BIBLIOGRAPHIC INFORMATION SYSTEM

Journal Full Title: Journal of Biomedical Research & Environmental Sciences
Journal NLM Abbreviation: J Biomed Res Environ Sci
Journal Website Link: https://www.jelsciences.com
Journal ISSN: 2766-2276
Category: Multidisciplinary
Subject Areas: Medicine Group, Biology Group, General, Environmental Sciences
Topics Summation: 130
Issue Regularity: Monthly
Review Process: Double Blind
Time to Publication: 21 Days
Indexing catalog: Visit here
Publication fee catalog: Visit here

DOI: 10.37871 (CrossRef)
Plagiarism detection software: iThenticate
Managing entity: USA
Language: English
Research work collecting capability: Worldwide
Organized by: SciRes Literature LLC

IndexCopernicus ICV 2020: 53.77

Manuscript should be submitted in Word Document (.doc or .docx) through

Online Submission

form or can be mailed to support@jelsciences.com

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JOURNAL OF BIOMEDICAL RESEARCH & ENVIRONMENTAL SCIENCES
issn: 2766-2276
SUBJECT AREA(S): PHARMACOLOGY | PHARMACEUTICA ANALYTICA ACTA
DOI: 10.37871

RESEARCH ARTICLE

NMN "Nicotinamide Mononucleotide" Activates Intracellular Energy and Approaches the Prevention and Improvement of Aging

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ABSTRACT

Aging was defined as one of the diseases by ICD-11. Preventing aging may avoid the risk of various diseases. However, it is difficult to simply prevent aging in daily life. The presence of nutrients is essential there. This time, we reviewed NMN "nicotinamide nucleotide", which is attracting attention as an anti-aging component, and conducted additional experiments using AMPK "AMP-activated protein kinase" and NAD + as indicators to determine whether or not it actually prevents aging gone. As a result, a significant increase in AMPK and NAD + was confirmed, suggesting that NMN may help prevent aging in the future.

INTRODUCTION

From ICD-11, aging has been treated as a disease [1]. Aging is said to be a physical and mental decline associated with aging [2,3]. Since aging is considered to be the cause of all diseases [4], countermeasures are urgently needed. In order to prevent aging, it is said that active intake of nutrients that are good for the body [5], good sleep [6], and moderate exercise [7], but if it is not done by one's own will, it is said. Is said to cause stress and generate active oxygen that causes aging [8]. It is said that active oxygen and aging are closely related [8]. Reactive oxygen species are generated by various external and internal factors [9], weaken normal mitochondrial function [10], and may be a risk of aging-related diseases such as cancer. There are four types of active oxygen in a nutshell [11], which can be divided into superoxide, hydroxyl radical, hydrogen peroxide, and singlet oxygen [12]. Normally, these active oxygens are produced by enzymes for removing active oxygen, such as SOD (superoxide dismutase) and catalase that exist in the living body [12,13]. In addition, active oxygen is also removed by antioxidants taken from the diet, such as vitamin C and vitamin E [14,15]. However, these reactive oxygen species are usually produced in small amounts in the body and are involved in functions such as maintenance of homeostasis, signal transduction, gene expression, and receptor activation in cells [16], so it is not possible to remove them altogether. It is

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DOI: 10.37871/jbres1480

Submitted: 17 May 2022

Accepted: 20 May 2022

Published: 21 May 2022

Keywords

- Aging
- NMN
- AMPK
- NAD+
- Functional food
- Vitamins

MEDICINE GROUP

PHARMACOLOGY | PHARMACEUTICA ANALYTICA ACTA

VOLUME: 3 ISSUE: 5 - MAY, 2022

How to cite this article: Kawakami S, Fukuzawa Y, Ichikawa H, Sato T, Ide T, Maeda Y, Yamamoto T. NMN "Nicotinamide Mononucleotide" Activates Intracellular Energy and Approaches the Prevention and Improvement of Aging. J Biomed Res Environ Sci. 2022 May 21; 3(5): 560-565. doi: 10.37871/jbres1480, Article ID: JBRES1480, Available at: https://www.jelsciences.com/articles/jbres1480.pdf

considered undesirable. Therefore, appropriate antioxidants are required. In addition to antioxidants, activation of AMPK (AMP-activated protein kinase) is also required to approach aging [17]. AMPK (AMP-activated protein kinase) is an energy sensor in the body and is a serine / threonine kinase that works to maintain homeostasis of sugar and lipid metabolism [18]. It is thought that aging can be prevented by activating, promoting autophagy, and enhancing mitochondrial function [19,20]. Therefore, in this study, the activity of AMPK was measured from the concept of nutrition using NMN "nicotinamide mononucleotide" [21], which is currently attracting attention as an anti-aging substance, and the expression level of NAD +, which is said to decrease with aging, is also measured. It was measured. NAD + decreases with age, and it is said that when NAD + decreases, age-related diseases are caused. It is considered that the presence of NAD + as well as AMPK is necessary to prevent aging. Therefore, it is considered that activation of both leads to prevention of aging. If NMN can mention the possibility of preventing aging in this study, it will be useful not only for aging-related diseases but also for maintaining / promoting health or preventing diseases in the future.

AMPK (AMP-activated protein kinase) is an energy sensor in the body and is a serine / threonine kinase that works to maintain homeostasis of glucose and lipid metabolism [18]. It is said that activation of AMPK regulates energy metabolism and maintains energy homeostasis, and is attracting attention as a potential therapeutic effect for metabolic diseases including type 2 diabetes and cancer [22]. The existence of energy is indispensable for human beings to live, and the energy source is ATP (adenosine triphosphate), and when ATP is hydrolyzed and converted to ADP (adenosine diphosphate). Occurs [23]. By regulating this ATP level, AMPK is expected to maintain homeostasis and be effective against metabolic diseases such as cancer, type II diabetes, and obesity [24-26].

In other words, it is expected that the increase in AMPK activity can be expected to prevent lifestyle-related diseases including cancer. It is also considered that AMPK regulates metabolism by inhibiting the ATP consumption pathway [27,28]. From that, the following effects can be expected. AMPK is known to have the following effects.

1. Adjust the balance of inflammation [29].

By suppressing chronic inflammation with AMPK, it approaches cancer and heart disease and contributes to the maintenance of health in the living body [30,31].

2. Improvement of insulin sensitivity and glucose tolerance [32].

It has been reported that activation of AMPK can suppress insulin resistance and high insulin status, which cause metabolic diseases33). In addition, by shifting to a state of fat burning, it induces a decrease in body fat mass and suppresses the secretion of inflammatory cytokines from excess body fat [33].

3. Promote autophagy [34].

AMPK activates cell autophagy.

4. Enhances mitochondrial function [35].

Restoring intracellular energy (ATP) levels is one of the main objectives of AMPK activation. AMPK may increase intracellular ATP levels by activating mitochondrial biosynthesis [36,37].

5. Immune system regulation [38].

When AMPK is activated, the immune monitoring function is strengthened, and the host's defense against pathogens is enhanced, which may enhance the immune function [38]. Autophagy is indispensable for innate immunity, which is the forefront of the immune system, and this autophagy is also activated by AMPK [34].

6. It acts on the sirtuin gene and may lead to longevity [39].

AMPK activates the production of longevity genes sirtuins and FOXO proteins associated with healthy longevity [40].

MATERIALS AND METHODS

NMN

NMN purchased from Wellness-One Co., Ltd. (Iwate, Japan) is adjusted to a final concentration of 1 mg/ml.

AMPK activity measurement

In this experiment, the CycLex® AMPK kinase assay kit (Institute of Medical Biology, Tokyo, Japan) was used to confirm the activity of AMPK using MCF-7 cells as usual. The group was divided into a PBS-added group and an NMN-added group (final concentration 1 mg/ml), each was compared, and the PBS group was used as a control for evaluation. With this kit, AMPK activity was measured 1 hour, 12 hours, and 24 hours after addition. The evaluation was performed by statistical processing software (IBM SPSS Statistics Ver.26). Statistical evaluation was performed by the Mann-Whitney U test).

NAD + measurement

In this experiment, NAD/NADH (DOJINDO LABORATORIES (Kumamoto Prefecture, Japan) was purchased and measured using MCF-7 cells according to the operation of the kit. Compared with the control group (PBS group), NMN Addition group (final concentration 1 mg/ml). The amount of NADH and total NAD +/NADH were measured at a wavelength of 450 nm using an absorber, and the amount of NAD + expressed was measured by subtracting the amount

of NADH from the total amount of NAD/NAD. The evaluation was performed by statistical processing software (IBM SPSS Statistics Ver.26). Statistical evaluation was performed by the Mann-Whitney U test).

RESULTS

AMPK activity measurement

In this experiment, the CycLex® AMPK Kinase Assay Kit (MEDICAL & BIOLOGICAL LABORATORIES CO., LTD. Tokyo, Japan) was used as per the standard method, and the PBS-added group was used for the AMPK activity in the NMN-added group (final concentration 1 mg/ml). It was compared and evaluated as a control. In this kit, in order to measure the current amount of AMPK activity, the activity of AMPK was confirmed 1 hour, 12 hours, and 24 hours after the addition. In addition, the evaluation was performed statistically by the Mann-Whitney U test using statistical processing software (IBM SPSS Statistics Ver.26) (Table 1, figure 1).

NAD + measurement

In this experiment, NAD/NADH (DOJINDO LABORATORIES (Kumamoto, Japan) was purchased and measured according to the operation contents of the kit. Compared with the control group (PBS group), the NMN-added group (final concentration 1 mg/ml)) NADH amount and total NAD +/NADH were measured at a wavelength of 450 nm with an absorptiometer, and the expression level of NAD + was measured by subtracting the NADH amount from the total NAD/NAD amount. The evaluation was performed by statistical processing software (IBM SPSS Statistics). Statistical evaluation was per-formed by Mann-Whitney U test using Ver.26) (Table 2, figure 2).

DISCUSSION

This time, we examined the mechanism at the in vitro level using the nutritional component "NMN (nicotinamide mononucleotide)" that is currently attracting attention. NMN is a substance contained in nicotinic acid (niacin), a coenzyme present in the cells of all living organisms, and is produced in the body [41]. In this study, we measured whether NMN increased the activity of AMPK. As a result, in the NMN-added group, when the control group was set to 100%, the activity increased by 1230.5% 1 hour after the addition, 506.5% after 12 hours, and 849.2% after 24 hours. In other words, the activity of AMPK was significantly observed even after 24 hours, suggesting that NMN is involved in the activity of AMPK. In addition, the activity

Table 1: Absorbance of an hourly control group and NMN-added group.

1. Absorbance					
1hr		12hrs		24hrs	
Cnt	NMN	Cnt	NMN	Cnt	NMN
0.285 ± 0.013	3.507 ± 0.136	0.352 ± 0.019	1.783 ± 0.311	0.264 ± 0.034	2.242 ± 0.182
2. AMPK activity increase rate when Cnt is 100%					
1hr		12hrs		24hrs	
Cnt	NMN	Cnt	NMN	Cnt	NMN
100%	1230.50%	100%	506.50%	100%	849.20%

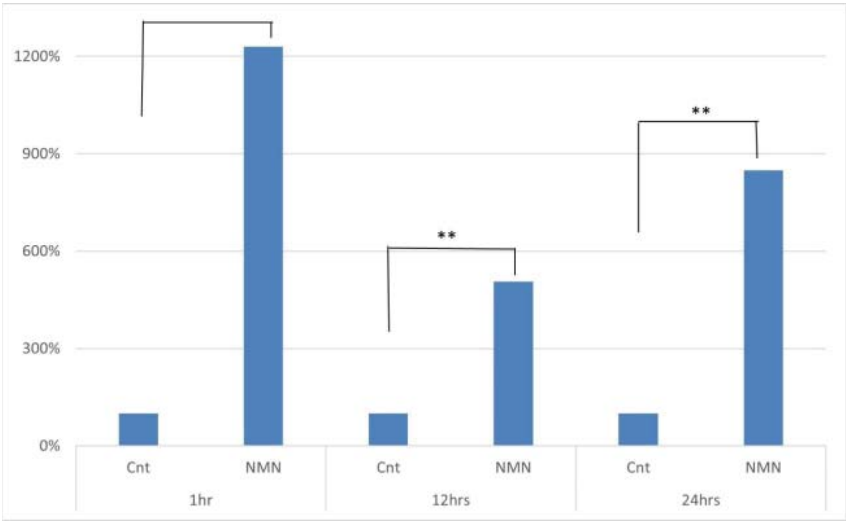

Figure 1 Rate of increase in AMPK activity in the control group and NMN-added group at each time (Mann-Whitney U test $p < 0.01$).

Table 2: Absorbance of NAD + in the control group and the NMN-added group.

Both of *n* = 3	Average of OD value
Control	0.177 ± 0.005 (100%)
Addition group of NMN(1 mg/ml)	0.544 ± 0.008 (307.3%)

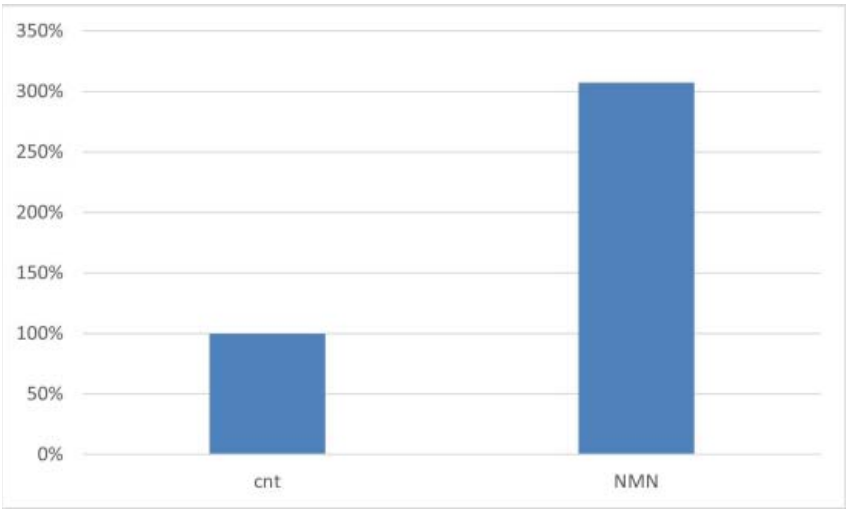

Figure 2 When the Mann-Whitney U test was performed, the expression level of NAD + in the NMN-added group increased significantly at $p < 0.01$.

was increased compared to the control group even after 24 hours had passed, so a sustained action was expected. By activating AMPK, the various effects mentioned above can be expected. Among them, autophagy and mitochondrial activation were observed [34,35], suggesting prevention and improvement of aging. In this study, we focused on the activity of mitochondria and measured NAD +, which is one of the indicators [35]. In the NMN-added group, a significant increase was observed compared to the control group. It was also suggested that the function would be improved. NMN is synthesized from vitamin B3 and is known as a precursor of NAD [41]. It is considered that administration of NMN efficiently promotes NAD + synthesis and further activates sirtuins [42]. Since it has been found that NAD + is reduced in many aged organs [43,44], it is considered important to maintain organ function by supplying NAD + [45]. NAD + is an important coenzyme involved in the redox reaction of major metabolic pathways in cells [46]. NAD exists in cells as oxidized NAD + and reduced NADH, and the balance between these two is essential for maintaining cell function [47]. In recent years, a causal relationship between a decrease in the amount of NAD + and diseases associated with aging has also been pointed out [48]. In this study, not only the activation of AMPK but also the amount of NAD + was confirmed to increase, suggesting that it can approach aging. In addition, at this stage, no report on the antioxidant activity, which is one of the causes of aging, can be seen in NMN. However, there are reports that it improves mitochondrial function and increases metabolism [49]. In addition, NMN is said to be deeply involved in the maintenance and promotion of health by acting on the sirtuin gene [50]. From the above, it was suggested that NMN may prevent aging in the future, leading to an extension of healthy life expectancy and ultimately an extension of life expectancy.

ACKNOWLEDGMENT

I would also like to express our deep gratitude to Dr. Yoshitaka Fukuzawa of Aichi Medical University for his useful advice in conducting this research.

Conflicts of Interest

The authors declare no conflict of interest.

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How to cite this article: Kawakami S, Fukuzawa Y, Ichikawa H, Sato T, Ide T, Maeda Y, Yamamoto T. NMN "Nicotinamide Mononucleotide" Activates Intracellular Energy and Approaches the Prevention and Improvement of Aging. J Biomed Res Environ Sci. 2022 May 21; 3(5): 560-565. doi: 10.37871/jbres1480, Article ID: JBRES1480, Available at: https://www.jelsciences.com/articles/jbres1480.pdf

NMN“nicotinamide mononucleotide” activates intracellular energy and approaches the prevention and improvement of aging.

NMN「ニコチンアミドモノヌクレオチド」は細胞内エネルギーを活性化し、老化の予防と改善効果をもたらす。

1. Introduction

ICD-11 より老化は疾病として取り扱われるようになった[1)]。老化は加齢に伴う身体的・精神的な衰えと言われている[2) 3)]。老化はあらゆる疾病の原因[4)]ともされているため、その対策は急務である。老化を予防するためには体に良い栄養素の積極的な摂取[5)]、質の良い睡眠[6)]、適度な運動[7)]とされているが、自分の意思で行われていないのであれば、それがストレスとなってしまい、老化の原因となる活性酸素を発生させると言われている[8)]。活性酸素と老化は密接な関係があると言われている[8)]。活性酸素は様々な外的･内的因子より発生し[9)]、正常なミトコンドリア機能を減弱させ[10)]、癌をはじめとする老化関連疾患のリスクとなる可能性が考えられる[11)]。一言で活性酸素と言っても大きく分けると 4 種類存在し[12)]、スーパーオキシド、ヒドロキシルラジカル、過酸化水素、一重項酸素に分けることができる[12)]。通常、これらの活性酸素は生体内に存在するＳＯＤ（スーパーオキシドディスムターゼ）やカタラーゼなど、活性酸素を除去するための酵素が作られている[13) 14)]。そのほか、ビタミンＣやビタミンＥなど、食事から摂取する抗酸化物質でも活性酸素を除去しています[15)16)]。しかしながら、これら活性酸素種は通常、体内で少量産生され、細胞における恒常性の維持、シグナル伝達、遺伝子発現、受容体の活性化などの機能に関与しているため[17)]、全て除去することは望ましくないと考えられる。ゆえに適度な抗酸化が必要となってくる。抗酸化に加え、老化に対してアプローチするためには AMPK（AMP-activated protein kinase）の活性化も必要である[18)]。AMPK（AMP-activated protein kinase）とは、生体内のエネルギーセンサーであり、糖・脂質代謝の恒常性維持に働くセリン/スレオニンキナーゼであり[19)]、AMPK を活性化することにより、好中球の活性化やオートファジーの促進、ミトコンドリアの機能を高めることによって老化を予防することができると考えられる[20)21)]。そこで本研究においては現在抗老化物質として注目されている NMN“nicotinamide mononucleotide”[22)]を使用して栄養の概念から AMPK の活性を測定し、加齢によって低下すると言われている NAD+の発現量も測定した。NAD+は加齢とともに減少し、NAD+が減少すると加齢に伴う疾患が引き起こされると言われている。老化の予防には AMPK のみならず NAD+の存在も必要であると考えられる。ゆえに、両者が活性化することで、老化の予防につながると考えられる。本研究において NMN が老化予防の可能性に言及できるのであれば、今後老化関連疾患のみならず健康の維持・増進または疾病の予防に役立てることができると考えられる。

2. What is AMPK ?

AMPK（AMP-activated protein kinase）とは、生体内のエネルギーセンサーであり、糖・脂質代謝の恒常性維持に働くセリン/スレオニンキナーゼである [19]。AMPK が活性化することにより、エネルギー代謝を調節し、エネルギーの恒常性を保つと言われており、2 型糖尿病やがんを含む代謝疾患の潜在的な治療効果が期待できるものとして着目されている [23]。
人間は生きていく上でエネルギーの存在が不可欠となっているが、そのエネルギー源は ATP（アデノシン三リン酸）であり、ATP が加水分解され、ADP（アデノシン二リン酸）に変化する際に発生する [24]。AMPK はこの ATP レベルの調節を行うことで、恒常性を保ち、がんや II 型糖尿病、肥満などの代謝疾患に対する効果が期待されている [25][26][27]。
つまり、AMPK の活性が上昇することによって、がんを含む生活習慣病の予防が期待できると考えられる。また AMPK は ATP の消費経路を阻害することによって代謝の調節を行っていると言うことが考えられる [28][29]。そのことから以下の効果が期待できる。

2- 1.炎症のバランスを調整する [30]。
AMPK により、慢性炎症を抑制することで、癌や心疾患などにアプローチし、生体における健康の維持に寄与する [31] [32]。
2-2.インスリン感受性と耐糖能の向上 [33]。
AMPK を活性化させると代謝性疾患の原因となるインスリン抵抗性や高インスリンの状態を抑制させることができると言う報告がある [33]。また、脂肪燃焼の状態に移行することで、体脂肪量の減少を誘導させ、過剰な体脂肪からの炎症性サイトカインの分泌を抑制させる [34]。
2-3.オートファジーを促進する [35]。
AMPK は細胞のオートファジー（自食作用）を活性化する。
2-4. ミトコンドリアの機能を高める [36]。
AMPK の活性化の主な目的の一つに細胞内エネルギー (ATP) レベルを回復させる。AMPK はミトコンドリアの生合成を活性化することで細胞内の ATP レベルを高める可能性がある [37][38]。
2-5. 免疫系の調整 [39]
AMPK が活性化すると免疫監視機能が強化され、病原体に対する宿主の防御力が高まることで、免疫機能が高まる可能性がある [39]。免疫系の最前線である自然免疫にはオートファジーが不可欠であるが、このオートファジーも AMPK により活性化する [35]。
2-6.サーチュイン遺伝子に作用し、長寿につながる可能性 [40]。
AMPK は健康長寿に関係する長寿遺伝子サーチュインと FOXO タンパク質の産生を活性化する [40]。

3. Materials and Methods

3-1. NMN

NMN purchased from Wellness-One Co., Ltd. (Iwate, Japan) is adjusted to a final concentration of 1 mg / ml.

3-2. AMPK activity measurement

In this experiment, the CycLex® AMPK kinase assay kit (Institute of Medical Biology, Tokyo, Japan) was used to confirm the activity of AMPK using MCF-7 cells as usual. The group was divided into a PBS-added group and an NMN-added group (final concentration 1 mg / ml), each was compared, and the PBS group was used as a control for evaluation. With this kit, AMPK activity was measured 1 hour, 12 hours, and 24 hours after addition. The evaluation was performed by statistical processing software (IBM SPSS Statistics Ver.26). Statistical evaluation was performed by the Mann-Whitney U test).

3-3. NAD + measurement

In this experiment, NAD / NADH (DOJINDO LABORATORIES (Kumamoto Prefecture, Japan) was purchased and measured using MCF-7 cells according to the operation of the kit. Compared with the control group (PBS group), NMN Addition group (final concentration 1 mg / ml) The amount of NADH and total NAD + / NADH were measured at a wavelength of 450 nm using an absorber, and the amount of NAD + expressed was measured by subtracting the amount of NADH from the total amount of NAD / NAD. The evaluation was performed by statistical processing software (IBM SPSS Statistics Ver.26). Statistical evaluation was performed by the Mann-Whitney U test).

4. Result

4-1. AMPK activity measurement (Table.1, Fig. 1)

In this experiment, the CycLex® AMPK Kinase Assay Kit (MEDICAL & BIOLOGICAL LABORATORIES CO., LTD. Tokyo, Japan) was used as per the standard method, and the PBS-added group was used for the AMPK activity in the NMN-added group (final concentration 1 mg / ml). It was compared and evaluated as a control. In this kit, in order to measure the current amount of AMPK activity, the activity of AMPK was confirmed 1 hour, 12 hours, and 24 hours after the addition. In addition, the evaluation was performed statistically by the Mann-Whitney U test using statistical processing software (IBM SPSS Statistics Ver.26).

Table.1 Absorbance of hourly control group and NMN-added group

1)Absorbance

1hr		12hrs		24hrs	
Cnt	NMN	Cnt	NMN	Cnt	NMN
0.285±0.013	3.507±0.136	0.352±0.019	1.783±0.311	0.264±0.034	2.242±0.182

2）AMPK activity increase rate when cnt is 100%

1hr		12hrs		24hrs	
Cnt	NMN	Cnt	NMN	Cnt	NMN
100%	1230.50%	100%	506.50%	100%	849.20%

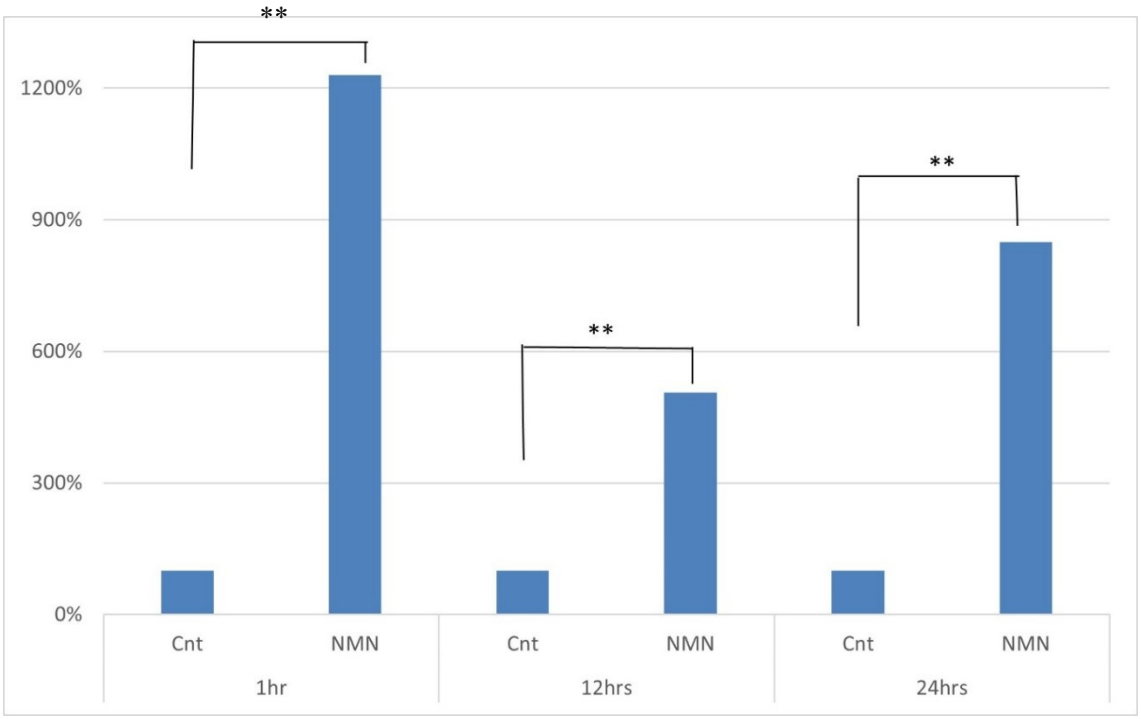

Fig. 1 Rate of increase in AMPK activity in the control group and NMN-added group at each time. (Mann-Whitney U test. P<0.01)

4-2. NAD + measurement (Table. 2, Fig. 2)

In this experiment, NAD / NADH (DOJINDO LABORATORIES (Kumamoto, Japan) was purchased and measured according to the operation contents of the kit. Compared with the control group (PBS group), the NMN-added group (final concentration 1 mg / ml)) NADH amount and total NAD + / NADH were measured at a wavelength of 450 nm with an absorptiometer, and the expression level of NAD + was measured by subtracting the NADH amount from the total NAD / NAD amount. The evaluation was performed by statistical processing software (IBM SPSS Statistics). Statistical evaluation was per-formed by Mann–Whitney U test using Ver.26).

Table.2 Absorbance of NAD + in the control group and the NMN-added group.

Both of n=3	Average of OD value
Control	0.177±0.005（100%）
Addition group of NMN(1mg/ml)	0.544±0.008（307.3%）

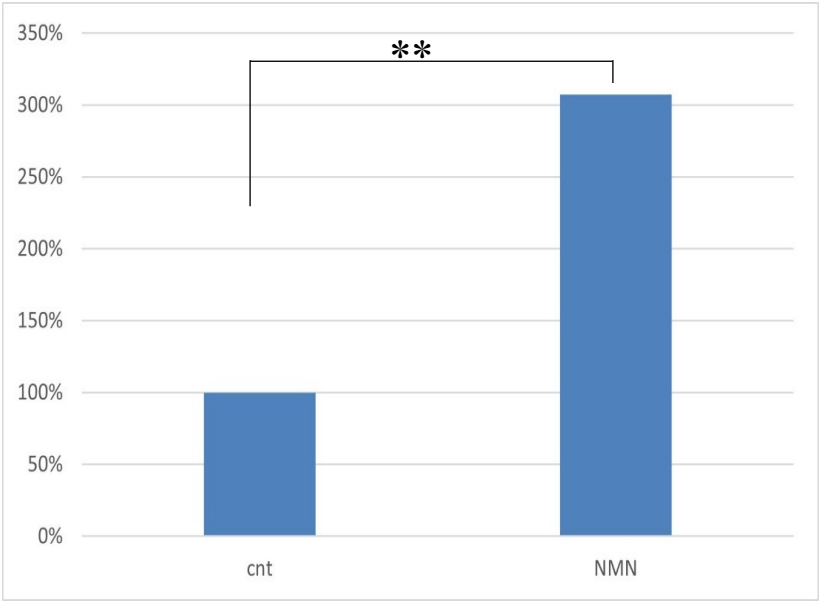

Fig. 2 When the Mann-Whitney U test was performed, the expression level of NAD + in the NMN-added group increased significantly at P <0.01.

5. Discussion

今回我々は現在注目されている栄養成分「NMN（ニコチンアミドモノヌクレオチド）」を用いて、in vitro レベルでのメカニズムを考察した。NMN は、ニコチン酸（ナイアシン）に含ま

れる物質で、すべての生物の細胞に存在する補酵素であり、体内で産生される。[41]本研究においてはNMNがAMPKの活性を上昇させるか否かについて測定を行った。その結果、NMN添加群ではコントロール群を100%としたとき、添加1時間後では1230.5%、12時間後では506.5%、24時間後では849.2%の活性度の上昇がみられた。つまり、24時間経過してもAMPKの活性が有意にみられたことから、NMNはAMPKの活性に関与することが示唆された。また、24時間経過した後でもコントロール群に比べ活性が上昇していると言うことから持続作用が期待された。AMPKを活性化することで前述した様々な効果が期待できる。中でもオートファジーやミトコンドリアの活性化などがみられるために[35][36]、老化の予防・改善を行うことが示唆された。また、本研究においてはミトコンドリアの活性に着目をし、その指標の一つであるNAD+の測定も行ったところ[36]、NMN添加群においてはコントロール群に比べ有意に増加が認められたため、ミトコンドリアの機能の向上についても示唆された。NMNはビタミンB_3から合成され、NADの前駆体として知られている[42]。NMN 投与により効率よくNAD+合成が促進され、さらにそこからサーチュインが活性化されると考えられる[43]。老化した多くの臓器でNAD+が低下していることが判明しているために[44][45]、NAD+を供給することによって臓器機能維持に重要であると考えられる[46]。NAD+は細胞内における主要な代謝経路の酸化還元反応に関与する重要な補酵素である[47]。NADは細胞内において酸化型のNAD+と還元型のNADHとして存在しているが、これら二つのバランスが細胞機能を維持する上で必須である[48]。近年、NAD+量の低下と老化に伴う疾患との因果関係も指摘されている[49]。本研究においてはAMPKの活性化のみならずNAD+の量に関しても増加が確認されたために、老化に対してアプローチできることが示唆された。また、現段階ではNMNにおいて、老化の一つの原因である抗酸化活性についての報告はみることが出来ない。しかしミトコンドリア機能を改善し、代謝を増加させるという報告がある[50]。また、NMNはサーチュイン遺伝子に作用することで、健康の維持・増進にも深くかかわっていると言われている[51]。以上のことから、NMNによって今後老化予防の可能性が示唆され、健康寿命の延伸、最終的には平均寿命の延伸につながる可能性が示唆された。

6. Conflicts of Interest:

The authors declare no conflict of interest.

7. Acknowledgments

I would also like to express our deep gratitude to Dr. Yoshitaka Fukuzawa of Aichi Medical University for his useful advice in conducting this research.

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