ORIGINAL ARTICLE

Nicotinamide mononucleotide supplementation mitigates osteopenia induced by modeled microgravity in rats

Yunfei Huang[1] · Yusheng Dou[2] · Bo Yang[1] · Baorong He[1] · Xuefang Zhang[1] · Ke Zhang[1] · Xiaobin Yang[1]

Abstract

Exposure to weightlessness causes severe osteopenia, resulting in raised fracture risk. The current study aimed to investigate whether nicotinamide mononucleotide (NMN) supplementation protected against the osteopenia in hindlimb unloading (HLU) rats in vivo and modeled microgravity-induced osteoblastic dysfunction in vitro. The 3-mo-old rats were exposed to HLU and intragastrically administered NMN every 3 days (500 mg/kg body weight) for 4 weeks. NMN supplementation mitigated HLU-induced bone loss, evidenced by greater bone mass and biomechanical properties and better trabecular bone structure. NMN supplementation mitigated HLU-induced oxidative stress, evidenced by greater levels of nicotinamide adenine dinucleotide and activities of superoxide dismutase 2 and lesser malondialdehyde levels. Modeled microgravity stimulation using rotary wall vessel bioreactor in MC3T3-E1 cells inhibited osteoblast differentiation, which was reversed by NMN treatment. Furthermore, NMN treatment mitigated microgravity-induced mitochondrial impairments, evidenced by lesser reactive oxygen species generation and greater adenosine triphosphate production, mtDNA copy number, and activities of superoxide dismutase 2 and Complex I and II. Additionally, NMN promoted activation of AMP-activated protein kinase (AMPK), evidenced by greater AMPKα phosphorylation. Our research suggested that NMN supplementation attenuated osteoblastic mitochondrial impairment and mitigated osteopenia induced by modeled microgravity.

Keywords Rotary wall vessel bioreactor · Osteoblast · Oxidative stress · MC3T3-E1 cells · Hindlimb unloading

Introduction

Osteopenia is one of the well documented phenomena for crew members during a long spaceflight or patients subjected to long-term bed rest, which results in increased fracture risk (Coulombe et al. 2020; Frings-Meuthen et al. 2019). Numerous investigations have demonstrated that impaired osteoblastic function played an important role in weightlessness or microgravity-induced osteopenia (Morey and Baylink 1978; Cao et al. 2021). Countermeasures including

Yunfei Huang and Yusheng Dou equally contributed to the current study.

✉ Xiaobin Yang
yangxb1128@sina.com

1 Department of Spine Surgery, Hong Hui Hospital, Xi'an Jiaotong University, No.99 Yanxiang Road, Xi'an 710054, Shaanxi Province, China

2 Department of Should and Elbow Joint, Hong Hui Hospital, Xi'an Jiaotong University, Xi'an, China

exercise regimes and supplementation of vitamin D, calcium and bisphosphonates developed to date are ineffective for prevention of bone loss following microgravity (Smith et al. 1999). Development of novel anti-osteoporotic drugs is required for space travelers and geriatric population.

As a crucial intermediate during biosynthesis of nicotinamide adenine dinucleotide (NAD^+), accumulating evidence revealed that nicotinamide mononucleotide (NMN) was able to reverse defects in mitochondrial homeostasis, redox state, cell survival, as well as DNA repair caused by deficiency of NAD^+ (Croteau et al. 2017). Recent preclinical studies have demonstrated NMN administration as a promising therapeutic compound to extend the lifespan and exert diverse pharmacological actions in various diseases (Covarrubias et al. 2021; Hong et al. 2020). Long-term NMN supplementation mitigated age-associated and aluminum-induced osteoporosis in animal experiments (Mills et al. 2016; Liang et al. 2019). In vitro, NMN treatment promoted osteogenesis and self-renewal of mesenchymal stromal cells (Song et al. 2019), and attenuated dexamethasone-induced osteogenic inhibition in bone mesenchymal stem cells (Huang and Tao 2020).

Our work aimed to test the therapeutic role of NMN supplementation on the osteopenia in hindlimb unloading (HLU) rats in vivo and osteogenic inhibition induced by stimulated microgravity in vitro and elucidate the possible mechanisms.

Material and methods

HLU model and NMN treatment

Three-month-old Wistar rats, obtained from Vital-River Animal Ltd (SCXK 2015–0002, Beijing, China), were fed with standard rodent chow and distilled tap water at an appropriate temperature (about 22 °C) with 12 h light/dark cycle. All experiments involving rats had been approved by the Institutional Animal Care and the Animal Ethics Committee of Xi'an Jiaotong University (No. 202104017).

Briefly, orthopedic adhesive tapes were applied along the proximal 1/3 of tails of animals and placed by using a metal ring, attaching with a metal bar on the top of the cage as previously described (Xin et al. 2015). Control animals were individually housed in same cage as HLU rats. NMN (every 3 days, 500 mg/kg) (Wan et al. 2021; Lee et al. 2016) or vehicle was intragastrically administered in both groups for 4 weeks. Animals were sacrificed with an intraperitoneal injection of 30 μg/g xylazine and 300 μg/g ketamine at the end of study. Then, blood and samples of femurs and tibiae were harvested for further measurements. The detail of methods was shown in the supplementary data.

Cell culture and study design

The murine MC3T3-E1 cell (an osteoblastic cell line, Chinese Academy of Sciences Cell Bank, Shanghai, China) was cultured using α-MEM (gentamicin, 100 μg/ml; fetal bovine serum, 10%). Microgravity of 10^{-2}G in vitro was modeled in cultured cells using the rotary wall vessel bioreactor (RWVB, Synthecon, Houston) as previously described (Zayzafoon et al. 2004).

Cells were cultured in osteogenic medium with 10 mM β-glycerophosphate under modeled microgravity or normal gravity and treated with NMN (0.1 or 1 mM) (Liang et al. 2019; Ryu et al. 2018) for 7 days. Activities of alkaline phosphatase (ALP) were determined using a kit (KA1642, Novus Biologicals, Littleton, CO, USA).

Cells were cultured under modeled microgravity with NMN or not for 96 h. The levels of osteopontin (OPN) and runt-related transcription factor 2 (Runx2) mRNA were determined using real-time polymerase chain reaction (RT-PCR).

Assessment of bone loss

The right femurs and tibiae were subjected to the dual energy X-ray test (Hologic, USA) to determine the bone mineral density (BMD) of the whole femurs and tibiae. Trabecular bone morphometry analysis in proximal tibiae (volume of interest: starting from the lowest point of the growth plate and extending toward the diaphysis for 1500 μm) was conducted by using μCT (Yang et al. 2015) with a 10.5-μm voxel size. The mechanical characteristics of midshaft of femurs were determined using a Bose Electro Force Testing System (ELF3510, Eden Prairie, USA).

Biochemical analysis

Urinary deoxypyridinoline (DPD) levels were determined with a kit (QUIDEL Corporation, CA, USA) to assess bone resorption and the final result of each sample was corrected for its creatinine concentration. Creatine levels in urine were assayed with a Creatinine Assay Kit (Colorimetric/Fluorometric, ab65339, Abcam, Cambridge, MA, USA). Serum levels of Ca^{2+} were assayed using the Hitachi 7170 autoanalyzer (Hitachi, Tokyo, Japan). Content of C-terminal cross-linked telopeptides of type I collagen (CTX, NBP2-82,444) and osteocalcin (NBP2-68,153) and activities of ALP (KA1642, Novus Biologicals) in serum and levels of malondialdehyde (MDA, KA1381) and activity of superoxide dismutase 2 (SOD2, DYC3419-2) in femurs were determined using kits obtained from Novus Biologicals (Littleton, CO, USA). The levels of NAD^+ were assayed using an assay kit (K958, BioVision, USA).

Quantitative RT-PCR measurement

Femurs and cultured cells were harvested and total RNAs were then extracted using TRIzol reagent (R0016, Beyotime Biotechnology). The total RNAs concentrations were measured through the Spectrophotometer (NanoDrop 2000, Thermo Fisher, MA, USA). RT-PCR measurement was done using a RT-PCR Kit (Beyotime Biotechnology, D7268M). Transcription levels of genes of interest were quantified by $2^{-\Delta\Delta Ct}$ method. The information of primers was shown in Table 1.

Measurement of mitochondrial function

Lucigenin-enhanced chemiluminescence was applied for detection of mitochondrial reactive oxygen species (ROS). Mitochondrial adenosine triphosphate (ATP) was quantified using a kit (BioVision). The complex I and II activities were assayed as described previously (Maher et al. 2007). The intracellular NAD^+ content was determined using the kit described above. The mitochondrial copy number was detected using PCR method as described previously (Zhai et al. 2019) using cyclooxygenase-1 (COX-1) and β-actin as marker of mitochondrial DNA and nuclear DNA, respectively. The primer pairs of COX-1 and β-actin were shown in Table 1.

Table 1 Primers sequences used in current study

Genes		Sequence (5'-3')
OPN (mouse)	Sense	GACCACAGGACGACGATG
	Antisense	TGGAACTTGCTTGACTATCGA
Runx2 (mouse)	Sense	GACTGTGGTTACCGTCATGGC
	Antisense	ACTTGGTTTTTCATAACAGCGGA
Osterix (mouse)	Sense	CTGCGGAAAGGAGGCACAAAGAAG
	Antisense	GGGTTAAGGGGAGCAAAGTCAGAT
Osteocalcin (mouse)	Sense	ACGGTATCACTATTTAGGACCTGTG
	Antisense	ACTTTATTTTGGAGCTGCTGTGAC
Col1a1 (mouse)	Sense	ATGTTCAGCTTTGTGGACCTC
	Antisense	CAGAAAGCACAGCACTCGC
COX-1 (mouse)	Sense	ATTGCCCTCCCCTCTCTACGCA
	Antisense	CGTAGCTTCAGTATCATTGGTGCCC
β-actin (mouse)	Sense	CCATGTTCCAAAACCATTCC
	Antisense	GGGCAACCTTCCCAATAAAT
GAPDH (mouse)	Sense	TATGTCGTGGAGTCTACTGGT
	Antisense	GAGTTGTCATATTCTCGTGG
ALP (rat)	Sense	GGGTCAAGGCCAACTACAAGA
	Antisense	CACTGGTCTAATCGAGCAGC
Osteocalcin (rat)	Sense	TCTCTGCTCACTCTGCTGG
	Antisense	GTGGTGCCATAGATGCGCT
TRAP (rat)	Sense	AATTGCCTACTCCAAGATCTCCAA
	Antisense	GCGGAACTTTGAAACGCAAA
GAPDH (rat)	Sense	TATCACTCTACCCACGGCAAG
	Antisense	ATACTCAGCACCAGCATCACC

Western blot

The concentration of protein preparation was detected using a Beyotime bradford protein assay kit (Beyotime). Protein preparations of 25 μg were separated on SDS–polyacrylamide gels, and transferred to PVDF membranes (0.2 μm), and incubated with antibodies. Antibodies used in our work included anti-p-AMPKα (Thr172) (1:800; #50,081, CST, USA), anti-AMPKα (1:800; #5831, CST), and anti-GAPDH (1:5000; ab8245, Abcam).

Statistics

Two-way ANOVA followed by Tukey–Kramer test was applied for our statistical work using software of GraphPad Prism 9.0 (San Diego, CA). Differences were considered significant statistically at P-values < 0.05. All data of current investigation were expressed as mean ± SD.

Results

Benefits of NMN supplementation on bone loss in HLU rats

Adult rats were challenged with HLU exposure and intragastrically administered NMN or vehicle for 4 weeks. As compared to control rats, HLU rats displayed lower femoral and tibial BMD (Fig. 1a, b). NMN supplementation mitigated HLU-induced bone loss, evidenced by greater femoral and tibial BMD.

As compared to control rats, HLU rats displayed impaired bone morphometry of proximal tibiae, evidenced by lesser bone volume/total volume (BV/TV, Fig. 1c), trabecular thickness (Tb.Th, Fig. 1d), trabecular number (Tb.N, Fig. 1e), and trabecular BMD (Fig. 1g), and greater trabecular separation (Tb.Sp, Fig. 1f), which was reversed by NMN supplementation, at least in part.

As compared to control rats, HLU rats displayed impaired biomechanical properties of femur midshaft, evidenced by lesser ultimate load (Fig. 1h), stiffness (Fig. 1i), and energy to max force (Fig. 1j), which was also mitigated by NMN supplementation.

Effects of NMN supplementation on the biomarkers of bone metabolism

When compared to control animals, HLU animals displayed lesser body weight (Fig. 2a) and levels of Ca^{2+} (Fig. 2b), activities of ALP (Fig. 2c), and levels of osteocalcin (Fig. 2d) in serum and greater urinary DPD levels (Fig. 2e) and serum CTX levels (Fig. 2f). Body weight, serum levels of Ca^{2+} or CTX, and urinary levels of DPD in HLU + NMN animals were similar with that in HLU animals. Serum levels of ALP activities and osteocalcin in HLU + NMN animals were greater than that in HLU animals.

As compared to control rats, ALP (Fig. 3a) and osteocalcin (Fig. 3b) mRNA levels were lesser and tartrate-resistant acid phosphatase (TRAP) mRNA level was greater (Fig. 3c) in femurs of HLU rats. The femoral mRNA levels of ALP and osteocalcin in HLU + NMN animals were greater than that in HLU animals. The mRNA levels of TRAP in femurs were similar between HLU + NMN animals and HLU animals.

Antioxidant effects of NMN supplementation in HLU rats

As compared to control rats, levels of NAD^+ (Fig. 4a) in femurs of HLU rats were lower. Femoral levels of NAD^+ were greater in both control and HLU animals after NMN supplementation.
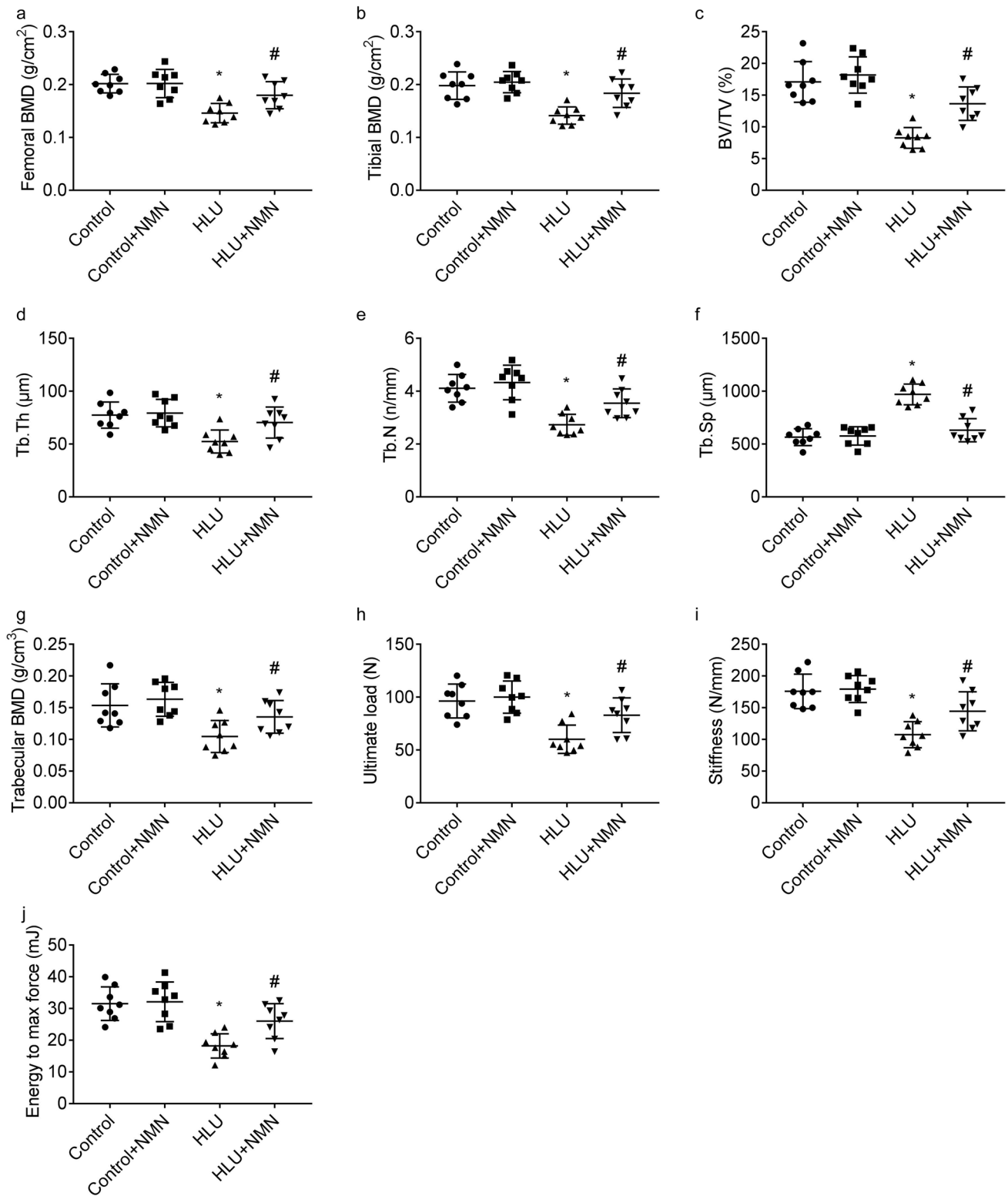
Fig. 1 Benefits of NMN on the osteoporosis-like phenotype in HLU rats. Femoral (**a**) and tibial (**b**) BMD, trabecular bone morphometry including BV/TV, Tb.Th, Tb.N, Tb.Sp, and BMD (**c-g**) of proximal tibiae, and biomechanical properties including ultimate load (**h**), stiffness (**i**), and energy to max force (**j**) of femur midshaft were presented. * $P < 0.05$ vs. Control animals treated with vehicle; # $P < 0.05$ vs. HLU animals treated with vehicle. $n = 8$ or 5/group
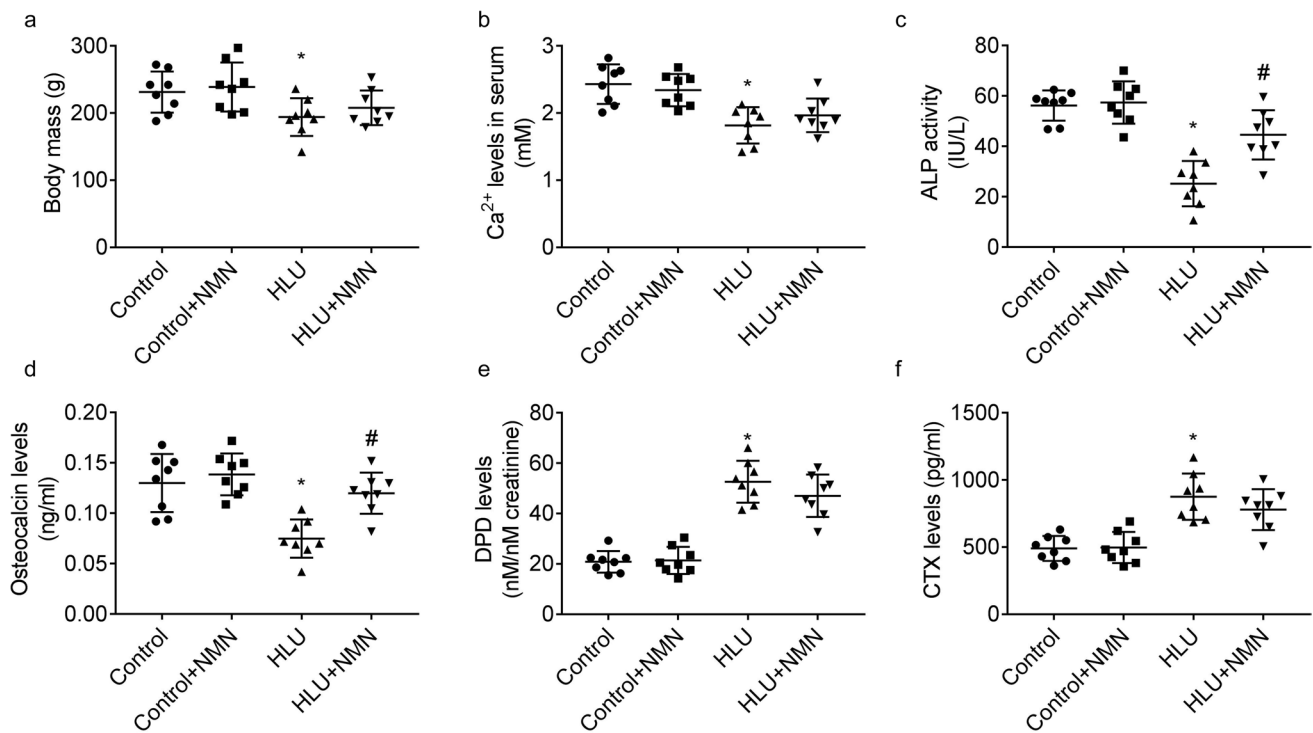
Fig. 2 Benefits of NMN on the biomarkers of bone metabolism in vivo. Graphs showed the body mass (**a**), serum levels of Ca^{2+} (**b**), ALP activity (**c**), osteocalcin (**d**) and CTX (**f**), and urinary lev- els of DPD (**e**). * $P < 0.05$ vs. Control animals treated with vehicle; # $P < 0.05$ vs. HLU animals treated with vehicle. $n = 8$/group
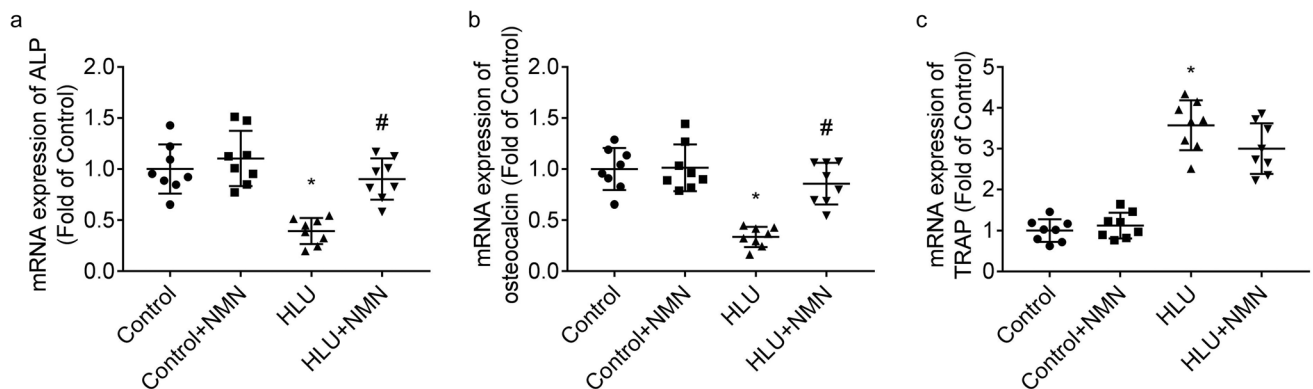
Fig. 3 Modulation of NMN on the biomarkers involved in osteoclas- togenesis and osteoblastogenesis. Graphs showed femoral mRNA lev- els of ALP (**a**), osteocalcin (**b**) and TARP (**c**). * $P < 0.05$ vs. Control animals treated with vehicle; # $P < 0.05$ vs. HLU animals treated with vehicle. $n = 8$/group

As compared to control rats, MDA production (Fig. 4b) in femurs of HLU rats were higher, which was reversed by NMN partly.

Activities of SOD2 (Fig. 4c) in femurs of HLU group were higher when compared to that of control group. Femo- ral SOD2 activities were greater in both control and HLU animals after NMN supplementation.

Benefits of NMN on osteoblastic differentiation

When compared to MC3T3-E1 cells cultured under normal gravity, mRNA levels of OPN (Fig. 5a), Runx2 (Fig. 5b), Osterix (Fig. 5c), and osteocalcin (Fig. 5d) and activities of ALP (Fig. 5f) were lesser under modeled microgravity, which was reversed by NMN treatment dose-dependently.
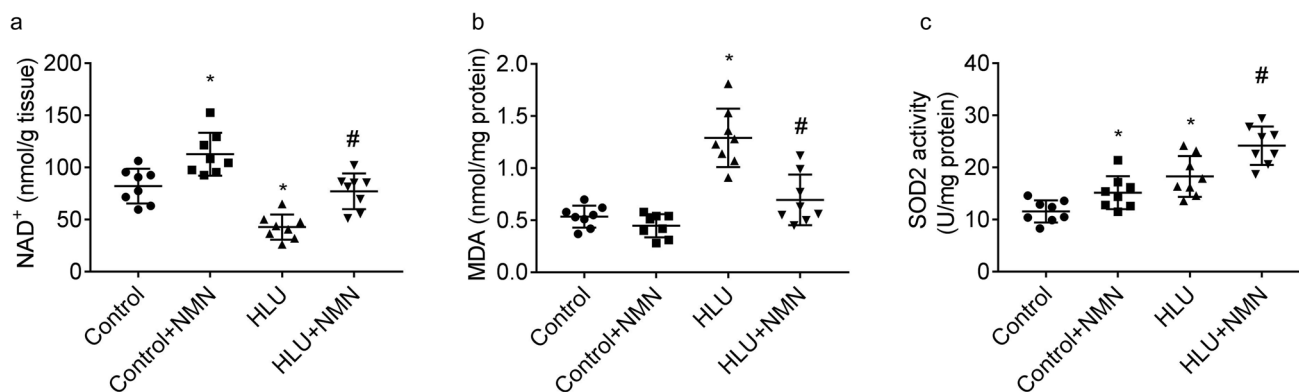
Fig. 4 Modulation of NMN on oxidative stress and NAD$^+$ levels. Graphs presented femoral NAD$^+$ content (**a**) and MDA content (**b**) and SOD2 activity (**c**). * $P < 0.05$ vs. Control animals treated with vehicle; # $P < 0.05$ vs. HLU animals treated with vehicle. $n = 8$/group

Modeled microgravity stimulation had no significant effect on Col1a1 mRNA expression (Fig. 5e). The mRNA expression of Col1a1 were higher in NMN-treated group than that in vehicle-treated group, under both modeled microgravity and normal gravity.

Benefits of NMN treatment on mitochondrial impairment

When compared to MC3T3-E1 cells cultured under normal gravity, intracellular NAD$^+$ levels (Fig. 6a) were lesser under

modeled microgravity. NAD$^+$ levels were greater in NMN-treated group than that in vehicle-treated group, under both modeled microgravity and normal gravity.

When compared to cells cultured under normal gravity, modeled microgravity stimulation led to a severe mitochondrial impairment, evidenced by greater mitochondrial ROS generation (Fig. 6b) and lesser ATP content (Fig. 6c) and Complex I and II activities (Fig. 6e, f), which was reversed by NMN treatment.

When compared to cells cultured under normal gravity, SOD2 activity (Fig. 6d), mtDNA copy number (Fig. 6g),
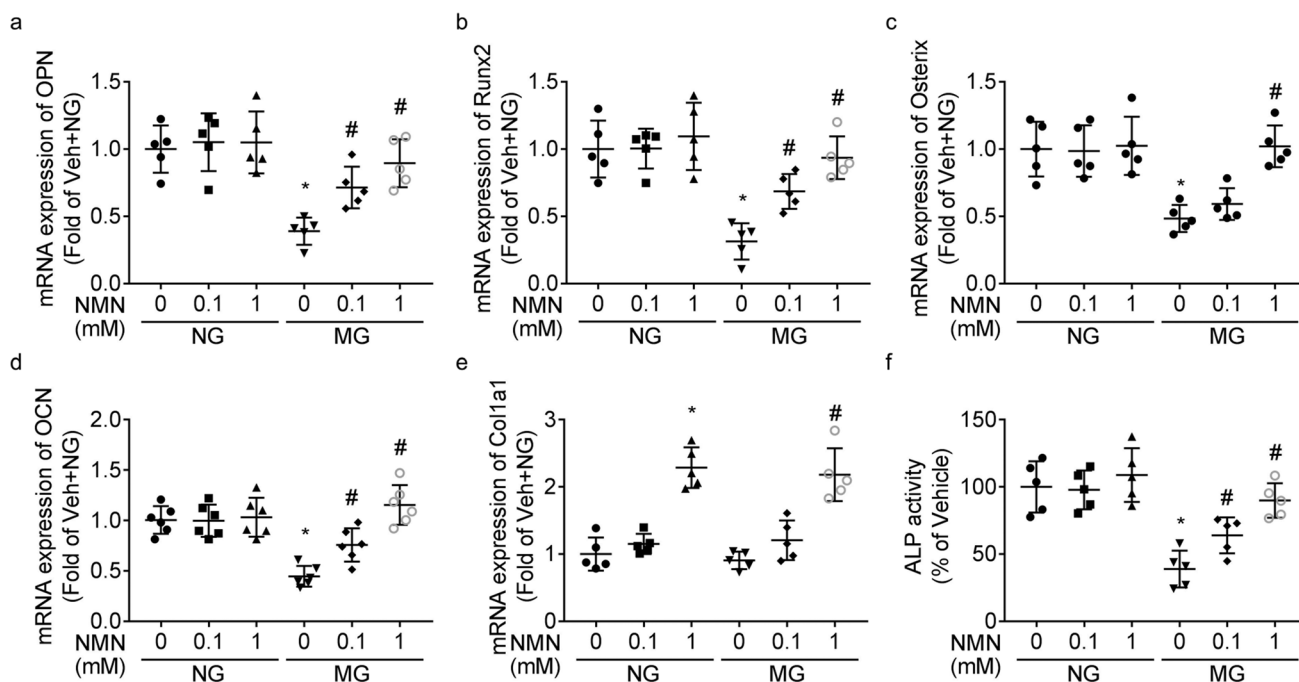
Fig. 5 Benefit of NMN on osteoblastic differentiation. The mRNA levels of genes of OPN (**a**), Runx2 (**b**), Osterix (**c**), osteocalcin (OCN, **d**), and Col1a1 (**e**) and activities of ALP (**f**) were shown. * $P < 0.05$

vs. group cultured under normal gravity (NG) and treated with vehicle; # $P < 0.05$ vs. group cultured under microgravity (MG) and treated with vehicle
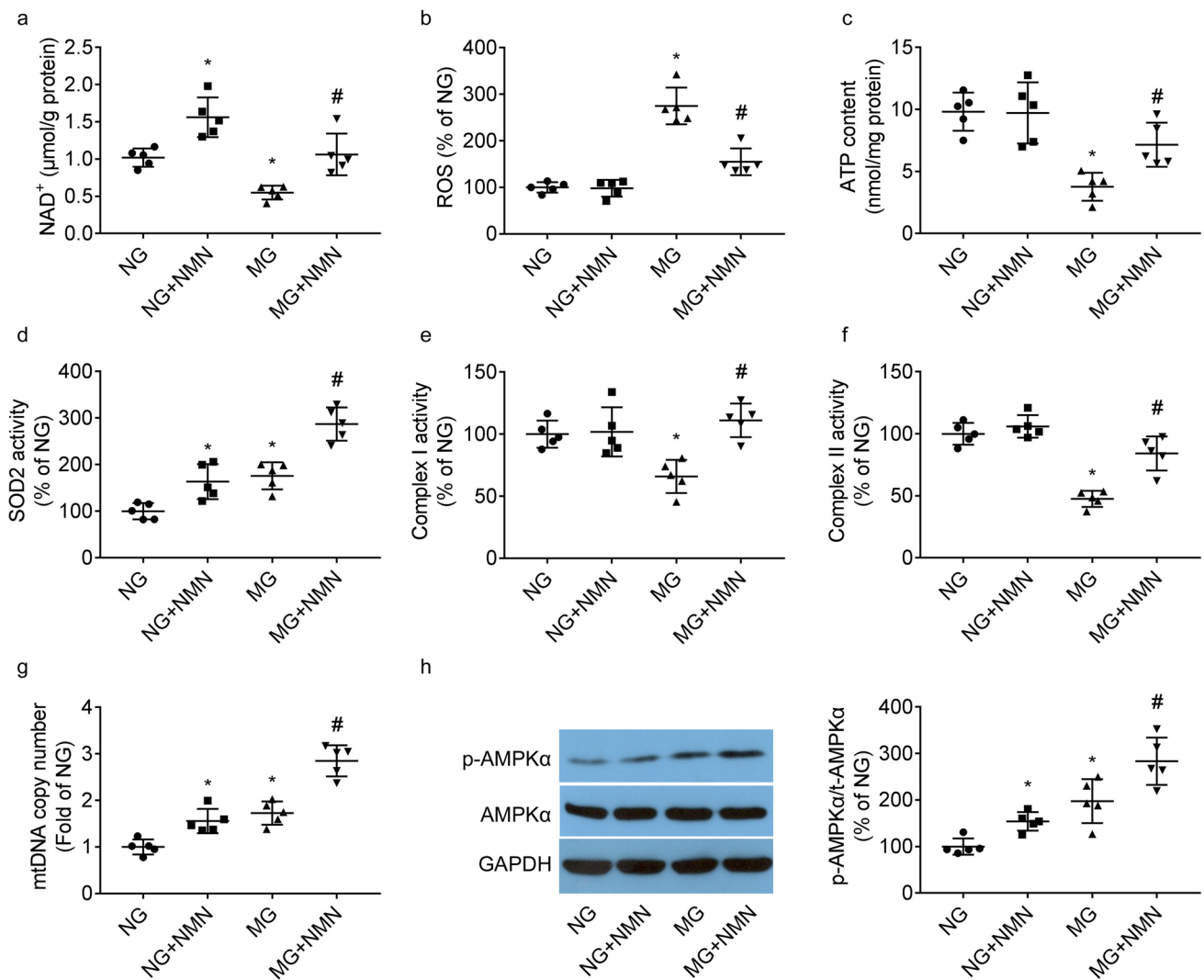
Fig. 6 Benefit of NMN on mitochondrial impairments. Graphs presented the intracellular levels of NAD$^+$ (**a**), mitochondrial ROS production (**b**), ATP content (**c**), activities of SOD2 (**d**) and Complex I and II (**e**, **f**), and mtDNA copy number (**g**). Western blot of AMPKα phosphorylation and responding quantification results (**h**) were shown. * $P < 0.05$ vs. group cultured under normal gravity (NG) and treated with vehicle; # $P < 0.05$ vs. group cultured under microgravity (MG) and treated with vehicle

and AMPKα phosphorylation (Fig. 6h) were greater under modeled microgravity. SOD2 activity, mtDNA copy number, and AMPKα phosphorylation were greater in NMN-treated group than that in vehicle-treated group, under both normal gravity and modeled microgravity.

Discussion

Our work demonstrated that NMN supplementation mitigated osteopenia in HLU rats. The 19.5-day space flight in rats strikingly inhibited bone formation (Morey and Baylink 1978). It was also observed that simulated microgravity in vitro

inhibited differentiation phenotypes of osteoblastic cells (Cao et al. 2021; Xin et al. 2015). Therefore, impairment of bone formation contributed to the osteopenia induced by microgravity. NMN treatment attenuated aluminum or dexamethasone-induced osteogenic inhibition in vitro (Liang et al. 2019; Huang and Tao 2020). In our investigation, serum levels of ALP activities and osteocalcin in HLU + NMN animals were greater than that in HLU animals. Furthermore, NMN treatment reversed the modeled microgravity-induced reduction of differentiation phenotypes in MC3T3-E1 cells. We demonstrated that NMN supplementation promoted osteoblastogenesis, which might explain its anti-osteoporotic action under modeled microgravity.

Oxidative stress is one of the most investigated events involved in bone tissues in response to the microgravity (Tian et al. 2017). Accumulating evidence reported that excessive ROS produced in bone intervened osteoblast differentiation and prevented osteoblast activity (Tao et al. 2020). NMN abated oxidative stress in various diseases involved in heart, aorta, tendon and brain (Yamaura et al. 2022; Wu et al. 2021; Tarantini et al. 2019; de Picciotto et al. 2016). The antioxidant property of NMN was linked to enhanced activities of antioxidant enzymes such as SOD (Wan et al. 2021). SOD was crucial for osteoblast differentiation and trabecular bone loss was reported in the mice of osteoblast lineage *Sod2* deficiency (Schoppa et al. 2022). In current work, SOD2 activities were greater and MDA levels were lesser in femurs of HLU + NMN animals than that of HLU animals. Thus, the antioxidant function of NMN might contributed to its protective effects against bone loss induced by microgravity.

Recently, a comprehensive multi-omics analysis demonstrated that mitochondrial dysfunction including impaired respiratory chain and increased mitochondrial ROS formation was a consistent phenotype for spaceflight biology (da Silveira et al. 2020). In mesenchymal stem cells, simulated microgravity increased mtDNA copy number, but inhibited mitochondrial oxidative phosphorylation (Liu et al. 2020). In current study, Complex I and II activities were lesser in cells when stimulated with modeled microgravity. Active mitochondria were required for osteoblastic differentiation (Shares et al. 2018). NAD^+ was required in glycolysis in mitochondria and NAD depletion resulted in mitochondrial dysfunction (Sims et al. 2018; Alano et al. 2010). In our work, NAD^+ levels were lesser in bone tissues and cells when exposed to modeled microgravity. NMN treatment improved osteoblastic mitochondrial function, which contributed to its benefit against microgravity-induced osteopenia.

AMPK played a key role in bone formation and AMPKα deficiency inhibited osteoblastic differentiation (Kanazawa et al. 2018). The crosstalk between mitochondria and AMPK, such as mitochondrial impairment causing abnormal activation of AMPK and AMPK activation preserving mitochondrial homeostasis (Wu and Zou 2020; Jornayvaz and Shulman 2010), have been demonstrated. In our work, AMPKα phosphorylation were greater in NMN-treated group than that in vehicle-treated group, under both normal gravity and modeled microgravity. It might also contribute to its protection against microgravity-induced mitochondrial dysfunction and bone loss.

It should be noted that NMN supplementation had good safety. No increased mortality rate or obvious side effects was reported in mice after NMN administration (300 mg/kg/day) for one year (Mills et al. 2016). In rats, oral NMN supplementation for 90 days at doses up to 1500 mg/kg/d did not generate serious toxicity as seen from biochemical parameters in blood as well as histological analysis of liver and kidney (Cros et al. 2021). These reports provided hope for translating the findings of our work to human beings.

As a ground-based analog for microgravity in animals, HLU model had several weaknesses including inconsistencies of stress reaction in HLU animals and continued loading of forelimbs and upper spine (Globus and Morey-Holton 2016). Therefore, further investigations are needed to confirm the efficacy of NMN in animals in spaceflight and humans in bed rest and spaceflight.

In summary, NMN supplementation attenuated osteoblastic mitochondrial impairment and mitigated osteopenia induced by modeled microgravity in rats. NMN supplementation may be a promising nutritional strategy for anti-osteoporotic therapy of astronauts and geriatric population.

Declarations

References

Alano CC, Garnier P, Ying W, Higashi Y, Kauppinen TM, Swanson RA (2010) NAD+ depletion is necessary and sufficient for poly(ADP-ribose) polymerase-1-mediated neuronal death. J Neurosci 30:2967–2978. https://doi.org/10.1523/JNEUROSCI.5552-09.2010

Cao Z, Zhang Y, Wei S, Zhang X, Guo Y, Han B (2021) Comprehensive circRNA expression profile and function network in osteoblast-like cells under simulated microgravity. Gene 764:145106. https://doi.org/10.1016/j.gene.2020.145106

Coulombe JC, Senwar B, Ferguson VL (2020) Spaceflight-Induced Bone Tissue Changes that Affect Bone Quality and Increase Fracture Risk. Curr Osteoporos Rep 18:1–12. https://doi.org/10.1007/s11914-019-00540-y

Covarrubias AJ, Perrone R, Grozio A, Verdin E (2021) NAD+ metabolism and its roles in cellular processes during ageing.

Nat Rev Mol Cell Biol 22:119–141. https://doi.org/10.1038/s41580-020-00313-x

Cros C, Cannelle H, Laganier L, Grozio A, Canault M (2021) Safety evaluation after acute and sub-chronic oral administration of high purity nicotinamide mononucleotide (NMN-C®) in Sprague-Dawley rats. Food Chem Toxicol 150:112060. https://doi.org/10.1016/j.fct.2021.112060

Croteau DL, Fang EF, Nilsen H, Bohr VA (2017) NAD+ in DNA repair and mitochondrial maintenance. Cell Cycle 16:491–492. https://doi.org/10.1080/15384101.2017.1285631

da Silveira WA, Fazelinia H, Rosenthal SB, Laiakis EC, Kim MS, Meydan C, Kidane Y, Rathi KS, Smith SM, Stear B, Ying Y, Zhang Y, Foox J, Zanello S, Crucian B, Wang D, Nugent A, Costa HA, Zwart SR, Schrepfer S, Elworth RAL, Sapoval N, Treangen T, MacKay M, Gokhale NS, Horner SM, Singh LN, Wallace DC, Willey JS, Schisler JC, Meller R, McDonald JT, Fisch KM, Hardiman G, Taylor D, Mason CE, Costes SV, Beheshti A (2020) Comprehensive Multi-omics Analysis Reveals Mitochondrial Stress as a Central Biological Hub for Spaceflight Impact. Cell 183:1185-1201.e20. https://doi.org/10.1016/j.cell.2020.11.002

de Picciotto NE, Gano LB, Johnson LC, Martens CR, Sindler AL, Mills KF, Imai S, Seals DR (2016) Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice. Aging Cell 15:522–530. https://doi.org/10.1111/acel.12461

Frings-Meuthen P, Bernhardt G, Buehlmeier J, Baecker N, May F, Heer M (2019) The negative effect of unloading exceeds the bone-sparing effect of alkaline supplementation: a bed rest study. Osteoporos Int 30:431–439. https://doi.org/10.1007/s00198-018-4703-6

Gao J, Feng Z, Wang X, Zeng M, Liu J, Han S, Xu J, Chen L, Cao K, Long J, Li Z, Shen W, Liu J (2018) SIRT3/SOD2 maintains osteoblast differentiation and bone formation by regulating mitochondrial stress. Cell Death Differ 25:229–240. https://doi.org/10.1038/cdd.2017.144

Globus RK (1985) Morey-Holton E (2016) Hindlimb unloading: rodent analog for microgravity. J Appl Physiol 120:1196–1206. https://doi.org/10.1152/japplphysiol.00997.2015

Hong W, Mo F, Zhang Z, Huang M, Wei X (2020) Nicotinamide Mononucleotide: A Promising Molecule for Therapy of Diverse Diseases by Targeting NAD+ Metabolism. Front Cell Dev Biol 8:246. https://doi.org/10.3389/fcell.2020.00246

Huang RX, Tao J (2020) Nicotinamide mononucleotide attenuates glucocorticoid-induced osteogenic inhibition by regulating the SIRT1/PGC-1α signaling pathway. Mol Med Rep 22:145–154. https://doi.org/10.3892/mmr.2020.11116

Jornayvaz FR, Shulman GI (2010) Regulation of mitochondrial biogenesis. Essays Biochem 47:69–84. https://doi.org/10.1042/bse0470069

Kanazawa I, Takeno A, Tanaka KI, Notsu M, Sugimoto T (2018) Osteoblast AMP-Activated Protein Kinase Regulates Postnatal Skeletal Development in Male Mice. Endocrinology 159:597–608. https://doi.org/10.1210/en.2017-00357

Lee CF, Chavez JD, Garcia-Menendez L, Choi Y, Roe ND, Chiao YA, Edgar JS, Goo YA, Goodlett DR, Bruce JE, Tian R (2016) Normalization of NAD+ Redox Balance as a Therapy for Heart Failure. Circulation 134:883–894. https://doi.org/10.1161/CIRCULATIONAHA.116.022495

Liang H, Gao J, Zhang C, Li C, Wang Q, Fan J, Wu Z, Wang Q (2019) Nicotinamide mononucleotide alleviates Aluminum induced bone loss by inhibiting the TXNIP-NLRP3 inflammasome. Toxicol Appl Pharmacol 362:20–27. https://doi.org/10.1016/j.taap.2018.10.006

Liu L, Cheng Y, Wang J, Ding Z, Halim A, Luo Q, Song G (2020) Simulated Microgravity Suppresses Osteogenic Differentiation of Mesenchymal Stem Cells by Inhibiting Oxidative Phosphorylation. Int J Mol Sci 21:9747. https://doi.org/10.3390/ijms21249747

Maher P, Salgado KF, Zivin JA, Lapchak PA (2007) A novel approach to screening for new neuroprotective compounds for the treatment of stroke. Brain Res 1173:117–125. https://doi.org/10.1016/j.brainres.2007.07.061

Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y, Redpath P, Migaud ME, Apte RS, Uchida K, Yoshino J, Imai SI (2016) Long-Term Administration of Nicotinamide Mononucleotide Mitigates Age-Associated Physiological Decline in Mice. Cell Metab 24:795–806. https://doi.org/10.1016/j.cmet.2016.09.013

Morey ER, Baylink DJ (1978) Inhibition of bone formation during space flight. Science 201:1138–1141. https://doi.org/10.1126/science.150643

Ryu KW, Nandu T, Kim J, Challa S, DeBerardinis RJ, Kraus WL (2018) Metabolic regulation of transcription through compartmentalized NAD+ biosynthesis. Science. 360:aan5780. https://doi.org/10.1126/science.aan5780

Schoppa AM, Chen X, Ramge JM, Vikman A, Fischer V, Haffner-Luntzer M, Riegger J, Tuckermann J, Scharffetter-Kochanek K, Ignatius A (2022) Osteoblast lineage Sod2 deficiency leads to an osteoporosis-like phenotype in mice. Dis Model Mech 15:dmm049392. https://doi.org/10.1242/dmm.049392

Shares BH, Busch M, White N, Shum L, Eliseev RA (2018) Active mitochondria support osteogenic differentiation by stimulating β-catenin acetylation. J Biol Chem 293:16019–16027. https://doi.org/10.1074/jbc.RA118.004102

Sims CA, Guan Y, Mukherjee S, Singh K, Botolin P, Davila A Jr, Baur JA (2018) Nicotinamide mononucleotide preserves mitochondrial function and increases survival in hemorrhagic shock. JCI Insight 3:e120182. https://doi.org/10.1172/jci.insight.120182

Smith SM, Wastney ME, Morukov BV, Larina IM, Nyquist LE, Abrams SA, Taran EN, Shih CY, Nillen JL, Davis-Street JE, Rice BL, Lane HW (1999) Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. Am J Physiol 277:R1-10. https://doi.org/10.1152/ajpregu.1999.277.1.r1

Song J, Li J, Yang F, Ning G, Zhen L, Wu L, Zheng Y, Zhang Q, Lin D, Xie C, Peng L (2019) Nicotinamide mononucleotide promotes osteogenesis and reduces adipogenesis by regulating mesenchymal stromal cells via the SIRT1 pathway in aged bone marrow. Cell Death Dis 10:336. https://doi.org/10.1038/s41419-019-1569-2

Tao H, Ge G, Liang X, Zhang W, Sun H, Li M, Geng D (2020) ROS signaling cascades: dual regulations for osteoclast and osteoblast. Acta Biochim Biophys Sin (shanghai) 52:1055–1062. https://doi.org/10.1093/abbs/gmaa098

Tarantini S, Valcarcel-Ares MN, Toth P, Yabluchanskiy A, Tucsek Z, Kiss T, Hertelendy P, Kinter M, Ballabh P, Süle Z, Farkas E, Baur JA, Sinclair DA, Csiszar A, Ungvari Z (2019) Nicotinamide mononucleotide (NMN) supplementation rescues cerebromicrovascular endothelial function and neurovascular coupling responses and improves cognitive function in aged mice. Redox Biol 24:101192. https://doi.org/10.1016/j.redox.2019.101192

Tian Y, Ma X, Yang C, Su P, Yin C, Qian AR (2017) The Impact of Oxidative Stress on the Bone System in Response to the Space Special Environment. Int J Mol Sci 18:2132. https://doi.org/10.3390/ijms18102132

Wan Y, He B, Zhu D, Wang L, Huang R, Zhu J, Wang C, Gao F (2021) Nicotinamide mononucleotide attenuates doxorubicin-induced cardiotoxicity by reducing oxidative stress, inflammation and apoptosis in rats. Arch Biochem Biophys 712:109050. https://doi.org/10.1016/j.abb.2021.109050

Wu S, Zou MH (2020) AMPK, Mitochondrial Function, and Cardiovascular Disease. Int J Mol Sci 21:4987. https://doi.org/10.3390/ijms21144987

Wu K, Li B, Lin Q, Xu W, Zuo W, Li J, Liu N, Tu T, Zhang B, Xiao Y, Liu Q (2021) Nicotinamide mononucleotide attenuates isoproterenol-induced cardiac fibrosis by regulating oxidative stress and Smad3 acetylation. Life Sci 274:119299. https://doi.org/10.1016/j.lfs.2021.119299

Xin M, Yang Y, Zhang D, Wang J, Chen S, Zhou D (2015) Attenuation of hind-limb suspension-induced bone loss by curcumin is associated with reduced oxidative stress and increased vitamin D receptor expression. Osteoporos Int 26:2665–2676. https://doi.org/10.1007/s00198-015-3153-7

Yamaura K, Mifune Y, Inui A, Nishimoto H, Kurosawa T, Mukohara S, Hoshino Y, Niikura T, Kuroda R (2022) Antioxidant effect of nicotinamide mononucleotide in tendinopathy. BMC Musculoskelet Disord 23:249. https://doi.org/10.1186/s12891-022-05205-z

Yang X, He B, Liu P, Yan L, Yang M, Li D (2015) Treatment with curcumin alleviates sublesional bone loss following spinal cord injury in rats. Eur J Pharmacol 765:209–216. https://doi.org/10.1016/j.ejphar.2015.08.036

Zayzafoon M, Gathings WE, McDonald JM (2004) Modeled microgravity inhibits osteogenic differentiation of human mesenchymal stem cells and increases adipogenesis. Endocrinology 145:2421–2432. https://doi.org/10.1210/en.2003-1156

Zhai Y, Behera J, Tyagi SC, Tyagi N (2019) Hydrogen sulfide attenuates homocysteine-induced osteoblast dysfunction by inhibiting mitochondrial toxicity. J Cell Physiol 234:18602–18614. https://doi.org/10.1002/jcp.28498