1 2	Carbohydrate ingestion during prolonged exercise and net skeletal muscle glycogen utilization: A meta-analysis
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Abstract

Background: While some studies report attenuated **net** muscle glycogenolysis with carbohydrate ingestion, others show no effect, possibly due to small sample sizes or methodological differences. Objective: To determine whether carbohydrate ingestion during endurance exercise reduces net skeletal muscle glycogen use, and to identify potential moderating factors.

Methods: A meta-analysis was conducted using data from 31 studies which included 48 unique effect sizes derived from crossover trials comparing carbohydrate vs. placebo ingestion during prolonged endurance exercise. Standardized mean differences (SMDs) in net muscle glycogen utilization were calculated. A multilevel random-effects model accounted for repeated estimates within studies. Subgroup and meta-regression analyses tested potential moderators. Sensitivity analyses were conducted using a range of plausible pre/post correlation values.

Results: Carbohydrate ingestion was associated with a small but statistically significant muscle glycogen-sparing effect (SMD = -0.16, 95% CI: -0.30 to -0.02, p = 0.021). Subgroup and moderator analyses revealed no significant effects of exercise mode, carbohydrate type, ingestion rate, or pre-exercise glycogen on the observed effect. Translating the standardized effect into absolute units, carbohydrate ingestion was estimated to spare \sim 24 mmol kg⁻¹ dry weight (95% CI: 4 to 45 mmol kg⁻¹) of muscle glycogen, relative to placebo, during \sim 100 min of exercise.

Conclusion: Carbohydrate ingestion during endurance exercise leads to a small but statistically significant reduction in net skeletal muscle glycogen utilization. Although no consistent moderating variables were identified, the direction of effect was consistent across studies, and the absolute magnitude of sparing may be physiologically meaningful during prolonged or repeated efforts.

Introduction

Carbohydrate ingestion during endurance exercise is a well-established strategy for enhancing performance (1–3). The mechanisms through which carbohydrate ingestion during endurance exercise improves performance are not completely understood. Skeletal muscle glycogen stores are limited and can be depleted to very low concentrations during prolonged, vigorous exercise (4–6), and this depletion has been mechanistically linked to fatigue (7–11). Similarly, the decline in blood glucose concentrations that can occur during prolonged exercise may also contribute to fatigue (12–15).

Alongside preservation of blood glucose concentrations, a plausible explanation for the performance benefit of carbohydrate ingestion is the ability to attenuate net skeletal muscle glycogenolysis by displacing endogenous carbohydrate use with oxidation of ingested carbohydrates. This is supported by evidence showing substantial reductions in endogenous carbohydrate oxidation when carbohydrates are ingested during exercise (16–20). However, while some studies have demonstrated a reduction in net skeletal muscle glycogen utilization with carbohydrate ingestion (21, 22), the majority have not (23–28). Various mechanisms have been posited for the discrepancies in the literature, including moderating effects of exercise modality and/or the specific muscle sampled, exercise intensity, the amount, type, and timing of carbohydrate ingestion, as well as pre-exercise nutritional and training status of study participants (29). However, many of these studies had relatively small sample sizes, potentially limiting their power to detect small-to-moderate effects. Thus, a meta-analytic approach may be better suited to evaluate whether carbohydrate ingestion during endurance exercise has a muscle glycogen-sparing effect.

Accordingly, the aim of this meta-analysis was to determine if carbohydrate ingestion during endurance exercise reduces net skeletal muscle utilization, thus preserving muscle glycogen

stores. We hypothesised that the existing literature would demonstrate a small magnitude skeletal muscle glycogen-sparing effect of carbohydrate ingestion during endurance exercise.

Methods

This systematic review with meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (30). The study protocol was pre-registered at the International Prospective Register of Systematic Reviews (PROSPERO; identification code: 627850). The protocol registration occurred after pilot searches but before any formal systematic searches were conducted.

Search strategy

The following Boolean search terms were used: "carbohydrate*" AND "muscle glycogen*" AND "run*" OR "cycl*" OR "bicycl*". The intervention term "carbohydrate*" was employed to capture studies that involved carbohydrate ingestion. The outcome term "muscle glycogen*" was employed to capture studies that quantified net muscle glycogen utilization, or provided data that could be used to quantify net muscle glycogen utilization. The population terms "run*", "cycl*", and "bicycle*" were used to capture studies that used running or cycling models. The search string was developed after through an optimisation process. Searches were performed in MEDLINE/EBSCO, PubMed, and Scopus.

Inclusion and exclusion criteria

Studies were included if they met the following inclusion criteria: (i) the study was published in English in a peer-reviewed journal, (ii) the study was performed in healthy, non-diabetic, non-obese humans, (iii) the study used a crossover design where exercise was performed with and without carbohydrate ingestion, (iv) the exercise protocol was >20 minutes, (v) the exercise performed was either cycling or running, (vi) the external work rate and exercise duration was controlled and fixed between the placebo and carbohydrate trials, (vii) net muscle glycogen utilization was quantified using either muscle biopsy or magnetic

resonance spectroscopy (studies measuring muscle glycogen oxidation using stable isotopes were excluded as glycogen synthesis is possible during exercise (31, 32), and stable isotope methods cannot accurately quantify oxidation in the first hour of exercise due to the slow turnover of the bicarbonate pool (33)), (viii) Muscle glycogen use was quantified from the start of the exercise protocol, (ix) muscle glycogen use was quantified in the vastus lateralis/quadriceps femoris or gastrocnemius, and (x) end-exercise muscle biopsy samples were obtained within 5 minutes of exercise cessation. Studies not meeting these criteria were excluded.

Text screening

Article titles and abstracts were independently screened by two researchers (HC, CS) using (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia). The full text of each paper was then assessed against the *a priori* inclusion and exclusion criteria by two researchers. Discrepancies were resolved by consensus with a third researcher (EM).

Quality of evidence assessment

Study quality was independently assessed by two researchers (HDR, JAR) and verified by a third researcher (EM). Specifically, methodological quality was assessed using the Cochrane Risk of Bias tool 2 for randomised clinical studies (34), with context-specific considerations for pre-intervention dietary control, adequacy of washout, clarity of carbohydrate drink composition, and internal consistency of reported glycogen outcomes (supplemental files).

Data extraction

The following data from studies satisfying the inclusion and exclusion criteria were extracted: sample size and participant age, sex, body mass, maximal oxygen consumption ($\dot{V}O_2$ max), exercise duration, exercise intensity (as continuous or interval, due to the homogeneity in protocols), pre-exercise carbohydrate ingestion (<4 h), carbohydrate ingestion rate during exercise, composition of ingested carbohydrates (as percent of total carbohydrate intake derived from glucose or glucose-containing compounds, e.g., glucose polymers, maltodextrin, sucrose), carbohydrate ingestion frequency, and mean and standard deviation of pre- and post-exercise glycogen content. Where numerical values were not provided in the text and only presented in figures, software (Digitizelt, Brunswick, Germany) was used for obtaining mean and standard deviation values.

Statistical analysis

Descriptive data are presented as mean ± standard deviation. All analysis was performed in RStudio (R version 4.3.1), using the esc and metafor packages. For each study, the between-condition standardized mean difference (SMD) in muscle glycogen utilization was calculated using group-level means, standard deviations, sample sizes, and the within-subject correlation between pre- and post-exercise measurements. Where this correlation was not available, a 0.5 correlation was assumed, based on the median of the 16 values calculated from the available raw data. Sensitivity analyses using a range of plausible correlation values (0.2–0.9) were conducted to evaluate the robustness of the results (35). No other imputation methods were used. The SMDs were calculated using the unbiased estimation method (vtype = "UB"), appropriate for paired-sample designs. Model parameters were estimated using restricted maximum likelihood, with effect sizes weighted by the inverse of their sampling variance.

A multilevel random-effects meta-analysis was conducted with a random intercept for study to account for the non-independence of multiple effect sizes within studies. Subgroup analyses were performed by exercise modality (run or cycle) and glycogen measurement

unit (dry weight or wet weight). Moderator analyses (i.e., meta-regressions) were used to assess whether study-level variables influenced the effect of carbohydrate ingestion on muscle glycogen utilization. The following variables were considered, with the number of effect sizes included for each: exercise intensity (continuous vs. interval exercise, n = 48), exercise duration (n = 48), participant $\dot{V}O_2$ max (n = 48), pre-exercise glycogen value (for studies reporting mmol kg^{-1} wet or dry weight, n = 45), glycogen measurement units (n = 48), carbohydrate ingestion type (n = 46), rate (n = 48), and frequency during exercise (n = 47). and carbohydrate ingestion within four hours prior to exercise (n = 48). For pre-exercise glycogen values, wet weight measures were converted to dry weight using a conversion factor of 4.35 (36), and the mean of the carbohydrate and placebo groups was calculated. Between-study heterogeneity was quantified using the estimated between-study variance (τ^2) (37), as well as I² (proportion of total variability due to heterogeneity), and H² (ratio of total to sampling variability), with Cochran's Q statistic and its associated p-value reported as a test for excess dispersion. The T² estimate was obtained using restricted maximum likelihood (REML), with additional sensitivity estimates from alternative estimators (DerSimonian-Laird, Sidik-Jonkman). While no universal benchmarks exist for interpreting τ², larger values reflect greater between-study heterogeneity and may indicate the presence of important moderators, methodological differences, or outliers. Standardized mean differences were interpreted as follows: small (0.20-0.49), moderate (0.50-0.79) and large (> 0.80) (38). Publication bias was assessed both visually, using funnel plots, and statistically using a multilevel Egger-type regression test, in which the standard error of the effect size was included as a predictor in a mixed-effects model (39). Statistical significance was defined as $P \le 0.05$.

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191	Results
192	A summary of the search process is shown in Figure 1.
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Study characteristics

***Insert Figure 1 around here

The 31 studies included 48 unique effect sizes (due to different study arms or measures at multiple time points, e.g., muscle glycogen measurements after 120 and 180 min of exercise), and 279 participants (273 male, 6 female, age 26.8 ± 6.0 years) (21–26, 28, 40–62). The number of effect sizes in cycling and running were 40 and 8, respectively. Exercise duration was 117.4 ± 51.6 min. Muscle glycogen was measured per unit dry weight (n = 22), wet weight (n = 23), protein (n = 1), and mmol/L (n = 2). All but one study extracted the muscle biopsy from the vastus lateralis, the remaining study reported sampling from the quadriceps femoris, but did not report the specific muscle (60). A summary of studies is shown in Figure 2.

***Insert Figure 2 around here

Methodological quality of the studies

Risk of bias was assessed using the ROB2 tool for crossover trials (34). Most studies were judged to have either low risk of bias (10 studies) or some concerns (19 studies), with only two studies rated as having an overall high risk of bias (Figure 3). The primary sources of bias related to randomization and blinding, which would not be expected to meaningfully influence the primary analysis, and lack of clarity regarding the carbohydrate composition of the test beverages.

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Net muscle glycogen utilization

A total of 48 effect sizes from crossover studies were included in the meta-analysis, comparing net muscle glycogen utilization between carbohydrate and placebo conditions during endurance exercise. The pooled SMD was -0.16 [95% CI: -0.30 to -0.02], p = 0.021, indicating a small but statistically significant muscle glycogen-sparing effect of carbohydrate ingestion (Figure 4). The direction of effect predominantly favoured carbohydrate ingestion, with most studies reporting numerically-lower glycogen depletion compared to placebo. However, individual effect sizes varied substantially, ranging from negative effects (e.g., -1.09) to positive effects (e.g., +0.93). Between-study heterogeneity was minimal across all model types, suggesting that the true effect size was relatively consistent across studies despite differences in protocols, measurement methods, and participant characteristics. In the multilevel model, the estimated between-study variance was $\tau^2 = 1.20 \times 10^{-10}$. A univariate fixed-effects model (assuming independence) produced a Cochran's Q(47) = 31.93, p = 0.95, with $I^2 = 0\%$ and $H^2 = 0.68$, indicating no excess dispersion beyond sampling variability. In the corresponding random-effects model (REML), the estimated T² was 0, with a 95% confidence interval of 0 to 0.018, and H² = 1.00, reflecting no betweenstudy heterogeneity. Alternative τ² estimators (e.g., Sidik-Jonkman) yielded slightly higher values ($\tau^2 = 0.079$), but all consistently indicated low heterogeneity. No evidence of smallstudy effects was detected (Egger test p = 0.989).

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Subgroup analyses revealed a statistically significant glycogen-sparing effect of carbohydrate ingestion during running trials (SMD = -0.38 [-0.74, -0.02], p = 0.036), with no observed between-study heterogeneity ($\tau^2 = 0$; $I^2 = 0\%$; Q(7) = 5.85, p = 0.557). In cycling

trials, no significant effect was observed (SMD = -0.12 [-0.28, 0.03], p = 0.107), and heterogeneity remained low (τ^2 = 0; I^2 = 0%; Q(39) = 24.4, p = 0.967). When stratified by glycogen measurement unit, there were now significant effects for dry weight measures (SMD = -0.20 [-0.41, -0.009], p = 0.060), with very low heterogeneity (τ^2 = 0.000007; I^2 = 0%; Q(21) = 18.4, p = 0.626) or wet weight measures (SMD = -0.10 [-0.30, 0.11], p = 0.347), with no between-study heterogeneity (τ^2 = 0; I^2 = 0%; Q(22) = 9.56, p = 0.990). Forest plots for sub-group analyses are shown in Supplemental Figures 1–4. These findings suggest that the glycogen-sparing effect of carbohydrate ingestion may be more detectable in running protocols, however, these null findings likely reflect limited statistical power within subgroups, increasing the risk of type II errors, rather than confirming a true absence of

moderation.

***Insert Figure 4 around here

Small-study effects were evaluated using a multilevel meta-regression with standard error (SE) as a predictor, an approach analogous to Egger's test adapted for multilevel models (39). The coefficient for SE was not statistically significant (β = 0.012, P = 0.989), indicating no evidence of small-study effects or funnel plot asymmetry (Figure 5).

***Insert Figure 5 around here

Moderation

None of the tested moderators (carbohydrate dose, ingestion frequency, exercise duration, exercise mode, starting glycogen value, or pre-exercise carbohydrate) were significantly associated with the effect size in univariable models (all P > 0.05). Therefore, a multivariable

267	model was not pursued. To illustrate the range of study characteristics, distributions of key
268	moderator variables are shown in Figure 6.
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270	***Insert Figure 6 around here
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272	Sensitivity Analysis
273	Sensitivity to imputed correlation coefficients was checked using values between 0.2 and
274	0.9. A significant effect was found for all correlation values, indicating an overall robustness
275	to imputed within-subject correlations (Figure 7).
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277	***Insert Figure 7 around here
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Discussion

The primary finding from this meta-analysis is that carbohydrate ingestion during exercise induces a small but statistically significant reduction in net skeletal muscle glycogen utilization. Subgroup and moderation analyses were conducted to explore whether study-level characteristics influenced this effect. Among subgroups, running-based studies showed a significant effect of carbohydrate ingestion, while cycling studies did not reach statistical significance. However, moderation testing indicated these differences were not statistically different. In line with this, none of the categorical or continuous moderators including exercise type or duration, carbohydrate intake rate, composition, or timing, or starting glycogen concentration were statistically significant in meta-regression models. These findings suggest that while a muscle glycogen-sparing effect is observed on average, no individual study-level factor consistently explains the variability in effect sizes across the included trials.

A meta-analysis by Areta and Hopkins (36) examined a broad range of studies reporting muscle glycogen at rest and during endurance exercise, regardless of whether carbohydrate was ingested. Among their subgroup analyses, they reported the effect of carbohydrate ingestion compared to no intake, finding a mean difference of -7 mmol/kg dry weight (90% CI: -23 to +9), which they classified as a trivial effect (36). While their analysis included a limited subset of studies with carbohydrate supplementation and yielded a wide confidence interval, the direction of effect is consistent with this analysis. Furthermore, they included a wider range of study protocols including non-crossover studies, studies using glucose infusion, and non-placebo-controlled interventions. Our findings, derived from a focused multilevel meta-analysis of placebo-controlled crossover studies, show a small but statistically significant reduction in net muscle glycogen utilization with carbohydrate ingestion during exercise (Figure 4).

Several physiological processes may help explain the observed muscle glycogen-sparing effect of carbohydrate ingestion during endurance exercise. Carbohydrate ingestion increases plasma glucose concentrations (13, 63, 64) and stimulates glucose transporter type 4 translocation to the plasma membrane in skeletal muscle (65). This increases skeletal muscle glucose uptake, and therefore plasma glucose oxidation rates (66). The data from our meta-analysis suggests that some of the additional plasma glucose oxidation that occurs when carbohydrate is ingested reduces the reliance on intramuscular glycogen to meet energy demands (Figure 4). The small size of the muscle glycogen-sparing effect that we report when carbohydrate is ingested during exercise reflects the simultaneous suppression of fatty acid oxidation (16–20, 67). Accordingly, assuming movement economy is unchanged with carbohydrate ingestion, the reduction in net muscle glycogen utilization must be smaller than the net increase in plasma glucose oxidation. Additionally, it is possible that increased muscle glycogen synthesis contributes to the small reduction in net muscle glycogen utilization during exercise with carbohydrate ingestion, as net increases in muscle glycogen content have been observed following low-intensity exercise with carbohydrate ingestion (31, 32). Together, these adaptations provide a mechanistic basis for how carbohydrate ingestion may preserve muscle glycogen stores.

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The reasons for the performance-enhancing effects of carbohydrate ingestion are not fully understood, but are believed to involve stimulation of the central nervous system through oral carbohydrate sensing, the provision of an additional fuel source for ATP production during exercise, improved TCA cycle flux, and its ability to reduce liver glycogen breakdown and maintain blood glucose concentrations (1, 15, 49, 68). Our analysis suggests that a small reduction in net muscle glycogen utilization should also be considered a plausible contributor to the commonly observed beneficial effects of carbohydrate ingestion during exercise on performance (2). Nearly all effect sizes (46 out of 48) included in our analysis were reported as non-significant (Figure 4). However, the average sample size across all

effect sizes was n = 8.8, which provides insufficient statistical power to detect small effects of carbohydrate ingestion on skeletal muscle glycogenolysis. Indeed, with a crossover design, a sample size of nine has 80% power to detect an effect size of 1.07, well above the effect sizes typically observed in this context. Furthermore, 309 participants would be needed to detect a significant difference between groups for our effect size of 0.16. This issue has recently been highlighted in the context of muscle metabolism research (69).

If the magnitude of the reduction in net muscle glycogen utilization with carbohydrate ingestion varies between subcellular compartments, the effect on performance could be larger or smaller than the overall effect on overall net muscle glycogen utilization observed here suggests (8). For example, intramyofibrillar glycogen appears to be associated with tetanic Ca²⁺ handling (9, 10), and is preferentially depleted during exercise (70, 71). Accordingly, if carbohydrate ingestion during exercise disproportionately preserves intramyofibrillar glycogen, it is possible that a meaningful effect on endurance performance would result. Investigation into the effect of carbohydrate ingestion during exercise on net utilization of specific subcellular muscle glycogen depots is therefore warranted.

To contextualize our overall effect size, we simulated an example scenario using studies that measured glycogen in mmol·kg□¹ dry weight. Assuming a starting concentration of 550 mmol·kg□¹ and exercise duration of 110 min (average of the included studies), the average decline in the placebo condition was 270 mmol·kg□¹, resulting in an end value of 280 mmol·kg□¹. In contrast, with carbohydrate ingestion, the average decline was 247 mmol·kg□¹, leading to an end value of 303 mmol·kg□¹. This reflects a muscle glycogen sparing effect of approximately 24 mmol·kg□¹ (95% CI: 4 to 45 mmol·kg□¹). While this difference is small, it could plausibly offer a meaningful advantage during prolonged or repeated efforts, particularly in light of suggestions that high-intensity performance may be

impaired when muscle glycogen drops below ~250-300 mmol·kg□¹ dry weight (72). However, the data presented here do not allow us to determine the practical significance of this effect.

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We also investigated potential moderating variables, including the type, amount, and frequency of carbohydrate ingestion, exercise type (i.e., interval vs. continuous), starting muscle glycogen concentration, and the unit of glycogen measurement, but none were statistically significant. In response to observations of professional cyclists routinely consuming carbohydrate at levels well above previous recommendations, recent studies have investigated the potential benefits of ingesting carbohydrate at rates as high as 120 g h ¹ (73, 74). Increased oxidation of exogenous carbohydrate has been reported with 120 g·h⁻¹ compared with 90 g h⁻¹, but endogenous carbohydrate oxidation rates were not different (73). In fact, glucose ingestion at rates that exceed intestinal transport capacity (90 vs. 60 g·h⁻¹) have also been reported to accelerate muscle glycogen oxidation (18). Likewise, no difference in glycogen use was observed when cyclists consumed either 45 or 90 g h-1 carbohydrate (47). Thus, any potential benefits of high-dose carbohydrate ingestion are unlikely related to further attenuations in net muscle glycogenolysis. However, it is important to note that very high carbohydrate intakes, such as those commonly consumed by professional cyclists (e.g., ≥100 g·h⁻¹), were not typically assessed in the included studies (Fig. 6A). Therefore, investigation of contemporary, optimised strategies such as individualized dosing on net muscle glycogen utilization, including in specific subcellular depots (8), are warranted (75). If these strategies do confer a performance advantage, it is possible that mechanisms beyond glycogen sparing are involved.

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Our finding of no effect of carbohydrate type aligns with previous studies showing no difference in glycogen breakdown between glucose and sucrose (49), or between various

combinations of maltodextrin, glucose, and fructose (25). The lack of moderating effect of exercise type is also consistent with multiple studies comparing continuous vs. variable intensity cycling which found no differences in total glycogen use, although fibre-type specific depletion patterns do occur (54, 76). It is well established that glycogen use during exercise is greater when starting from higher glycogen concentrations (77). Mechanistically, low initial muscle glycogen reduces substrate availability by limiting glycogen phosphorylase activity and indirectly downregulates carbohydrate oxidation via reduced activation of pyruvate dehydrogenase (78). Based on this, one could hypothesise that the carbohydrate ingestion-induced reduction in net muscle glycogen utilization is more evident with higher starting glycogen levels, due to higher overall rates of net muscle glycogen utilization (79). However, pre-exercise glycogen concentration was not a significant moderator in our analysis. Taken together, these results suggest that the glycogen-sparing effect of carbohydrate ingestion during exercise appears consistent across a range of protocols and conditions, without clear influence from specific study-level characteristics.

Effect size calculations for within-subject designs require estimates of the pre-post correlation. We were able to obtain participant-level data for the recent studies through author correspondence; however, many included studies were published 30–40 years ago, making data retrieval unfeasible. In such cases, a conservative correlation value of 0.5 is commonly used (80). We instead chose to use the median of the observed correlations from available datasets, which was also 0.50. Our results were robust across a range of imputed correlation values, but it is noteworthy that the observed pre-post correlations varied widely (Figure 7). This variation may reflect small differences in biopsy site, tissue handling and weighing, muscle fibre-type composition, and/or individual differences in substrate utilization (81–83).

Among subgroups, running-based studies showed a significant effect of carbohydrate ingestion, while cycling studies did not reach statistical significance. A key consideration is that all studies included in our analysis used muscle samples taken from the quadriceps and not the gastrocnemius. The gastrocnemius sees greater net muscle glycogen utilization during level-gradient running than the quadriceps (84). Therefore, it is possible that our exercise modality subgroup analysis was influenced by net glycogen utilization being assessed in primary versus secondary muscle groups. Nevertheless, moderation testing indicated these differences were not statistically different (p = 0.195), which is likely to mean sampling variability and lower statistical power in the smaller subgroups account for differences in the sub-group analysis, rather than true physiological differences. This is supported by a small (n = 7) individual study reporting no difference in the effect of carbohydrate ingestion on net muscle glycogen utilization between running and cycling (24). Muscle-specific investigation of the effects of carbohydrate ingestion during exercise on net muscle glycogen utilization are warranted.

Nearly all included studies involved young males. Consequently, the effect of carbohydrate ingestion during exercise on net muscle glycogen utilization in females cannot be ascertained. Consideration of biological sex in this context is important, given that sex differences in substrate metabolism are well-documented. For instance, Tarnopolsky et al. (85) found that males had ~25% greater muscle glycogen utilization than females during prolonged treadmill running, which they attributed to higher carbohydrate and protein oxidation rates. However, other studies have reported similar glycogen utilization between sexes (17, 86, 87), highlighting the complexity of sex-related responses. These findings underscore the need for research specific to females, and, more specifically, investigation into the effect of carbohydrate ingestion during exercise on net muscle glycogen utilization in females.

Conclusion

In summary, this meta-analysis provides evidence that carbohydrate ingestion during exercise induces a small but statistically significant reduction in net skeletal muscle glycogen utilization. While subgroup and moderator analyses did not identify any consistent study-level characteristics that significantly influenced the magnitude of this effect, the direction of results was generally consistent across protocols. Importantly, the estimated glycogen sparing effect, although small, may offer a meaningful physiological advantage during prolonged or repeated efforts, particularly when muscle glycogen stores approach critically low levels. These findings reinforce current carbohydrate intake guidelines for endurance performance and underscore the importance of contextualizing carbohydrate strategies within the demands of specific exercise tasks. Future research should prioritize diverse populations and investigate the effects of high-dose carbohydrate feeding protocols that more closely reflect contemporary elite athlete practices.

- Code Availability: Raw data and code use in this analysis is posted at
- 453 https://osf.io/vjwsu/

- **Supplemental materials**
- **Supplemental Figs. S1-S4: DOI 10.6084/m9.figshare.30483119.**
- **Supplemental Tables S1-S2: DOI 10.6084/m9.figshare.30483119.**

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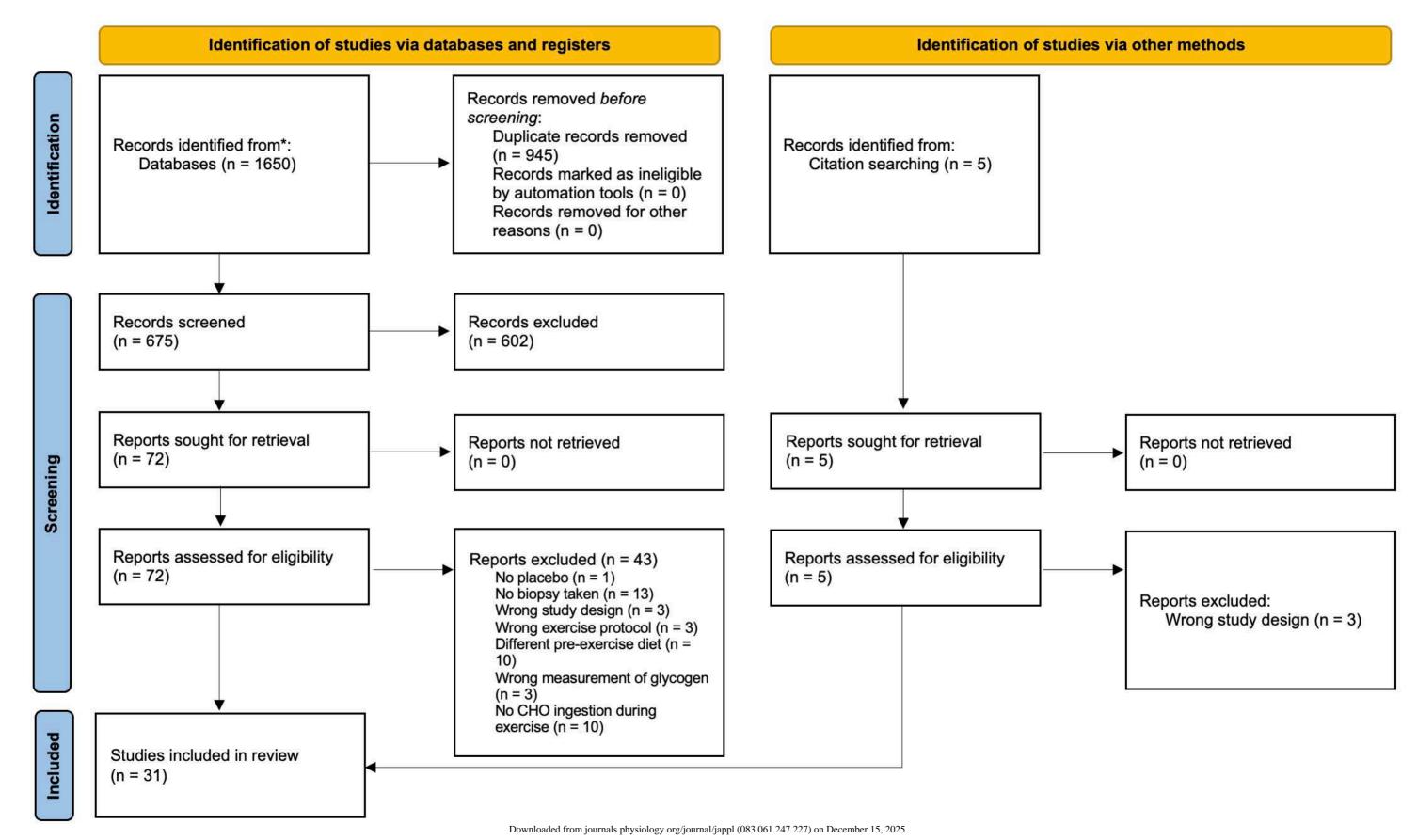
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747

749 Captions

- 750 **Figure 1**. Literature search flow chart. n = number of studies.
- 751 **Figure 2**. Study characteristics included in the meta-analysis. Protocol characteristics
- 752 include carbohydrate intake rate (CHO, g·h⁻¹), percent CHO from glucose or glucose-
- 753 containing compounds including glucose polymers, maltodextrin, sucrose (Glu %), ingestion
- frequency (Freq, bolus·h⁻¹), exercise mode (run or cycle), type of exercise (continuous vs.
- 755 Interval), and the units used for reporting muscle glycogen concentration. Exercise intensity
- 756 is shown as $\%VO_{2max}$, for studies prescribing intensity as percentage of VO_{2max} . Missing
- 757 values for glucose percentage indicate unclear reporting, missing values for ingestion
- 758 frequency indicates continuous sipping. The analysis includes 31 studies with 48 unique
- 759 effect sizes. Exercise modality included cycling (n = 23 studies), running (n = 7 studies), and
- 760 both cycling and running (n = 1 study).
- 761 **Figure 3.** Risk of bias assessment for all included studies.
- 762 Figure 4. Forest plot from multilevel meta-analysis showing standardized mean differences
- 763 (SMD) in muscle glycogen utilization between carbohydrate ingestion and placebo
- conditions during endurance exercise. Each line represents an individual study arm or time
- point, with squares sized by inverse variance weighting and horizontal lines indicating 95%
- 766 confidence intervals. Letters following study name correspond to studies with multiple
- 767 comparison arms or time points, defined in supplemental file. Negative values reflect
- 768 reduced glycogen utilization (i.e., glycogen sparing) with carbohydrate ingestion. The
- diamond at the bottom shows the overall random effects estimate from the multilevel model.
- 770 Between-study heterogeneity was negligible ($\tau^2 = 0$).
- 771 Figure 5. Funnel plot of standardized mean differences and standard errors. The plot shows
- 772 no clear asymmetry, and a multilevel meta-regression (Egger-type test) confirmed the
- absence of small-study effects (β = 0.012, P = 0.989). Note: Because multiple effect sizes
- per study were modelled using a multilevel approach, interpretation of the funnel plot should
- be made with caution.
- 776 Figure 6. Meta-regression plots examining the relationship between study-level variables
- 777 and standardized mean difference (SMD) in muscle glycogen utilization between
- 778 carbohydrate and placebo conditions. Negative SMD relates to a glycogen sparing effect.
- 779 Each point represents an effect size from an individual study arm, with point size scaled by
- 780 the inverse of its sampling variance (i.e., precision). For (G), values reflect the mean of the
- 781 pre-exercise carbohydrate and placebo groups. Blue lines represent simple linear
- 782 regressions for visualization only; moderator effects were formally tested using multilevel
- 783 meta-regression models accounting for study-level clustering and inverse-variance
- weighting. No significant moderators were identified (all P > 0.05).
- 785 **Figure 7**. Sensitivity of standardized effect size to imputed pre/post correlation values.
- 786 Standardized mean differences (SMD) in glycogen change between carbohydrate and
- 787 placebo conditions are shown across a range of imputed within-condition pre/post
- 788 correlations (r = 0.2 to 0.9). Diamonds represent the estimated effect sizes from a multilevel
- 789 random-effects meta-analysis, with horizontal bars indicating 95% confidence intervals. The
- 790 vertical dotted line at 0 represents the null effect. A violin plot on the left displays the
- 791 distribution of observed pre/post correlation values calculated from raw data (where
- 792 available), with individual values shown as points. The red diamond highlights the effect
- 793 estimate derived using the median of the observed correlations, which was used in the
- 794 primary analysis.



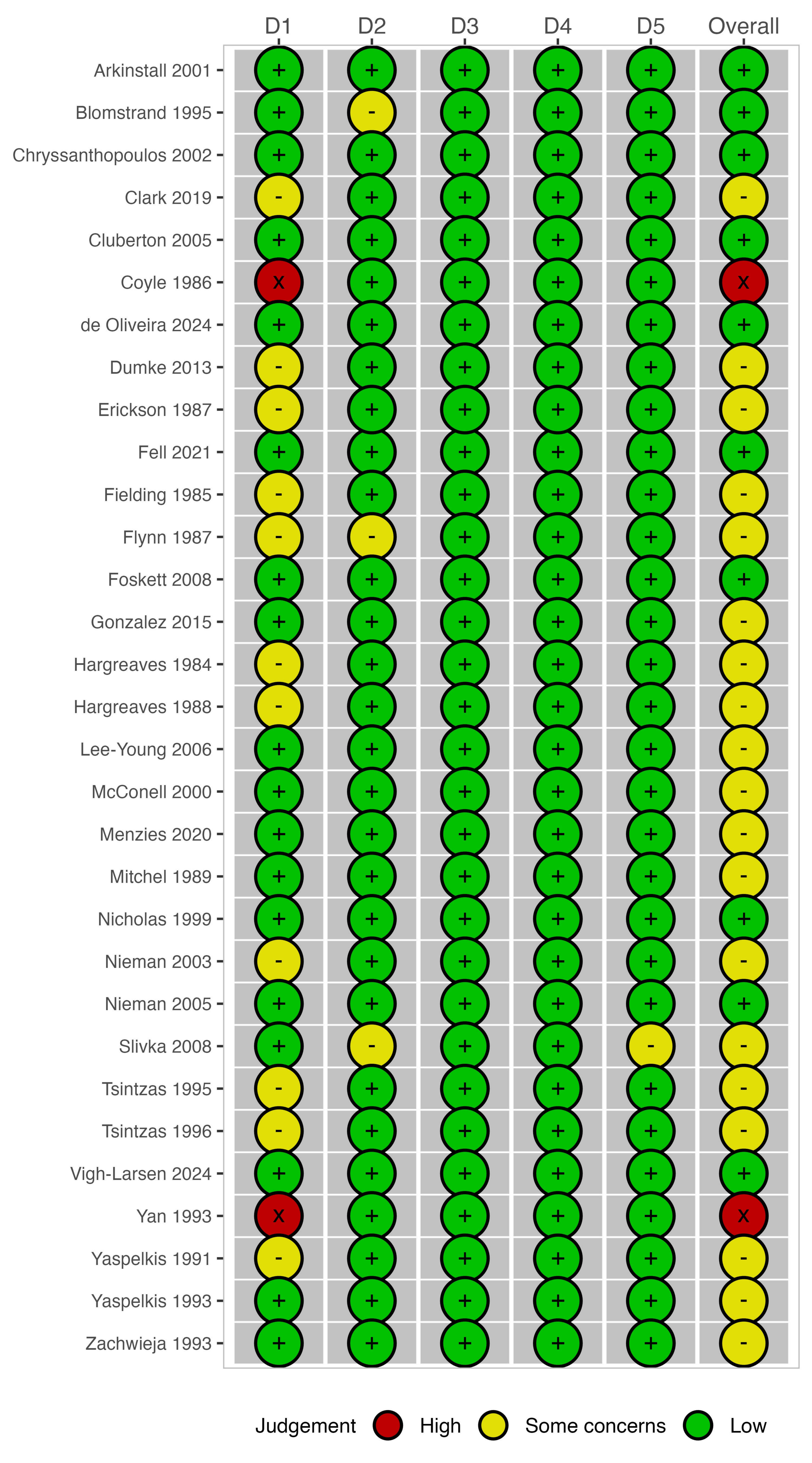
Study	Protocol					VO ₂ r	VO ₂ max (ml·kg ⁻¹ ·min ⁻¹)			Duration	(min)	Moderators		
		CHO (g·h	¹) Glu (%)	Freq (h	Intensity							Exercise mode	Туре	Glycogen units
Arkinstall et al., 2001 Cycle	7	64	100	3	69		52		60			Cycling	Continuous	dry wt
Arkinstall et al., 2001 Run	7	64	100	3	78		54		60			Running	Continuous	dry wt
Blomstrand et al., 1995	5	40		4	75			69	60			Cycling	Continuous	wet wt
Chryssanthopoulos et al., 2002	8	46	100	3	70		5	9	60			Running	Continuous	dry wt
Clark et al., 2019	16	60	56	4	65		52			120		Cycling	Continuous	dry wt
Cluberton et al., 2005	6	20	100		74		46		60			Cycling	Continuous	dry wt
Coyle et al., 1986 part 1	5	148	100	3	71			65		105		Cycling	Continuous	wet wt
Coyle et al., 1986 part 2	7	108	100	3	72			70			80	Cycling	Continuous	wet wt
de Oliveira et al., 2024	13	60	100	3			57			120		Cycling	Interval	dry wt
Dumke et al., 2013	8	37	100	2			53		60			Cycling	Interval	wet wt
Erickson et al., 1987	5	48	100	2.7	68			65	9			Cycling	Continuous	wet wt
Fell et al., 2021	8	45	88	2	64			60			80	Cycling	Continuous	dry wt
Fielding et al., 1985	9	22	50	2			50				24	Cycling	Interval	wet wt
Flynn et al., 1987	8	22	100	3				64		120		Cycling	Interval	wet wt
Foskett et al., 2008	6	90	100	4				60	9			Running	Interval	dry wt
Gonzalez et al., 2015	9	102	100	4				60			80	Cycling	Continuous	mmol/L
Hargreaves & Briggs 1988	5	60	100	2	70			68		120		Cycling	Continuous	wet wt
Hargreaves et al., 1984	10	43	50					62			24	Cycling	Interval	wet wt
Lee-Young et al., 2006	8	64	100	4	65		48			120		Cycling	Continuous	dry wt
McConell et al., 2000	6	81	100	4	83			66)	73)			Cycling	Continuous	dry wt
Menzies et al., 2020	6	60	50	12	77			61	75)			Running	Continuous	dry wt
Mitchell et al., 1989	10	74	71	4	70			63		105		Cycling	Continuous	wet wt
Nicholas et al., 1999	6	44	73	4			56			105		Running	Interval	dry wt
Nieman et al., 2003	16	60	54	4	70		45				80	Running	Continuous	dry wt
Nieman et al., 2005	15	74	54	4	74		54			150		Cycling	Continuous	wet wt
Slivka et al., 2008	9	60		2				60		120		Cycling	Continuous	wet wt

Continuous

dry wt

Running

Tsintzas et al., 1995



Domains:

D1: Bias due to randomisation

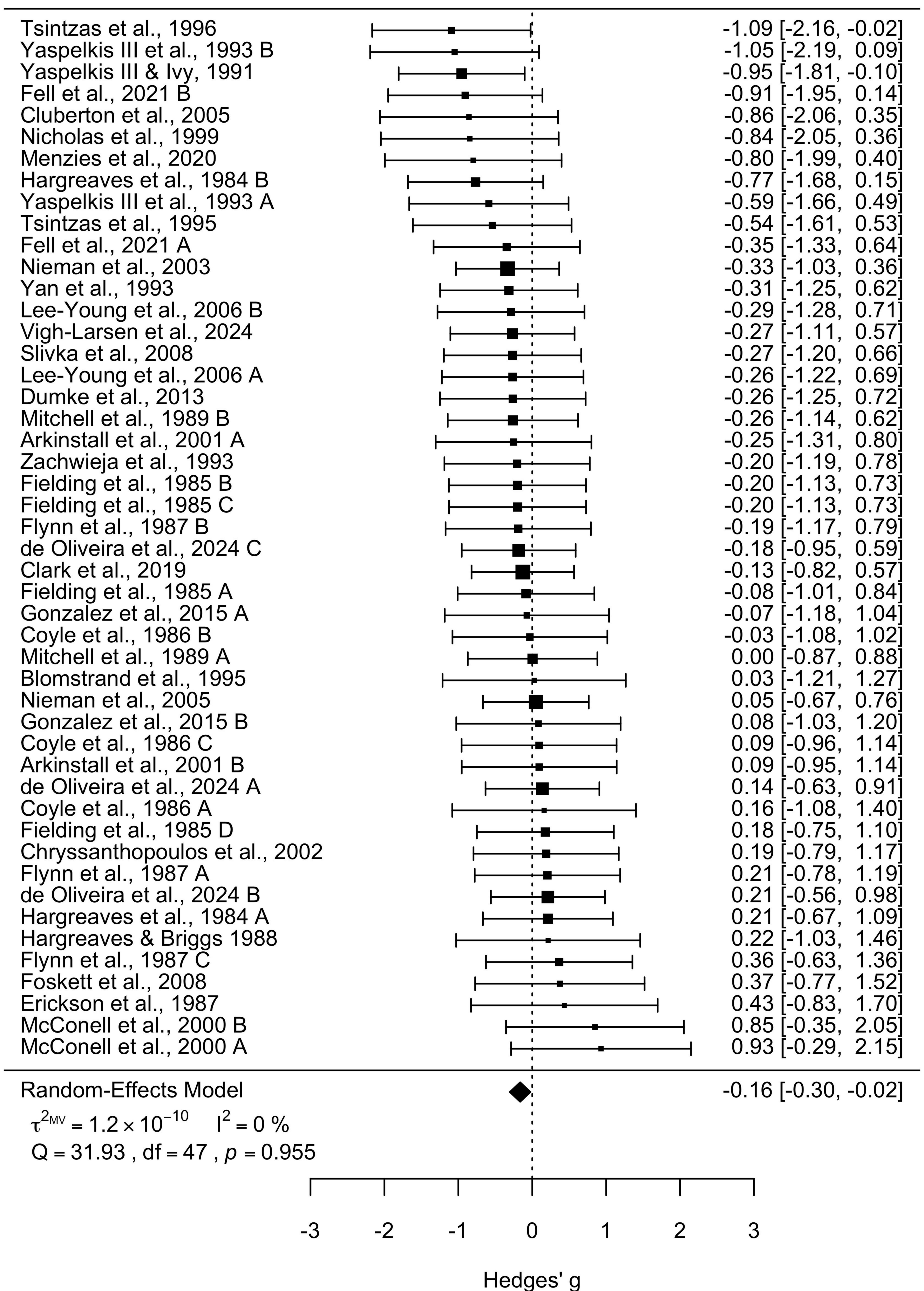
D2: Bias due to deviations from intended intervention

D3: Bias due to missing data

D4: Bias due to outcome measurement D5: Bias due to selection of reported result

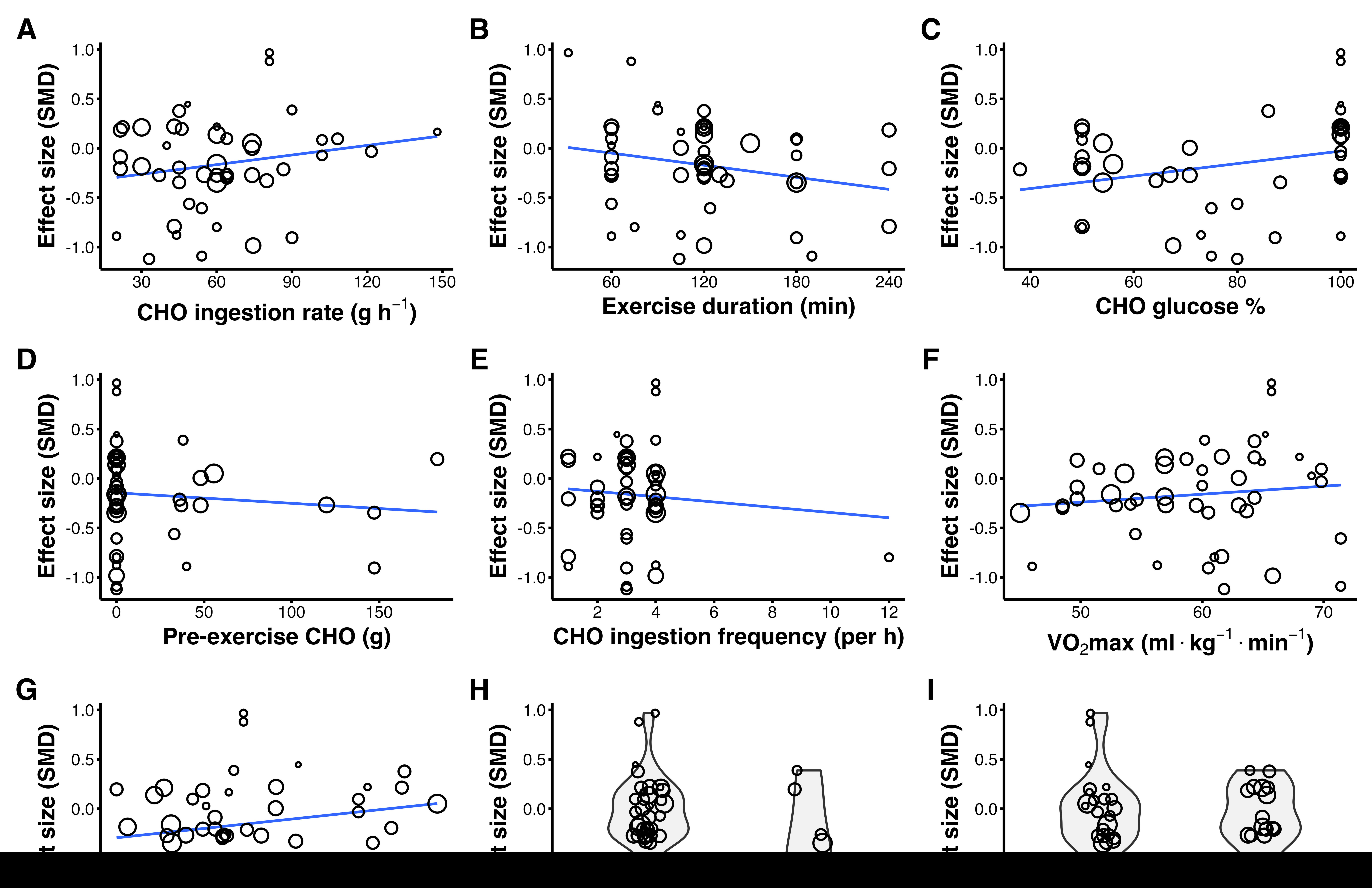
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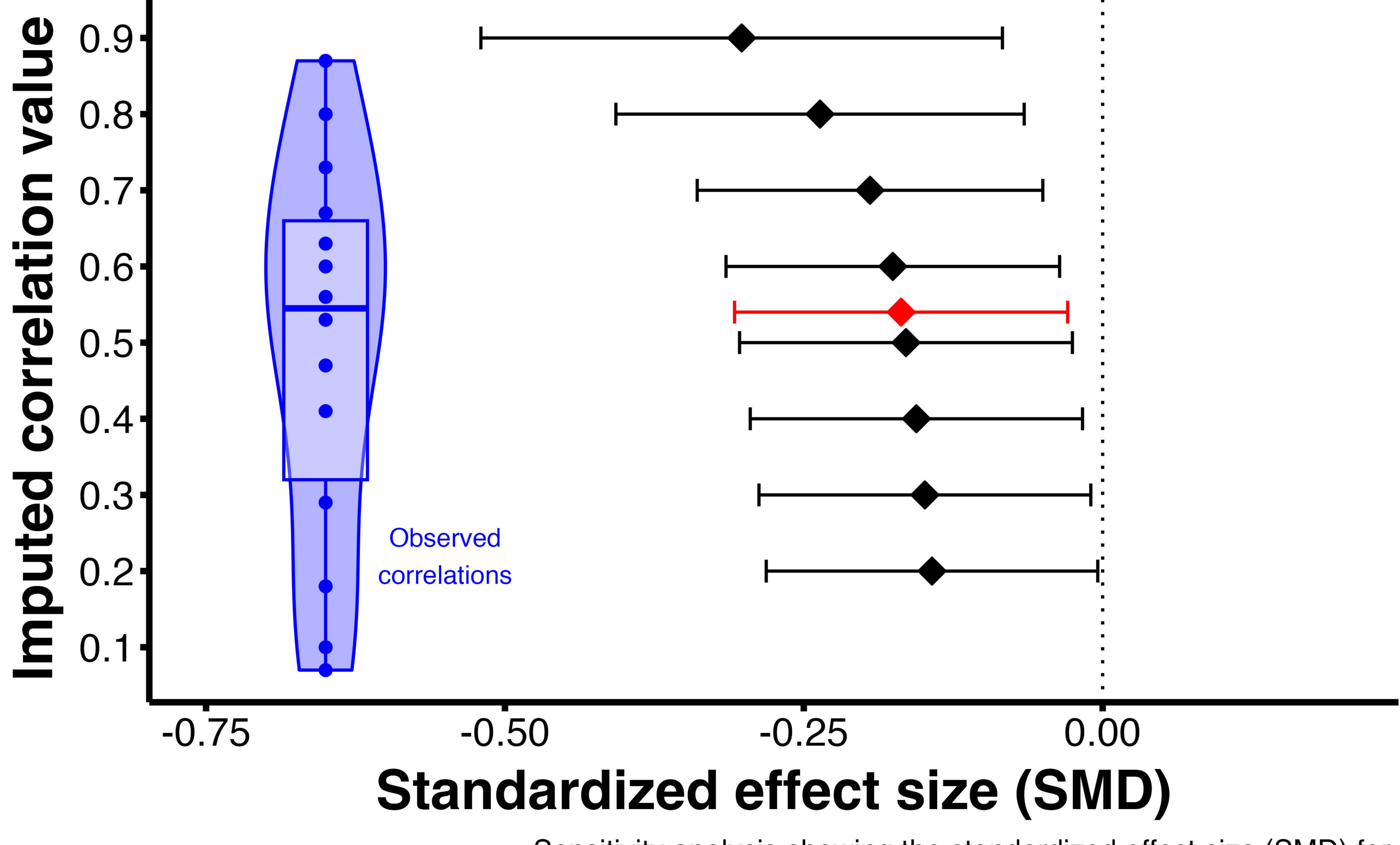
Study SMD [95% CI]



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Sensitivity analysis showing the standardized effect size (SMD) for glycogen change across a range of imputed pre/post correlations (r)

Carbohydrate ingestion during prolonged exercise and skeletal muscle glycogen utilization: A meta-analysis

METHODS



48 comparisons from 31 crossover studies where cycling or running was performed with and without carbohydrate ingestion.



279 participants (273 m, 6 f)



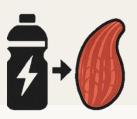
Exercise duration 117.4 ± 51.6 min



Muscle glycogen use quantified using muscle biopsy or magnetic resonance spectroscopy

RESULTS

- ✓ Carbohydrate ingestion had a muscle glycogen-sparing effect (SMD = −0.16, p = 0.021)
- ✓ Sub-group analysis showed a significant effect for running (p = 0.036) but not cycling (p = 0.107) trials
- ✓ No moderating effects of carbohydrate type, ingestion rate, or pre-exercise glycogen



CONCLUSION Carbohydrate ingestion during endurance exercise leads to a modest but statistically significant sparing of skeletal muscle glycogen. Although no consistent moderating variables were identified, the direction of effect was consistent across studies, and the absolute magnitude of sparing may be physiologically meaningful during prolonged or repeated efforts.