Note

Consideration of Diurnal Variations in Human Blood NAD and NADP Concentrations

Tsutomu FUKUWATARI and Katsumi SHIBATA

Department of Food Science and Nutrition, School of Human Cultures, The University of Shiga Prefecture,
2500 Hassaka, Hikone, Shiga 522–8533, Japan

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Summary The sum of the urinary excretion of nicotinamide and its catabolites, which are metabolites of NAD and NADP, were observed to have clear diurnal variations in human urine. Then, we examined whether NAD and NADP in blood also showed the diurnal variation. All subjects were housed in the same facility and given the same diet during the experiment. In addition, we examined whether diurnal variations were affected by the intakes of dietary nicotinamide or not. As a result, neither the NAD nor the NADP content of the blood shows the diurnal variation regardless of the administered amount of nicotinamide. The concentrations of NAD and NADP did not increase according to the intake of nicotinamide. The existence of a mechanism by which NAD and the NADP levels of the blood are constantly maintained by the adjustment of the amount of excretion to the urinary bladder, was suggested.

Key Words diurnal variation, NAD, blood, human, vitamin

We reported a diurnal variation in the NAD and NADP catabolites *N*¹-methylnicotinamide, *N*¹-methyl-2-pyridone-5-carboxamide, and *N*¹-methyl-4-pyridone-3-carboxamide was observed (1) and an elevation of blood NAD level after moderate exercise (2). In the present study, we examined whether NAD and NADP in blood also showed the diurnal variation. In addition, we examined whether diurnal variations were affected by the intakes of dietary nicotinamide or not.

Ten healthy male Japanese college students participated in the present experiment. They did not have regular use of medications or dietary supplements, or habitual alcohol or cigarette consumption. Their age, body weight, height and body mass index (mean±SD) were 22.1±2.3 y old, 63.6±5.2 kg, 174.0±4.6 cm, and 21.0±1.6 kg/m², respectively. This study was reviewed and approved by The University of Shiga Prefecture.

All subjects (*n*=10) were housed in the same facility and given the same diet. The experimental period was 4 wk. The diet consisted of bread, margarine, ham, yoghurt, tomato, lettuce, and milk as breakfast; rice, toasted and seasoned laver, luncheon meat, boiled egg, raw cabbage, *miso*-soup, and Japanese tea as lunch; rice, soy sauce-seasonal Pacific saury, *tofu* (soybean curd), spinach (leaves, boiled), kiwifruit and Japanese tea as dinner; and cheese and jelly fruit mix as a mid-night snack. The nutrient elements are shown in Table 1. Nutrients were calculated by using Standard Tables of Food Composition in Japan, Fifth revised and enlarged edition (3).

The subjects took the diet on days 1 to 5 of each week (experimental period, 4 wk). But, they lived freely to soften the restraint on days 6 and 7 of each week. Approximately 1-, 3- and 6-fold of the synthesized water-soluble vitamin mixture as vitamin mixture A, B and C shown in Dietary Reference Intakes for Japanese, 2005, were made (Table 2). They were given the diet only for the 1st week, the diet with vitamin mixture A for the 2nd week, the diet with vitamin mixture B for the 3rd week, and the diet with vitamin mixture C for the 4th week. One third of the dose was put into a small gelatinous capsule, and the capsule was administered three times daily after breakfast, lunch and dinner. A 10-μL of finger blood was periodically taken at 08:00, 12:00, 18:00, and 21:00 on day 5 of each week and 32:00 which means 08:00 on day 6. The blood (10-μL) was immediately put into a micro tube containing 200-μL of cold 50 mM potassium phosphate buffer (pH 6.0) containing 100 mM nicotinamide and the mixture was immersed in a hot water bath at 90°C for exactly 1.5 min, cooled on ice, and then centrifuged using an Eppendorf (15,000 rpm, 10 min, 4°C). The supernatant was stored at -20°C until needed. The treatment was done within 1 h after the blood collection. The NAD (NAD⁺+NADH) and NADP (NADP⁺+NADPH) were measured by the methods of Shibata and Murata (4) and Shibata and Tanaka (5), respectively.

Diurnal variations in the blood NAD and NADP were determined by two-way repeated measures analysis of variance with Bonferroni's post hoc test for multiple comparisons. STATVIEW software (version 5.0; Abacus Concepts, Berkeley, CA) was used for all analysis.

Figure 1 shows the diurnal variations of blood NAD

E-mail; fukkie@shc.usp.ac.jp

Table 1. The composition of the diet.

Energy and nutrients	Amount
Energy (kcal)	2,648
Protein (g)	97.5
Fat (g)	86.7
Carbohydrates (g)	361
Water-soluble vitamins ¹	
Vitamin B ₁ (mg as thiamin)	1.12
Vitamin B ₂ (mg as riboflavin)	1.72
Vitamin B ₆ (mg as pyridoxine)	1.12
Vitamin B ₁₂ (μ g as cyanocobalamin)	13.3
Niacin equivalent ² (mg)	28.8
Pantothenic acid (mg)	6.75
Folates (μ g as pteroylmonoglutamic acid)	367
Biotin (μ g)	—
Vitamin C (mg as L-ascorbic acid)	125

¹ Nutrients are calculated by using the Standard Tables of Food Composition in Japan (3).

² The niacin equivalent intake was calculated as follows: the average tryptophan content in food protein is 1.1% and 1/60 (on a weight basis) of tryptophan taken was converted into niacin in the body.

$97.5 \times 1,000 \times (1.1/100) \times (1/60) = 17.9$ mg.

17.9 (biosynthesized from tryptophan) + 10.8 (niacin from food) = 28.8.

Table 2. The vitamin contents in the vitamin mixtures for 3 capsules per day.

	V. mix. A	V. mix. B	V. mix. C
Thiamin	1.4 mg/d	4.2 mg/d	8.4 mg/d
Riboflavin	1.6 mg/d	4.8 mg/d	9.6 mg/d
Pyridoxine	1.4 mg/d	4.2 mg/d	8.4 mg/d
Cyanocobalamin	2.4 μ g/d	7.2 μ g/d	14.4 μ g/d
Nicotinamide	15 mg/d	45 mg/d	90 mg/d
Pantothenic acid	6 mg/d	18 mg/d	36 mg/d
Pteroylmonoglutamic acid	240 μ g/d	720 μ g/d	1,440 μ g/d
Biotin	50 μ g/d	150 μ g/d	300 μ g/d
Ascorbic acid	100 mg/d	300 mg/d	600 mg/d

and NADP. Neither the NAD nor the NADP content of the blood shows the diurnal variation regardless of the administered amount of nicotinamide. The blood concentrations of NAD in the 1st, 2nd, 3rd, and 4th week were 29.9 ± 4.2 (mean $\pm$ SD for 50 samples), 27.7 ± 4.1 , 31.4 ± 4.1 , and 30.0 ± 4.9 nmol/mL, respectively. The blood concentrations of NADP in the 1st, 2nd, 3rd, and 4th week were 10.0 ± 2.1 (mean $\pm$ SD for 50 samples), 9.6 ± 1.4 , 10.3 ± 1.7 , and 10.4 ± 1.2 nmol/mL, respectively. The concentrations of NAD and NADP did not increase according to the intake of nicotinamide and diurnal variations were not observed. So, we calculated the mean $\pm$ SD by using whole values ($n=200$). The blood NAD content was 29.7 ± 4.6 nmol/mL, and the blood NADP content was 10.1 ± 1.6 nmol/mL. These values were almost the same as reported previously (2). Recently, Creeke et al. (6) reported that blood NAD and NADP concentrations were not depressed in subjects

Fig. 1. The alterations in time of day of the blood NAD (A) and NADP (B) concentrations. ●, 1st-week (basal diet); ○, 2nd-week (basal diet + V. mix. A); ■, 3rd-week (basal diet + V. mix. B); □, 4th-week (basal diet + V. mix. C). Values are means $\pm$ SD for 10 subjects.

with clinical pellagra. Their reported values in pellagrins were 29.7 nmol/mL in NAD, and 26.1 nmol/mL in NADP vs in healthy subjects 24.3 nmol/mL in NAD and 19.6 nmol/mL in NADP (6). They also reported that pellagrins were cured by administering 100 mg of nicotinamide, but increases in the levels of NAD and NADP were not observed (6). On the other hand, Fukuwatari et al. (2) revealed that exercise affects the blood NAD and NADP levels in humans and mice. In that case, to deal with acute energy expenditure, the salvage pathway of NAD would be accelerated by decreasing the catabolism of NAD. Generally, the blood concentrations of NAD and NADP are regulated by the reaction of nicotinamide phosphoribosyltransferase which is inhibited by NAD (7). We think that this is the main reason why the blood concentrations of NAD and NADP did not increase even when the intake of nicotinamide increased. The existence of a mechanism where by NAD and NADP levels of the blood were maintained constantly without diurnal variation, by the adjustment of the amount of the urinary excretion, was suggested.

Erythrocyte produces ATP by glycolysis. The TCA cycle doesn't exist. Therefore, we think that maintaining the concentration of NAD is equivalent to maintaining the ATP production ability constantly. On the other hand, NADP is an end product of the antioxidation system of the erythrocyte membrane. Therefore, we think that it has the mechanism maintained constantly.

We permitted that the urinary excretion of nicotinamide and its catabolites was not permitted until the blood NAD was saturated, and that the urinary excretion of nicotinamide catabolites increased for the first time after the blood NAD was saturated (8). Furthermore, we reported that the blood NAD did not change,

though the urinary excretion of nicotinamide and its catabolites decreased when rats were fasted (9). Therefore, urine is a better sample than blood in order to evaluate nutritional status.

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