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REVIEW ARTICLE

Much Ado About Zone 2: A Narrative Review Assessing the Efficacy of Zone 2 Training for Improving Mitochondrial Capacity and Cardiorespiratory Fitness in the General Population

Kristi L. Storoschuk[1] · Andres Moran-MacDonald[1] · Martin J. Gibala[2] · Brendon J. Gurd[1]

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Abstract
Popular media has recently positioned Zone 2 training—defined as low-intensity exercise below the lactate threshold—as the optimal intensity for improving mitochondrial and fatty acid oxidative capacity, thereby supporting cardiometabolic health and chronic disease prevention. These recommendations largely stem from observational data of elite endurance athletes who engage in large volumes of Zone 2 training and possess high mitochondrial and fatty acid oxidative capacity. However, we challenge the broad endorsement of Zone 2 training for members of the general public, as it contradicts substantial evidence supporting the use of high-intensity exercise for improving mitochondrial capacity and cardiometabolic health. This narrative review critically examines the current evidence on Zone 2 training and mitochondrial and fatty acid oxidative capacity outcomes to assess the appropriateness for a public recommendation. We conclude that current evidence does not support Zone 2 training as the optimal intensity for improving mitochondrial or fatty acid oxidative capacity. Further, evidence suggests prioritizing higher exercise intensities (> Zone 2) is critical to maximize cardiometabolic health benefits, particularly in the context of lower training volumes.

1 Introduction

Skeletal muscle mitochondrial capacity (a broad term used to include common indices of mitochondrial content and function) is an important determinant of metabolic health and athletic performance [1, 2]. Mitochondrial capacity is also linked to the capacity for glucose and fatty acid oxidation (i.e., metabolic flexibility) [3, 4], as well as aging [2] and the pathophysiology of insulin resistance [5]. Exercise prescription to enhance mitochondrial capacity is generally modeled on elements of the Frequency, Intensity, Time, and Type (FITT) principle [6]. While all elements are important, the 'optimal' intensity to elicit mitochondrial responses has emerged as a topic of considerable debate.

An increasingly prominent narrative, advanced by influential health and fitness commentators including leading podcasters, is that Zone 2 training should be prioritized over higher exercise intensities to optimize improvements in mitochondrial capacity [7]. Specifically, the popular

Key Points

Zone 2 training is touted by influential commentators including on podcasts and in popular and social media as the optimal training intensity for improving mitochondrial and fat oxidative capacity, thereby supporting metabolic health and chronic disease prevention.

Our review failed to uncover substantive evidence supporting claims that Zone 2 is superior to higher exercise intensities for improving mitochondrial and fat oxidative capacity, a result possibly driven by the lack of studies explicitly examining Zone 2 training as it is commonly characterized.

Zone 2 may fall below the moderate- to vigorous-intensity range recommended by physical activity guidelines, and thus advising the general public to forgo higher exercise intensities in place of Zone 2 may limit the health benefits of exercise.

✉ Brendon J. Gurd
gurdb@queensu.ca

[1] School of Kinesiology and Health Studies, Queen's University, 28 Division St., Kingston, ON KZL 1A1, Canada

[2] Department of Kinesiology, McMaster University, Hamilton, ON L8S 4K1, Canada

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narrative claims "Zone 2 exercise intensity is the best at stimulating mitochondrial function and fat oxidation" [8]. Owing to the link between mitochondria and metabolic health, Zone 2 training is therefore purported to "play(s) a crucial role in preventing chronic disease by improving the health and efficiency of your mitochondria" [7]. Finally, Zone 2 prescriptions shared through popular media emphasize that exceeding Zone 2 intensity is to be avoided to achieve the unique benefits of Zone 2 on mitochondrial capacity [7]. Zone 2 in these contexts is generally referred to as low- to moderate-intensity exercise coinciding with: (1) the maximal rate of fat oxidation (Fat_{max}) [9]; (2) blood lactate concentration ([BLa]) just below the first lactate threshold (LT1; ~ 1.7–2.0 mmol/L; Fig. 1) [7]; and (3) the capacity to maintain a comfortable conversation (i.e., Talk Test [10]).

Proponents of Zone 2 training (as the term is generally defined above) commonly cite observations that high-level endurance athletes perform large volumes of low-intensity (i.e., Zone 2) training [11–15] and possess high mitochondrial and fatty acid oxidative (FAO) capacities [1]. However, conflating the training habits of endurance athletes and the optimal exercise dose for improving mitochondrial capacity in non-athletes may be misplaced for two reasons: (1) endurance athletes perform high volumes of both low- (Zone 2) and high-intensity training [12, 13, 16], making claims of a causal relationship between low-intensity training and mitochondrial capacity tenuous and (2) the total training volumes undertaken by endurance athletes, often > 20 h per week [17], are substantially greater than physical activity targets set by public health guidelines [18]. These caveats make it challenging to confidently infer that Zone 2 training is optimal for eliciting improvements in mitochondrial capacity, especially in populations performing total training volumes consistent with physical activity guidelines (i.e., ~ 150 min per week).

The advocacy for Zone 2 exercise over higher exercise intensities to improve mitochondrial capacity, and health, also contradicts experimental evidence and physical activity recommendations in exercise science. When compared with an equivalent volume of moderate-intensity exercise, high-intensity exercise (HIE) generally results in greater mitochondrial signaling and adaptations [19, 20], cardiorespiratory fitness (CRF) [21, 22], and other indices of cardiometabolic health [23, 24]. Further, the American College of Sports Medicine (ACSM) physical activity guidelines acknowledge that higher intensities may be *required* to improve CRF [6]. We do not question the health benefits of physical activity, which includes that done at low intensity or characteristic of Zone 2 exercise. However, given the known physiological and health benefits associated with HIE [25], the general consensus in exercise science that high intensities lead to greater health and fitness outcomes [26], and the widespread interest in Zone 2 training, a critical evaluation of claims that Zone 2 training is superior for improving mitochondrial capacity is needed.

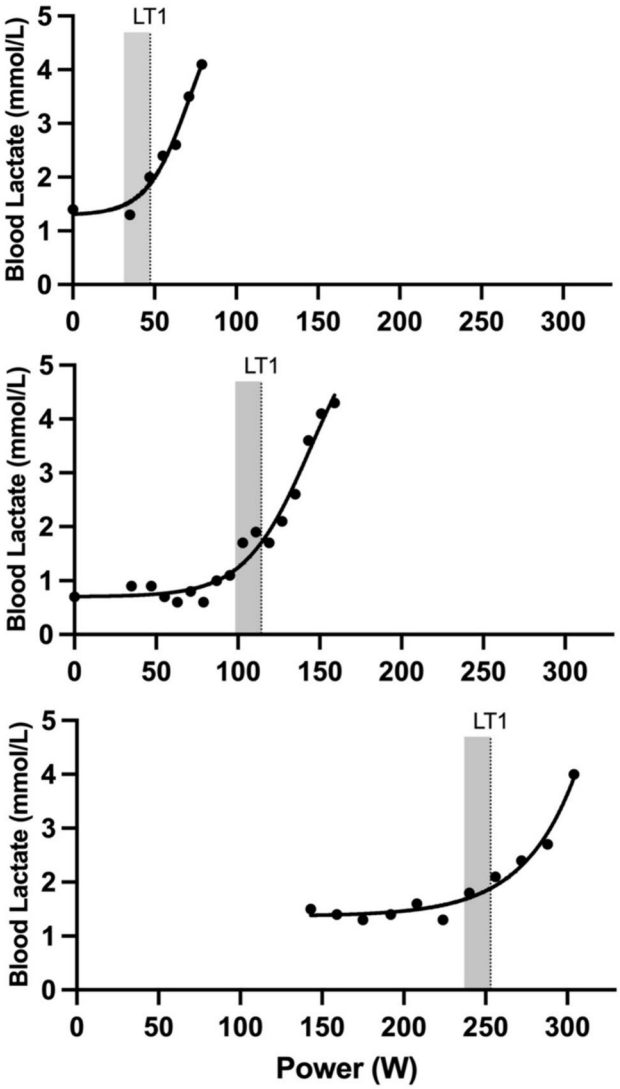

Fig. 1 Graphical representation of the first lactate threshold [LT1] (*dashed line*; [BLa] = 2.0 mmol/L) and corresponding Zone 2 (*shaded region*; blood lactate concentration [BLa] = ~ 1.7–2.0 mmol/L) derived from a graded exercise test. **A** Sex: female; age: 20 years; WR_{peak}: 204W; LT1: 23% WR_{peak}. **B** Sex: female; age: 18 years; WR_{peak}: 262W; LT1: 45% WR_{peak}. **C** Sex: male; age 24 years; WR_{peak}: 441W; LT1: 57% WR_{peak}. Data obtained from our laboratory during a graded cycling test. Briefly, participants A and B began cycling at a load-less intensity followed by a step increase to 36 watts (W) for an additional 5 min and subsequent 8-W increments every 5 min until [BLa] reached 4.0 mmol/L. Participant C's test began at 33% WR_{peak} and increased by 16 W every 3 min until [BLa] reached 4.0 mmol/L. WR_{peak}: highest average 30-s average power (W) achieved during an incremental cycling test consisting of 1-min increases of 24 W/min until volitional fatigue beginning at 80 W

Thus, the purpose of this narrative review is to critically evaluate the evidence supporting the efficacy of Zone 2

training for improving mitochondrial capacity in the general population (i.e., non-endurance-trained individuals who are insufficiently active or meeting physical activity guidelines). We focus on two foundational claims regarding Zone 2 training: (1) Zone 2 training is optimal for improving mitochondrial capacity and (2) Zone 2 training is optimal for improving FAO capacity. As Zone 2 training is ultimately recommended for reducing the risk of chronic disease, we additionally address whether Zone 2 training is optimal for improving health and fitness.

2 Methods

2.1 Literature Search

A systematic literature search was not utilized to obtain Zone 2 exercise studies. Articles were obtained by searching databases using search terms related to "low-intensity exercise," "endurance training," "continuous training," "lactate threshold", "ventilatory threshold," and "Fat_{max}". We used additional articles from reference lists, including relevant systematic reviews, articles shared through social media, and relevant literature known to authors.

2.2 Inclusion Criteria

For the current review, because most definitions of Zone 2 place it within the moderate-intensity domain, and we were able to find few studies that explicitly prescribed Zone 2 exercise, we considered exercise performed at intensities below LT1, or demonstrating physiological responses consistent with the moderate-intensity domain ([BLa] < 2.0 mmol/L, below ventilatory threshold 1, below Fat_{max}, < 45% maximum rate of oxygen consumption [$\dot{V}O_2max$]) when assessing the evidence regarding the potential benefits of Zone 2. It is important to note that there are many definitions of LT1 and methods for assessing the threshold [27]. We chose the 2.0-mmol/L threshold because it is a widely used threshold for determining LT1 [27, 28] and the definition most commonly cited within social/popular media [7]. Studies that did not meet these criteria were generally not included in our evaluation of the acute responses and chronic adaptations to Zone 2 training. For the sake of brevity, we have not provided specific detail of studies we judged to be "Zone 2 exercise" within the text of our review; however, full exercise prescription details for all studies discussed below are included in Table 1 of the Electronic Supplementary Material.

3 Defining Zone 2 Training

In a performance context, exercise intensity is divided into three domains, moderate, heavy, and severe, each exhibiting distinct physiological responses [29, 30]. Notably, this characterization and the associated terminology differs somewhat from the classification system commonly used in physical activity and exercise prescription guidelines [31]. The moderate-intensity domain, typically defined as exercise below LT1 [32], is characterized by a relatively high reliance on FAO, relatively low rates of glycogen depletion [33, 34], adenosine monophosphate (AMP)/adenosine diphosphate (ADP) accumulation [35], and phosphocreatine (PCr) breakdown [33, 36], and a mono-exponential increase in oxygen consumption [29, 37–39]. Zone 2 training, based on the definition provided via popular media as well as the low-intensity training practices of endurance athletes [11, 16, 31, 40], positions Zone 2 exercise within the moderate-intensity domain.

The upper boundary of Zone 2 (i.e., the threshold between the moderate- and heavy-intensity domain) ranges from ~ 24% to 80% of $\dot{V}O_2max$ depending on fitness and training status [34, 41–44]. Exercise within Zone 2 can thus range from an approximately four-fold resting metabolic rate in sedentary individuals (e.g., walking at a normal pace or cycling at – or well below – 100W) [45–47] or exceed an approximately ten-fold resting metabolic rate in endurance athletes (e.g., cycling at ~ 300 watts [W] for an endurance athlete) [46]. These data demonstrate that the absolute intensities associated with Zone 2 can be vastly different between athletes and members of the general public.

4 Does Zone 2 Training Improve Mitochondrial Capacity?

Remodeling and expansion of the mitochondrial reticulum, increased mitochondrial capacity, and improved maintenance of intracellular energy homeostasis are classic adaptations to endurance training (ET) [48, 49]. Adaptive responses to training in muscle are triggered by contraction-induced increases in the AMP/ADP:adenosine triphosphate (ATP) ratio, intramuscular calcium ($[Ca^{2+}]_i$), reactive oxygen species, and redox balance (NAD^+:NADH) [50] and the subsequent activation of cellular signaling molecules that include AMP-activated protein kinase (AMPK) and calcium/calmodulin serine/threonine kinase (CaMKII) [51]. In the following sections, we review the available literature examining the impact of Zone 2 exercise on mitochondrial biogenesis by focusing on: (1) intramuscular signals; (2) signaling response and gene expression; and (3) mitochondrial

capacity. Because long-duration low-intensity exercise has also been proposed to act primarily via calcium signaling [52], we also distinctly address the impact of Zone 2 training on the calcium signaling pathway.

4.1 Impact of Zone 2 on Intramuscular Metabolites

The available evidence demonstrates minimal changes in the muscle AMP/ADP:ATP ratio and/or indicators of energetic stress (e.g., reduced muscle [PCr] and increased muscle [lactate]) following Zone 2 exercise. For example, 200 min of Zone 2 exercise does not change ATP, ADP, or AMP in muscle of untrained adults [53]. The AMP:ATP ratios were also unaltered during and following Zone 2 exercise performed to exhaustion in young active men [54]. However, 2 h of Zone 2 exercise can induce small but statistically significant increases in the AMP/ADP:ATP ratio in endurance-trained men [55].

Decreases in [PCr], driven by increases in intracellular [ADP] [56], are sometimes absent during Zone 2 exercise [57]. However, declines in muscle [PCr] occur following both short (5 min) and long (120–211 min) durations of Zone 2 exercise [33, 54, 55, 58, 59]. Small increases in muscle lactate concentrations, indicative of elevated AMP/ADP and increased rates of glycolytic flux, are sometimes [55, 59] but not always [33, 53, 58] observed during Zone 2 exercise. Interestingly, and consistent with classic demonstrations of glycogen oxidation during low-intensity exercise [60, 61], prolonged Zone 2 exercise (2–3.5 h) decreases muscle glycogen [33, 53–55]. Because glycogen depletion is a mediator of AMPK activation [62], these results raise the possibility that Zone 2 may activate AMPK in the absence of large increases in AMP and/or ADP.

To our knowledge, changes in cellular redox potential and reactive oxygen species production during Zone 2 exercise have not been reported. Thus, our understanding of intramuscular signals in response to Zone 2 exercise is limited to relatively small and/or inconsistent changes in AMP/ADP, PCr, lactate/glycolysis, and intramuscular glycogen.

4.2 Impact of Zone 2 on Mitochondrial Biogenic Signaling and Gene Expression

The activation of AMPK in response to increased AMP/ADP [63], and potentially reduced muscle glycogen [62], is a primary signaling pathway involved in the initiation of mitochondrial biogenesis [64–66]. Exercise intensities that do not impose energetic disturbances (i.e., no change in AMP/ADP) appear to not increase AMPK signaling [35]. Thus, the negligible-to-small changes in the AMP/ADP:ATP ratio in response to Zone 2 exercise described above suggest that Zone 2 exercise may be below the intensity required to activate AMPK. In agreement with this suggestion, Zone 2 exercise does not increase AMPK activity in endurance-trained men [55]. Zone 2 exercise also failed to alter the phosphorylation of AMPK or class II histone deacetylases, downstream targets of AMPK [67–69]. Conversely, although AMPK activity was unchanged following 2 h of Zone 2, it was elevated at exhaustion (~3.5 h) [54]. Interestingly, the phosphorylation of acetyl-CoA carboxylase, another downstream target of AMPK, increased 1 h into Zone 2 exercise before returning to baseline levels at later timepoints [54]. Similar increases in phosphorylation of acetyl-CoA carboxylase, in the absence of increased p-AMPK, were also observed following 65 min of exercise just above Zone 2 [69].

The effect of Zone 2 exercise on PGC-1α, a downstream target of AMPK and a key transcriptional regulator of mitochondrial biogenesis [64], is unclear. No change in PGC-1α gene expression was reported following 30 min [68, 70] and ~90 min [70] of Zone 2 exercise. Popov et al. also failed to observe changes in mitochondrial transcription factor A, mitochondrial transcription factor B2, as well as the mitochondrial genes citrate synthase (CS) and cytochrome C oxidase subunit II [68]. The well-trained status ($\dot{V}O_2$max of 59 mL/min/kg) of participants in this study may partly explain the absence of a mitochondrial biogenic response. Supramaximal intensities may be required to induce mitochondrial adaptations in well-trained populations [71]. In contrast, increases in PGC-1α messenger RNA expression — and additional genes involved in mitochondrial biogenesis — can occur following Zone 2 exercise [67–69, 72]. Duration-mediated effects have been reported for PGC-1α gene expression with 60 and 90 min, but not 30 min of Zone 2 increasing expression [68]. In the only report examining the impact of Zone 2 on muscle protein synthesis we are aware of, a single bout of exercise failed to increase rates of mitochondrial protein synthesis [73].

Although the collective evidence is mixed (Fig. 2), Zone 2 exercise does appear capable of activating signaling pathways that initiate mitochondrial adaptations. However, future research is required to further clarify the impact of Zone 2 exercise on mitochondrial signaling pathways, including those beyond AMPK (e.g., sirtuin 1 and P38 mitogen-activated protein kinase).

4.3 Does Zone 2 Training Improve Mitochondrial Capacity Via Calcium Signaling?

High volumes of low-intensity/Zone 2 training are proposed to induce mitochondrial adaptations through calcium signaling, while high-intensity training acts through differential mechanisms (i.e., AMPK signaling) [11, 52, 74]. This contention appears to be based on the theory [52] that repeated muscle contractions increase $[Ca^{2+}]_i$ and activate

mitochondrial biogenesis via CaMKII [75]. However, there is strong evidence that HIE activates both AMPK and CaMKII signaling pathways [20]. Thus, while Zone 2 exercise may activate mitochondrial adaptations primarily via CaMKII signaling, the idea that HIE initiates mitochondrial adaptations via distinct signaling pathways (i.e., only AMPK) is not supported by available evidence.

We are unaware of studies examining changes in $[Ca^{2+}]_i$ during or following Zone 2 exercise. There also appear to be few studies that have measured calcium signaling in response to Zone 2 exercise. Of the limited studies we could find, 65–70 min of Zone 2 training failed to increase p-CaMKII [67, 69]. However, mixed results are reported for downstream targets of CaMKII with phosphorylation of cyclic-AMP response element and p38 mitogen-activated protein kinase being increased [67] and unchanged [68, 69], respectively. Thus, it is unclear if or how Zone 2 training acutely activates calcium signaling.

A lack of data does not disprove the contention that elite endurance athletes benefit from high volumes of low-intensity exercise via calcium-mediated adaptation [74]. However, recommendations that the general population forgo higher intensity exercise in favor of Zone 2 appear to be largely based on an unsubstantiated theory. Further research is needed to comprehensively define the impact of Zone 2 training on calcium signaling and elucidate the importance, if any, of calcium signaling in Zone 2-mediated mitochondrial adaptations in both endurance athletes and the general population.

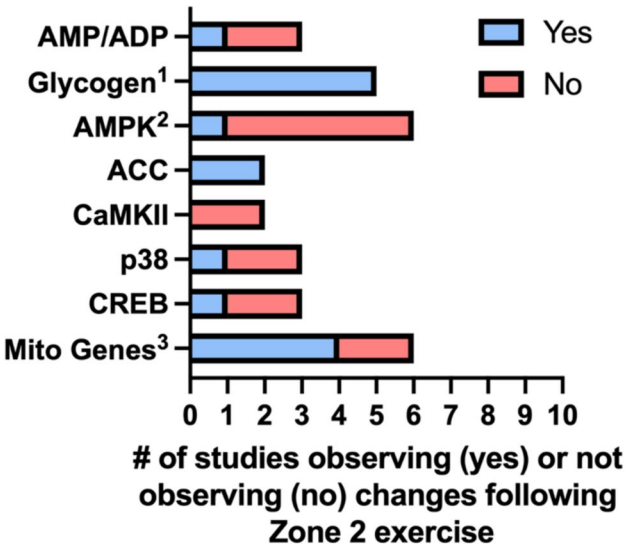

Fig. 2 Changes in intramuscular signals and mitochondrial biogenic signaling in response to Zone 2 exercise. Blue (yes) indicates an increase in the indicated outcome. Red (no) indicates no change or decrease in the indicated outcome. [1]Glycogen depletion observed with exercise > 120 min. [2]Wojtaszewski et al., included for both "No" (2 h) and "Yes" (3.5 h) [54]. [3]Popov et al., included for both "No" (30 min) and "Yes" (60 min, 90 min) [68]. *ACC* acetyl-CoA carboxylase, *ADP* adenosine diphosphate, *AMP* adenosine monophosphate, *AMPK* AMP-activated protein kinase, *CaMKII* calcium/calmodulin serine/threonine kinase II, *CREB* cyclic AMP response element-binding protein

4.4 Does Zone 2 Training Improve Mitochondrial Capacity?

Few studies have explicitly investigated the impact of Zone 2 training on mitochondrial outcomes and the available evidence is mixed. In support of Zone 2 training improving mitochondrial capacity, 10 weeks of twice-weekly training at an exercise intensity corresponding to FAT_{max}—possibly above Zone 2 as discussed below—increased CS activity and mitochondrial respiration in obese individuals with type 2 diabetes mellitus [76]. Additionally, 12 weeks of Zone 2 cycling three times-weekly low-intensity cycling that is characteristic of Zone 2 improved PCr recovery rates—a marker of mitochondrial capacity assessed via magnetic resonance spectroscopy—in both healthy male individuals and male individuals with type 2 diabetes [77]. In contrast, several studies do not support the ability of Zone 2 training to improve mitochondrial capacity. Four weeks of Zone 2 training did not increase CS activity or mitochondrial respiration in recreationally active men [78]. Five months of primarily (86% of training volume) Zone 2 training 7 days/week also failed to improve CS activity or succinate dehydrogenase activity in elite endurance athletes [79]. Finally, although exercise intensity was not confirmed with blood lactate measures, a 42-day skiing expedition requiring 6 h/day at ~ 60% maximum heart rate (HR_{max}) [a daily duration that strongly suggests Zone 2 intensity] reduced CS activity and mitochondrial respiration [80]. Similarly, following a 50-day ski expedition involving 5.5 h/day skiing at ~ 45% $\dot{V}O_2$max, increases in CS were limited to arm muscles, with no effect observed in legs [81]. Importantly, results from a detailed meta-analysis of exercise intensity and mitochondrial adaptations suggest that exercise performed below 60% maximum work rate (an intensity likely equivalent to or above Zone 2 in most non-endurance-trained individuals) is not expected to improve mitochondrial content or mitochondrial respiratory capacity [19].

4.5 Is Zone 2 Training Optimal for Improving Mitochondrial Capacity?

Despite ongoing debate surrounding the role of intensity as a key mediator of exercise-induced mitochondrial adaptations [82, 83], it is well established that high-intensity interval

training induces robust mitochondrial adaptations [25, 84, 85]. Exercise above Zone 2 results in greater changes in the AMP/ADP:ATP ratio [35, 86], declines in [PCr] [33, 55, 58, 59], increases in intramuscular lactate, and decreases in pH [33, 59]. Although long durations (~ 120–211 min) of Zone 2 exercise result in glycogen depletion [33, 55], rates of glycogen depletion increase with increasing exercise intensity [60, 61, 87].

Downstream mitochondrial signaling pathways are also activated to a greater extent following exercise intensities above Zone 2 [67, 69, 88]. Some studies demonstrate that *only* exercise performed above Zone 2 results in increased AMPK activation [55, 67, 69] and mitochondrial biogenic signaling is rapidly activated by HIE [89–91]. Furthermore, phosphorylation of phospholamban, a downstream target of CaMKII, does not increase following low- to moderate-intensity exercise (35% and 60% $\dot{V}O_2$peak), but does increase with HIE (85% $\dot{V}O_2$peak) [92]. Greater activation of CaMKII also occurs in response to "all out" sprints than 50 min of continuous exercise at 70% $\dot{V}O_2$max [89]. Compared with Zone 2, work-matched exercise performed above Zone 2 induces greater increases in PGC-1α gene expression and additional mitochondrial genes [70, 72]. Further, 5 months of high-intensity, but not Zone 2 training, increases succinate dehydrogenase activity in elite endurance athletes [79].

It is important to highlight that moderate-intensity continuous training (MICT) can induce improvements in mitochondrial capacity. For example, a recent meta-analysis showed improvements in mitochondrial content following ET that were only slightly less than those induced by higher intensity interval training [71]. However, because this meta-analysis defined ET as training conducted below an intensity equivalent to the second ventilatory threshold (i.e., below the severe-intensity domain), it remains unclear whether all ET, including that characteristic of Zone 2—or only that performed in the heavy-intensity domain (i.e., above Zone 2)—improves mitochondrial capacity. Results from another meta-analysis of 56 training studies suggest that exercise performed below 60% maximum work rate (an intensity likely equivalent to or above Zone 2 in most non-endurance-trained individuals) is not expected to improve mitochondrial content or mitochondrial respiratory capacity [19]. This meta-analysis also suggests that HIE (> 90% maximum work rate) and sprint interval training are most effective for increasing mitochondrial respiratory capacity [19, 82]. These results suggest that exercise performed above Zone 2 may be superior for inducing mitochondrial adaptations, directly contradicting the notion that Zone 2 training is the optimal intensity for such outcomes. It may be that the high mitochondrial capacity of elite endurance athletes is more related to their training spent above Zone 2 rather than their large volumes of Zone 2 training per se.

5 Does Zone 2 Training Improve Fatty Acid Oxidative Capacity?

Increased FAO capacity is a well-established adaptation to ET [48, 93]. Higher FAO reduces reliance on carbohydrate metabolism [50, 94, 95], is positively associated with athletic performance [96, 97], and correlates with greater cardiometabolic health [46, 98]. Thus, interventions that improve FAO capacity are of interest to athletes targeting endurance performance and to members of the general public seeking to prevent and/or treat cardiometabolic disease.

Zone 2 training has been positioned as the optimal intensity for improving FAO capacity and cardiometabolic health. This section first reviews studies examining the impact of Zone 2 training on the mechanism underlying improvements in FAO before addressing the question of whether Zone 2 training improves FAO capacity.

5.1 Impact of Zone 2 Training on Determinants of Fatty Acid Oxidative Capacity

Improved FAO following training is related to increases in: (1) mitochondrial capacity [97, 102]; (2) skeletal muscle capillary density [103, 104]; (3) intramuscular triglyceride (IMTG) storage and breakdown [105, 106]; (4) proportion of oxidative type I muscle fibers [104, 107]; and (5) enzymes involved in lipid metabolism and transport [97, 108, 109]. Because the impact of Zone 2 training on the induction of mitochondrial biogenesis and changes in mitochondrial capacity is limited (discussed in Sect. 4), any improvements in FAO capacity in response to Zone 2 training would presumably occur through alternative mechanisms (i.e., mechanisms 2–5 above).

Capillary density increases after 6 weeks of Zone 2 training in untrained non-obese men [110], whereas no change in skeletal muscle capillary density was observed after 42 days of large volumes of daily exercise at 60% HR_{max} [80]. No change in capillary density was also observed in leg muscles after 50 days of large volumes of skiing at an intensity equivalent to Zone 2, but was found in arm muscles [81]. Importantly, a recent meta-analysis concluded that low-intensity ET, likely encompassing Zone 2, does not increase capillary density or the capillary-to-fiber ratio [111]. We were unable to find studies directly investigating the impact of Zone 2 training on IMTG breakdown. However, low-intensity training characteristic of Zone 2 training tended to increase intramyocellular lipid content in male individuals with type 2 diabetes [77], and utilization of non-plasma fatty acid oxidation (which includes IMTGs) increased after 12 weeks of Zone 2 training in women [112] and men [113] with obesity. We found mixed results for the impact of Zone 2 training on type I (oxidative) muscle fiber percentage, with

one study demonstrating a positive effect (42 days of high-volume training at 60% HRmax [80]) and another failing to observe an effect after 6 weeks of Zone 2 training [110]. Similar to changes in capillary density, IMTGs, and fiber distribution, the impact of Zone 2 training-mediated changes on enzymes involved in the transport and utilization of fatty acids is equivocal. Four weeks of Zone 2 training induced non-significant ($p = 0.07$) increases in resting skeletal muscle lipoprotein lipase activity [114] and 42 days of training at 60% HRmax failed to increase hydroxyacyl-CoA dehydrogenase (HAD) [80]. Although 50 days of large volumes of skiing at an intensity equivalent to Zone 2 (45% $\dot{V}O_2$max) training increased HAD in arm muscles, Zone 2 training did not increase HAD in leg muscles [81]. Finally, 4 months of Zone 2 training did not change the protein content of collagen type I receptor (CD36), a key fatty acid transporter [115]. Thus, although Zone 2 can improve intramuscular determinants of FAO capacity, the literature in this area is limited and equivocal.

5.2 Does Zone 2 Training Improve Fatty Acid Oxidative Capacity?

Fatty acid oxidative capacity (typically calculated from the respiratory exchange ratio) is quantified as either the maximal rate of fatty acid oxidation (MFO) and/or the exercise intensity associated with MFO (FAT_{max}) [101, 116]. We were surprised to find only one study that measured rates of FAO following confirmed Zone 2 training ([BLa] < 2.0 mmol/L). This study demonstrated increased FAT_{max} and MFO in previously sedentary adults following 1 year of Zone 2 training [117]. Similarly, albeit in studies where [BLa] was not assessed, 12 weeks of cycling at 40% $\dot{V}O_2$max reduced the exercise respiratory exchange ratio and increased total fat oxidation rates in men with obesity [113] and in women with lower, but not upper, body obesity [112]. Although failing to reach significance ($p = 0.06$), a tendency for increased rates of fat oxidation was also observed in healthy non-obese men following 12 weeks of training at 40% $\dot{V}O_2$max [114]. Although maximal mitochondrial respiratory capacity, ex vivo quantification, and mitochondrial content decreased, the contribution of fatty acid substrates to maximal mitochondrial respiration increased after 42 days of training at 60% HR_{max}, possibly suggesting improved FAO capacity [80].

In addition to the above data supporting the ability of Zone 2 and/or very low intensity training to improve FAO capacity, there is a body of work utilizing FAT_{max} as an anchor for exercise prescription. It is important to note that although Zone 2 occurs at FAT_{max} in healthy active populations [118], prescribing exercise at FAT_{max} in sedentary populations may result in exercise above Zone 2 [119]. Thus, studies prescribing exercise anchored to FAT_{max} may not necessarily support/refute effects of Zone 2 training per se. Nonetheless, 2–12 weeks of training at FAT_{max} in overweight/obese adults increases MFO [120], increases FAT_{max} [76, 120–122], and lowers the respiratory exchange ratio during exercise [76, 123]. Similarly, 10–12 weeks of training at FAT_{max} improves FAT_{max} and increases fat oxidation rates during exercise in obese men with type 2 diabetes [76] and insulin-resistant men [121]. Thus, Zone 2 training does appear to increase fat oxidative capacity, although increases are likely limited to sedentary/untrained populations.

5.3 Is Zone 2 Training Optimal for Improving Fat Oxidative Capacity?

The evidence above suggests that Zone 2 training can increase FAO capacity in overweight, obese, and type 2 diabetic individuals. That said, this is also true of endurance exercise training at intensities greater than Zone 2 [93, 124–126]. However, unlike the body of evidence supporting exercise intensity-dependent improvements in mitochondrial capacity, the optimal training intensity for improving FAO capacity is less clear [116].

Regarding the mechanisms of FAO capacity besides mitochondrial capacity, 12 weeks of Zone 2 training, but not higher intensity training, increases non-plasma FAO during exercise in obese men [113], suggesting that Zone 2 training may be superior for increasing the contribution of IMTG to FAO during exercise. Conversely, a recent meta-analysis revealed that only exercise intensities above 50% $\dot{V}O_2$max are expected to increase capillarization in sedentary subjects [111], suggesting that Zone 2 training in some individuals may fall below the threshold for inducing increases in capillarization. Finally, although no direct comparisons to Zone 2 training have been made, HIE increases IMTG storage and oxidation during submaximal exercise [127, 128] and enzymes and proteins involved in fatty acid transport and oxidation including CD36 [126, 129, 130], carnitine palmitoyl transferase-1 [126, 131], $FABP_{pm}$ [129, 130, 132], and β-HAD [129, 132, 133].

Training studies comparing exercise intensities above Zone 2 to those possibly equivalent to Zone 2 (FAT_{max} and < 45% $\dot{V}O_2$max) yield equivalent effects on FAO with some studies favoring Zone 2 training [113, 123, 134] and others higher exercise intensities [120, 135]. Two recent meta-analyses reported no differences in FAO capacity following high-intensity interval training and MICT [136], and small, but greater improvements with high-intensity interval training/sprint interval training compared with MICT [137] in obese and overweight adults but not normal weight adults. Importantly, it is likely that many of the MICT protocols including in these meta-analyses utilized intensities above Zone 2, highlighting the need for studies directly comparing Zone 2 to HIE.

An older meta-analysis demonstrating no effect of Zone 2 training on LT1—a proxy for FAO capacity [46, 118, 119] — suggests Zone 2 training may produce too minimal of a training stimulus to improve FAO capacity in trained individuals [138]. This meta-analysis suggests that trained individuals require high exercise intensities to improve LT1. At present, it remains unclear if there is an optimal exercise prescription for improving FAO capacity, and we are unaware of convincing data to support the contention that Zone 2 training produces greater increases in FAO capacity than higher exercise intensities.

6 Is Zone 2 Training Optimal for Health and Fitness?

Proponents of Zone 2 cite improvements in mitochondrial capacity as a main driver of improved cardiometabolic health [7, 100]. However, although mitochondrial capacity is linked to performance and cardiometabolic health [1–3], its links to cardiovascular disease and all-cause mortality risk are less established. Conversely, the evidence linking maximal aerobic capacity ($\dot{V}O_2$max; CRF) with cardiometabolic disease and all-cause mortality risk is robust [139–145].

When impacts of Zone 2 and higher intensities of exercise on CRF are compared in untrained populations, there is no difference in improvements [146–153], greater improvements with higher exercise intensities [113, 120, 154–156], or only improvements with higher exercise intensities [114, 135]. Similarly, CRF only increases with training intensities above Zone 2 in healthy active [157, 158] and trained athletes [159–161]. Furthermore, for a fixed amount of exercise, higher exercise intensities result in greater improvements in CRF [21, 154] and additional markers of cardiometabolic health (e.g., glucose tolerance) [23].

It is important to note that although zone-based training is used by athletes and coaches for optimizing performance, governing bodies targeting health in the general public traditionally do not ascribe to zone-based exercise prescriptions (see Coates et et al. [31] for a comprehensive summary and comparison of many classification models). Rather, public health exercise and physical activity recommendations classify exercise intensities as very light, light, moderate, vigorous, and near-maximal/maximal intensity exercise [162, 163]. The ACSM guidelines recommend accumulating a minimum of 150 min per week of moderate-intensity physical activity (3–5.9 metabolic equivalent of tasks [METs]) [6]. Because Zone 2 exercise can represent a wide range of relative and absolute exercise intensities (Fig. 1), with a potential range from light to vigorous depending on population fitness and training status, it is possible that Zone 2 training in unfit populations could involve exercising at an intensity that fails to meet the minimum intensity recommended by ACSM physical activity guidelines [31]. Importantly, the ACSM guidelines recognize that 150 min per week of moderate-intensity exercise (e.g., Zone 2) may not be sufficient for improving CRF, suggesting that exercise intensity and/or duration be augmented when improvements in CRF are targeted [6].

Thus, despite Zone 2 training being positioned as the optimal exercise intensity for reducing the risk of chronic disease via its proposed benefits on mitochondrial health and FAO capacity [7, 100], the available evidence refutes the use of Zone 2 training as the optimal intensity for improving CRF—one of the strongest predictors of cardiometabolic disease risk [141]. Further, prioritizing higher intensities is unlikely to jeopardize mitochondrial benefits as exercise training protocols that increase $\dot{V}O_2$max also increase mitochondrial and FAO capacity [41, 104, 129, 164–166]. Thus, prioritizing HIE appears critical when designing exercise programs to improve CRF and reduce chronic disease risk, especially when training time is limited. Most importantly, given the clear intensity-dependent effect of exercise on CRF, members of the general public who replace HIE with Zone 2 exercise may risk minimizing the benefits of exercise on long-term health.

7 Summary and Conclusions

Evidence from acute studies demonstrates small and inconsistent activation of mitochondrial biogenic signaling following Zone 2 exercise. Further, the majority of the available evidence argues against the ability of Zone 2 training to increase mitochondrial capacity, a fact that refutes the current popular media narrative that Zone 2 training is optimal for mitochondrial adaptations. Left unchecked, the recommendations for members of the general public to prioritize Zone 2 training over higher exercise intensities—including continuous training in the heavy-intensity domain—limits potential improvements in mitochondrial capacity gained from accumulating volumes above Zone 2. Many gaps remain in our understanding of whether and how mitochondria adapt to Zone 2 training and more research directly studying the impact of Zone 2 training on mitochondrial capacity is required.

Zone 2 does appear to improve FAO capacity in untrained populations; however, pooled analyses suggest that higher exercise intensities may be favorable in untrained and potentially required in trained individuals. Importantly, major gaps in the literature exist, including the mechanisms by which Zone 2 training improves FAO and whether Zone 2 training improves FAO capacity across populations with different training and health status. We provide evidence that both low and high exercise training intensities improve FAO capacity, aligning with

converging mechanisms to explain improvements in FAO capacity in response to different exercise intensities. Thus, claims that Zone 2 training is optimal for improving FAO capacity are not supported by strong evidence and it does not appear that Zone 2 training elicits unique benefits for FAO capacity that cannot be achieved by HIE.

A major finding of the current review is that few studies have explicitly examined the effect of Zone 2 training. We therefore made our best attempt to include papers that aligned with the definition of Zone 2 as exercise within the moderate-intensity domain when writing this review. This limitation highlights the need for studies specifically designed to test hypotheses around Zone 2. For members of the general public attempting to meet physical activity guidelines, we believe prioritizing higher exercise intensities is critical to improve health [145, 167].

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Conflicts of Interest/Competing Interests Martin J. Gibala is an advisor to and holds equity in Longevity League, Ltd., a US-based company whose services in part relate to exercise. Kristi L. Storoschuk, Andres Moran-MacDonald, and Brendon J. Gurd have no conflicts of interest that are directly relevant to the content of this article.

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Authors' Contributions KLS, MJG, and BJG conceived the idea for this review, KLS and BJG wrote the first draft, and AMM and MJG contributed to the writing of subsequent drafts and provided helpful suggestions and edits. All authors read and approved the final version.

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