Protein turnover rates of two human subjects during an unassisted crossing of Antarctica

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During the Austral summer of 1992–3, two men, MS and RF, walked 2300 km across Antarctica in 96 d, unassisted by other men, animals or machines. During the journey they ate freeze-dried rations, towed on sledges, that contained an average of 21·3 MJ/d of which 56·7% was fat, 35·5% carbohydrate and 7·8% protein (98·8 g). Despite this high energy intake both men lost more than 20 kg in body weight due to their extremely high energy expenditures. Studies of protein turnover using [^{15}N]glycine by the single-dose end-product method were made before, during and after the journey, and these demonstrated considerable differences in the metabolic responses of the two men to the combined stresses of exercise, cold and undernutrition. However, both men maintained high and relatively stable levels of protein synthesis during the expedition despite the great exertion and the onset of considerable debilitation. This stability indicates the vital physiological function of protein synthesis.

Protein turnover: Exercise: Undernutrition

During the Austral summer of 1992–3 two men made the first crossing of Antarctica, unassisted by other men, animals or machines. They travelled on foot, hauling sledges for 10–12 h every day. Each sledge had a starting weight of 222 kg and contained 100 days' supplies of food and fuel. The journey, which has been described by Stroud (1993), started at the Weddell Sea and ended after 96 d at the Ross ice-shelf. Overall, they covered about 2300 km with a climb of 3500 m to the highest part of the Antarctic polar plateau.

During the course of the journey the men consumed an average of 21·3 MJ/d which, in order to save weight, was derived mainly from fat. Based on measurements of energy expenditure made on an expedition of similar nature (Stroud, 1987) it was expected that loss of body weight would be limited to 10 kg. In the event this was an underestimate and weight losses exceeded 20 kg. At the end of the journey the two men were on half-rations.

In the course of the expedition a number of scientific studies were made including measurements of whole-body protein turnover, since little is known about the response of this key function to very severe exertion continued over a long time, especially when combined with negative energy balance. Protein turnover was measured 1 week before the expedition; five times during the expedition on days 9/10, 19/20, 49/50, 76/77, and 92/96 (the two subjects being tested on different days); and twice following the journey, 2 and 3 weeks after return. The present paper reports the results of these measurements.

METHODS
Subjects
The subjects, MS and RF, were fit men aged 37 and 48 years respectively. Both men gave informed written consent for all experiments.

Energy intake
Before and after the expedition normal foods were consumed *ad libitum*. The amount of each food was estimated and recorded, but not weighed. These semi-quantitative records were analysed independently by two dietitians.

During the expedition the subjects ate a specially formulated pre-packed diet (Be-Well Nutrition, Stamford, Lincs.) containing a high proportion of fat in order to provide a high energy:weight ratio. This consisted of freeze-dried meals supplemented with butter, chocolate bars, biscuits, soups and hot chocolate drinks. Breakfast, consisting of hot cereal and butter, was eaten at 07.30 hours; soup and chocolate bars were eaten at intervals throughout the day; and the main meal was taken at 21.00 hours. Analysis was performed before departure with food values taken from manufacturers' nutritional information about their products. All food was weighed and packaged into daily ration bags at the beginning of the expedition and, at the end of the expedition, mean daily energy intakes were calculated making an allowance for any unused food.

Energy expenditure
Mean daily energy expenditure was estimated for the first 50 d and the last 45 d of the expedition by combining mean dietary energy intakes with the mean daily energy deficits calculated from losses of lean tissues and fat over each period of the expedition.

Body masses were recorded in the UK (Salter KR120 scales, West Bromwich, East Midlands) before and after the expedition, and were also recorded at 10 d intervals during the journey with a miniature load-cell portable scale (Miniscale, Raviv-Aran, Israel). On each occasion the measurements were made after overnight fast, defaecation and the voiding of urine. Body composition estimates were calculated on days 0 and 50 of the expedition from the dilution space of the stable isotope ^{18}O and on day 95 of the expedition from the dilution space of ^{2}H. These isotopes were given to the subjects in order to measure energy expenditure using the doubly-labelled water method, a study that is to be reported elsewhere (Stroud *et al.* 1996). Measurements of body composition were also made 10 d before and 7 d after the expedition using underwater weighing (UWW) with measurements of residual lung volumes by He-dilution spirometer (Gould Pulmonet III). Lean-tissue losses were assumed to be 73% water and 27% protein, whereas fat losses were considered to be 100% fat. The energy value of protein was taken as 18·39 kJ/g and that of fat was taken as 39·7 kJ/g (Brouwer, 1965).

Protein turnover
Protein turnover was measured with $[^{15}N]$glycine by the single-dose end-product method (Fern *et al.* 1981). The bladder was emptied at 07.00 hours and the subject then took 100 mg $[^{15}N]$glycine (Promochem, Welwyn Garden City, Herts.; 99 atom per cent ^{15}N) dissolved in water. Urine was collected in containers with thymol crystals as a preservative until 19.00 hours. A second urine collection was made from 19.00 hours to 07.00 hours the next morning. Urine volumes were measured and a sample retained for later analysis.

Urinary creatinine was determined by the Jaffre reaction and urinary NH_3 and urea were measured by the Berthelot reaction. NH_3 and urea-N were removed stepwise from urine

samples for mass spectrometry. The amount of urine estimated to contain 2 mg NH_3-N was made up to 14 ml with water and 10 M-NaOH to adjust the pH to 11–12. This was then aerated into 15 ml of 0·005 M-$(NH_4)_2SO_4$ for 3 h before 1–2 ml was used for mass spectrometry. The volume of NH_3-free urine estimated to contain 2 mg urea-N was then treated with urease (*EC* 3.5.1.5) at pH 7·8 at 37° for 3 h in EDTA buffer, before the resulting NH_3 was collected by further alkaline aeration. The ^{15}N abundances in the NH_3 collections were measured in a triple collector mass spectrometer (SIRA 10; VG Isogas, Middlewich, Ches.) after liberation of N_2 by LiOH.

In each test ^{15}N abundances were calculated after subtracting the background abundance in the urine sample collected before the dose of labelled glycine. The flux Q was calculated from the general equation:

$$Q(gN/12 h) = d.E/e,$$

where d is the dose of ^{15}N (mg) (Waterlow *et al.* 1978*b*).
For the flux based on NH_3 (Q_A):

$$E_A(12) = NH_3 - N \text{ excreted in 12 h (g)},$$

and

$$e_A(12) = {}^{15}N \text{ excreted in } NH_3 \text{ in 12 h (mg)}.$$

For the flux based on urea (Q_U):

$$E_U(12) = \text{urea N excreted in 12 h (g)}.$$

Since blood samples were not taken, it was assumed that there was no significant change in the size of the body urea pool. The value of e_U, the isotope excreted in urea, needed to be corrected for the isotope retained as urea in the body at 12 h. Since this could not be measured directly, it was assumed that all the labelled urea remaining in the body at 12 h had been excreted by 24 h (Grove & Jackson, 1995). Therefore the appropriate value for e_U is the ^{15}N excreted in 24 h. Hence

$$Q_U(12) = d.E_U(12)/e_U(24).$$

The error introduced by this assumption is considered later.

Rates of protein synthesis (S) and breakdown (B) were obtained from the usual equation:

$$Q = S + E = B + I,$$

where I is the intake and E is the total urinary N excretion, for which $E_U + E_A + N$ loss in creatinine is taken as a proxy. Because the measurement of Q_A is more open to error, synthesis rates were calculated only from Q_U and were determined only for the 12 h periods following the dose since it could not be assumed that rates of protein synthesis and breakdown remained the same during the night, when only one meal was eaten, as during the day, when the bulk of the food was consumed.

RESULTS

The dietary intake provided an average over the whole journey of 21·3 MJ/d of which 56·7% came from fat, 35·5% from carbohydrate and 7·8% from protein. Over the first 50 d the average daily intake was 19·9 MJ consisting of 95 g protein, 293 g fat, and 425 g carbohydrate. Over the second part of the expedition the average daily intake was 22·2 MJ consisting of 103 g protein, 338 g fat, and 557 g carbohydrate.

Despite the high energy intake, both men lost considerable weight and became significantly debilitated by starvation towards the end of the walk. In the later stages they also developed frostbite of the hands and feet which became chronically infected. The

Table 1. *Body weight and body composition of two men, before, during and after an unassisted crossing of Antarctica, measured using isotope dilution or underwater weighing*

Subject	Age (years)	Body weight (kg)					Body fat (%)				
		Day −10	Day 0	Day 50	Day 95	Day +7	Day −10*	Day 0	Day 50	Day 95	Day +7*
MS	37	69·0	74·8	59·3	53·0	58·8	16·3	22·8	8·3	0·0	2·5
RF	48	89·9	95·6	72·8	71·0	76·2	17·4	22·3	2·1	0·0	1·9

* Body fat determined using underwater weighing.

estimates of body composition from the stable isotopes and UWW are shown in Table 1. The isotope data indicated that MS lost 9·8 kg fat and 5·7 kg lean tissue during the first 50 d, whereas RF lost 22·9 kg fat and actually gained 0·1 kg lean tissues. Similar differences were also seen during the latter 45 d of the expedition when MS lost 5·5 kg fat and 0·8 kg lean tissues, whereas RF lost 2·2 kg fat and gained 0·4 kg lean tissue. At the end of the expedition the isotope data in both men suggested that fat content was zero, although in both men the isotope dilution spaces were exceptionally large suggesting that there had been considerable increases in the water content of the lean body mass. The UWW values for body fat at 7 d post-expedition, after considerable food intake and weight gain, were also exceptionally low in both men and hence tend to support the fact that fat stores were essentially zero on the last day of the journey.

Combining the intake data with the estimated changes in body composition yielded energy expenditures during the first 50 d of 28·3 MJ/d in MS and 38·1 MJ/d in RF, whilst during the last 45 d they were 26·6 MJ/d in MS and 23·9 MJ/d in RF. These values for the first 50 d were similar to those estimated using isotope-labelled water which gave values of 29·1 MJ/d in MS and 35·5 MJ/d in RF. In the latter 45 d there was less good agreement with isotope values of 18·8 MJ/d in MS and 23·1 MJ/d for RF (Stroud *et al.* 1996).

Table 2 shows the timing of the protein turnover studies, and gives body weights and estimated energy and protein intakes at the time of each test. It also contains some general comments on the condition of the subjects and their situation at the time. As discussed in the Methods section, the pre- and post-expedition values are less accurate than those made during the expedition. Table 3 shows the 24 h urine volume, N intake, output and the apparent N balance, together with the output of creatinine. It also shows the percentages of the dose of ^{15}N excreted in NH_3 during the first 12 h and in urea from 0–12 and from 12–24 h. If the urea pool turns over with a half-life of about 10 h (Waterlow *et al.* 1978*a*), it can be calculated that about 8 % of the [^{15}N]urea formed will still be retained in the body at 24 h. Fern *et al.* (1981), by direct measurement with [^{15}N]urea, obtained a value of 10 % for retention at 24 h and so values of total [^{15}N]urea excreted, corrected by adding 10 %, are also shown in Table 3.

There were two cases in which excretion of [^{15}N]urea was suspiciously low in the period 0–12 h: MS4 and RF3. These results suggest that the urine collections were incomplete and the NH_3 values bear this out. An incomplete collection, even if the loss is small, will be far more serious if urine is lost in the first few hours after the dose than if it is lost later on. In an attempt to clarify these possible losses, Table 3 also shows the excretion during the 12 h daytime period compared with that during the night (D:N) for a number of variables: N, creatinine, ^{15}N in urea (e_U) and ^{15}N in NH_3 (e_A). The results suggests incomplete collection in MS4, RF3, and possibly MS7. The fact that some of the D:N ratios may be

Table 2. *Position, conditions and body weights of two men, MS and RF, before, during and after an unassisted crossing of Antarctica on test days with estimates of energy and protein intakes for 07.00–19.00 hours (D), 19.00–07.00 hours (N) and total 24 h period (T)*

Test no.	Day*	Body weight (kg)			Energy intake (MJ/d)		Protein intake (g/d)		General conditions
		MS	RF		MS	RF	MS	RF	
0	Pre 7	71·7	92·0	T	31·04	29·59	248	213	Gaining weight before departure
1	9, 10	72·0	95·0	D	12·25	10·95	54	50	Position – 79 deg 37 min South. Good
				N	6·60	6·60	34	34	physical condition. Temperature −15°
				T	18·85	17·56	88	84	
2	19, 20	68·8	91·5	D	12·25	11·58	54	57	Position – 81 deg 37 min South. Reached
				N	5·39	6·60	42	34	land and beginning to climb. Onset of
				T	17·64	18·18	96	91	extreme work. Temperature −18°
3	49, 50	59·3	72·8	D	15·22	15·22	66	66	Position – 86 deg 58 min South. Edge of
				N	6·27	6·65	34	31	Polar plateau at 8000 feet. Marked
				T	21·49	21·86	100	97	fatigue and onset of hunger.
									Temperature −28°
4	77, 76	56·3	71·1	D	17·97	17·97	76	72	Position – 87 deg 57 min South. Beyond
				N	8·32	8·03	39	49	Pole on plateau at 12400 feet.
				T	26·29	26·00	115	121	Constantly cold and hungry. RF
									frostbitten. Temperature −48°
5	92, 96	53·3	71·0	D	9·74	9·82	39	39	Position – 83 deg 10 min South. Back
				N	4·14	3·72	17	17	down on Pacific ice-shelf. Episodic
				T	13·88	13·50	56	56	hypoglycaemia, marked muscle pains. RF
									and MS frostbitten. Temperature −35°
6	Post 14	58·8	76·2	T	25·42	24·49	203	185	Recovering in UK
7	Post 20	61·5	78·9	T	22·17	26·35	144	162	Recovering in UK

* The first number is for RF and the second for MS.

low when that for creatinine is not particularly reduced may be due to a small loss in an early urine specimen having a large effect on the isotope output, particularly in e_A, with little effect on creatinine.

The estimates of flux based on urea (Q_U) and NH_3 (Q_A) are shown in Table 4 (with Q_U corrected for an estimated 10 % retention of e_U at 24 h) along with synthesis rates derived from Q_U by subtraction of the 12 h N output. High synthesis rates were maintained throughout the expedition by both men.

DISCUSSION

It is evident from the weight losses alone that this expedition entailed extremely hard work, maintained for a period of over 3 months. The first part of the journey involved climbing 3500 m and in many places the terrain was made difficult and dangerous by crevasses and ridges of iced snow. Both subjects at times experienced symptoms of hypoglycaemia and RF had severe frostbite of the feet, while MS had frostbitten fingers. At the end of the journey, by the time of test 5 (days 92 and 96), they had been on half rations for 8 (RF) and 12 (MS) d. When they reached the ice-shelf of Ross Bay, having successfully completed the crossing of the Antarctic continent, they were so exhausted that they would probably have died if they had tried to reach open water.

Table 3. *Urine volume; nitrogen intake and output; apparent nitrogen balance; creatinine excretion; percentage of ^{15}N excreted in ammonia; percentage of ^{15}N dose excreted in urea 07.00–19.00 hours (D), 19.00–07.00 hours (N) and total (T); and D:N excretion values for total nitrogen, creatinine, ^{15}N isotope in urea (e_U) and ^{15}N isotope in ammonia (e_A) in two men (MS and RF) making an unassisted crossing of Antarctica*

Test no.	Urine volume (ml)	Nitrogen (g/d) Intake	Nitrogen (g/d) Output*	Nitrogen (g/d) Balance	Creatinine (g/d)	^{15}N excreted in ammonia (% of dose)	^{15}N excreted in urea (% of dose) D	N	T	Corrected T	Total N D:N	Creatinine D:N	e_U D:N	e_A D:N
MS 0	2450	39.7	17.3	15.0	2.24	1.13	8.1	4.3	12.4	13.6	0.49	1.13	1.88	7.05
MS 1	1120	14.1	12.2	−2.9	1.39	0.66	5.6	5.8	11.4	12.5	0.43	1.27	0.96	6.0
MS 2	1840	15.4	15.6	−6.3	1.88	0.90	7.4	7.8	15.2	16.7	0.44	1.10	0.95	7.5
MS 3	2980	16.0	14.9	−5.1	1.09	0.76	6.7	7.0	13.7	15.1	0.45	0.97	0.96	5.4
MS 4†	4860	18.4	12.4	2.1	1.25	0.35	3.8	7.8	11.6	12.6	0.36	1.03	0.48	1.75
MS 5	4320	9.0	13.9	−10.3	1.26	0.83	6.2	7.3	13.5	14.9	0.42	1.13	0.85	16.6
MS 6	3130	32.5	12.5	12.9	1.50	0.43	11.5	7.8	19.3	15.7	0.63	0.99	3.03	7.2
MS 7	3320	23.0	20.3	−2.5	1.82	0.59	8.2	11.2	19.4	21.3	0.29	0.93	0.73	0.6
RF 0	2450	34.1	12.0	22.7	1.90	1.29	5.5	3.3	8.8	9.7	1.47	1.00	1.55	8.0
RF 1	2370	13.5	16.7	−1.7	2.23	0.73	4.2	4.8	9.0	9.9	0.72	0.87	0.87	3.8
RF 2	2900	14.5	14.0	1.2	2.11	0.56	7.8	4.8	12.6	13.9	1.52	1.15	1.64	3.7
RF 3†	3000	15.5	12.9	3.3	2.16	0.12	1.85	7.5	9.3	10.2	0.37	0.96	0.68	1.2
RF 4	3700	18.4	16.7	2.3	1.79	0.21	6.6	4.6	11.2	12.3	1.09	1.15	1.30	1.5
RF 5	5010	5.7	12.4	−6.2	1.69	0.24	5.6	2.6	8.2	9.0	1.59	0.79	1.36	9.0
RF 6	1720	29.6	19.5	10.6	1.96	0.56	11.8	5.9	17.1	19.5	1.32	1.08	2.03	24.4
RF 7	1210	25.9	13.1	13.2	1.83	0.30	5.4	3.2	8.6	9.5	1.25	1.69	1.70	3.3

* Not corrected for non-absorbed nitrogen.
† Urine collection suspected to be incomplete.

Table 4. *Nitrogen losses 07.00–19.00 hours; nitrogen flux estimated from urea (Q_U) and ammonia (Q_A); and protein synthesis rates estimated from Q_U (S) for two men (MS and RF) making an unassisted crossing of Antarctica*

Test no.	N loss (g/12 h)	N loss (mg N/kg per h)	Q_U (mg N/kg per h)	Q_A (mg N/kg per h)	Q_U:Q_A	S (mg N/kg per h)	S (g protein/kg per 12 h)
MS 0	8·57	9·97	66·5	28·2	2·35	56·5	4·24
MS 1	5·24	6·07	45·3	31·5	1·44	39·2	2·94
MS 2	6·91	8·37	46·5	21·1	2·21	38·1	2·86
MS 3	6·80	9·55	59·0	26·3	2·25	49·4	3·71
MS 4*	5·14	7·60	47·5	76·4	—	—	—
MS 5	6·07	9·49	57·2	35·6	1·61	47·7	3·58
MS 6	8·07	11·46	61·7	68·0	0·91	50·2	3·77
MS 7	6·12	8·29	35·2	34·8	1·01	26·9	2·02
Mean (MS 1–5)	—	—	—	—	—	—	3·27 (SD 0·43)
RF 0	7·01	6·35	59·8	20·7	2·88	53·4	4·01
RF 1	7·23	6·34	57·3	23·1	2·48	50·9	3·82
RF 2	8·83	8·04	51·0	36·3	1·40	43·0	3·22
RF 3*	4·04	4·63	35·5	63·0	—	—	—
RF 4	9·42	11·04	78·5	64·3	1·22	67·5	5·06
RF 5	8·12	9·52	92·8	31·3	2·96	83·3	6·23
RF 6	11·12	12·21	59·0	37·6	1·57	46·7	3·51
RF 7	1·25	7·61	75·4	42·6	1·77	67·8	5·09
Mean (RF 1–5)	—	—	—	—	—	—	4·58 (SD 1·34)

* Urine collections were suspected to be incomplete.

Under these conditions accurate collection, recording and storing of specimens is extremely difficult and collection of urine in the daytime even risked penile frostbite. It is, therefore, almost surprising that, as far as we can tell from the internal evidence, only two tests, one for each subject, had to be rejected because of losses of urine. This does not imply a large loss, but one that must have occurred at a critical time, soon after the dose of isotope had been given. The results must be examined with this in mind. However, because the men were constantly hungry during the expedition, there was essentially no plate wastage and with pre-weighed, pre-packed, simple daily rations, the estimates of dietary intake are likely to be very accurate.

During the course of the expedition, MS tended to be in negative apparent N balance, whereas RF tended to be in positive balance, although the outputs took no account of faecal and other losses. In principal, the amounts of urea, NH_3 and other N sources excreted are irrelevant to the calculation of flux which depends only on ^{15}N abundance in the end-products. However, the estimates of protein synthesis rates will have inaccuracies dependent on the additional N excreted and it is therefore of interest to examine the likely scale of such unaccounted N losses. The rates of change of lean tissue can be calculated from the body composition data and for the first 50 d there were losses of 4·9 g N/d for MS and gains of 0·7 g N/d for RF. These values are almost identical to the mean of the three values of apparent N balance for the same period (MS1–3, RF1–3) which gave losses of 4·8 g N/d for MS and gains of 0·9 g N/d for RF. This would suggest that non-urinary N losses during the period were very small, presumably due to efficient colonic salvage. For the second 45 d period the body composition data suggested a similar pattern of changes with losses of 0·8 g N/d for MS and gains of 0·4 g N/d for RF. However, in this case it would be misleading to make a comparison with the arithmetic mean of the two pertinent measurements (MS4 and 5, RF4 and 5), since the men reduced to half rations on day 84. Instead, a better mean for the latter 45 d period must be calculated by assuming that N balance during the 34 d up to day 84 was that measured in MS4 and RF4, while for 11 d beyond the switch to half rations it was that measured in MS5 and RF5. This approach gives mean losses of 0·9 g N/d for MS and gains of 0·2 g N/d for RF, which are also remarkably close to the values derived from estimates of body composition changes.

These comparisons of N balance as assessed by two independent techniques suggest that non-accounted N losses must have been very small during the expedition. However, there must have been some additional losses via the gastrointestinal (GI) tract, the skin and non-measured urinary uric acid. GI losses of unabsorbed dietary N and other faecal losses normally range from 1–10 % of dietary intake depending on the type of diet. In the case of this expedition the low intake of dietary fibre and the probability of enhanced colonic salvage would make it likely that GI losses were at the lower end of this scale and hence that less than 1 g N/d was lost by this route. Skin losses are usually about 20 mg N/kg body weight and so would amount to a maximum of 1·8 g N/d. The urinary losses accounted for in this study included those in urea, NH_3 and creatinine, but not those in uric acid which may account for 3–5 % of urinary N for individuals on a diet of about 70 g protein/d. However, the actual amount of uric acid excreted does not normally rise with an increased protein intake and hence for the present subjects the value of 3 % of urinary N losses being in this form would seem most likely. In this case maximal additional urinary N losses would be of the order of 0·6 g/d. Overall, therefore, we believe that total unaccounted N losses are unlikely to have exceeded 4 g/d and would have led to overestimates of protein synthesis rates of only 5–10 %.

It is interesting that it was MS who was in apparent negative N balance from both body-composition studies and estimates of N intake and urinary losses. Garrow (1974) reported that when obese subjects lose weight through dietary restriction, approximately 25 % of

losses are lean, and hence energy deficits alone could have been responsible for the 10·9 % lean loss in MS. However, if this was the case one might have expected similar lean losses in RF. The dietary protein requirements for men undertaking endurance exercise of this nature are unclear. In a review, Lemon (1991) concluded that protein requirements in very active individuals may be as high as 160 % of the recommended dietary allowance. However, he also noted that there is a decline in this exercise-induced protein requirement with continued training, and Millward *et al.* (1994) concluded that in fully trained individuals, N losses associated with physical activity are minimal. However, both these reviews assume that individuals are in energy balance and during this expedition this was not the case. It seems likely, therefore, that protein oxidation was needed to fuel exertion, particularly during moderate-to-high-intensity exercise when it is likely to have provided 5–10 % of energy supply. Since MS, although much smaller, was pulling a sledge of equal weight to RF, the difference in N balance results might reflect the relatively higher exercise intensities at which MS must have worked for some of the journey.

Whatever the cause, the differences in N balance between the two men suggest that they behaved very differently in their metabolic responses to the stresses of the expedition and this individuality was also evident in the pattern of their N excretion. For example, MS excreted more N and more ^{15}N in urea during the night than the day, whereas the opposite tended to be the case for RF. The D:N ratios for creatinine were close to unity in both men, an observation that was also made during an expedition to the Andes, where day and night collections were made for 24 d (J. C. Waterlow, unpublished results). The D:N ratios for ^{15}N in NH_3 were more varied but probably have little meaning, since far more NH_3 is excreted by day and hence small fluctuations in night-time excretion have a big effect on ratios.

The main purpose of the present study was to examine how the basic biological process of protein turnover responded to conditions of extreme stress and negative energy balance imposed over a long period. There is evidence that acute physical exercise leads to an increase in body protein breakdown and a decrease in synthesis (e.g. Wolfe *et al.* 1984), but nothing is known about the effects of very-long-term exertion. In subjects exposed to infection or trauma, both components of protein turnover, synthesis and breakdown, are increased, with breakdown predominating to produce a negative N balance (Tomkins *et al.* 1983; Jeevanandam *et al.* 1986). On the food side, perhaps the best analogy is the severely malnourished child who for a long time has been short of energy and protein. In such children the rate of protein turnover is reduced (Waterlow, 1992). The Antarctic expedition provided a scenario in which all three of these elements affecting protein turnover were combined: intense exertion, some degree of infection and trauma, and food deficit.

The end-product method of measuring protein turnover with [^{15}N]glycine rests on a number of assumptions which have been justly criticized, but there is no other method that can be used in the field. The two estimates of flux that are obtained from the end-products urea and NH_3, Q_U and Q_A, are not additive, but are separate estimates of whole-body N flux. It is suggested that Q_U is biased towards the metabolism in the splanchnic bed because urea is synthesized in the liver, whereas Q_A is biased towards the peripheral tissues because the main source of glutamine, the precursor of urinary NH_3, is probably the muscles. The ratio $Q_U:Q_A$ may, therefore, give us a glimpse of the relative rates of protein synthesis in these two parts of the body. Values obtained for this ratio in normal subjects are in the range 1·25–1·30 (Fern *et al.* 1981; Fern & Garlick, 1983). In both RF and MS $Q_U:Q_A$, although variable, was almost always higher than this, and for the successful collection periods during the expedition it averaged 2·05 in RF and 1·87 in MS, perhaps indicating a relatively greater flux through the visceral organs with high protein feeding. Following the expedition, in recovery tests 6 and 7, $Q_U:Q_A$ in MS was well below the normal level,

perhaps indicating that restoration of lost muscle was taking priority, although in RF low values were not seen at this time.

For the best estimate of total N flux it has been proposed that an average of Q_U and Q_A, either arithmetic or harmonic, should be used (Fern & Garlick, 1983; Fern et al. 1985 a, b). However, since values of Q_A in the present study were very variable, probably because Q_A is subject to a number of influences such as changes in acid–base status, the synthesis rates given in Table 4 were derived only from Q_U. The mean values for protein synthesis in normal human subjects are between 2 and 3 g protein/kg per 12 h (Fern et al. 1981; Fern & Garlick, 1983) and hence the values for the pre-expedition tests were markedly raised in both men, probably due to overfeeding, which might also account for the high values seen in the first post-expedition assessments. However, average values for the four successful measurements made during the course of the expedition were also higher than might have been expected at 3·3 g protein/kg per 12 h for MS and 4·6 g protein/kg per 12 h for RF, and we believe, therefore, that the overall message from these findings is that even in the face of great exertion, marked negative energy balance and serious physical deterioration, protein synthesis can be maintained. This indicates the vital role of protein synthesis in physiological function as long as life is not extinct.

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