Effect of Season and Exercise on Dermal Nitrogen Losses and Their Relation to Urinary Nitrogen Excretion

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Summary The present study was designed to estimate dermal nitrogen losses in summer and winter under the conditions of minimal daily activities, on a diet of standard Japanese protein intake level and to determine whether the increased dermal nitrogen losses induced by hot climate or exercise were compensated for by the decrease in urinary nitrogen excretion. Six healthy male university students served as the subjects. The daily dermal nitrogen losses (mean ± SD) were 0.22 ± 0.07 g or 3.10 ± 0.58 mg/kg in winter and 0.44 ± 0.19 g or 6.35 ± 2.46 mg/kg in summer, showing significantly higher dermal nitrogen losses in summer than in winter. On the contrary, urinary nitrogen excretion tended to be larger in winter than in summer. Thus, renal compensation seemed to exist for the seasonal changes in dermal nitrogen losses. In the summer experiment, the subjects took light exercise besides the minimal daily activities for a 2-day exercise period. The pooled mean of daily dermal nitrogen losses during the exercise period was significantly larger than that during the sedentary period, while the urinary nitrogen excretion was almost the same in the two periods. No compensatory reduction in the urinary nitrogen excretion during the exercise period was observed under the conditions of the present study.

Key Words dermal nitrogen loss, urinary nitrogen excretion, nitrogen balance, season, exercise

One of the physiological approaches for estimating nitrogen requirement is the nitrogen balance method, which is based on measurements of the minimal nitrogen intake needed to maintain nitrogen equilibrium. In general, dermal nitrogen losses have been ignored in most nitrogen balance studies, because of their small values...
compared with urinary nitrogen. However, Cuthbertson and Guthrie (1), Mitchell and Hamilton (2) and Consolazio et al. (3, 4) reported fairly high dermal nitrogen losses, especially under profuse sweating conditions, such as high environmental temperature and/or heavy physical exercise. Mitchell and Hamilton (2) proposed that dermal nitrogen losses had to be included as part of the total daily output in nitrogen balance studies. Mitchell and Edman (5) and Consolazio et al. (3) stressed that protein requirements should be increased under the conditions that produced profuse sweating, because the increased dermal nitrogen losses were not compensated for by a decrease in urinary nitrogen excretion even after acclimatization. On the other hand, Bost and Borgstrom (6) and Huang et al. (7, 8) suggested the presence of renal compensation for changes in dermal nitrogen losses.

The present study was designed to determine the extent of dermal nitrogen losses in summer and winter under the dietary conditions of standard Japanese protein intake levels and to determine whether the increased dermal nitrogen losses induced by hot climate or exercise were compensated for by the decrease in urinary nitrogen excretion.

MATERIALS AND METHODS

Six healthy male university students well-trained in physical exercise served as the subjects. Their physical characteristics are shown in Table 1. The subjects were given an experimental diet in both summer (August) and winter (January) throughout the experimental periods, which consisted of 8 days of adjustment period and 4 days of sedentary period. The subjects were engaged in their minimal daily activities during the sedentary period. In the summer experiment the sedentary period was followed by an additional 2 days of exercise, in which the subjects exercised on a bicycle ergometer for 20 min at the rate of 800 kpm/min twice a dy. Energy expenditure for the exercise was 75 kcal/day.

 Constituents of the experimental diet were well-milled rice, dried whole egg, skim milk, soda cracker, processed cheese, katsuobushi (frigate mackerel, dried strip), miso, kōri-dōfu (freeze-dried soybean curd), wheat flour, dehydrated mashed potatoes, onions, Japanese radish, carrot, cabbage, cucumber, bananas, soy sauce, takuan-zuke (pickled radish), corn starch, sucrose, margarine and NaCl. Nitrogen contents of all the foods were determined by the macro Kjeldahl method. The daily nitrogen intake is shown in Table 5. The average daily intake of protein (N × 6.25) per kg of body weight (mean ± SD) was 1.21 ± 0.04 g with the range of 1.14–1.26 g. Energy intake calculated from Standard Tables of Food Composition in Japan (9) was approximately 42–44 kcal/kg/day. The composition of the experimental diet, daily nitrogen and energy intake of each subject was the same in both summer and winter experiments. No extra energy was added during the exercise period.

 Daily urinary excretion of nitrogen and creatinine were determined for 4 days of the sedentary period in both summer and winter experiments and also for 2 days of the exercise period in the summer experiment.

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Dermal nitrogen losses were measured every day during both sedentary and exercise periods in the summer experiment, however, the collected samples were pooled for 4 days of the sedentary period, and mixed and analyzed for nitrogen content in the winter experiment.

The following method was used for the collection of sweat and desquamated cells to analyze the nitrogen content. The subject bathed body and scalp thoroughly, rinsed himself in deionized water and towel-dried, and then put on cotton underwear consisting of a long-sleeved vest and long johns, and cotton socks. He was given towels for wiping away sweat and for drying the face and hands. He put on a cotton hat and cotton gloves during exercise in the summer experiment. The underwear and socks were changed after exercise according to the request of the subject. All these clothes, including sheets, had been washed and soaked in 0.1% acetic acid solution for 48 h, and then rinsed in deionized water and dried prior to use. After a 24-h period, the entire body of the subject was well scrubbed with a washrag and warm deionized water was poured over his head repeatedly. The collected water was made up to a certain volume, mixed well and part of it was removed for analysis of the nitrogen content. The clothing such as underwear, socks, towels and so on were soaked for 48 h in several liters of 0.1% acetic acid solution and rinsed six times in deionized water. All the soaking and rinsing solutions were mixed together and a sample of the mixed solution was taken for the analysis of nitrogen. The dermal nitrogen losses in the present study did not include losses due to hair and nails which have been estimated as small as 24 mg/day and 27–65 mg/day by Sirbu et al. (10) and Calloway et al. (11) respectively.

Feces were pooled for 5 consecutive days during the experimental period in a freezer, the pooled samples then being homogenized. One-tenth of the homogenate of each sample was mixed together and subjected to determination of the average daily fecal nitrogen excretion.

Urinary, fecal and dermal nitrogen were determined by the semi-micro Kjeldahl method and urinary creatinine by the Jaffe reaction (12).

RESULTS

Although the initial body weight of each subject slightly differed between the summer and winter experiments because of a 6-month interval, it was maintained almost constant during each of the experimental periods (Table 1).

Daily urinary creatinine was almost the same in winter and in summer during the sedentary period, but was significantly higher during the exercise period than the sedentary period in the summer experiment. The mean values for each subject are shown in Table 5. Daily urinary nitrogen excretions are shown in Table 2. There was daily fluctuation in the urinary nitrogen of each subject, but the mean value for 6 subjects showed no significant daily changes in both summer and winter experiments. The pooled mean and SD for the sedentary period (n=24) was 11.19±1.64 g in winter and 10.87±1.56 g in summer, showing a slightly higher
Table 1. Characteristics of subjects.

| Subject | Age | Height (cm) | Weight (kg) |
|---------|-----|-------------|-------------|
|         |     | Summer      | Winter      |
| A       | 21  | 182.0       | 80.6        | 82.8        |
| B       | 21  | 174.6       | 63.5        | 64.0        |
| C       | 21  | 176.5       | 80.5        | 82.0        |
| D       | 21  | 161.0       | 54.5        | 54.0        |
| E       | 22  | 174.0       | 64.0        | 65.5        |
| F       | 21  | 176.0       | 68.2        | 68.0        |

Table 2. Urinary nitrogen excretion (g/day).

| Day | Subject | Mean ± SD |
|-----|---------|-----------|
|     | A       | B         | C         | D         | E         | F         |         |
| 1   | 11.70   | 9.69      | 12.99     | 10.20     | 9.79      | 12.41     | 11.13±1.43 |
| 2   | 12.78   | 10.27     | 11.56     | 8.84      | 12.51     | 12.64     | 11.43±1.58 |
| 3   | 14.00   | 9.62      | 14.27     | 9.12      | 9.74      | 10.89     | 11.27±2.29 |
| 4   | 10.62   | 10.82     | 12.80     | 9.41      | 9.20      | 12.77     | 10.94±1.57 |
| Mean| 12.28   | 10.10     | 12.91     | 9.39      | 10.31     | 12.18     | 11.19±1.64 |

* Pooled mean (n=24).  b Pooled mean (n=12).

value in winter than in summer, but the difference was not statistically significant. The pooled mean for the exercise period (n=12) in the summer experiment was 10.80±1.38 g, indicating almost the same value as that for the sedentary period.

Daily dermal nitrogen losses in the summer experiment are shown in Table 3. The pooled mean was 0.43±0.20 g/day for the sedentary period and 0.78±
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Table 3. Daily dermal nitrogen loss in summer experiment (g/day).

| Subject | Day | A   | B   | C   | D   | E   | F   | Mean ± SD     |
|---------|-----|-----|-----|-----|-----|-----|-----|--------------|
| Sedentary | 1   | 0.24| 0.18| 0.52| 0.17| 0.51| 0.55| 0.36 ± 0.18  |
|         | 2   | 0.39| 0.16| 0.70| 0.23| 0.67| 0.58| 0.46 ± 0.23  |
| Exercise | 2   | 0.37| 0.13| 0.80| 0.49| 0.45| 0.50| 0.46 ± 0.22  |
|         | 4   | 0.21| 0.30| 0.85| 0.50| 0.48| 0.43| (0.43 ± 0.20) |

*Pooled mean for sedentary period (n=24), b Pooled mean for exercise period (n=12). Significant difference: a vs. b (p<0.001).

Table 4. Mean daily dermal nitrogen loss.

| Subject | N (g/day) | Mean ± SD   |
|---------|-----------|-------------|
| Sedentary W* | 0.27 | 0.16 | 0.34 | 0.14 | 0.19 | 0.21 | 0.22 ± 0.07d |
| Sedentary S^b | 0.30 | 0.19 | 0.72 | 0.35 | 0.53 | 0.52 | 0.44 ± 0.19e |
| Exercise S^c | 0.68 | 0.53 | 1.31 | 0.78 | 0.69 | 0.74 | 0.79 ± 0.27f |

N (mg/kg/day)

| Subject | Sedentary W* | 3.26 | 2.52 | 4.14 | 2.63 | 2.90 | 3.13 | 3.10 ± 0.58d |
|---------|-------------|------|------|------|------|------|------|--------------|
| Sedentary S^b | 3.75 | 3.02 | 8.94 | 6.39 | 8.37 | 7.62 | 6.35 ± 2.46e |
| Exercise S^c | 8.41 | 8.36 | 16.42 | 14.29 | 10.85 | 10.87 | 11.53 ± 3.23f |

*Winter experiment, mean for 4 days. b Summer experiment, mean for 4 days. c Summer experiment, mean for 2 days. Significant difference: d vs. e (p<0.05), e vs. f (p<0.005).

0.27 g/day for the exercise period, showing significantly larger dermal nitrogen losses during the exercise period than during the sedentary period. The mean dermal nitrogen losses are shown in Table 4 in terms of g/day and mg/kg/day. The mean body weight for the experimental period was used for the calculation of mg/kg/day. The mean value of dermal nitrogen losses under the sedentary condition was 0.22 ± 0.07 g/day or 3.10 ± 0.58 mg/kg/day in winter and 0.44 ± 0.19 g/day or 6.35 ± 2.46 mg/kg/day in summer. The value in summer was twice as high as that in winter and the difference was statistically significant.

Fecal nitrogen output and the apparent digestibility of protein in the experi-
Table 5. Fecal nitrogen, digestibility and urinary creatinine excretion.

| Subject | N intake (g/day) | Fecal N (g/day) | AD (% ) | Urinary creatinine (g/day) |
|---------|-----------------|-----------------|---------|---------------------------|
|         | Wb              | Sd              | W       | S       | W             | S       | SEa       |
| A       | 15.15           | 1.79            | 2.01    | 2.54   | 86.7          | 83.2    | 2.20      | 2.36     | 2.42    |
| B       | 12.45           | 1.48            | 1.79    | 1.54   | 85.6          | 87.6    | 1.66      | 1.66     | 1.69    |
| C       | 15.14           | 1.48            | 2.06    | 1.88   | 86.4          | 87.6    | 2.19      | 2.22     | 2.26    |
| D       | 10.74           | 1.39            | 1.08    | 1.62   | 89.9          | 84.9    | 1.43      | 1.44     | 1.46    |
| E       | 12.87           | 1.39            | 2.14    | 2.21   | 83.4          | 82.8    | 1.69      | 1.62     | 1.67    |
| F       | 13.73           | 1.39            | 2.09    | 1.65   | 84.8          | 88.0    | 1.80      | 1.81     | 1.86    |
| Mean    | 13.35           | 1.86            | 1.86    | 1.91   | 86.1          | 85.7    | 1.83      | 1.85     | 1.89    |
| ±(SD)   | (1.70)          | (0.40)          | (2.2)   | (0.39) | (0.31)        | (0.36)  | (0.37)    |           |         |

*a Apparent digestibility. *b Winter, sedentary period, mean for 4 days. *c Summer, sedentary period, mean for 4 days. *d Summer, exercise period, mean for 2 days. *e Significant difference between sedentary period and exercise period (p < 0.005).

Table 6. Nitrogen balance (g/day).

| Subject | Winter        | Summer        |
|---------|---------------|---------------|
|         | Sedentary     | Exercise      |
|         |               | Sedentary     | Exercise     |
| A       | +0.59         | +0.37         | -0.05        |
| B       | +0.40         | +0.76         | +0.55        |
| C       | -0.17         | -0.29         | -0.09        |
| D       | +0.13         | +0.15         | -0.40        |
| E       | +0.23         | +0.06         | -0.17        |
| F       | -0.75         | -0.26         | -0.74        |
| Mean ±SD| +0.07 ± 0.48  | +0.13 ± 0.40  | -0.15 ± 0.43 |

mental diet are shown in Table 5. No seasonal difference was observed in fecal nitrogen output, and the mean digestibility was almost the same in winter and in summer.

Nitrogen balance data are shown in Table 6. Dermal nitrogen losses were included in the calculation of the nitrogen balance. Mean nitrogen balance was not significantly different between summer and winter under the sedentary condition. Nitrogen balance in each subject except for Subject C was smaller during the exercise period than the sedentary period in the summer experiment. The same tendency was found in the mean value for the six subjects, but the difference was not statistically significant.

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DISCUSSION

It is well known that physical exercise increases energy expenditure. However, no extra energy was given during the exercise period in the summer experiment, because it was assumed that 42–44 kcal/kg/day was sufficient even during the exercise period because of the light intensity and the short duration of the physical exercise. The almost constant body weight throughout the experiment suggested a sufficient supply of energy.

A wide range of values has been reported for dermal nitrogen losses. This may be due mainly to the difference in the experimental conditions, because it has been observed that dermal nitrogen losses vary depending upon environmental temperature (2, 3, 8, 13, 14), physical activity (4, 6, 10, 15) and dietary protein level (1, 4, 10, 11, 15, 16). The difference in the methods for the collection of sweat also can be one of the reasons.

The mean values obtained in the present study during the sedentary period were 6.35 ± 2.46 mg/kg/day in summer and 3.10 ± 0.58 mg/kg/day in winter (Table 4). The average outdoor temperature at noon was 24.5°C in the summer experiment and 5.5°C in the winter experiment, and the buildings of our institute were air-conditioned. Inoue et al. (14) reported mean dermal nitrogen losses of 12.7 ± 1.9 mg/kg/day in summer and 3.6 ± 1.7 mg/kg/day in winter with subjects who lived in a metabolic ward on a diet supplying about 45 kcal/kg/day of energy and about 1.2 g/kg/day of protein and who were allowed only 1 h of routine work or light sports activity per day. Mean values of 9.26 ± 3.22 mg/kg/day in summer and 5.67 ± 1.42 mg/kg/day in winter were reported respectively by Yoshimura and Yamada (17) and Yamada and Yoshimura (18) with subjects who consumed a diet providing 1.20 g/kg/day of protein and 45 kcal/kg/day of energy and who were engaged in normal light school activities. The mean dermal nitrogen loss obtained in the present study in winter was almost the same as that reported by Inoue et al. (14), but lower than that reported by Yamada and Yoshimura (18). The mean value in summer was lower than the values reported by Inoue et al. (14) and Yoshimura and Yamada (17). The reasons are not clear why our results are lower than theirs under almost the same experimental conditions. There might be slight differences in environmental conditions and physical activities, and also, differences in the method to collect sweat may be a contributory factor.

The mean daily dermal nitrogen losses obtained in our laboratory were 220 ± 70 mg (with the range of 160–340 mg) in winter and 440 ± 190 mg (190–720 mg) in summer during the sedentary period, and 790 ± 270 mg (520–1,310 mg) during the exercise period in summer (Table 4). Our results may be compatible with the following data reported, when the experimental conditions are taken into consideration. Bost and Borgstrom (6) reported daily dermal nitrogen losses of 613 and 900 mg in two subjects who worked actively in hot conditions. Voit (19) calculated daily dermal loss of nitrogen in nonsweating subjects to be 333 mg. Cuthbertson and Guthrie (1) found that dermal nitrogen losses were
215–412 mg per day in subjects whose protein intakes were 67–77 g/day at the average room temperature of 15.0–17.8°C. Freyberg and Grant (20) gave average daily nitrogen loss through the skin to be 372 mg under nonsweating conditions. Mitchell and Hamilton (2) calculated mean daily dermal nitrogen loss to be 360 mg under comfortable conditions. Darke (21) reported the mean daily dermal nitrogen loss of 243 mg for male adult Africans engaged in sedentary activities in a hot climate. Ashworth and Harrower (22) obtained mean values of 482 and 494 mg per day for heavily sweating subjects eating a low-normal protein diet (8 g N/day). Sirbu et al. (10) measured dermal nitrogen losses in sedentary men in a comfortable environment, and the mean daily value for subjects on the control diet (12.3 g N/day) was 119 mg. Calloway et al. (11) obtained mean value of 149 mg in sedentary subjects who consumed a normal protein diet (12 g N/day).

On the other hand, Mitchell and Hamilton (2) reported high dermal nitrogen losses, i.e. averaging 173 mg/h under a hot, humid environment and on a diet containing 13 g/day of nitrogen. On a 24-h basis the value became 4.15 g. However, they did not collect sweat for 24 h, but for 7.5 h per day. To obtain the concentration of nitrogen in sweat they took the net loss in body weight as a measure of total sweat loss, and the amount of nitrogen removed from the body by washing was divided by the sweat loss. Consolazio et al. (3, 4) also reported considerably high dermal nitrogen losses, i.e. averaging 1.78 and 1.89 g/day under sedentary conditions on a daily protein intake of 1.44 g/kg. However, the high values can be ascribed partly to their arm-bag method for the collection of sweat. Consolazio et al. (23) showed a fairly good agreement in nitrogen excretion between arm and total body sweat, but Costa et al. (24) concluded that arm-bag sweat cannot be used to predict body losses of nitrogen.

As regards the relationship between urinary nitrogen excretion and dermal nitrogen losses under different environmental and/or exercise conditions, many contradictory results have been reported.

Borgstrom and Bost (25) reported a lower urinary nitrogen output in warm weather than in cold weather, and Bost and Borgstrom (6) suggested that this is a compensatory factor for cutaneous nitrogen excretion. Huang et al. (7) found that obligatory urinary nitrogen loss was significantly lower in summer than in winter, and then observed in a further experiment (8) that when dermal nitrogen losses increased during high temperatures, urinary nitrogen excretion decreased gradually and total nitrogen losses did not increase. They concluded that the protein requirements of people living in tropical areas possibly were not higher than those living in temperate zones, since there was renal compensation for changes in dermal nitrogen losses.

In the case of increased sweat nitrogen losses mainly induced by exercise, Consolazio et al. (4) reported essentially unchanged urinary nitrogen excretion, showing no compensatory decrease in urinary nitrogen excretion. Weiner et al. (15) reported that urinary nitrogen excretion remained constant in spite of a small increase in intake, whereas sweat nitrogen output increased by about 20% during
the exercise period. Yamada (26) observed decreased urinary nitrogen excretion during profuse sweating induced by hard exercise in well-trained men. Ashworth and Harrower (22) indicated that their fully acclimatized men excreted less urinary nitrogen during the sweating exercise period than during the nonsweating sedentary period. Both Ashworth and Harrower (22) and Weiner et al. (15), however, pointed out that nitrogen losses in sweat were minute compared with those in urine, and therefore it was difficult to determine whether there was any compensatory decrease in urinary nitrogen excretion as a result of sweating.

In the present study, the dermal nitrogen losses were significantly larger in summer than in winter (Table 4), while urinary nitrogen excretion was higher in winter than in summer not only for the pooled mean but also the mean for 4 days of each subject (Table 2), although the difference was not statistically significant. Consequently, nitrogen balance was not significantly different between winter and summer under the sedentary condition (Table 6). Our results showed the same tendency as the findings of Huang et al. (8), who mentioned the existence of a compensatory relationship between dermal nitrogen losses and urinary nitrogen excretion. In our summer experiment, the dermal nitrogen loss was significantly larger during the exercise period than during the sedentary period (Tables 3, 4), while the urinary nitrogen excretion was almost the same between the two periods (Table 2). The results were coincident with those of Consolazio et al. (4), who were unable to observe a compensatory reduction of urinary nitrogen when sweat nitrogen losses increased.

Thus, in our present study the compensatory change in the urinary nitrogen excretion was observed for the seasonal change in dermal nitrogen loss, but not for the exercise-induced change in dermal nitrogen loss. We can point out the following assumption relating to the factors responsible for the different trend of changes in urinary nitrogen excretion between season and exercise. The subjects acclimatized well to the seasonal changes in environmental conditions, but may not have adapted to the very short term exercise conditions. It is well known that stress induces increased body protein catabolism and results in increased urinary nitrogen excretion. But neither environmental nor exercise conditions were likely to be very stressful in the present study. Body protein catabolism is also enhanced by energy deficit and exercise increases energy expenditure. But energy supply seemed to be sufficient as seen by the almost constant body weight maintained during the experimental periods.

Further investigations are necessary taking into consideration experimental conditions such as duration of experimental period, dietary nitrogen intake level, energy intake level, and intensity and duration of exercise.

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