Accepted Manuscript **[Published after patent application]**

Regular Article

Effects of nicotinamide mononucleotide and paprika xanthophyll on endurance performance: a randomized, placebo-controlled, double-blind, parallel group study

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Number of tables and figures: 8 tables, 5 figures

Running title: Effect of NMN and Paprika Xanthophyll on Endurance

Abstract

Efficient energy production is essential for endurance performance, and thus it is important to promote mitochondrial ATP production. Nicotinamide mononucleotide (NMN) is converted to the coenzyme nicotinamide adenine dinucleotide (NAD^+), which contributes to mitochondrial ATP production. Meanwhile, paprika xanthophyll (PX), an oil-based extract from ripe red paprika, improves the oxygen-delivery capacity of red blood cells by increasing their membrane flexibility. This study was a randomized, placebo-controlled, double-blind, parallel-group trial that investigated the effects of intake of NMN (500 mg/day), PX (9 mg/day), or their combination for 8 weeks on endurance performance. The participants were male collegiate track-and-field athletes aged 18 years or older. As endurance performance, running velocity at lactate threshold, running velocity at the onset of blood lactate accumulation, and heart rate were evaluated using the lactate curve test. The results showed that NMN did not effectively improve endurance performance after intake for 8 weeks, which was the primary endpoint of this study. However, the results after intake for 4 weeks as well as stratified analyses by blood NAD^+ levels before intake suggested that the combination of NMN and PX might improve endurance performance. Thus, both the increase in NAD^+ induced by NMN intake and sufficient oxygen delivery induced by PX intake may be beneficial for promoting mitochondrial ATP production, thereby leading to high endurance performance.

Keywords: endurance, lactate curve test, nicotinamide mononucleotide, paprika xanthophyll, mitochondria

ニコチンアミドモノヌクレオチドおよびパプリカキサントフィルによる持久パフォーマンスへの影響：ランダム化プラセボ対照二重盲検並行群間比較試験

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抄録

持久パフォーマンスには効率的なエネルギー産生が不可欠であるため，ミトコンドリアの ATP 産生を促進することがアスリートの持久パフォーマンス向上の鍵となる．ニコチンアミドモノヌクレオチド（NMN）は，補酵素ニコチンアミドアデニンジヌクレオチド（NAD^+）に変換され，ミトコンドリアの ATP 産生に寄与する．また，赤パプリカ由来の油性抽出物であるパプリカキサントフィル（PX）は，赤血球膜の柔軟性を高めることで酸素運搬能力を向上する．本研究では，8 週間の NMN（500 mg/day），PX（9 mg/day），またはその併用摂取が持久パフォーマンスに与える影響を，ランダム化プラセボ対照二重盲検並行群間比較試験で検討した．対象者は，大学陸上部に所属する 18 歳以上の男性ア

スリートであった. 持久パフォーマンスとして, 乳酸作業性閾値, 乳酸蓄積開始点および心拍数を乳酸カーブテストによって評価した. その結果, 主要評価項目である摂取開始 8 週間後の NMN 群の持久パフォーマンス向上効果は認められなかった. しかし, 摂取開始 4 週間後の結果および摂取前の血中 NAD^+量による層別解析から, NMN と PX の併用によって持久パフォーマンスが向上する可能性が示唆された. したがって, NMN 摂取による NAD^+の増加と, PX 摂取による十分な酸素供給の両方が, ミトコンドリアの ATP 産生を促進し, 高い持久パフォーマンスを発揮することに重要かもしれない.

Introduction

For athletes to achieve high performance, factors such as endurance, explosive strength, flexibility, and muscular strength are necessary[1]. Endurance performance is the ability to sustain exercise over an extended period and is an important factor that directly affects athletic performance, especially in endurance sports such as marathons, triathlons, and soccer. Efficient energy production is essential for endurance performance, as prolonged muscle contraction demands a large and continuous supply of energy[2]. Energy is produced primarily in the mitochondria through oxidative phosphorylation, a process that relies on oxygen to convert glucose and fatty acids into adenosine triphosphate (ATP). Oxygen plays a critical role in the electron transport chain, enabling the efficient production of ATP. Therefore, enhancing mitochondrial function and promoting efficient ATP production are key to improving endurance performance.

One food ingredient that activates mitochondrial ATP production is nicotinamide mononucleotide (NMN). A type of vitamin B3, NMN is a precursor of nicotinamide adenine dinucleotide (NAD^+), which is involved in various biological reactions, including cellular redox regulation, metabolism, and DNA repair[3]. Accordingly, NMN intake is expected to yield health benefits. A decline in NAD^+ levels with aging leads to diminished biological functions[4], which has prompted active research into the anti-aging effects of supplying NAD^+ through NMN intake[5]. Given that NAD^+ also contributes to ATP production as a cofactor in the tricarboxylic acid cycle and oxidative phosphorylation in mitochondria[6], NMN intake may enhance endurance performance by activating mitochondrial ATP production. In fact, studies have shown that NMN intake improves mitochondrial function[7], and that the oxygen

uptake capacity of middle-aged amateur runners increased after NMN intake for 6 weeks combined with exercise training[8]. From this, we hypothesized that NMN intake enhanced endurance performance, and that this effect would be stronger in individuals with low NAD^+ levels in their bodies.

Meanwhile, oxygen supply is also essential for mitochondrial ATP production, and it was reported that endurance performance declined in environments with insufficient oxygen supply[9]. Oxygen delivery to mitochondria in peripheral tissues, including skeletal muscle, is provided by red blood cells (RBCs). However, RBCs are vulnerable to membrane phospholipid damage caused by reactive oxygen species, and the phospholipid composition of the membrane affects the flexibility of RBCs, which is critical for effective oxygen delivery to peripheral tissues[10]. Xanthophylls, natural pigments found in vegetables, have strong antioxidant properties and are recognized as the main dietary antioxidants. Paprika xanthophylls (PX), an oil-based extract from ripe red paprika, contains seven xanthophylls: β-cryptoxanthin, zeaxanthin, capsanthin, cucurbitaxanthin A, cryptocapsin, capsanthin 3,6 epoxide, and capsorbin[11]. Previous studies reported that PX intake increased xanthophyll localization to the membrane of RBCs[11] and allowed collegiate athletes to perform exercise at a set intensity with lower oxygen uptake and maximum heart rate[12,13]. This suggests that PX intake may improve endurance performance by increasing the flexibility of RBCs, thereby improving oxygen delivery capacity to peripheral tissues, including skeletal muscle. Accordingly, we hypothesized that endurance performance could be synergistically enhanced by NMN intake, thereby improving mitochondrial ATP production through increased NAD^+ level, as well as PX intake, thereby improving oxygen delivery to mitochondria. Although previous studies have suggested that NMN and PX intake independently

enhanced endurance performance, the combined effect of these supplements on endurance performance has not been investigated.

Therefore, the present study aimed to elucidate the effects of NMN and/or PX intake on endurance performance in endurance athletes as well as the impact of NAD^+ level, through a clinical trial.

Materials and Methods

1. Research ethics

This study was commissioned by EUPHORIA Co., Ltd. (Tokyo, Japan). The study protocol was approved by the Shiba Palace Clinic Ethics Review Committee (approval number: 152152_rn-35003) and complied with the Declaration of Helsinki and the Ethical Guidelines for Life Sciences and Medical Research Involving Human Subjects. The study was registered in advance with the University Hospital Medical Information Network Center, a system for registering clinical trials (ID: UMIN000050969).

2. Study design

The study design was a randomized, placebo-controlled, double-blind, parallel-group study involving healthy young Japanese male athletes. The study was conducted from mid-May to mid-September 2023.

A schematic of this study is shown in Figure 1. Participants were measured a total of three times after a 2-week washout period: before the test foods intake (pre), after intake for 4 weeks (4 weeks), and after intake for 8 weeks (8 weeks). Between pre-measurement and the start of test foods intake, approximately 4 weeks were allowed for organizing measurement results and randomization. Based on pre-measurement

endurance performance [running velocity at lactate threshold (vLT) and running velocity at the onset of blood lactate accumulation (vOBLA)] and blood NAD^+ levels, participants were randomly assigned to one of four groups: the placebo group (n=55), the NMN group (n=55), the PX group (n=55), and the NMN+PX group (n=54). The test foods were taken daily for 8 weeks. Throughout the study period, participants kept a web diary to record their test food intake, physical condition, sleep status, and physical activity. Figure 1

Stratified randomization was performed using the following stratification factors: affiliated team, NAD^+, and vLT. Participants were enrolled, randomized, and allocated to the appropriate groups by the investigators. The participants, research staff, and those assessing the outcomes were blinded throughout the study.

3. Participants

We recruited healthy Japanese male collegiate track-and-field athletes aged 18 years and older. During the study period, they carried out group training sessions, such as long-distance running, interval training, and jogging, in addition to individual training, totaling approximately 3 to 7 times per week. Those with serious diseases such as diabetes mellitus, liver disease, renal disease, cardiac disease, or cardiovascular disease (including those under treatment) or with pre-existing conditions of such diseases; those unable abstain from health foods such as dietary supplements, quasi-drugs, and over-the-counter drugs during the study period; those who could not abstain from smoking during the study period; those who were likely to change their lifestyle during the study period, except for training camps and other activities conducted as part of their practice;

and those deemed unsuitable for the study by the investigator or the study sponsor for any other reason were excluded.

The sample size was estimated based on VO_2@VT, an endurance performance measure. In a previous study evaluating the oxygen uptake capacity–enhancing effects of NMN on middle-aged amateur runners, the effect size (Cohen's *d*) for VO_2@VT between the placebo and NMN groups was 1.45. Using this as a reference, a sample size of 26 participants per group was calculated with G-power to achieve at least 80% power ($\beta \geq 0.8$) and statistical significance ($\alpha \leq 0.05$) in an independent *t*-test. Given that stratified analyses by blood NAD^+ levels would be performed and that a certain number of participants might withdraw from the study after enrollment, the target number of participants was set at 60 per group in order to detect the effects on endurance performance.

4. Test foods

During the study period, participants were instructed not to change their usual diet or daily routines and to refrain from taking other supplements. Participants were randomly assigned to each group in a double-blind manner. The test foods, which were identical in size and shape, for each group are shown in Table 1. Participants took each test food once daily and recorded their daily intake in a web diary.

Table 1

In this study, the dosage of NMN was set at 500 mg/day. The previous study on amateur runners examined doses of 300, 600, and 1200 mg/day, reporting improvements in the oxygen uptake capacity starting at the medium dose of 600 mg/day[8]. Because NAD^+ levels decline with age, it was considered that NAD^+ levels of collegiate athletes participating in this study would be comparable to or higher than those of participants in

120 the previous study. Furthermore, the safety of a single 500 mg dose of NMN has already
121 been confirmed[14]. Considering that the participants of this study were young athletes, a
122 dose of 500 mg/day was established to balance efficacy and safety. On the other hand,
123 the dosage of PX was set at 9 mg/day. This dose was established based on the previous
124 studies in which oxygen uptake and heart rate were reduced during exercise[12,13].
125 NMN was obtained from Coach Boueki Co., Ltd. (Osaka, Japan). PX was obtained
126 from Glico Nutrition Co., Ltd. (Osaka, Japan) as PapriX-nano, a commercial paprika
127 xanthophyll formulation containing 1% PX, which included 5 mg of capsanthin and 0.5
128 mg of β-cryptoxanthin within a total of 9 mg of xanthophylls.
129
130 5. Measurements
131 Measurements were taken three times: pre, 4 weeks, and 8 weeks. On the
132 measurement days, body composition and endurance performance were measured, and
133 biological samples were taken.
134
135 5-1. Body composition
136 Body weight, body fat percentage, and muscle mass were measured using a
137 direct segment multi-frequency bioelectrical impedance analyzer (InBody Dial H20N:
138 InBody Japan Inc., Tokyo, Japan).
139
140 5-2. Endurance performance
141 Endurance performance was measured using the lactate curve test, which
142 evaluates running speed as corresponding to blood lactate concentrations of 2 mmol/L
143 (lactate threshold: LT) and 4 mmol/L (onset of blood lactate accumulation: OBLA).

Participants jogged on a treadmill at 8 km/h for 5 min. After jogging, they rested for 5 min and blood lactate concentration was measured as at the starting point. Running velocity at the first stage was decided based on the individual best time of the 5-km time trial[15]. The running speed was then increased in 1.2-km/h increments up to seven stages, with each stage consisting of 3 min of running followed by a 1-min break on a treadmill set at a 3% incline[16]. During each break, a fingertip blood sample was taken, and blood lactate concentration was measured using the Lactate Pro 2 LT-1730 (Arkray Inc., Kyoto, Japan). During the test, the participants' heart rate was measured using the wearable Polar H10 N (Polar Electro Japan, Tokyo, Japan), and was used to calculate the slope of the change in heart rate relative to running speed (HR slope), with reference to previous studies[17].

5-3. Blood NAD^+ level

Blood samples were collected in blood collection tubes containing ethylenediaminetetraacetic acid, frozen at −80°C, and analyzed by Shimadzu Techno-Research, Inc. (Kyoto, Japan). Then, 50 µL of blood and 300 µL of 5 µg/mL trichloroacetic acid solution were mixed and centrifuged at 10,000 rpm at 4°C for 5 minutes. The upper aqueous phase was transferred to a new tube and filtered through a centrifugal filter. Then, 100 µL of filtrate was mixed with 5 µL of 1 M ammonium carbonate solution to make the sample. NAD^+ was analyzed using the MS system Triple Quad 5500+ (AB Sciex Pte. Ltd., Tokyo, Japan) in combination with a high-performance liquid chromatography system (Nexera X3; Shimadzu Corporation, Kyoto, Japan). The analytes were analyzed using mobile phase A (100 mM ammonium acetate) and mobile phase B (acetonitrile) at a flow rate of 250 µL/min and a column

temperature of 40°C (Shim-pack Scepter PFPP-120, 2.1 × 150 mm, particle size 3 μm; Shimadzu Corporation, Kyoto, Japan). The programmed mobile phase gradients were as follows: 0–0.5 min, 1%; 0.5–10.5 min, 90%; 10.5–27.5 min, 1%.

5-4. Sleep-quality questionnaire

Throughout the study period, a brief sleep-quality questionnaire was administered using a web diary. Participants answered the questionnaire by selecting one of the following four options (0: slept very well, 1: slept somewhat well, 2: slept somewhat poorly, 3: did not sleep well at all) for their self-assessment of sleep quality on that day. The total score was tabulated for each period: from the washout period to the day before the test food intake (the before-intake period), from the start of intake to after intake for 4 weeks (the 0–4-week period), and from after intake for 4 weeks to after intake for 8 weeks (the 4–8-week period).

6. Primary endpoint

The primary endpoint was endurance performance (vLT, vOBLA, HR slope) of the NMN group at the 8 week-measurement.

7. Secondary endpoints

Secondary endpoints were endurance performance (vLT, vOBLA, HR slope) excluding the primary endpoint, blood NAD^+ levels, and sleep-quality questionnaire results.

8. Statistical analyses

Analyses were conducted in the per-protocol set (PPS, n=139), which included participants from the full analysis set (FAS, n=194) who did not deviate from the protocol and had no potential to influence the study analyses. For example, participants who had a low frequency in taking the test foods, consumed medications during the study period, or were unwell or injured during the study period were excluded.

All statistical analyses were performed using R (ver. 4.3.2). Statistical significance was set at P-value <0.05 for all analyses. For endurance performance and blood NAD^+ levels, linear mixed-effects model analyses were performed with time course and test-food contents, baseline values for each measurement item, fixed effects for the interaction between time course and test-food contents, and random effects for each participant. The model estimation method was restricted to the maximum likelihood estimation, and the method for computing degrees of freedom was Satterthwaite. Estimated means, differences in estimated means, and 95% confidence intervals (CIs) from the time of pre-measurement for each group to each time point were estimated for the above models. For body composition and results of the sleep-quality questionnaire, means and standard deviations were calculated for each group and each time point. Because the results of the sleep-quality questionnaire were nonparametric data, the Mann–Whitney U test was used for between-group comparisons, and the Wilcoxon signed rank test was used for within-group comparisons. Multiplicity adjustment for the primary endpoint was performed using a step-down approach. Both the analyses of the results for the secondary endpoints and the stratified analyses by blood NAD^+ levels before intake were performed in an exploratory manner, and no multiplicity adjustment was performed.

Results

1. Participants for analyses

A total of 219 athletes participated in this study and consumed the test foods. After excluding dropouts and those who violated the exclusion criteria, 139 participants were included in the analyses (Figure 2). The baseline characteristics of the participants are shown in Table 2. One-way analysis of variance confirmed that there were no significant differences between the groups. By the way, there were no significant differences between the groups in the average running distance per participant for each period, such as the before-intake period, the 0–4-week period, and the 4–8-week period.

Figure 2

Table 2

2. Endurance performance

The results of endurance performance as measured by the lactate curve test are shown in Table 3 and Figure 3. The improvement in endurance performance in the NMN group compared with the placebo group at 8 weeks, which was set in a step-down approach as the primary endpoint, was not observed in vLT, vOBLA, and HR slope. At 8 weeks, all participants, including those in the placebo group, showed a significant improvement in endurance performance compared with before intake. This result suggested that vLT increased in the NMN+PX group at 4 weeks, although a significant difference cannot be discussed as an adjustment for multiplicity in a step-down approach.

Table 3

Figure 3

3. Blood NAD^+ levels

The results of blood NAD^+ levels are shown in Figure 4. Blood NAD^+ levels increased significantly in the NMN group and the NMN+PX group compared with the placebo group at 4 and 8 weeks.

Figure 4

4. Stratified analyses by blood NAD^+ levels before intake

To test the first hypothesis that the effect of NMN intake on endurance performance can be expected to be stronger in people with low NAD^+ levels, stratified analyses were performed. Based on the median blood NAD^+ levels of all participants before intake, each group was divided into two segments, a low segment and a high segment. The baseline for the participants in each group is shown in Table 4. Student's *t*-test confirmed that there were no significant differences between the low and high segments of each group in any index other than blood NAD^+ levels. Table 4

The results of the stratified analyses of endurance performance are shown in Tables 5 and 6. Regardless of blood NAD^+ levels before intake, the blood NAD^+ levels of the NMN group and the NMN+PX group were significantly increased compared with the placebo group at 4 and 8 weeks. In the low segments of blood NAD^+ levels before intake, vOBLA was significantly improved in the NMN+PX group compared with the placebo group at 4 weeks. In addition, there was a trend toward an improvement in vLT compared with the placebo group at 4 weeks, and in vOBLA compared with the placebo group at 8 weeks. The PX group also showed a significant improvement in vLT compared with the placebo group at 4 weeks, and in vOBLA compared with the placebo group at 8 weeks. However, the NMN group showed no improvement in endurance performance compared with the placebo group. There were no differences between groups in the high segments of blood NAD^+ levels before intake. Table 5, 6

5. Sleep-quality questionnaire

From the results described earlier, it is indicated that the intake of the test foods may enhance mitochondrial energy production. Based on this, it was hypothesized that the increased energy production could positively effect sleep quality by facilitating

recovery. To examine this hypothesis, the effect of the test foods on sleep quality was evaluated by a questionnaire. The results of the sleep-quality questionnaire are shown in Table 7. The sleep quality score for the 0–4-week and 4–8-week periods significantly improved compared with the before-intake period only in the NMN+PX group. Furthermore, in the NMN+PX group, the sleep quality score for the 4–8-week period improved significantly compared with the placebo group.

6. Adverse events

Participants took NMN and/or PX throughout the study period, and no participants reported any adverse events.

Discussion

During endurance exercise such as marathon running, an athlete's performance is influenced by the activity of energy production in skeletal muscle mitochondria. This study examined the effects on endurance performance of intake of NMN (500 mg/day), PX (9 mg/day), or their combination for 8 weeks. The main finding of this study was that the combination of NMN, which increases the energy production capacity by raising the NAD^+ level, and PX, which increases the oxygen delivery capacity of RBCs, might activate mitochondrial ATP production and improve endurance performance.

Combination of NMN and PX improved endurance performance

As exercise intensity increases, lactate accumulates because of increased energy supply from the glycolytic system. The vLT and vOBLA measured by the lactate curve test represent thresholds at which blood lactate levels begin to rise rapidly with

increasing exercise intensity, and these are well-known indicators of endurance performance. Previous studies have reported that vLT correlates with the race pace of long-distance runners[18] and is improved by high-intensity exercise training[19].

Only the NMN+PX group showed an improvement in vLT at 4 weeks, with no significant difference in the NMN group and the PX group compared with the placebo group (Table 3). This suggests the possibility that the combination of NMN and PX improves endurance performance. To improve vLT, increasing the efficiency of pyruvate uptake by mitochondria to produce ATP is essential. NAD^+ plays a key role as a cofactor in tricarboxylic acid cycle and oxidative phosphorylation, both of which are ATP-producing pathways in mitochondria. NMN supplementation has been reported to increase blood NAD^+ levels[20], and in the present study, NMN and NMN + PX intake significantly increased blood NAD^+ levels (Figure 4). NMN may contribute to improving vLT by activating mitochondrial metabolism through conversion to NAD^+. However, not only the coenzyme NAD^+ but also oxygen is essential for ATP production in mitochondria. PX intake has been shown to improve oxygen delivery capacity of RBCs by localizing carotenoids in the membranes of RBCs[12]. No improvement in vLT was seen in the NMN group despite a sufficient increase in NAD^+ levels, whereas vLT was significantly improved in the NMN+PX group (Table 3, Figure 4). This implies that both the increase in NAD^+ by NMN intake as well as sufficient oxygen delivery by PX intake are crucial for activating mitochondrial energy production, thereby leading to high endurance performance.

However, this study did not statistically evaluate the effect of the combined intake of NMN and PX on endurance performance improvement as the primary endpoint. Further studies should aim to improve the study design to confirm the effects

of the test foods more effectively. For example, tighter control over the content and amount of training during the study period could be implemented, such as prohibiting all training.

NMN and PX alone were not effective in improving endurance performance

NAD^+ is a key cofactor in mitochondrial metabolism, and NMN intake has been shown to activate mitochondrial energy production. Additionally, it was reported that NMN intake for 6 weeks combined with exercise training improved oxygen uptake in middle-aged amateur runners[8]. It has also been reported that PX intake enabled athletes to perform exercise at a set intensity with lower oxygen uptake and maximum heart rate[12,13]. However, in the present study, there were no significant differences in vLT, vOBLA, or HR slope between the NMN group or the PX group and the placebo group (Table 3). One possible explanation for this discrepancy lies in the differences in trainability due to the endurance levels of participants in previous studies versus those in the present study. The participants in the previous NMN study were middle-aged amateur runners[8], while those in the previous PX studies were track-and-field athletes, including short-distance runners and jump event athletes[12], and athletes in a variety of disciplines, not just track-and-field[13]. In contrast, those in this study were competitive level long-distance runners who likely already had high endurance at baseline. It was therefore suggested that NMN and PX alone had less of an effect on the participants of this study because individuals with a higher baseline endurance experienced smaller gains in endurance performance. Moreover, the effect of NMN on oxygen uptake has been suggested to be dose-dependent[8], raising the possibility that the required intake might vary according to each athlete's endurance level.

Because NAD^+ levels naturally decline with age, NMN is thought to have stronger effects in older people. In this study, the lack of significant results with NMN alone may be attributable to the younger age of the participants, whose NAD^+ levels were likely sufficient.

Therefore, future research should explore whether the effects of NMN and PX on endurance performance vary according to age and baseline endurance levels.

Participants' overall endurance performance improved at 8 weeks.

In this study, there were no differences between groups in endurance performance at 8 weeks (Table 3). Observations of the variability of vLT and vOBLA over time in each group showed that vLT and vOBLA increased significantly during the 4–8-week period in most groups, including the placebo group. This suggests that the test foods had no effect on vLT and vOBLA. One of the reasons for this result is that the participants had performed higher-load exercises during the 4–8-week period compared with their usual training, leading to a significant improvement in overall exercise capacity. For the participants of this study, who were all long-distance athletes belonging to a collegiate track-and-field team, the 4–8-week period coincided with a time when they had several training camps, which likely increased their total training volume. A study on the running distance of a collegiate relay race team reported that running distance increased significantly from August to September, which corresponds to the 4–8-week period in this study compared with the previous month[21]. In fact, in the present study, when the daily mileage for the before-intake period, the 0–4-week period, and the 4–8-week period were tabulated, the increase during the 4–8-week period was greater than that during the other periods (Supplemental Table 1). Therefore, it was

362 suggested that changes in training volume because of training camps and other factors
363 have affected the results, making it difficult to confirm the effects of each test food.
364 Although data were not obtained in this study, it was suggested that changes in the
365 quality and quantity of the diet because of changes in training volume have also affected
366 the results. The overlap of the study period with training camps is a limitation of this
367 study. Therefore, in future studies, conducting research during periods when training
368 volume and diet quality and quantity are more stable would be expected to more
369 accurately capture the effects of the test foods on endurance performance.
370 Supplemental Table 1
371 Effects of supplementation on endurance performance were stronger in participants with
372 low NAD^+ levels
373 To test the second hypothesis that NMN intake improves endurance
374 performance, with stronger effects in those with lower NAD^+ levels, stratified analyses
375 were performed based NAD^+ levels before intake (Tables 5 and 6). The results showed
376 that the combination of NMN and PX improved endurance performance only in the low
377 segments of blood NAD^+ levels before intake test foods. However, even in those
378 segments, the NMN group did not show significant improvement in endurance
379 performance. These results suggest that NMN intake is more effective in individuals
380 with low NAD^+ levels, while both coenzyme NAD^+ and oxygen delivery by PX are
381 important for mitochondrial energy production and endurance performance.
382 Additionally, the effect of PX on improving endurance performance was observed in the
383 low segments of blood NAD^+ levels before intake of the test foods. It was suggested
384 that individuals with low NAD^+ levels also experience suppressed mitochondrial
385 metabolism because of insufficient oxygen delivery and that PX helps activate

mitochondrial energy production, thereby improving endurance performance. Elucidating the detailed mechanisms through which the combination of NMN and PX or PX alone may have stronger effects in individuals with low NAD^+ levels will be a focus of future research.

Combined intake of NMN and PX improved sleep quality

The results of the sleep-quality questionnaire showed that only the combined intake of NMN and PX improved sleep quality (Table 7). Humans require a lot of energy for brain and body recovery, not only during daytime activity but also during sleep. It was reported that brain activity and energy demand were higher in the deep sleep state[22], and energy supply is important for good quality sleep. In athletes' sleep, it is assumed that a larger amount of energy is needed to repair tissues damaged by intense exercise. Therefore, activation of mitochondrial energy production by the combination of NMN and PX can promote recovery from exercise-induced fatigue by providing sufficient energy during sleep and improving sleep quality.

NMN has been reported to cross the blood-brain barrier and increase brain NAD^+ levels[23]. So, NMN and PX may activate mitochondrial energy production in the brain as well as in peripheral tissues such as skeletal muscle. It was also reported that brain mitochondrial function is linked to serotonergic activity, a neurotransmitter involved in sleep quality[24]. Therefore, it was suggested that the activation of brain mitochondrial energy production by the combined intake of NMN and PX has improved sleep quality through neurotransmitter regulation.

The detailed mechanism through which the combination of NMN and PX, rather than NMN or PX alone, improves sleep quality is an area that warrants further research.

Conclusions

The results of this study suggest that the combined intake of NMN and PX may improve endurance performance. NMN intake provides coenzyme NAD^+ at levels sufficient for mitochondrial metabolism, and PX intake improves oxygen delivery capacity of RBCs, thereby ensuring an adequate oxygen supply to skeletal muscle. The combination of both effects may activate actual mitochondrial energy production in skeletal muscle and improve endurance performance. Additionally, the combination of NMN and PX appeared to improve sleep quality, suggesting that this combination might contribute not only to endurance performance but also to recovery from fatigue in athletes.

Acknowledgment

We would like to express our sincere appreciation to the team members involved in this research. The authors declare that this study received funding from Ezaki Glico Co., Ltd.

Conflicts of Interest

NK, ET, KY, and HK are employees of Ezaki Glico Co., Ltd. KN, ET, KY, and HK have applied for patents (JP2024-087242) based on the results of this research. The purpose of this patent is to commercialize the techniques and findings from the research.

Contributions

Experiment conception, design, and experiment implementation: KN, ET, KY, and HK. Data analysis: KN. Paper composition: KN. Analyzing and writing advisory: ET, KY, and HK. All authors have read and agreed to the final version of the manuscript.

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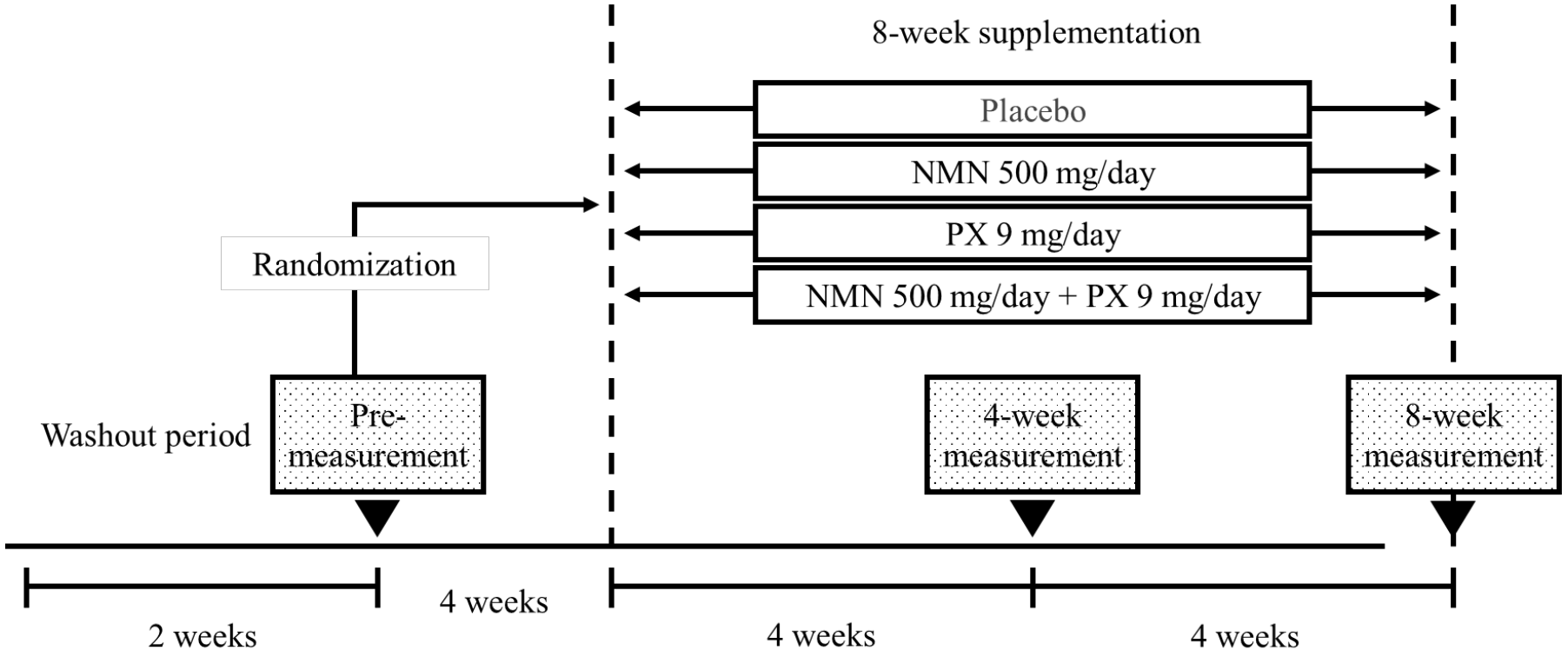


Figure 1. Study design

NMN, nicotinamide mononucleotide; PX, paprika xanthophyll

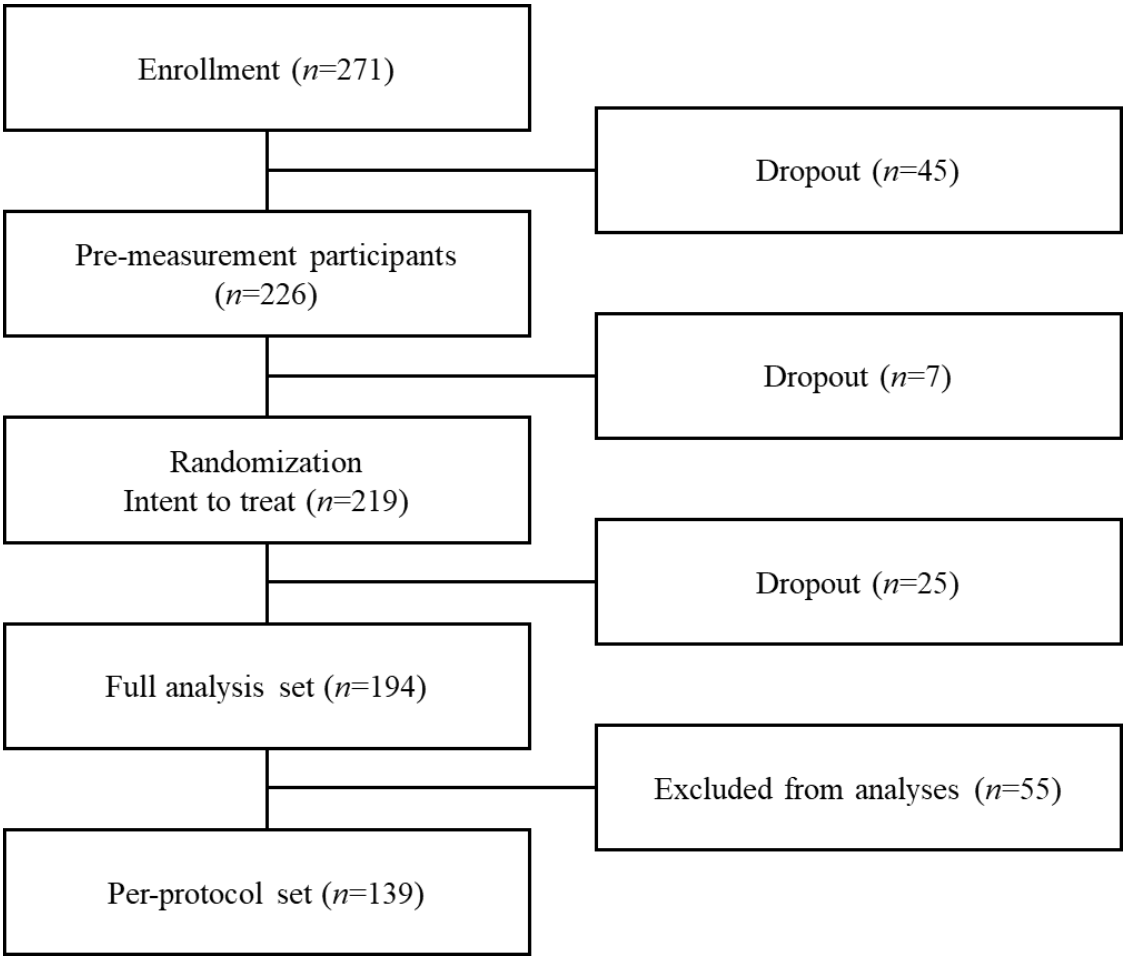


Figure 2. Study flow diagram

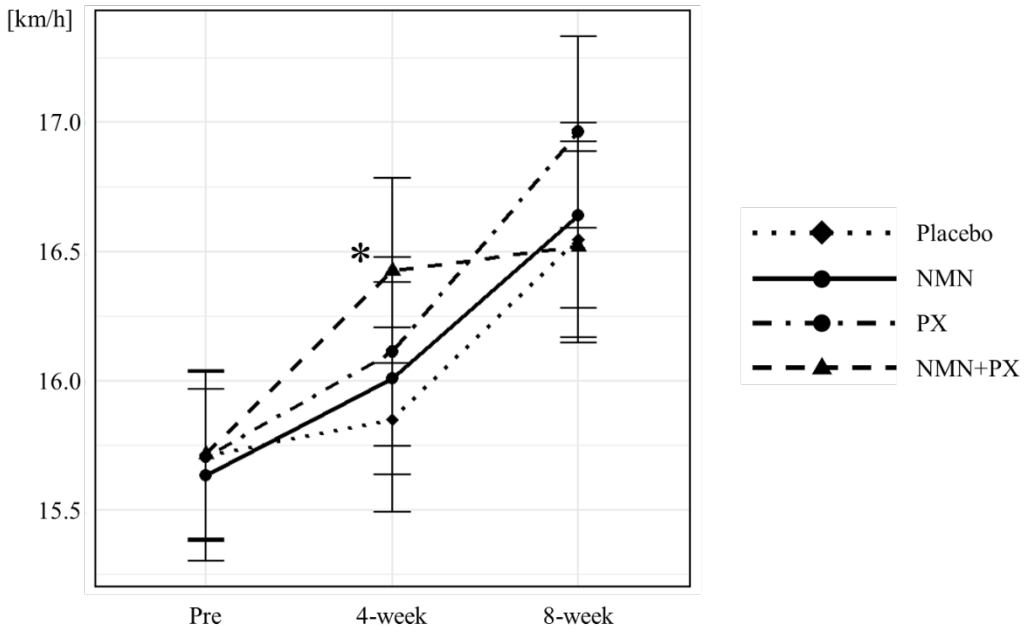


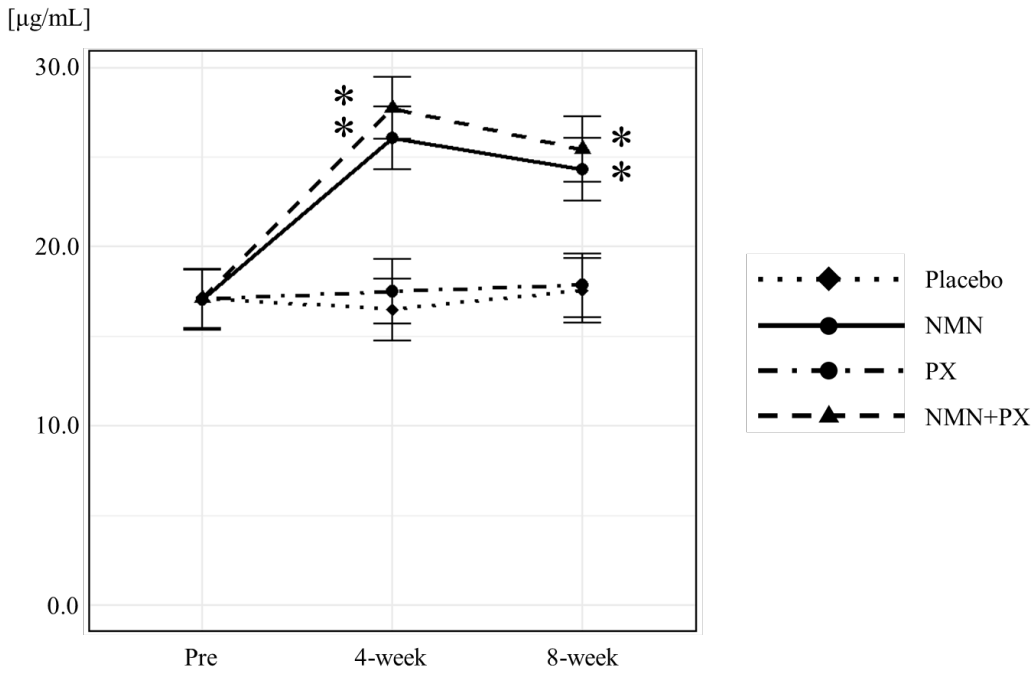
Figure 3. Endurance performance (vLT)

Data are shown as adjusted mean, and the I bars indicate 95% confidence intervals.

For the adjusted means, a linear mixed effect model analysis was performed using group, measurement time, baseline value, and the interaction between the group and measurement time point as fixed effects; participants as random effects; and baseline values as covariates.

NMN, nicotinamide mononucleotide; PX, paprika xanthophyll; vLT, running velocity at lactate threshold

*p<0.05 vs. placebo



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549 Figure 4. Blood NAD^+ levels

550 Data are shown as adjusted mean, and the I bars indicate 95% confidence intervals.

551 For the adjusted means, a linear mixed effect model analysis was performed using

552 group, measurement time, baseline value, and the interaction between the group and

553 measurement time point as fixed effects; participants as random effects; and baseline

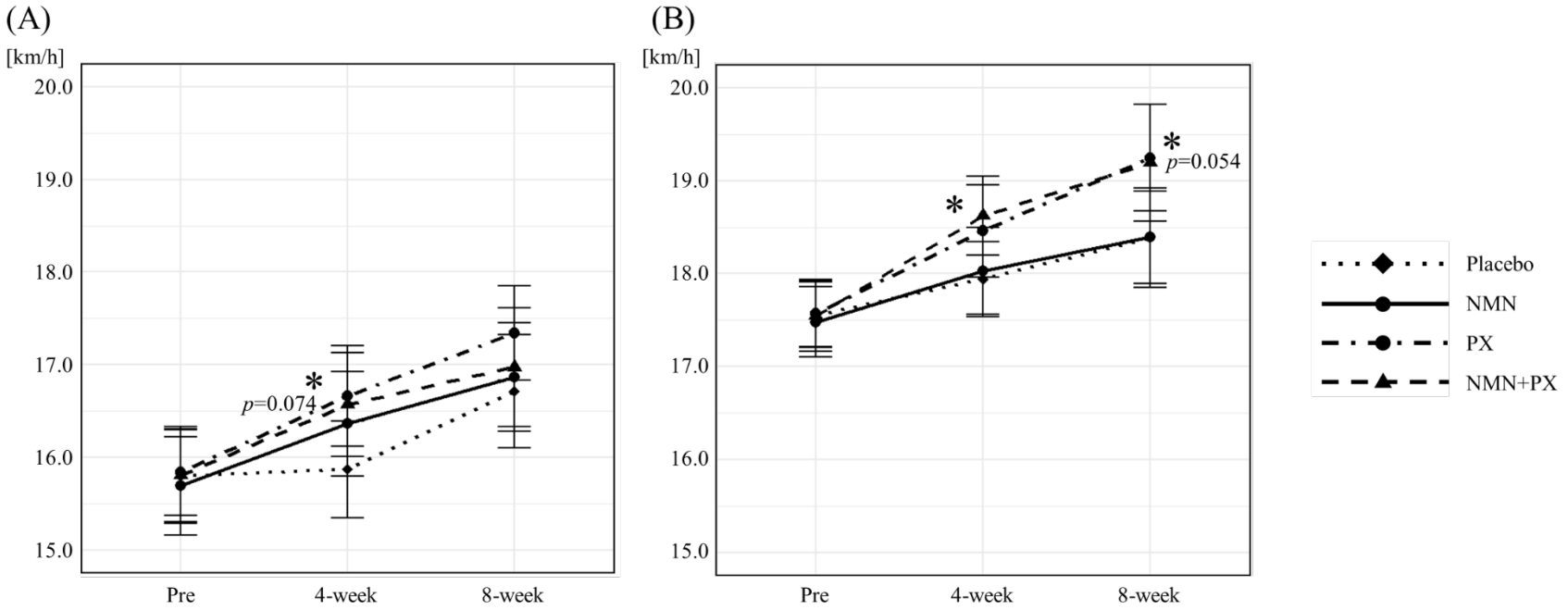
554 values as covariates.

555 NMN, nicotinamide mononucleotide; PX, paprika xanthophyll; CI, confidence interval;

556 NAD, nicotinamide adenine dinucleotide

557 *p<0.001 vs. placebo

558



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560 Figure 5. Endurance performance results for the low segments of blood NAD^+ levels before intake

561 (A) vLT; (B) vOBLA

562 Data are shown as adjusted mean, and the I bars indicate 95% confidence intervals.

563 For the adjusted means, a linear mixed effect model analysis was performed using group, measurement time, baseline value, and the

564 interaction between the group and measurement time point as fixed effects; participants as random effects; and baseline values as covariates.

NMN, nicotinamide mononucleotide; PX, paprika xanthophyll; CI, confidence interval; vLT, running velocity at lactate threshold; vOBLA, running velocity at the onset of blood lactate accumulation

*p<0.05 vs. placebo

569 Table 1. Contents of the test foods in this study

Group	Test foods
Placebo	placebo hard capsule, placebo soft capsule
NMN	NMN hard capsule (500 mg/day), placebo soft capsule
PX	placebo hard capsule, PX soft capsule (9 mg/day)
NMN+PX	NMN hard capsule (500 mg/day), PX soft capsule (9 mg/day)

570

571 NMN, nicotinamide mononucleotide; PX, paprika xanthophyll

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573 Table 2. Baseline characteristics of the participants

	Total (*n*=139)	Placebo (*n*=35)	NMN (*n*=34)	PX (*n*=35)	NMN+PX (*n*=35)
Age (years)	19.7±1.4	19.3±1.3	19.8±1.5	19.7±1.0	19.9±1.8
Height (cm)	171.5±5.2	171.4±5.7	171.9±5.0	171.0±4.7	171.6±5.5
Body mass (kg)	56.9±4.6	57.5±5.4	56.9±4.1	56.3±3.7	56.9±5.1
Body mass index (kg/m^2)	19.4±1.2	19.5±1.1	19.3±1.2	19.3±1.5	19.3±1.1
Body fat (%)	13.6±2.6	13.7±2.3	13.5±2.7	13.9±2.9	13.4±2.7
Muscle mass (kg)	27.4±2.5	27.7±2.9	27.4±2.1	26.9±2.0	27.5±2.7
Blood NAD (μg/mL)	17.15±3.09	17.35±3.38	16.86±3.05	16.96±2.91	17.42±3.08
vLT (km/h)	15.72±1.77	15.81±1.69	15.46±1.86	15.79±1.83	15.83±1.76
vOBLA (km/h)	17.64±1.46 (*n*=125)	17.76±1.45 (*n*=32)	17.46±1.54 (*n*=32)	17.58±1.53 (*n*=30)	17.78±1.33 (*n*=31)
HR slope	6.38±1.05	6.63±1.04	6.15±1.03	6.38±1.00	6.36±1.14

574

575 Data are shown as means ± standard deviation.

576 *n* is the number of participants.

577 NMN, nicotinamide mononucleotide; PX, paprika xanthophyll; NAD, nicotinamide adenine dinucleotide;

578 vLT, running velocity at lactate threshold; vOBLA, running velocity at the onset of blood lactate accumulation; HR, heart rate

579

Table 3. Endurance performance

		Placebo (*n*=35)		NMN (*n*=34)	Difference (NMN-placebo)	PX (*n*=35)	Difference (PX-placebo)	NMN+PX (n=35)	Difference (NMN+PX-placebo)
	Week	*n*	Adjusted mean [95% CI]	*n*	Adjusted mean [95% CI]	Adjusted mean [95% CI] *P* value	*n*	Adjusted mean [95% CI]	Adjusted mean [95% CI] *P* value	*n*	Adjusted mean [95% CI]	Adjusted mean [95% CI] *P* value
vLT (km/h)	4	29	15.85 [15.49, 16.21]	27	16.01 [15.64, 16.38]	0.16 [−0.36, 0.67] *P*=0.544053	28	16.11 [15.75, 16.48]	0.26 [−0.24, 0.77] *P*=0.310003	29	16.43 [16.07, 16.78]	0.58 [0.07, 1.08] ***P*=0.025760 ***
	8	26	16.55 [16.17, 16.92]	29	16.64 [16.28, 17.00]	0.09 [−0.42, 0.61] *P*=0.723525	27	16.96 [16.59, 17.33]	0.42 [−0.11, 0.94] *P*=0.123459	27	16.52 [16.15, 16.89]	-0.03 [-0.56, 0.50] *P*=0.914330
vOBLA (km/h)	4	24	17.97 [17.68, 18.26]	21	17.94 [17.62, 18.26]	−0.03 [−0.45, 0.40] *P*=0.906445	19	18.01 [17.69, 18.34]	0.04 [−0.39, 0.48] *P*=0.841814	21	18.26 [17.95, 18.57]	0.29 [-0.13, 0.72] *P*=0.174447
	8	19	18.19 [17.86, 18.51]	23	18.37 [18.07, 18.67]	0.18 [−0.26, 0.63] *P*=0.416915	13	18.69 [18.29, 19.09]	0.50 [−0.01, 1.02] ***P*=0.054948**	18	18.43 [18.11, 18.76]	0.25 [-0.21, 0.71] *P*=0.295557
HR slope	4	31	6.00 [5.70, 6.30]	30	6.15 [5.85, 6.45]	0.15 [−0.27, 0.57] *P*=0.484615	29	6.05 [5.75, 6.36]	0.05 [−0.37, 0.47] *P*=0.815683	31	5.96 [5.66, 6.25]	-0.04 [-0.46, 0.37] *P*=0.834222
	8	29	6.24 [5.94, 6.55]	30	6.49 [6.19, 6.79]	0.25 [−0.18, 0.68] *P*=0.251546	30	6.33 [6.03, 6.63]	0.09 [−0.34, 0.52] *P*=0.681629	28	6.31 [6.00, 6.62]	0.07 [-0.36, 0.51] *P*=0.750814

Data are shown as adjusted means [95% CIs] unless otherwise indicated.

n is the number of 4- or 8-week–measurement participants.

For the adjusted means, a linear mixed effect model analysis was performed using group, measurement time, baseline value, and the interaction between the group and measurement time point as fixed effects; participants as random effects; and baseline values as covariates.

NMN, nicotinamide mononucleotide; PX, paprika xanthophyll; CI: confidence interval; vLT, running velocity at lactate threshold; vOBLA, running velocity at the onset of blood lactate accumulation; HR, heart rate

Table 4. Baseline characteristics of the participants (stratified analyses by blood NAD^+ levels before intake)

	Total (*n*=139)		Placebo (*n*=35)		NMN (*n*=34)		PX (*n*=35)		NMN+PX (*n*=35)	
NAD levels	low (*n*=66)	high (*n*=73)	low (*n*=17)	high (*n*=18)	low (*n*=15)	high (*n*=19)	low (*n*=19)	high (*n*=16)	low (*n*=15)	high (*n*=20)
Age (years)	19.6±1.4	19.8±1.5	19.1±1.2	19.6±1.4	19.5±0.9	19.9±1.9	19.5±1.1	19.9±1.0	20.2±2.1	19.8±1.4
Height (cm)	170.6±4.5	172.2±5.7	170.1±5.3	172.7±5.9	171.5±4.8	172.2±5.3	169.8±3.6	172.4±5.5	171.4±4.4	171.6±6.4
Body mass (kg)	56.3±4.4	57.5±4.7	56.4±5.7	58.4±5.0	56.1±3.0	57.5±4.8	55.7±4.1	57.0±3.2	56.9±4.7	56.9±5.5
Body mass index (kg/m^2)	19.3±1.2	19.4±1.3	19.5±1.2	19.6±1.0	19.1±1.1	19.4±1.3	19.3±1.4	19.2±1.7	19.3±1.2	19.3±1.1
Body fat (%)	13.2±2.5	14.1±2.7	13.4±2.7	14.1±1.8	12.5±2.3	14.2±2.8	13.6±2.4	14.4±3.4	13.1±2.8	13.6±2.6
Muscle mass (kg)	27.2±2.4	27.5±2.5	27.3±3.1	28.0±2.8	27.4±1.6	27.5±2.4	26.6±2.2	27.2±1.8	27.6±2.5	27.4±2.9
Blood NAD (μg/mL)	14.66±1.60	19.40±2.26*	14.55±1.56	19.99±2.33*	14.45±1.93	18.76±2.34*	14.81±1.52	19.51±1.88*	14.81±1.54	19.38±2.41*
vLT (km/h)	15.77±1.68	15.68±1.86	15.82±1.79	15.82±1.64	15.38±1.82	15.51±1.92	15.97±1.40	15.58±2.26	15.85±1.84	15.81±1.74
vOBLA (km/h)	17.63±1.28 (*n*=59)	17.65±1.61 (*n*=66)	17.74±1.43 (*n*=16)	17.77±1.52 (*n*=16)	17.24±1.35 (*n*=14)	17.63±1.71 (*n*=18)	17.82±1.02 (*n*=16)	17.32±1.97 (*n*=14)	17.69±1.34 (*n*=13)	17.83±1.36 (*n*=18)
HR slope	6.31±1.04	6.45±1.08	6.51±1.16	6.75±0.94	6.01±0.92	6.25±1.11	6.44±1.02	6.30±1.00	6.21±1.04	6.48±1.22

Data are shown as means ± standard deviation.

n is the number of participants.

NMN, nicotinamide mononucleotide; PX, paprika xanthophyll; NAD, nicotinamide adenine dinucleotide;

vLT, running velocity at lactate threshold; vOBLA, running velocity at the onset of blood lactate accumulation; HR, heart rate

*$p<0.05$ vs. the low segment of each group

Table 5. Results for the low segments of blood NAD^+ levels before intake

	Week	Placebo (*n*=17)		NMN (*n*=15)	Difference (NMN-placebo)	PX (*n*=19)	Difference (PX-placebo)	NMN+PX (*n*=15)	Difference (NMN+PX-placebo)
		n	Adjusted mean [95% CI]	*n*	Adjusted mean [95% CI]	Adjusted mean [95% CI] *P* value	*n*	Adjusted mean [95% CI]	Adjusted mean [95% CI] *P* value	*n*	Adjusted mean [95% CI]	Adjusted mean [95% CI] *P* value
vLT (km/h)	4	15	15.87 [15.34, 16.39]	13	16.36 [15.80, 16.92]	0.49 [−0.27, 1.26] *P*=0.209414	14	16.66 [16.12, 17.20]	0.79 [0.04, 1.54] ***P*=0.040069 ***	13	16.57 [16.01, 17.13]	0.70 [-0.06, 1.46] ***P*=0.074260**
	8	11	16.71 [16.10, 17.33]	12	16.87 [16.28, 17.45]	0.15 [−0.69, 1.00] *P*=0.720286	16	17.34 [16.83, 17.85]	0.63 [−0.17, 1.42] *P*=0.123128	10	16.97 [16.33, 17.62]	0.26 [-0.62, 1.14] *P*=0.560820
vOBLA (km/h)	4	13	17.94 [17.54, 18.35]	10	18.03 [17.56, 18.50]	0.09 [−0.52, 0.70] *P*=0.772338	9	18.46 [17.96, 18.96]	0.52 [−0.12, 1.15] *P*=0.111910	12	18.62 [18.20, 19.05]	0.68 [0.11, 1.26] ***P*=0.022338 ***
	8	7	18.39 [17.85, 18.92]	9	18.39 [17.90, 18.89]	0.01 [−0.71, 0.73] *P*=0.985706	7	19.25 [18.67, 19.82]	0.86 [0.09, 1.63] ***P*=0.031338 ***	5	19.19 [18.56, 19.82]	0.81 [-0.01, 1.62] ***P*=0.054423**
HR slope	4	17	5.82 [5.42, 6.22]	15	6.02 [5.60, 6.45]	0.20 [−0.38, 0.78] *P*=0.492009	15	5.94 [5.52, 6.37]	0.12 [−0.46, 0.70] *P*=0.680068	14	6.10 [5.66, 6.54]	0.28 [-0.31, 0.87] *P*=0.358586
	8	14	6.02 [5.58, 6.46]	13	6.41 [5.95, 6.87]	0.39 [−0.24, 1.02] *P*=0.222250	17	6.19 [5.79, 6.59]	0.17 [−0.42, 0.76] *P*=0.569904	10	5.99 [5.47, 6.51]	-0.03 [-0.70, 0.65] *P*=0.933168
Blood NAD (μg/mL)	4	17	15.66 [13.68, 17.64]	15	23.07 [20.96, 25.17]	7.41 [4.54, 10.28] ***P*<0.001 ***	15	15.79 [13.71, 17.87]	0.13 [−2.71, 2.98] *P*=0.926928	14	26.09 [23.92, 28.27]	10.44 [7.52, 13.36] ***P*<0.001 ***
	8	14	15.74 [13.58, 17.90]	13	23.28 [21.03, 25.52]	7.54 [4.45, 10.62] ***P*<0.001 ***	17	16.53 [14.56, 18.50]	0.79 [−2.11, 3.70] *P*=0.592650	10	21.48 [18.95, 24.00]	5.74 [2.44, 9.03] ***P*<0.001 ***

Data are shown as adjusted means [95% CI] unless otherwise indicated.

n is the number of 4- or 8-week–measurement participants.

For the adjusted means, a linear mixed effect model analysis was performed using group, measurement time, baseline value, and the interaction between the group and measurement time point as fixed effects; participants as random effects; and baseline values as covariates.

NMN, nicotinamide mononucleotide; PX, paprika xanthophyll; CI, confidence interval; vLT, running velocity at lactate threshold; vOBLA, running velocity at the onset of blood lactate accumulation; HR, heart rate

609 Table 6. Results for the high segments of blood NAD^+ levels before intake of the test foods

	Week	Placebo (*n*=18)		NMN (*n*=19)	Difference (NMN-placebo)	PX (*n*=16)	Difference (PX-placebo)	NMN+PX (*n*=20)	Difference (NMN+PX-placebo)
		n	Adjusted mean [95% CI]	*n*	Adjusted mean [95% CI]	Adjusted mean [95% CI] *P* value	*n*	Adjusted mean [95% CI]	Adjusted mean [95% CI] *P* value	*n*	Adjusted mean [95% CI]	Adjusted mean [95% CI] *P* value
vLT (km/h)	4	14	15.85 [15.36, 16.34]	14	15.67 [15.18, 16.17]	−0.18 [−0.87, 0.52] *P*=0.620060	14	15.58 [15.09, 16.07]	−0.27 [−0.96, 0.42] *P*=0.450451	16	16.30 [15.84, 16.76]	0.45 [−0.22, 1.12] *P*=0.188432
	8	15	16.39 [15.91, 16.86]	17	16.47 [16.02, 16.91]	0.08 [−0.57, 0.73] *P*=0.810981	11	16.50 [15.95, 17.06]	0.11 [−0.61, 0.84] *P*=0.759657	17	16.22 [15.78, 16.67]	−0.16 [−0.81, 0.48] *P*=0.619999
vOBLA (km/h)	4	11	17.99 [17.61, 18.38]	11	17.87 [17.46, 18.28]	−0.12 [−0.68, 0.43] *P*=0.665750	10	17.65 [17.24, 18.06]	−0.34 [−0.90, 0.22] *P*=0.231955	9	17.82 [17.39, 18.25]	−0.17 [−0.75, 0.40] *P*=0.553939
	8	12	18.07 [17.68, 18.46]	14	18.37 [18.01, 18.73]	0.30 [−0.23, 0.82] *P*=0.266629	6	18.13 [17.60, 18.65]	0.06 [−0.59, 0.70] *P*=0.860846	13	18.14 [17.78, 18.50]	0.07 [−0.45, 0.59] *P*=0.793784
HR slope	4	14	6.20 [5.74, 6.65]	15	6.27 [5.83, 6.71]	0.07 [−0.56, 0.70] *P*=0.828835	14	6.17 [5.71, 6.62]	−0.03 [−0.67, 0.60] *P*=0.917089	17	5.85 [5.44, 6.26]	−0.35 [−0.96, 0.26] *P*=0.263342
	8	15	6.46 [6.02, 6.90]	17	6.56 [6.15, 6.97]	0.10 [−0.50, 0.70] *P*=0.740601	13	6.49 [6.02, 6.96]	0.03 [−0.61, 0.67] *P*=0.925438	18	6.52 [6.12, 6.92]	0.06 [−0.53, 0.65] *P*=0.846711
Blood NAD (μg/mL)	4	14	16.82 [13.87, 19.77]	15	28.99 [26.14, 31.84]	12.17 [8.08, 16.26] ***P*<0.001 ***	14	19.00 [16.04, 21.96]	2.18 [−1.96, 6.33] *P*=0.303493	17	29.28 [26.60, 31.96]	12.46 [8.50, 16.41] ***P*<0.001 ***
	8	15	19.25 [16.38, 22.11]	17	25.52 [22.82, 28.23]	6.28 [2.36, 10.20] ***P*<0.002032 ***	13	18.68 [15.62, 21.74]	−0.56 [−4.72, 3.59] *P*=0.790263	18	28.47 [25.85, 31.08]	9.22 [5.36, 13.08] ***P*<0.001 ***

610

611 Data are shown as adjusted means [95% CIs] unless otherwise indicated.

612 *n* is the number of 4- or 8-week–measurement participants.

For the adjusted means, a linear mixed effect model analysis was performed using group, measurement time, baseline value, and the interaction between the group and measurement time point as fixed effects; participants as random effects; and baseline values as covariates.

NMN, nicotinamide mononucleotide; PX, paprika xanthophyll; CI, confidence interval; vLT, running velocity at lactate threshold; vOBLA, running velocity at the onset of blood lactate accumulation; HR, heart rate

Table 7. Sleep-quality questionnaire

	Period	Placebo (*n*=35)		NMN (*n*=34)		PX (*n*=35)		NMN+PX (*n*=35)	
		n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
Sleep quality	Before-intake period	35	0.87±0.43	34	0.95±0.54	35	0.91±0.55	35	0.90±0.58
	0–4-week period	35	0.88±0.52	34	0.94±0.58	35	0.88±0.51	35	0.79±0.60**
	4–8-week period	28	0.86±0.50	29	0.88±0.59	28	0.82±0.45	28	0.59±0.58#*

n is the number of participants at each period.

*p<0.05 vs. the before-intake period, **p<0.01 vs. the before-intake period, #p<0.05 vs. placebo

NMN, nicotinamide mononucleotide; PX, paprika xanthophyll; SD, standard deviation

Supplemental Table 1. Running distance for each period

	Period	Placebo (*n*=35)		NMN (*n*=34)		PX (*n*=35)		NMN+PX (*n*=35)	
		n	Mean	*n*	Mean	*n*	Mean	*n*	Mean
Running distance (km/day)	Before-intake period	35	15.5	34	13.9	35	15.3	35	15.6
	0–4-week period	35	16.2	34	14.4	35	15.3	35	14.8
	4–8-week period	28	18.3	29	16.0	28	17.2	28	17.5

n is the number of participants in each period.

NMN, nicotinamide mononucleotide; PX, paprika xanthophyll