



Review Article



Potential Role of Natural Compounds Modulating Bone Formation by Mitochondrial Oxidative Phosphorylation

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Abstract

Mitochondria are the main cell organelles responsible for adenosine triphosphate production through cellular respiration. They also have roles in regulating other cellular processes, including reactive oxygen species, apoptosis, and others. The function and number of mitochondria are important for the maintenance of bone homeostasis. In recent years, the regulation of bone homeostasis by mitochondria has attracted particular interest. In addition, some natural compounds have been demonstrated to modulate mitochondria functions, such as resveratrol, quercetin, and curcumin, *etc.* Here, we review the recent discoveries concerning mitochondrial oxidative phosphorylation and bone formation, as well as the effects of some natural compounds (resveratrol, quercetin, and curcumin) on oxidative phosphorylation, and discuss their therapeutic implications in treating bone disorders.

Introduction

Mitochondria are the main cell organelles that convert nutrients into adenosine triphosphate (ATP) by cellular respiration.¹ Previous studies have demonstrated that cellular ATP level is involved in cell growth, apoptosis, necrosis, *etc.*^{2–4} There are four stages of cellular respiration, including glycolysis, pyruvate oxidation, citric acid cycle, and oxidative phosphorylation. Oxidative phosphorylation is the most efficient stage for ATP synthesis. The defects of the mitochondrial respiratory chain have been proven to be involved in many diseases, such as tumors, cardiomyopathy, Fanconi's syndrome, seizures, ophthalmoplegia, *etc.*^{5–11}

Bone is a tissue that mechanically supports the body, protects vital organs, and impacts endocrine regulation. Currently, four types of cells have been identified in bone, osteoblasts, osteocytes, bone lining cells, and osteoclasts.¹² Osteoblasts and osteocytes

are derived from stem cells known as mesenchymal stem cells (MSCs).^{13–15} The function and number of mitochondria are important factors in the maintenance of bone cells, including osteoblasts and osteoclasts,^{16–18} as both bone formation and resorption depend strongly on energy expenditure.¹⁹ An imbalance between bone formation mediated by osteoblasts and bone resorption mediated by osteoclasts has been shown to directly contribute to the onset of many bone diseases such as osteoporosis, osteolysis, arthritis, *etc.*^{20,21} Mutations of mitochondrial DNA (mtDNA) that accumulate with age may play a significant role in bone homeostasis.²² Accumulating mutations of mtDNA ultimately lead to mitochondrial and cellular dysfunction.²³ A recent case-control study showed that m.3243A>G, a mitochondrial point mutation, was associated with decreased bone mineral density and bone strength.²⁴ In aged mice, osteoclasts had reduced levels of ATP and mtDNA with increased bone-resorbing activity.²⁵ Osteoclast-specific deletion of mitochondrial transcription factor A, which regulates mtDNA copy number, leads to ATP depletion and increased bone resorption.²⁵ Mitochondrial dysfunction induced by mtDNA polymerase gamma deletion has been reported to impair osteogenesis, increase osteoclast activity, and accelerate age-related bone loss.²⁶ Superoxide dismutase 2 deletion in osteocytes increases reactive oxygen species (ROS) production and bone loss in an age-dependent manner.²⁷

In this review, we discuss recent discoveries concerning mitochondrial oxidative phosphorylation and bone formation, as well as the effects of some natural compounds (including resveratrol, quercetin, and curcumin) on oxidative phosphorylation, and whether optimizing mitochondria function could improve bone

Keywords: Mitochondria; Oxidative phosphorylation; Bone; Natural compounds.

Abbreviations: ATP, adenosine triphosphate; BMP, bone morphogenetic protein; LRP, low-density lipoprotein receptor-related protein; MSC, mesenchymal stem cell; mtDNA, mitochondrial DNA; NAD⁺, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; Nrf2, nuclear factor erythroid 2-related factor 2; PGC, peroxisome proliferator-activated receptor-gamma coactivator; RUNX2, runt-related transcription factor 2; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle; Wnt, Wingless-type MMTV integration site family.

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quality or even reverse bone disorders.

Oxidative phosphorylation and bone formation

Oxidative phosphorylation is defined as the process by which electrons from reduced nicotinamide adenine dinucleotide and reduced flavine adenine dinucleotide are transferred to O₂ molecules through a series of electron carrier/protein complexes to generate ATP. It provides most of the ATP for the cell's energy needs. Defects in this process are a frequent cause of mitochondrial energy metabolism disturbance, which occurs with a prevalence of at least 1 in 7600.²⁸ Up to now, accumulating evidence has indicated that oxidative phosphorylation is involved in the regulation of cell fate decisions.^{29–31} *In vitro* studies have indicated that oxidative phosphorylation plays a crucial role in osteogenesis. Upon osteogenic induction, energy metabolism can shift from glycolysis to mitochondrial oxidative phosphorylation with the increase of respiratory enzymes, copy number of mitochondrial DNA, oxygen consumption rate, and intracellular ATP content.³² Other studies have also demonstrated that oxidative phosphorylation is increased during the osteogenesis of MSCs, while glycolysis is preferred in undifferentiated stem cells.^{33,34} The increased β -catenin acetylation and activity might be one of the important underlying mechanisms that account for the effect of oxidative phosphorylation on the osteogenesis of MSCs.³⁵ Bone morphogenetic protein 2 has a critical role in regulating bone formation, a recent study reported that bone morphogenetic protein 2 triggered rapid metabolic adaptation in MSCs, which is characterized by the successive activation of glycolysis and oxidative phosphorylation.³⁶

Nicotinamide adenine dinucleotide (NAD⁺) is an obligatory substrate for the reaction of glycolysis and oxidative phosphorylation. Its intracellular level and NAD⁺/nicotinamide adenine dinucleotide ratio are essential for mitochondrial function, which links cellular metabolism to changes in transcriptional events and signaling pathways.³⁷ Nicotinamide mononucleotide (NMN), a key natural NAD⁺ intermediate, has been found to promote osteogenesis and reduce adipogenesis of MSCs, as well as stimulate osteogenesis of endogenous MSCs, and protect bone from aging and irradiation-induced damage in mice.³⁸ A recent study by Boer Li *et al.* reported that osteogenesis-committed MSCs have increased oxidative phosphorylation activity and elevated NAD⁺ levels. Suppression of NAD⁺ impairs mitochondrial fusion, leading to mitochondria dysfunction and reduced activity of oxidative phosphorylation, which subsequently blocks osteogenesis and bone fracture healing.³⁹

As we know, aging is associated with mitochondria dysfunction, as well as decreased bone mass and accumulated marrow fat. It is reported that, during aging, the content of NAD⁺ and ATP has a progressive, age-dependent decline.⁴⁰ Up to now, mitochondrial metabolic failure has been considered an important hallmark of aging.^{41,42} Mills *et al.* conducted a 12-month administration of NMN, demonstrating that NMN effectively mitigates age-associated physiological decline in mice, including increasing energy metabolism, bone density, *etc.*⁴³ NMN supplementation could also attenuate senescent cell induction in growth plates, partially prevent osteoporosis, and accelerate bone healing in osteoporotic mice.⁴⁴ In addition, upregulation of oxidative phosphorylation activity and ATP production by mitochondria transfer could enhance the osteogenesis of MSCs and accelerate bone defect healing after transplantation of mitochondria-recipient MSCs *in situ*.⁴⁵ On the other hand, a recent study by Suh *et al.* found that changes in mitochondrial morphology had a more significant impact on os-

teogenic activity than moderate alterations in mitochondrial ATP production, as enhancing mitochondrial fission by overexpression of Fis1 accelerates osteogenesis but results in mild but significant decreases of ATP production.⁴⁶ Hence, it would be safe to assume that improvement of mitochondrial metabolism would enhance the osteogenesis of MSCs and bone formation, as well as hinder the progression of osteoporosis, especially senile osteoporosis.

Reactive oxygen species (ROS)

ROS are mainly formed as byproducts of oxidative phosphorylation in aerobic organisms. The ROS that have been found in living organisms include superoxide anions, hydroxyl radicals, nitric oxide, *etc.* The evidence so far has suggested that ROS serve as a double-edged sword for the cells. On one hand, ROS at low levels function as signaling molecules that are necessary to maintain cell viability, differentiation, self-renewal ability, migration, *etc.*⁴⁷ Studies of *Candida elegans* and yeast have shown that ROS were required for the increase of lifespan.^{48,49} On the other hand, an excess of ROS is harmful as it can induce oxidative stress and cell death.⁵⁰ The levels of ROS are increased in dysfunctional mitochondria and senescent cells, implicating ROS are involved in various cellular damages.⁵¹ The osteogenic differentiation of bone osteoblastic cells (MC3T3-E1), marrow stromal cells (M2-10B4), and MSCs were greatly suppressed by ROS, and adipogenesis was increased.^{32,52–54} Many studies have shown that ROS may be involved in the pathogenesis of osteoporosis.^{55,56}

Hopefully, the destructive effects of ROS can be inhibited by antioxidant agents, such as vitamin K, glutathione, and its precursor N-acetyl cysteine and other natural compounds. The cells have intrinsic mechanisms that coordinate changes in oxidative phosphorylation and antioxidant enzymes without generating excess ROS. For example, during the osteogenesis of MSCs, although ROS are produced by increased oxidative phosphorylation, they are quickly removed by the upregulation of antioxidant enzymes, such as manganese-dependent superoxide dismutase and catalase.³² N-acetyl cysteine has beneficial effects that prevent osteoporosis by reducing stimuli of the loss of bone mass, osteoblast apoptosis, oxidative stress, and osteoclastogenesis after gonadectomy.^{57,58} The effects of some natural compounds like quercetin, resveratrol, and curcumin on oxidative phosphorylation or mitochondria are discussed below.

Quercetin

Quercetin is an abundant polyphenolic flavonoid and has beneficial effects in many diseases. The main molecular mechanism responsible for its protective effects is the capacity to quench ROS-induced oxidative damage. However, studies about the effect of quercetin on mitochondrial function appear contradictory. It was previously shown to inhibit mitochondrial ATP synthase, inhibit oxidative phosphorylation, and decrease mitochondrial membrane potential.^{59–61} However, recent studies have shown that quercetin treatment improved mitochondrial quality and reduced oxidative stress.⁶² Qiu *et al.* found that quercetin not only enhanced mitochondrial membrane potential, oxygen consumption, and ATP in mitochondria, but also increased the mitochondrial copy number in rat chondrocytes.⁶³ Direct evidence from Fukaya *et al.* demonstrates that quercetin significantly increased oxygen consumption and ATP production in hepatocytes.⁶⁴ In addition, quercetin protects against bone loss by stimulating bone formation and inhibiting bone resorption.^{65–67} The underlying mechanisms include

Wingless-type MMTV integration site family, bone morphogenetic protein, extracellular regulated protein kinases, nuclear factor erythroid 2-related factor 2 (Nrf2), and smad-dependent signaling pathways, *etc*. Nrf2 was maintained in an inactive state in the cytosol by binding to kelch-like ECH-associated protein-1, which was inhibited by quercetin, leading to nuclear translocation of Nrf2 and increased expression of antioxidative genes.⁶⁸ Pang *et al*. reported that quercetin promoted bone MSCs proliferation and osteogenic differentiation by stimulating the bone morphogenetic protein signaling pathway.⁶⁹ However, the effect of quercetin on oxidative phosphorylation has not been confirmed in bone-forming cells, including osteoblasts or MSCs. It would be interesting to find out whether quercetin protects bone by increasing oxidative phosphorylation or improving mitochondrial function or not.

Resveratrol

Resveratrol (3,4,5-trihydroxystilbene) is an edible polyphenolic phytoalexin with anti-oxidant and anti-aging activity.⁷⁰ Lagouge *et al*. found that resveratrol improved mitochondrial function by inducing genes for oxidative phosphorylation, mitochondrial biogenesis, and an increase in the activity of peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 α .⁷¹ Under normal conditions, PGC-1 α is inactivated by acetylation by acetyltransferase, general control of nucleotide synthesis 5.⁷² Resveratrol activates the NAD⁺-dependent histone deacetylase Sirt1. Sirt1 mediates the deacetylation of PGC-1 α , leading to the stimulation of genes involved in oxidative phosphorylation.⁷³ The effect of resveratrol is also related to mitofilin, a mitochondrial inner membrane protein that is required for mitochondrial homeostasis and osteogenesis of MSCs.⁷⁴ However, resveratrol cannot normalize oxidative phosphorylation activity in cells with oxidative phosphorylation defects,⁷⁵ implying the effect of resveratrol on mitochondrial activity is dependent on the induction of genes for oxidative phosphorylation. Moon *et al*. reported that resveratrol treatment (at 5 μ M) promoted osteogenic differentiation of periosteum-derived MSCs, and increased both mitochondrial mass and mtDNA copy number, supporting the application of resveratrol for treating osteoporotic fractures.⁷⁶ In an ovariectomized rat model of osteoporosis, Feng *et al*. demonstrated that resveratrol prevented bone loss by upregulating FoxO1 transcription activity.⁷⁷ Tresguerras *et al*. found that resveratrol (10 mg/kg/day) increased bone microstructure and bone mechanical properties in old male rats, indicating resveratrol hindered the progression of senile osteoporosis.⁷⁸ A meta-analysis of randomized controlled trials of resveratrol and bone-health biomarkers showed that resveratrol supplementation increased some key bone biomarkers, such as alkaline phosphatase and bone alkaline phosphatase.⁷⁹ It should be noted that the effect of resveratrol on mitochondria is tissue- or cell-specific. Resveratrol also inhibits oxidative phosphorylation and mitochondrial metabolic pathways in mitochondria isolated from rat brains,⁸⁰ and cancer cells.^{81,82}

Curcumin

Curcumin is a natural phenolic biphenyl compound isolated from the plant *Curcuma longa*. The evidence so far has shown that curcumin is a protonophoric uncoupler, causing a decrease in ATP biosynthesis in mitochondria.⁸³ In *Escherichia coli*, the ATP synthase F1 sector is inhibited by curcumin and its analogs.⁸⁴ One study indicated that curcumin preserved mitochondrial function, with increased membrane potential and complex III, which is in-

involved in oxidative phosphorylation. It ultimately ameliorated oxidative stress-induced apoptosis in osteoblasts.⁸⁵

Evidence from published studies indicates the effects of curcumin on osteogenesis and bone formation are contradictory. For example, it has been demonstrated that curcumin inhibited the osteogenesis of human adipose derived-MSCs in a dose-dependent manner.⁸⁶ However, another study showed that curcumin promoted osteogenesis and bone repair activity of human periodontal ligament stem cells under oxidative stress.⁸⁷ To reveal its relationship with mitochondria, the controversial roles of curcumin in mitochondrial function deserve further study, especially in MSCs or osteoblasts.

Conclusions

Oxidative phosphorylation, which produces most of the ATP in cells, is closely correlated with mitochondrial function and many diseases. The role of oxidative phosphorylation in bone formation and the effect of natural compounds are shown in Figure 1. Herbal medicines have been traditionally used to combat diseases. They contain thousands of natural compounds, some of which may be the material base for their therapeutic functions. However, few natural compounds have been found to modulate mitochondrial function, and new ones need to be identified in the near future. Resveratrol has been found to induce the expression of genes for oxidative phosphorylation, and subsequent mitochondrial biogenesis, which would be useful for combating bone diseases by increasing bone formation. We should note that oxidative phosphorylation is not the only pathway by which natural compounds influence the function of mitochondria. Dietary lipids have been observed in some studies to regulate the transfer of mitochondria to macrophages, which determines whether mitochondria from adipocytes are released into the systemic circulation to aid the metabolic adaptation of distant organs.^{88,89} Mitochondrial transport may also be regulated by natural compounds or herbal medicines to impact bone formation or other biological processes. This is an interesting issue to be addressed in future studies. So far, natural compounds have many effects on mitochondria, but some data are contradictory. The discrepancies may arise from different cell models and under different conditions. There is an urgent need to clarify the discrepancies in using natural compounds that are beneficial for mitochondria to improve bone quality.

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Conflict of interest

LLX has been an editorial board member of *Future Integrative Medicine* since November 2022. The authors declare no other conflict of interests related to this publication.

Author contributions

Wrote the draft (MLD, QQZ), conceived the original idea, and

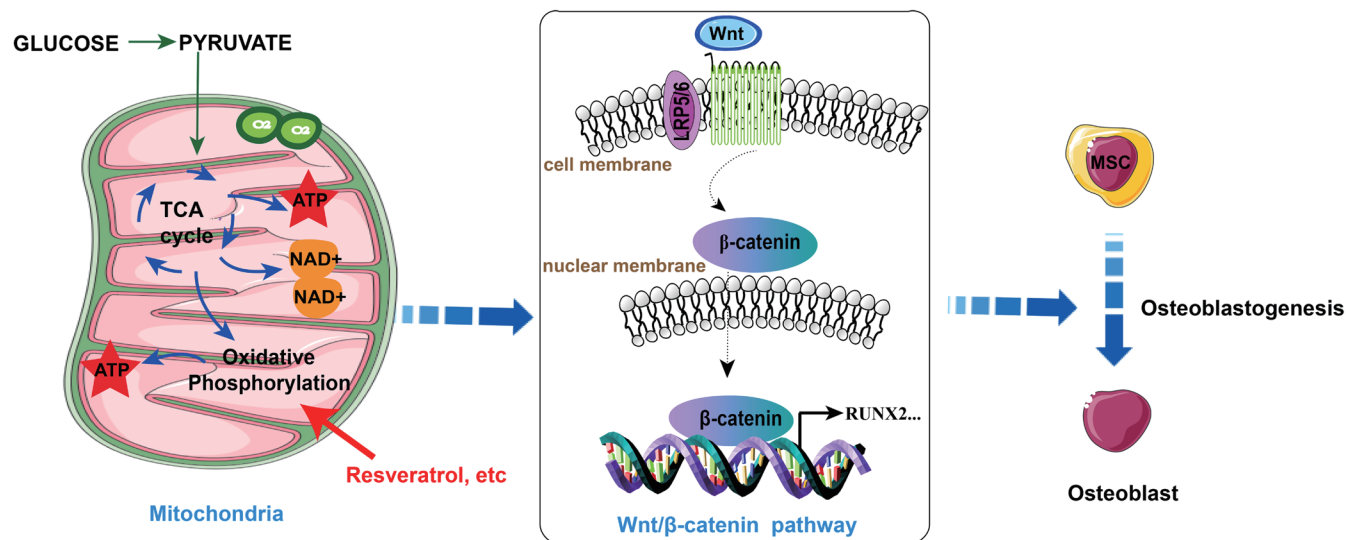


Fig. 1. Natural compounds regulate bone formation through oxidative phosphorylation. Osteoblasts derived from MSCs are cells required for bone formation. Oxidative phosphorylation produces a lot of ATP and other products including NAD⁺ and ROS. These factors play important roles in the regulation of osteogenesis through different mechanisms, such as Wnt/β-catenin signaling. Natural compounds including resveratrol and others modulate osteogenesis by regulating oxidative phosphorylation in mitochondria. ATP, adenosine triphosphate; LRP, low-density lipoprotein receptor-related protein; MSC, mesenchymal stem cell; NAD⁺, nicotinamide adenine dinucleotide; ROS, reactive oxygen species; RUNX2, runt-related transcription factor 2; TCA, tricarboxylic acid cycle; Wnt, Wingless-type MMTV integration site family.

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