

# Skeletal Muscle Glycogen Content at Rest and During Endurance Exercise in Humans: A Meta-Analysis

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## Abstract

**Background** Skeletal muscle glycogen is an important energy source for muscle contraction and a key regulator of metabolic responses to exercise. Manipulation of muscle glycogen is therefore a strategy to improve performance in competitions and potentially adaptation to training. However, assessing muscle glycogen in the field is impractical, and there are no normative values for glycogen concentration at rest and during exercise.

**Objective** The objective of this study was to meta-analyse the effects of fitness, acute dietary carbohydrate (CHO) availability and other factors on muscle glycogen concentration at rest and during exercise of different durations and intensities.

**Data Source and Study Selection** PubMed was used to search for original articles in English published up until February 2018. Search terms included muscle glycogen and exercise, filtered for humans. The analysis incorporated 181 studies of continuous or intermittent cycling and running by healthy participants, with muscle glycogen at rest and during exercise determined by biochemical analysis of biopsies.

**Data Analysis** Resting muscle glycogen was determined with a meta-regression mixed model that included fixed

effects for fitness status [linear, as maximal oxygen uptake ( $\dot{V}O_{2max}$ ) in  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ] and CHO availability (three levels: high,  $\geq 6 \text{ g}\cdot\text{kg}^{-1}$  of CHO per day for  $\geq 3$  days or  $\geq 7 \text{ g}\cdot\text{kg}^{-1}$  CHO per day for  $\geq 2$  days; low, glycogen depletion and low-CHO diet; and normal, neither high nor low, or not specified in study). Muscle glycogen during exercise was determined with a meta-regression mixed model that included fixed effects for fitness status, resting glycogen [linear, in  $\text{mmol}\cdot\text{kg}^{-1}$  of dry mass (DM)], exercise duration (five levels, with means of 5, 23, 53 and 116 min, and time to fatigue), and exercise intensity (linear, as percentage of  $\dot{V}O_{2max}$ ); intensity, fitness and resting glycogen were interacted with duration, and there were also fixed effects for exercise modes, CHO ingestion, sex and muscle type. Random effects in both models accounted for between-study variance and within-study repeated measurement. Inferences about differences and changes in glycogen were based on acceptable uncertainty in standardised magnitudes, with thresholds for small, moderate, large and very large of 25, 75, 150 and  $250 \text{ mmol}\cdot\text{kg}^{-1}$  of DM, respectively.

**Results** The resting glycogen concentration in the vastus lateralis of males with normal CHO availability and  $\dot{V}O_{2max}$  (mean  $\pm$  standard deviation,  $53 \pm 8 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) was  $462 \pm 132 \text{ mmol}\cdot\text{kg}^{-1}$ . High CHO availability was associated with a moderate increase in resting glycogen ( $102, \pm 47 \text{ mmol}\cdot\text{kg}^{-1}$ ; mean  $\pm$  90% confidence limits), whereas low availability was associated with a very large decrease ( $-253, \pm 30 \text{ mmol}\cdot\text{kg}^{-1}$ ). An increase in  $\dot{V}O_{2max}$  of  $10 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  had small effects with low and normal CHO availability ( $29, \pm 44$  and  $67, \pm 15 \text{ mmol}\cdot\text{kg}^{-1}$ , respectively) and a moderate effect with high CHO availability ( $80, \pm 40 \text{ mmol}\cdot\text{kg}^{-1}$ ). There were small clear increases in females and the gastrocnemius muscle. Clear

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modifying effects on glycogen utilisation during exercise were as follows: a 30%  $\dot{V}O_{2\max}$  increase in intensity, small ( $41, \pm 20 \text{ mmol}\cdot\text{kg}^{-1}$ ) at 5 min and moderate ( $87\text{--}134 \text{ mmol}\cdot\text{kg}^{-1}$ ) at all other timepoints; an increase in baseline glycogen of  $200 \text{ mmol}\cdot\text{kg}^{-1}$ , small at 5–23 min ( $28\text{--}59 \text{ mmol}\cdot\text{kg}^{-1}$ ), moderate at 116 min ( $104, \pm 15 \text{ mmol}\cdot\text{kg}^{-1}$ ) and moderate at fatigue ( $143, \pm 33 \text{ mmol}\cdot\text{kg}^{-1}$ ); an increase in  $\dot{V}O_{2\max}$  of  $10 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ , mainly clear trivial effects; exercise mode (intermittent vs. continuous) and CHO ingestion, clear trivial effects. Small decreases in utilisation were observed in females (vs. males:  $-30, \pm 29 \text{ mmol}\cdot\text{kg}^{-1}$ ), gastrocnemius muscle (vs. vastus lateralis:  $-31, \pm 46 \text{ mmol}\cdot\text{kg}^{-1}$ ) and running (vs. cycling:  $-70, \pm 32 \text{ mmol}\cdot\text{kg}^{-1}$ ).

**Conclusion** Dietary CHO availability and fitness are important factors for resting muscle glycogen. Exercise intensity and baseline muscle glycogen are important factors determining glycogen use during exercise, especially with longer exercise duration. The meta-analysed effects may be useful normative values for prescription of endurance exercise.

### Key Points

Muscle glycogen concentration may modify endurance performance and muscle adaptive response to training, but assessment of muscle glycogen concentration in the field is impractical.

This meta-analysis of studies of human muscle biopsies provides normative values for glycogen at rest and during exercise that may be useful for prescription of endurance exercise.

Important factors affecting resting muscle glycogen are dietary carbohydrate availability and fitness, while those for glycogen use during exercise are resting muscle glycogen and exercise intensity.

It was then associated with the capacity of maintaining high work rates during the later stages of prolonged endurance competitions [6]. The early studies on exercise and muscle glycogen set the framework for the following decades of a research area that would grow through hundreds of scientific studies further characterising the relationships between muscle glycogen, diet, work capacity and performance. Subsequently, sports nutrition guidelines have evolved from advising carbohydrate (CHO) availability be maximised and glycogen stores be increased for competitions and all training sessions [7], to unravelling that training with low muscle glycogen may be beneficial to enhance the skeletal muscle response to endurance training [8] and ultimately performance [9]. Hence, our understanding of the role of skeletal muscle glycogen has expanded from energy storage for muscle contraction to also being a key intracellular ‘gauge’ with potential for modulating the molecular adaptive response towards an enhanced oxidative phenotype when its concentration is low [10–13]. Furthermore, it has recently been suggested that there is a glycogen threshold to promote training adaptation [14]. However, estimating glycogen content in the field is difficult.

The only current accurate non-invasive technique for assessment of muscle glycogen is magnetic resonance spectroscopy [15], which relies on large and expensive equipment and is not practical for use with the general population or athletes. The difficulties of measuring muscle glycogen with the biopsy technique have not represented a major limiting factor in allowing research in this area to flourish, but factors affecting muscle glycogen concentration at rest and during exercise remain poorly characterised. Some authors have assessed the specific effects of exercise duration [5, 16, 17], intensity [17–20] and baseline muscle glycogen [21, 22] as important factors for determining muscle glycogen utilisation during exercise. These apply only to specific experimental settings, and normative values have not been established. The main aim of this analysis was, therefore, to meta-analyse factors affecting the concentration of skeletal muscle glycogen and its dynamics in exercise.

## 1 Introduction

Skeletal muscle glycogen has been recognised as a key intracellular metabolite for exercising humans since the introduction of the Bergström biopsy needle in the 1960s [1] and biochemical analysis of muscle. First, it was determined to be utilised during exercise [2–4], responsive to dietary macronutrient composition and directly related to the capacity to perform physical exercise to exhaustion [5].

## 2 Methods

### 2.1 Criteria for Study Consideration

#### 2.1.1 Population

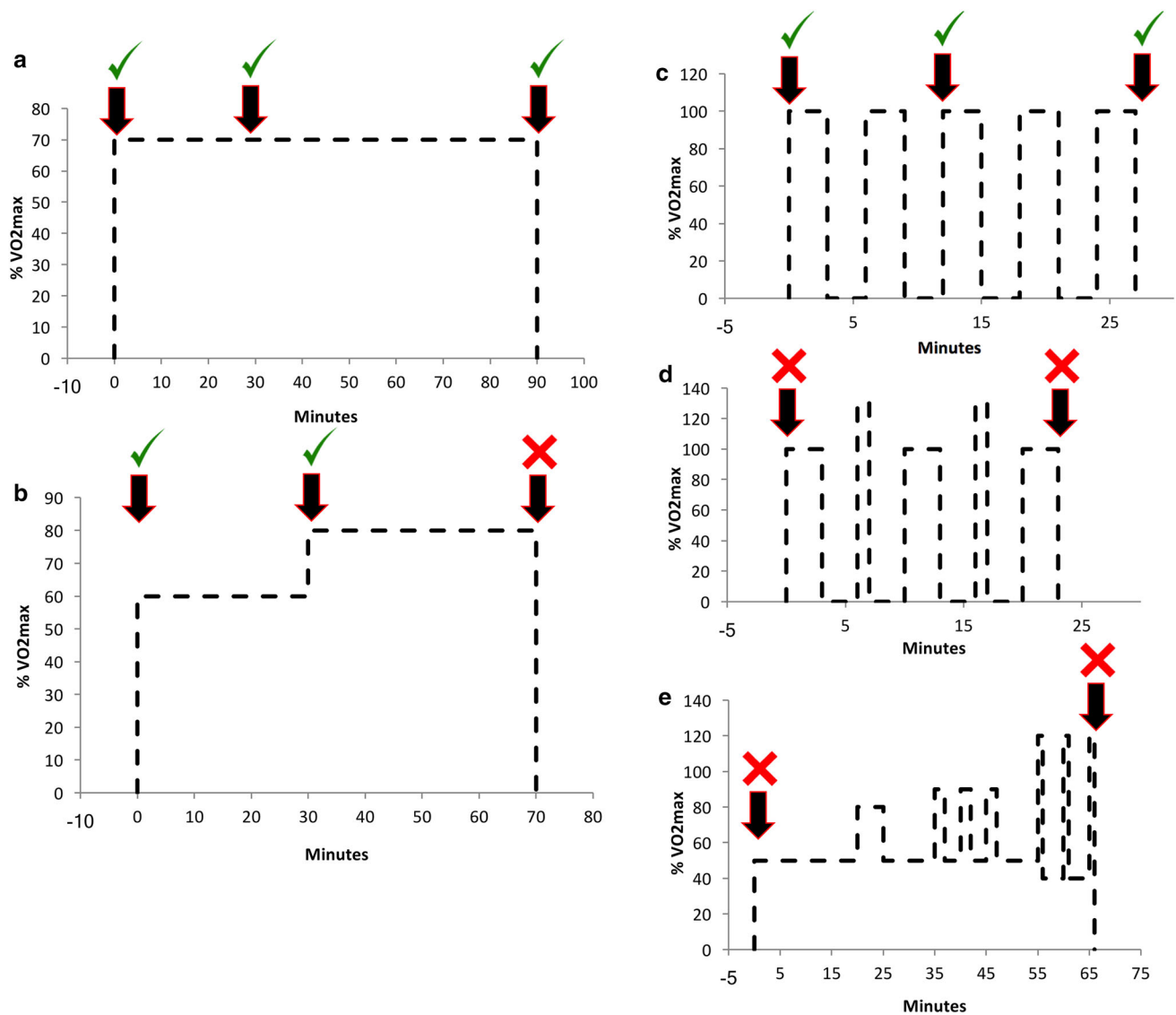
Studies of participants of any age, sex and level of physical activity were included. Studies of participants with metabolic disorders were excluded.

### 2.1.2 Type of Exercise

Studies of running or cycling (upright, double-legged) at fixed intensity in either continuous or intermittent fashion (with rest periods of fixed duration) were included (Fig. 1). Studies using any other type of exercise were excluded. The intensity of exercise had to be reported as a percentage of subjects' maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) or calculable as such. For intermittent exercise, cumulative time in exercise bouts was recorded. For studies with changes of exercise intensity, only the first period of measurement was included. For exercise continuing to fatigue, data at the point of fatigue were included.

### 2.1.3 Skeletal Muscle Glycogen Measurement

Muscle glycogen was recorded when reported as a consequence of biochemical determination of total (whole) muscle glycogen. When reported in units other than  $\text{mmol}\cdot\text{kg}^{-1}$  of dry mass (DM), values were transformed to this unit as per conversion factors shown in Table 1. Conversion of wet mass to DM assumes 77% water content of muscle [23–25]. Conversion of grams of glycogen to millimoles was based on a molecular weight of 180.16 for glucose. It was recorded whether the concentration was originally reported relative to muscle wet mass or DM. Studies reporting only fibre-type specific glycogen



**Fig. 1** Examples of common exercise intensity patterns found in the studies screened. Arrows indicate examples of possible muscle biopsy timepoints. Ticks and crosses above arrows indicate timepoint included or not in the analysis, respectively. **a** Continuous exercise with no variation in intensity. **b** Continuous exercise with one

increase in intensity. **c** Intermittent exercise with no variations in intensity. **d** Intermittent exercise with variations of intensity. **e** Representation of a generic glycogen depletion protocol.  $\dot{V}O_{2\max}$  maximal oxygen uptake

**Table 1** Conversion between commonly reported units for skeletal muscle glycogen

WM vs. DM		
1 mmol·kg <sup>-1</sup> WM	→	4.35 mmol·kg <sup>-1</sup> DM
1 mmol·kg <sup>-1</sup> DM	→	0.23 mmol·kg <sup>-1</sup> WM
1 g·100 g <sup>-1</sup> vs. 1 mmol·kg <sup>-1</sup> , WM and DM		
1 g·100 g <sup>-1</sup> WM	→	55.5 mmol·kg <sup>-1</sup> WM
1 g·100 g <sup>-1</sup> DM	→	241.5 mmol·kg <sup>-1</sup> DM
1 mmol·kg <sup>-1</sup> WM	→	0.018 g·100 g <sup>-1</sup> WM
1 mmol·kg <sup>-1</sup> DM	→	0.00414 g·100 g <sup>-1</sup> DM

DM dry mass, WM wet mass

concentration or relative concentration (e.g. periodic acid Schiff) were not included. Studies assessing muscle glycogen concentration only indirectly [using tracers, magnetic resonance imaging (MRI), etc.] were not included.

#### 2.1.4 Muscle Groups

There was no exclusion criterion based on specific leg muscles, but studies only of vastus lateralis or gastrocnemius met the other selection criteria.

#### 2.1.5 Fitness Status

$\dot{V}O_{2\max}$  was used to assess aerobic fitness status. Participants' relative  $\dot{V}O_{2\max}$  [mL·min<sup>-1</sup>·kg<sup>-1</sup> of body mass (BM)] were recorded as group averages. If only absolute  $\dot{V}O_{2\max}$  was reported as absolute for the group, relative  $\dot{V}O_{2\max}$  was calculated using the groups' average absolute  $\dot{V}O_{2\max}$  and BM. It was recorded whether the original information was reported as relative or absolute  $\dot{V}O_{2\max}$  for the group. If information reported was not sufficient to estimate groups' relative  $\dot{V}O_{2\max}$ , the study was not included.

#### 2.1.6 Nutritional Status and Carbohydrate Availability

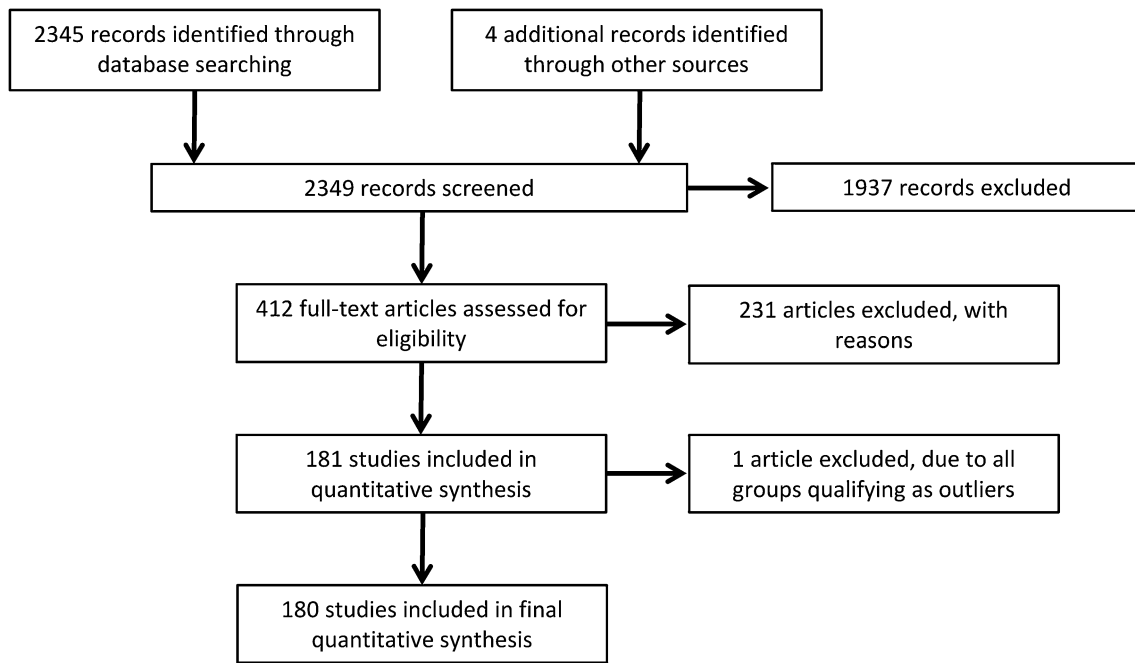
Participants experienced various pre-trial nutritional and/or exercise interventions that changed muscle glycogen concentration. We discretised these into three types of 'CHO - availability' groups: high, low and normal. High was defined by a diet containing either  $\geq 6$  g·kg<sup>-1</sup> of CHO per day for  $\geq 3$  days, or  $\geq 7$  g·kg<sup>-1</sup> CHO per day for  $\geq 2$  days. Low was defined as interventions using exercise for depleting muscle glycogen followed by low dietary CHO (as defined by the authors) during  $\geq 1$  day. Normal was defined as when neither the high nor low conditions were met or if specific details on nutrition intervention were not reported.

## 2.2 Search Strategy and Study Identification

A computerised search of the literature was performed last on 7 February 2018 using the PubMed database (<http://www.ncbi.nlm.nih.gov/pubmed/>). The search terms were "Muscle Glycogen" AND "Exercise" filtered for "humans". We also included articles identified through reference lists. Abstracts from conferences, commentaries, reviews or duplicated data in publications were not included in this analysis. The preliminary search yielded 2345 articles, of which 181 were included in the analysis. An outline of the flow of articles is depicted in Fig. 2 and the list and details of studies included is outlined in Electronic Supplementary Material Table S1.

## 2.3 Meta-Analytic Models

Muscle glycogen concentration was meta-analysed using the general linear mixed-model procedure (Proc Mixed) in the Statistical Analysis System<sup>®</sup> (SAS<sup>®</sup> version 9.4, SAS Institute, Cary, NC, USA). Estimates from each study were weighted by the inverse of the square of their standard errors. For the analysis of mean resting glycogen, the standard errors were simply the between-subject standard deviation (SD) divided by the square root of the sample size. Meta-analysis of the SD of resting glycogen was performed on the log-transformed between-subject SD; from first principles, the standard errors were given by  $1/\sqrt{2(\text{sample size}-1)}$ . For analyses of change in glycogen and rate of glycogen utilisation, standard errors of the mean change scores were derived where possible from confidence limits (CLs), SDs of change scores or exact *p* values. Where none of these data were provided, standard errors were imputed with a prediction model for SDs of change scores, on the assumption that studies with similar characteristics would have similar errors of measurement. Non-missing standard errors were converted to SDs of change scores, the logarithm of which was predicted with a mixed linear model in which the fixed effects were CHO availability (three levels: low, normal and high), the log of baseline glycogen concentration and a cubic function of the



**Fig. 2** Flow of scientific publications for the meta-analysis

log of the mean change in glycogen concentration, while the random effect was simply the residual, but allowing for a different variance for each of the three baseline conditions. This prediction model was developed empirically to provide visual uniformity in plots of residuals versus predicted values. For studies with missing standard errors, predicted values of SDs of change scores were then converted to standard errors.

In all meta-analytic models, the residual variance was set to unity to estimate between- and within-study variances [26]. Details of the models are given in Sects. 2.3.1–2.3.4.

### 2.3.1 Mean of Resting Skeletal Muscle Glycogen

The dependent variable was the raw mean glycogen concentration ( $\text{mmol}\cdot\text{kg}^{-1}\text{DM}$ ). Fixed effects were CHO availability, fitness status (linear, as  $\dot{V}\text{O}_{2\text{max}}$  in  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) interacted with CHO availability, muscle type (two levels: gastrocnemius and vastus lateralis) and sex (two levels: male and female). The random effects allowed for differences between studies, repeated measurement within studies within the same subjects and repeated measurement within studies between different subjects. A set of random effects were estimated for each of the three conditions of CHO availability.

### 2.3.2 Standard Deviation of Resting Skeletal Muscle Glycogen

The meta-analytic model was the same as for mean muscle glycogen, except that the dependent variable was the log of the between-subject SD for each group of subjects in each study. Predicted SD were back-transformed to raw concentrations ( $\text{mmol}\cdot\text{kg}^{-1}\text{DM}$ ). Estimates of observed between-subject SD in the three conditions of CHO availability and for  $\dot{V}\text{O}_{2\text{max}}$  of 40–70  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  were derived by adding the between-study estimate variance from the analysis of resting mean glycogen with the square of the predicted between-subject SD squared and taking the square root. These observed SDs represent the expected differences between subjects with the given characteristics if the subjects were drawn randomly from different studies.

### 2.3.3 Effect of Exercise on Muscle Glycogen

The dependent variable was the raw mean change in glycogen concentration from baseline concentration. Fixed effects were exercise time (five levels, with means of 5, 23, 53 and 116 min, and time to fatigue); exercise time interacted with baseline glycogen concentration (linear), exercise intensity (linear, as percentage of  $\dot{V}\text{O}_{2\text{max}}$ ) and fitness status (linear, as  $\dot{V}\text{O}_{2\text{max}}$ ); binary variables representing exercise mode (intermittent and continuous), exercise type (running and cycling), supplementation (CHO ingestion and no ingestion) and muscle type (gastrocnemius and vastus lateralis); and, finally, proportion of females in the

**Table 2** Effects of carbohydrate availability, fitness status, sex and muscle type on resting skeletal muscle glycogen concentration

Condition	Effect (mmol·kg <sup>-1</sup> DM) (mean, ± 90% CL)	Inference <sup>a</sup>
Carbohydrate availability		
High vs. normal	<b>102, ± 47</b>	Moderate ↑****
Low vs. normal	– <b>253, ± 30</b>	Very large ↓****
High vs. low	<b>356, ± 53</b>	Very large ↑****
Fitness ( $\dot{V}O_{2max}$ , per ↑10 mL·kg <sup>-1</sup> ·min <sup>-1</sup> )		
With low CHO availability	29, ± 44	Small ↑*
With normal CHO availability	<b>67, ± 15</b>	Small ↑****
With high CHO availability	<b>123, ± 42</b>	Moderate ↑****
Sex (female vs. male)	34, ± 47	Small ↑*
Muscle (gastrocnemius vs. vastus lateralis)	29, ± 68	Small ↑

Data in bold represent clear effects at the 99% level

For comparison, predicted resting glycogen in the vastus lateralis of males with normal carbohydrate availability and  $\dot{V}O_{2max}$  of 50 mL·kg<sup>-1</sup>·min<sup>-1</sup> was 462 ± 132 mmol·kg<sup>-1</sup>DM (mean ± between-subject SD; data from Fig. 3). The CHO content of diet was defined as high, CHO ≥ 6 g·kg<sup>-1</sup> per day for ≥ 3 days or ≥ 7 g·kg<sup>-1</sup> per day for ≥ 2 days; low, glycogen depletion and low-CHO diet; or normal, neither high nor low or not specified in study

CHO carbohydrate, CL confidence limit, DM dry mass, SD standard deviation,  $\dot{V}O_{2max}$  maximal oxygen uptake, ↑ indicates increase, ↓ indicates decrease

<sup>a</sup>Likelihood for clear substantial effects: \* possibly; \*\*\*\* most likely

sample (linear, range 0–1). The random effects allowed for differences between studies, repeated measurement within studies between timepoints, and repeated measurement within studies within the same timepoint.

#### 2.3.4 Time to Fatigue

Time to fatigue was modeled for exercise intensity of 70% of  $\dot{V}O_{2max}$ , since there were insufficient studies at other intensities. The dependent variable was the log of time to fatigue. Fixed effects were baseline glycogen concentration (linear), fitness status (linear, as  $\dot{V}O_{2max}$ ), and binary variables representing exercise mode (intermittent and continuous), exercise type (running and cycling), supplementation (CHO ingestion and no ingestion) and muscle type (gastrocnemius and vastus lateralis); and, finally, proportion of females in the sample (linear, range 0–1). The random effects allowed for repeated measurement within studies, but data for estimation of standard errors were not recorded, so only a fixed-effects meta-analysis was performed.

Uncertainty in the true effects of the predictors was evaluated using non-clinical magnitude-based inference [27]. The estimate of observed between-subject SD of resting (baseline) glycogen with normal CHO availability and  $\dot{V}O_{2max}$  of 50 mL·kg<sup>-1</sup>·min<sup>-1</sup> provided the SD (132 mmol·kg<sup>-1</sup>DM) to derive magnitude thresholds via standardisation: 0.2, 0.6, 1.2 and 2.0 of the SD were the thresholds for small, moderate, large and very large, respectively. With rounding, the corresponding changes in

glycogen concentration were 25, 75, 150 and 250 mmol·kg<sup>-1</sup>DM. Inferences about glycogen changes were based on acceptable level of uncertainty: changes were deemed clear if the 90% confidence interval did not include positive and negative substantial values; clear effects with 99% CLs are highlighted in bold in Tables 2 and 3 to address concerns about inflation of Type-1 error. Clear effects were reported with a qualitative likelihood that the true effect was either substantial or trivial (whichever probability was greater) using the following scale: < 0.5% most unlikely, 0.5–5% very unlikely, 5–25% unlikely, 25–75% possibly, 75–95% likely, 95–99.5% very likely and > 99.5% most likely.

## 3 Results

### 3.1 Resting Skeletal Muscle Glycogen

The observed average mean ± SD resting muscle glycogen across studies was 484 ± 144 mmol·kg<sup>-1</sup>DM (range 150–928 mmol·kg<sup>-1</sup>DM). Figure 3 depicts the predicted measured average resting muscle glycogen values for individual group means with a  $\dot{V}O_{2max}$  of 40–70 mL·kg<sup>-1</sup>·min<sup>-1</sup> and low, normal and high CHO availability. The meta-analysed effects of fitness status, CHO availability, sex and muscle type are detailed in Table 2. Compared with normal CHO availability, low and high CHO availability had very large negative effects and moderate positive effects, respectively. The effects of an increase of  $\dot{V}O_{2max}$  of

**Table 3** Effects of exercise intensity, baseline glycogen, fitness status, intermittent exercise, carbohydrate supplementation, sex, muscle type and exercise mode on the change from baseline of skeletal muscle glycogen at various times during exercise

Effect	Mean, $\pm$ 90% CL (mmol·kg <sup>-1</sup> )	Inference <sup>a,b</sup>
Intensity (per 30% $\dot{V}O_{2max}$ )		
At 5 $\pm$ 3 min	<b>41, <math>\pm</math> 20</b>	Small $\uparrow^{**}$
At 23 $\pm$ 7 min	<b>87, <math>\pm</math> 29</b>	Moderate $\uparrow^{****}$
At 54 $\pm$ 9 min	<b>122, <math>\pm</math> 37</b>	Moderate $\uparrow^{****}$
At 116 $\pm$ 35 min	<b>134, <math>\pm</math> 38</b>	Moderate $\uparrow^{****}$
At fatigue	115, $\pm$ 133	Moderate $\uparrow^{**}$
Baseline glycogen (per 200 mmol·kg <sup>-1</sup> DM)		
At 5 $\pm$ 3 min	<b>28, <math>\pm</math> 16</b>	Small $\uparrow^*$
At 23 $\pm$ 7 min	<b>31, <math>\pm</math> 15</b>	Small $\uparrow^{**}$
At 54 $\pm$ 9 min	<b>59, <math>\pm</math> 13</b>	Small $\uparrow^{****}$
At 116 $\pm$ 35 min	<b>104, <math>\pm</math> 15</b>	Moderate $\uparrow^{****}$
At fatigue	<b>143, <math>\pm</math> 33</b>	Moderate $\uparrow^{****}$
Fitness (per 10 mL·kg <sup>-1</sup> ·min <sup>-1</sup> $\dot{V}O_{2max}$ )		
At 5 $\pm$ 3 min	3, $\pm$ 24	Trivial <sup>††</sup>
At 23 $\pm$ 7 min	- 1, $\pm$ 17	Trivial <sup>†††</sup>
At 54 $\pm$ 9 min	- <b>30, <math>\pm</math> 15</b>	Small $\downarrow^*$
At 116 $\pm$ 35 min	- <b>17, <math>\pm</math> 15</b>	Trivial <sup>††</sup>
At fatigue	- 12, $\pm$ 28	Trivial <sup>††</sup>
Exercise mode (intermittent vs. continuous)	21, $\pm$ 30	Trivial <sup>†</sup>
CHO supplementation (yes vs. no)	- <b>7, <math>\pm</math> 16</b>	Trivial <sup>†††</sup>
Sex (female vs. male)	- <b>30, <math>\pm</math> 29</b>	Small $\downarrow^{**}$
Muscle (gastrocnemius vs. vastus lateralis)	- 31, $\pm$ 46	Small $\downarrow^*$
Exercise type (running vs. cycling)	- <b>70, <math>\pm</math> 32</b>	Small $\downarrow^{***}$

Results in bold represent clear effects at the 99% level

For comparison, predicted baseline glycogen in the vastus lateralis of males with normal carbohydrate availability and  $\dot{V}O_{2max}$  of 50 mL·kg<sup>-1</sup>·min<sup>-1</sup> was 462  $\pm$  132 mmol·kg<sup>-1</sup>DM (mean  $\pm$  between-subject SD; data from Fig. 3)

CHO carbohydrate, CL confidence limit, DM dry mass, SD standard deviation,  $\dot{V}O_{2max}$  maximal oxygen uptake,  $\uparrow$  indicates increase,  $\downarrow$  indicates decrease

<sup>a</sup>Likelihood for clear substantial effects: \* possibly, \*\* likely, \*\*\* very likely, \*\*\*\* most likely

<sup>b</sup>Likelihood for clear trivial effects:  $\uparrow$  possibly,  $\uparrow\uparrow$  likely,  $\uparrow\uparrow\uparrow$  very likely

10 mL·kg<sup>-1</sup>·min<sup>-1</sup> were small with normal CHO availability, moderate with high CHO availability and trivial with low CHO availability. Muscle type (gastrocnemius vs. vastus lateralis) had unclear effects, while there was a small positive effect in females compared with males.

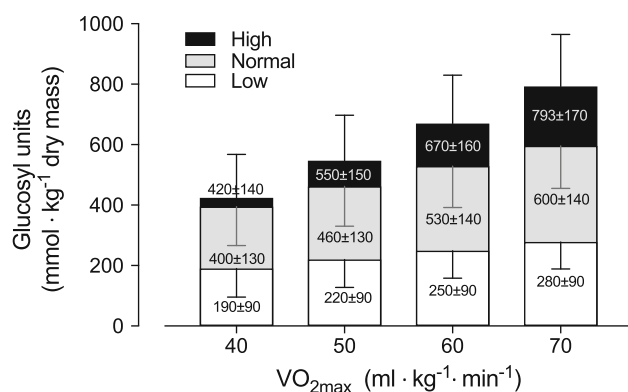
Estimates of random effects as SD (in mmol·kg<sup>-1</sup>DM  $\pm$  90% CL) were 106  $\pm$  16 for between studies, 55  $\pm$  7 within subjects within studies and 44  $\pm$  37 between subjects within studies.

### 3.2 Effect of Exercise on Skeletal Muscle Glycogen Change from Baseline

Exercise intensity, baseline muscle glycogen and, to a minor extent, fitness status were shown to be important factors affecting muscle glycogen concentration as a

function of exercise duration (Table 3). In addition, we showed that intermittent exercise had a possibly trivial effect on glycogen utilisation compared with continuous exercise, CHO ingestion had no effect on sparing muscle glycogen during exercise, running is associated with moderately less use of muscle glycogen than cycling, and that there is small magnitude effect in gastrocnemius muscle and females using less glycogen than vastus lateralis and males, respectively.

The magnitude of the fixed effects of exercise intensity, baseline muscle glycogen and fitness status became, as a general rule, larger with a longer exercise duration than baseline. An increase in intensity of 30% of  $\dot{V}O_{2max}$  (Table 3, Fig. 4) showed small effects at 5 min and moderate effects from 23 min onwards. An increase in fitness status of 10 mL·kg<sup>-1</sup>·min<sup>-1</sup> showed trivial effects at all exercise durations, except at 54 min, where a small effect



**Fig. 3** Predicted resting glycogen concentration in vastus lateralis of males with  $\dot{V}O_{2max}$  of 40–70 mL · kg<sup>-1</sup> · min<sup>-1</sup> in conditions of low, normal and high carbohydrate availability. Data are predicted means and between-subject standard deviations expected if subjects were drawn randomly from different studies. The carbohydrate content of the diet was defined as high, carbohydrate  $\geq 6$  g · kg<sup>-1</sup> per day for  $\geq 3$  days or  $\geq 7$  g · kg<sup>-1</sup> per day for  $\geq 2$  days; low, glycogen depletion and low-carbohydrate diet; and normal, neither high nor low or not specified in study.  $\dot{V}O_{2max}$  maximal oxygen uptake

is observed (Table 3, Fig. 5). An increase in baseline muscle glycogen of 200 mmol · kg<sup>-1</sup> DM showed small effects up until 54 min and moderate effects at 116 min and at fatigue (Table 3, Fig. 6).

### 3.3 Time to Fatigue

The majority of the studies incorporating time to fatigue protocols were found to utilise exercise intensities in the proximity of 70% of  $\dot{V}O_{2max}$  ( $71 \pm 16\%$ , mean  $\pm$  SD). Therefore, predicted values for time to fatigue and glycogen concentration at time to fatigue were only generated for 70%  $\dot{V}O_{2max}$  intensity groups and shown in figures only (Figs. 4, 5, 6 and Electronic Supplementary Material figures).

## 4 Discussion

In this first meta-analytic review of research quantifying skeletal muscle glycogen concentration at rest and during endurance exercise, we have reported normative values for skeletal muscle glycogen concentration for individuals differing in sex, fitness status and dietary CHO availability both at rest and in running or cycling exercise of various intensities and durations. Resting muscle glycogen showed a clear direct effect of CHO availability and fitness status. During exercise, baseline glycogen concentration and exercise intensity showed clear direct effects on muscle glycogen utilisation and fitness status showed a small inverse effect.

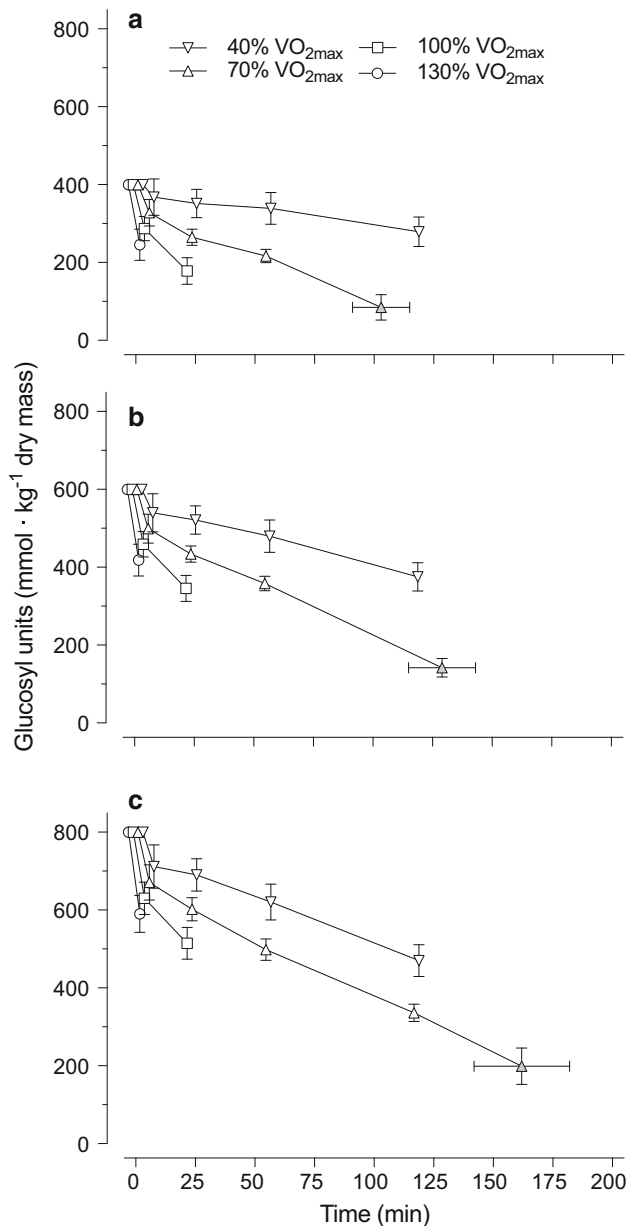
The effects on resting muscle glycogen by dietary CHO availability and fitness status were clear and direct, as shown in Table 2 and Fig. 3. High CHO availability resulted in moderate increases in resting muscle glycogen with higher fitness status. In contrast, low and normal CHO availability resulted in small increases with increasing fitness status. These findings are dependent on our criteria for ‘normal’, ‘low’ and ‘high’ CHO availability and are limited for estimating the effect of CHO loading on resting muscle glycogen. In such practice, large amounts of CHOs ( $> 8$  g · kg<sup>-1</sup> BM) are typically ingested for 2 days or more coupled with low training load [28], resulting in large amounts of resting muscle glycogen ( $> 800$  mmol · kg<sup>-1</sup> BM) in some [29, 30] but not all studies [31, 32]. These amounts of ingested CHOs are above the criteria defined in our analysis and would possibly result in higher values than those predicted for the high CHO availability group. Additionally, we have not considered the changes in muscle glycogen concentration after short-term training in an untrained population [33, 34] or after a single glycogen-depleting session [2, 35].

The predictive values for resting glycogen, nonetheless, agree with reported values in a trained population even when glycogen is assessed over consecutive days of training following a moderate- or high-CHO diet [36]. However, it is possible that in cases of repeated days of exercise to exhaustion or involving maximal exercise, skeletal muscle glycogen may fail to supercompensate [37] or even return to normal values [38] independent of dietary CHO availability. Further research is needed to understand the dynamics of muscle glycogen stores in relation to repeated sessions of glycogen-depleting exercise, and the applicability of our findings to those scenarios. Nevertheless, we expect that the findings of the current meta-analysis for glycogen utilisation during exercise will provide new insights and are applicable to a wide range of scenarios.

To this end, we report findings for the effects of exercise intensity, baseline glycogen and fitness on skeletal muscle glycogen utilisation during exercise as a function of time. Additionally, we show the modifying effects of intermittent versus continuous exercise, CHO ingestion, sex, muscle (gastrocnemius vs. vastus lateralis) and exercise type (cycling vs. running).

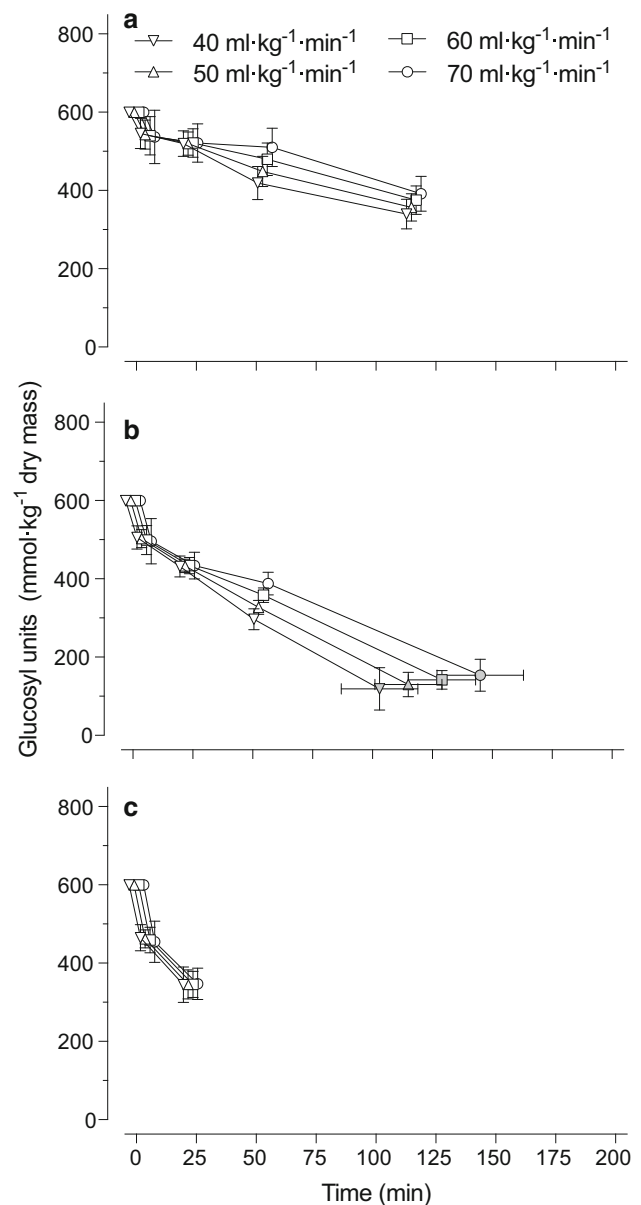
The effects of exercise intensity reported (Table 3, Fig. 4 and Electronic Supplementary Material Figure S1) are in line with those specifically assessed by individual studies [17–20]. We show a clear direct effect of an increase in 30% of  $\dot{V}O_{2max}$ , which is small during early exercise ( $\sim 5$  min) but moderate during all subsequent stages of exercise (23 min and later). The main effects of exercise intensity at  $54 \pm 9$  min are remarkably close to





**Fig. 4** Predicted skeletal muscle glycogen in vastus lateralis during continuous cycling exercise at four exercise intensities (40, 70, 100 and 130%  $\dot{V}O_{2max}$ ) for males with a  $\dot{V}O_{2max}$  of  $60 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  and different baseline muscle glycogen: **a** 400, **b** 600 and **c**  $800 \text{ mmol}\cdot\text{kg}^{-1}\text{DM}$ . Timepoints are 0, 4.7, 22.7, 53.5 and 116 min, nudged for enhanced visualisation. The grey-shaded timepoint at 70% of  $\dot{V}O_{2max}$  represents time to fatigue. Bars are 90% confidence limits. Predicted values for all combinations between baseline glycogen, fitness status and exercise intensity can be found in the Electronic Supplementary Material (Figure S1). *DM* dry mass,  $\dot{V}O_{2max}$  maximal oxygen uptake

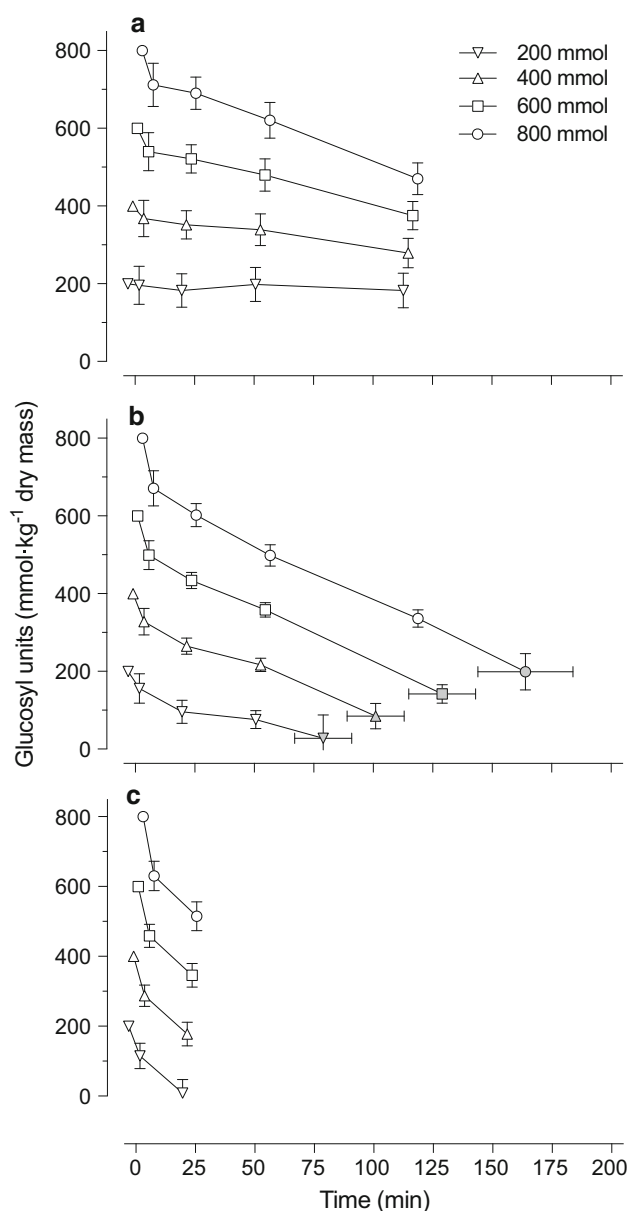
those reported in classical study by Hermansen et al. [17] over a period of 1 h at a range of intensities 28–74% of  $\dot{V}O_{2max}$ , and at  $116 \pm 35$  min are close to those reported



**Fig. 5** Predicted skeletal muscle glycogen in vastus lateralis for males with baseline muscle glycogen of  $600 \text{ mmol}\cdot\text{kg}^{-1}\text{DM}$  and four levels of fitness ( $\dot{V}O_{2max}$  of 40, 50, 60 and  $70 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) during continuous cycling exercise at three intensities: **(a)** 40%, **(b)** 70% and **(c)** 100%  $\dot{V}O_{2max}$ . Timepoints are 0, 4.7, 22.7, 53.5 and 116 min, nudged for enhanced visualisation. The grey-shaded timepoints at 70% of  $\dot{V}O_{2max}$  represent time to fatigue. Bars are 90% confidence limits. Predicted values for all combinations between baseline glycogen, fitness status and exercise intensity can be found in the Electronic Supplementary Material (Figure S2). *DM* dry mass,  $\dot{V}O_{2max}$  maximal oxygen uptake

over a period of 2 h at 31 and 64% of  $\dot{V}O_{2max}$  by Gollnick et al. [18].

The use of  $\dot{V}O_{2max}$  as a reference parameter for intensity during exercise can be considered both a strength and a weakness of the current meta-analysis. This variable is a



**Fig. 6** Predicted skeletal muscle glycogen in vastus lateralis for males with  $\dot{V}O_{2\max}$  of  $60 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  and four values of baseline muscle glycogen (200, 400, 600 and  $800 \text{ mmol}\cdot\text{kg}^{-1}\text{DM}$ ) during continuous cycling exercise at three intensities: **a** 40%, **b** 70% and **c** 100%  $\dot{V}O_{2\max}$ . Timepoints are 0, 4.7, 22.7, 53.5 and 116 min, nudged for enhanced visualisation. The grey-shaded timepoints at 70% of  $\dot{V}O_{2\max}$  represent time to fatigue. Bars are 90% confidence limits. Predicted values for all combinations between baseline glycogen, fitness status and exercise intensity can be found in the Electronic Supplementary Material (Figure S3). *DM* dry mass,  $\dot{V}O_{2\max}$  maximal oxygen uptake

key parameter for determining aerobic capacity and is in widespread use in the literature. However, muscular oxidative capacity can vary between individuals with similar  $\dot{V}O_{2\max}$  values [39, 40]. Instead, lactate threshold (LT) represents a better predictor of muscle oxidative

capacity when compared to  $\dot{V}O_{2\max}$  [41]. Therefore, intensity relative to LT is a better predictor of substrate use [41] and glycogen utilisation [42] during exercise in physically active and well-trained populations. Consequently, an analysis using LT as a reference for intensity would likely provide more precise estimates of glycogen utilisation. However, LT has rarely been used as a parameter for determining exercise intensity in the studies measuring muscle glycogen during exercise, and ‘lactate threshold’ is conceptualised in different ways by different authors [43], resulting in different measures of intensity at LT and it not being possible to use it as a common parameter for intensity across studies. It is, therefore, important to understand that differences in individuals’ glycogen concentration from reported mean values should be expected due to the variability of the muscular oxidative capacity in individuals with similar  $\dot{V}O_{2\max}$  values. For example, it would be expected that individuals with high muscle oxidative capacity, and therefore an LT as percentage of  $\dot{V}O_{2\max}$  that is higher than the average population, would display lower reliance on muscle glycogen during exercise than that predicted in the current meta-analysis.

An increase of fitness shown as a  $\dot{V}O_{2\max}$  of  $10 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  resulted in mostly trivial effects on muscle glycogen utilisation (Table 3, Fig. 5, Electronic Supplementary Material Figure S2). However, larger increases in  $\dot{V}O_{2\max}$  ( $20\text{--}30 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) would result in small or moderate negative effects after 54 min of exercise. This is in line with the idea that more aerobically fit individuals with higher oxidative capacity rely less on glycogen utilisation, as discussed earlier. If that is the case, it would be an indirect effect of  $\dot{V}O_{2\max}$  in muscle glycogen use that would explain these findings. A population with higher  $\dot{V}O_{2\max}$  is likely to be more trained [44, 45] than their lower  $\dot{V}O_{2\max}$  counterparts. This would in turn result in higher muscle oxidative capacity and lower reliance on CHOs at the same intensity relative to  $\dot{V}O_{2\max}$  [39, 41].

Baseline muscle glycogen, instead, showed small and moderate effects after  $\sim 5$  and  $\sim 55$  min, respectively, when increased by  $200 \text{ mmol}\cdot\text{kg}^{-1}\text{DM}$  (Table 3, Fig. 6, Electronic Supplementary Material Figure S3). The effects reported here at an  $\sim 1 \text{ h}$  timepoint are lower than those studies specifically assessing the effect of baseline muscle glycogen [21, 22]. While we cannot explain the discrepancy between our values and those reported previously in studies specifically assessing the effect of this variable, our findings support the concept that baseline muscle glycogen is important when considering glycogen utilisation during exercise. As such, this represents an important remark for those studies aiming to assess the effect of interventions on

glycogen utilisation and the importance of controlling diet and exercise to ensure comparable baseline muscle glycogen values in treatment groups. Moreover, in studies reporting glycogen utilisation it should be considered insufficient to report only pre-post exercise differences; reports of absolute values should be considered essential, more so when considering the rates of glycogen utilisation during the early stages of exercise.

Continuous exercise was the main type of exercise in study groups that matched our selection criteria, but endurance exercise is typically also done in an intermittent (work/rest) and variable intensity fashion. Interestingly, we found a trivial effect of intermittent exercise when compared with continuous exercise. This is in line with studies directly comparing time- and work-matched exercise protocols of continuous- or variable-intensity exercise for durations of 50–40 min in untrained [46], active [47] and trained [48] populations, which have shown no differences in the rates of glycogen utilisation. This suggests that the average intensity of a session is more important than the changes in intensity, at least when the changes in intensity are close to the average work rate and do not incorporate supramaximal exercise periods.

An important final consideration of the current meta-analysis is that data were collected, analysed and reported as group means and not individual values. For this reason, the degree of uncertainty on predicting individual values would be higher than those reported herein. The meta-regression approach used here has also been inappropriately labelled “daft”, because of “the risk of ecological bias, whereby [modifying effects] at the aggregate level might not reflect the true [modifying effects] at the individual participant level” [49]. We have mitigated the risk of such bias by including potentially confounding study and mean subject characteristics in a single meta-regression [50].

Considering the findings of the current meta-analysis for depleting glycogen during continuous exercise, it seems evident that baseline exercise with normal muscle glycogen would require  $\sim 2$  h at  $\sim 70\%$  of  $\dot{V}O_{2\max}$ —on average—to utilise  $\sim 60\%$  of glycogen stores and reach low levels of muscle glycogen ( $\sim 250$  mmol $\cdot$ kg $^{-1}$ DM). In line with the points discussed, in the case of highly trained individuals with muscle oxidative capacity that is above average, it is possible that the intensity would need to be higher to reach the same amount of glycogen depletion in that period of time. These findings are of particular importance in the context of the recently proposed glycogen threshold of 100–300 mmol $\cdot$ kg $^{-1}$ DM [14] for enhancing the training response, and they would allow us to better determine nutrition interventions, exercise intensity and duration to achieve optimal muscle glycogen to maximise adaptation to training.

## 5 Conclusion

We report novel normative and predictive values for resting muscle glycogen in different conditions of CHO availability in individuals of different fitness status, and the modifying effects of exercise intensity, baseline muscle glycogen and fitness status throughout endurance (cycling and running) exercise. Exercise intensity and baseline muscle glycogen are shown to have a higher magnitude of effect on muscle glycogen during exercise than fitness status when exercise intensity is considered as a percentage of maximal aerobic capacity. We expect that our findings will allow better understanding of skeletal muscle glycogen utilisation dynamics and facilitate the development of a guide for estimation of pre-/post-exercise muscle glycogen status based on a few key parameters.

### Compliance and Ethical Standards

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**Conflict of Interest** José L. Areta and Will G. Hopkins declare that they have no conflicts of interest relevant to the content of this review.

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