

CROSSTALK

CrossTalk opposing view: Exercise training volume is more important than training intensity to promote increases in mitochondrial content

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Since the pioneering work of Professor John Holloszy, it has been known that exercise training can increase the total protein content of mitochondria in skeletal muscle (Holloszy, 1967). This seminal work inspired others to investigate the effects of different volumes and intensities of exercise on mitochondrial biogenesis using a range of techniques (as reviewed in Granata *et al.* 2018a). The ‘gold standard’ method for assessing mitochondrial content is transmission electron microscopy (TEM), which can be used to calculate mitochondrial volume density (Mito_{VD}) or mitochondrial fractional area (Larsen *et al.* 2012); however, this technique requires specialised equipment and expertise not available in all laboratories. Therefore, biochemical measurements are often used as a surrogate of mitochondrial content (reviewed in more detail in Bishop *et al.* 2019). Due to its strong correlation with measurements of mitochondrial fractional area (as assessed by TEM) at rest (Larsen *et al.* 2012), the most widely used biomarker for mitochondrial content in skeletal muscle

is the activity of citrate synthase (CS), an enzyme exclusively located in the mitochondria (Tonkonogi & Sahlin, 1997). While the validity of this biomarker to assess changes in mitochondrial content has been questioned (Jacobs *et al.* 2016; Meinild Lundby *et al.* 2018; pooled correlation between training-induced changes in CS activity and Mito_{VD} was found to be $r = 0.246$, $P > 0.05$), it continues to be the most common marker used in exercise training studies.

Whether mitochondrial content is assessed via CS activity or TEM, there is good evidence in both rodents (Bishop *et al.* 2014) and humans (Granata *et al.* 2018b) that training volume is an important determinant of increases in skeletal muscle mitochondrial content. We have also shown that these changes in mitochondrial content are not always associated with changes in functional measures, such as mitochondrial respiration (Granata *et al.* 2016b). However, while the importance of training volume to increase mitochondrial content seems clear, the question for this CrossTalk debate is whether training volume is *more* important than exercise intensity to promote increases in mitochondrial content.

When evaluating research findings, the strongest level of evidence is typically assigned to pooled analyses of all relevant studies. For this reason, we pooled the results from 56 training studies and observed that greater training volumes were associated with greater increases in CS activity ($r = 0.59$; 95% CI, 0.41, 0.72; $P < 0.001$; Fig. 1A). This association was stronger when removing studies employing sprint interval training (SIT) ($r = 0.71$; 95% CI, 0.54; 0.83; $P < 0.001$). Pooling the results of the six studies (which included 10 training groups) that have assessed mitochondrial content using TEM, we also observed a strong relationship between training volume and training-induced

changes in Mito_{VD} ($r = 0.91$; CI, 0.66, 0.98; $P = 0.003$) (Hoppeler *et al.* 1985; Turner *et al.* 1997; Tarnopolsky *et al.* 2007; Montero *et al.* 2015; Montero & Lundby, 2017; Meinild Lundby *et al.* 2018). There was, however, no significant association between exercise intensity and changes in mitochondrial content (as assessed by either CS activity or TEM; Granata *et al.* 2018b). Thus, whether mitochondrial content is assessed via CS activity or TEM, the available evidence indicates that training volume is *more* important than training intensity to promote increases in mitochondrial content (especially when relative exercise intensity is less than 100% of the power at $\dot{V}_{O_{2max}}$, i.e. \dot{W}_{max}).

Although the evidence indicates that training volume is *more* important than training intensity to increase mitochondrial content, this does *not* imply that training intensity is unimportant. It is possible there is a minimal training intensity that is required to increase mitochondrial content (although increases in CS activity of ~25% have been reported in two studies that used exercise intensities of 45–50% of \dot{W}_{max} ; Gorostiaga *et al.* 1991; Gillen *et al.* 2016). Furthermore, training at a higher intensity induces similar increases in mitochondrial content for a smaller total training volume; when comparing the 27 studies that used moderate-intensity continuous training (MICT) (average exercise intensity = $66 \pm 9\%$ \dot{W}_{max} ; range = 45–80% \dot{W}_{max}) with the nine studies that used high-intensity interval training (HIIT) (average exercise intensity = $80 \pm 12\%$ \dot{W}_{max} ; range = 65 to 90% \dot{W}_{max}), there was no significant difference for the average increase in CS activity ($28 \pm 16\%$ vs. $27 \pm 13\%$; $P > 0.05$) despite an ~60% lower average training volume in the studies that used HIIT (Granata *et al.* 2018b) (see inset in Fig 1A). However, once again, when examining just

David Bishop and Javier Botella (both from Victoria University), and Cesare Granata (previously at Victoria University and now at Monash University) collaborate to investigate how the exercise prescription (e.g. manipulating exercise intensity and volume) alters molecular pathways that affect mitochondrial biogenesis (i.e. the generation of new mitochondrial components leading to changes in mitochondrial content and function). The ultimate goal of this research is to uncover novel biological mechanisms for the effects of different ‘doses’ of exercise on mitochondrial biogenesis, and to actively translate this new knowledge into more individualised exercise prescriptions to improve performance and to better prevent and manage diseases strongly linked to physical inactivity.



the nine studies that used HIIT, there was a strong correlation between total training volume and changes in CS activity (Fig 1B).

There are limitations in using pooled data (e.g. methodological and participant

differences between studies). However, only a few studies have investigated changes in markers of mitochondrial content in response to different types of training within the one study (Green *et al.* 1991;

LeBlanc *et al.* 2004; Burgomaster *et al.* 2008; Granata *et al.* 2016a,b; MacInnis *et al.* 2017; Montero & Lundby, 2017; Shepherd *et al.* 2017). In the only study to investigate the effect of different volumes

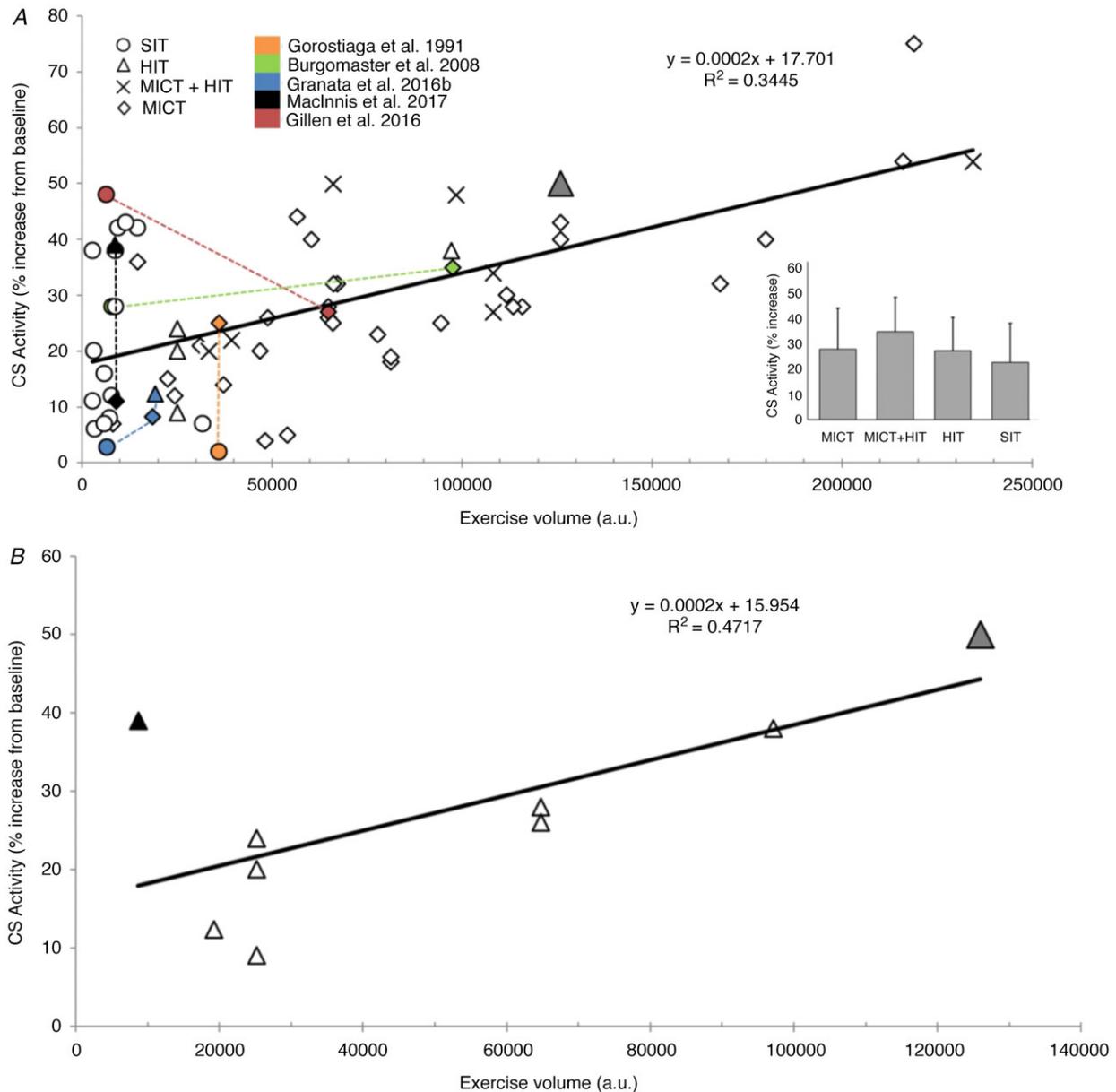


Figure 1. Relationship between training volume and training-induced changes in citrate synthase (CS) activity measured in the vastus lateralis muscle of healthy human participants

A, the results from 56 training studies that included Sprint Interval Training (SIT), High-Intensity Interval Training (HIIT), Moderate-Intensity Continuous Training (MICT), or MICT + HIIT. Dashed, colored lines connect groups that performed different types of training within the one study. Insert: average change in CS activity for all studies using the nominated type of training. See Granata *et al.* (2018b) for individual studies included in these analyses. B, relationship between training volume and training-induced changes in CS activity in studies that used HIIT. The small black triangle identifies a study that used one-legged training. The larger grey triangle identifies the same study in both figure A and B. In both figures, a linear correlation analysis was used to calculate the correlation coefficient between training volume and training-induced changes in CS activity, according to Pearson's product moment (r) (Systat Software, Inc., San Jose, CA, USA). The level of statistical significance was set at $P < 0.05$. a.u., arbitrary units.

of training on Mito_{VD} , increasing training volume (by increasing days of weekly training from 1 to 4) led to greater increases in Mito_{VD} (there was no further increase when training volume was increased further by training 5 days per week) (Montero & Lundby, 2017). In the only study to directly compare the effects of different training intensities, Mito_{VD} increased by 47% following SIT and 88% following MICT (although this difference between groups was not significant; Shepherd *et al.* 2017). In the three studies that have directly compared different volumes of training within the one study, greater training volumes were always associated with greater increases in CS activity (Green *et al.* 1991; LeBlanc *et al.* 2004; Granata *et al.* 2016a). When training volume was matched between groups, one study has reported greater increases in CS activity following HIIT versus MICT (39 vs. 11%; respectively, $P < 0.05$) (MacInnis *et al.* 2017). However, the total training volume performed in this study was very low (see black rhomboid in Fig 1A) and it may be that when total training volume is low, exercise intensity is more important to promote increases in mitochondrial content (as assessed by CS activity).

Building on this last point, some studies have reported large increases in CS activity with low-volume SIT performed at intensities well above \dot{W}_{max} (see results indicated by circles in Fig 1A). Nonetheless, when analysing the pooled results of the research to date, the average change in CS activity following SIT ($23 \pm 16\%$) is not significantly greater than that observed following MICT ($28 \pm 16\%$), MICT + HIIT ($35 \pm 14\%$), or HIIT ($27 \pm 13\%$) (see inset in Fig 1A). Furthermore, average increases in CS activity were greater following the five studies with the highest training volumes when compared with the five studies with the highest training intensities ($51 \pm 16\%$ vs. $29 \pm 17\%$). We are only aware of two studies that have directly compared changes in CS activity following MICT versus SIT matched for training volume. The first reported a greater increase following MICT (25 vs. 2%; Gorostiaga *et al.* 1991) and the second reported no significant difference after SIT or MICT (48 vs. 27%; Gillen *et al.* 2016). While more studies are required that directly compare different volumes and intensities of exercise within the one study, especially measuring mitochondrial content with TEM, there does not seem to be any compelling evidence that exercise intensity

is more important than exercise volume to promote increases in mitochondrial content.

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Additional information

Competing interests

The authors declare no conflict of interest.

Author contributions

All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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