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The Agreement between Fat_{max} and the Gas-Exchange and Blood Lactate Thresholds: A Systematic Review and Meta-Analysis

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ABSTRACT

Background: The relationship between the exercise intensity at which maximal fat oxidation occurs (Fat_{max}) and the lactate threshold/gas-exchange threshold (LT/GET) has gained interest in both sport performance and health-related communities, yet the absolute agreement between these two “thresholds” remains inconsistent. This review aims to systematically assess the available literature to determine whether Fat_{max} occurs at the same exercise intensity as the LT/GET and to examine whether aspects of study design moderated this relationship. **Methods:** PubMed, MEDLINE, Embase and Scopus were systematically searched from inception to 20th January 2025. Studies were included if they reported Fat_{max} and measures of the LT/GET in healthy adults. Exercise intensities corresponding to these thresholds were analysed using a random effects multilevel meta-analysis. Univariate moderator analyses were performed to identify potential methodological or participant-related moderators of the effect size (ES). **Results:** The meta-analysis ($N = 42$ studies) revealed that Fat_{max} occurred at a significantly lower exercise-intensity than LT/GET, by approximately 5.78% $\dot{V}O_{2max}$, 4.75 ml/min/kg $\dot{V}O_2$, 22 watts, or 11 beats per minute (ES=0.74, 95% CI=0.32–1.16, $P=0.001$). Moderator analyses indicated that differences between Fat_{max} and LT/GET varied according to participant characteristics (e.g., age, sex, fitness level), nutritional status, study design, testing protocols and analytical methods. **Conclusions:** Although Fat_{max} typically occurs at a lower exercise intensity than the LT/GET, this difference appears smaller in sedentary individuals (<20 years), when testing is performed with <6 hour fasting period, and when treadmill-based, two separate sessions protocols are employed. Under these conditions, the LT/GET could serve as a surrogate for Fat_{max} when direct substrate oxidation measurement is not feasible. **Key Words:** MAXIMAL

FAT OXIDATION, LACTATE, VENTILATORY THRESHOLD, FUEL UTILISATION,
INDIRECT CALORIMETRY, EXERCISE TESTING

ACCEPTED

INTRODUCTION

The absolute rate of fat oxidation increases with exercise-intensity from low- to moderate-intensity exercise (~25-65% maximal oxygen consumption; $\dot{V}O_{2\max}$), before declining rapidly at higher intensities (i.e., ~85-95% $\dot{V}O_{2\max}$) (1, 2, 3). Thus, at an intermediate submaximal exercise-intensity, there occurs a point of maximal fat oxidation (MFO). The exercise-intensity corresponding to MFO is termed the Fat_{\max} (2). Training at an individual's Fat_{\max} elicits physiological and metabolic adaptations, enhancing fat oxidation capacity, endurance performance, and overall metabolic health (4, 5, 6, 7, 8, 9). Certainly, training at this exercise-intensity elicits a 44% increase in fat oxidation rates, compared with no changes when interval training is performed (5). Furthermore, endurance training at Fat_{\max} improves body composition (6, 7, 8, 9), $\dot{V}O_{2\max}$ (6, 7, 8), insulin sensitivity (6, 8, 9), and lipid profiles (7, 8) in both paediatric and adult clinical populations.

Seeing that the capacity to oxidize fat during exercise has been associated with endurance performance success (10, 11), and markers of metabolic health (12), the ability to identify Fat_{\max} in a time-efficient manner has become increasingly important (5). Some researchers have suggested that Fat_{\max} can be estimated from a single, graded exercise test using the moderate-to-heavy intensity transition as a surrogate (13, 14, 15). Whereas, other investigators have observed significant differences between this exercise-intensity transition and Fat_{\max} , suggesting these two “thresholds” should be determined using separate protocols (16). The lactate threshold (LT), the Gas Exchange Threshold (GET), and the ventilatory threshold (VT) are well-established markers of the moderate-to-heavy intensity transition (17, 18), therefore, this transition point will be referred to as the LT/GET throughout (19).

A recent meta-analysis (20) including 14 studies reported no significant differences between Fat_{max} (effect size (ES) = -0.23, 95% Confidence Interval (CI) = -0.56–0.09, $P > 0.05$) and markers of the moderate-to-heavy intensity transition (i.e., LT_1 , GET or VT_1). A follow-up review from the same group was conducted to assess the agreement between Fat_{max} and LT/GET , with LT/GET intensities frequently falling outside the predefined $\pm 10\%$ MFO range (21). Although a moderate association was observed, no consistent directional bias was identified, indicating that LT/GET cannot reliably serve as a surrogate for Fat_{max} at the individual level. However, the results must be interpreted with caution, given that the authors systematically searched only two databases (MEDLINE and Google Scholar) using a date-range spanning January 2001 to April 2021 (i.e., most recent 20 years). Despite the technical term “ Fat_{max} ” not being introduced until 2001 (22), researchers have measured peak fat oxidation during exercise prior to this year. For example, Romijn, Coyle (3) and Astorino (23) examined fat and carbohydrate oxidation across varying exercise intensities. The inclusion criteria stated by Peric, Nikolovski (20) were that studies must report Fat_{max} , LT/GET , and the statistical correlation between those two parameters, resulting in the potential exclusion of studies where the two thresholds were not primary outcomes, nor were correlations between these two variables performed.

In turn, it remains unknown whether a more comprehensive search of the literature (since inception across multiple databases) would yield a greater number of eligible studies for inclusion in a meta-analysis. Therefore, this systematic review aimed to create a synthesis from existing literature and determine whether Fat_{max} occurs at the same intensity as LT/GET within healthy participants. It is clear from a preliminary search of the literature that studies measuring both thresholds have included a wide range of participant preparatory conditions (13, 16, 24),

acute nutritional status conditions (23, 25), exercise modes and protocols (15, 26, 27), and data analysis methods (15, 28). Therefore, a secondary aim was to determine whether various aspects of study design were moderators of the reported effect size between exercise intensities corresponding to Fat_{max} and LT/GET.

METHODS

This systematic review and meta-analysis was performed under the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (29). The protocol for this review was registered with PROSPERO (ID CRD42022346457).

Search strategy

Four electronic databases (PubMed, Medline, Embase, Scopus) were systematically searched from inception to the 9th July 2022, with an update on 20th January 2025. The search strategy involved combining the following keywords: “lactate” OR “ventilat*” OR “gas exchange threshold” OR “metabolic threshold” OR “aerobic threshold” OR “anerobic threshold” OR “anaerobic threshold” AND “fat max” OR “Fatmax” OR “maximal fat oxidation” OR “peak fat oxidation” OR “lipomax” OR “fuel utili*”.

Eligibility criteria

Studies were considered eligible for inclusion if they: (1) were original research articles published in peer-reviewed journals; (2) recruited non-obese (body mass index $< 30 \text{ kg}\cdot\text{m}^{-2}$) ostensibly healthy human participants without known cardiometabolic diseases, cancer, or receiving medications affecting substrate metabolism; (3) were cross-sectional by design or, in the case of intervention studies, included a control group or baseline measurement time point; (4) assessed the moderate-to-heavy intensity transition during graded exercise via any of its surrogate markers, including blood or plasma lactate, GET or VT; (5) provided an assessment of

Fat_{max} during a graded exercise protocol; and if they (6) reported the mean, and a measure of dispersion around the mean (i.e., standard deviation, SD; or standard error, SE) for the exercise intensities corresponding to LT/GET and Fat_{max}.

If descriptives were not reported in the study, the corresponding data was requested by contacting the study authors. Studies were excluded if either the LT, GET and VT, Fat_{max}, or $\dot{V}O_{2max}$ were obtained via prediction equations, reference values, and/or extrapolated from submaximal exercise data. To facilitate comparison between LT/GET and Fat_{max}, studies were only included if each threshold was reported in identical units of exercise-intensity. Acceptable units of exercise-intensity were: $\dot{V}O_2$ reported in absolute (L/min) or relative (e.g., ml/kg/min) units or expressed as a percent of $\dot{V}O_{2max}$; work rates reported in absolute (W) and relative (e.g., W/kg) units, or expressed as a percent of maximum W (%W_{max}); and HR in absolute (beats/min) units or expressed as a percentage of maximum HR (%HR_{max}) or HR reserve (HRR). Studies that calculated and defined LT as 4 mmol/L were excluded given that this discrete point has been shown to represent the maximal lactate steady state (MLSS) (30, 31).

Study screening and selection process

Search results were exported from each database into Endnote reference management software (Endnote 20.3), and then imported into Covidence systematic review software (Veritas Health Innovations, Melbourne, Australia) (32). Duplicates were removed, and each title and abstract screened independently by two reviewers (CH, KR, TC, MT) according to the eligibility criteria. In the case of conflict, a third reviewer (KR, TC) decided, and consensus was reached in all cases. Full-text copies of each included study were obtained and further screened by two reviewers (CH, KR, TC, AB, PP), and any conflicts were resolved by a third reviewer (KR, TC).

Data extraction

Two authors (CH, MT, PP) independently extracted data using a customised Microsoft Excel™ spreadsheet (see Supplemental Digital Content 1, <http://links.lww.com/MSS/D419> and Supplemental Digital Content 3, <http://links.lww.com/MSS/D423>). Descriptive statistics were recorded including means and SD. In the case where a study included a clinical population, only the data from the healthy control group were extracted. If a study implemented an intervention of any kind, only pre-intervention data were extracted.

Risk of bias assessment

Risk of bias was assessed by using the relevant tool depending on the study design that was agreed upon between two independent reviewers (CH, MT, PP), and any discrepancies were resolved with a third reviewer (MT, PP). Inter-rater reliability between reviewers was assessed using Cohen's Kappa coefficient (κ). Results are displayed graphically using the *Robvis* statistical package in R language and environment for statistical computing (Version 4.3.1) (33). See Supplemental Digital Content 2 (Supplemental Digital Content 2, <http://links.lww.com/MSS/D420>) for specific details on tools used for each study design.

Multi-level meta-analysis

A random-effects multilevel meta-analysis was performed in R using the *meta*, *metafor* and *dmetar* statistical packages. The hierarchical structure nested in the three-level model was: (1) each individual participant within a sample; (2) within-study heterogeneity (i.e., each ES); and (3) between study heterogeneity (i.e., each study). Model parameters were calculated using the restricted maximum likelihood estimation. The standardised mean difference (SMD) using Hedge's G was used to calculate the pooled ES and associated 95% confidence intervals (CI). A forest plot was generated to visually depict the studies pooled SMD and associated 95% CI. The

heterogeneity across levels was assessed using a multi-level version of the *I*-square (I^2) test, where $\leq 25\%$ was low, $\leq 50\%$ moderate, and $> 75\%$ was considered high heterogeneity (34). Funnel plots, contoured enhanced funnel plots, an Egger's regression test (35), and the trim and fill procedure (36) were used to consider publication bias. Sensitivity analysis was performed using the leave-one-out method (37), whereby each study was sequentially removed and the meta-analysis re-run to assess the influence of individual studies on the overall effect. Univariate moderator analyses relating to participant characteristics, participant acute nutritional status, study design, protocol characteristics and equipment, and analysis methods, were performed to separately explore whether each moderator influenced the pooled ES for the exercise intensities corresponding to Fat_{max} and LT/GET. Statistical analyses were considered significant if $P < 0.05$. A detailed description of the statistical analyses performed in this study is provided in Supplemental Digital Content 3, <http://links.lww.com/MSS/D420> (Supplemental Digital Content 2, <http://links.lww.com/MSS/D420>).

RESULTS

Study selection

The systematic identification, screening and selection process of relevant literature is summarised in Figure 1. There were 66 potential studies identified. Of these studies, a total of 34 authors were contacted for provision of incomplete data, and ten authors responded. Thus, 24 studies were excluded from further analysis. In total, 42 studies were included in our systematic review which, in turn, yielded a total of 158 estimates of ES (e.g., median number of ES was 2 per study).

Study and participant characteristics

The included studies are summarised in Supplemental Digital Content 4, <http://links.lww.com/MSS/D420> (Supplemental Digital Content 2, <http://links.lww.com/MSS/D420>). The included studies ranged across 18 different countries, the majority being from Europe ($n = 27$, 64%). There were 22 studies (52%) that included a male-only sample, 13 studies (29%) that included both sexes and only three studies (7%) that included female-only samples. There was a total of 1,505 participants, of which 101 participants' sex were not recorded. Of the remaining participants there were 958 males (68%), 402 females (29%), and 44 (3%) were reported as combined males and females. Mean age ranged from 13.9-48.5 years. The average age of participants of the included studies was distributed as follows: 15% were < 20 years, 40% between 20-29 years, 41% between 30-39 years, and 4% between 40-49 years. Across the included participants ($N = 1,505$), the pooled weighted mean age was 28.1 ± 10.9 years. We assigned a participant fitness category to each study according to author descriptions of the physical activity and exercise levels of their participants, and any instances where participant $\dot{V}O_{2max}$ were compared to normative values according to sex, age and exercise modality (38). Accordingly, participants fitness level comprised of: elite= 448 participants (30%), trained = 525 participants (35%), active= 407 participants (27%), sedentary= 125 participants (8%).

Acute nutritional status

Fasting time was specified in 32 studies (76%) and ranged from 2 hours on the morning of testing to an overnight fast of 10-12 hours. Most studies ($n = 20$, 48%) implemented an overnight fast of 10-12, or 7-10 hours. In ten studies (24%) it was unclear what fasting protocol was implemented prior to the exercise tests. Participants were instructed to maintain their regular

dietary habits in 19 studies (45%). In 12 studies (29%) participants consumed a carbohydrate rich diet ranging from the dinner prior to three days pre-testing. Standardised meals were prescribed by a nutritionist ($n = 7$, 17%) for dinner, or breakfast the day of testing, or 24 hours prior. A food recall was obtained in 10 studies (24%). A 3-day food recall was most commonly implemented (4 studies) (39, 40, 41, 42), while a 4-day (43), 5-day (44) and 7-day (45) also used.

Menstrual cycle considerations and oral contraception use

Out of the 16 studies that included female participants, seven studies (44%) did not record any details regarding the menstrual cycle or contraception use. Four studies (25%) reported that menstrual cycle was not controlled for, but participants were eumenorrheic, while five studies (31%) reported participants completed the study during the follicular phase, or the luteal phase. Five studies (31%) provided details about oral contraception usage. Females in four studies (80%) were not using oral contraception, and one study (20%) examined the differences between females prescribed oral contraception and others who had not used any for at least 12 months (46).

Protocol characteristics

Across the included studies, there were 21 different metabolic carts used to measure pulmonary gas-exchange (see Table 1), while one study used Douglas bags with the Servomex 1400 (Sussex, UK). The most commonly used metabolic cart ($n = 9$, 21%) was the Oxycon Pro (Jaeger, Würzburg, Germany). There were two types of ergometers used: a cycle-ergometer ($n = 30$, 71%) and a treadmill ($n = 12$, 29%). All included studies (with the exception of two (26, 44)) were maximal graded exercise tests. The majority of studies ($n = 26$, 62%) performed a single graded exercise test to exhaustion to estimate both outcomes including LT/GET and Fat_{max} . Out of these studies, most performed the graded exercise test to exhaustion ($n = 10$, 38%) with stage

intervals of 3 minutes. Other intervals used included 2-, 4-, 5-, 6-, and 10-min stages. Nine studies (30%) performed 2-, 3-, or 5-min stages until the respiratory exchange ratio (RER) > 1.0 then all studies increased intensity each minute until exhaustion.

Sixteen studies (38%) performed two separate exercise tests, one to identify the $\dot{V}O_{2\max}$ of participants followed by a sub-maximal exercise test to estimate substrate oxidation. Out of these studies, the majority ($n = 8$, 50%) used 1-min stages in the $\dot{V}O_{2\max}$ test. Other intervals included 2- or 3-min stages, and two studies performed a ramp protocol. For the additional test to estimate substrate oxidation (i.e., Fat_{\max} test) the most commonly used stage length was 6-mins ($n = 5$, 31%). Other intervals performed were 2-, 3-, 5-, 6-, 9-, 10-, 15-, and 20-mins.

Methods of estimating fat oxidation

There were eight different stoichiometric equations utilised to estimate the carbohydrate and fat oxidation (see Table 2a). The equations proposed by Frayn (47) were most commonly used across 20 studies (48%). The methods used to estimate MFO and Fat_{\max} varied across the studies (see Table 2b). Nine different methods were identified, while two studies did not report how data analysis was performed. The majority of studies ($n = 24$, 57%) used the maximum observed values obtained during the graded exercise tests.

Methods of estimating the lactate thresholds and gas-exchange thresholds

Twenty studies used LT as the marker of LT/GET and the methods for determining LT are summarised in Table 2c. Twelve different methods were used for estimating LT across these 20 studies. The first increase in lactate concentration above baseline (as described by Coyle et al. (48), Hagbehg & Coyle (49)) was the most common method of estimation used by six studies

(14%). The 13 different referenced methods used to estimate the GET across the 26 studies are summarised in Table 2d. Four studies measured both LT and GET.

Risk of bias assessment

Based on rating 766 items across identified studies, inter-rater reliability for the two reviewers attained a high agreement level ($k = 0.90$, $P < 0.0001$). Risk of bias for all included studies is depicted in Figure 2. All 11 cross-over RCTs had low risk of bias. While the three pre-post studies had moderate risk of bias (see Figure 2c). The cross-sectional tool did not require an overall judgement, however, all 28 studies clearly stated the study aims/objectives. In contrast, sample size was justified in only one study (40). Approximately 65% of the cross-sectional studies did not provide clearly specify whether the sample was representative of the target population under investigation.

Fat_{max} versus lactate thresholds and gas-exchange thresholds

To evaluate the appropriateness of a three-level meta-analysis model compared to a traditional two-level model, an ANOVA was conducted. The results indicated that the three-level model had lower AIC and BIC values compared with a two-level model (i.e., AIC = 425 v 513, BIC = 434 v 519, respectively). Thus, a three-level meta-analysis was the preferred (superior) model. Furthermore, a significant likelihood ratio test ($\chi^2 = 89.65$, $P < 0.0001$) showed the three-level model provided a significant improvement in model fit over the two-level model.

There was a substantially unequal variance across the levels, with 12.3% attributed to level 2 (within studies) and the largest variance of 82.7% attributed to level 3 (between studies). Total heterogeneity was considered high with $I^2=95.0\%$. Figure 3 shows the forest plot displaying the multi-level meta-analysis for LT/GET versus Fat_{max}. There was a significant difference between the exercise intensities corresponding to the LT/GET and Fat_{max}. A large

positive ES (ES=0.74, 95% CI = 0.32 – 1.16, $P=0.0010$) was seen, indicating that Fat_{max} typically occurred at a lower exercise-intensity than the LT/GET.

Funnel plots are summarised in Supplemental Digital Content 5, <http://links.lww.com/MSS/D420> (Supplemental Digital Content 2, <http://links.lww.com/MSS/D420>). The Egger's regression test was non-significant (intercept=1.77, $t=1.14$, $P=0.13$) which indicated no potential publication bias. To confirm there was no publication bias, the trim and fill procedure showed no missing studies, indicating the funnel plot was symmetrical and there were no changes to the overall effect. Sensitivity analyses indicated that the overall ES was robust, with the pooled standardised mean difference remaining consistent (0.651–0.807) and all 95% confidence intervals overlapping the main ES, suggesting that no single study disproportionately influenced the meta-analytic results.

Moderator analysis

A summary of the 23 moderators included in the analysis is provided in Table 3. Among participant characteristics, the ES between Fat_{max} and LT/GET significantly differed from zero in both males ($P < 0.001$) and females ($P < 0.001$), participants aged ≥ 20 years ($P < 0.01$), and those with an active to elite fitness level ($P < 0.05$). However, no significant difference was found when sex was unclear, participants were < 20 years, or participants were classified as sedentary. In females, when it was unclear if menstrual cycle or OC was controlled for, Fat_{max} occurred at a lower exercise intensity relative to the LT/GET ($P < 0.01$).

Moderator analysis revealed that participant acute nutritional status influenced the effect between Fat_{max} and the LT/GET. In studies where participants maintained a regular diet ($n = 38$, ES = 0.214, 95% CI = -0.476-0.904, $P = 0.53$), consumed standardised meals ($n = 27$, ES = 0.745, 95% CI = -0.445-1.935, $P = 0.21$), completed a food recall ($n = 31$, ES = 0.670, 95% CI =

-0.412-1.752, $P = 0.22$), or where dietary carbohydrate intake was clearly reported ($n = 87$, $ES = 0.348-0.765$, $95\% \text{ CI} = -0.259-1.746$, $P > 0.05$), no significant difference was observed between the exercise intensities at which Fat_{max} and LT/GET occurred. However, when the macronutrient breakdown was unclear ($n = 71$, $ES = 0.913$, $95\% \text{ CI} = 0.317-1.509$, $P = 0.22$), or a fasting period of 7-12 hours was implemented ($n = 63$, $ES = 1.069$, $95\% \text{ CI} = 0.496-1.641$, $P = 0.0005$) Fat_{max} occurred at a lower exercise intensity relative to the LT/GET ($P < 0.01$).

When considering study design, Fat_{max} occurred at a lower exercise intensity compared to the LT/GET, regardless of how LT/GET was estimated (GET; $n = 80$, $ES = 0.905$, $P < 0.001$ or LT; $n = 78$, $ES = 0.524$, $P < 0.05$), or when the exercise intensities corresponding to Fat_{max} and LT/GET were reported in units of $\dot{V}O_2$, power output (PO) or HR ($P < 0.01$).

Various protocol characteristics influenced the ES between Fat_{max} and LT/GET. Fat_{max} occurred at a significantly lower exercise intensity than LT/GET when a cycle ergometer ($n = 84$, $ES = 0.945$, $95\% \text{ CI} = 0.484-1.407$, $P = 0.0002$), a single exercise test ($n = 123$, $ES = 0.683$, $P = 0.01$), or two separate exercise tests (i.e., a $\dot{V}O_{2max}$ and Fat_{max} test) were used ($n = 35$, $ES = 0.841$, $P = 0.03$), as well as when protocols included constant workload increments ($P < 0.01$), or short stage durations (i.e., ≤ 3 -mins; $P < 0.001$). This difference was also observed in studies using breath-by-breath indirect calorimetry ($P < 0.01$), spirometry ($P < 0.05$), or multiple calorimeters within a study ($P < 0.01$). In contrast, no significant difference between Fat_{max} and LT/GET was found when treadmill-based protocols were implemented, constant workload increments were not used (e.g., 3 min increments until $RER < 1.0$, then 1 min until exhaustion), or when long stage durations were performed. Similarly, no significant differences between Fat_{max} and LT/GET were seen when portable indirect calorimeters or mixing chambers were used.

When substrate oxidation was estimated using the stoichiometric equations by Frayn, 1983 (47) Fat_{max} occurred at a significantly lower exercise intensity than LT/GET ($n = 84$, $ES = 0.945$, $95\% \text{ CI} = 0.484\text{-}1.407$, $P = 0.0002$). This difference was also evident when MFO and Fat_{max} were derived using measured values or a polynomial regression ($n = 35$, $ES = 1.109$, $95\% \text{ CI} = 0.555\text{-}1.664$, $P = 0.0003$), as well as when LT_1 and GET were determined using logarithmic/curve fitting ($n = 18$, $ES = 0.720$, $95\% \text{ CI} = 0.471\text{-}0.970$, $P < 0.0001$) or the v-slope method ($n = 16$, $ES = 1.442$, $95\% \text{ CI} = 0.678\text{-}2.206$, $P = 0.0007$), respectively. All other stoichiometric equations, methods to determine Fat_{max} , LT and GET found no significant difference between Fat_{max} and LT/GET (refer to Table 3 for specific analysis methods).

DISCUSSION

Summary of key findings

The primary aim of this systematic review and multi-level meta-analysis was to comprehensively search the broader literature and identify whether LT/GET occurred at the same relative exercise-intensity as Fat_{max} . This review included 42 studies and 158 ES, incorporating 30 additional studies not identified in previous reviews (20, 21). Across a large sample including 1,505 participants, the primary finding of our review was that Fat_{max} and LT/GET do not occur at the same exercise-intensity within the apparently healthy population. Our results showed that Fat_{max} occurred at a lower relative exercise-intensity than LT/GET by approximately 5.78% $\dot{V}O_{2max}$ ($95\% \text{ CI} = 2.50\text{-}9.06$); 4.75 ml/min/kg $\dot{V}O_2$ ($95\% \text{ CI} = 2.05\text{-}7.45$); 22 W ($95\% \text{ CI} = 9\text{-}34$); or 11 bpm ($95\% \text{ CI} = 5\text{-}18$). Our secondary aim was to explore the potential moderating variables related to participant characteristics, study design and analytical methods. All 23 moderators assessed had at least one sub-group that significantly influenced the ES between Fat_{max} and LT/GET. These findings highlight the considerable variability in how both Fat_{max} and

LT/GET are measured across studies, influenced by factors such as study design, participant preparation, and analysis methods. This variability presents challenges when comparing results across studies or even within individuals pre- and post-testing, for example if acute nutritional conditions differed between tests. Additionally, our findings contrast the recent meta-analysis by Peric, Nikolovski (20) which reported a non-significant small effect with no difference between Fat_{max} and the LT/GET (ES= -0.23, 95% CI= -0.56–0.09, $P>0.05$) (20).

Practical implications

Researchers, clinicians and coaches should carefully consider study design when using LT/GET as a surrogate for Fat_{max} . When considering the population that is being tested, results consistently show that Fat_{max} occurs at a lower exercise intensity than LT/GET in both males and females. This suggests the two thresholds are not equivalent and should not be used interchangeably without careful consideration. In females, menstrual cycle phase does not appear to influence the exercise intensities Fat_{max} and LT/GET occur at. Although the impact of oral contraceptive use remains unclear, our results indicate that in women not using oral contraception, LT/GET may align with Fat_{max} . Only one study included oral contraceptive users, therefore, we could not include this sub-group in the moderator analysis and future research is warranted. Researchers are recommended to record and report usage within their sample.

We found that Fat_{max} and LT/GET occurred at the same exercise-intensity when fasting was <6 hours, however, with a 7–12-hour fast Fat_{max} occurred at significantly lower intensity than LT/GET. The fasting period in this review ranged from 90 minutes to an overnight 10-12 hour fast. Previous research indicates that consuming glucose pre-exercise significantly reduces MFO and Fat_{max} (50, 51, 52). Astorino et al., (53) and Croci et al., (54) proposed standardising diet 48 hours pre-testing, to increase reliability of estimating MFO and Fat_{max} . In this review,

when participants consumed habitual diets, standardised meals or completed food recalls, alignment between the respective exercise intensities of the two thresholds was found. . Based on this evidence, we recommended standardising nutritional consumption 48 hours prior and ensuring an overnight fast (10-12 hours) for studies using fasting protocols. Similarly, carbohydrate-rich meals did not appear to affect these intensities. This suggests that pre-test meals can be based on individual dietary habits as long as they reflect typical intake. While long-term dietary habits were not assessed in this review, they remain an important topic for future research.

Regardless of whether GET or LT – both differ significantly from Fat_{max} . However, the ES was larger when using GET ($P < 0.001$), compared to a moderate ES observed with LT ($P < 0.05$). This suggests that the discrepancy may be more pronounced depending on the LT/GET marker chosen. Protocols using separate tests for $\dot{V}O_{2max}$ and Fat_{max} with stage durations of 4-mins or longer tended to produce exercise intensities for LT/GET and Fat_{max} that were comparable. However, limitations in the current literature should be acknowledged. None of the included studies accounted for the mean response time of gas-exchange kinetics, despite using ramp or short-stage protocols. This omission likely contributed to greater divergence between Fat_{max} and LT/GET in studies using short stage intervals to estimate GET and longer stage intervals to determine Fat_{max} . While the choice of indirect calorimeter often depends on institutional availability, using a portable system or a mixing chamber resulted in no significant differences between the two thresholds. In contrast, breath-by-breath systems – although the most commonly used in the studies reviewed – were associated with Fat_{max} occurring at a significantly lower exercise intensity than LT/GET.

Thirteen different stoichiometric equations were used across studies (Table 2a). Prior work has highlighted that such variation limits between study comparisons, with differences of up to 6% for carbohydrate and 3% for fat oxidation rates (26, 55, 56). Much of this variation reflects whether equations are based on glucose (e.g., Frayn (47))—appropriate at rest—or glycogen, which is predominate during exercise. Consequently, studies using Frayn’s (47) equations showed Fat_{max} occurring at a lower intensity than LT/GET, whereas those using Jeukendrup and Wallis (57) found no difference. Given these systematic differences, and because the Jeukendrup and Wallis (57) equations were developed specifically for exercise and account for substrate shifts across intensities, we recommend their use for exercise-based fat oxidation research.

For estimating MFO and Fat_{max} a sine model or other mathematical models (see Table 2b for specific methods) reduced the divergence between the thresholds. Although half the studies used measured values, this approach can introduce error equivalent to the stage increments. Previous research has found the RER method to be less accurate than 3rd order polynomial or sine curve fitting (58), thus, we recommend the use of either of these methods. The logarithmic or curve-fitting method was the only approach for identifying LT that showed a significant difference to Fat_{max} , whereas all other methods showed no significant differences. LT_1 is often estimated using arbitrary methods, making standard recommendations challenging. These variations should be considered in future research and between study comparisons. Similarly, the v -slope was the only GET method that consistently showed significant differences between Fat_{max} and the LT/GET and these differences in GET method need to be carefully considered in future research.

Physiological Implications

From a physiological perspective, some of the interest in Fat_{max} and LT/GET has been based on the concept that lactate may inhibit fat oxidation. The rapid decrease in fat oxidation at

moderate intensities has been linked to the rise in blood lactate concentrations that occurs as a result of an increase in glycolytic flux during moderate exercise (51, 59, 60). Recently, lactate metabolism has been shown to act as a signalling molecule with hormone like behaviours and has been referred to as a “lactormone” (61, 62, 63). The mechanisms by which lactate inhibits fat oxidation have been investigated in animal models and *in vitro*. Lactate has been shown to limit substrate availability by activating an orphan G-protein coupled receptor (GPR81), which in turn downregulates adipose lipolysis, decreasing circulating free fatty acid concentrations (64, 65).

While our findings indicate that Fat_{max} typically occurs below the moderate-to-heavy intensity transition, this temporal sequence does not negate the possibility that lactate contributes to reductions in fat oxidation. Importantly, the moderate-to-heavy intensity transition is defined using blood lactate responses, whereas the proposed inhibitory mechanisms such as GPR81-mediated suppression of adipose lipolysis, are driven by intramuscular lactate accumulation (64, 65). These two signals do not necessarily increase concurrently (61, 66). Furthermore, our data cannot determine whether lactate contributes to additional suppression of fat oxidation at intensities above this transition. Therefore, our results suggest that factors other than blood lactate accumulation likely contribute to the initial decline in fat oxidation below the transition, while lactate may still exert additive inhibitory effects at higher intensities.

Review strengths and limitations

Firstly, this review analysed a large dataset, across 42 studies and 1,505 participants. The appropriate use of the multi-level meta-analysis allowed the multiple ES nested within studies, to be accounted for reducing statistical bias and improving estimate precision. Continuous variables such as age were not inputted into the moderator analysis in an attempt to reduce ecological biases. Despite the strengths present within this review, it is not without limitations. One

limitation is the review included only apparently healthy populations, therefore the current findings should not be generalized to populations with clinical conditions including obesity. The reader is directed to a recent review on optimizing fat oxidation during exercise in obesity (67). The high heterogeneity that remained once looking at sub-groups needs to be considered. Due to the inconsistent nature of the approach to measuring Fat_{max} and LT/GET, univariate analysis had to be performed. Covariates could not be inputted together into a single model to provide the effect of all covariates on the ES between Fat_{max} and LT/GET. When considering age, it cannot be confirmed what effect older adults (> 55 years) would have, as there were limited studies that included this age range, so we used the category of > 30 years. Given that our meta-analysis used study-level summary data (means and SD/SEM), we were unable to assess individual-level agreement between Fat_{max} and LT/GET. Consequently, our results reflect average group-level relationships rather than variability at the individual level.

CONCLUSIONS

This review found that Fat_{max} occurred at significantly lower exercise intensity relative to the LT/GET. Subsequently, several factors – including participant characteristics, participant preparation, study design, and analytical methods – were shown to influence the magnitude of the difference. Although the overall findings confirm that Fat_{max} occurred at a lower exercise intensity than LT/GET, the review also identified specific methodological conditions under which Fat_{max} may occur at similar exercise intensities as the LT/GET. While our review cannot determine the underlying cause, it is possible that these instances reflect methodological artefacts (e.g., protocol design, stage duration, or threshold determination methods) rather than true physiological alignment. These insights provide important guidance for researchers, clinicians and coaches aiming to use LT/GET as a surrogate for Fat_{max} , particularly in settings where direct

measurement of substrate oxidation is not feasible. Standardising key elements of participant preparation and protocols may help reduce the inconsistency in the current approaches and improve comparability between studies and within participants.

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FIGURE LEGENDS

Figure 1. PRISMA flow diagram summarising study identification and selection process.

Figure 2. Risk of bias assessments of included studies.

- a. Cochrane RoB2 for crossover trials
- b. National Institute of Health – Pre-post studies with no control group
- c. Appraisal tool for cross-sectional studies (AXIS)

Figure 3. Forest plot for the multi-level meta-analysis random-effects model from included studies that determined AT and Fat_{max} during exercise.

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: AT_Fatmax_Meta-analysis_Supp_1.xlsx

SDC 2: AT_Fatmax_Meta-analysis_Supp.docx

SDC 3: AT_Fatmax_Meta-analysis_Supp_6.xlsx

Table 1. Metabolic carts used during exercise testing across the included studies.

Metabolic Cart	% of Included Studies	Studies
Oxycon Pro (Jaeger, Würzburg, Germany)	21.4	[13, 25, 26, 38, 41, 61, 70, 72, 80]
Oxycon Pro-Delta (Jaeger, Würzburg, Germany)	2.4	[81]
Oxycon Alpha (Jaeger, Würzburg, Germany)	2.4	[15]
Oxycon CPX (Jaeger, Würzburg, Germany)	2.4	[80]
Quark PFT (Cosmed, Rome, Italy)	9.5	[16, 60, 71, 76]
Quark PFT2 (Cosmed, Rome, Italy)	2.4	[69]
Quark CPET (Cosmed, Rome, Italy)	7.1	[39, 78, 80]
Quark B2 (Cosmed, Rome, Italy)	2.4	[64]
K5 Portable System (Cosmed, Rome, Italy)	2.4	[23]
Metalyzer (Cortex, Leipzig, Germany)	2.4	[63]
Metalyzer II (Cortex, Leipzig, Germany)	2.4	[24]
Metalyzer 3B (Cortex, Leipzig, Germany)	7.1	[37, 40, 73]
Metamax 3B (Cortex, Leipzig, Germany)	4.8	[68, 82]
Metamax II (Cortex, Leipzig, Germany)	2.4	[77]
Vmax 229 (SensorMedics, California, USA)	2.4	[14]
2900 Metabolic Cart (SensorMedics, California, USA)	4.8	[22, 62]
CPX Ultima CardiO2 (Medical Graphics Corp, Minnesota, USA)	2.4	[67]
TrueOne 2400, (ParvoMedics, Sandy, Utah, USA)	2.4	[66]
Ergocard,(Medisoft, Dinant, Belgium)	2.4	[74]
Vyntus CPX (Jaeger, Würzburg, Germany)	2.4	[83]
AE-310S (Minato Medical Science, Osaka, Japan)	2.4	[42]
Douglas bags and Servomex 1400 (Taylor Instrument Analytics, Sussex, United Kingdom)	2.4	[65]

Not recorded	2.4	[79]
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Table 2a. Stoichiometric equations used to estimate fat and carbohydrate oxidation across the included studies.

Stoichiometric Equations	% of Included Studies	Studies
Frayn, 1983 [1] $FAT_{Ox} = 1.67 \times \dot{V}O_2 - 1.67 \times \dot{V}CO_2$ $CHO_{Ox} = 4.55 \times \dot{V}CO_2 - 3.21 \times \dot{V}O_2$	50.0	[2-22] [23, 24]*
Jeukendrup & Wallis, 2005 [25] $FAT_{Ox} = 1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2 - 1.77$ CHO_{Ox} 0-40% = $4.585 \times \dot{V}CO_2 - 3.226 \times \dot{V}O_2$ 40-50% = $4.344 \times \dot{V}CO_2 - 3.061 \times \dot{V}O_2$ 50-75% = $4.210 \times \dot{V}CO_2 - 2.962 \times \dot{V}O_2$	23.8	[26-35]
Peronnet & Massicotte, 1991 [36] $FAT_{Ox} = 1.695 \times \dot{V}O_2 - 1.701 \times \dot{V}CO_2$ $CHO_{Ox} = 4.585 \times \dot{V}CO_2 - 3.226 \times \dot{V}O_2$	4.8	[37, 38]
Elia & Livesey, 1992 [39] $FAT\% = 1.0 - CHO\%$ $CHO\% = (5.045 \times RQ - 3.582) / (0.36 \times RQ + 1.103)$	2.4	[40]
Brouwer, 1957 [41] $FAT_{Ox} = 1.718 \times \dot{V}O_2 - 1.718 \times \dot{V}O_2 - 0.315 \times p$ (g/min) Ferranini, 1988 [42] CHO_{Ox} : Glucose = $4.55 \times \dot{V}CO_2 - 3.21 \times \dot{V}O_2$ (low-medium) Glycogen = $4.09 \times \dot{V}CO_2 - 2.88 \times \dot{V}O_2$ (medium-high)	2.4	[43]
Mader's approach (see Mader [44], Mader [45])	2.4	[46]
Calories, CHO_{Ox} and FAT_{Ox} were calculated from the steady-state $\dot{V}O_2$ adjusted for substrate metabolism using the RER value.	2.4	[47]

No details	11.9	[23, 24, 48-50]
<p>Table notes: CHO_{ox} = carbohydrate oxidation; FAT_{ox} = fat oxidation; g = grams; min = minute; p = protein; RER = respiratory exchange ratio; RQ = respiratory quotient; $\dot{V}CO_2$ = volume of CO₂ expired; $\dot{V}O_2$ = oxygen consumption.</p> <p>*[23, 24] cited Consolazio, 1963 [51] and MacRae, Noakes & Dennis, 1995 [52] however, no equations were provided and it seems as though the equations are identical to Frayn, 1983 [1] but this remains unclear.</p>		

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Table 2b. Methods used to estimate MFO and Fat_{max} across the included studies.

Method of estimation for MFO and Fat _{max}	% of Included Studies	Studies
Measured values.	57.1	[2, 4, 6, 7, 9, 11-14, 16, 17, 20-22, 26-33, 40, 47]
Third-degree polynomial regression.	11.9	[3, 10, 19, 34, 35]
Second-degree polynomial regression.	7.1	[8, 15, 43]
Smoothing of the PO vs fat oxidation rate curve.	4.8	[37, 38]
Sine model [53].	4.8	[18, 23]
Two different linear relationships: 1-RQ and $\dot{V}O_2$ vs PO were derived to find the point where the value of the derived equation equals zero.	2.4	[24]
Mader's approach (see Mader [44], Mader [45])	2.4	[46]
Highest point of the binomial parabola formed by the fat expenditure-velocity data.	2.4	[50]
Visual inspection by two investigators.	2.4	[5]
No details.	4.8	[48, 49]
<i>Table notes:</i> Fat _{max} = exercise-intensity at which maximal fat oxidation occurs; MFO = maximal fat oxidation; PO = power output; RQ = respiratory quotient; $\dot{V}O_2$ = oxygen consumption.		

Table 2c. Definitions and methods used to estimate LT₁ across the included studies.

LT₁ definition and method of estimation	% of LT₁ Studies	Studies
First increase in lactate concentration above baseline (Hagberg and Coyle,1983) [54, 55].	14.3	[2, 6, 7, 13, 17, 38]
Log-log method : The lactate curve was divided into two segments and the intersection point of the two lines with the lowest residuals sum of squares was taken as the LT ₁ [56].	7.1	[15, 22, 34]
Highest exercise-intensity before onset blood lactate concentration (Davis et al., 1976) [57].	4.8	[8, 43]
Calculated using a computer program with the lactate concentration vs workload curve (Stegmann et al., 1981) [58].	2.4	[31]
The intersection of blood lactate vs $\dot{V}O_2$ (ml/kg/min) for both the lower and higher intensities.	2.4	[4]
2 mmol/L calculated using a polynomial equation (blood lactate concentration vs % $\dot{V}O_2$ peak).	2.4	[28]
2 mmol/L and 4 mmol/L calculated using least squares (lactate concentration vs power output).	2.4	[24]
Break point in blood lactate concentration vs power output, representing a departure of blood lactate concentration from a linear baseline, using an R script (Lactate-R) [59].	2.4	[26]
Minimum blood lactate concentration measured during the test + 1.5 mmol/L [60]	2.4	[9]
The highest power output and $\dot{V}O_2$ obtained just prior to the curvilinear increase in blood lactate.	2.4	[14]
Mader's approach (see Mader [44], Mader [45])	2.4	[46]
Visual inspection	2.4	[35]
<i>Table notes:</i> kg = kilogram; L = litre; LT ₁ = first lactate threshold; min = minute; ml = millilitre; $\dot{V}O_2$ = oxygen consumption.		

Table 2d. Definitions and methods used to estimate GET across the included studies.

GET definition and method of estimation	% of GET Studies	Studies
The first maintained rise in $VE/\dot{V}O_2$ while $VE/\dot{V}CO_2$ remained at a plateau (Meyer et al., 2005) [61].	11.9	[23, 29, 37, 40, 50]
Ventilatory equivalent method described by Wasserman et al., 1990 [62].	9.5	[21, 34, 48, 49]
V-slope method (as described by Beaver et al. 1986) [56].	7.1	[12, 18, 47]
The increase in $VE/\dot{V}O_2$ with the absence of changes in the $VE/\dot{V}CO_2$ (Lucía et al., 2000) [63].	4.8	[3, 33]
Manually identified as the point at which the plot $\dot{V}O_2$ vs. VE deviated from linearity (Skinner & McLellan, 1980; McLellan & Skinner, 1982) [64, 65].	4.8	[10, 16]
The increase in $VE/\dot{V}O_2$ and PET_{O_2} with the absence of changes in the $VE/\dot{V}CO_2$.	4.8	[11, 38]
the power output where a systematic increase in $VE/\dot{V}O_2$ appeared N. A. Jamnick, R. W. Pettitt, C. Granata, D. B. Pyne, and D. J. Bishop,	2.4	[35]
A significant non-linear increase of VE and $VE/\dot{V}O_2$ determined by two independent and experienced investigators (Wasserman et al., 1973) [66].	2.4	[47]
Disproportionate increase in expired CO_2 relative to $\dot{V}O_2$ and the $VE/\dot{V}O_2$ and $VE/\dot{V}CO_2$ (Wasserman et al., 2005)[67].	2.4	[5]
$VE/\dot{V}O_2$ vs $\dot{V}O_2$ was plotted to identify the point during exercise where this curve has minimum value (Hollmann, 2001) [68].	2.4	[27]
When the RER rapidly exceeded more than 1.00 and was detectable by breaking the linearity of $\dot{V}CO_2$ vs $\dot{V}O_2$ curve by the V-Slope method (Schneider et al., 1993) [69].	2.4	[32]
Plotted VE and FeO_2 vs relative $\dot{V}O_2$ to identify VT_1 , by identifying the work rate or $\dot{V}O_2$ just before the appearance of a nonlinear increase in "IC" in combination with a rapid increase in FeO_2 and RER. Visual inspection by two investigators.	2.4	[4]
The individual anaerobic threshold was determined using a modified v-slope method.	2.4	[30]
No definition or method of estimation provided	2.4	[19]
Table notes: CO_2 = carbon dioxide; FeO_2 = fraction of expired oxygen; GET = gas exchange threshold; RER = respiratory exchange ratio; $\dot{V}CO_2$ = volume of CO_2 expired; VE = minute ventilation; $VE/\dot{V}CO_2$ = ventilatory equivalent for carbon dioxide; $VE/\dot{V}O_2$ = ventilatory equivalent for oxygen; $\dot{V}O_2$ = oxygen consumption.		

Table 3. A summary of the protocol characteristic moderator analysis for the multi-level meta-analysis of the markers of LT/GET and Fat_{max}.

Moderators	Effects (k)	#ES	Overall ES	95% CI	σ^2 Level 12	σ^2 Level 13	$F(df_1, df_2)$	P
Participant characteristics								
Sex	Male Female Not recorded Combined	103 32 20 3	0.855 0.979 -0.612 -	0.413 – 1.297** * 0.493 – 1.464** * -2.171 – 0.947 -	1.55 2	0.24 6	$F(3, 37) = 5.786$	0.002**
Age	< 20 years 20 – 29 years ≥ 30 years	36 72 50	0.206 0.943 0.663	-0.372 – 0.7833 0.289 – 1.597** 0.296 – 1.030** *	1.60 0	0.24 2	$F(3, 39) = 5.508$	0.003**
Fitness	Elite Trained Active Sedentary	85 43 17 13	0.720 0.600 0.835 0.886	0.299 – 1.141** 0.069 – 1.131* 0.258 – 1.412** -0.357 – 2.129	1.66 3	0.24 5	$F(4, 38) = 4.480$	0.005**
Menstrual cycle	Controlled Uncontrolled Unclear N/A	11 13 11 123	1.370 0.326 1.099 -	-0.003 – 2.743 -0.771 – 1.423 0.411 – 1.787** -	0.92 2	0.23 5	$F(3, 13) = 5.657$	0.011*
Oral contraception	Excluded Unclear	12 22	0.944 0.815	-0.189 – 2.078	0.55 9	0.22 0	$F(2, 14) = 7.979$	0.005**

	Included N/A	1 123	- -	0.326 – 1.305** - -				
Participant acute nutritional status								
Fasting period	< 6 hours 7-12 Unclear	52 63 43	0.374 1.069 0.538	-0.273 – 1.021 0.496 – 1.641** * -0.601 – 1.676	1.63 1	0.23 9	$F(3, 39) = 5.503$	0.003**
Regular diet	No Yes Unclear	68 38 52	1.083 0.214 1.219	0.376 – 1.791** -0.476 – 0.904 0.570 – 1.868** *	1.47 2	0.24 0	$F(3, 39) = 8.141$	0.0002** *
Carbohydrate rich	No Yes Unclear	16 71 71	0.348 0.765 0.913	-0.259 – 0.954 -0.216 – 1.746 0.317 – 1.509**	1.66 3	0.24 0	$F(3, 39) = 4.480$	0.009**
Standardised meals	No Yes	131 27	0.741 0.745	0.286 – 1.196** -0.445 – 1.935	1.66 6	0.24 0	$F(2, 40) = 6.209$	0.005**
Food recall	No Yes	127 31	0.763 0.670	0.312 – 1.214** -0.412 – 1.752	1.66 3	0.24 0	$F(2, 40) = 6.619$	0.003**
Study design								
Measure	GET/VT LT	80 78	0.905 0.524	0.384 – 1.427** * 0.020 – 1.029*	1.67 6	0.23 5	$F(2, 40) = 6.455$	0.004**

Units	$\dot{V}O_2$ PO HR Mmol/L Km/h SmO ₂ %	82 37 29 8 1 1	0.747 0.750 0.769 - - -	0.309 – 1.186** 0.257 – 1.243** 0.280 – 1.258** - - -	1.63 1	0.18 4	$F(3, 39) = 4.112$	0.013*
Protocol characteristics and equipment								
Mode	Cycle ergometer Treadmill	135 23	0.748 0.724	0.238 – 1.258** -0.061 – 1.509	1.66 6	0.24 0	$F(2, 40) = 6.128$	0.005**
Fat_{max} test	Combined Additional	123 35	0.683 0.841	0.175 – 1.190** 0.078 – 1.604*	1.66 6	0.24 0	$F(2, 40) = 6.177$	0.005**
Constant increments	Yes No N/A	85 38 35	0.693 0.654 -	0.183 – 1.203** -0.514 – 1.822 -	1.46 8	0.25 5	$F(2, 24) = 4.602$	0.020*
$\dot{V}O_{2max}$ test protocol	Short Long	126 32	0.869 -0.543	0.404 – 1.333** * -1.468 – 0.383	1.99 4	0.07 8	$F(2, 40) = 11.134$	0.0001** *
Fat_{max} test protocol	Short Long	103 55	1.241 -0.108	0.690 – 1.792** * -0.824 – 0.609	2.20 3	0.07 7	$F(2, 40) = 11.314$	0.0001** *
Indirect calorimetry	Breath by breath Portable Spirometry Mixing chamber Multiple Unclear	90 30 21 8 6 3	0.843 -0.468 1.694 0.348 1.149 -	0.283 – 1.403** -1.720 – 0.784 0.354 – 3.034*	1.54 3	0.24 2	$F(5, 36) = 4.903$	0.002**

				-0.628 – 1.323 0.299 – 1.999** -				
Analysis methods								
Stoichiometric equations	Frayn, 1983 Jeukendrup & Wallis, 2005 Alternative equations Unclear	84 26 19 29	0.945 0.998 0.403 -0.483	0.484 – 1.407** * -0.100 – 2.096 -0.892 – 1.697 -1.728 – 0.763	1.55 4	0.23 9	$F(4, 38) = 5.398$	0.002**
Fat_{max} method	Measured values Polynomial Sine model Other mathematical method Unclear Visual	80 35 8 9 25 1	0.824 1.109 0.708 0.072 0.103 -	0.207 – 1.440* 0.555 – 1.664** * -0.093 – 1.510 -1.985 – 2.128 -0.580 – 0.786 -	1.78 6	0.23 9	$F(5, 36) = 5.422$	0.0008** *
LT₁ method	First increase above baseline Maximal intensity before rise Logarithmic/curve fitting Fixed lactate concentrations Intersection Visual N/A	25 24 18 8 2 1 80	0.624 0.461 0.720 2.001 - - -	-0.136 – 1.383 -0.377 – 1.299 0.471 – 0.970** * -0.557 – 4.559 - - -	0.77 8	0.40 1	$F(4, 13) = 11.565$	0.0003** *
GET method	Ventilatory equivalents V-slope	63 16 1	0.439 1.442 -	-0.366 – 1.244 -	1.94 6	0.07 3	$F(2, 23) = 8.255$	0.002**

	No method provided N/A	78	-	0.678 – 2.206** * - -				
<p>Table notes: # ES = number of effect sizes; CI = confidence intervals; df = degrees of freedom; GET = gas exchange threshold; HR = heart rate; LT₁ = first lactate threshold; N/A = not applicable; PO = power output; SmO₂ % = muscle oxygen saturation percentage; $\dot{V}O_2$ = oxygen consumption; $\dot{V}O_{2max}$ = maximal oxygen consumption; VT = ventilatory threshold; σ^2 Level 2 = variance between effect sizes; σ^2 Level 3 = variance between studies; *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$.</p>								

ACCEPTED

Figure 1

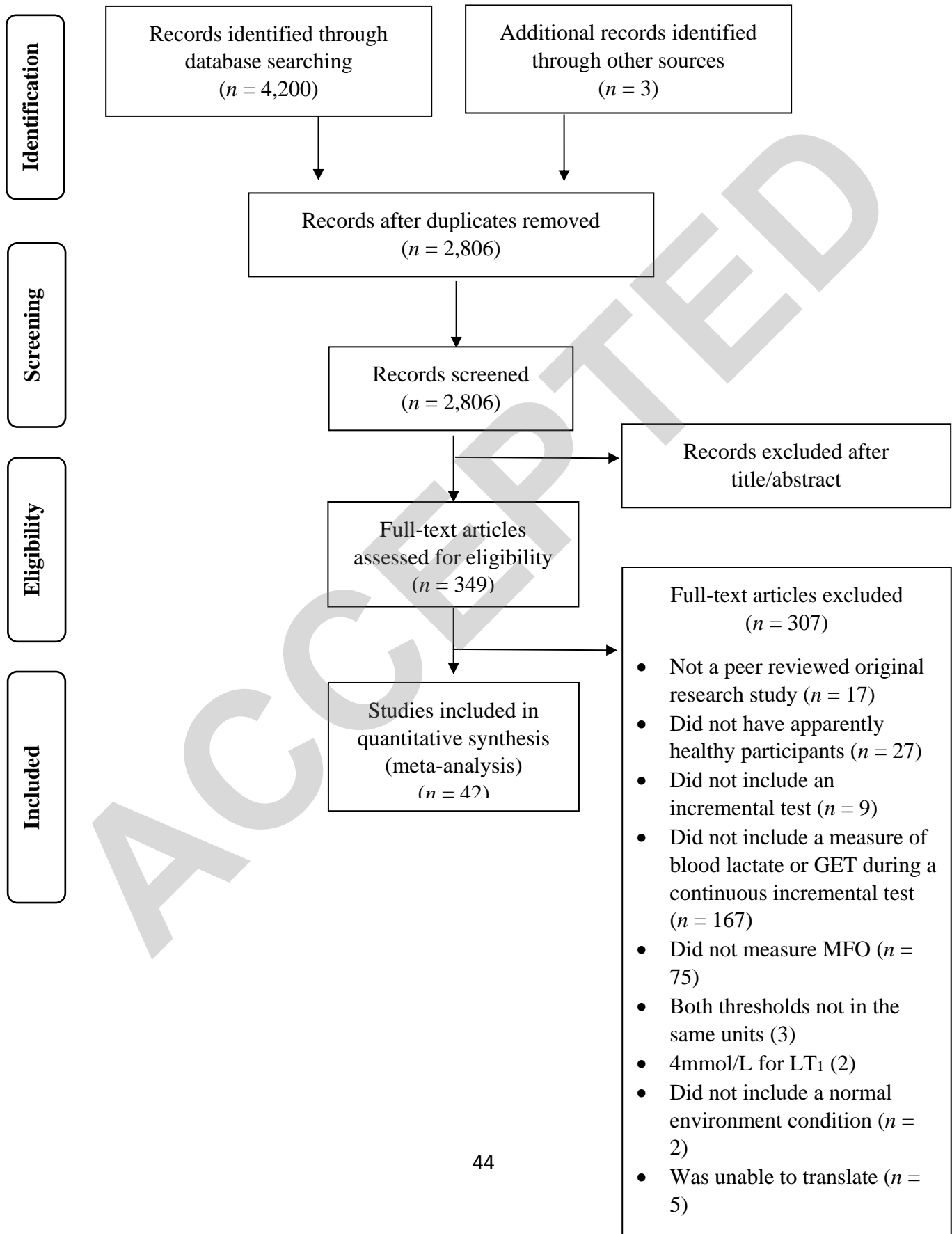


Figure 2

a.

		Risk of bias						
		D1	D2	D3	D4	D5	D6	Overall
Study	Amaro-Gahete 2019	+	+	-	+	+	+	+
	Benitez-Munoz 2025	-	+	+	+	+	+	+
	Bircher 2005	+	-	X	+	+	+	+
	Ghazzagh 2024	+	+	+	+	+	+	+
	Ghorbani 2014	+	-	-	+	+	+	+
	Gonzalez-Haro 2015	+	+	X	+	+	+	+
	He 2019	-	+	-	+	+	+	+
	Jimenez-Redondo 2024	+	+	-	+	+	+	+
	Michalik 2021	+	+	X	+	+	+	+
	Mohebbi 2015	+	+	X	+	+	+	+
	Nosaka 2020	+	+	+	+	+	+	+

D1: Bias arising from the randomisation process
 D2: Bias arising from period and carryover effects
 D3: Bias due to deviations from the intended interventions
 D4: Missing outcome data
 D5: Bias in measurement of the outcome
 D6: Bias in selection of the reported result

Judgement
 X High
 - Unclear
 + Low

b.

		Risk of bias												
		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	Overall
Study	Barker 2014	+	+	X	?	?	+	+	+	?	+	+	○	-
	Mcswiney 2021	+	+	X	?	?	+	+	+	?	+	X	○	-
	Martinez Nogueira 2022	+	+	X	?	?	X	+	X	?	+	X	○	-

D1: Was the study question or objective clearly stated?
 D2: Were eligibility/selection criteria for the study population prespecified and clearly described?
 D3: Were the participants representative of those who would be eligible for the intervention in the general or clinical population of interest?
 D4: Were all eligible participants that met the prespecified entry criteria enrolled?
 D5: Was the sample size sufficiently large to provide confidence in the findings?
 D6: Was the test/service/intervention clearly described and delivered consistently across the study population?
 D7: Were the outcome measures prespecified, clearly defined, valid, reliable, and assessed consistently across all study participants?
 D8: Were the people assessing the outcomes blinded to the participants' exposures/interventions?
 D9: Was the loss to follow-up after baseline 20% or less? Were those lost to follow-up accounted for in the analysis?
 D10: Did statistical methods examine changes in outcome measures and provide p values from pre-to-post?
 D11: Were outcome measures of interest taken multiple times before the intervention and multiple times after the intervention?
 D12: If the intervention was conducted at a group level did the statistical analysis take into account the use of individual-level data to determine effects at the group level?

Judgement
 X High
 - Moderate
 + Low
 ? No information
 ○ Not applicable

c.

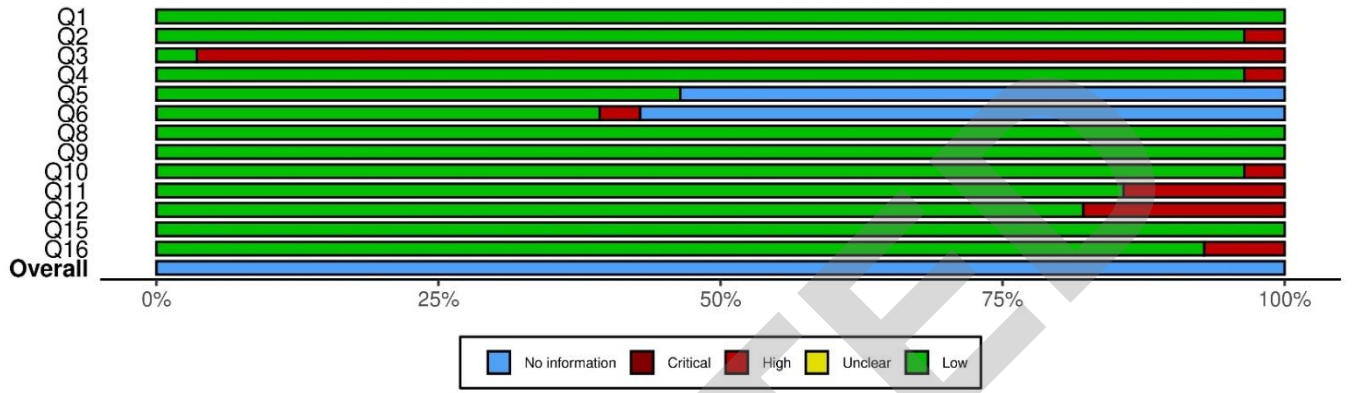
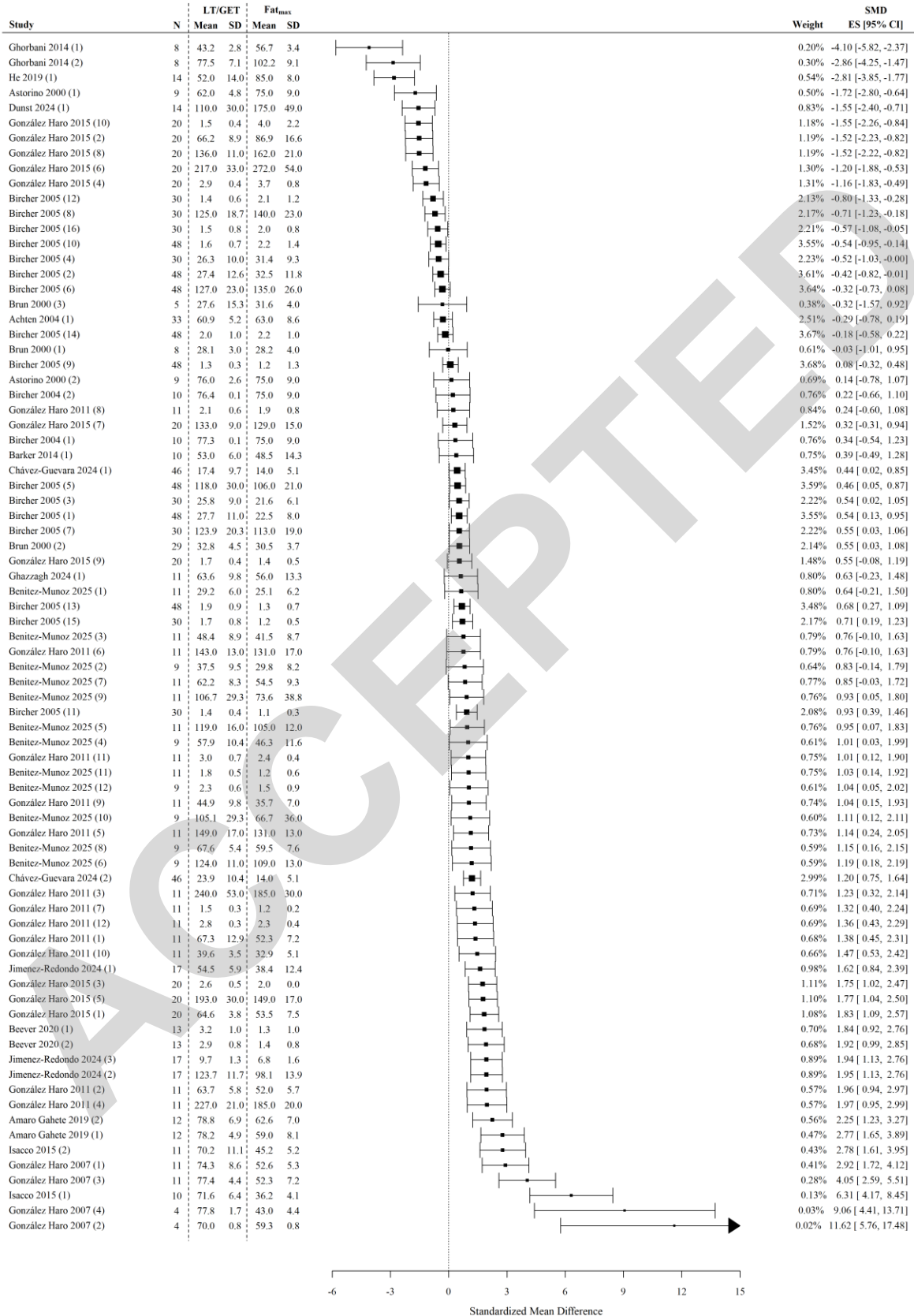


Figure 3



Study	N	LT/GET		Fat _{max}		Weight	SMD	
		Mean	SD	Mean	SD		ES [95% CI]	
Vasquez-Bonilla 2023 (7)	40	42.0	13.0	50.0	12.0	1.95%	-0.63	[-1.08, -0.18]
Martinez Noguera 2022 (4)	20	153.0	33.1	173.0	38.5	0.99%	-0.55	[-1.18, 0.09]
Martinez Noguera 2022 (1)	20	50.0	4.8	52.5	7.6	1.00%	-0.39	[-1.01, 0.24]
Martinez Noguera 2022 (3)	20	2.0	0.4	2.1	0.5	1.02%	-0.24	[-0.86, 0.38]
Martinez Noguera 2022 (2)	20	128.0	10.9	131.0	13.7	1.02%	-0.24	[-0.86, 0.38]
Peric 2018 (1)	57	46.0	10.2	47.5	10.6	2.91%	-0.15	[-0.51, 0.22]
Peric 2018 (2)	57	23.9	5.3	24.7	5.5	2.91%	-0.14	[-0.51, 0.22]
Michalik 2021 (2)	57	44.5	9.7	45.1	9.9	2.92%	-0.06	[-0.43, 0.31]
Marin Pagán 2021 (18)	15	181.3	22.0	181.6	37.6	0.77%	-0.01	[-0.73, 0.71]
Marin Pagán 2021 (21)	15	2.8	0.4	2.8	0.6	0.77%	0.00	[-0.72, 0.72]
Meucci 2021 (3)	29	115.0	11.0	115.0	11.0	1.48%	0.00	[-0.51, 0.51]
Kang 2007 (1)	11	60.4	7.9	60.1	10.9	0.56%	0.03	[-0.81, 0.86]
Meucci 2021 (1)	29	17.2	3.9	17.1	3.6	1.48%	0.03	[-0.49, 0.54]
Meucci 2021 (5)	29	1.1	0.3	1.1	0.3	1.48%	0.03	[-0.48, 0.54]
Marin Pagán 2021 (19)	24	2.5	0.3	2.5	0.6	1.23%	0.09	[-0.47, 0.66]
Michalik 2021 (1)	57	45.0	9.3	43.9	9.5	2.91%	0.12	[-0.25, 0.48]
Marin Pagán 2021 (15)	15	35.7	4.1	35.1	5.4	0.77%	0.12	[-0.59, 0.84]
Kang 2007 (2)	11	58.3	9.6	56.7	11.7	0.56%	0.15	[-0.69, 0.98]
Nikolovski 2021 (3)	22	112.0	19.0	109.0	15.0	1.12%	0.17	[-0.42, 0.76]
Marin Pagán 2021 (3)	15	52.2	5.2	51.2	5.4	0.76%	0.18	[-0.53, 0.90]
Nikolovski 2021 (1)	22	46.8	10.2	45.0	7.7	1.12%	0.19	[-0.41, 0.78]
Nikolovski 2021 (2)	22	22.1	4.8	21.3	3.6	1.12%	0.19	[-0.41, 0.78]
Marin Pagán 2021 (16)	24	152.7	20.8	147.7	30.1	1.22%	0.19	[-0.38, 0.76]
Rynders 2011 (2)	74	12.7	5.8	11.4	7.5	3.77%	0.19	[-0.13, 0.52]
Robinson 2016 (1)	57	61.0	12.0	58.0	17.0	2.90%	0.20	[-0.17, 0.57]
Meucci 2021 (6)	29	0.9	0.2	0.8	0.2	1.48%	0.21	[-0.30, 0.73]
Rynders 2011 (1)	74	15.4	5.7	14.0	7.3	3.77%	0.21	[-0.11, 0.54]
Nikolovski 2021 (4)	22	63.4	9.8	61.5	7.2	1.12%	0.21	[-0.38, 0.81]
Meucci 2021 (2)	29	17.1	4.3	16.2	4.0	1.48%	0.21	[-0.30, 0.73]
Meucci 2021 (4)	29	126.0	14.0	123.0	13.0	1.48%	0.22	[-0.30, 0.74]
Marin Pagán 2021 (6)	15	139.7	11.8	136.6	11.0	0.76%	0.26	[-0.45, 0.98]
Marin Pagán 2021 (12)	15	2.3	0.2	2.2	0.4	0.76%	0.31	[-0.41, 1.03]
Tolfrey 2010 (1)	8	41.0	6.0	39.0	6.0	0.40%	0.32	[-0.67, 1.30]
Marin Pagán 2021 (9)	15	72.6	5.4	70.9	4.3	0.76%	0.34	[-0.38, 1.06]
Tolfrey 2010 (3)	8	64.0	7.0	61.0	7.0	0.40%	0.41	[-0.58, 1.40]
Marin Pagán 2021 (20)	22	2.7	0.4	2.5	0.5	1.10%	0.45	[-0.15, 1.05]
Michallet 2008 (1)	14	1.6	0.4	1.4	0.4	0.70%	0.49	[-0.27, 1.24]
Marin Pagán 2021 (17)	22	179.2	22.3	163.9	35.1	1.09%	0.51	[-0.09, 1.11]
Michallet 2008 (2)	14	1.7	0.6	1.4	0.4	0.69%	0.57	[-0.18, 1.33]
Zurbuchen 2020 (4)	11	30.4	7.0	26.2	5.8	0.54%	0.63	[-0.23, 1.48]
Marin Pagán 2021 (10)	24	2.1	0.3	1.9	0.3	1.17%	0.66	[0.07, 1.24]
Martinez-Navarro 2022 (1)	19	71.8	6.1	64.9	10.7	0.90%	0.78	[0.12, 1.43]
Marin Pagán 2021 (1)	24	56.8	4.3	51.2	9.0	1.14%	0.78	[0.19, 1.37]
Marin Pagán 2021 (4)	24	149.5	12.2	137.9	16.1	1.14%	0.80	[0.21, 1.39]
Schwindling 2014 (1)	16	3.5	0.7	2.8	0.9	0.76%	0.82	[0.10, 1.54]
Marin Pagán 2021 (2)	22	54.9	5.4	48.9	8.5	1.04%	0.83	[0.21, 1.44]
Tolfrey 2010 (4)	11	62.0	4.0	56.0	9.0	0.52%	0.83	[-0.04, 1.70]
Marin Pagán 2021 (11)	22	2.4	0.3	2.1	0.4	1.04%	0.83	[0.22, 1.45]
Tolfrey 2010 (2)	11	37.0	7.0	32.0	4.0	0.52%	0.84	[-0.03, 1.72]
Marin Pagán 2021 (14)	22	37.0	5.1	32.2	5.8	1.03%	0.86	[0.25, 1.48]
Vasquez-Bonilla 2023 (4)	40	139.0	13.0	126.0	16.0	1.87%	0.88	[0.42, 1.34]
Vasquez-Bonilla 2023 (2)	40	200.0	41.0	162.0	43.0	1.86%	0.90	[0.44, 1.36]
Marin Pagán 2021 (7)	24	76.1	5.6	70.4	6.6	1.11%	0.92	[0.32, 1.51]
Marin Pagán 2021 (13)	24	35.4	3.2	31.7	4.6	1.11%	0.92	[0.32, 1.51]
Vasquez-Bonilla 2023 (1)	40	37.0	6.2	31.0	6.2	1.84%	0.96	[0.50, 1.42]
Zurbuchen 2020 (2)	11	61.5	7.7	53.2	8.3	0.50%	1.00	[0.11, 1.88]
Marin Pagán 2021 (8)	22	74.9	5.9	66.6	9.5	0.99%	1.03	[0.40, 1.66]
Marin Pagán 2021 (5)	22	149.7	13.2	131.8	19.2	0.99%	1.07	[0.44, 1.70]
Vasquez-Bonilla 2023 (3)	40	55.0	10.0	44.0	10.0	1.78%	1.09	[0.62, 1.56]
Vasquez-Bonilla 2023 (5)	40	67.0	7.0	58.0	8.0	1.74%	1.19	[0.71, 1.66]
Venables 2005 (2)	143	67.0	12.5	52.0	12.5	6.21%	1.19	[0.94, 1.45]
Johari 2017 (1)	8	51.3	7.3	40.1	9.5	0.34%	1.25	[0.18, 2.32]
Vasquez-Bonilla 2023 (6)	40	57.0	6.0	48.0	7.0	1.66%	1.37	[0.88, 1.85]
Ozdemir 2019 (1)	14	18.2	4.1	13.7	1.9	0.58%	1.39	[0.57, 2.22]
Martinez-Navarro 2022 (2)	13	72.1	4.4	63.4	7.3	0.53%	1.40	[0.54, 2.26]
Nosaka 2020 (1)	30	89.3	29.8	51.2	22.4	1.22%	1.43	[0.86, 1.99]
Venables 2005 (1)	157	63.0	12.5	45.0	12.5	6.39%	1.43	[1.19, 1.68]
Klaris 2024 (1)	12	278.0	28.0	236.0	26.0	0.48%	1.50	[0.59, 2.41]
Jimenez-Redondo 2024 (5)	17	3.5	0.7	2.4	0.7	0.67%	1.53	[0.77, 2.30]
Zurbuchen 2020 (3)	11	43.1	4.5	33.9	6.6	0.43%	1.57	[0.61, 2.52]
Waldman 2023 (1)	12	55.0	6.3	42.0	7.5	0.44%	1.81	[0.86, 2.76]
Waldman 2023 (2)	13	65.0	6.3	52.0	7.5	0.47%	1.82	[0.90, 2.73]
Jimenez-Redondo 2024 (4)	17	195.0	30.5	125.9	41.6	0.61%	1.85	[1.05, 2.65]
Zurbuchen 2020 (1)	11	66.4	4.7	52.1	9.3	0.39%	1.87	[0.87, 2.87]
Klaris 2024 (2)	12	285.0	22.0	236.0	26.0	0.41%	1.96	[0.99, 2.94]
McSwiney 2021 (1)	9	70.1	6.4	48.4	12.4	0.30%	2.09	[0.94, 3.24]
Mohebbi 2015 (1)	9	65.0	7.0	44.0	6.0	0.21%	3.07	[1.70, 4.43]
Ozdemir 2019 (2)	14	120.0	4.4	103.1	4.1	0.25%	3.83	[2.58, 5.08]
Mohebbi 2015 (2)	9	126.0	9.0	84.0	6.0	0.10%	5.23	[3.29, 7.17]

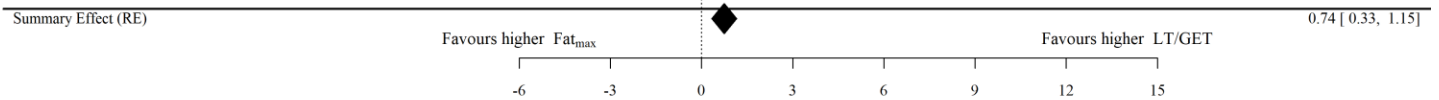


Figure notes: AT = Anaerobic Threshold; CI = confidence intervals; ES = effect size; Fat_{max} = exercise-intensity at which maximal fat oxidation occurs at; SD = standard deviation; SMD = standardised mean difference; Study name (number) = Numerous effect sizes per study are entered into the model which are accounted for in the nested hierarchical structure of the meta-analysis, see supplementary material 6 for specifics in the data sheet.

ACCEPTED