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Translational

Curcumin treatment enhances the effect of exercise on mitochondrial biogenesis in skeletal muscle by increasing cAMP levels

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ABSTRACT

Background. In response to physiologic stressors, skeletal muscle has the potential to elicit wide variety of adaptive responses, such as biogenesis of mitochondria and clearance of damaged mitochondria to promote healthy muscle. The polyphenol curcumin, derived from the rhizome *Curcuma longa L.*, is a natural antioxidant that exhibits various pharmacological activities and therapeutic properties. However, the effect of curcumin on the regulation of mitochondrial biogenesis in skeletal muscle remains unknown. The present study aimed to examine the effects of combination of endurance training (eTR) and curcumin treatment on the expression of AMPK, SIRT1, PGC-1α, and OXPHOS subunits, mitochondrial DNA copy number, and CS activity in rat skeletal muscle. Furthermore, the present study also examined the effect of exercise and curcumin treatment on the levels of cAMP and downstream targets of PKA including phosphorylated CREB and LKB-1.

Methods. Ten-week-old male Wistar rats were randomly divided into non-eTR and eTR groups. Low doses (50 mg/kg-BW/day) or high doses (100 mg/kg-BW/day) of curcumin dissolved in dimethyl sulfoxide (DMSO) were injected intraperitoneally in all animals for 28 days to investigate the effect of curcumin alone and the combined effect of curcumin with eTR. Western blotting (WB) and immunoprecipitation (IP) were performed to detect the presence of proteins.

Results. Our results demonstrated that combination of curcumin treatment and eTR increased the expression of COX-IV, OXPHOS subunits, mitochondrial DNA copy number and CS activity in the gastrocnemius (Gas) and soleus (Sol) muscles. In addition, this combination increased AMPK phosphorylation, NAD^+/NADH ratio, SIRT1 expression, and

Abbreviations: AMPK, 5′ adenosine monophosphate-activated protein kinase; BW, body weight; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element binding protein; COX-IV, cytochrome c oxidase subunit IV; CS, citrate synthase; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; eTR, endurance training; Gas, gastrocnemius muscle; IP, immunoprecipitation; LKB-1, liver kinase B1; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide hydrogen; OXPHOS, oxidative phosphorylation; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1alpha; PKA, protein kinase A; SIRT1, sirtuin 1; Sol, soleus muscle; WB, western blotting; GLUT4, Glucose transporter 4; NRF1/2, nuclear respiratory factor1/2; GA, guanine adenine; Tfam, transcription factor A; mRNA, messenger ribonucleid acid; ADP, adenosine diphosphate; MMP, mitochondrial membrane potential; ATP, adenosine triphosphate; ROS, reactive oxygen species; NOS, nitric oxide synthase.

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PGC-1α deacetylation. Furthermore, curcumin treatment as well as exercise also increased levels of cAMP and downstream target of PKA including phosphorylation CREB and LKB-1 which are involved in the regulation of mitochondrial biogenesis.

Conclusion. Taken together, these results suggest that the combination of curcumin treatment and eTR has the potential to accelerate mitochondrial biogenesis in skeletal muscle by increasing cAMP levels.

1. Introduction

Skeletal muscle represents one of the largest organs in the body, with a wide variety of adaptive responses to physiological stressors. The biogenesis of mitochondria and the clearance of damaged mitochondria promote healthy muscle and this turnover can prevent metabolic imbalances, which could predispose individuals to the development of obesity, diabetes, cardiovascular disease, and accelerated aging [1,2]. Muscle adaptation occurs in the mitochondria following exercise training. Endurance exercise training has the potential to enhance metabolic characteristics in the skeletal muscle, including mitochondrial biogenesis and the expression of glucose transporter 4 (GLUT4) [3]. Although such adaptations have been investigated for several decades, their underlying mechanisms remain to be elucidated. Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α) has been implicated as a master regulator of mitochondrial biogenesis by interacting with nuclear respiratory factor 1 (NRF1) and nuclear respiratory factor 2 (NRF-2/GA-binding protein-A). Both PGC-1α and NRF-1 activate the mitochondrial transcription factor A (Tfam), which is responsible for transcribing nuclear-encoded mitochondrial genes, as well as the transcription, translation, and repair of proteins involved in mitochondrial DNA (mtDNA) [4,5]. The expression levels of PGC-1α, (both mRNA and protein) increase following acute endurance exercise [6–9] and endurance exercise training [10,11], thus suggesting that PGC-1α is a potential regulator of metabolic adaptations after endurance exercise.

Mammalian sirtuins, such as sirtuin 1 (SIRT1), are members of a conserved family of NAD^+-dependent deacetylases and ADP-ribosyltransferases, which are involved in numerous fundamental cellular processes, including gene silencing, DNA repair, and metabolic regulation [12,13]. SIRT1 promotes mitochondrial biogenesis via the deacetylation of PGC-1α. Another important metabolic sensor, AMP-activated protein kinase (AMPK), also activates PGC-1α. Both SIRT1 expression [14] and activity [15] increase after endurance exercise. SIRT1 is also activated by acute endurance exercise [16] and it has been proposed that AMPK activates SIRT1 indirectly by increasing the intracellular levels of its co-substrate, NAD^+. Similar to AMPK, SIRT1 is widely associated with energy metabolism, suggesting that activators of these enzymes could enhance mitochondrial biogenesis and function and thereby enhance the endurance capacity. Collectively, these findings suggest that metabolic adaptations resulting from endurance exercise training could result, at least in part, from an AMPK-mediated increase in the expression of PGC-1α.

In both mammalian cells and yeast, the regulation of mitochondrial biogenesis clearly involves the cyclic adenosine monophosphate (cAMP) signaling pathway. Indeed, it has been shown that treatment of human preadipocytes with forskolin, which leads to an over-activation of the cAMP pathway, increased the copy number of mtDNA [17]. Protein kinase A (PKA), also known as cAMP-dependent enzyme, is a well studied, downstream effector of cAMP and is activated only in the presence of cAMP. Activated PKA phosphorylates a number of other proteins including cAMP response element binding protein (CREB) and liver kinase B1 (LKB-1), which induces PGC-1α to regulate mitochondria biogenesis ([18,19]. Previous studies have provided evidence that exercise, including swimming and running increase cAMP levels in skeletal muscle and myocardium [20] and the mitochondrial biogenesis seen after exercise may be attributable to this increase.

Several polyphenols have been shown to activate cAMP, and are currently under intense investigation as potential inducers of mitochondrial biogenesis [21,22]. Curcumin, a compound with medicinal properties found in *Curcuma longa L*, and turmeric, a popular culinary spice used in both vegetarian and non-vegetarian foods are examples of polyphenols. The anti-oxidant activities of curcumin have been reported to be more potent than those of another well-studied polyphenol, resveratrol [23] and the long-term effects of dietary curcumin on various markers of mitochondrial biogenesis have been investigated. Five months of curcumin as a dietary supplement in senescence-accelerated mouse-prone 8 (SAMP8), a fast-aging mouse strain, up-regulated PGC-1α protein expression thereby improving the mitochondrial membrane potential (MMP) and ATP levels and restoring mitochondrial fusion in the brain [24]. The effects of curcumin on the regulation of mitochondrial biogenesis in skeletal muscle however, have yet to be elucidated.

In the present study, we investigated the mechanisms by which curcumin affects mitochondrial biogenesis in rats as reflected by the levels of cAMP and downstream targets of PKA including phosphorylated CREB and LKB-1. The primary purpose of the present study was to determine the combined effects of curcumin treatment together with 24 days of endurance training (eTR) on the regulation of mitochondrial biogenesis in skeletal muscles (Gas and Sol). We predicted that treatment with curcumin combined with eTR may additively or synergistically enhance the effect of exercise. The results showed that curcumin treatment and eTR have additive effect on increasing mitochondrial biogenesis in skeletal muscle.

2. Material and Methods

2.1. *Animals Experiments*

All procedures performed in the present study were approved by the Ethics Committee on Animal Experimentation of