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SYMPOSIUM REVIEW

Physiological adaptations to interval training and the role of exercise intensity

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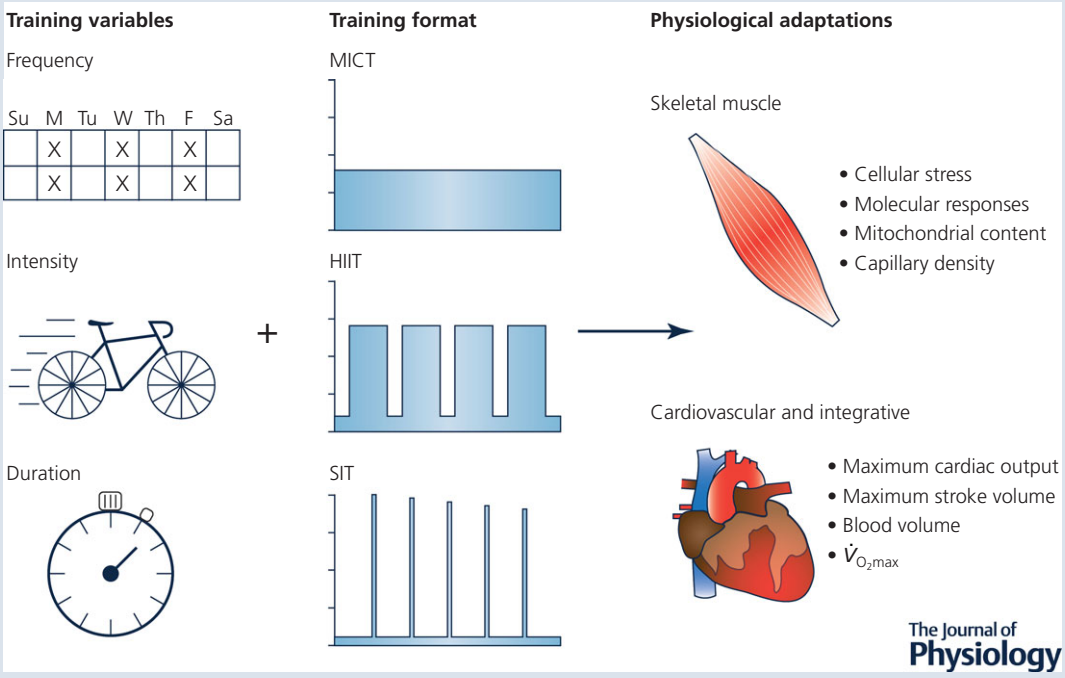

Abstract Interval exercise typically involves repeated bouts of relatively intense exercise interspersed by short periods of recovery. A common classification scheme subdivides this method into high-intensity interval training (HIIT; 'near maximal' efforts) and sprint interval training (SIT; 'supramaximal' efforts). Both forms of interval training induce the classic physiological adaptations characteristic of moderate-intensity continuous training (MICT) such as increased aerobic capacity ($\dot{V}_{O_2max}$) and mitochondrial content. This brief review considers the role of exercise intensity in mediating physiological adaptations to training, with a focus on the capacity for aerobic energy metabolism. With respect to skeletal muscle adaptations, cellular stress and the resultant metabolic signals for mitochondrial biogenesis depend largely on exercise intensity, with

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limited work suggesting that increases in mitochondrial content are superior after HIIT compared to MICT, at least when matched-work comparisons are made within the same individual. It is well established that SIT increases mitochondrial content to a similar extent to MICT despite a reduced exercise volume. At the whole-body level, $\dot{V}_{O_2max}$ is generally increased more by HIIT than MICT for a given training volume, whereas SIT and MICT similarly improve $\dot{V}_{O_2max}$ despite differences in training volume. There is less evidence available regarding the role of exercise intensity in mediating changes in skeletal muscle capillary density, maximum stroke volume and cardiac output, and blood volume. Furthermore, the interactions between intensity and duration and frequency have not been thoroughly explored. While interval training is clearly a potent stimulus for physiological remodelling in humans, the integrative response to this type of exercise warrants further attention, especially in comparison to traditional endurance training.

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Abstract figure legend Physiological responses to acute and chronic exercise are mediated by characteristics of the training programme. Training volume is the product of the frequency, intensity, and duration of exercise. The format of the training programme, although often complex, can generally be characterized as moderate-intensity continuous training (MICT), high-intensity interval training (HIIT), or sprint interval training (SIT). Characteristics of the training programme influence the magnitude of the skeletal muscle, cardiovascular and integrative adaptations to exercise. In particular, there is strong evidence that exercise intensity mediates mitochondrial adaptations to exercise and improvements in maximum aerobic capacity ($\dot{V}_{O_2max}$); however, the influence of exercise intensity is uncertain for specific cardiovascular adaptations, including capillarization, maximum cardiac output and stroke volume, and blood volume.

Abbreviations AMPK, AMP-activated protein kinase; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CS, citrate synthase; CK, creatine kinase; COXIV, cytochrome *c* oxidase subunit IV; ETC, electron transport chain; PHOS, glycogen phosphorylase; HIIT, high-intensity interval training; $\dot{V}_{O_2max}$, maximum aerobic capacity; MICT, moderate-intensity continuous training; OXPHOS, oxidative phosphorylation; $\dot{V}_{O_2peak}$, peak aerobic capacity; p38 MAPK, p38 mitogen-activated protein kinase; PGC-1α, peroxisome proliferator-activated receptor γ 1-α; ROS, reactive oxygen species; SIT, sprint interval training; SDH, succinate dehydrogenase; VEGF, vascular endothelial growth factor.

Introduction

Exercise is traditionally defined as either endurance or strength or viewed as a continuum anchored by these common descriptors (Coffey & Hawley, 2007; Hawley *et al.* 2014). In accordance with the principle of training specificity, endurance training is associated with an improved capacity for aerobic energy metabolism and fatigue resistance, whereas strength training is linked to muscle hypertrophy and increased force-generating capacity (Baar, 2006; Egan & Zierath, 2013; Hawley *et al.* 2014). Interval training, which can be simply defined as intermittent periods of intense exercise separated by periods of recovery (Fox *et al.* 1973), occupies a sort of middle ground. Depending on the specific protocol employed, this type of training can elicit adaptations resembling endurance or strength training, or a mix of the two. For example, interval training using repeated Wingate tests is a potent stimulus to increase mitochondrial content and peak aerobic capacity ($\dot{V}_{O_2peak}$; MacDougall *et al.* 1998) while interval training using body-weight resistance exercise increases $\dot{V}_{O_2peak}$ and muscular strength (McRae *et al.* 2012). This review will focus on the potential for interval training to elicit physiological adaptations that enhance aerobic energy metabolism.

Interval training terminology. Exercise prescription is obviously complex and involves numerous variables that can be manipulated, as evidenced by detailed reviews that have characterized interval training from the perspective of performance enhancement (Seiler, 2010; Tschakert & Hofmann, 2013; Buchheit & Laursen, 2013*a*,*b*). For simplicity and consistency, this review will employ the nomenclature put forward by Weston *et al.* (2014*a*) to differentiate two basic types of interval training based on exercise intensity (Fig. 1). High-intensity interval training (HIIT) is defined as 'near maximal' efforts generally performed at an intensity that elicits ≥ 80% (but often 85–95%) of maximal heart rate. In contrast, sprint interval training (SIT) is characterized by efforts performed at intensities equal to or greater than the pace that would elicit $\dot{V}_{O_2peak}$, including 'all-out' or 'supramaximal' efforts. The term moderate intensity continuous training (MICT) is used for comparative purposes to describe exercise that is

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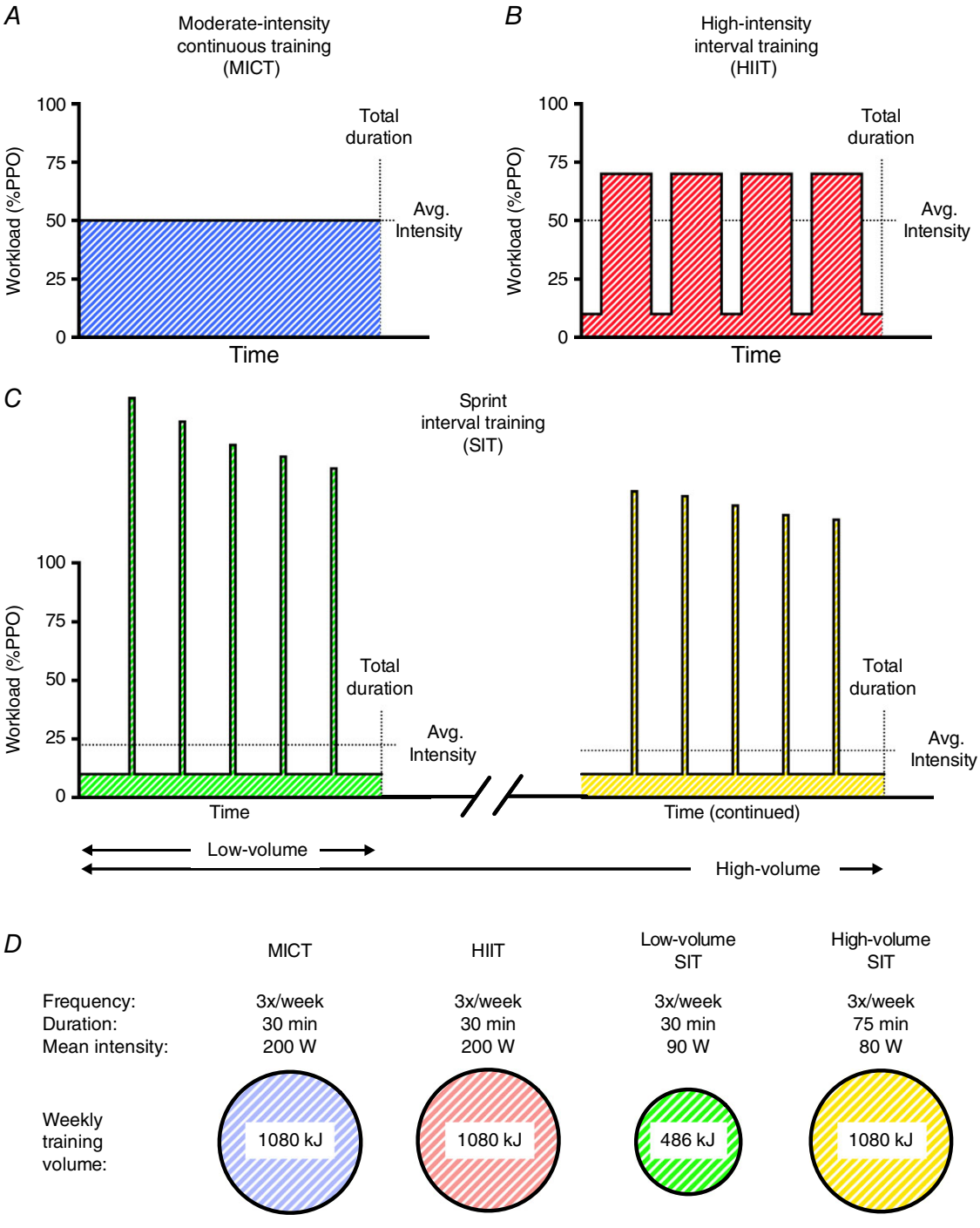

Figure 1. A graphical depiction of the main types of aerobic exercise
A–C, representative examples of moderate intensity continuous training (MICT), high-intensity interval training (HIIT), and low and high volumes of sprint interval training (SIT). The intensity is depicted as a percentage of the peak power output (PPO) obtained during a standard ramp $\dot{V}_{O_2peak}$ test (e.g. MacInnis *et al.* 2016). Note that most recent studies of SIT used a low-volume protocol (e.g. Burgomaster *et al.* 2006, 2008), whereas earlier studies of SIT used a high-volume protocol (e.g. Saltin *et al.* 1976). *D*, the training volume associated with each protocol based on the durations and training frequencies provided. The MICT and HIIT protocols shown in *A* and *B* are work-matched when performed for the same duration and at the same frequency. The low-volume SIT protocol requires less total work to complete relative to HIIT and MICT, whereas performing three sessions of the high-volume SIT protocol matches the training volume in the MICT and HIIT protocols.

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performed in a continuous manner and at lower intensities than HIIT. While imperfect, this general classification scheme is nonetheless suitable for our purposes here.

Assessing physiological adaptations to interval training. A fundamental and longstanding focus of exercise physiology has been the elucidation of the mechanisms underlying training adaptations. Of particular relevance to the present discussion, improvements in aerobic energy metabolism are primarily linked to peripheral adaptations, including increased skeletal muscle mitochondrial content and capillary density (Holloszy & Coyle, 1984), and central factors such as increased maximal stroke volume, maximal cardiac output and blood volume (Blomqvist & Saltin, 1983; Bassett & Howley, 2000). These variables generally respond to changes in exercise volume, which is the product of exercise intensity (i.e. work per unit time), exercise duration (i.e. time per session), and training frequency (i.e. sessions per week), as shown in Fig. 1. This review will consider the role of exercise intensity and its interaction with duration and frequency in mediating physiological adaptations to interval training in humans. Given our primary expertise and the data available, emphasis will be placed on skeletal muscle remodelling with a focus on the regulation of mitochondrial biogenesis. A large body of research has evaluated the effect of interval training on maximum aerobic capacity ($\dot{V}_{O_2max}$; Bacon *et al.* 2013; Gist *et al.* 2013; Sloth *et al.* 2013), but data are limited regarding the underlying cardiovascular mechanisms involved. Finally, given our focus on physiological adaptations in healthy (albeit sometimes sedentary or overweight) populations, the reader is directed elsewhere for recent reviews regarding the effect of interval training on health-related markers and disease risk (Gibala *et al.* 2012; Kessler *et al.* 2012; Weston *et al.* 2014*a*; Jelleyman *et al.* 2015; Ramos *et al.* 2015).

Skeletal muscle adaptation to interval exercise training

Mitochondrial responses to exercise and their time course. Skeletal muscle mitochondrial density regulates substrate metabolism during submaximal exercise, with increased mitochondrial content promoting a greater reliance on fat oxidation and a proportional decrease in carbohydrate oxidation (Holloszy & Coyle, 1984; Egan & Zierath, 2013). As a result, exercise training lessens glycogen degradation and lactate production at a given intensity, while increasing the lactate threshold and allowing individuals to exercise for longer durations and at greater percentages of their $\dot{V}_{O_2max}$ (Joyner & Coyle, 2008). Thus, given its central role in exercise performance, there is considerable interest in the factors mediating exercise-induced mitochondrial adaptations (Bishop *et al.* 2014).

A variety of methods are available for investigating the effects of exercise on skeletal muscle mitochondria in humans. In general, acute studies measure changes in the phosphorylation state of signalling proteins (e.g. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), AMP-activated protein kinase (AMPK), p38 mitogen activated protein kinase (p38 MAPK)), gene expression (e.g. peroxisome proliferator-activated receptor γ 1-α (PGC-1α)), or mitochondrial protein synthesis rates. Training studies usually assess the volume or area of mitochondria via microscopy, the activity or protein content of mitochondrial enzymes (e.g. citrate synthase (CS) and succinate dehydrogenase (SDH)), or respiration in permeabilized muscle fibres or isolated mitochondria (e.g. oxidative phosphorylation (OXPHOS) capacity). While mitochondrial respiration is sometimes considered a measure of mitochondrial function and not content, OXPHOS capacity is a biomarker of mitochondrial density (Larsen *et al.* 2012). Furthermore, enzyme activity (CS or COX) and OXPHOS capacity generally increase similarly in training studies, suggesting that mitochondrial function (i.e. respiration per unit of mitochondria) is not altered in the short-term (Jacobs *et al.* 2013; MacInnis *et al.* 2016). In contrast, mitochondrial function correlates with aerobic capacity in cross-sectional studies (Jacobs & Lundby, 2013), indicating a potential long-term effect of training. The reader is directed elsewhere for detailed reviews on the signalling pathways associated with mitochondrial biogenesis (Scarpulla *et al.* 2012; Egan & Zierath, 2013) and the methodology available for assessing mitochondrial responses to exercise in humans (Larsen *et al.* 2012; Miller & Hamilton, 2012).

The relatively rapid rate at which mitochondrial content responds to training permits relatively short-term studies of mitochondrial adaptations in humans. Similar to MICT, a single session of HIIT or SIT activates signalling pathways associated with mitochondrial biogenesis, such as the phosphorylation of AMPK and p38 MAPK and the expression of PGC-1α mRNA (Gibala *et al.* 2009; Little *et al.* 2011; Metcalfe *et al.* 2015). The regular and repeated activation of these pathways leads to increases in mitochondrial density (Coffey & Hawley, 2007). A comprehensive study by Perry *et al.* (2010) examined the early time course of adaptations to HIIT and showed that mRNA expression (e.g. PGC-1α) was acutely and transiently increased following each session of HIIT. CS protein content and enzyme activity increased steadily over the seven sessions, with CS maximal activity significantly increased above baseline after the third session. Other studies have reported significant increases in CS activity 24 h after a single session of SIT (Little *et al.* 2011) or MICT (Egan *et al.* 2013). Numerous studies have demonstrated that mitochondrial content (measured with CS or COX activity) increased by ~25–35% after six to seven sessions of HIIT (Talanian *et al.* 2006; MacInnis *et al.* 2016) or

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SIT (Burgomaster *et al.* 2006; Gibala *et al.* 2006). When the intensity and duration of exercise are held constant, mitochondrial content has been shown to plateau after ~5 days of training (Egan *et al.* 2013); however, when the intensity is increased progressively, mitochondrial content continues to rise for at least several weeks (Henriksson & Reitman, 1977).

Exercise intensity mediates acute mitochondria-related responses to exercise. The molecular and cellular events that underpin adaptations to exercise are fundamental aspects of exercise biology (Egan & Zierath, 2013; Hawley *et al.* 2014), and the responsiveness of these pathways to divergent exercise stimuli is essential for understanding the process through which humans adapt to exercise (Baar, 2006; Coffey & Hawley, 2007; Baar, 2009). Cellular stress occurs in proportion to exercise intensity (Egan & Zierath, 2013), and there is strong evidence that higher intensities of exercise elicit a greater metabolic signal than moderate intensities (Fig. 2). Firstly, ATP turnover is greater for higher intensities of exercise (Howlett *et al.* 1998), which also rely more on carbohydrate oxidation and utilize more glycogen than do lower intensities of exercise (Gollnick *et al.* 1974; Vøllestad & Blom, 1985; Romijn *et al.* 1993; van Loon *et al.* 2001). Consequently, the accumulation of intracellular lactate, creatine, AMP and ADP increases with exercise intensity (Howlett *et al.* 1998; van Loon *et al.* 2001), as do the activity of AMPK (Wojtaszewski *et al.* 2000; Egan *et al.* 2010; Kristensen *et al.* 2015) and CaMKII (Rose & Hargreaves, 2003; Rose *et al.* 2006; Egan *et al.* 2010). The greater activation of these particular kinases, which was elicited by high- compared to low-intensity exercise matched for total work, was associated with greater expression of mRNA for PGC-1α, a major regulator of mitochondrial biogenesis (Egan *et al.* 2010). Finally, and downstream of the myriad metabolic signals described above, mitochondrial protein synthesis was greater in response to continuous exercise performed at a higher intensity relative to work-matched exercise performed at a lower intensity (Di Donato *et al.* 2014), signifying a greater rate of mitochondrial biogenesis when a given volume of exercise is performed at a higher intensity.

With respect to low-volume SIT in particular, two recent studies suggest that the activation of mitochondrial biogenesis in response to all-out exercise may be linked in part to the production of reactive oxygen species (ROS; Fig. 2). Six bouts of Wingate-based SIT induced ROS-dependent fragmentation of the ryanodine receptor (RyR), which was implicated in the post-exercise increase in intracellular Ca^{2+} concentration, a signal for mitochondrial biogenesis (Place *et al.* 2015). Additionally, Larsen *et al.* (2016) reported that 2 weeks of Wingate-based SIT inhibited aconitase activity through an increase in ROS. The inhibition of this tricarboxylic acid cycle enzyme was associated with reduced respiration in isolated mitochondria, which the authors hypothesized was compensated through an increase in mitochondrial content in the vastus lateralis muscle. Whether different durations or intensities of exercise are capable of activating these ROS-dependent mechanisms to similar extents, or at all, remains unknown.

The on-and-off pattern characteristic of interval training (i.e. rest–work cycles) could partially explain skeletal muscle responses to this type of exercise. AMPK phosphorylation was greater when a session of moderate-intensity exercise was divided into 1 min intervals, interspersed with rest, compared to when it was performed as a continuous 30 min session (Combes *et al.* 2015); however, whether or not these acute differences in signalling patterns would translate to different chronic effects is unclear. Research from our group suggests that the intermittent nature of interval training plays a role in the magnitude of the adaptations. We demonstrated that CS maximal activity was unchanged by performing a single 4 min all-out bout of cycling 3 days $week^{-1}$ for 6 weeks, despite being increased in response to approximately the same volume of work performed as four 30 s all-out bouts, interspersed with recovery periods (Cochran *et al.* 2014).

The role of exercise intensity in mediating mitochondrial adaptations to training. Training volume has been suggested to be a primary determinant of the exercise-induced increase in mitochondrial content in humans (Bishop *et al.* 2014). Supporting evidence in this regard was derived mainly from correlations based on studies that measured CS maximal activity before and after exercise programmes of different lengths rather than work-matched HIIT and MICT protocols or studies that compared relatively low volumes of SIT and high volumes of MICT. Given the limitations inherent to making inferences based on a small pool of studies with methodological differences, the authors called for additional research in humans comparing different training stimuli within the same study. The training studies included in this section are described in greater detail in Table 1.

We recently examined the role of exercise intensity in determining mitochondrial adaptations to short-term training. Cognizant of the classic study design by Saltin and colleagues (1976), we employed single-leg cycling as a model to examine the effect of two different training interventions within the same individual. Participants performed six training sessions with each leg over 2 weeks, with one leg performing HIIT and the other leg performing MICT (MacInnis *et al.* 2016). A weight was affixed to the contralateral crank arm during exercise, providing the 'feel' of two-legged cycling, even though participants trained with only one leg at a time (Abbiss *et al.* 2011; Burns *et al.* 2014). Importantly, the volume

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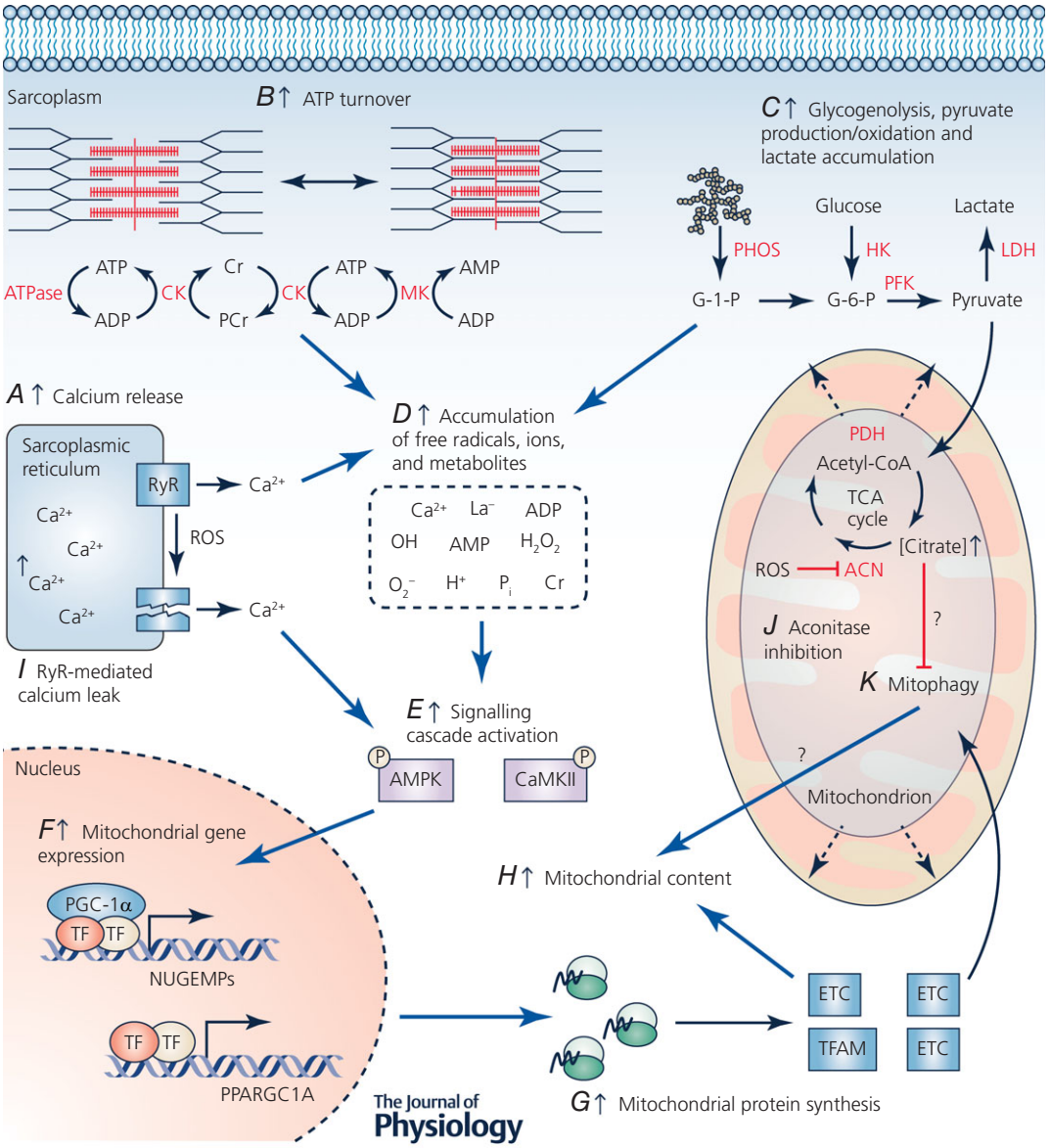

Figure 2. A schematic diagram of the putative mechanisms through which high-intensity exercise may elicit greater mitochondrial adaptations to aerobic training compared to lower intensities of exercise
Exercising at a higher intensity increases calcium release (*A*), requires greater ATP turnover (*B*), and leads to greater use of carbohydrates for fuel (*C*), compared to exercising at a lower intensity. As a result, there is a greater accumulation of metabolites, ions, and free radicals (*D*), which increase the activation of signalling proteins (*E*), including the kinases Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and AMP-activated protein kinase (AMPK). The increased activity of these protein kinases causes greater rates of gene expression for PGC-1α (encoded by *PPARGC1A*), which in turn acts as a transcriptional co-activator for nuclear genes encoding mitochondrial proteins (NUGEMPs; *F*). In turn, mitochondrial protein synthesis rates are greater for high-intensity exercise (*G*), leading to a greater increase in mitochondrial content (*H*), relative to exercise at a lower intensity. Two additional ROS-mediated mechanisms explaining the potency of low-volume SIT have recently been reported. Firstly, through a ROS-dependent mechanism, low-volume SIT led to the fragmentation of the ryanodine receptor (RyR) of the sarcoplasmic reticulum and increased the intracellular calcium concentration (*I*), a signal for mitochondrial biogenesis. Similarly, two weeks of low-volume SIT was associated with the inhibition of aconitase in the tricarboxylic acid cycle (TCA) and an increased intracellular citrate concentration, which was suggested to increase mitochondrial content via a reduction in mitophagy (*J*). For specific references, see 'Exercise intensity mediates acute mitochondria-related responses to exercise' in text. ACN, aconitase; ATPase, adenosine triphosphatase; CK, creatine kinase; ETC, electron transport chain; PHOS, glycogen phosphorylase; HK, hexokinase; LDH, lactate dehydrogenase; MK, myosin kinase; PFK, phosphofructokinase; PDH, pyruvate dehydrogenase; TFAM, transcription factor A, mitochondria.

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Table 1. Description of studies that have compared mitochondrial adaptations to different training protocols in healthy humans

Author	*n* (M/F)	Activity level; rel. $\dot{V}_{O_2max}$ (ml kg^{-1} min^{-1})	Duration; frequency; mode	Protocols	Mitochondria-related measurements
Bækkerud *et al.* (2016)	12/18	NR; ∼34	6 weeks; 3 days week^{-1}; running and walking	MICT: 45 min at 70% HR_{max}† HIIT: 10 min at 70% HR_{max}; 4× (4 min at 85–95% and 3 min at 70% HR_{max})† SIT: 10× (1 min at ∼90% and active recovery)†	CS activity
Burgomaster *et al.* (2008)	10/10	Moderately active; 41	6 weeks; 3–5 days week^{-1}; cycling	MICT: 40–60 min at ∼65% $\dot{V}_{O_2peak}$ SIT: 4–6× (30 s Wingate test and 4.5 min at 30 W)	CS activity, substrate oxidation
Daussin *et al.* (2008)	7/4	Sedentary; ∼30	8 week; 3 days week^{-1}; cycling	MICT: 20–35 min at 61% W_{max}† SIT: 4–7× (4 min at 54% and 1 min at 90% of W_{max})†	Mass-specific respiration
Gibala *et al.* (2006)	16/0	Moderately active; 50	2 week; 3 days week^{-1}; cycling	MICT: 90–120 min at ∼65% $\dot{V}_{O_2peak}$ SIT: 4–6× (30 s Wingate test and 4.0 min at 30 W)	COX activity, ETC complex 4 protein content
Gillen *et al.* (2016)	25/0	Sedentary; ∼32	12 week; 3 days week^{-1}; cycling	MICT: 2 min at 50 W; 45 min at 70% HR_{max}; 3 min at 50 W SIT: 2 min at 50 W; 3× (20 s 'modified' Wingate test and 2 min at 50 W); 1 min at 50 W	CS activity, ETC complexes 1–5 protein content
Gorostiaga *et al.* (1991)	3/9	Moderately active or sedentary; 36	8 week; 3 days week^{-1}; cycling	MICT: 30 min at 50% W_{max}† SIT: 30× (30 s at 100% W_{max} and 30 s rest)†	CS activity

(*Continued*)

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Table 1. Continued

Author	*n* (M/F)	Activity level; rel. $\dot{V}_{O_2max}$ (ml kg^{-1} min^{-1})	Duration; frequency; mode	Protocols	Mitochondria-related measurements
Granata *et al.* (2015)	29/0	Moderately active; 46	4 week; 3 days week^{-1}; cycling	MICT: 20–36 min 65% W_{max}† HIIT: 4–7× (4 min at ∼90% W_{max} and 2 min at 60 W)† SIT: 4–10× (30 s Wingate test and 4 min rest)	CS activity, ETC complexes 1–5 protein content, mass- and mitochondria-specific respiration
Henriksson & Reitman (1976)	9/0	NR; ∼47	7-8 week; 3 days week^{-1}; cycling	MICT: ∼27 min at 79% $\dot{V}_{O_2max}$† HIIT: 5× (4 min at 101% $\dot{V}_{O_2max}$ and 2 min rest)†	SDH activity
MacInnis *et al.* (2016)	10/0	Moderately active; 46	2 week; 3 days week^{-1}; cycling	MICT: 5 min at 25 W; 30 min at 50% W_{max}† HIIT: 5 min at 25 W; 4× (5 min at 65% and 2.5 min at 20% W_{max})†	CS activity, ETC complex 4 protein content, mass- and mitochondria-specific respiration
Saltin *et al.* (1976)	13/0	Not regularly training; 46	4 week; 4–5 days week^{-1}; cycling	MICT: 35–45 min at 75% single-leg $\dot{V}_{O_2max}$† SIT: 20–30× (40–50 s at 150% single-leg $\dot{V}_{O_2max}$ and 90 s rest)†	SDH activity
Scribbans *et al.* (2014)	16/3	Moderately active; 48	6 week; 4 days week^{-1}; cycling	MICT: 30 min at 65% $\dot{V}_{O_2max}$ SIT: 8× (20 s at 170% $\dot{V}_{O_2max}$ and 10 s rest)	SDH activity
Shepherd *et al.* (2012)	16/0	Sedentary; 42	6 week; 3–5 days week^{-1}; cycling	MICT: 40–60 min at ∼65% $\dot{V}_{O_2max}$ SIT: 4–6× (30 s Wingate test and 4.5 min at 30 W)	ETC complex 4 protein content, substrate oxidation

Note that the classic Wingate test consists of a 30 s 'all-out' cycling effort against a resistance equivalent to 7.5% of body weight, whereas the 'modified' Wingate test involved a 20 s effort against a resistance of 5.0% of body weight. †Protocols that had the same oxygen cost or were work-matched. CS, citrate synthase; ETC, electron transport chain; HR_{max}, maximum heart rate; HIIT, high-intensity interval training; MICT, moderate-intensity continuous training; NR, not reported; SDH, succinate dehydrogenase; SIT, sprint interval training; $\dot{V}_{O_2max}$, maximum aerobic capacity; W_{max}, peak power output.
BMI and age ranged from 22-30 kg/m^2 and 21-45 years, respectively

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of training was identical for each leg. We showed that HIIT compared to MICT elicited a greater increase in mitochondrial content, assessed by CS maximal activity and OXPHOS capacity in permeabilized muscle fibres (Fig. 3). Mitochondrial function (i.e. OXPHOS capacity normalized to CS maximal activity) was unchanged with training.

Our recent findings are supported by a crossover study that compared work-matched HIIT and MICT in the same individuals (Daussin *et al.* 2008). In that study, the high-intensity programme elicited an increase in skeletal muscle mitochondrial respiration whereas the moderate-intensity programme did not. In the three comparisons of work-matched HIIT and MICT that used parallel-group designs, increases in mitochondrial content were similar (Henriksson & Reitman, 1976; Bækkerud *et al.* 2016) or not observed (Granata *et al.* 2015). Work-matched comparisons addressing the effect of intensity on lactate threshold generally report similar increases across groups as well (Poole & Gaesser, 1985; Helgerud *et al.* 2007; Granata *et al.* 2015). Sample sizes were relatively small in all of the above work-matched studies; however, within-subject comparisons provide greater control for potential sources of variation (e.g. diet, sleep, stress) and greater statistical power than between-subject comparisons. We suggest that the lack of consensus among these studies is partly due to differences in experimental design.

Supporting evidence for the role of exercise intensity is derived from studies reporting that a small volume of exercise performed at a very high intensity can elicit similar skeletal muscle adaptations compared to a large volume of moderate-intensity exercise. Comparable increases in mitochondrial content were reported for low-volume SIT and MICT after 2 (Gibala *et al.* 2006), 6 (Burgomaster *et al.* 2008; Shepherd *et al.* 2012; Scribbans *et al.* 2014) and 12 (Gillen *et al.* 2016) weeks of training. In the longest of these comparisons, we demonstrated that three weekly sessions of SIT (1 min of intense exercise performed over a 10 min session) elicited similar increases in CS maximal activity compared to a MICT protocol that involved 150 min of weekly exercise (Gillen *et al.* 2016). Furthermore, low-volume SIT induced similar improvements relative to MICT for multiple aspects of fat and carbohydrate metabolism (Gibala *et al.* 2006; Burgomaster *et al.* 2008; Shepherd *et al.* 2012; Scribbans *et al.* 2014). When low-volume SIT was compared to a higher volume of HIIT, an increase in mitochondrial respiration was only apparent following SIT (Granata *et al.* 2015). Interestingly, CS activity did not increase for either condition in that study. Finally, low-volume SIT increased lactate threshold, a variable strongly associated with skeletal muscle mitochondrial content (Ivy *et al.* 1980), to a similar extent relative to greater volumes of MICT and HIIT (McKay *et al.* 2009; Granata *et al.* 2015).

Observations from studies comparing high-volume SIT and MICT suggest that there are diminishing returns with increased durations of SIT (i.e. number of bouts per session). Comparisons of work-matched SIT and MICT resulted in similar increases in SDH maximal activity after 4 weeks (Saltin *et al.* 1976) or greater increases in CS maximal activity for MICT relative to SIT after 8 weeks of training (Gorostiaga *et al.* 1991). The total duration of sprint exercise performed in both of these studies

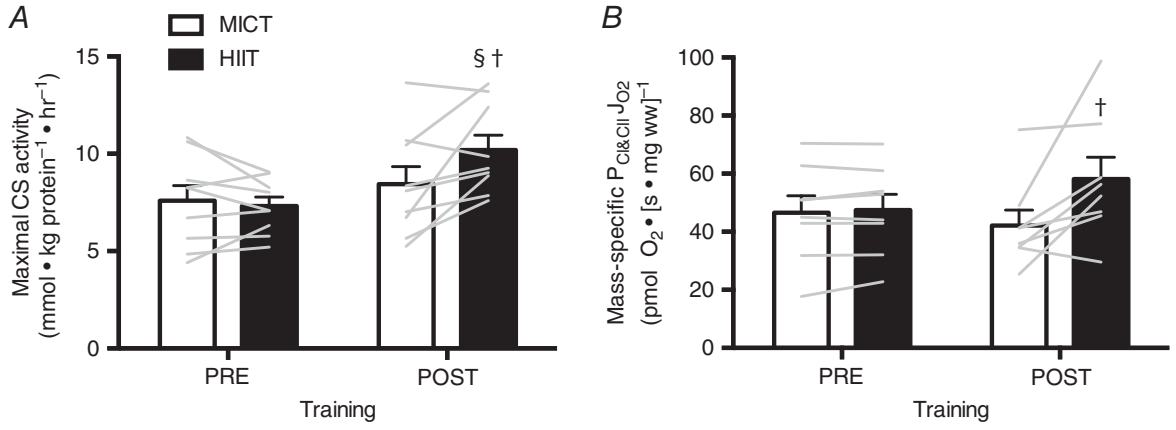

Figure 3. Changes in mitochondrial content in response to 2 weeks of high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT), matched for total work
Subjects performed six sessions of single-leg cycling with each leg, completing either a HIIT or MICT protocol. The greater increase in mitochondrial content elicited by HIIT as compared to MICT was evident from post-training differences in maximal citrate synthase activity (*A*) and mitochondrial respiration (J_{O_2}), specifically oxidative phosphorylation capacity through complexes I and II ($P_{CI\&CII}$, *B*). Bars represent the mean responses for each group, whereas lines refer to the responses of individual subjects. Symbols indicate a significant difference from the within-group, pre-training mean (§), and significant differences between groups at the post-training mean (†). Error bars represent 1 standard error of the mean. For *A*, $n = 9$ and for *B*, $n = 8$ subjects.

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(10–25 min; 20–30 bouts) was much higher than recent studies (∼1–3 min; 3–6 bouts). Note that in both studies, the two protocols elicited similar decreases in blood lactate concentrations during submaximal exercise, and in the latter study, CS maximal activity was numerically greater in the SIT group post-training. The hypothesis of diminishing returns with increased number of bouts is supported by acute studies of SIT. Parolin *et al.* (1999) demonstrated that AMP and ADP concentrations were greatest after the first of three Wingate tests, and glycogenolysis and lactate accumulation were strongly depressed during the third bout relative to the first bout of exercise, suggesting that the metabolic signal was not enhanced with further bouts. Furthermore, the expression of genes related to mitochondrial biogenesis was similar for SIT and MICT, whether work was matched (Wang *et al.* 2009) or not (Psilander *et al.* 2010).

The potential effects of exercise duration and frequency on mitochondrial adaptation. Studies performed in rodents have demonstrated that increasing the training volume by raising the duration (Fitts *et al.* 1975; Dudley *et al.* 1982) or frequency (Hickson, 1981) of exercise augments mitochondrial adaptations to aerobic exercise; however, insufficient data are available to fully ascertain the roles of these variables in mediating mitochondrial adaptations to exercise in humans.

The effect of duration seems to depend on the intensity of the exercise. Green and colleagues (2012, 2013) examined 'metabolic strain' (a proxy for mitochondrial content) during steady-state exercise following 10 days of cycling for 30 or 60 min day^{-1} at a low, moderate, or high intensity (60, 70 and 86% of $\dot{V}_{O_2max}$, respectively). Following training at the two higher intensities, but not the lowest intensity, the accumulation of AMP and ADP and the depletion of phosphocreatine and glycogen during steady-state exercise were reduced more following the 60 min day^{-1} as compared to the 30 min day^{-1} programmes, suggesting increases in mitochondrial content (Holloszy & Coyle, 1984). Thus, the effect of duration was augmented at higher exercise intensities. In a relatively large comparison of two 11-week training programmes consisting of mixed exercise modes and intensities, the high dose (∼3800 kcal week^{-1}) and the moderate dose (∼2000 kcal week^{-1}) groups exhibited similar increases in markers of mitochondrial content (Reichkendler *et al.* 2013; Rosenkilde *et al.* 2015), which could be explained by the relatively low average intensity (∼67% of $\dot{V}_{O_2max}$). Note that neither study compared two durations of interval training.

Limited data are available to ascertain whether weekly training frequency influences mitochondrial adaptations in humans. Costill *et al.* (1991) reported a greater increase in CS maximal activity for well-trained swimmers who trained twice each day *versus* once each day at high intensities. Similarly, performing SIT twice per week *versus* once per week augmented the improvement in lactate threshold following 6 weeks of training (Dalleck *et al.* 2010). The increase in CS maximal activity was also similar (Parra *et al.* 2000) or blunted (Hatle *et al.* 2014) when exercise was performed at a high (7–8 sessions per week) compared to moderate frequency (2–3 sessions per week); however, subjects performed the same number of sessions over different lengths of time across protocols, preventing conclusions related to the effect of training frequency.

Recently, Granata *et al.* (2016) reported that increasing the volume of HIIT (by augmenting duration and frequency while maintaining intensity) increased mitochondrial content, providing evidence that increases in the volume of high-intensity exercise can augment mitochondrial content; however, the relative importance of frequency or duration in mediating this adaptation cannot be determined from this study.

The role of skeletal muscle recruitment pattern and fibre type. Evidence from rodent studies suggests that mitochondrial adaptations to exercise occur in a fibre type-specific manner (Dudley *et al.* 1982; Taylor *et al.* 2005); however, the interaction between fibre type and exercise intensity has received less attention in humans, as most studies examine adaptations at the whole-muscle level. Skeletal muscle recruitment occurs in proportion to exercise intensity (Vøllestad & Blom, 1985; Sale, 1987), implying that higher intensities of exercise could elicit greater responses in type II fibres relative to lower intensities of exercise.

In our recent comparison of work-matched HIIT and MICT (MacInnis *et al.* 2016), we hypothesized that changes in mitochondrial content (measured with cytochrome *c* oxidase subunit IV (COXIV) protein content) would be greater in type II fibres following HIIT as compared to MICT. We demonstrated an effect of training on COXIV in whole muscle, but we were unable to demonstrate that response in either fibre type. In contrast, greater increases in SDH maximal activity in type II fibres have been reported following HIIT relative to work-matched MICT (Henriksson & Reitman, 1976), and type II fibre activation and AMPK activity were greater following an acute session of HIIT relative to a comparable session of MICT (Kristensen *et al.* 2015). In rodent muscle, increases in exercise intensity led to a plateau in mitochondrial content in red quadriceps muscle, whereas relatively high intensities of exercise were necessary to increase the mitochondrial content of white quadriceps muscle (Dudley *et al.* 1982; Taylor *et al.* 2005). Comparisons of low-volume SIT and MICT demonstrated similar increases in COX expression (Shepherd *et al.* 2012) and SDH activity (Scribbans *et al.* 2014) across fibre types despite the differences in training volume and the expected differences in muscle recruitment between SIT and MICT.

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Thus, data regarding the potential for different training programmes to induce fibre type-specific mitochondrial responses to exercise in humans is inconclusive.

Interval training and skeletal muscle capillary density. Skeletal muscle capillarization requires weeks to months to manifest in response to exercise training (Andersen & Henriksson, 1977; Hoppeler *et al.* 1985), and changes in capillary density appear to be blunted at higher exercise intensities (see Gliemann, 2016). Low-volume SIT induced similar or greater increases in the expression of several angiogenesis-related mRNAs relative to MICT, including greater vascular endothelial growth factor (VEGF) expression; however, the concentration of muscle interstitial VEGF protein and the proliferation of cultured endothelial cells were lower following the SIT session (Hoier *et al.* 2012). These acute differences corresponded with the increased and unchanged capillary density following the 4-week preconditioning period of MICT and the 4 weeks of SIT, respectively. In the one comparison of work-matched HIIT and MICT we are aware of, skeletal muscle capillarization increases were greatest following MICT (Daussin *et al.* 2008); however, two separate studies reported similar increases in capillary density with 6 weeks of low-volume SIT or MICT (Cocks *et al.* 2013; Scribbans *et al.* 2014). With the limited data, it is difficult to reconcile the inconsistent results, but in all cases MICT was more or equally as effective for increasing capillary density compared to HIIT/SIT. To our knowledge, there are no human data addressing the role of exercise duration or training frequency on skeletal muscle capillarization; however, given the relationship between capillary density and exercise performance (Coyle *et al.* 1988; Iaia *et al.* 2011), this area of research is deserving of more attention.

Cardiovascular adaptations to interval exercise training

Time course of cardiovascular adaptations to exercise training in humans. Improvements in $\dot{V}_{O_2max}$ typically manifest as early as 2–4 weeks after initiating training (Henriksson & Reitman, 1976; Andersen & Henriksson, 1977), but $\dot{V}_{O_2max}$ can increase after 1 week (Hickson *et al.* 1977). The latter study reported the largest mean increase in $\dot{V}_{O_2max}$ in humans, a 44% increase over 10 weeks in response to a high volume of intense interval and continuous training. In cross-sectional studies, the variation in $\dot{V}_{O_2max}$ is predominately attributable to variation in maximum stroke volume (and cardiac output) as opposed to the arteriovenous O_2 difference (Bassett & Howley, 2000; Montero *et al.* 2015*b*), and training studies generally reach the same conclusion (e.g. Ekblom *et al.* 1968). The increase in maximum cardiac output observed after several weeks of endurance training was related to exercise-induced haematological adaptations, as phlebotomizing subjects returned cardiac output and $\dot{V}_{O_2max}$ to baseline values (Bonne *et al.* 2014; Montero *et al.* 2015*a*). Although plasma and blood volumes increase after relatively few exercise sessions (Convertino *et al.* 1980; Green *et al.* 1987; Graham *et al.* 2016), contributing to increased stroke volume and decreased heart rate during submaximal exercise (Green *et al.* 1990; Goodman *et al.* 2005), changes in maximum stroke volume and cardiac output seem to require more time to manifest. Improvements in maximum stroke volume have been reported after 2–6 weeks of training in some (e.g. Warburton *et al.* 2004; Esfandiari *et al.* 2013; Bonne *et al.* 2014; Montero *et al.* 2015*a*) but not all training protocols (e.g. Macpherson *et al.* 2011; Jacobs *et al.* 2013).

The role of exercise intensity in mediating improvements in $\dot{V}_{O_2max}$. In a meta-analysis comparing the effects of interval and continuous training on $\dot{V}_{O_2max}$ in healthy adults, Milanovic *et al.* (2016) reported a greater response to interval training relative to continuous training whether training volume was equal or not. This conclusion is supported by an analysis from Bell & Wenger (1988), which demonstrated a linear improvement in $\dot{V}_{O_2max}$ as training intensity increased from 50 to 100% of $\dot{V}_{O_2max}$, and a meta-analysis from Bacon *et al.* (2013), which reported a greater increase in $\dot{V}_{O_2max}$ for high-intensity training relative to values typically reported in large studies of MICT. Similarly, a meta-analysis by Weston *et al.* (2014*a*) concluded that HIIT was more effective than work-matched MICT for improving $\dot{V}_{O_2max}$ in patients with lifestyle-induced cardiometabolic disease. Finally, a recent, large randomized control trial of different intensities of continuous exercise with obese adults supports these meta-analyses: greater increases in $\dot{V}_{O_2max}$ were demonstrated in response to 24 weeks of exercise performed at 75% of $\dot{V}_{O_2max}$ relative to isocaloric exercise performed at 50% of $\dot{V}_{O_2max}$, with differences apparent after 8 weeks of training (Ross *et al.* 2015).

The importance of exercise intensity in improving $\dot{V}_{O_2max}$ is further evident from comparisons of MICT and much lower volumes of SIT/HIIT. Low-volume SIT/HIIT performed for 2–16 weeks increased $\dot{V}_{O_2max}$ (Gist *et al.* 2013; Sloth *et al.* 2013; Weston *et al.* 2014*b*), with improvements elicited by the high-intensity protocols being equal to improvements from MICT protocols when compared (Gist *et al.* 2013). In agreement with these analyses, we recently demonstrated that low-volume SIT increased $\dot{V}_{O_2peak}$ to the same extent as MICT over a 12-week period, despite a fivefold difference in training volume (Gillen *et al.* 2016).

Relatively few studies have investigated changes in $\dot{V}_{O_2max}$ in response to different durations or frequencies of interval training; however, both variables appear to have relatively small effects on $\dot{V}_{O_2max}$ compared to the effect of exercise intensity. For example, the improvements in

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$\dot{V}_{O_2max}$ were not different in subjects who performed either one or four bouts of 4 min intervals (Tjønna *et al.* 2013) or either 2 or 4 days per week of interval training (Fox *et al.* 1975). In a series of classic studies for which subjects trained for 10 weeks and then continued to perform a lower volume of exercise for 15 weeks, Hickson and colleagues reported that maintaining intensity (Hickson *et al.* 1985) was more important to preserve the training-induced increase in $\dot{V}_{O_2max}$ than maintaining duration (Hickson *et al.* 1982) or frequency (Hickson & Rosenkoetter, 1981).

While the effect of interval training duration on $\dot{V}_{O_2max}$ is unclear, two meta-analyses suggested that longer interval bouts increased $\dot{V}_{O_2max}$ to a greater extent than shorter interval bouts (Bacon *et al.* 2013; Milanović *et al.* 2016). In contrast, Knuttgen *et al.* (1973) and Helgerud *et al.* (2007) both reported similar increases in $\dot{V}_{O_2max}$ when comparable volumes of high-intensity exercise were performed as short (15 s) or long (3–4 min) intervals.

The role of exercise intensity in cardiac output and blood volume responses to training. Despite the compelling evidence that intensity has a strong influence on the exercise-induced increase in $\dot{V}_{O_2max}$, relatively few studies are available to understand the effects of exercise intensity on maximum stroke volume and cardiac output or blood volume. In work-matched comparisons, maximum stroke volume increased more (Helgerud *et al.* 2007; Daussin *et al.* 2008; Bækkerud *et al.* 2016) or similarly (Warburton *et al.* 2004) following HIIT relative to lower intensities of continuous exercise; however, when assessed, changes in haematological parameters did not explain the differences in stroke volume between groups (Helgerud *et al.* 2007; Bækkerud *et al.* 2016). In contrast, the increase in maximum stroke volume in response to MICT was greater than or similar to the responses elicited by low-volume SIT (Macpherson *et al.* 2011) and low-volume HIIT (Esfandiari *et al.* 2013), respectively. It is tempting to suggest that, similar to the findings for $\dot{V}_{O_2max}$, high-intensity exercise has a greater effect on central adaptations than moderate-intensity exercise; however, it is difficult to draw strong conclusions on the importance of exercise intensity for eliciting cardiovascular adaptations based of the limited number of comparisons in healthy individuals, particularly given the heterogeneity in the subjects, training programmes and methods of the studies.

Conclusions

The relative importance of the intensity, duration and frequency of interval training has not been established for many key physiological adaptations to exercise. For skeletal muscle mitochondrial adaptations and $\dot{V}_{O_2max}$, exercise intensity mediates responses to training: relative to MICT, physiological adaptations to interval training are seemingly greater when training volumes are equal or similar when the volume of interval training is lower. For other physiological variables, the effect of intensity is unclear, and it is uncertain whether interval training is advantageous compared to MICT. Given the relative lack of data regarding the influences of exercise duration and training frequency on physiological adaptations to exercise, particularly for interval exercise, more research is needed to understand how these training variables impact peripheral and central adaptations to interval exercise. Specifically, we are unable to determine whether performing longer durations (i.e. a greater numbers of bouts per session) or greater frequencies of interval training would have beneficial effects for any of the variables in question. In summary, interval training is a powerful stimulus to elicit improvements in mitochondrial content and $\dot{V}_{O_2max}$; however, we know relatively little regarding the influences of exercise intensity, duration, and frequency on other components of the integrative physiological response to interval training.

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Additional information

Competing interests

The authors have no competing interest to declare.

Author contributions

M.M. and M.G. conceived of the review, identified and interpreted relevant studies for inclusion, wrote the manuscript, and critically revised the manuscript. Both authors approved of the final manuscript and agreed to be accountable for all aspects of the work. Both persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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