Effects of Stress on the Urinary Excretory Pattern of Niacin Catabolites, the Most Reliable Index of Niacin Status, in Humans

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Summary Urinary output of water-soluble vitamins has been used as an indices for vitamin nutrition. It has been pointed out that the coefficient variance of these values is high, especially for niacin catabolites. Thus, we investigated what kinds of stress affect the catabolism using female subjects. The effects of cold exposure (as a typical physical stress), calculation exercise (a typical mental stress) and dark exposure (a typical emotional stress) on the metabolism of niacin were investigated. Of the stresses, cold exposure significantly increased urinary excretory output of the niacin metabolites. The biosynthesis of nicotinamide from Trp seemed to be increased by cold exposure.

Key Words niacin catabolism, stress, cold exposure, calculation exercise, dark exposure

The urinary output of water-soluble vitamins has been used as an indices for the vitamin nutrition (1). It has been pointed out that this sign has a problem that the coefficient of variance is very high, especially for niacin catabolites. MNA, 2-Py and 4-Py (2). This variance might be the result of various stresses. We investigated the effects of typical stresses such as cold exposure (a typical physical stress) (3), calculation exercises (a typical mental stress) (3) and dark exposure (a typical emotional stress) (3) on the metabolism of Nam.

The average weight of 12 females 20.9±2.1 y-old (mean±SD) and 162.6±4.5 cm tall was 54.67±5.43 kg at the beginning of the experiment, and 54.49±4.89 kg at the end of the experiment. This study was reviewed and approved by the Ethical Committee of the National Institute of Health and Nutrition.

All females were housed in the same facility for 12 d (July 24 to August 4, 1999). The daily schedule during the experiment was as follows: Sleeping time, from the time a subject lays down on their bed until the time the subject gets off the bed; the lights were turned off at 22:00 and turned on at 06:00. We did not check when the subjects indeed slept and awakened. The first 4 d were environmental adaptation. Days 5, 6, 7, 9, 10 and 11 were experimental days. Day 8 was an intermission day. Four daily menus were devised and fed in rotation. Niacin, Trp and energy intake were calculated based on the Table of Food Composition in Japan (4). The niacin intake from Trp was calculated by assuming that 1 mg niacin is converted from 60 mg Trp (5). The sum of the amount of niacin in the diet and the niacin converted from dietary Trp is the niacin equivalent (NE) intake. Energy, niacin and NE of the experimental diet were 7,903±512 kJ, 13.3±1.07 mg and 25.0±1.01 mg NE, respectively.

Urine samples were collected and pooled during five specific periods (06:30 to 08:30, 08:30 to 13:00, 13:00 to 18:30, 18:30 to 21:30 and 21:30 to 06:30 am of the next day). Though subjects were allowed to urinate whenever they wished, they had an obligation to urinate at just before 06:30, 08:30, 13:00, 18:30 and 21:30. The volume of each urine sample was measured, collected in a bottle containing 1 mL of 1 mol/L HCl and then stored at −25°C until needed.

A complete cross-over design in the sequence of stresses was used to avoid the influence of diet with that of stress.

Cold exposure is a typical physical stress (3). The area of the cold room was 5.4×4.5×2.4 m (W×D×H) and a temperature of 2 to 6°C was maintained. The subjects for cold exposure wore ski suits, including ski gloves, wool caps and double socks, over their normal winter clothes. The application of stress was carried out from 09:30 to 12:30 and from 14:00 to 17:00. The subjects sat on chairs in the cold room, read books and played crossword games. The control subjects wore self-selected clothes, sat on chairs in front of the cold room, read books and played crossword games, and monitored the conditions of the subjects in the cold room.

Calculation exercise is a typical mental stress (3). The area of the calculation room was 7.3×7.3×2.2 m (W×D×H). The room temperature was maintained at around 25°C. Subjects for treatment and non-treat-
ment wore self-selected clothes and sat on chairs in the room. The problems for the calculation exercise were of the third-grade elementary school level in Japan. For example, they calculated addition (double figures added to double figures), subtraction (double figures subtracted from three figures), multiplication (double figures multiplied with single figures) and division (four figures divided by a single figure). Calculation exercises were carried out from 09:30 to 12:30 and from 14:00 to 17:00. The control subjects checked every page to determine whether or not the answers provided were correct. When the answer was not correct, the mistakes were indicated and the pages were immediately corrected by the calculation subjects.

Dark exposure is a typical emotional stress (3). Dark exposure was carried out from 09:30 to 12:30 and from 14:00 to 17:00. The area of the darkroom and room temperature were exactly the same as that of the calculation room. The subjects for the dark exposure groups wore self-selected clothes and sat on stools in the room. The subjects for dark exposure wore eye masks and were restricted in movement and prohibited from talking to each other. The control subjects sat on chairs and read books and monitored whether or not the stress subjects were sleeping.

Urinary MNA was measured by the HPLC method of Shibata (6). The 2-Py and 4-Py contents in urine were simultaneously measured by the HPLC method of Shibata et al. (7). Urinary creatinine was measured by the HPLC method of Shibata and Matsuo (8).

Cold exposure affected the niacin metabolism as shown in Fig. 1. During the day of cold exposure, MNA outputs were higher in the urine samples collected from 13:00 to 18:30, from 18:30 to 21:30 and from 21:30 to 06:30 am the next day than during the respective control day. Outputs of 2-Py, 4-Py and the sum of specified measurements in the urine samples collected from 08:30 to 13:00 and 18:30 to 21:30 were significantly higher during the cold exposure day. The ratio of (2-Py + 4-Py)/MNA (Fig. 1E) was not affected by cold exposure.

The calculation exercise (Fig. 2A) and dark exposure (anxiety stress, Fig. 2B, in contrast to cold exposure, had no significant effect on the Nam metabolism. Cold exposure significantly increased the urinary output of Nam metabolites, MNA, 2-Py, 4-Py and the sum of specified measurements during the periods of exposure (Fig. 1). These findings suggest that cold exposure caused an increase in the metabolic rate and energy expenditure for thermogenesis, and that as a result, the turnover ratio of the NAD cycle was immediately activated. High urinary output of MNA and 2-Py

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**Fig. 1.** Effect of cold exposure on the urinary excretion of Nam metabolites, MNA (A), 2-Py (B), 4-Py (C), sum of specified measurements (D), and the excretory ratio of (2-Py+4-Py)/MNA (E). Each urine sample was collected into a bottle containing 1 mL of 1 mol/L HCl from 06:30–08:30, 08:30–13:00, 13:00–18:30, 18:30–21:30, 21:30–06:30 the next day, 06:30–08:30 the next day, and then stored at −25°C until needed. The cold control group data obtained from day 5 of group 3, day 6 of group 2, day 7 of group 1, day 9 of group 5, day 10 of group 4 and day 11 of group 6 were combined. The cold exposure data obtained from day 5 of group 5, day 6 of group 4, day 7 of group 6, day 9 of group 3, day 10 of group 2 and day 11 of group 1 were combined. Each point is mean ± SD for 12 urine samples. ○, cold-control; ●, cold-exposure. An asterisk (*) means significantly different at p<0.05 in a comparison of the same time as determined by paired t-test in the two-tailed p value.
also occurs in the instance of burned or scalded patients (9), indicating a high need for NAD in skin repair. Stress may also be a factor.

Calculation exercise had no significant effect on the metabolism of niacin (Fig. 2A). However, in patients with primary affective disorders (10) and autism (11), disturbances of Nam metabolism were reported.

In summary, the Nam catabolism was affected by only the physical stress of cold exposure. Cold exposure raised energy expenditure (12), resulting in activated biosynthesis of Nam from Trp and increased Nam metabolism.

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REFERENCES