## INTRODUCTION

The location of both the Arctic and Antarctic at high latitudes endows them with unique environments featuring cold climates, considerable changes in the length of nights and days, and greatly differing strengths of sunlight throughout the year.

There are some reports on seasonal changes in serum 25-hydroxyvitamin D [25(OH)D] at high latitudes (including the Antarctic and the Arctic)$^{1-5)}$ and how these affect bone metabolism in Eskimos$^{6-9)}$ and other people$^{10)}$ in the northern hemisphere. It is very difficult to surmise whether or not these changes are really caused by only the environment as these peoples have been living there since prehistoric times while preserving a peculiar life style, including their diet.

In contrast, recently many non-native people have come to reside in the Antarctic for up to one year, but their life styles and diet remain similar to those of their native countries except for the environment. To clarify the effect of the Antarctic environment on bone turnover, it is essential to investigate the differences between people who temporarily reside in the Antarctic and people who are native to high latitudes in the northern hemisphere.

One of the authors (I.O.) was a wintering member of the 35th Japanese Antarctic Research Expedition (35th JARE, 1993-1995) and previously reported changes in body weight and the daily number of steps$^{11)}$, and ultrasound parameters of the right os calcis$^{12)}$, and examined the human calcium metabolism in the Antarctic. This report consolidates these results and additional data on blood and urinary biochemical markers.

## MATERIALS AND METHODS

### Subjects

Forty wintering members of the 35th JARE were observed during the period of the expedition. Informed consent was obtained from all members prior to the start of this study.

### Period of the expedition

The 35th JARE left Japan on board the Icebreaker SHIRASE on 14 November 1993, staying in Australia (Fremantle) from 28 November to 2 December, and reached the Antarctic at the end of December 1993, leaving the Antarctic at the beginning of February 1995.

### Location and natural environment

The base camp of JARE in the Antarctic is Syowa Station (69°00' S, 39°35' E) located on Ongul island about 5 km from the Antarctic continent in Fig. 1.

At Syowa Station, the sun does not set for about 40 days in summer (from 10 December to 20 January) and does not rise for about 40 days in winter (from 1 June to 10 July). The average number of hours of sunshine per day, the monthly average temperature, and the monthly mean value of UV-B radiation at Syowa Station are shown in Fig. 2$^{13)}$.

### Intracontinental travel

During this expedition, three intracontinental trips were made from Syowa Station to a relay point (about 650 km south of Syowa Station; altitude 3,300 m; autumn and spring trips), and to Dome Fuji Station (about 1,000 km south of Syowa Station; altitude 3,800 m; summer trips).

### Groups

Some 20 members aged 24 to 51 (31.3±7.3 years old) stayed at Syowa Station and did not travel intracontinentally for more than one month. They were called the Station Group.

The other group consisted of the members who went on the intracontinental excursions and was named the Travelers Group. Seven members (34.4±5.0 years old) who went on the autumn travel were named the Autumn TG (Traveler Group). Nine members (35.9±5.9 years old) made up the Spring TG and 9 members (32.6±4.7 years old) formed the Summer TG. One member went on all three trips, one member went on the autumn and spring trips, and four members went on the autumn and summer trips. Two members went for about half of the summer trip; their data was not included.

### Measurement of biochemical markers

In the Station Group, blood samples were taken on three mornings while staying at Syowa Station and once after coming back to Japan. The blood samples were centrifuged and the serum was separated. The first urine sample taken after waking up was obtained three times (the same day as the blood sampling) at Syowa Station. In the Travelers Group, blood and urine were sampled before and after their trip, once in autumn and twice on the summer trip. During the trip, the blood samples were also centrifuged and the serum was separated and stored at natural temperatures (about -68℃ to -5℃) with

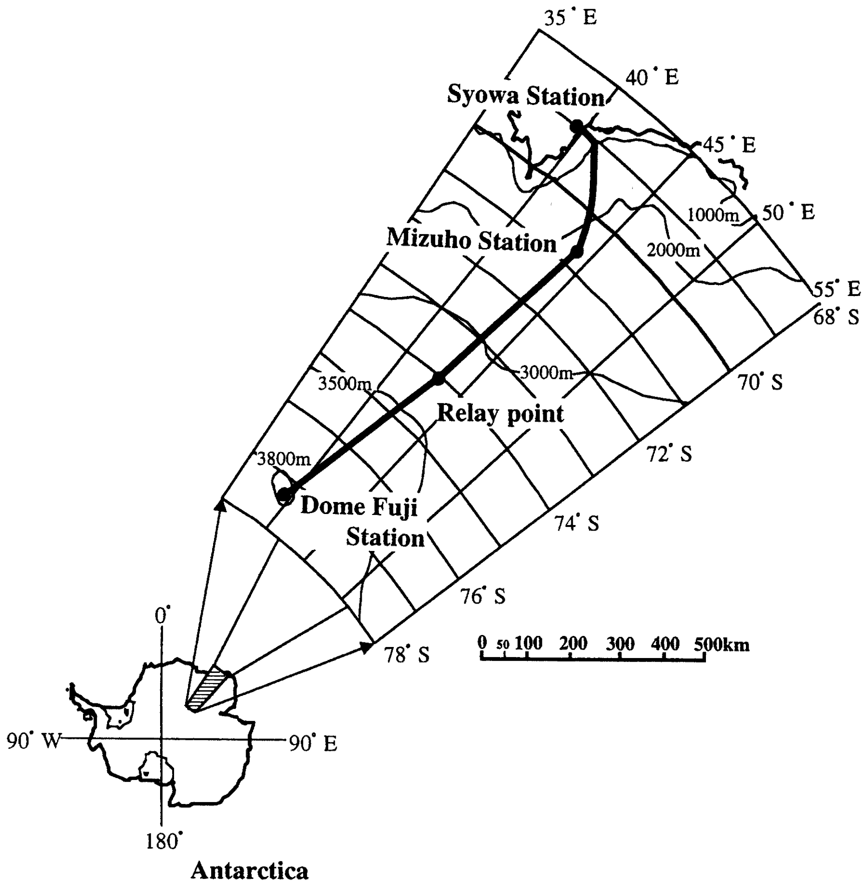

Fig. 1. Intracontinental route between Syowa Station and Dome Fuji Station.

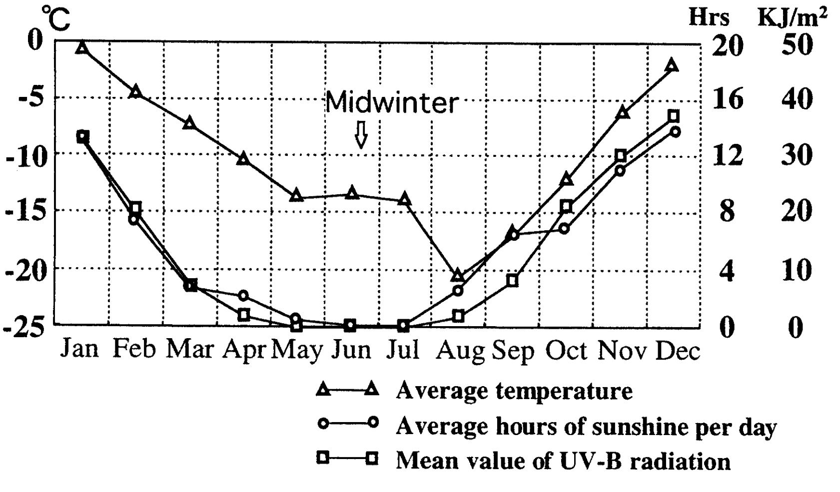

Fig. 2. Temperature, sunshine, and UV-B radiation at Syowa station. △-△: Average temperature (℃), ○-○: Average hours of sunshine per day (Hrs), □-□: Monthly mean value (daily integrated) of UV-B radiation (KJ/m$^2$) at Syowa Station in 1994.

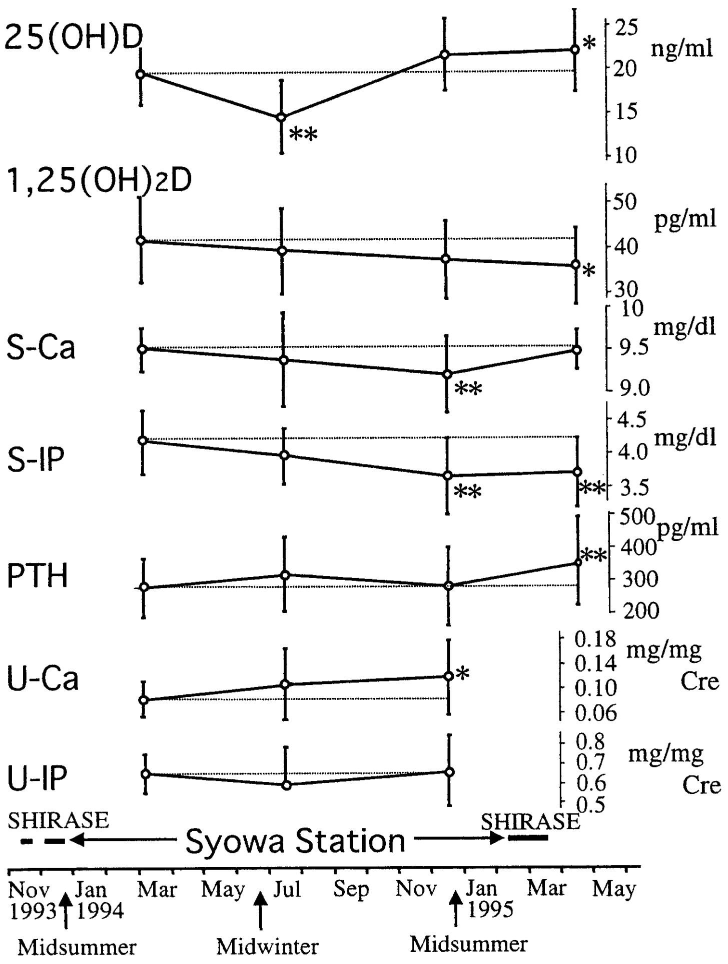

**Fig. 3.** Serum 25(OH)D, 1,25(OH)$_2$D, Ca, IP, PTH, and urinary Ca and IP in the Station Group (n=20; Mean±SD; **: $p<0.05$, **: $p<0.01$)

**Table 1.** SOS, BUA and stiffness in the Station Group

| | SOS | BUA | Stiffness |
|---|---|---|---|
| 1993. Nov | 1559.6 ± 27.3 | 129.0 ± 8.3 | 109.8 ± 12.7 |
| 1994. Feb | 1562.2 ± 27.2 | 126.8 ± 8.1 | 108.5 ± 11.8 |
| Mar | 1562.3 ± 28.2 | 126.8 ± 7.9 | 108.6 ± 12.1 |
| May | 1562.5 ± 25.7 | 125.8 ± 8.6 | 107.9 ± 11.4 |
| Jun | 1560.7 ± 26.3 | 125.6 ± 8.2 | 107.4 ± 11.7 |
| Aug | 1561.2 ± 27.1 | 126.8 ± 8.1 | 108.5 ± 11.6 |
| Sep | 1565.3 ± 26.8 | 126.6 ± 7.8 | 109.6 ± 11.7 |
| 1995. Jan | 1564.5 ± 25.2 | 127.2 ± 8.9 | 110.0 ± 11.6 |
| Feb | 1566.7 ± 27.9 | 125.2 ± 7.0 | 109.4 ± 11.9 |

(n=20; mean±SD)

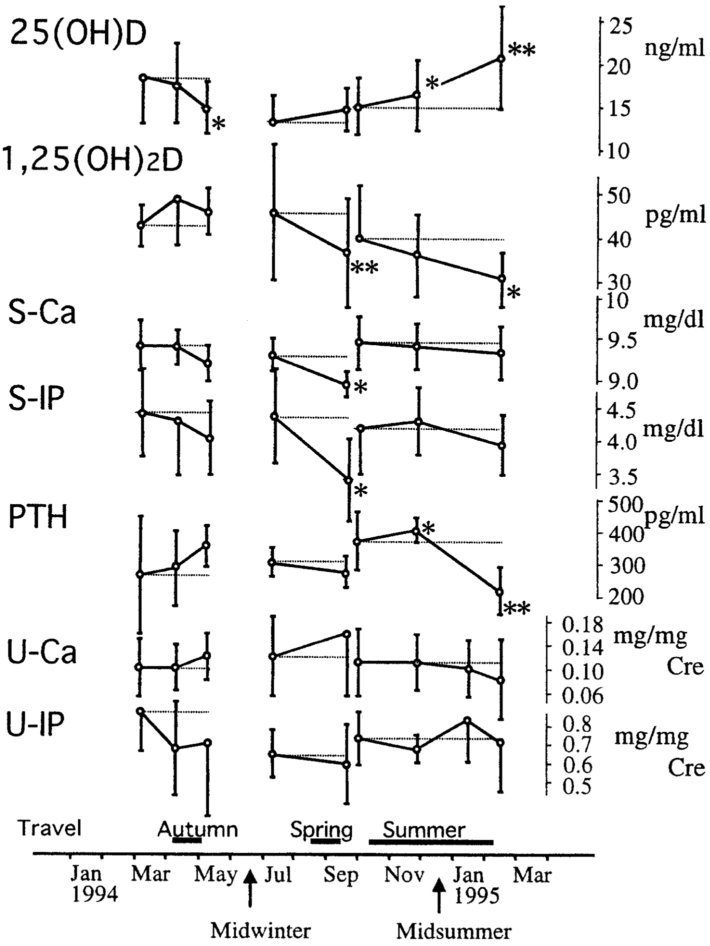

**Fig. 4.** Serum 25(OH)D, 1,25(OH)$_2$D, Ca, IP, PTH, and urinary Ca and IP in the Travelers Group, Autumn TG (n=7), Spring TG (n=9) and Summer TG (n=9). (Mean±SD; *: $p<0.05$, **: $p<0.01$)

**Table 2.** Changes in SOS, BUA and stiffness in the Travelers Group before and after intracontinental travel

| TG | n | Before | After | p value |
|---|---|---|---|---|
| SOS | | | | |
| Autumn | 7 | 1549.6 ± 22.0 | 1552.7 ± 21.6 | 0.344 |
| Spring | 9 | 1552.0 ± 29.6 | 1563.6 ± 27.7 | 0.012* |
| Summer | 9 | 1553.6 ± 33.3 | 1567.4 ± 33.3 | 0.021* |
| BUA | | | | |
| Autumn | 7 | 125.0 ± 8.2 | 127.9 ± 8.4 | 0.233 |
| Spring | 9 | 124.3 ± 10.7 | 125.6 ± 11.8 | 0.343 |
| Summer | 9 | 124.1 ± 8.3 | 124.7 ± 6.9 | 0.343 |
| Stiffness | | | | |
| Autumn | 7 | 105.6 ± 12.4 | 109.3 ± 13.9 | 0.027* |
| Spring | 9 | 107.3 ± 16.4 | 111.7 ± 16.9 | 0.027* |
| Summer | 9 | 105.4 ± 15.8 | 10.2 ± 14.5 | 0.033* |

mean±SD *: $p<0.05$

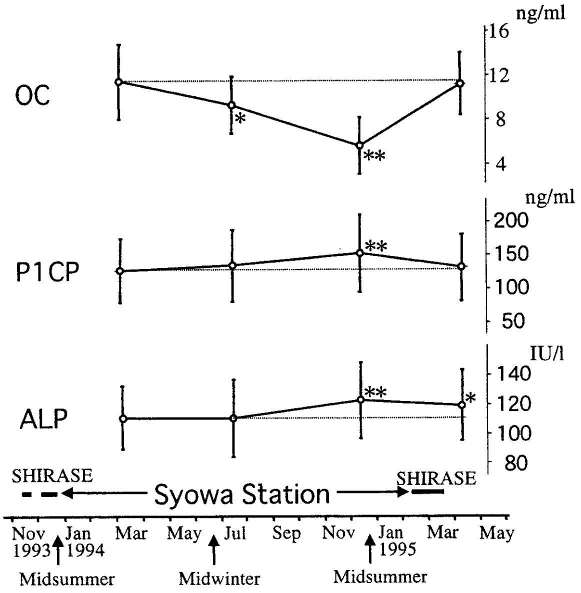

**Fig. 5.** Bone forming markers in the Station Group (n=20; Mean±SD,*: p<0.05, **: p<0.01)

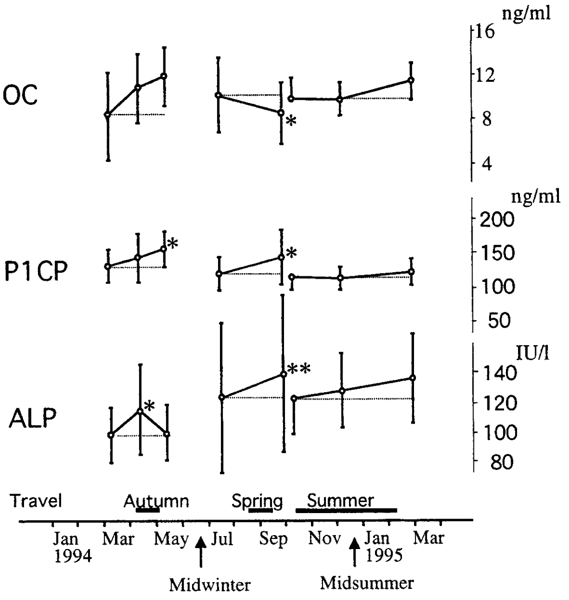

**Fig. 6.** Bone forming markers in the Travelers Group, Autumn TG (n=7), Spring TG (n=9) and Summer TG (n=9). (Mean±SD; *: p<0.05, **: p<0.01)

the urine samples and brought back to Syowa Station. The samples of the serum and the urine were stored at about -25℃ in Syowa Station and in the Icebreaker SHIRASE, to be measured after coming back to Japan.

The following are the measurements made: 25(OH) D by competitive protein binding assay, serum 1,25-dihydroxyvitamin D[1,25(OH)$_2$D] by radioreceptor assay, serum calcium (s-Ca), serum inorganic phosphate (s-IP), serum alkaline phosphatase (ALP) and serum resistant acid phosphatase (TRAP) by spectrophotometry, serum high sensitive parathyroid hormone (PTH), serum tartrate procollagen type 1 carboxyterminal extension peptide (PlCP), and serum procollagen type 1 C-terminal propeptide (1CTP) by radioimmunoassay, serum osteocalcin (OC) by immuno radiometric assay, and urinary calcium (u-Ca), urinary inorganic phosphate (u-IP) and urinary creatinine (u-Cre) by spectrophotometry, urinary pyridinoline (u-Pyr) and urinary deoxypyridinoline (u-D-Pyr) by high performance liquid chromatography. These measurements were performed in cooperation with SRL Inc.
(Tokyo, Japan)

### Calcaneal ultrasonometry

Ultrasound parameters of the right os calcis were measured using the Achilles Ultrasound Bone Densitometer (Lunar Corp. USA) nine times between November 1993 and February 1995 for expedition members who stayed at Syowa Station and before and after traveling with the intracontinental Travelers Group. The Achilles Densitometer measures the speed of sound (SOS), the broadband ultrasound attenuation (BUA) and the stiffness index (stiffness, calculated by the computer program provided by the manufacturer from the combined SOS and BUA values). The precision error in measuring a standard phantom during this study was 0.1% CV for SOS and 0.9% CV for BUA.

### Nutrition

Daily meals were the same as with all annual JARE. The average oral intake while wintering from the 21st to the 31st JARE were as follows: calories 2737 kcal/day, protein 106.1 g/day and Ca 481.8 mg/day (food working group of the National Institute of Polar Research, unpublished). Besides the meals, no Vitamin D, Ca or other nutrient supplements were used.

### Exercise

It was reported earlier that wintering members of the JARE had to stay inside the small station during the winter season, and the average number of steps per

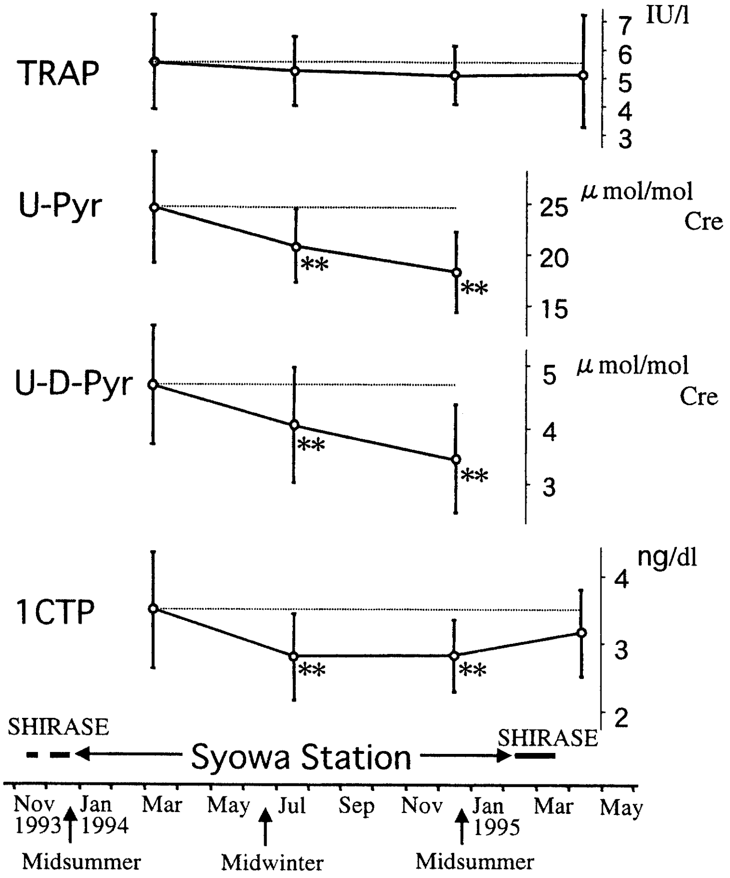

**Fig. 7.** Bone resorbing makers in the Station Group (n=20; Mean± SD; *: $p<0.05$, **: $p<0.01$)

person was significantly lower compared with that of the summer season[11]. However, the numbers of steps of the wintering members has been increasing yearly due to the expanded size of the Syowa Station. In the 10th JARE, the minimum number of steps per day was about 6000 in winter[14], while in the 35th JARE, there were at least 8550[11].

The number of steps could not be counted while on intracontinental trips because the snow vehicles shook greatly on the rugged snow surfaces and pedometers were not useful, resulting in false counts. On the summer trip, the members went to Dome Fuji Station and constructed a new station. The route going to and from Dome Fuji was in the same condition as on other trips, and during construction, the members' number of steps were equivalent or slightly more than the Station Group.

### Statistical analysis

Statistical analysis was performed using the Wilcoxon signed-ranks test and the Two Factor ANOVA. Differences were considered significant at $p<0.05$.

## RESULTS AND DISCUSSION

The seasonal changes in serum 25(OH)D, serum $1,25(OH)_2D$, s-Ca, s-IP and PTH of the Station and Traveler Groups are shown in Figs. 3 and 4. Serum 25(OH)D of both the Station Group and the Traveler Group showed seasonal changes, decreasing in winter and increasing in summer. While working outdoors in the Antarctic, the members usually exposed only their faces. A seasonal change in serum 25(OH)D was reported both at normal latitudes[15,16] and at high latitudes[1,3]. One of the reasons for statistically high values in April 1995 was exposure to an extensive

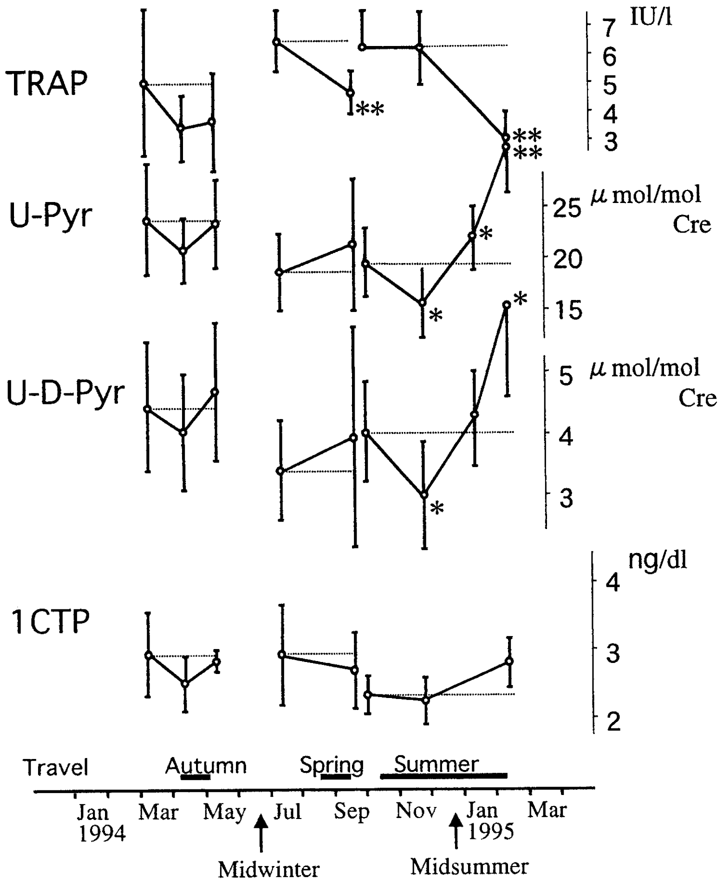

**Fig. 8.** Bone resorbing makers in the Traveler Group, Autumn TG (n=7), Spring TG (n=9) and Summer TG (n=9). (Mean±SD; *: $p < 0.05$, **: $p < 0.01$)

amount of sunlight in summer during the Australian holidays for one week on the way back to Japan from the Antarctic.

Although serum 25(OH)D varied depending on the season, serum 1,25(OH)$_2$D of both groups decreased gradually, being statistically low in April 1995 (Figs. 3 and 4). These results suggest that the level of serum 1,25(OH)$_2$D was under considerable hormonal control or some other factors.

It is known that a decrease in the s-Ca level induces an increase in the serum PTH level. This factor directly increases bone resorption and re-uptake from the kidneys, and is indirectly activated to produce 1,25(OH)$_2$D from 25(OH)D in the kidneys; 1,25(OH)$_2$D increases uptake of Ca and IP from the intestines. In the Station Group, s-Ca decreased gradually and showed statistically low values in December 1994, then returned to nearly the same values as the previous wintering in April 1995, while serum PTH showed no remarkable changes in December 1994 but was statistically high in April 1995. The decrease in s-Ca levels in winter triggered an increase in PTH in April 1995, followed by inducing an increase in s-Ca between December 1994 and April 1995.

There are reports that the high phosphorus content of meat apparently minimizes the hypercalciuric effect[17,18] and that the low levels of s-IP lead to the hypercalciuric. In addition, the excess protein intake in winter might cause increases in the excretion of Ca from the urine[19,20,21]. The deficiency of Ca and the high phosphorus content of meat intake induced a gradual decrease in s-Ca until the arrival of the next expedition members with fresh food supplies. After returning to Japan with sufficient Ca intake, the s-Ca value returned to the same level as before wintering.

As for the bone forming markers in the Station Group, OC decreased significantly to approximately

50% in July and December 1994, but P1CP and ALP showed significant increases (Fig. 5). The decrease in the OC indicates that osteoblastic activity was lower during winter but later recovered. Bone forming markers in the Travelers Group showed an increase or at least a tendency to do so (Fig. 6). This suggests that osteoblastic activity was activated during and after the trips.

As for the bone resorbing markers in the Station Group, u-Pyr, u-D-Pyr and 1CTP significantly decreased in July and December 1994 (Fig. 7). These results suggest that osteoclastic activity was lower during the winter.

The s-Ca decreased significantly after the intracontinental trip in the Spring TG (Fig. 4). The amount of markers of osteoclasts did not increase, while TRAP showed a statistically significant decrease (Fig. 8). It is very difficult to explain the difference between the decrease in TRAP and the increase in u-Pyr, u-D-Pyr and 1CTP in the Summer TG.

The wintering members who stayed at Syowa Station were affected by the environment (weaker and shorter exposure to sunlight, namely UV, and insufficient intake of Ca from food), suggesting that bone remodeling was suppressed during wintering. Ultrasonometric parameters in the right os calcis did not change significantly (Table 1). Stiffness after all three TG's and SOS after the Spring and Summer TG's increased statistically (Table 2). The amount of steps, Ca from food intake, and the amount of UV exposure while the traveling group was moving were the same or less than the Station Group. One of the environmental differences between the Travelers Group and the Station Group was the altitude. Dome Fuji Station is 3,800 m above sea level and even the relay point is 3,300 m. Tanaka et al. reported[22] that, after Himalayan mountaineering, s-Ca, serum $1,25(OH)_2D$ and bone mineral density at the 1/6 radial distal portion measured by single photon absorptiometry decreased significantly. This study exhibited a significant decrease in s-Ca and serum $1,25(OH)_2$ D on every trip and an increase in ultrasound parameters in the Travelers Group. The high altitude (the main reason for hypoxia) and protracted riding on snow vehicles that over rough terrain about 10 to 13 hours a day for about 30 days on each trip may have influenced bone metabolism. Indeed, recent evidence from animal and human studies suggest that mechanical vibration is sufficient to inhibit bone loss[23] and influence bone, muscle, and hormones[24,25].

Further investigation under conditions with a sufficient intake of Ca and moderate intake of protein and phosphate will be mandatory in order to confirm the pure effects of the atmosphere in the Antarctic on bone metabolism. It will also be important to evaluate bone metabolism in those who are staying for longer periods in the Antarctic.

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