are attenuated by carbohydrate intake before exercise, due to the inhibition of lipolysis mediated by insulin[6]. It has also been suggested that increases in insulin concentrations can directly inhibit the transfer of fat through the muscle cell membrane and/or mitochondrial membranes[8]. Therefore, as a consequence of lower availability of NEFA and the inhibition of oxidation of IMTG, exercise performed in the fed state shows reduced fat oxidation[7].

Apart from diet, use of energy substrates during exercise depends on factors such as intensity, duration and level of training[4]. It has been shown that fat oxidation, rather than the use of carbohydrate as a substrate, tends to be higher at low-to-moderate intensities of exercise, no >60–65 % VO_{2max}, but is likely to decrease at an intensity >75 % VO_{2max}[60,61]. These data corroborate the findings of the present study, in which most of the interventions relating to fat oxidation during exercise
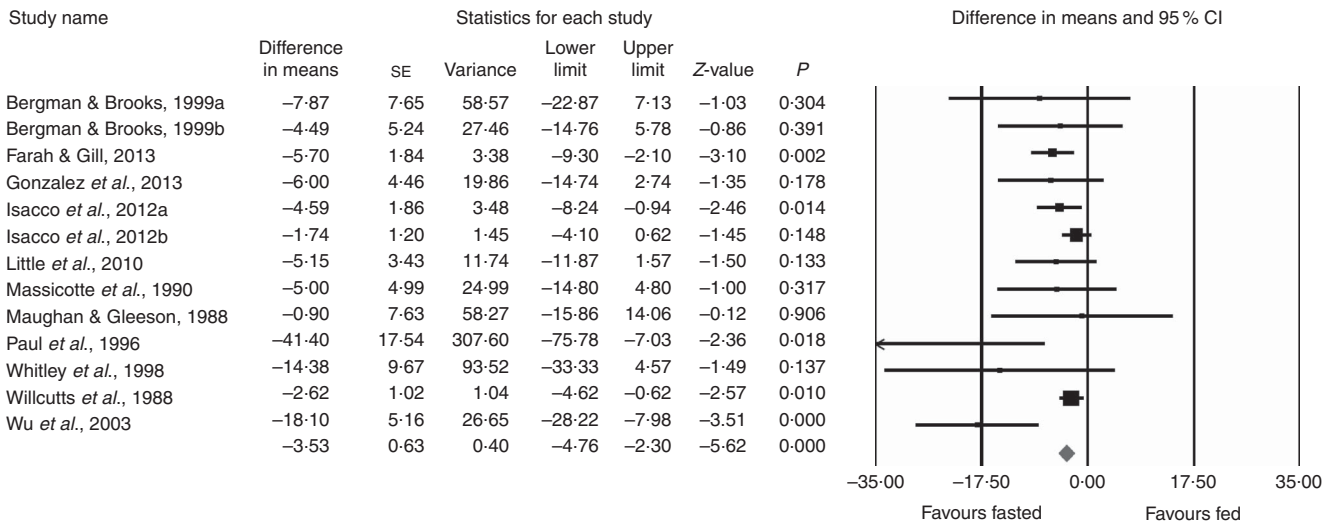
Study name	Statistics for each study							Difference in means and 95 % CI
	Difference in means	SE	Variance	Lower limit	Upper limit	Z-value	P	
Bergman & Brooks, 1999a	−7·87	7·65	58·57	−22·87	7·13	−1·03	0·304	
Bergman & Brooks, 1999b	−4·49	5·24	27·46	−14·76	5·78	−0·86	0·391	
Farah & Gill, 2013	−5·70	1·84	3·38	−9·30	−2·10	−3·10	0·002	
Gonzalez et al., 2013	−6·00	4·46	19·86	−14·74	2·74	−1·35	0·178	
Isacco et al., 2012a	−4·59	1·86	3·48	−8·24	−0·94	−2·46	0·014	
Isacco et al., 2012b	−1·74	1·20	1·45	−4·10	0·62	−1·45	0·148	
Little et al., 2010	−5·15	3·43	11·74	−11·87	1·57	−1·50	0·133	
Massicotte et al., 1990	−5·00	4·99	24·99	−14·80	4·80	−1·00	0·317	
Maughan & Gleeson, 1988	−0·90	7·63	58·27	−15·86	14·06	−0·12	0·906	
Paul et al., 1996	−41·40	17·54	307·60	−75·78	−7·03	−2·36	0·018	
Whitley et al., 1998	−14·38	9·67	93·52	−33·33	4·57	−1·49	0·137	
Willcutts et al., 1988	−2·62	1·02	1·04	−4·62	−0·62	−2·57	0·010	
Wu et al., 2003	−18·10	5·16	26·65	−28·22	−7·98	−3·51	0·000	
	−3·53	0·63	0·40	−4·76	−2·30	−5·62	0·000	

Fig. 4. Fat oxidation (g) during exercise performed in the fasted state *v.* fed state. ■, Study-specific estimates: ◆, pooled estimates of fixed-effects meta-analyses.
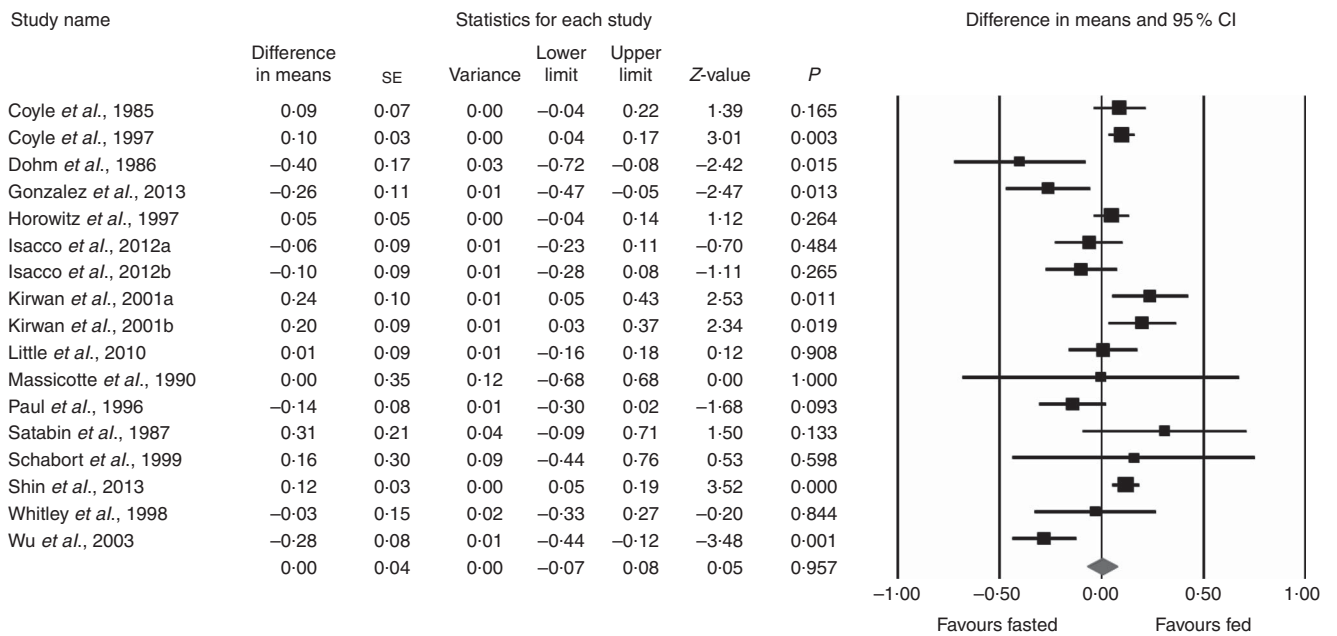
Study name	Statistics for each study							Difference in means and 95 % CI
	Difference in means	SE	Variance	Lower limit	Upper limit	Z-value	P	
Coyle et al., 1985	0·09	0·07	0·00	−0·04	0·22	1·39	0·165	
Coyle et al., 1997	0·10	0·03	0·00	0·04	0·17	3·01	0·003	
Dohm et al., 1986	−0·40	0·17	0·03	−0·72	−0·08	−2·42	0·015	
Gonzalez et al., 2013	−0·26	0·11	0·01	−0·47	−0·05	−2·47	0·013	
Horowitz et al., 1997	0·05	0·05	0·00	−0·04	0·14	1·12	0·264	
Isacco et al., 2012a	−0·06	0·09	0·01	−0·23	0·11	−0·70	0·484	
Isacco et al., 2012b	−0·10	0·09	0·01	−0·28	0·08	−1·11	0·265	
Kirwan et al., 2001a	0·24	0·10	0·01	0·05	0·43	2·53	0·011	
Kirwan et al., 2001b	0·20	0·09	0·01	0·03	0·37	2·34	0·019	
Little et al., 2010	0·01	0·09	0·01	−0·16	0·18	0·12	0·908	
Massicotte et al., 1990	0·00	0·35	0·12	−0·68	0·68	0·00	1·000	
Paul et al., 1996	−0·14	0·08	0·01	−0·30	0·02	−1·68	0·093	
Satabin et al., 1987	0·31	0·21	0·04	−0·09	0·71	1·50	0·133	
Schabort et al., 1999	0·16	0·30	0·09	−0·44	0·76	0·53	0·598	
Shin et al., 2013	0·12	0·03	0·00	0·05	0·19	3·52	0·000	
Whitley et al., 1998	−0·03	0·15	0·02	−0·33	0·27	−0·20	0·844	
Wu et al., 2003	−0·28	0·08	0·01	−0·44	−0·12	−3·48	0·001	
	0·00	0·04	0·00	−0·07	0·08	0·05	0·957	

Fig. 5. Weighted mean difference of NEFA concentrations (mmol/l) relative to exercise performed in the fasted state *v.* fed state. ■, Study-specific estimates: ◆, pooled estimates of random-effects meta-analyses.

included in the meta-analysis were performed with intensities between 40 and 65 % VO_{2max}[30,31,39,41,44,47,51,52]. Studies that used greater intensities, and where exact values were given in the published report, did not surpass 70 % VO_{2max}[40,43]. Concerning the duration of exercise, the studies included were evaluated up to 120 min. It is suggested that after 2 h of exercising, the substrate utilisation patterns become similar between fasted and fed states[45]. That is, an increase in fat oxidation may also occur in 'fed state' individuals after a certain interval, and may be caused by a reduction in muscle glycogen that occurs in the advanced stages of prolonged exercise[4]. Furthermore, the majority of included studies were conducted with physically active individuals[30,40,41,43,44,47,50–52], and the literature

indicates that fat oxidation during sub-maximal exercise is improved with aerobic physical training[14,60].

As exercise intensity can influence the utilisation of energy substrates during exercise, sensitivity analyses were performed according to this criterion. It is well established in the literature that the contribution of carbohydrate to energy supply increases incrementally with exercise intensity (>65 % VO_{2max}), whereas the fat oxidation peak occurs at lower intensities (45–65 % VO_{2max}), which may be influenced by sex, training status, VO_{2max} and diet[60]. The present analysis showed that during exercises at intensities <70 % VO_{2max}, fat oxidation was higher in the fasted state (approximately 3·45 g), but that there was no difference in this variable between
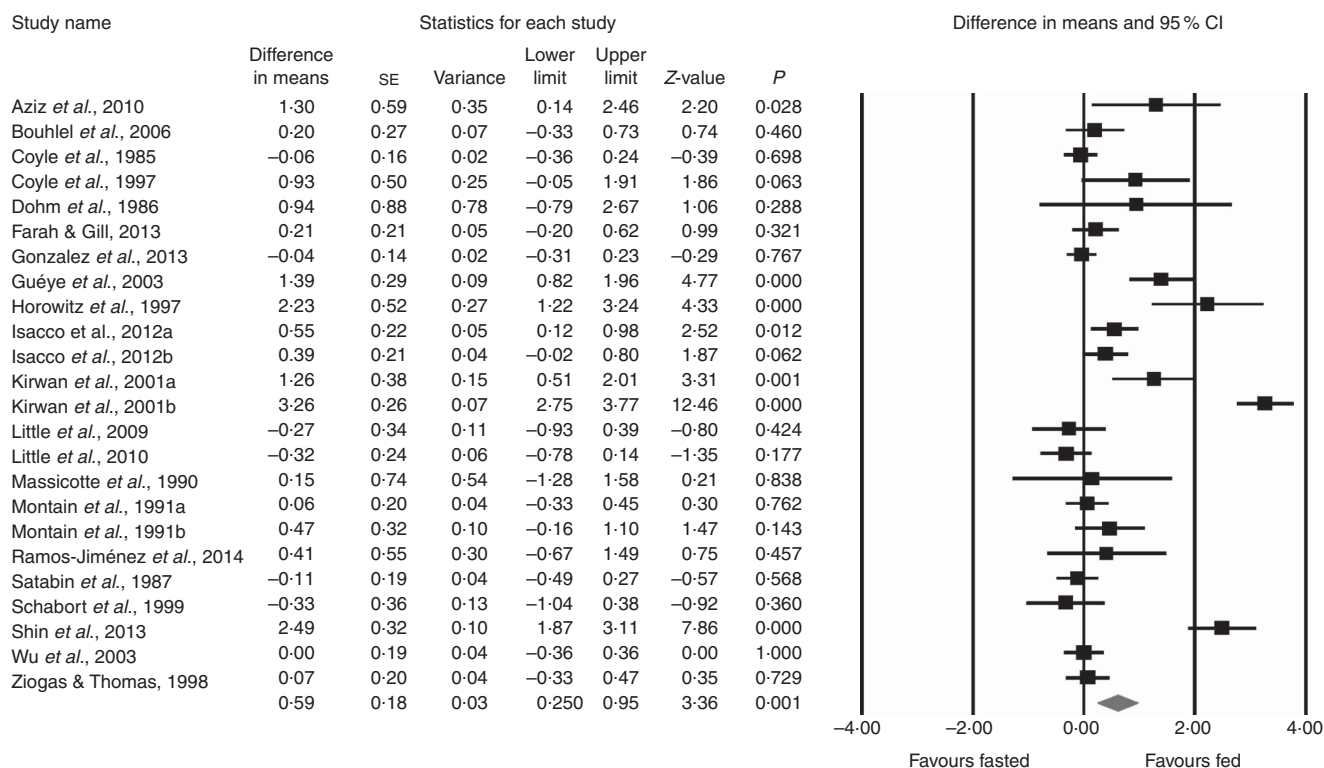
Fig. 6. Weighted mean difference of glucose concentrations (mmol/l) relative to exercise performed in the fasted state *v.* fed state. ■, Study-specific estimates; ◆, pooled estimates of random-effects meta-analyses.

Study name	Difference in means	SE	Variance	Lower limit	Upper limit	Z-value	P
Aziz et al., 2010	1·30	0·59	0·35	0·14	2·46	2·20	0·028
Bouhlel et al., 2006	0·20	0·27	0·07	−0·33	0·73	0·74	0·460
Coyle et al., 1985	−0·06	0·16	0·02	−0·36	0·24	−0·39	0·698
Coyle et al., 1997	0·93	0·50	0·25	−0·05	1·91	1·86	0·063
Dohm et al., 1986	0·94	0·88	0·78	−0·79	2·67	1·06	0·288
Farah & Gill, 2013	0·21	0·21	0·05	−0·20	0·62	0·99	0·321
Gonzalez et al., 2013	−0·04	0·14	0·02	−0·31	0·23	−0·29	0·767
Guéye et al., 2003	1·39	0·29	0·09	0·82	1·96	4·77	0·000
Horowitz et al., 1997	2·23	0·52	0·27	1·22	3·24	4·33	0·000
Isacco et al., 2012a	0·55	0·22	0·05	0·12	0·98	2·52	0·012
Isacco et al., 2012b	0·39	0·21	0·04	−0·02	0·80	1·87	0·062
Kirwan et al., 2001a	1·26	0·38	0·15	0·51	2·01	3·31	0·001
Kirwan et al., 2001b	3·26	0·26	0·07	2·75	3·77	12·46	0·000
Little et al., 2009	−0·27	0·34	0·11	−0·93	0·39	−0·80	0·424
Little et al., 2010	−0·32	0·24	0·06	−0·78	0·14	−1·35	0·177
Massicotte et al., 1990	0·15	0·74	0·54	−1·28	1·58	0·21	0·838
Montain et al., 1991a	0·06	0·20	0·04	−0·33	0·45	0·30	0·762
Montain et al., 1991b	0·47	0·32	0·10	−0·16	1·10	1·47	0·143
Ramos-Jiménez et al., 2014	0·41	0·55	0·30	−0·67	1·49	0·75	0·457
Satabin et al., 1987	−0·11	0·19	0·04	−0·49	0·27	−0·57	0·568
Schabort et al., 1999	−0·33	0·36	0·13	−1·04	0·38	−0·92	0·360
Shin et al., 2013	2·49	0·32	0·10	1·87	3·11	7·86	0·000
Wu et al., 2003	0·00	0·19	0·04	−0·36	0·36	0·00	1·000
Ziogas & Thomas, 1998	0·07	0·20	0·04	−0·33	0·47	0·35	0·729
	0·59	0·18	0·03	0·250	0·95	3·36	0·001

Fig. 7. Weighted mean difference of insulin concentrations (pmol/l) relative to exercise performed in the fasted state *v.* fed state. ■, Study-specific estimates; ◆, pooled estimates of random-effects meta-analyses.
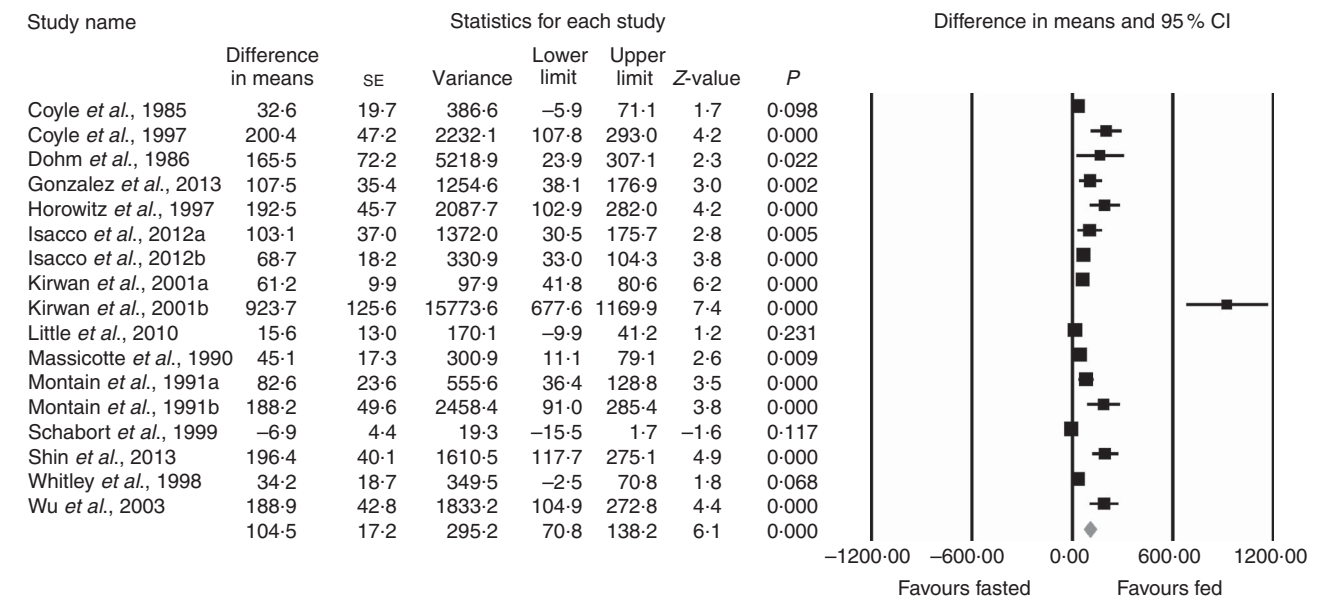
Study name	Difference in means	SE	Variance	Lower limit	Upper limit	Z-value	P
Coyle et al., 1985	32·6	19·7	386·6	−5·9	71·1	1·7	0·098
Coyle et al., 1997	200·4	47·2	2232·1	107·8	293·0	4·2	0·000
Dohm et al., 1986	165·5	72·2	5218·9	23·9	307·1	2·3	0·022
Gonzalez et al., 2013	107·5	35·4	1254·6	38·1	176·9	3·0	0·002
Horowitz et al., 1997	192·5	45·7	2087·7	102·9	282·0	4·2	0·000
Isacco et al., 2012a	103·1	37·0	1372·0	30·5	175·7	2·8	0·005
Isacco et al., 2012b	68·7	18·2	330·9	33·0	104·3	3·8	0·000
Kirwan et al., 2001a	61·2	9·9	97·9	41·8	80·6	6·2	0·000
Kirwan et al., 2001b	923·7	125·6	15773·6	677·6	1169·9	7·4	0·000
Little et al., 2010	15·6	13·0	170·1	−9·9	41·2	1·2	0·231
Massicotte et al., 1990	45·1	17·3	300·9	11·1	79·1	2·6	0·009
Montain et al., 1991a	82·6	23·6	555·6	36·4	128·8	3·5	0·000
Montain et al., 1991b	188·2	49·6	2458·4	91·0	285·4	3·8	0·000
Schabort et al., 1999	−6·9	4·4	19·3	−15·5	1·7	−1·6	0·117
Shin et al., 2013	196·4	40·1	1610·5	117·7	275·1	4·9	0·000
Whitley et al., 1998	34·2	18·7	349·5	−2·5	70·8	1·8	0·068
Wu et al., 2003	188·9	42·8	1833·2	104·9	272·8	4·4	0·000
	104·5	17·2	295·2	70·8	138·2	6·1	0·000

fasted and fed states during exercises at intensities ≥70 % VO_{2max}. These findings confirm the results reported by Bergman & Brooks[30], in which the effect of intensity and previous feeding on energy substrate used during exercise was verified. Higher fat oxidation was observed in the fasted state, compared with the fed state, during exercises with intensities up to 59 % VO_{2peak}, but not at the intensity of 75 % VO_{2peak}.

The literature reports that physical training is able to reduce insulin resistance[14] related to excessive accumulation of IMTG in sedentary individuals[62]. This effect seems to be due to increased fat oxidation[15], mainly coming from fatty acids

derived from IMTG[63]. The acute effects of exercise in the fasted state are able to reduce the content of IMTG by approximately 60 %[5,64], which does not seem to occur in the fed state[5] and, in the long term, seems to be more effective in improving insulin sensitivity[65].

Venables & Jeukendrup[14] demonstrated that an increase in fat oxidation of approximately 3 g during 30 min of aerobic exercise was able to enhance insulin sensitivity in obese and sedentary men. The present meta-analysis indicates that aerobic exercise performed in the fasted state provides an increase in fat oxidation of about 3·08 g during the session compared with the fed state. Therefore, it is suggested that exercising in the fasted state can be an alternative to increase the use of fat as the energy source and the increase of oxidised fats by 3·08 g during an exercise session may be sufficient to induce improvements in insulin sensitivity.

As previously described, it is well established that plasma concentrations of NEFA are higher in the fasted states compared with fed states[4,66,67]. However, our results indicate that the magnitude of variation, from before to after exercise, does not appear to differ between fasted *v*. fed states. In general, during the first 15 min of exercise, plasma NEFA concentrations decrease, as the utilisation rate in the muscles exceeds the lipolysis-driven release rate. After this period, the release rate exceeds use in the muscles and the fatty acid concentrations in the plasma rise[4]. On the basis of the results provided in this meta-analysis, this event seems to occur in similar ways in both fasted and fed states.

Although the weighted mean difference of NEFA showed no significant differences, the present study demonstrates that variation in glucose and insulin concentrations before and after exercise was significantly higher during exercise performed in the fed state. One possible explanation for this finding in relation to insulin is that carbohydrate ingestion before exercise can result in a considerable increase in insulin concentrations[68], which may remain high for about 3 h after consuming a meal[51], and tend to return to basal values when exercise is performed[45,47]. In this case, it is noteworthy that the majority of studies included in the meta-analysis offered meals up to 180 min before exercising[6,7,31,32,37,38,47–52]. Hence, it is probable that insulin concentrations remained high at the beginning of exercise and decreased over the course of the exercise for 'fed state' participants.

Regarding glucose concentrations, the highest variation generated by exercise performed in the fed state is attributed to increased glucose concentrations in plasma, due to the intake of carbohydrates before exercise, and subsequent fall in glucose concentration due to the combined effects of hyperinsulinaemia and glucose uptake for use as an energy substrate in muscle contractile activity[6,69]. On the other hand, fasting causes increases in glycerol release through hydrolysis of TAG molecules from fat cells; this is a valuable precursor for hepatic gluconeogenesis, thus contributing to the availability of glucose[2]. These principles can be used to explain the greater variations in plasma glucose concentration in the fed state relative to the fasted state. When sensitivity analyses were performed for this variable according to the criterion 'quantity of carbohydrate consumed in the pre-exercise meal ≥100 g', meta-analysis did not demonstrate a significant difference in the variation of glucose between fasted *v*. fed states.

A possible hypothesis for this is that the intake of carbohydrate-rich meals increases the availability of glucose during exercise[68].

Although this systematic review with meta-analysis was performed with the maximum methodological rigour possible, some limitations should be highlighted. First was the inclusion of different intensities and durations of exercise, sex, times between meals and exercise, types of meals and quality and quantity of carbohydrates: in spite of methodological differences between the studies under review, we sought to maximise standardisation in the data examined. Second, as many relatively old publications were included in this study, certain methodological limitations and flaws were noted in the presentation of data. We would like to emphasise in particular that the large number of trials with results presented exclusively in graphs, and the lack of provision of these data (means and standard deviations) from authors, limited the accuracy of data extraction. Third, most of the studies included in the analyses of glucose concentrations assessed glucose levels using venous blood sampling. Previous studies have reported[70,71] that the arterial sampling would be more recommended to assess glucose levels; however, venous blood sampling is the most commonly used method and it is widely accepted. In addition, high heterogeneity was identified in meta-analyses related to blood molecular concentrations, necessitating caution in interpreting these data.

Is worth mentioning that, although our results have shown increased fat oxidation during exercise performed in the fasted state, it is necessary to take care when prescribing this strategy in practice, as this meta-analysis was performed using only data assessing the acute effects of exercise during fasting *v*. fed states. The findings should not be extrapolated as long-term effects, especially with the aim of reducing body fat, as there is insufficient evidence of effectiveness and safety.

Conclusion

This systematic review with meta-analysis suggests that aerobic exercise at low-to-moderate intensity, performed in the fasted state, induces an increase in fat oxidation, when compared with exercise performed following consumption of a carbohydrate-containing meal. Despite high heterogeneity of the data, no difference appears to exist between exercising in the fasted or fed states in relation to variations in NEFA concentrations before and after exercise. In contrast, variation in relation to glucose and insulin concentrations appears to be higher in the fed states. Future meta-analyses and randomised clinical trials, inclusive of an evaluation of the long-term effects of aerobic exercise on fat and carbohydrate metabolism in the fasted and fed states, will be necessary to confirm the findings of the present review, as well as to identify their real benefits or consequences for long-term health.

Acknowledgements

Karen Soo, Donald Chisholm and Katarina Borer, all of whom answered our questions by email.

Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114516003160

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