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Nicotinamide mononucleotide induces lipolysis by regulating ATGL expression via the SIRT1-AMPK axis in adipocytes

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ABSTRACT

Nicotinamide adenine dinucleotide (NAD^+) -dependent protein deacetylase SIRT1 plays an important role in the regulation of metabolism. Although the administration of nicotinamide mononucleotide (NMN), a key NAD^+ intermediate, has been shown to ameliorate metabolic disorders, such as insulin resistance and glucose intolerance, the direct effect of NMN on the regulation of lipid metabolism in adipocytes remains unclear. We here investigated the effect of NMN on lipid storage in 3T3-L1 differentiated adipocytes. Oil-red O staining showed that NMN treatment reduced lipid accumulation in these cells. NMN was found to enhance lipolysis in adipocytes since the concentration of glycerol in the media was increased by NMN treatment. Western blotting and real-time RT-PCR analysis revealed that adipose triglyceride lipase (ATGL) expression at both protein and mRNA level was increased with NMN treatment in 3T3-L1 adipocytes. Whereas NMN increased SIRT1 expression and AMPK activation, an AMPK inhibitor compound C restored the NMN-dependent upregulation of ATGL expression in these cells, suggesting that NMN upregulates ATGL expression through the SIRT1-AMPK axis. NMN administration significantly decreased subcutaneous fat mass in mice on a high-fat diet. We also found that adipocyte size in subcutaneous fat was decreased with NMN treatment. Consistent with the alteration of fat mass and adipocyte size, the ATGL expression in subcutaneous fat was slightly, albeit significantly, increased with NMN treatment. These results indicate that NMN suppresses subcutaneous fat mass in diet-induced obese mice, potentially in part via the upregulation of ATGL. Unexpectedly, the reduction in fat mass as well as ATGL upregulation with NMN treatment were not observed in epididymal fat, implying that the effects of NMN are site-specific in adipose tissue. Thus, these findings provide important insights into the mechanism of NMN/NAD^+ in the regulation of metabolism.

1. Introduction

Adequate volumes of fat mass are important for the regulation of whole-body energy metabolism [1,2]. Fat mass is primarily defined by adipocyte size, which is regulated by the balance between lipogenesis and lipolysis [3,4]. Adipose triglyceride lipase (ATGL) is considered the main lipolytic enzyme, catalyzing the first step of lipolysis and converting intracellular triglyceride to diacylglycerol and free fatty acids [5,6]. It has been shown that ATGL expression is decreased in white adipose tissue of obese animals and humans [7–9]. Moreover, systemic or adipose tissue-specific ATGL deficiency in mice results in an increased fat mass [10,11], while adipose tissue-specific overexpression of this enzyme in mice improves diet-induced obesity and related metabolic disorders [12]. Thus, decreased ATGL expression is thought to be involved in the pathogenesis of obesity and related metabolic disorders, and this enzyme is considered to be a potential therapeutic target for these diseases. Although several food ingredients have been reported to reduce fat mass through the induction of ATGL expression in adipose tissue in obese mice [13,14], the mechanism underlying the regulation of ATGL expression by these nutrients is not yet fully understood.

Nicotinamide adenine dinucleotide (NAD^+) is involved in a number of cellular redox reactions as a coenzyme, as well as in mitochondrial energy production. NAD^+ is also used as a substrate for sirtuins and poly-ADP-ribose polymerases [15]. In mammals, NAD^+ is predominantly

Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; ATGL, adipose triglyceride lipase; BMI, body mass index; DMEM, Dulbecco's modified Eagle's medium; NAD^+, nicotinamide adenine dinucleotide; NAMPT, nicotinamide phosphoribosyltransferase; NMN, nicotinamide mononucleotide; SE, standard error; SIRT1, sirtuin 1.

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synthesized from nicotinamide through nicotinamide mononucleotide (NMN). The conversion of nicotinamide to NMN, a rate-limiting step on NAD^+ synthesis, is catalyzed by nicotinamide phosphoribosyltransferase (NAMPT). Recent studies have shown that NAMPT expression is decreased in the white adipose tissue of obese animals and humans [16–20], suggesting that the decrease in the amount of NAD^+ by NAMPT downregulation may be involved in the pathogenesis of obesity and related disorders. As the NAD^+ precursor NMN is not regulated by NAMPT, the administration of NMN can enhance cellular NAD^+ levels [18,21,22]. In fact, the pharmacological effects of NMN on various diseases such as Alzheimer's disease, cerebral and cardiac ischemia, and obesity and related disorders have been reported in animal studies [23,24] while the safety as well as efficacy of NMN for these diseases have not been well established in human trials [25,26]. NMN treatment has been shown to improve obesity and related disorders such as glucose intolerance and dyslipidemia, independently of food intake, in mouse studies [18,27–29]. However, the mechanism by which NMN reduces fat mass remains unknown.

To clarify the mechanism of anti-obesity effect by NMN, we here investigated the direct effect of NMN on the regulation of lipid metabolism in adipocytes. NMN was found to upregulate ATGL expression, thereby inducing lipolysis in adipocytes. We also investigated the molecular mechanism by which NMN increases ATGL expression in adipocytes, as well as the effect of NMN treatment on adipose tissue mass and adipocyte size in mice fed a high-fat diet.

2. Material and methods

2.1. Cell culture

3T3-L1 pre-adipocytes were obtained from the Japanese Collection of Research Bioresources Cell Bank (IFO50416) (Osaka, Japan) and maintained in low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum, 100 units/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Differentiation into mature adipocytes was performed as previously described [30]. Briefly, 100% confluent cells were maintained for two days and changed to differentiation medium (high-glucose DMEM containing 10% fetal bovine serum, 10 μg/mL insulin, 1 μM dexamethasone, and 500 μM 3-isobutyl-1-methylxanthine). After two days, the media were replaced with DMEM containing 10% fetal bovine serum and refreshed every other day for an additional six days. NMN (Sigma Aldrich, St. Louis, MO, USA) was added to the media after differentiation to a final concentration of 0.5 mM.

2.2. Oil red O staining

Differentiated 3T3-L1 adipocytes were treated with NMN for four days and stained with Oil red O using modified conventional method [31]. In brief, the cells were washed twice with PBS and fixed using 4% paraformaldehyde for 10 min at room temperature. After washing with deionized water and replacing with 60% isopropanol for 1 min, the cells were stained with the Oil red O solution in 60% isopropanol for 20 min. The stained adipocytes were washed with 60% isopropanol and deionized water and observed under a microscope. Oil red O was extracted from the cells with 100% isopropanol and quantified by its absorbance at 500 nm.

2.3. MTT cell viability assay

Twenty-four hours after the addition of NMN or vehicle to differentiated 3T3-L1 adipocytes, the medium was replaced with DMEM containing 0.5 mg/mL MTT and maintained for 4 h at 37 °C. Subsequently, the medium was removed, and the generated formazan was solubilized with DMSO. The absorbance at 535 nm of each solution was measured.

2.4. Lipolysis assay

Differentiated 3T3-L1 adipocytes were treated with NMN or vehicle for 24 h, and the level of free glycerol released into the medium was measured using EnzyChrom™ Adipolysis Assay Kit (Bio Assay Systems, Hayward, CA, USA), according to the manufacturer's protocol.

2.5. Real-time RT-PCR

Total RNA was isolated from cells using RNAiso Plus (Takara Bio, Shiga, Japan). Subsequently, cDNA was synthesized using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA), according to the standard method. SYBR green-based quantitative real-time PCR was performed using the synthesized cDNA and an appropriate primer set for each target gene with a StepOne™ real-time PCR system (Applied Biosystems).

2.6. Western blotting

Western blotting was performed as previously described [32]. Briefly,the tissues and cells were lysed in cold RIPA buffer supplemented with a protease inhibitor and phosphatase inhibitor cocktail. Extracted protein was separated using SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with Blocking One (Nacalai Tesque, Kyoto, Japan) for 30 min at room temperature and treated with each antibody overnight at 4 °C. Then, the membrane was incubated with an appropriate secondary antibody for 1 h at room temperature and visualized using a luminescent image analyzer (ImageQuant LAS-500; GE Healthcare, Tokyo, Japan). The intensity of signal was quantified using ImageJ (version 1.52).

2.7. Animal treatment

Male 5-week-old C57BL/6J mice were purchased from CLEA Japan (Tokyo, Japan) and housed at 23 ± 1 °C on a 12 h light/dark cycle. Mice were provided free access to water and normal chow. At six weeks, the mice were randomly divided into three groups and fed normal diet (ND, n = 6), high-fat diet (HFD32; CLEA Japan) (HFD, n = 8) or a high-fat diet containing 500 mg/kg body weight/day of NMN (HFD + NMN, n = 8) ad libitum for eight weeks. NMN was provided by Nordeste Co., Ltd. (Tokyo, Japan). After the experimental period, the mice were euthanized under anesthesia using isoflurane. Then, subcutaneous and epididymal adipose tissue samples were harvested. All animal experiments were approved by and performed in accordance with the guidelines of the animal ethics committees of University of Shizuoka (approval number. 226581).

2.8. Hematoxylin and eosin staining

The tissue was fixed with 4% paraformaldehyde and embedded in paraffin. The tissue section was stained hematoxylin and eosin.

2.9. Statistical analysis

Values are expressed as the mean ± standard error (SE). Statistical significance was determined using one-way analysis of variance, followed by Tukey's multiple comparison test or by Student's t-test. $P < 0.05$ was considered significant. All analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. NMN treatment induces lipolysis by increasing ATGL expression in differentiated 3T3-L1 adipocytes

To determine the effect of NMN on lipid storage in matured white

adipocytes, we treated differentiated 3T3-L1 adipocytes with NMN and performed Oil red O staining. Treatment with 0.5 mM NMN for four days was found to significantly reduce lipid accumulation in differentiated 3T3-L1 adipocytes (Fig. 1A), while this concentration of NMN did not affect the viability of the cells (Fig. 1B). The concentration of glycerol in the media of 3T3-L1 adipocytes was increased by NMN treatment (Fig. 1C). These results suggest that NMN reduces lipid accumulation in 3T3-L1 adipocytes by enhancing lipolysis.

To identify the mechanism by which NMN regulates lipolysis, we examined the expression of genes related to lipolysis. *Atgl* mRNA expression in 3T3-L1 adipocytes was found to increase after NMN treatment, while *Hsl* mRNA expression was unchanged (Fig. 1D). Consistent with the alteration of mRNA expression, the protein level of ATGL was found to be increased by NMN treatment (Fig. 1E). In contrast, the expression of genes related to lipogenesis, including that of *Srebf1c* and *Pparγ*, was not altered by NMN treatment (Fig. 1D). These results suggest that NMN induces lipolysis by increasing ATGL expression in 3T3-L1 adipocytes.

3.2. NMN treatment enhances ATGL expression through SIRT1/AMPK axis in 3T3-L1 adipocytes

Next, we investigated the mechanism by which NMN upregulates the expression of ATGL in 3T3-L1 adipocytes. Consistent with the previous studies [22,33], NMN treatment significantly increased sirtuin 1 (*Sirt1*) expression in 3T3-L1 adipocytes (Fig. 2A). AMPK is a key molecule in the regulation of energy metabolism [34] and has been shown to be activated by SIRT1-mediated enhancement of LKB1 activity via deacetylation of LKB1 [35]. In line with these results, NMN treatment increased AMPK phosphorylation in 3T3-L1 adipocytes (Fig. 2B). Compound C, an AMPK inhibitor, restored the NMN-dependent increase in ATGL expression at both mRNA and protein levels (Fig. 2C and D). These findings suggest that the SIRT1-AMPK axis is involved in the regulation of ATGL expression by NMN.

3.3. NMN treatment suppresses adipocyte hypertrophy in subcutaneous adipose tissue of diet-induced obese mice

To determine the effect of NMN on obese adipose tissue, mice fed a high-fat diet were treated with NMN for eight weeks. Body weight was significantly decreased in mice treated with NMN compared to that of the control mice (Fig. 3A). Consistent with the alteration of body weight, subcutaneous fat mass was significantly decreased by NMN treatment, while epididymal fat mass was not altered (Fig. 3B). Histological examination showed that adipocyte size in subcutaneous adipose tissue was decreased by NMN treatment (Fig. 3C), suggesting that treatment with NMN improved adipocyte hypertrophy. Consistent with the alteration of fat mass and adipocyte size, the ATGL protein levels were slightly, albeit significantly, increased by NMN treatment (Fig. 3D). These results indicate that NMN suppresses subcutaneous fat mass in diet-induced obese mice, potentially in part via the upregulation of ATGL.

4. Discussion

In this study, we investigated the direct effect of NMN on 3T3-L1 adipocytes and found that NMN treatment increased ATGL expression through the SIRT1-AMPK axis, thereby inducing lipolysis in these cells. ATGL is a major lipolytic enzyme that catalyzes the first step of lipolysis. Genetic studies using adipose tissue-specific ATGL deficient or transgenic mice revealed ATGL as a therapeutic target for obesity and related metabolic disorders [10–12]. In addition to its lipase activity, a recent study demonstrated that ATGL possesses transacylase activity to synthesize the fatty acid esters of hydroxy fatty acids (FAHFAs) [36], which has been shown to improve glucose tolerance and insulin sensitivity in obese mice fed a high-fat diet [37]. Although NMN is known to exert favorable effects, such as improving glucose tolerance and insulin sensitivity, in dietary- or age-induced diabetic mice [18,27–29], our results indicate that the favorable effects of NMN in the metabolic regulation may be due in part to the upregulation of ATGL in adipose tissue.

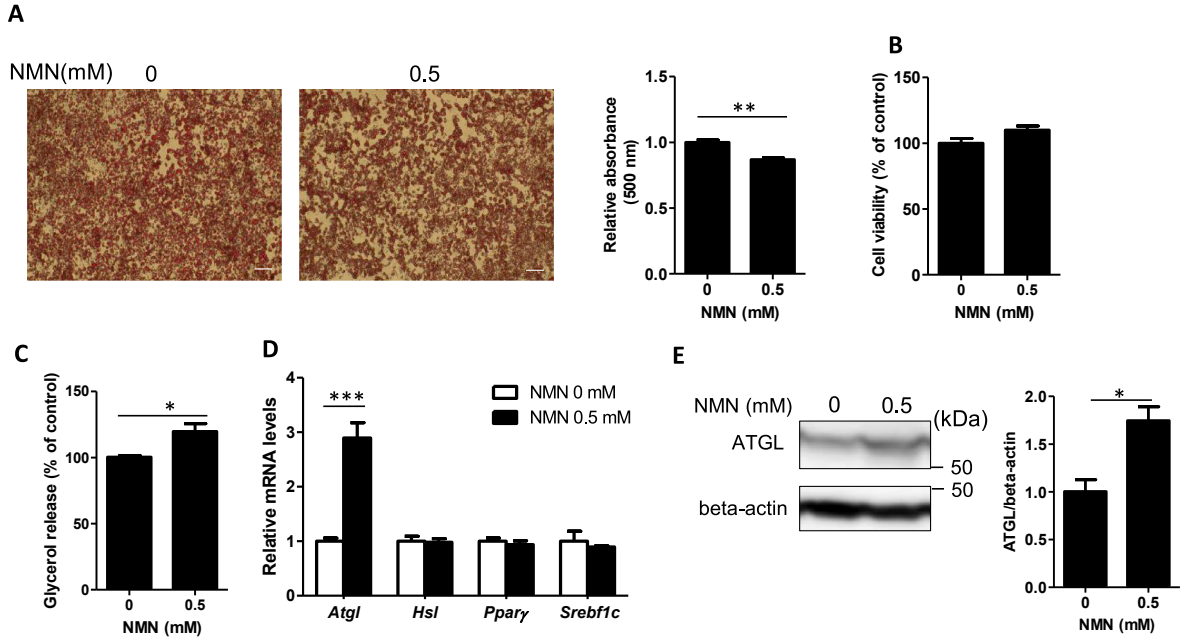

Fig. 1. NMN induces lipolysis by increasing ATGL expression in 3T3-L1 adipocytes. (A) Oil red O staining of 3T3-L1 differentiated adipocytes treated with (right panel) or without (left panel) 0.5 mM NMN for four days (Magnification, 10 × . Scale bar, 100 μm). Right graph shows Oil red O contents determined colorimetrically. (B, C) Cell viability and the amount of glycerol release in media of 3T3-L1 differentiated adipocytes treated with NMN for 24 h. (D, E) *Atgl*, *Hsl*, *Pparγ*, and *Srebp1c* mRNA expression and ATGL protein expression in 3T3-L1 differentiated adipocytes treated with NMN for 24 h. Each value represents the mean ± SE (n = 3–4). $^{*}p < 0.05$, $^{***}p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

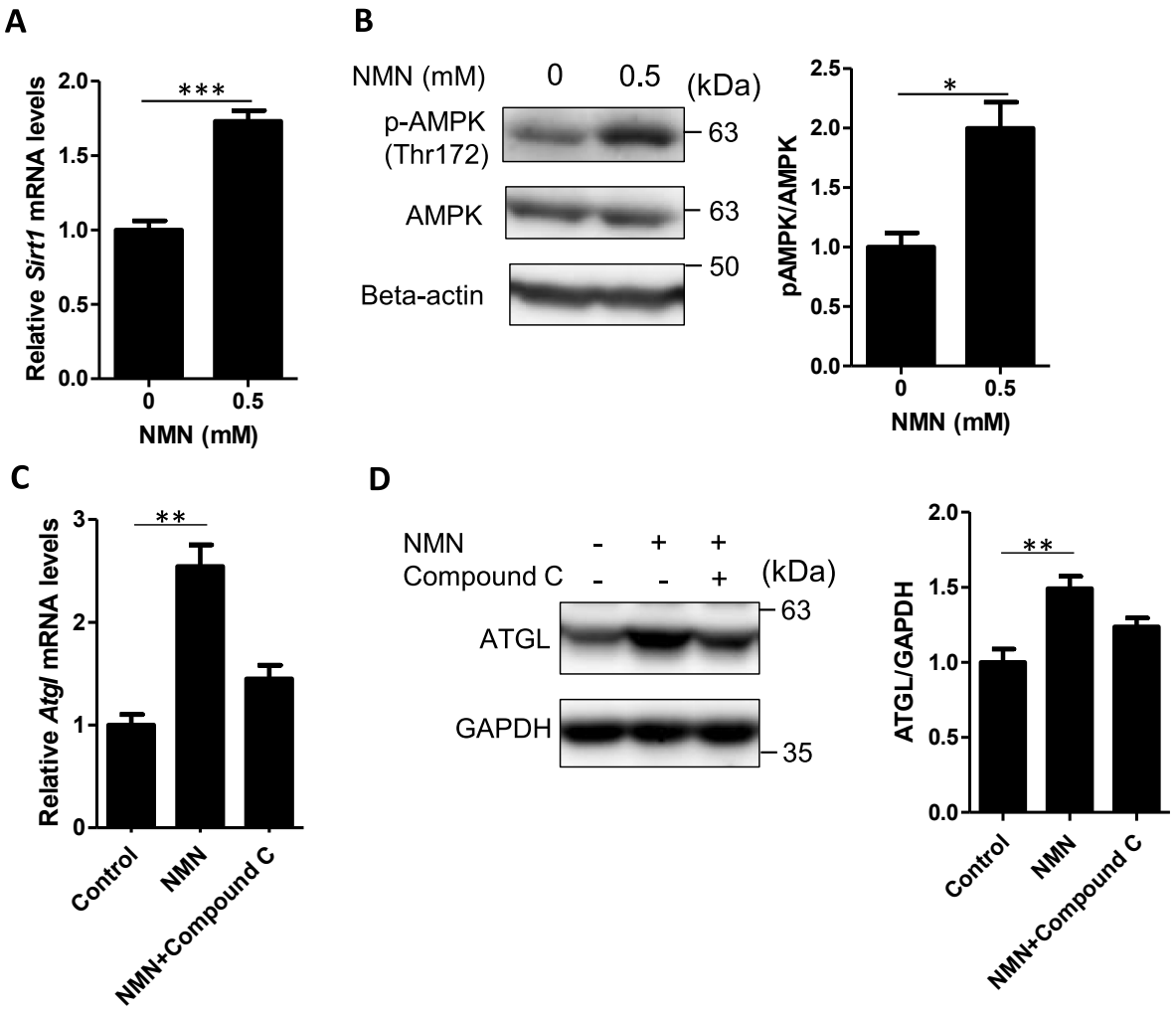

Fig. 2. NMN increases ATGL expression through the SIRT1-AMPK axis in 3T3-L1 adipocytes. (A, B) *Sirt1* mRNA expression and AMPK phosphorylation in 3T3-L1 differentiated adipocytes treated with or without NMN for 24 h. (C, D) *Atgl* mRNA and protein levels in 3T3-L1 differentiated adipocytes treated with 0.5 mM NMN in the absence or presence of 10 μM compound C, an AMPK inhibitor for 24 h. Each value represents the mean ± SE (n = 3–4). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Consistent with the previous studies showing the importance of SIRT1 in the regulation of lipolysis in adipocytes [38–41], we have here shown that NMN enhances lipolysis in 3T3-L1 adipocytes. We found that NMN treatment increased AMPK activity, as well as SIRT1 expression, in 3T3-L1 adipocytes. We also found that compound C, an inhibitor of AMPK, restored the NMN-dependent increase in ATGL expression in these cells, which is consistent with the results in previous studies [42–44]. Given that AMPK has been shown to be activated by the SIRT1-mediated enhancement of LKB1 activity via the deacetylation of LKB1 [35,45], the regulation of ATGL by NMN is likely to be mediated by AMPK activation via an increased SIRT1 expression. Although recent studies showed that plant-derived extracts such as berberine and genistein as well as nicotinamide ribosides, an NAD^+ precursor, activate the SIRT1-AMPK axis and ameliorate ectopic fat accumulation in the liver and skeletal muscle [46–48], the molecular mechanism by which activation of the SIRT1-AMPK axis leads to improvement of ectopic fat accumulation remained unclear. We have here shown for the first time that upregulation of ATGL expression through the SIRT1-AMPK axis mediates the lipolytic effects of NMN in adipocytes.

We examined the effect of NMN in the adipose tissue of mice fed a high-fat diet and found that NMN treatment suppressed adipocyte hypertrophy, thereby reducing subcutaneous fat mass. Unexpectedly, the reduction of fat mass was observed only in subcutaneous but not in epididymal adipose tissue. This site-specific effect of NMN on adipose tissue was accompanied by alterations in ATGL expression specific to each adipose tissue. The reason for this difference is unknown. Interestingly, a study in humans found that NAMPT mRNA expression in subcutaneous adipose tissue was negatively associated with body mass index (BMI), whereas that in visceral adipose tissue was positively associated with BMI [20]. In addition, both NAMPT and SIRT1 expression have been reported to decrease only in subcutaneous adipose tissue, while its recovery has been reported in obese subjects after weight loss [49]. Therefore, the effect and its' mechanism of NMN/NAD^+ in adipose tissue may vary depending on the site of this tissue. However, the current study does not provide sufficient information on the mechanism of site-specific effect of NMN on adipose tissue mass. Further studies will be needed to reveal the mechanism of the site-specific regulation of ATGL to gain insights into the significance of NMN/NAD^+ in the regulation of metabolism.

The pharmacological effects of NMN on various diseases have been attributed at least in part to its anti-oxidative and anti-inflammatory effects [25]. Recent studies have reported that the combination therapies with NMN and antioxidants such as resveratrol, ginsenosides, and melatonin are more effective than NMN monotherapy in some pathological conditions [50–52]. Although the effects of combination therapy with NMN and antioxidants on obesity and the related disorders have not been reported, an antioxidant resveratrol has been shown to improve these pathological conditions through reliving oxidative stress

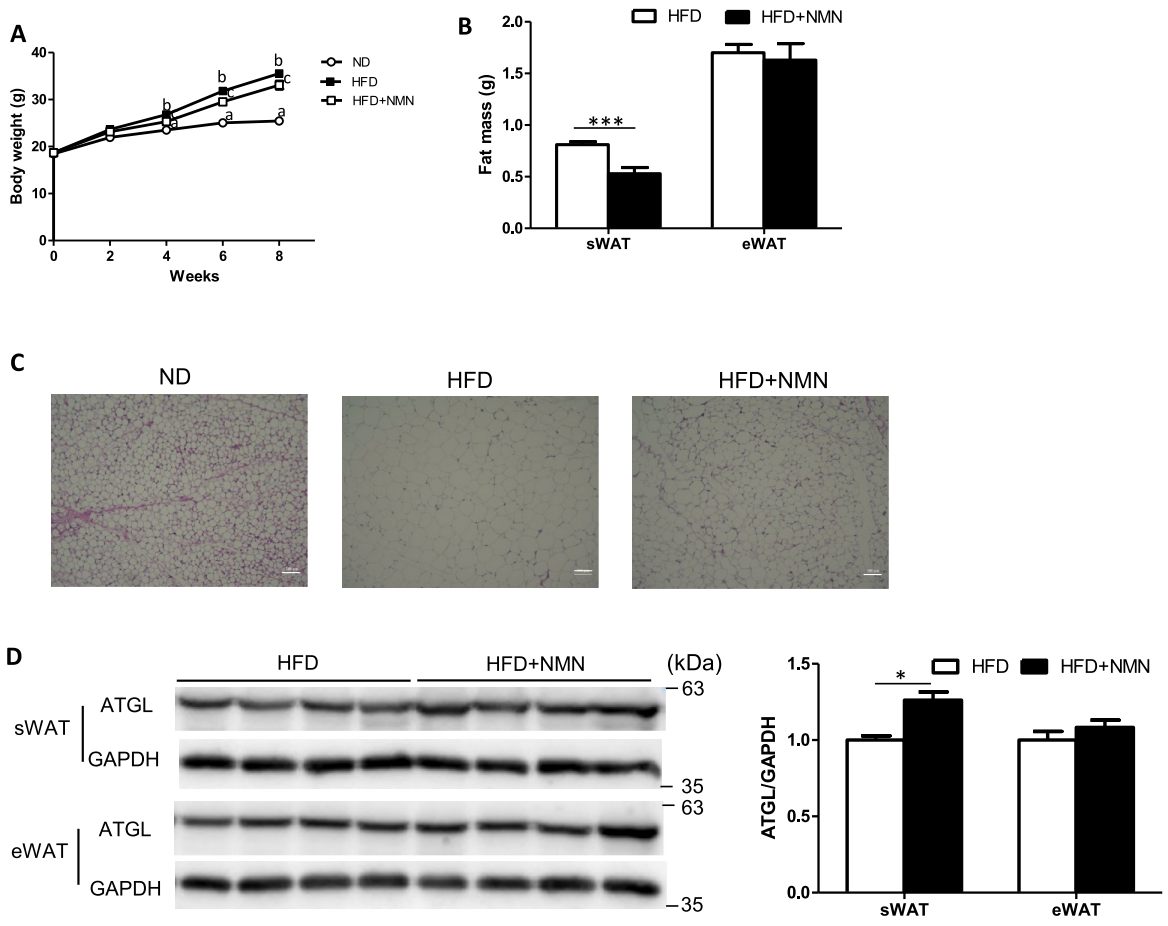

Fig. 3. NMN treatment reduces adipocyte size and fat mass in subcutaneous fat of mice fed a high-fat diet. Mice were fed a normal diet (ND), a high-fat diet (HFD), or a high-fat diet containing NMN (HFD + NMN) for 8 weeks. (A) Body weight curve during the experimental period. (B) The mass of subcutaneous adipose tissue (sWAT) and epididymal adipose tissue (eWAT). (C) Representative images of hematoxylin and eosin staining of sWAT (left panel; ND, middle panel; HFD, right panel; HFD + NMN). Magnification, 10 × . Scale bar, 100 μm. (D) ATGL protein levels in sWAT and eWAT. Each value represents the mean ± SE (n = 4–8). Significant differences are indicated using different superscript letters in (A) ($p < 0.05$) or asterisks in (B, D) (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

and chronic inflammation [53,54]. Given that oxidative stress and chronic inflammation, especially in adipose tissue, play important roles in the pathogenesis of obesity and the related disorders [55,56], combination therapies with NMN and antioxidants may be useful in the treatment of these conditions by additively enhancing anti-oxidative and anti-inflammatory effect.

5. Conclusion

In this study, we have shown that NMN administration increases ATGL expression via the SIRT1-AMPK axis in adipocytes. This mechanism may be involved in the inhibitory effect of NMN on adipocyte hypertrophy in subcutaneous adipose tissue. The mechanism uncovered in this study may serve as a therapeutic target for obesity and related metabolic disorders.

Author contributions

Y.I. and T.H. designed the study. Y.I., R.A., N.K., and Y.O. conducted the experiments. Y.I. and T.H. drafted the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2023.101476.

References

[1] C.R. Kahn, G. Wang, K.Y. Lee, Altered adipose tissue and adipocyte function in the pathogenesis of metabolic syndrome, J. Clin. Invest. 129 (2019) 3990–4000.
[2] A. Garg, Clinical review#: lipodystrophies: genetic and acquired body fat disorders, J. Clin. Endocrinol. Metab. 96 (2011) 3313–3325.
[3] Q.A. Wang, C. Tao, R.K. Gupta, P.E. Scherer, Tracking adipogenesis during white adipose tissue development, expansion and regeneration, Nat Med 19 (2013) 1338–1344.
[4] E.T. Chouchani, S. Kajimura, Metabolic adaptation and maladaptation in adipose tissue, Nat Metab 1 (2019) 189–200.
[5] R. Zimmermann, J.G. Strauss, G. Haemmerle, G. Schoiswohl, R. Birner-Gruenberger, M. Riederer, A. Lass, G. Neuberger, F. Eisenhaber, A. Hermetter, R. Zechner, Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase, Science 306 (2004) 1383–1386.

[6] G. Haemmerle, A. Lass, R. Zimmermann, G. Gorkiewicz, C. Meyer, J. Rozman, G. Heldmaier, R. Maier, C. Theussl, S. Eder, D. Kratky, E.F. Wagner, M. Klingenspor, G. Hoefler, R. Zechner, Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase, Science 312 (2006) 734–737.
[7] J. Li, L. Gong, S. Liu, Y. Zhang, C. Zhang, M. Tian, H. Lu, P. Bu, J. Yang, C. Ouyang, X. Jiang, J. Wu, Y. Zhang, Q. Min, C. Zhang, W. Zhang, Adipose HuR protects against diet-induced obesity and insulin resistance, Nat. Commun. 10 (2019) 2375.
[8] Q. Xu, E.C.M. Mariman, N.J.T. Roumans, R.G. Vink, G.H. Goossens, E.E. Blaak, J. W.E. Jocken, Adipose tissue autophagy related gene expression is associated with glucometabolic status in human obesity, Adipocyte 7 (2018) 12–19.
[9] L. Cruz-Color, Z.H. Hernandez-Nazara, M. Maldonado-Gonzalez, E. Navarro-Muniz, J.A. Dominguez-Rosales, J.R. Torres-Baranda, E.D.C. Ruelas-Cinco, S.M. Ramirez-Meza, B. Ruiz-Madrigal, Association of the PNPLA2, SCD1 and leptin expression with fat distribution in liver and adipose tissue from obese subjects, Exp. Clin. Endocrinol. Diabetes 128 (2020) 715–722.
[10] A.J. Hoy, C.R. Bruce, S.M. Turpin, A.J. Morris, M.A. Febbraio, M.J. Watt, Adipose triglyceride lipase-null mice are resistant to high-fat diet-induced insulin resistance despite reduced energy expenditure and ectopic lipid accumulation, Endocrinology 152 (2011) 48–58.
[11] J.W. Wu, S.P. Wang, S. Casavant, A. Moreau, G.S. Yang, G.A. Mitchell, Fasting energy homeostasis in mice with adipose deficiency of desnutrin/adipose triglyceride lipase, Endocrinology 153 (2012) 2198–2207.
[12] M. Ahmadian, R.E. Duncan, K.A. Varady, D. Frasson, M.K. Hellerstein, A. L. Birkenfeld, V.T. Samuel, G.I. Shulman, Y. Wang, C. Kang, H.S. Sul, Adipose overexpression of desnutrin promotes fatty acid use and attenuates diet-induced obesity, Diabetes 58 (2009) 855–866.
[13] N. Arias, M.T. Macarulla, L. Aguirre, I. Milton, M.P. Portillo, The combination of resveratrol and quercetin enhances the individual effects of these molecules on triacylglycerol metabolism in white adipose tissue, Eur. J. Nutr. 55 (2016) 341–348.
[14] M.S. Lee, C.T. Kim, Y. Kim, Green tea (-)-epigallocatechin-3-gallate reduces body weight with regulation of multiple genes expression in adipose tissue of diet-induced obese mice, Ann. Nutr. Metab. 54 (2009) 151–157.
[15] S. Imai, L. Guarente, NAD+ and sirtuins in aging and disease, Trends Cell Biol. 24 (2014) 464–471.
[16] J. Mercader, N. Granados, A. Caimari, P. Oliver, M.L. Bonet, A. Palou, Retinol-binding protein 4 and nicotinamide phosphoribosyltransferase/visfatin in rat obesity models, Horm. Metab. Res. 40 (2008) 467–472.
[17] X. Wei, R. Jia, G. Wang, S. Hong, L. Song, B. Sun, K. Chen, N. Wang, Q. Wang, X. Luo, J. Yan, Depot-specific regulation of NAD(+)/SIRTs metabolism identified in adipose tissue of mice in response to high-fat diet feeding or calorie restriction, J. Nutr. Biochem. 80 (2020), 108377.
[18] J. Yoshino, K.F. Mills, M.J. Yoon, S. Imai, Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice, Cell Metabol. 14 (2011) 528–536.
[19] M. Kovacikova, M. Vitkova, E. Klimcakova, J. Polak, J. Hejnova, M. Bajzova, Z. Kovacova, N. Viguerie, D. Langin, V. Stich, Visfatin expression in subcutaneous adipose tissue of pre-menopausal women: relation to hormones and weight reduction, Eur. J. Clin. Invest. 38 (2008) 516–522.
[20] V. Varma, A. Yao-Borengasser, N. Rasouli, A.M. Bodles, B. Phanavanh, M.J. Lee, T. Starks, L.M. Kern, H.J. Spencer 3rd, R.E. McGehee Jr., S.K. Fried, P.A. Kern, Human visfatin expression: relationship to insulin sensitivity, intramyocellular lipids, and inflammation, J. Clin. Endocrinol. Metab. 92 (2007) 666–672.
[21] A.P. Gomes, N.L. Price, A.J. Ling, J.J. Moslehi, M.K. Montgomery, L. Rajman, J. P. White, J.S. Teodoro, C.D. Wrann, B.P. Hubbard, E.M. Mercken, C.M. Palmeira, R. de Cabo, A.P. Rolo, N. Turner, E.L. Bell, D.A. Sinclair, Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging, Cell 155 (2013) 1624–1638.
[22] N.E. de Picciotto, L.B. Gano, L.C. Johnson, C.R. Martens, A.L. Sindler, K.F. Mills, S. Imai, D.R. Seals, Nicotinamide mononucleotide supplementation reverses vascular dysfunction and oxidative stress with aging in mice, Aging Cell 15 (2016) 522–530.
[23] W. Hong, F. Mo, Z. Zhang, M. Huang, X. Wei, Nicotinamide mononucleotide: a promising molecule for therapy of diverse diseases by targeting NAD+ metabolism, Front. Cell Dev. Biol. 8 (2020) 246.
[24] J. She, R. Sheng, Z.H. Qin, Pharmacology and potential implications of nicotinamide adenine dinucleotide precursors, Aging Dis 12 (2021) 1879–1897.
[25] H. Nadeeshani, J. Li, T. Ying, B. Zhang, J. Lu, Nicotinamide mononucleotide (NMN) as an anti-aging health product - promises and safety concerns, J. Adv. Res. 37 (2022) 267–278.
[26] A. Loreto, C. Antoniou, E. Merlini, J. Gilley, M.P. Coleman, NMN: the NAD Precursor at the Intersection between Axon Degeneration and Anti-ageing Therapies, Neurosci Res, 2023.
[27] G.M. Uddin, N.A. Youngson, B.M. Doyle, D.A. Sinclair, M.J. Morris, Nicotinamide mononucleotide (NMN) supplementation ameliorates the impact of maternal obesity in mice: comparison with exercise, Sci. Rep. 7 (2017), 15063.
[28] G.M. Uddin, N.A. Youngson, D.A. Sinclair, M.J. Morris, Head to head comparison of short-term treatment with the NAD(+) precursor nicotinamide mononucleotide (NMN) and 6 Weeks of exercise in obese female mice, Front. Pharmacol. 7 (2016) 258.
[29] K.F. Mills, S. Yoshida, L.R. Stein, A. Grozio, S. Kubota, Y. Sasaki, P. Redpath, M. E. Migaud, R.S. Apte, K. Uchida, J. Yoshino, S.I. Imai, Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice, Cell Metabol. 24 (2016) 795–806.
[30] Y. Imi, N. Yabiki, M. Abuduli, M. Masuda, H. Yamanaka-Okumura, Y. Taketani, High phosphate diet suppresses lipogenesis in white adipose tissue, J. Clin. Biochem. Nutr. 63 (2018) 181–191.
[31] J.L. Ramirez-Zacarias, F. Castro-Munozledo, W. Kuri-Harcuch, Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O, Histochemistry 97 (1992) 493–497.
[32] Y. Imi, K. Shibata, Nutritional factors that affect the formation of 5-aminolevulinic acid, a key intermediate of heme biosynthesis, J. Nutr. Sci. Vitaminol. 67 (2021) 339–350.
[33] Y. Guan, S.R. Wang, X.Z. Huang, Q.H. Xie, Y.Y. Xu, D. Shang, C.M. Hao, Nicotinamide mononucleotide, an NAD(+) precursor, rescues age-associated susceptibility to AKI in a sirtuin 1-dependent manner, J. Am. Soc. Nephrol. 28 (2017) 2337–2352.
[34] D. Garcia, R.J. Shaw, AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance, Mol Cell 66 (2017) 789–800.
[35] N.B. Ruderman, X.J. Xu, L. Nelson, J.M. Cacicedo, A.K. Saha, F. Lan, Y. Ido, AMPK and SIRT1: a long-standing partnership? Am. J. Physiol. Endocrinol. Metab. 298 (2010) E751–E760.
[36] R. Patel, A. Santoro, P. Hofer, D. Tan, M. Oberer, A.T. Nelson, S. Konduri, D. Siegel, R. Zechner, A. Saghatelian, B.B. Kahn, ATGL is a biosynthetic enzyme for fatty acid esters of hydroxy fatty acids, Nature 606 (2022) 968–975.
[37] M.M. Yore, I. Syed, P.M. Moraes-Vieira, T. Zhang, M.A. Herman, E.A. Homan, R. T. Patel, J. Lee, S. Chen, O.D. Peroni, A.S. Dhaneshwar, A. Hammarstedt, U. Smith, T.E. McGraw, A. Saghatelian, B.B. Kahn, Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects, Cell 159 (2014) 318–332.
[38] P. Chakrabarti, T. English, S. Karki, L. Qiang, R. Tao, J. Kim, Z. Luo, S.R. Farmer, K. V. Kandror, SIRT1 controls lipolysis in adipocytes via FOXO1-mediated expression of ATGL, J. Lipid Res. 52 (2011) 1693–1701.
[39] T. Shan, Y. Ren, Y. Wang, Sirtuin 1 affects the transcriptional expression of adipose triglyceride lipase in porcine adipocytes, J. Anim. Sci. 91 (2013) 1247–1254.
[40] C. Xu, B. Bai, P. Fan, Y. Cai, B. Huang, I.K. Law, L. Liu, A. Xu, C. Tung, X. Li, F. M. Siu, C.M. Che, P.M. Vanhoutte, Y. Wang, Selective overexpression of human SIRT1 in adipose tissue enhances energy homeostasis and prevents the deterioration of insulin sensitivity with ageing in mice, Am J Transl Res 5 (2013) 412–426.
[41] N. Wang, D. Wang, G. Luo, J. Zhou, Z. Tan, Y. Du, H. Xie, L. Liu, X. Yang, L. Hao, Lutein attenuates excessive lipid accumulation in differentiated 3T3-L1 cells and abdominal adipose tissue of rats by the SIRT1-mediated pathway, Int. J. Biochem. Cell Biol. 133 (2021), 105932.
[42] C.J. Liou, X.Y. Lai, Y.L. Chen, C.L. Wang, C.H. Wei, W.C. Huang, Ginkgolide C suppresses adipogenesis in 3T3-L1 adipocytes via the AMPK signaling pathway, Evid Based Complement Alternat Med 2015 (2015), 298635.
[43] J. Hao, C. Hao, L. Zhang, X. Liu, X. Zhou, Y. Dun, H. Li, G. Li, X. Zhao, Y. An, J. Liu, G. Yu, OM2, a novel oligomannuronate-chromium(III) complex, promotes mitochondrial biogenesis and lipid metabolism in 3T3-L1 adipocytes via the AMPK-PGC1alpha pathway, PLoS One 10 (2015), e0131930.
[44] M.P. Gaidhu, S. Fediuc, N.M. Anthony, M. So, M. Mirpourian, R.L. Perry, R. B. Ceddia, Prolonged AICAR-induced AMP-kinase activation promotes energy dissipation in white adipocytes: novel mechanisms integrating HSL and ATGL, J. Lipid Res. 50 (2009) 704–715.
[45] L. Jia, W. Li, J. Li, Y. Li, H. Song, Y. Luan, H. Qi, L. Ma, X. Lu, Y. Yang, Lycium barbarum polysaccharide attenuates high-fat diet-induced hepatic steatosis by up-regulating SIRT1 expression and deacetylase activity, Sci. Rep. 6 (2016), 36209.
[46] L. Zhu, J.J. Xu, H.D. Li, J.J. Li, M. Cheng, X.N. Niu, P.C. Jia, J.Y. Liu, C. Huang, X. W. Lv, J. Li, Berberine ameliorates abnormal lipid metabolism via the adenosine monophosphate-activated protein kinase/sirtuin 1 pathway in alcohol-related liver disease, Lab. Invest. 103 (2023), 100041.
[47] Z. Jiang, H. Wang, Y. Yang, Y. Yao, H. Ma, Genistein activated SIRT1-AMPK signaling pathway mediated by ERbeta-FOXO1-Nampt to reduce fat accumulation in chicken hepatocytes, Life Sci. 312 (2023), 121259.
[48] A. Serrano, A. Palou, M.L. Bonet, J. Ribot, Nicotinamide riboside supplementation to suckling male mice improves lipid and energy metabolism in skeletal muscle and liver in adulthood, Nutrients 14 (2022).
[49] E. Rappou, S. Jukarainen, R. Rinnankoski-Tuikka, S. Kaye, S. Heinonen, A. Hakkarainen, J. Lundbom, N. Lundbom, V. Saunavaara, A. Rissanen, K. A. Virtanen, E. Pirinen, K.H. Pietilainen, Weight loss is associated with increased NAD(+)/SIRT1 expression but reduced PARP activity in white adipose tissue, J. Clin. Endocrinol. Metab. 101 (2016) 1263–1273.
[50] A.J. Khosroshahi, B. Mokhtari, R. Badalzadeh, Combination of nicotinamide mononucleotide and troxerutin induces full protection against doxorubicin-induced cardiotoxicity by modulating mitochondrial biogenesis and inflammatory response, Mol. Biol. Rep. 49 (2022) 8209–8218.
[51] L. Hosseini, M.S. Vafaee, R. Badalzadeh, Melatonin and nicotinamide mononucleotide attenuate myocardial ischemia/reperfusion injury via modulation of mitochondrial function and hemodynamic parameters in aged rats, J. Cardiovasc. Pharmacol. Therapeut. 25 (2020) 240–250.
[52] L.B. Bai, L.F. Yau, T.T. Tong, W.H. Chan, W. Zhang, Z.H. Jiang, Improvement of tissue-specific distribution and biotransformation potential of nicotinamide mononucleotide in combination with ginsenosides or resveratrol, Pharmacol Res Perspect 10 (2022), e00986.
[53] Z.M. Lv, Q. Wang, Y.H. Chen, S.H. Wang, D.Q. Huang, Resveratrol attenuates inflammation and oxidative stress in epididymal white adipose tissue: implications for its involvement in improving steroidogenesis in diet-induced obese mice, Mol. Reprod. Dev. 82 (2015) 321–328.

[54] L. Aguirre, A. Fernandez-Quintela, N. Arias, M.P. Portillo, Resveratrol: anti-obesity mechanisms of action, Molecules 19 (2014) 18632–18655.
[55] L. Marseglia, S. Manti, G. D'Angelo, A. Nicotera, E. Parisi, G. Di Rosa, E. Gitto, T. Arrigo, Oxidative stress in obesity: a critical component in human diseases, Int. J. Mol. Sci. 16 (2014) 378–400.
[56] E. Maury, S.M. Brichard, Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome, Mol. Cell. Endocrinol. 314 (2010) 1–16.