Plasma free metanephrine and normetanephrine levels correlated to plasma catecholamine after acute running in amateur runner

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A B S T R A C T

Background: Catecholamine is a typical index of exercise intensity, but it is difficult to detect. Plasma metanephrine (MN) and normetanephrine (NMN) levels are more stable than those of catecholamines. This study aimed to investigate plasma MN and NMN levels during acute exercise running in amateur runners.

Methods: Samples were collected from eight healthy male participants. They were either sedentary or running at low or high intensity for 30 min. Blood samples were collected under these conditions. Measurements taken included plasma adrenaline, noradrenaline, MN, and NMN.

Results: Plasma adrenaline levels increased after high-intensity exercise compared with sedentary subjects. Plasma noradrenaline, MN, and NMN levels increased after both low- and high-intensity exercise compared with sedentary subjects. In addition, these levels were also significantly higher at high intensity than at low intensity. Plasma adrenaline and noradrenaline levels were positively correlated with plasma free MN and NMN levels after acute running, respectively.

Conclusion: This study revealed that plasma MN and NMN levels transiently increased depending on exercise intensity in amateur runners. In addition, plasma NMN levels are better markers than plasma MN levels because of their stronger correlation with plasma catecholamine levels.

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Introduction

Running has a significant positive effect on the body by improving cardiorespiratory function and by preventing and treating various diseases.1 As a result, and the number of people involved in running has increased worldwide. However, strenuous endurance exercises can cause immune system disorders and damage various tissues including muscles, as a result of metabolic and mechanical stress.2,3 Therefore, there is a need for biomarkers that can identify body conditions during and/or after endurance exercises. Moreover, biomarkers that can be measured via minimally invasive methods for continuous monitoring of internal conditions are important to prevent harmful effects.

To date, available biomarkers including lactate, free fatty acid, and glucose, have been investigated for various exercises.4,5 Among them, catecholamine is a typical index of exercise intensity.6 Catecholamine, including adrenaline and noradrenaline, regulates heart rate, blood pressure, and glycolysis.7 It is well recognized that adrenaline and noradrenaline increase with increasing exercise intensities.6 Epinephrine is almost completely synthesized and released from the adrenal medulla in response to adrenocorticotrophic hormone of the sympathetic nervous system, while norepinephrine synthesis occurs primarily within the ends of sympathetic nerve fibers and in the adrenal glands to a lesser extent. It occurs within the chromaffin cells of the medulla.8 Catechol-O-methyltransferase (COMT), an enzyme that metabolizes catecholamines,
occurs within the chromaffin cells of the medulla. COMT is abundantly present in the cytoplasm of liver and renal cells, changing blood adrenaline and noradrenaline to metanephrine (MN) and normethanephrine (NMN), which are inactivated by this enzyme. Subsequently, MN and NMN in the blood are rapidly sulfated and excreted from the kidney by phenol sulfotransferases (PST), which mainly distribute the hydroxyl group at the 4-position in the living body. As a result, free metanephrine is the form of the substance mainly distribute the hydroxyl group at the 4-position in the living body. However, it is unclear whether these evaluations considered exercise intensity. The purpose of this study was to investigate plasma MN and NMN levels depending on exercise intensity.

Patients and methods subjects

Subjects

Eight healthy male subjects who underwent aerobic exercises at least twice per week were recruited. Subject characteristics are provided in Table 1. The subjects were instructed not to drink alcohol, ensure sufficient amounts of sleep, and avoid binge eating before the experiment. This study was approved by the Ethical Committee of the Faculty of Medicine at the University of Tsukuba (approval number: 274). All subjects received an explanation and written documentation in advance regarding the purpose of the experiment, its contents, and safety issues, and provided informed consent.

Experimental design

The experimental period is shown in Fig. 1. To set the exercise intensity level, the subjects performed exercises on a treadmill until exhaustion. In this study, subjects’ $V\text{(·)O}_2$ was maximal because the respiratory expenditure rates were greater than 1.0. The maximal perceived exertion and observation plateau of $V\text{(·)O}_2$ was assessed using an incremental test (data not shown), as described by Tokinoya et al. (2020). Subjects performed an incremental test in advance to calculate the ventilatory threshold (VT) intensity in each subject. VT was determined by an exercise physiologist. They were assessed under three conditions: sedentary, low-intensity (85% VT), or high-intensity exercise (115% VT). All conditions were performed within a period of at least 2 days. The experimental protocol is described as follows: The subjects were allowed to warm up for 30 min and then drank water (500 mL). After warming up, they ran for 30 min at VT speeds of 85% or 115%.

Plasma catecholamine, metanephrine (MN) and normethanephrine (NMN) levels

Plasma catecholamine levels were measured using high-performance liquid chromatography (HPLC). Plasma MN and NMN levels were measured using liquid chromatography mass spectrometry (LC/MS/MS) method. These analyzes were outsourced to Tsukuba i-Laboratory LLP (Tsukuba, Japan).

Statistical analysis

All results are presented as the mean ± standard deviation. GraphPad Prism 7 software (GraphPad, Inc., La Jolla, CA, USA). All data were analyzed using one-way analysis of variance (ANOVA) or t-test. All results were analyzed using the Bonferroni multiple comparison method after a one-way ANOVA analysis. Correlation analysis was conducted using the Pearson product-moment correlation coefficient method. The significance level was set at $P < 0.05$.

Results

Acute running for 30 min

The sedentary and exercise conditions were completed at a temperature of $14.9 ± 1.4°C$ and $6.4 ± 1.5°C$, with a humidity level of $50.3 ± 5.9%$ and $72.0 ± 17.6%$, using the WBGT measurement (KYOTO ELECTRONICS MANUFACTURING CO., LTD., Japan). The HR reserve (HRR) was calculated from the results of each exercise condition and all tests. HRR of low-intensity exercises was lower than that of high-intensity exercises (72.5 ± 9.3 vs. 88.6 ± 9.1, $P < 0.01$). The HRR during low-intensity exercise was lower than that during high-intensity exercise. Therefore, there was a significant difference between exercise intensities.

Table 1

Participants’ characteristics in this study.

|         | Age (year) | Height (cm) | Weight (kg) | Body fat (%) | BMI | V(·)O₂max (ml/kg⁻¹·min) |
|---------|------------|-------------|-------------|--------------|-----|-----------------------|
| AVG     | 21.6       | 173.0       | 61.8        | 9.9          | 20.6| 64.3                  |
| SD      | 1.5        | 5.9         | 7.1         | 3.0          | 3.0 | 8.1                   |
Plasma catecholamine, metanephrine (MN) and normethanephrine (NMN) levels

Plasma catecholamine, metanephrine (MN), and normethanephrine (NMN) levels are shown in Fig. 2. Plasma adrenaline levels only increased after high-intensity exercise compared with sedentary exercise ($p < 0.01$). Plasma noradrenaline, MN, and NMN levels increased after both low- and high-intensity exercises compared with sedentary subjects ($p < 0.05$, and $p < 0.01$, respectively). In addition, the subjects engaged in high-intensity exercises also showed significantly higher levels than those who engaged in low-intensity exercises ($p < 0.05$, and $p < 0.01$, respectively). Moreover, there were many significant correlations between plasma adrenaline and free MN levels after acute running ($p < 0.01$).

Fig. 3. In addition, plasma noradrenaline levels were significantly correlated with plasma free NMN levels ($p < 0.01$).

Discussion

This study investigated the plasma MN and NMN levels under high and low exercise intensities in amateur runners. It is important for us to research various biomarkers of exercise intensity because exercise intensities are different from effects on health benefits in previous studies. Our findings showed that plasma MN and NMN levels were increased by acute running for 30 min, depending on the exercise intensity. In addition, plasma adrenaline and noradrenaline levels were positively correlated with plasma MN and NMN levels, respectively.
Plasma catecholamines, involving adrenaline and noradrenaline, have been shown to decrease after exercise training. In other words, the baseline plasma catecholamine level in trained individuals tended to be lower than that in untrained individuals. Plasma catecholamine levels increased after 30 minutes of acute running. In this study, the target subjects were those who were regularly trained in aerobic exercises. In previous studies, plasma adrenaline and noradrenaline reportedly increased depending on exercise intensities. Therefore, these levels were significantly correlated with plasma MN and NMN in this study. In a previous study, blood MN and NMN levels were the most sensitive markers among the other markers, including blood and urine catecholamines and urine MN and NMN, in the diagnosis of pheochromocytoma. Moreover, plasma MN and noradrenaline levels were more correlated than plasma MN and adrenaline levels. Danese et al. (2018) reported that plasma NM levels were positively related to running performance. In addition, they found that the plasma NMN level was increased by acute cycling under various conditions. Therefore, it is possible that NMN level is an index of exercise intensity under various conditions in addition to exercise performance.

In conclusion, plasma MN and NMN levels transiently increased after acute running in amateur runners. In addition, plasma catecholamine levels also increased depending on the exercise intensity. It is possible that plasma MNM level might be a better biomarker than plasma MN level because of its stronger correlation with plasma catecholamine levels in this study. In future studies, it will be necessary to investigate the effects of sex, age, and style of exercise on plasma MN and NMN levels to further investigate their suitability as exercise biomarkers.

Authorships

K Tokinoya, YS, YA and K Takekoshi contributed to the design. K Tokinoya, YS, AA, YY, TS, YN, and K Takekoshi performed data acquisition. K Tokinoya, YS, TS, YN, and K Takekoshi analyzed and interpreted the data. K Tokinoya and K Takekoshi performed the statistical expertise. K Tokinoya drafted the manuscript. All authors supervised and edited the manuscript draft. All authors reviewed the manuscript. All authors provided final approval of this version of the manuscript for publication and agreed to be accountable for all aspects of the work.

Declaration of competing interest

There are no conflicts of interest to disclose.

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