

Article

Administration of Nicotinamide Mononucleotide Mitigates the HIV Nef-Induced Metabolic and Pathological Changes in the Heart

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Abstract

Due to the application of antiretroviral therapy, HIV has become a manageable chronic disease, and people living with HIV/AIDS (PLWHA) experience several comorbidities, including cardiovascular disease. Although antiretroviral therapy suppresses the viral load to an undetectable level, HIV proteins can still be detected in the circulation and in different organs. In our previous study, we found that the expression of the Nef protein causes cardiac dysfunction and heart failure in a transgenic mouse model. We also observed inhibition of autophagy along with the upregulation of the senescence marker Bcl2. To further understand the metabolic changes related to Nef in cardiac tissue, we examined nicotinamide adenine dinucleotide (NAD) metabolism in the heart. Our metabolic study with cardiac tissue revealed that Nef expression decreases NAD⁺ levels in the heart. Additionally, we explored whether replenishing cellular NAD⁺ could be a potential therapeutic target for HIV-associated cardiovascular disease. Interestingly, our study found that NMN treatment can improve cellular autophagy, decrease the senescence marker Bcl2, and reduce fibrosis in the heart. Overall, our study suggests that NMN could serve as a promising therapeutic molecule for the treatment of HIV-associated cardiovascular comorbidities.

Keywords: NAD; NMN; HIV; Nef; fibrosis; comorbidities; cardiomyopathy; aging



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1. Introduction

According to a recent report by the World Health Organization (WHO), more than 40 million people were living with human immunodeficiency virus (HIV) in 2025, and 1.3 million people acquired HIV in 2024 [1]. The presence of a replication-competent latent viral reservoir during ART is an existing challenge to the development of a cure for HIV. People living with HIV (PLWH) show a higher life expectancy, and HIV has become a manageable chronic disease. The pathogenesis of HIV-associated cardiovascular disease is multifactorial and not fully understood. Clinical studies suggest that HIV-associated CVDs result from persistent inflammation, metabolic disturbances, and oxidative stress [2–5]. The heart is a metabolically active organ and requires a high level of energy to continuously pump blood throughout the body.

Nicotinamide mononucleotide (NMN) is one of the naturally occurring cellular metabolites found in almost every cell and functions as a cofactor for enzymes in energy metabolism [6]. In the cell, NMN is converted to NAD, which participates in oxidation–reduction reactions. Importantly, it was noted that NAD levels significantly decreased with age and in comorbidities associated with chronic disease, such as obesity, diabetes, neurological dysregulation, muscle loss, and the development of CVDs [7–9].

The cardiac muscle primarily relies on the NAD salvage pathway to produce NAD and regulate heart function [10]. Nicotinamide phosphoribosyl transferase (NAMPT) is a rate-limiting enzyme in the salvage pathway that generates NAD. Further studies have shown that NAMPT expression can vary during cardiac diseases, such as dilated cardiomyopathy and cardiac hypertrophy. Additionally, it was found that overexpression of NAMPT can have some beneficial effects on the heart in a mouse model of heart failure [11,12].

Growing evidence suggests that increasing cellular NAD⁺ levels can enhance heart function and prolong lifespan [9,13–15]. Beyond its well-known role in metabolic disorders, NAD⁺ also participates in inflammatory processes and contributes to antiviral defense [16–18]. In the past, Murray et al. demonstrated that NAD⁺ is a critical player in host–pathogen interactions of HIV, with levels decreasing in HIV-infected cells [19]. Later research reported a significantly decreased level of total NAD in the skeletal muscles of HIV-infected individuals compared to the general population [18]. Notably, NAD⁺ depletion in people living with HIV/AIDS (PLWHA) was strongly linked to viral coinfections such as hepatitis C virus (HCV) and cytomegalovirus (CMV), indicating that inflammation impacts NAD⁺ homeostasis [18]. However, the exact cause and mechanism of NAD reduction in HIV-infected cells remain unclear. Additionally, studies reveal an intriguing crosstalk between NAD⁺ and autophagy. On one side, autophagy regulates cellular NAD⁺, and on the other, NAD⁺ is required for autophagy progression [20–22]. Since NAD⁺ is closely connected to cellular energy metabolism, autophagy, and viral infection, it can be a therapeutic target for the treatment of HIV-associated cardiovascular disorders [7].

Previously, we reported that Nef expression causes cellular toxicity in cardiomyocytes and leads to cardiac dysfunction in Nef transgenic mice through the inhibition of autophagy. Moreover, we found that Bcl2 expression was significantly increased in Nef-expressing cells [23,24]. Bcl2 plays an important role in the cell through the regulation of cellular autophagy and cell death, and its expression is notably increased in the heart during cardiomyopathy conditions [25]. However, a pharmacological target for neutralizing Nef-induced cellular toxicity and improvement of CVDs still needs to be identified.

Recent studies have demonstrated that the administration of β -nicotinamide mononucleotide (NMN) can increase cellular NAD levels [9,26]. To test whether boosting the NAD⁺ pool can improve heart function in Nef-expressing mice, we used previously generated Nef transgenic mice [24]. We hypothesized that NMN administration would improve heart function in Nef mice. To test our hypothesis, we administered NMN or PBS daily for 1 month to Nef or wild-type (WT) mice. We found that 30 days of NMN treatment robustly modulates autophagy markers and reduces Bcl2 expression. We also observed that NMN treatment prevents cardiac remodeling in Nef mice.

2. Materials and Methods

2.1. Animal Model and Ethics

In the current study, we used Nef transgenic mice previously generated in our laboratory and their nontransgenic littermates as a control group [24]. The mice were housed in ventilated cages with free access to water and food on a 12-h light-dark cycle with standard temperature conditions. We performed experiments based on our approved protocol by the Institutional Animal Care and Use Committee of the University of Central Florida

(IACUC: IPROTO202300054) and guidelines provided by the Guide for the Care and Use of Laboratory Animals.

2.2. NAD (NADH and NAD⁺) Determination Assay

The total amount of NADH and NAD⁺ was detected using the NAD/NADH assay kit (Fluorometric) (Abcam, Cambridge, UK) according to the manufacturer's instructions. In brief, 20 mg of Nef-expressing adult mouse heart tissue was homogenized in 400 µL lysis buffer using a bead mill 24 tissue homogenizer (ThermoFisher Scientific, Waltham, MA, USA) [24]. The homogenized tissue samples were centrifuged, and the supernatant was transferred to a new corresponding tube and used for the assay. An aliquot of 25 µL of the samples was used to add the corresponding NAD/NADH control, NADH, or NAD extraction solution. After 15 min of incubation at 37 °C, 25 µL of NAD/NADH control, NAD or NADH extraction solution was added to a corresponding well to neutralize NADH or NAD extracts. Then, 75 µL of NADH reaction mixture was added to every reaction to make the final total assay volume 150 µL per well. The reactions were set up in a 96-well, transparent-bottom, black plate (Thermo Fisher, Waltham, MA, USA) at room temperature. Fluorescence intensity was measured at Ex/Em = 540/590 nm using a Synergy H1 plate reader (Agilent BioTek, Santa Clara, CA, USA). The level of NAD, NAD⁺, and NADH in heart tissue lysate was calculated as relative fluorescence units.

2.3. NMN Administration

For the NMN drug treatment study, Nef mice (TG33) and their non-transgenic littermates (WT) aged 10–13 weeks were randomized to receive either NMN (Sigma, St. Louis, MO, USA) or phosphate-buffered saline (PBS) (Sigma). Four groups were formed: two groups (Nef and WT) received 100 mg/kg/day of NMN subcutaneously (SQ) in a total volume of 100 µL of sterile PBS, and the other two groups (Nef and WT) received sterile PBS (Vehicle) [26]. All injections were administered daily for 30 days. After treatment, mice were euthanized, and their hearts were collected and frozen for molecular biology analysis. Additionally, hearts were perfused for histological examination.

2.4. Echocardiography

Heart function of mice was measured by transthoracic echocardiography according to the protocol described earlier [24]. Heart function was determined in Nef and WT mice by ultrasonography using Vevo 3100 (FujiFilm VisualSonic, Toronto, ON, Canada). For evaluation of heart function, echocardiographic parameters of the left ventricle, including ejection fraction (EF), fractional shortening (FS), cardiac output (CO), stroke volume (SV), and the thickness of the anterior (LVAW) and posterior wall (LVPW) during systole and diastole, were calculated using Vevo LAB software (version 5.6.1) [24]. Heart function was compared between the NMN and PBS treatment groups.

2.5. Histology

For histological evaluation, the mouse hearts were perfused with 10% Neutral Buffered Formalin (NBF) (Epredia, Kalamazoo, MI, USA) and stored in 10% NBF. The longitudinal sections of the heart were prepared and fixed overnight in 10% NBF (Epredia). The next day, tissue sections were processed and embedded in paraffin. For histological staining, paraffin-embedded heart tissues were sectioned at 5 µm thickness. Mouse heart tissue sections were stained with Hematoxylin and Eosin (H&E) (Sigma) and Masson's trichrome stain (Sigma) to determine the tissue structure and collagen deposition of the heart [24]. Images of the stained tissue sections were captured using a fluorescence microscope (BZ-X800, Keyence, Osaka, Japan). Images of Masson's trichrome-stained tissue sections were analyzed using BZ-X800 software (Keyence, version 1.1.1.8) [24].

2.6. Western Blotting

The expression of different signals in heart tissue was determined by Western blot as described previously [24]. In brief, 25 mg of heart tissue was homogenized in cold 0.1% SDS RIPA buffer containing a 1X protease inhibitor cocktail (Sigma). Homogenized tissue lysates were centrifuged at 12,000 rpm for 30 min at 4 °C, and supernatants were collected and used for Western blotting.

Protein concentration in the supernatant was determined by the Bradford protein assay (Sigma). Protein samples were prepared in 1X Laemmli buffer and resolved on a sodium dodecyl sulfate–polyacrylamide gel (SDS-PAGE) along with a protein ladder (Li-COR, Lincoln, NE, USA) and transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA). After incubation in blocking buffer (Li-COR) for 2 h at room temperature, the membranes were exposed to primary antibodies in 2.5% nonfat dry milk (Bio-Rad) in PBST (0.1% Tween 20) at 4 °C overnight. Then, membranes were washed with 1X PBST and 1X PBS and incubated with secondary antibodies labeled with IRDye (Li-COR) in 2.5% nonfat dry milk blocking buffer (Bio-Rad). Membranes were scanned using the Odyssey Infrared Imaging System (Li-COR) and quantified using Image Studio software (ver. 4.0, Li-COR). The primary antibodies used for the Western blot were HIV-1 Nef (AIDS reagent, Manassas, VA, USA), LC3 (Sigma), Beclin-1, Bcl2, NAMPT, GAPDH, and β -actin (Proteintech Group, Rosemont, IL, USA), and p62/SQSTM1 (Abnova, Taipei City, Taiwan, China). Protein expression on the Western blot was normalized to GAPDH or β -actin.

2.7. Statistical Analysis

Statistical analysis in various experiments was performed using GraphPad Prism 9.4 software (Dotmatics, Boston, MA, USA). To assess differences between experimental groups, either a *t*-test or one-way ANOVA was used. Data are presented as mean $\pm$ standard deviation. A *p*-value of ≤ 0.05 was considered statistically significant.

3. Results

3.1. Nef Protein Causes Dysregulation of Cellular NAD Metabolism

In our previous study, we found that Nef protein expression induces cardiotoxicity [23], heart failure, and upregulates senescence markers in the heart [24]. NAD plays a critical role in the cell in maintaining cellular energy metabolism and redox balance by maintaining the cellular NAD⁺/NADH ratio. Depletion of NAD⁺ levels and a decrease in the NAD⁺/NADH ratio have been reported in the aging heart and in heart failure [10,27]. NAD⁺ depletion was also observed in HIV-1-infected cells [19]. To further understand the impact of the Nef protein on cellular NAD metabolism, we evaluated NAD levels and NAMPT expression in the hearts of Nef mice. We measured the levels of NAD, NAD⁺, and NADH using heart tissue lysates through biochemical assays. Our study found that the total NAD level did not change in the heart tissue lysates in the presence of Nef protein (Figure 1A). However, levels of NAD⁺ and NADH were significantly altered in Nef-expressing heart tissue. Additionally, we observed that an indicator of redox imbalance, the NAD⁺/NADH ratio, was significantly decreased in the heart tissue in the presence of Nef protein (Figure 1B). Further, to understand the cause of dysregulation of the NAD⁺/NADH ratio, we detected the expression of NAMPT in the heart tissue by Western blot. Our study found that Nef expression slightly decreased NAMPT levels in the hearts of Nef mice (Figure 1C,D). However, these changes are not statistically significant. Further, we checked the expression of NAMPT in HEK293 cells in the presence of Nef protein. Cells were transfected with the Nef and control plasmids [24]. Our analysis showed that Nef expression did not change the NAMPT expression (Figure S1). These observations

indicate that the HIV protein Nef may alter cellular energy metabolism and redox balance by inhibiting cellular NAD⁺.

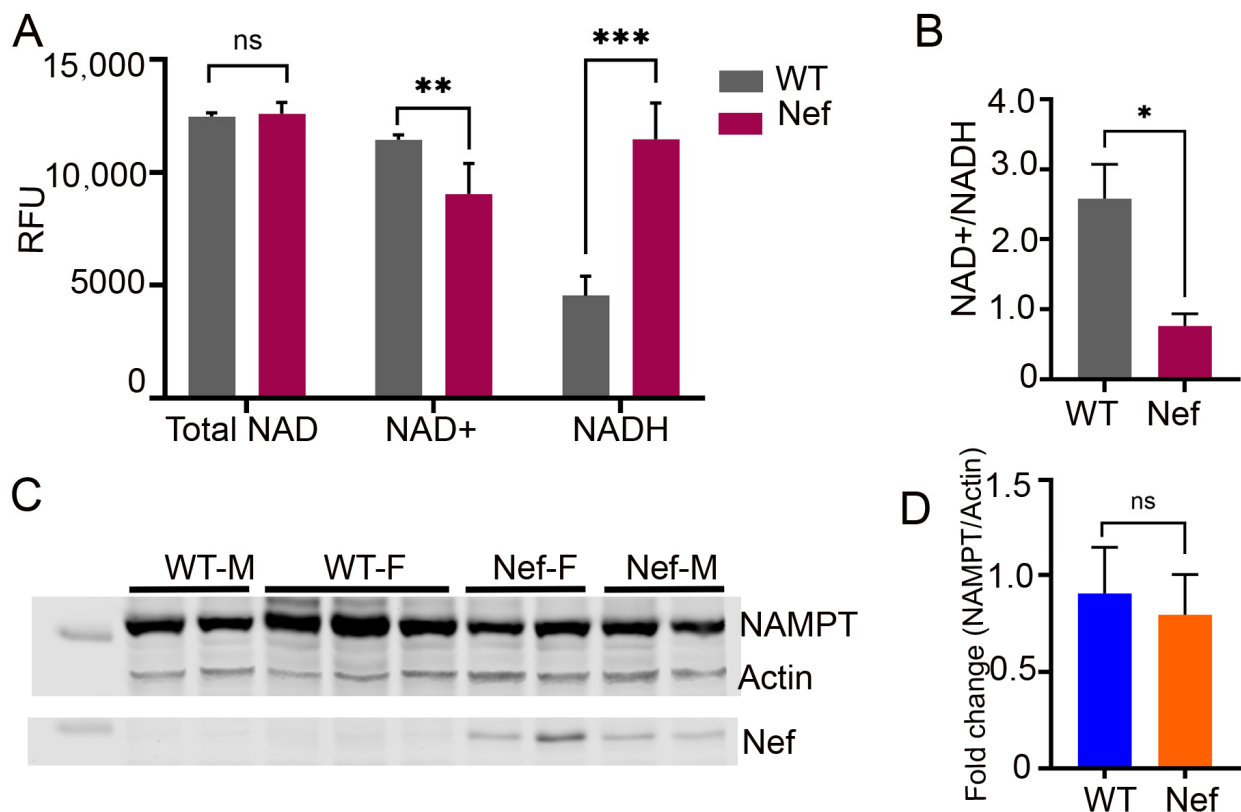


Figure 1. Nef protein expression is associated with decreased cellular NAD⁺ levels in cardiac tissue. (A) The biochemical assay was performed with tissue lysates isolated from Nef-expressing hearts at 8–9 weeks of age, including both male and female mice. The graphs show the levels of the total cellular NAD, NAD⁺, NADH, and (B) NAD⁺ to NADH ratio (WT ($n = 3$) and Nef ($n = 5$)). (C) Representative Western blot images showing the expression of NAMPT in the heart tissue of WT and Nef mice at 11–13 weeks. Data represent pooled results from both male and female mice (WT-M ($n = 11$), WT-F ($n = 11$), Nef-M ($n = 5$), Nef-F ($n = 6$)). (D) Graph showing the quantification of NAMPT expression. Expression of NAMPT was normalized to the endogenous β -actin. Data are presented as mean with standard deviation. Statistical significance was assessed between WT and Nef groups using the t -test (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = non-significant).

3.2. Administration of NMN Improves Heart Function in Nef Mice

In our study, we found that the NAD⁺ level was significantly altered due to Nef protein expression. Therefore, we tested the beneficial effect of NAD⁺ on heart function through the administration of NMN. NMN injections were given SQ to Nef and WT mice at 10–12 weeks of age. Age- and gender-matched Nef and WT mice treated with PBS were used as a control. The treatment was performed daily for 30 days. Heart function was assessed on the last day of injection. Consistent with our previous study, we noticed that Nef mice have decreased heart function (Figure 2A–C). Interestingly, we observed that heart function in NMN-treated Nef mice improved after drug treatment compared with PBS-treated Nef mice. All these data together suggest that NMN might be capable of preserving cardiac function in the Nef-expressing heart (Table 1, Figure 2A–C).

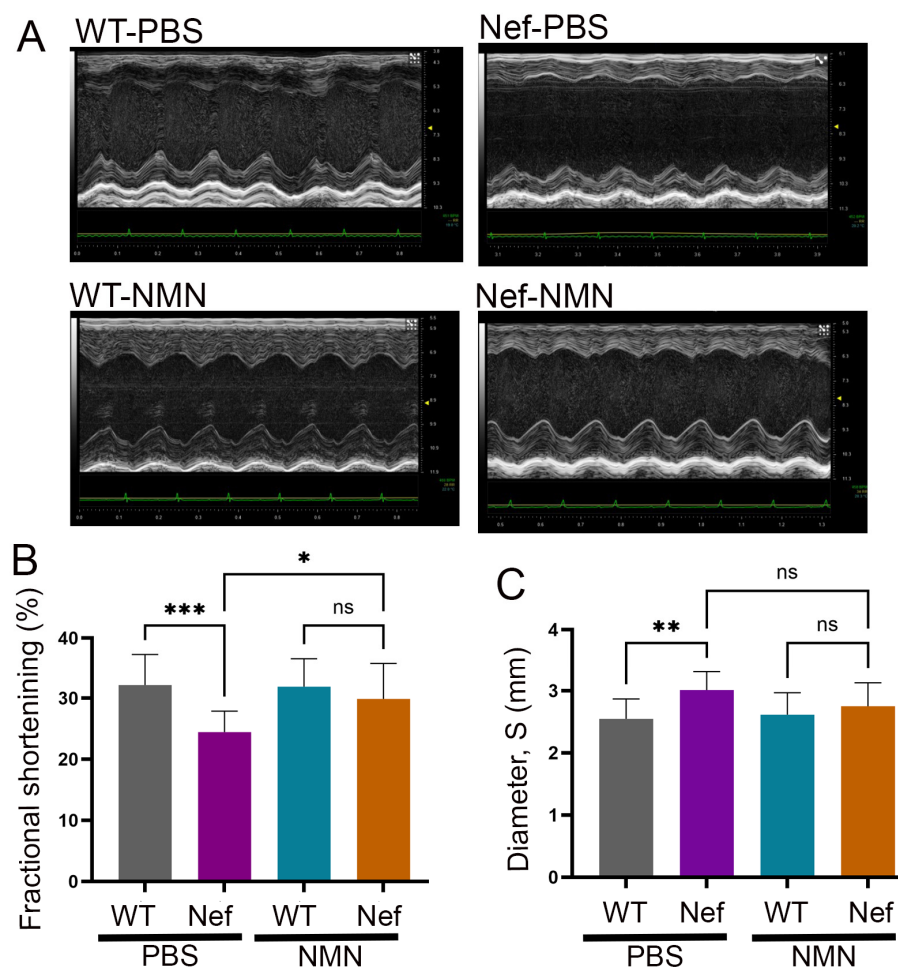


Figure 2. NMN administration improves heart function in Nef mice. (A) Representative images showing M-mode echocardiography of the left ventricle post-NMN treatment at 15–17 weeks. (B,C) Graphs showing the quantification of fractional shortening and diameters of the left ventricle during systole. Data represent pooled results from both male and female mice (WT-PBS ($n = 18$), Nef-PBS ($n = 17$), WT-NMN ($n = 17$), and Nef-PBS ($n = 12$)). Data are presented as mean with standard deviation. Statistical significance was determined between WT and Nef mice treated with PBS or NMN (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, ns = non-significant).

Table 1. Heart function analysis by echocardiography. Values shown are mean $\pm$ standard deviation. Statistical significance was determined between WT and Nef mice treated with PBS (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$) or Nef mice treated with PBS or NMN (# $p \leq 0.05$, ## $p \leq 0.01$).

	WT-PBS ($n = 18$)	Nef-PBS ($n = 17$)	WT-NMN ($n = 17$)	Nef-NMN ($n = 12$)
LV DIAMs (mm)	2.5470 $\pm$ 0.3205	3.0125 $\pm$ 0.2943 **	2.616 $\pm$ 0.3519	2.751 $\pm$ 0.3495
LV DIAMd	3.763 $\pm$ 0.3221	4.017 $\pm$ 0.3165	3.835 $\pm$ 0.3857	3.896 $\pm$ 0.2826
LV VOLs (μ L)	24.01 $\pm$ 7.172	36.62 $\pm$ 8.291 ***	25.78 $\pm$ 8.054	29.22 $\pm$ 8.322
LV VOLd (μ L)	61.20 $\pm$ 12.55	69.52 $\pm$ 11.47	64.27 $\pm$ 15.01	66.37 $\pm$ 11.29
SV (ul)	35.75 $\pm$ 5.84	35.28 $\pm$ 7.545	38.49 $\pm$ 8.793	37.15 $\pm$ 5.509
EF (%)	60.65 $\pm$ 7.454	48.91 $\pm$ 5.700 ****	60.35 $\pm$ 6.602	56.83 $\pm$ 8.097 #
FS (%)	32.13 $\pm$ 5.194	24.44 $\pm$ 3.433 ***	31.88 $\pm$ 4.751	29.89 $\pm$ 5.966 #
CO (mL/min)	15.76 $\pm$ 3.503	15.99 $\pm$ 3.358	17.79 $\pm$ 3.260	17.83 $\pm$ 2.456
LV mass (mg)	131.15 $\pm$ 28.02	129.55 $\pm$ 15.73 *	141.7 $\pm$ 20.05	128.35 $\pm$ 16.46

Table 1. Cont.

	WT-PBS (<i>n</i> = 18)	Nef-PBS (<i>n</i> = 17)	WT-NMN (<i>n</i> = 17)	Nef-NMN (<i>n</i> = 12)
LVAWs (mm)	1.471 ± 0.1712	1.201 ± 0.2180 ***	1.544 ± 0.1115	1.335 ± 0.2038
LVAWd (mm)	0.989 ± 0.1140	0.8864 ± 0.1298 *	1.084 ± 0.1145	0.8642 ± 0.0707
LVPWs (mm)	1.134 ± 0.1150	1.0628 ± 0.1687	1.202 ± 0.1530	1.274 ± 0.1910 ##
LVPWd (mm)	0.8231 ± 0.1553	0.7964 ± 0.1383	0.8400 ± 0.1565	0.9256 ± 0.1938
HR (bpm)	425.1 ± 17.25	453.6 ± 5.357 **	464.6 ± 23.66 ****	482.16 ± 40.93 #

3.3. NMN Administration Prevents the Progression of Cardiac Remodeling in Nef Mice

Previously, we found that Nef protein expression increased cardiac fibrosis, heart failure, and early mortality [24]. To investigate the effect of NMN administration on Nef-induced cardiac tissue remodeling, we performed histological analysis of heart tissue after NMN treatment in WT and Nef mouse groups. For the histological analysis, heart tissues were perfused with 10% NBF, and paraffin tissue sections were prepared. Tissue sections were stained with H&E, Masson's trichrome, and WGA. Our study found that PBS-treated Nef mice have enlarged hearts, increased heart weight-to-body weight ratio, increased fibrosis, and increased cross-sectional area compared to PBS-treated wild-type mice (Figure 3A–F, Figure S2). Interestingly, we noticed that NMN treatment significantly reduces fibrosis and cross-sectional area in Nef mice compared to the PBS-treated Nef group (Figure 3C–F).

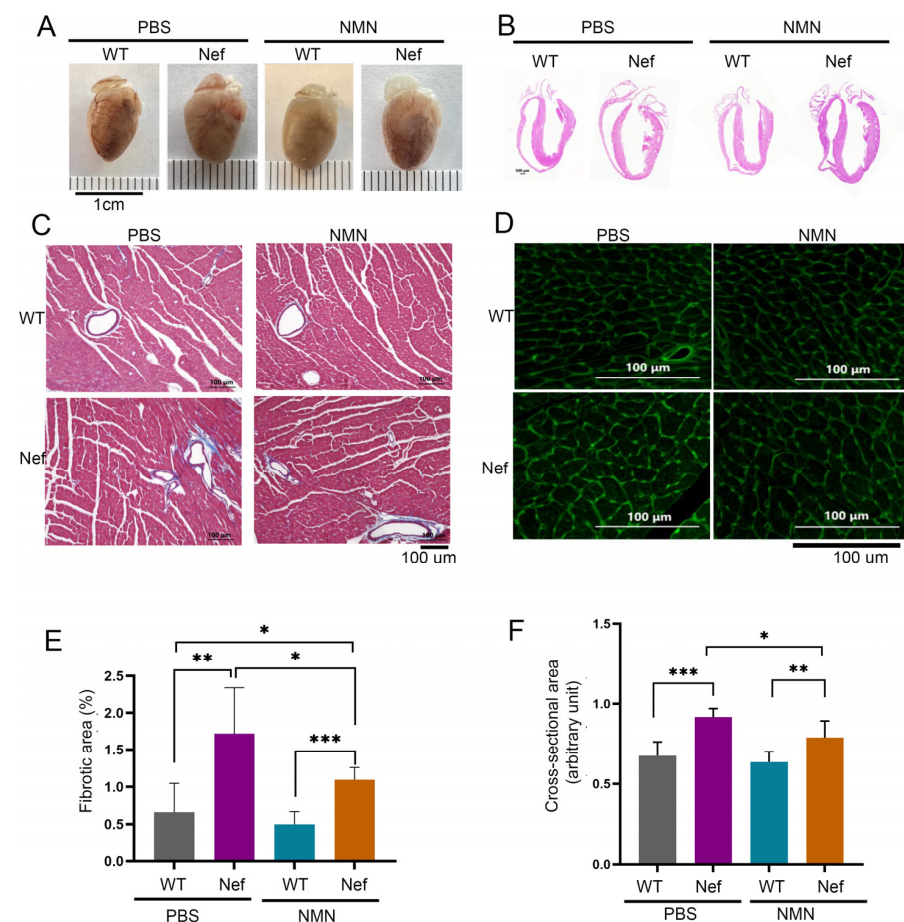


Figure 3. NMN administration can prevent the progression of Nef-induced cardiac fibrosis. For histological analysis, hearts were perfused and fixed with 10% NBF, and thin paraffin sections were

prepared after PBS and NMN treatment at 16–17 weeks. (A,B) Representative images showing whole hearts and butterfly sections of the heart stained with H&E. (C,D) Representative images showing Masson's trichrome and WGA staining of cardiac tissue sections. (E,F) Graphs showing quantification of fibrotic tissue and cross-sectional area in the heart. Data represent pooled results from both male and female mice (WT-PBS ($n = 6$), Nef-PBS ($n = 7$), WT-NMN ($n = 6$), and Nef-NMN ($n = 6$)). Statistical significance was measured between WT and Nef mice treated with PBS or NMN (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

3.4. NMN Administration Restored Autophagy in the Hearts of Nef Mice

In our previous study with Nef mouse hearts, we found that Nef protein expression dysregulates autophagy, with reduced autophagy marker LC3 II and increased p62 expression [23,24]. In this study, we examined the beneficial effects of NMN administration on cardiac muscle autophagy. Western blot analysis was conducted using autophagy markers LC3 and p62 in the heart tissue of Nef mice treated with NMN or PBS. Consistent with our previous findings, the Western blot results show decreased LC3 II (Figure 4A,B) and increased p62 (Figure 4A,B) protein levels in PBS-treated Nef mice compared to PBS-treated WT littermates. Interestingly, the level of LC3 II/LC3I expression was significantly higher in the hearts of NMN-treated Nef mice compared to PBS-treated Nef mice (Figure 4A,C). Additionally, Western blot data indicated a moderate decrease in p62 protein expression in the hearts of NMN-treated Nef mice compared to PBS-treated Nef mice (Figure 4D). Our findings demonstrate that short-term NMN treatment can enhance cardiac autophagy in Nef mice.

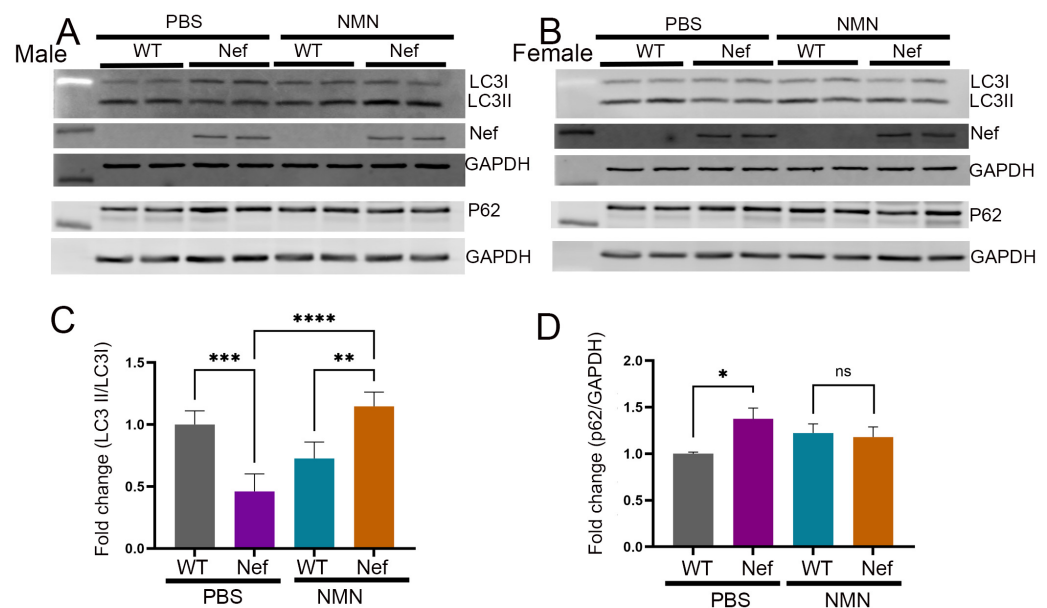


Figure 4. NMN treatment restored autophagy in Nef mouse hearts. (A,B) Representative Western blots showing the expression of the autophagy markers LC3 and p62 in heart tissue at 15–17 weeks. For Western blot analysis, total protein lysate was prepared from left ventricular heart tissue treated with PBS or NMN for 4 weeks. Data represent pooled results from both male and female mice (WT-PBS ($n = 4$), Nef-PBS ($n = 4$), WT-NMN ($n = 4$), Nef-NMN ($n = 4$)). (C,D) Graphs showing quantification of LC3 and P62. GAPDH was used as a loading control. Statistical significance was measured between WT and Nef mice treated with PBS or NMN using a *t*-test (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, ns = non-significant).

3.5. Expression of Bcl2 Was Significantly Reduced in the Cardiac Tissue of NMN-Treated Nef Mice

Bcl-2 is a stress-induced anti-apoptotic protein, and its expression is significantly increased in cardiac diseases. Additionally, upregulation of Bcl2 slows the autophagy process

within cells by binding to Beclin1 and promoting the formation of Beclin1 dimers [28]. In our previous study, we observed that Nef protein inhibits autophagy [24]. We also found that Bcl2 and Nef regulate each other's expression. To explore the potential mechanism of NMN-induced autophagy in Nef mice, we assessed Bcl2 and Beclin1 protein levels by Western blot. Protein expression analysis shows that NMN treatment significantly lowers Bcl2 levels in the hearts of Nef mice compared to those treated with PBS (Figure 5A,B). Furthermore, NMN treatment decreases Beclin1 expression in Nef mice to levels seen in PBS-treated WT mice (Figure 5C,D). These findings suggest that NMN promotes autophagy by reducing Bcl2 expression in the hearts of Nef mice. Overall, these results indicate that NMN treatment has beneficial effects on the Nef transgenic mouse heart through the regulation of autophagy and the suppression of cardiac remodeling.

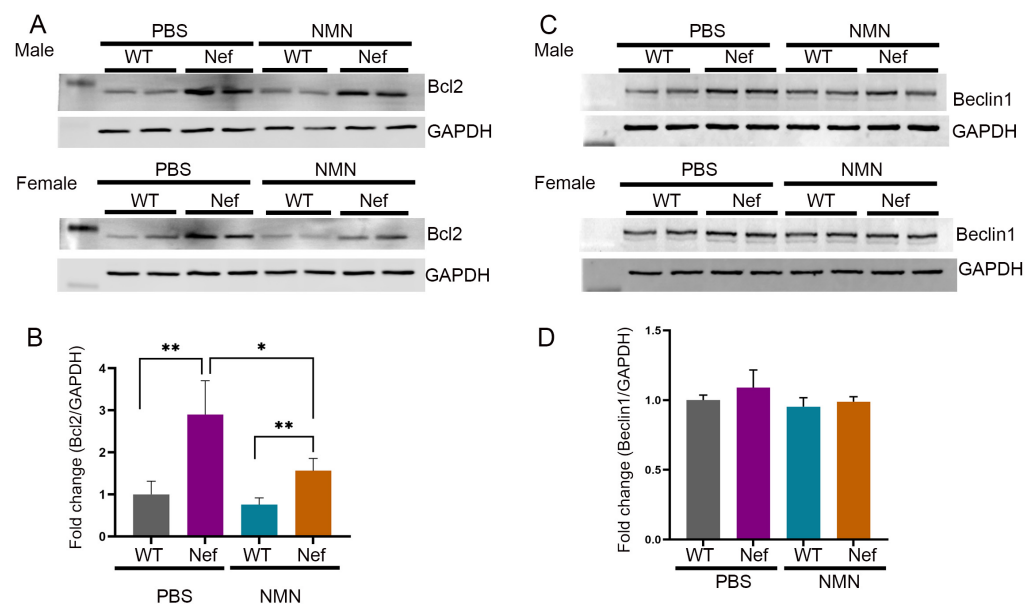


Figure 5. NMN treatment downregulates Bcl2 expression in Nef mouse hearts. (A,C) Representative Western blot images showing the expression of Bcl2 and Beclin1 in the heart of adult mice after PBS or NMN treatment. Data represent pooled results from both male and female mice (WT-PBS ($n = 4$), Nef-PBS ($n = 4$), WT-NMN ($n = 4$), Nef-NMN ($n = 4$)). Western blots were conducted using the total protein lysate of left ventricular tissue. Graphs show the quantification of (B) Bcl2 and (D) Beclin1. GAPDH was used as a loading control. Statistical significance was measured between WT and Nef mice treated with PBS or NMN using a t -test (* $p \leq 0.05$, ** $p \leq 0.01$).

4. Discussion

Multiple studies have been conducted to determine the correlation between the NAD⁺ cellular level and cardiovascular disease. There is conclusive evidence that NAD⁺ depletion is a core hallmark of cardiovascular disease [4,6,7]. Interestingly, a decline in cellular NAD⁺ content was reported in the failing mouse heart [10,29], as well as in the skeletal muscles of people with HIV [18]. In addition, NAMPT protein downregulation was observed in failing human hearts [12]. To investigate the possible effect of Nef protein on cellular NAD⁺ content and its role in Nef-induced cardiovascular dysfunction, we determined NAD metabolism in heart tissue in the presence of Nef protein. Our cellular NAD detection assay found that the total NAD level remains the same in Nef and WT mice. However, the level of NAD⁺ significantly decreased with a concomitant increase in NADH levels in the presence of Nef in heart tissue. In addition, expression of the NAD⁺ salvage pathway enzyme NAMPT was reduced in the hearts of Nef mice compared to WT mice. These findings indicate that HIV-1 Nef protein might directly or indirectly modulate myocardial NAD⁺ levels, resulting in metabolic stress that may negatively impact heart function. The

cellular NAD⁺/NADH ratio is an important factor to maintain cellular redox balance and proper function of the heart. Studies suggest that disruption of the cellular redox balance is often observed during the progression of cardiovascular disease and the development of heart failure [5,27,29].

In addition, redox state imbalance in monocytes and cerebrospinal fluid was reported in PLWH with or without ART, resulting in a decline in cognitive abilities [30]. Furthermore, it has been shown that not only HIV but also antiretroviral drugs can alter the redox balance, making PLWH vulnerable to HIV-related comorbidities [31]. Consistent with these clinical studies, a decreased NAD⁺/NADH ratio was observed in Nef-expressing heart tissue, which indicates redox imbalance in the cardiomyocytes of Nef mice.

Autophagy is known for its central role in the regulation of cellular homeostasis and maintenance of redox balance [20,21,32]. In the past, several studies have identified crosstalk between autophagy and cellular NAD⁺ levels. These studies noted that depletion of cellular NAD⁺ is associated with the inhibition of autophagy. Mechanistically, it was shown that NAD-consuming enzymes (NADases) such as SIRT6, PARPs, and CD38 can consume more NAD during stress, which can reduce cellular NAD and impact autophagy. On the other hand, defective autophagy may also reduce cellular NAD⁺ levels [22]. Consistent with our findings, several metabolic diseases, including the neurodegenerative disorder Alzheimer's disease, show poor organ function, mitochondrial dysfunction, and cell death due to dysregulation of the cellular autophagy–NAD⁺ axis [33,34].

Previously, we showed that the Nef protein suppressed autophagy in the hearts of Nef mice [24]. We therefore proposed that Nef-inhibited autophagy might lead to a redox state imbalance in the hearts of these mice, leading to cardiac dysfunction. It has been reported that NMN administration restores NAD⁺ levels and normalizes the redox state in cardiomyocytes of mice with heart failure [10]. Furthermore, NMN treatment has been shown to prevent the development of heart failure in various mouse models of cardiomyopathy [13,35]. Considering the depleted NAD⁺ levels in the hearts of Nef mice, we hypothesized that restoring the NAD⁺ pool in cardiomyocytes might improve Nef-induced cardiac dysfunction through autophagy modulation. To test this, we administered NMN daily for four weeks in Nef mice. Our echocardiography analysis revealed that short-term NMN treatment prevented the progression of Nef-induced cardiac dysfunction. It is known that increased cardiac fibrosis plays a critical role in the development of heart failure in PLWH [36]. Thus, we examined whether NMN influences Nef-induced morphological changes in heart tissue. We found that NMN prevents the progression of cardiac fibrosis in Nef mice. Consistent with our results, Yagi et al. demonstrated that NMN improved heart function and reversed cardiac fibrosis in a mouse model of heart failure [35].

NMN has been reported to enhance autophagy in mice with cardiac dysfunction [35]. In our previous study, we observed autophagy inhibition in the heart tissue of Nef mice [24]. Specifically, we found decreased levels of LC3 II and accumulation of p62 proteins in hearts expressing Nef protein. Interestingly, in this study, NMN administration increased LC3 II levels and decreased p62 protein levels in Nef mice compared with PBS-treated Nef mice. These findings suggest that NMN has a beneficial effect on the hearts of Nef-expressing mice through modulation of autophagy. Studies indicate that Bcl2 expression is significantly upregulated during cardiac stress, and Bcl-2, an anti-apoptotic protein, is associated with mitochondria [25,37]. Additionally, Bcl2 binds to Beclin1, a key regulator of autophagosome formation and fusion with lysosomes, rendering Beclin1 inactive and leading to autophagy inhibition [28]. Previously, we reported that Nef protein causes Bcl2 upregulation in the heart tissue of Nef mice [24]. A prior study suggested that NAMPT expression can also modulate cellular Bcl2 levels [12]. This study highlights a link between cellular NAD metabolism and Bcl2 expression. In our research, we observed decreased NAMPT

expression and increased Bcl2 protein levels in the heart tissue of Nef mice. Notably, NMN administration significantly reduced Bcl2 protein levels in the hearts of Nef mice compared to those treated with PBS. These findings suggest that NMN may regulate autophagy in cells through modulation of Bcl2 protein expression.

5. Conclusions

In conclusion, the current study has elucidated the potential cardioprotective effect of NMN in Nef mice via modulation of autophagy. However, additional studies need to be conducted to confirm the efficacy and safety of NMN in the treatment of HIV-induced cardiovascular disease.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cells15050444/s1>, Figure S1: NAMPT expression analysis in HEK293 cells in the presence of Nef; Figure S2: The graph shows the heart weight (HW) to body weight (BW) ratio in WT and Nef mice treated with PBS or NMN.

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Abbreviations

The following abbreviations are used in this manuscript:

AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
CO	Cardiac output
CVD	Cardiovascular disease
EF	Ejection fraction
EV	Extracellular vesicle
FS	Fractional shortening
GFP	Green fluorescent protein
H&E	Hematoxylin and eosin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Heart rate

IACUC	Institutional Animal Care and Use Committee
LV DIAMd	Diastolic left ventricular diameter
LV DIAMs	Systolic left ventricular diameter
LVAWd	Diastolic left ventricular anterior wall thickness
LVAWs	Systolic left ventricular anterior wall thickness
LVPWd	Diastolic left ventricular posterior wall thickness
LVPWs	Systolic left ventricular posterior wall thickness
NAD/NADH	Nicotinamide adenine dinucleotide
NAM	Nicotinamide
NAMPT	Nicotinamide phosphoribosyltransferase
NBF	Neutral Buffered Formalin
Nef	Negative Factor
NMN	Nicotinamide mononucleotide
PBS	Phosphate-buffered saline
PLWHA	People living with HIV / AIDS
ROS	Reactive oxygen species
SQ	Subcutaneously
SV	Stroke volume
TG	Transgenic
WGA	Wheat germ agglutinin
WT	Wild type

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