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Narrative Review

Effect of caffeine on mitochondrial biogenesis in the skeletal muscle – A narrative review

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SUMMARY

Caffeine is one of the most widely used substances as recreational drug for performance-enhancement in sport, underpinned by a strong evidence base. Although the effects of caffeine are widely investigated within the scope of performance physiology, the molecular effects of caffeine within skeletal muscle remain unclear. Evidence from *in vitro* and *in vivo* models suggest that caffeine regulates the glucose metabolism in the skeletal muscle. Moreover, caffeine seems to stimulate CaMKII, PPARδ/β, AMPK and PGC1α, classical markers of exercise-adaptations, including mitochondrial biogenesis and mitochondrial content. This review summarizes evidence to suggest caffeine-effects within skeletal muscle fibers, focusing on the putative role of caffeine on mitochondrial biogenesis to explore whether caffeine supplementation might be a strategy to enhance mitochondrial biogenesis.

1. Introduction

Caffeine (1,3,7-trimethylxanthine) is a popular ergogenic substance that is widely utilized as a method to improve athletic performance [1] increase alertness [2] and accelerate metabolism [3]. Alongside these well-established effects on health and performance, recent scientific studies have focused on potential for caffeine to enhance exercise training adaptations [1], potentially via mitochondrial biogenesis [4].

Mitochondrial biogenesis within muscle is associated with health; for instance, age-associated reductions in muscle function occur with a concurrent decrease in mitochondrial content [5]. Additionally, mitochondrial biogenesis is an important adaptive component to aerobic exercise, driving many of the positive health outcomes associated with this exercise type [6]. Accordingly, the development of strategies to support and improve mitochondrial biogenesis is potentially important for the maintenance of quality of life in the context of aging, and to physical performance at all ages. As such, the purpose of this review is to explore whether caffeine supplementation might be a strategy to enhance mitochondrial biogenesis.

2. Mitochondrial biogenesis

Mitochondrial biogenesis is a fine-tuned, regulated process that requires the coordination of various cellular events, including coordinated transcription of two genomes (nuclear and mitochondrial), the synthesis of lipids and proteins [7], with isolated or combined biosynthesis enzymes [8], and increased volume densities in the human muscle [9]. These complex events induce an expansion of the total muscle mitochondrial volume and the enlarged mitochondrial components and ability of the cell to match ATP production with ATP hydrolysis [7].

Although, there is a lack of consensus on how to evaluate mitochondrial biogenesis [10–13], mitochondrial biogenesis is coupled with other processes such as mitochondrial remodeling (fusion and fission), as well as catabolic events as acting as

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mitochondrial protein breakdown, mitophagy and apoptosis (see Refs. [5,14–16]. To comprehensively evaluate mitochondrial biogenesis, it is necessary to couple measurements of the synthesis rates of mitochondrial proteins along with abundance of transcriptional factors for mitochondrial genes and the mRNAs encoding mitochondrial proteins. Of the nearly 1200 proteins that build mitochondria, mitochondrial DNA (mtDNA) is only responsible for the transcription only of 13 genes [17].

The study of this biological feature, requiring the finely tuned coordination of both mtDNA and nDNA genomes to regulate organelle content, has offered considerable insight into the understanding of mitochondrial remodeling. Although there are other transcription of mitochondrial genes, the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is considered master regulator of mitochondrial biogenesis [18]. Discovered for the first time in 1998 by Puigserver and coworkers, PGC-1α was found in abundance in brown adipose tissue and in skeletal muscle of mice allocated in cold conditions (4 °C) [19]. PGC-1α and its family members, PGC-1β and PGC-related coactivator, work together to increase gene transcription by docking with transcription factors and additional proteins on DNA promoters to control nuclear genes encoding mitochondrial proteins [20]. PGC-1α is involved in many metabolic processes, including liver gluconeogenesis, thermogenesis, and fiber-type specialization in skeletal muscle [18]. PGC-1α expression is stimulated by different conditions, such as nutrient starvation, hypoxia, oxidative stress, cyclic adenosine monophosphate activation, physical exercise and or pharmacological manner [21]. Specifically, physical exercise triggers an array of signals that direct PGC-1α in the muscle. Such signals include the activation of calcium/calmodulin-dependent protein kinase (CaMK) through increased intracellular Ca^{2+} concentration [22], and activation of p38 mitogen-activated protein kinase (p38 MAPK), which is sensitive to a variety of stressors including reactive oxygen species (ROS) [23]. When exercising, the AMP to ATP ratio is increased within the muscle, modulating the energy status of the cell and thus activating AMP-activated protein kinase (AMPK). AMPK activation results in direct phosphorylation of PGC-1α, which appears to enhance its transcriptional activity [24]. Additionally, AMPK activation enhances PGC-1α promoter activity [25]. The activation of PGC-1α can also interact with transcription factors, such as nuclear respiratory factor (NRF)-1/2, which induce the expression of mitochondrial transcription factor A (Tfam) [26] and also, different sirtuins [27,28]. Tfam plays an important role in the organelle and serves as the most important transcription factor in increasing the transcription of mitochondrial DNA derived proteins [20,29]. The protein kinase p38 MAPK is one of the most likely kinases involved in PGC-1α regulation, that phosphorylates and activates PGC-1α(23). This plays important role in matching the expression of mitochondrially encoded genes to the changing expression of the nuclear genome [26]. Additionally, caffeine may induce the PGC-1α RNA and protein augmenting the mitochondrial content [4].

The large majority of the remaining mitochondrial proteins requires transcription into the nucleus and import into their suitable organelle compartments through mitochondrial chaperones and protein import channels. Taken together, improved mitochondrial biogenesis is vital to the support of metabolism. In particular, maintenance and functional mitochondrial biogenesis within skeletal muscle fibers is important to health-span [30], with skeletal muscle mitochondrial dysfunction associated with several disease states, such as muscular dystrophy, atrophy, type 2 diabetes, and aging related sarcopenia, among many others [5]. Additionally, it is well established that several markers of mitochondrial biogenesis are associated with improvements in exercise performance outcomes, at least in animal and *in vitro* models [6,31–33]. Thus, strategies to boost mitochondrial biogenesis are likely sine qua non to healthy aging, and here, by a review of the scientific evidence, we propose that caffeine intake could be a potentially strategy to augment mitochondrial biogenesis in the skeletal muscle.

3. Proposed mechanisms of caffeine induced-mitochondrial biogenesis

Ojuka and colleagues [22] demonstrated that caffeine may stimulate mitochondrial biogenesis. They analyzed the mitochondrial content after treating L6 myotubes cell line with caffeine (5 mM) for 5 h per day for 5 days. The results showed that caffeine administration increased both peroxisome proliferator-activated receptor gama (PPARγ) and PGC-1α and mitochondrial transcription factor A (mtTFA) protein expression. Furthermore, it also enhanced the binding of NRF-1 and NRF-2 to DNA. Finally, caffeine exposure increased mitochondrial marker enzyme proteins. As a result, raising Ca^{2+} levels through caffeine appears to induce exercise-like mimetic mitochondrial biogenesis. Later, the same research group showed in ex vivo incubated adult rat epitrochlearis muscle that exposure to caffeine for 6 h raised intracellular Ca^{2+} followed by augmented PGC-1α, cytochrome oxidase sub-unit I (COXI), aminolevulinate synthase (ALAS) COXI, ALAS mRNAs levels [34].

It has also been reported that exercise can induce p38 MAPK within skeletal muscle [35]. Interestingly, caffeine has been shown to augment muscular p38 phosphorylation, suggesting that p38 MAPK is downstream of CAMKII in a signal transduction pathway by which increases in cytosolic Ca^{2+} lead to increased PGC-1α expression and mitochondrial biogenesis in skeletal muscle [34]. AMPK further controls mitochondrial biogenesis through PGC-1α and NRF-1 modulation [36]. Schnuck et al. demonstrated that caffeine exposure lasting 15- and 30-min in C2C12 myotubes increased AMPK phosphorylation and improvements in the expression of metabolic genes involved in mitochondrial biogenesis [37]. It is important to highlight that even with lower physiological caffeine exposures (50 μM and 100 μM during 3, 6, 9 and 12 h) when compared to previous studies, the expression of PGC-1α, NRF1, sirtuin-3 (SIRT-3) and TFAM were increased. In addition, Barrès et al. demonstrated that caffeine administration elevated PGC-1α, TFAM, PPARβ/δ and MEF2A expression in myotubes [38]. Furthermore, they reported that caffeine exposure induced gene hypomethylation concomitant with an increase in the respective mRNA levels. Next, co-incubation of L6 myotubes with caffeine and dantrolene, a Ryanodine receptor calcium release inhibitor, during 60, 120 and 180 min, dramatically inhibited gene expression and suppressed promoter hypomethylation [38]. Collectively, these results show, that skeletal muscle cell signaling responses after caffeine administration are increased in the mitochondrial biogenesis pathway (Fig. 1).

Although here we have focused on mitochondrial biogenesis within skeletal muscle, it is important to note that caffeine can also induce mitochondrial biogenesis in other cell types. Chouchani et al. [39]; demonstrated that caffeine stimulated brown adipose tissue (BAT) function *in vivo* and *in vitro,* and increased mitochondrial biogenesis. Stem cell-derived adipocytes exposed to caffeine can increase uncoupling protein 1 (UCP1), a classical marker for thermogenesis and energy expenditure. Additionally, in healthy subjects with body mass index (BMI) (mean of 23 kg/m^2), that the consumption of coffee (Nescafe© Original 1.8 g sachet ~65 mg caffeine dissolved in 200 ml water at 22 °C) stimulated thermogenesis when compared to a control group [40].

Favorable mitochondrial adaptations in muscle can only be achieved if training is performed at a sufficient frequency, intensity

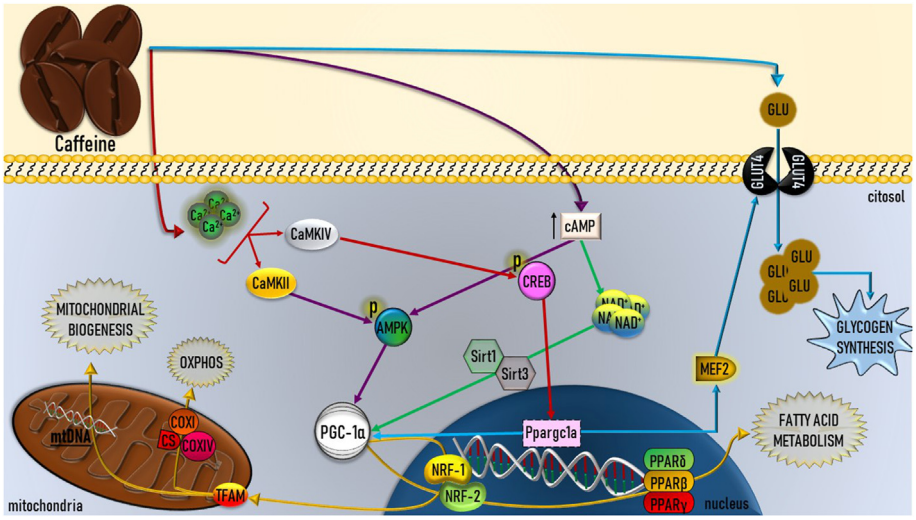

Fig. 1. The Effects of Caffeine Intake on Mitochondrial Metabolism in Skeletal Muscle Cells. Caffeine-induced Adenosine-2 Receptors (A2R) activate Ryanodine Channels (RyR) to release Calcium (Ca^{2+}) into the cytosol of skeletal muscle cells. This phenomenon improves the kinases CAMKII and CAMKIV, enhancing mitochondrial metabolism by two different signaling pathways: CAMKIV phosphorylates CREB, improving the mRNA levels of Ppargc1alpha; while CAMKII phosphorylates AMPK. Both pathways augment the protein levels of the major regulator of mitochondrial metabolism, PGC-1α. Caffeine intake improves the levels of cAMP, which also enhances AMPK and the NAD+ levels into the cytosol, activating PGC-1α and SIRTdeacetylases-dependent pathway. PGC-1α co-activates several mitochondrial-related proteins as [1]: PGC-1 gene (Ppargc1a) augment the levels of MEF2, a protein related with GLUT-4 dependent glucose uptake and glycogen synthesis in skeletal muscle cells [2]; PGC-1α improves the protein levels of NRF-2, which augment the levels of PPARs enhancing the fatty acid metabolism and oxidation into mitochondria [3]; PGC-1αdependent NRF-1 activation, improves the protein levels of TFAM, a major regulator of mitochondrial biogenesis, activating the OXPHOS complexes and the levels of mitochondrial DNA (mtDNA), leading to mitochondrial biogenesis in skeletal muscle cells.

and duration, and for an adequate length of time [8]. There is an ongoing debate within the scientific community about whether training volume or intensity are more important in enhancing mitochondrial adaptations. The highest level of evidence comes from a pooled analysis of all relevant studies [41], which indicates that training volume is more important than training intensity in promoting improvements in mitochondrial content, as evaluated through citrate synthase expression or transmission electron microscopy [41].

Although evidence suggests that training volume or intensity per se are effective to stimulate mitochondrial content, but one cannot exclude the other. It has been established that, when the total training volume is low, exercise intensity is more important to promote increases in mitochondrial content, as assessed by Citrate Synthase activity [42]. This seems reasonable to propose that training volume and intensity converge into the same stimulus of mitochondrial biogenesis, with the degree of metabolic stress being a likely candidate. Supporting this concept, Fiorenza et al. compared repeated sprint exercise (18 × 5 s all out with 30 s recovery), work-matched sprint endurance (6 × 20 s all out 120 s recovery), and traditional continuous moderate intensity exercise (50 min at 70% VO_{2max}), and found metabolic stress, to predict skeletal muscle mRNA responses associated with mitochondrial biogenesis [35]. This study also found an elevation in skeletal muscle CaMKII and p38 MAPK phosphorylation, as well as a greater rise in muscle lactate and plasma adrenaline levels during speed endurance; indicative of higher metabolic stress and consequently enhanced transcriptional responses [35]. Notably, caffeine ingestion in capsules (6 mg/kg) 1 h before a cycling endurance test (65% of the expected maximal heart rate), increased lactate and

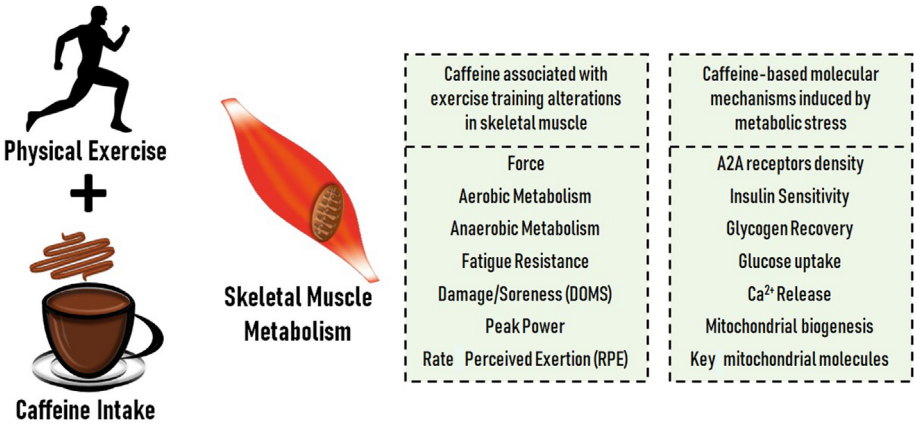

Fig. 2. The Possible Effects of Association of Caffeine Intake and Physical Exercise on Skeletal Muscle Metabolism and Physical Performance. Caffeine intake likely improves physical performance by activating physiological mechanisms related with [1]: Anaerobic metabolism (i.e.: improve strength, sprint, jump, peak power, etc.); and [2] Aerobic metabolism (i.e.: higher fatigue resistance, lower muscle damage/soreness (DOMS) and perceived effort (RPE). These accumulated effects are due to improvements in several molecular mechanisms induced by caffeine intake, such as: Adenosine type-2 Receptors (A2R), insulin sensitivity; glycogen metabolism; calcium release to cytosol; mitochondrial biogenesis and OXPHOS complexes. *Note: These effects are dependent on the amount of caffeine consumed, fitness status, habitual caffeine intake, individual physiology and metabolism.

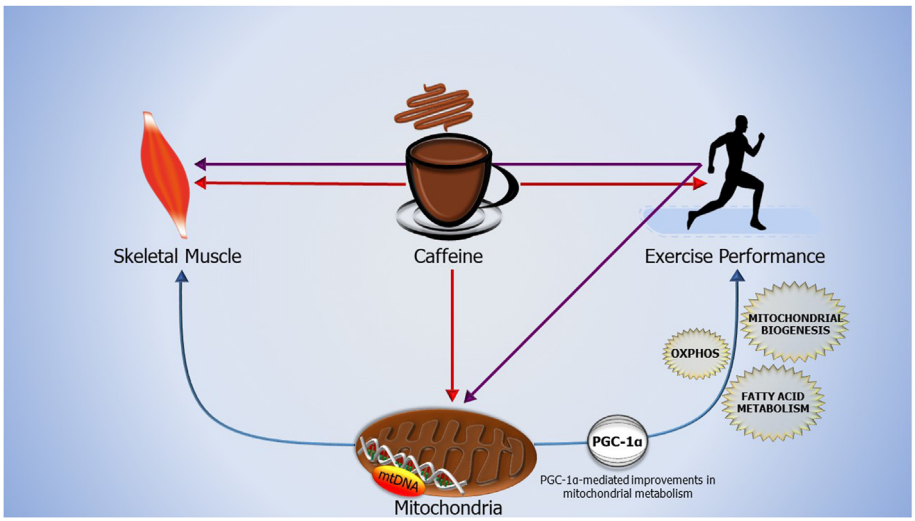

Fig. 3. Futures perspective. Over the past decade, studies exploring the effects of caffeine on skeletal muscle metabolism and physical performance have rapidly increased in number. To date, the scientific literature suggests that caffeine exerts several beneficial effects on muscle-related performance, such as improved anaerobic (e.g.: improvements in strength and power) and aerobic metabolism (e.g.: fatigue resistance). Crucially, it has emerged that the effects of caffeine on physical performance are not dependent only on skeletal muscle, with effects exerts across a variety of other tissues, including brain, liver and adipose tissue. In the context of multiple integrative systems to regulate the exercise-mediated responses within an organism, scientists and clinicians are increasingly interested in exploring the molecular mechanisms by which caffeine induces its beneficial effects on physical performance. Within this area, mitochondrial metabolism has garnered attention, with mitochondrial adaptations representing one of the central hubs of exercise adaptation. Practical implications in human around caffeine intake and mitochondrial metabolism, exploring the possible molecular mechanisms by which caffeine affects both mitochondrial metabolism and biogenesis are necessary to understand if or how caffeine modulates mitochondrial metabolism. Finally, clinical perspectives for future studies to address physical performance and non-pharmacological therapeutic interventions.

epinephrine during exercise in health elderly subjects when compared to the placebo control group [43]. Taken together, if skeletal muscle metabolic stress determines mitochondrial biogenesis, might concurrent caffeine-induced-metabolic stress than further boost mitochondrial biogenesis? Malek et al. examined the effects of one dose of caffeine administration (201 mg of caffeine) during 8 weeks of aerobic training on VO peak, time to exhaustion at 90% $VO_{2peak,}$ body weight and body composition [44]. Surprisingly, the caffeine supplementation did not augment the aerobic training adaptations [44]. Thus, more studies are required to explore and elucidate the effects of the combination of exercise and caffeine supplementation on skeletal muscle adaptations, with particular focus on dosage of caffeine, separating light and heavy caffeine consumers. Furthermore, it would be beneficial to further test whether chronic caffeine intake actually impairs mitochondrial biogenesis by organismal habituation, as the majority of studies investigate the acute effects of caffeine on performance outcomes [45]. Taken together, these observations make us speculate that caffeine supplementation may be a viable strategy, to maximize exercise-induced mitochondrial adaptations, particularly in combination with exercise comprising high training volume and intensity. However, the isolated effects of caffeine without exercise on mitochondrial biogenesis providing similar adaptations as to high volume and intensity training require further investigations.

Its have been show that exercise can modulates muscle redox homeostasis resulting in the peroxidation of lipids. One important molecule that contribute to redox homeostasis during exercise conditions is the reactive oxygen species (ROS) content. The ROS generation alters cellular environment according to the type of exercise and or intensity. Notably, skeletal muscle contractions and relaxing may cause ROS activity leading lipids oxidative lipid degradations. However, still there is no consensus evidence showing that ROS production by physical exercise may modulate mitochondrial adaptations (for more details see ref [46]. Interestingly, studies have shown that caffeine can modulate intracellular ROS and lipid peroxidation [47–49]. However, caffeine-induced intracellular ROS associate with exercise is still lacking in the literature.

Caffeine ingestion improves exercise performance across a broad range of exercise tasks. Caffeine is ergogenic for different aspects of exercise performance, including aerobic endurance, muscle strength, muscular endurance, power, jumping performance and exercise speed [45,50]. At the meta-analysis level, the ergogenic effect sizes of caffeine are generally larger on aerobic endurance than on anaerobic performance tests [45]. As aerobic exercise is a significant trigger for mitochondrial biogenesis, and likely the best method to improve oxidative metabolism, there is the possibility that caffeine might mimic some of the exercise-induced effects. However, this possibility remains poorly explored in the research literature to date.

One of the main mechanisms believed to contribute to the ergogenic effects of caffeine is by competitive inhibition of adenosine receptors [51]. Exercise training has been shown to increase adenosine A_{2A} receptor densities, with Mizuno et al. reporting that trained men have greater adenosine A_{2A} receptor in cardiac and skeletal muscle when compared to untrained subjects [52]. However, the effects of adenosine signaling into the skeletal muscle with different intensity of exercise in acute or chronic manner remain unsolved. Accordingly, the increased adenosine receptor density in trained individuals might allow a greater inhibitory effect of caffeine on adenosine receptor signaling, enhancing the magnitude of the acute improvements in exercise performance in response to caffeine ingestion. However, this idea is still speculative and requires further investigation. A summary of the potential effects of physical exercise combined with caffeine ingestion on skeletal muscle function are shown in Fig. 2.

4. Conclusion

In summary, several *in vitro* studies have demonstrated that caffeine can enhance mitochondrial biogenesis in skeletal muscle.

However, the effects of caffeine on mitochondrial biogenesis require further exploration *in vivo* either alone and/or in combination with exercise training. In addition, although some studies have demonstrating that caffeine acts via RyR Ca2+ release and AMPK pathway, there is no consensus in the literature about these effects, most of the studies were in cell line or animal models and studies are necessary to elucidate this hypothesis in human. Further studies in humans with double-blinded randomized controlled studies is also necessary, to exclude possible placebo effects. An important area for exploration is to elucidate whether caffeine can boost mitochondrial biogenesis in skeletal muscle with exercise-mimicking effects, and whether combining caffeine and exercise enhances human mitochondrial biogenesis above exercise alone (Fig. 3). Finally, the identification of putative molecular pathways combined with metabolomics, proteomics, transcriptomics and epigenomics will provide novel insights of how caffeine can remodel mitochondria.

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Author contributions

Yamada, A.K and Silva, V.R.R. conceived the idea and were responsible for writing the article. Cordeiro, A.V made the figures. Pimentel, GD and Pickering, C, help with discussion and data interpretation, checking, proofreading and English corrections. All the authors contributed to the preparation, editing of this manuscript for intellectual content and approved the final version.

Declaration of competing interest

The authors declare no conflict of interest.

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