Obligatory urinary and faecal nitrogen losses in young Chilean men given two levels of dietary energy intake

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(*Received 27 January 1981 – Accepted 11 August 1981*)

1. The obligatory nitrogen losses were measured in young adult males of the low socio-economic group, consuming an N-free diet at 192 kJ (46 kcal)/kg per d from day 1 to 10 and 243 kJ (58 kcal)/kg per d from day 11 to 18.

2. All subjects, except one, lost weight compatible with N loss.

3. A kinetic evaluation of the results showed that the asymptotically derived urinary N loss after stability had been reached was 35·8 mg N/kg per d. The mean time to stability was 6·5 d. The subjects showed a trend toward decline in N loss while consuming the high-energy N-free diet.

4. The obligatory faecal N loss for days 1–10 was 16·1 mg N/kg per d and 8 mg N/kg per d for days 11–18.

5. Based on the factorial approach the total obligatory N loss of our subjects, for the initial 10 d, was 57·5 mg N/kg per d.

To estimate the minimum protein requirements of man the 1965 and 1973 Joint FAO/WHO Expert Groups (Joint FAO/WHO Expert Group on Protein Requirements, 1965; Joint FAO/WHO ad hoc Expert Committee on Energy and Protein Requirements, 1973) and the (US) National Research Council (1968) adopted the factorial approach. This involves measuring obligatory urinary and faecal-nitrogen losses and adding integumental losses to obtain the minimum protein-N which must be replenished in the individual. Corrections are made for decreased utilization and individual variability. This constituted the basis for establishing presently recommended human daily dietary protein safe allowances. This approach has been heavily criticized in recent years.

The measurement of such obligatory N losses has been made so far only in groups of individuals of Caucasian and oriental ethnic origin. The information obtained has been considered valid for all populations and used to formulate recommendations for all men, irrespective of their ethnic and environmental characteristics (Nicol & Phillips, 1976). In a study conducted by Scrimshaw *et al.* (1972) in eighty-three Caucasian male university students, the average value for obligatory urinary-N loss was $37{\cdot}2 \pm 5{\cdot}5$ mg N/kg body-weight and the average value for obligatory faecal-N loss was 9 ± 2 mg N/kg body-weight. In the study by Huang *et al.* (1972) with fifty Chinese male university students a value of $33{\cdot}4 \pm 4{\cdot}2$ mg N/kg body-weight was found for obligatory urinary-N loss and a value of $13{\cdot}1 \pm 2{\cdot}5$ mg N/kg body-weight was found for obligatory faecal-N loss. The latter values are significantly different from those obtained from the Caucasian subjects. These results suggest that there may be racial differences in the amount of N lost while consuming a protein-free diet. If this is true, the basis for establishing dietary protein allowances applicable to population groups on a global scale should be re-examined.

In the present study eight Chilean young males, aged 24–31 years, were studied to obtain information from subjects of low socio-economic background living under the conditions of a developing country. The results would provide a more realistic basis for establishing daily protein allowances.

Table 1. *Physical characteristics of the subjects*

Subject	Age (years)	Wt (kg)	Height (m)	W/H	Energy intake (kJ (kcal)/kg) Period 1*	Period 2*
JA	26	55·0	1·61	0·90	213 (51)	268 (64)
JB	25	74·2	1·77	1·04	176 (42)	222 (53)
OG	25	61·7	1·74	0·89	201 (48)	251 (60)
SL	28	60·5	1·66	0·94	209 (50)	259 (62)
HR	25	61·8	1·71	0·91	188 (45)	238 (57)
ER	31	63·9	1·70	0·95	197 (47)	247 (59)
RE	31	69·9	1·69	1·04	180 (43)	226 (54)
NA	24	70·6	1·70	1·06	176 (42)	222 (53)
Mean	26·9	64·7	1·70	0·96	192 (46)	242 (58)
SD	2·8	6·3	0·05	0·069	14·7 (4)	17·5 (4)

W/H, weight: height index.
* For details, see p. 12.

MATERIALS AND METHODS

Subjects

Eight young male adults, whose ages ranged from 24–31 years, belonging to the low socio-economic group participated in the study. Their physical characteristics are given in Table 1. All subjects were in good health as indicated by physical examination and medical history. They slept in the INTA Metabolic Unit for the entire duration of the experiment, and they were asked to maintain their usual daily activities refraining from unusual physical exercise. The experimental procedure was approved by the INTA Ethics Committee which supervises the use of humans as experimental subjects, and by the Research Committee. The subjects received complete information about the nature of the study and signed consent forms stating that their participation in the study was on a voluntary basis and they were free to leave at any time. During the entire duration of the study the men were under the supervision of a physician and a nurse.

Diets

The habitual protein intake of the subjects before consuming the experimental diets was estimated to be approximately 1 g/kg body-weight on the basis of a 15 d dietary history and a prospective observation period. The daily energy intake of each subject was calculated in the same way, and also based on their energy expenditure according to the pattern of their usual energy intake and activity. Table 2 lists the components of the experimental diets. As shown, this was an essentially N-free diet providing less than 2 mg N/kg body-weight. Two successive experimental periods were conducted. From day 1 to day 10 the subjects were given the N-free diet at an adequate dietary energy level (period 1). From day 11 to day 18 the level of dietary energy was raised by 25% (period 2; Table 1).

Three isoenergetic meals were provided at 08.00, 13.00 and 19.00 hours and consumed under the supervision of a dietitian. A vitamin and mineral supplement was given each day at lunch to meet or exceed the (US) National Research Council Recommended Dietary Allowances (1974). Supplements of calcium and zinc were also given.

Collection procedure and analytical methods

At 07.30 hours before breakfast the men were weighed with minimal clothing and after voiding. Complete 24 h urine collections were made throughout the study. Samples were

Table 2. *Composition of protein-free diets used for study of obligatory nitrogen losses*

Ingredient (g)	Period 1*	Period 2*
Sugar	102	112
Honey	30	55
Maize starch	224	230
Margarine	68	83
Vegetable oil	103	150
Orange-flavoured beverage	30	50
Soup flavouring	2	4
Baking powder	8	7
Carbonated beverage (ml)	414	414
Apple sauce	98	97
Alphacel	6	6
Water (ml)	1522	1722
Vitamin-mineral supplement†		
Dietary energy (%)		
Carbohydrate	50·2	44·5
Fat	49·6	55·3
Energy intake (MJ)	12·55	15·69

Food preparations: protein-free cookies, maize-starch soup, maize-starch bread, maize-starch dessert, apple sauce; the intake is given for a 63 kg subject.

*For details, see p. 12.

† Vitamin–mineral supplement (Polyterra), Laboratories Pfizer de Chile, Santiago, Chile. One tablet supplies: retinol 1500 μg, cholecalciferol 25 μg, thiamin 1 mg, riboflavin 2 mg, pyridoxine 1 mg, cyanocobalamin 2 μg, ascorbic acid 50 mg, nicotinamide 12 mg, calcium pantothenate 2 mg, copper (as CuO) 70 mg, iodine (as KI) 50 μg, iron 1 mg, potassium (as KI) 16 μg, manganese (as $MnCO_3$) 29 μg, magnesium (as MgO) 108 μg, zinc (as ZNO) 71 μg. In addition each subject received daily a 15 mg Zn supplement (as $ZnCl_2$) and a tablet of Calcium Sandoz Forte providing 500 mg of Ca.

collected in plastic containers to which 10 ml sulphuric acid (100 ml/l) were added. Each 24 h collection was made up to 3000 ml with distilled water and thoroughly mixed. Portions were analysed immediately for total N (Association of Official Analytical Chemists, 1970), urea and creatinine (Tietz, 1970). Another sample was frozen for subsequent analysis. Faeces were collected daily in plastic containers and kept in a freezer until analysed. Composites were made for each subject from the faecal pools for the entire duration of each experimental period. Capsules containing brilliant blue or carmine red dyes were used as markers (Lutwak & Burton, 1964). Blood samples were drawn from the antecubital vein after an overnight fast of 12 h at days 1, 10 and 18 and analysed for serum total protein, albumin, urea, aminotransferases and complete blood count. In addition anthropometric measurements which included height, body-weight, waist, gluteal and mid-upper arm circumference, triceps fat fold thickness and subscapular fat fold thickness were made on days 1, 10 and 18.

Statistical analyses

The results were analysed by standard procedures (Snedecor & Cochran, 1967). The analysis of obligatory urinary-N loss was made according to the approach suggested by Rand *et al.* (1976). The model used for this purpose was that of a single exponential model:

$$y = P_1 e^{-P_2 t} + P_3$$

where, y is urinary N excretion, P_1 is difference between y at time 0 and at P_3, P_2 is rate of change in N excretion, P_3 is y value at the asymptote of the curve, and t is time (d).

The parameters of this curve are P_1, P_2 and P_3 and an additional index calculated was the time required for the stabilization of P_3 (t_s). The time to stability is defined as the

Table 3. *Body-weight changes (kg) of the young male subjects studied*

Subject	Day of study 0	10	18	Total change (kg)
JA	55·0*	55·0	54·2	−0·8
JB	73·8	72·9	72·8	−1·0
OG	61·7	61·7	61·7	0·0
SL	60·2	60·2	59·1	−1·1
HR	61·0	60·4	60·6	−0·4
ER	63·2	61·6	61·6	−1·6
RE	69·9	69·7	69·6	−0·3
NA	70·6	69·8	69·6	−1·0
Mean	64·4	63·9	63·6	−0·8
SD	6·4	6·1	6·4	0·5

* Mean wt for 3 d.

Table 4. *Anthropometric measurements of young male subjects given a protein-free diet at two levels of energy intake*

Variable	Day of study 1 Mean	1 SD	10 Mean	10 SD	18 Mean	18 SD
Height (m)	1·698	0·048	1·698	0·048	1·698	0·048
Wt (kg)	64·4	6·4	63·9	6·1	63·6	6·4
Waist (mm)	832·8	53·0	818·1	51·8	808·8	50·7
Gluteal circumference (mm)	911·9	34·0	906·3	36·8	898·8	38·1
Mid-upper right arm circumference (mm)	289·4	22·9	285·4	23·4	281·9	23·4
Mid-upper left arm circumference (mm)	282·5	25·5	279·4	24·4	276·9	25·0
Right triceps fat fold (mm)	7·6	2·6	7·5	2·6	7·3	2·6
Left triceps fat fold (mm)	7·6	2·6	7·4	2·6	7·3	2·6
Right subscapular fat fold (mm)	11·9	3·5	11·7	3·4	11·5	3·3
Left subscapular fat fold (mm)	11·8	3·3	11·6	3·2	11·4	3·2

time taken for y to achieve a value which is not significantly different from $P_3 \pm$ SD. The mathematical calculations were carried out at the INTA Biometrics Unit with the aid of a non-linear least-squares fits programme, using the Marquardt algorithm from the Public Library of IBM System 370, APL language.

RESULTS

Characteristics of subjects and individual energy intakes during both experimental periods are shown in Table 1. The mean weight for height index (W/H) was slightly below ideal and significantly below those observed in young adults from developed regions. During period 1 subjects received a mean of 192 kJ/kg per d and during period 2 energy intake was increased by 25%. Tables 3 and 4 show anthropometric changes observed during the study. All subjects, except one, lost weight to an extent compatible with N loss. During the first

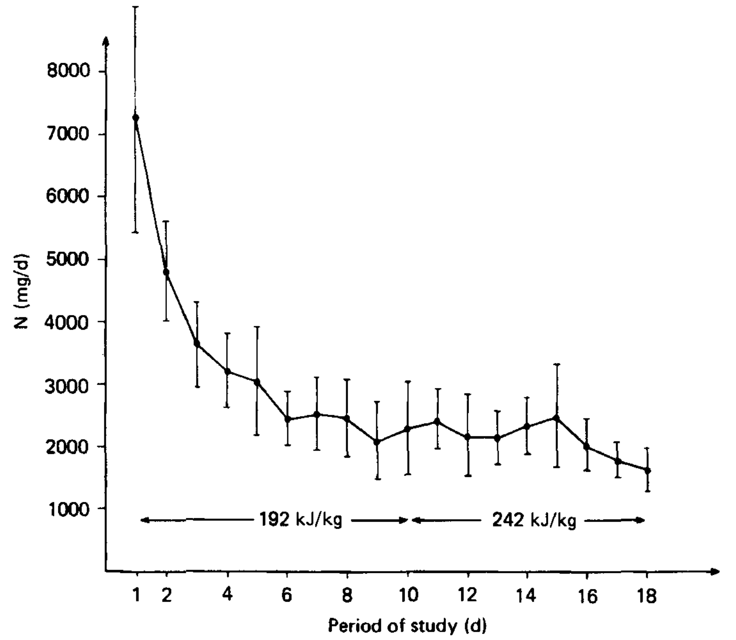

Fig. 1. Obligatory urinary nitrogen excretion in Chilean young adult male subjects given a protein-free diet at two levels of dietary energy intake. Points are mean values with their standard deviations represented by vertical bars.

10 d individuals experienced a mean loss of 0·5 kg. For the following 8 d only 0·3 kg were lost. The remaining anthropometric indices evaluated mainly the fat compartment and did not show significant changes.

Fig. 1 depicts the mean daily total urinary-N loss (mg/d) during both experimental periods.

Table 5 summarizes the individual daily N excretion (mg N/kg) while energy intake of the subjects was at maintenance levels. Subject ER on day 6 did not comply with the experimental procedure and exercised vigorously. The abnormally high value obtained for that day was eliminated and for computational purposes it was replaced with the mean value obtained from days 5 and 7. Based on methodology described by Rand *et al.* (1976) a kinetic evaluation of the values is presented in Table 5. Subject SL did not reach stability by day 10; the values obtained were not included in calculating the mean parameters of the equations. The asymptotically-derived urinary-N loss (P_3) after stability had been reached was 35·8 mg N/kg per d. The mean time to stability (t_S) was 6·5 d. The daily N excretions during the excess energy period are summarized in Table 5. Missing values for subject JB represented a lost sample, and for subjects SL and ER the fact that they completed the study on day 17. Values for subject HR on day 15 and subject ER on day 13 were not included in the pooled regression and daily means because these abnormally-elevated values corresponded to days on which the conditions of the experimental procedure were not fully met. The regression analysis of N loss *v.* time (d) with excess energy showed a trend toward decline in N loss with a negative correlation coefficient of $-0·39$ ($P < 0·01$) for the 9 d period increasing to a value of $-0·46$ if only the last 4 d were analysed.

Table 6 shows the mean urinary creatinine, urea-N and total N lost daily for the last 5 d. A significant decrease was noted for total urinary N. Table 6 also shows the mean daily

Table 5. *Kinetics analysis of daily nitrogen excretion (mg/kg per d) in young male subjects given a protein-free diet at two levels of energy intake for 18 d*

Subject... Day of study	JA	JB	OG	SL*	HR	ER	RE	NA	Mean	SD
				Dietary energy intake 192 kJ (46 kcal)/kg per d						
1	115·7	134·4	100·8	92·2	117·7	161·0	82·5	94·6	115·2	26·3
2	76·5	68·4	69·7	97·6	81·6	92·9	56·2	67·4	73·2	11·7
3	53·2	54·6	44·5	57·9	69·3	74·3	44·4	54·4	56·4	11·5
4	44·9	57·3	42·9	54·9	65·0	52·1	42·4	41·5	49·4	9·0
5	41·6	47·3	31·0	56·3	43·9	77·3	37·8	44·1	46·1	14·7
6	36·6	37·2	31·0	51·6	43·5	65·1†	32·5	34·0	40·0	11·8
7	39·9	36·3	26·7	53·4	43·9	54·1	32·7	28·7	37·5	9·5
8	29·8	32·4	32·7	53·4	45·7	54·1	27·4	33·9	36·6	9·7
9	34·8	29·0	24·2	21·4	43·5	35·2	27·4	30·0	32·0	6·4
10	31·6	33·8	25·2		65·0	33·9	27·4	27·4	34·9	13·7
P_1	159·0	229·5	135·8	92·0	136·8	244·4	93·3	106·9	158·0	58·2
P_2	0·6591	0·8488	0·5996	0·1500	0·6668	0·7885	0·5454	0·4819	0·6557	0·1295
P_3	33·46	36·19	26·27	13·29	47·50	49·91	28·44	28·60	35·77	9·47
SD	1·369	3·188	1·731	49·72	4·109	5·867	1·507	1·920		
t_s	7·2	5·0	7·3	*	5·3	4·7	7·6	8·3	6·5	1·4
				Dietary energy intake 242 kJ (58 kcal)/kg per d						
11	36·6	35·1	31·1	42·7	48·3	51·8	27·6	32·7	38·2	8·5
12	36·6	—	26·7	43·9	48·8	30·0	27·7	30·9	34·9	8·5
13	35·1	32·6	23·7	57·8	42·3	90·7‡	27·5	28·9	35·4	11·5
14	33·5	30·3	29·5	46·4	50·3	46·1	31·6	28·9	37·1	8·9
15	30·2	31·5	26·6	44·8	69·7‡	46·0	30·3	31·6	34·4	7·7
16	29·2	27·6	25·1	49·4	36·3	44·6	27·6	32·9	34·1	8·8
17	35·4	26·3	20·8	31·0	25·6	29·7	26·3	31·5	28·3	4·5
18	37·3	22·7	17·9	—	27·2	—	19·7	27·6	25·4	7·0

Regression equation: (*a*) for obligatory urinary N loss from days 1–10

$$y = 158{\cdot}0\, e^{-0{\cdot}6557\, t} + 35{\cdot}77\ (y = P_1\, e^{-P_2 t} + P_3)$$

P_1, difference between y at time 0 and at P_3 (mg N/kg per d); P_2, rate of change in N excretion; P_3, y value at the asymptote of the curve; t_s, time to stability defined as time taken for y to achieve a value which is not significantly different from $P_3 \pm$ SD (d);

(*b*) for values from days 11–18 using the complete set of values; $y = -1{\cdot}50x + 55{\cdot}4$ r $-0{\cdot}39$, $P < 0{\cdot}01$.

Using values from days 15–18; $y = -3{\cdot}31x + 85{\cdot}2$ r $-0{\cdot}46$, $P < 0{\cdot}05$.

* Subject SL did not reach a stable N loss during this period; values obtained were not included in the means.

† Mean value between days 5 and 7.

‡ Value not included in the calculation because it deviates from expected values.

Table 6. *Obligatory nitrogen losses (g/d) in young male subjects given a protein-free diet at two levels of energy intake*

Days of study...	6–10		14–18		Statistical significance of difference (paired *t* test): *P*
Energy intake (kJ (kcal)/kg per d)...	192 (46)		242 (58)		
	Mean	SD	Mean	SD	
Creatinine	1·33	0·25	1·22	0·29	NS
Urea N	1·41	0·68	1·54	0·46	NS
Total urinary N	2·365	0·477	2·107	0·388	< 0·01
Faecal N	1·029	0·194*	0·562	0·141†	< 0·001

NS, not significant.
* Faecal-N losses for days 1–10.
† Faecal-N losses for days 11–18.

Table 7. *Plasma biochemical measurements for young male subjects given a protein-free diet at two levels of energy intake*

Day of study...	1		10		28		Statistical significance of difference (ANOVA)	
	Mean	SD	Mean	SD	Mean	SD	*P*	LSD
Protein (g/l)	74	5	69	5	69	6	NS	—
Albumin (g/l)	50	3	46	3	42	3	< 0·001	0·61
Glucose (mg/l)	814	87	783	85	866	64	NS	—
Urea (mg/l)	249	52	102	26	125	39	< 0·001	7·7
Urea N (mg/l)	116	34	47	12	58	18	< 0·001	3·6
SGOT (U/l)*	138	52·8	110·9	52·3	185·3	52·8	< 0·05	11·4
SGPT (U/l)*	82·1	49	66·7	46·1	73·9	69·6	NS	—
Creatinine (mg/l)	6·5	1	7·9	1	7·2	1	< 0·05	0·01
Triglycerides (mg/l)	528	251	449	115	491	125	NS	—
Cholesterol (mg/l)	1749	381	1608	422	1346	143	NS	—

NS, not significant; LSD, Least Significance Difference; SGOT, serum glutamic-oxaloacetic transaminase (*EC* 2.6.1.1); SGPT, serum glutamic-pyruvate transaminase (*EC* 2.6.1.2).
* Standard units.

faecal-N excretion for both experimental periods. A marked decline in N lost by this route was documented.

Table 7 summarizes biochemical measurements obtained initially and at the end of each period. Significant declines were observed in albumin, urea-N and serum urea-N. We also observed a significant rise in serum glutamic-oxaloacetic transaminase (SGOT) (*EC* 2.6.1.1) on day 18.

Based on the factorial approach we calculated and present in Table 8 the total obligatory N losses during the initial 10 d obtaining a value of 57·5 mg N/kg per d. Table 8 includes FAO/WHO reported values for comparison. Based on our results the protein requirement to satisfy most of the population would be 0·62 g protein/kg per d which agrees with other reported values.

Table 8. *Factorial nitrogen losses (mg/kg per d) for young male subjects given a protein-free diet*

Subject	P_3*	Urinary N†	Faecal N‡	Total obligatory N losses‖	Correction factor 1·3
JA	33·5	34·5	13·9	53·4	69·4
JB	36·2	33·7	16·0	54·7	71·1
OG	26·3	28·0	18·9	51·9	67·5
SL	13·3	45·0	14·0	64·0	83·2
HR	47·5	48·3	18·0	71·3	92·7
ER	49·9	48·5	20·2	73·7	95·8
RE	28·4	29·5	13·7	48·2	62·6
NA	28·6	30·8	13·7	49·5	64·4
Mean	35·8	36·2	16·1	58·4	75·8
SD	9·5	8·6	2·6	9·9	12·9
FAO/WHO (1973)	—	37	12	54	70

* Urinary-N loss (mg/kg per d) after asymptotic stabilization.
† Obligatory urinary-N losses for days 6–10.
‡ Obligatory faecal-N losses for days 1–10.
‖ Assuming 5 mg N/kg per d for integumental and miscellaneous losses.

DISCUSSION

Our results indicate that the capacity to adapt to an essentially protein-free diet is inherent to mammalian metabolic regulation independent of ethnic and environmental conditions. The results are in accordance with previous results obtained in North American and Taiwanese subjects which served as a basis for establishing daily protein allowances by the factorial method.

The sum of N losses while consuming a protein-free diet represents the minimum N required and appears to be a reliable consistent measurement (FAO/WHO ad hoc Expert Committee on Energy and Protein Requirements, 1973). The correction factors for decreased utilization and individual variability at higher intakes closer to maintenance have been insufficiently studied, weakening the validity of the factorial approach accurately to predict protein requirements for N equilibrium. The factorial method has been shown to underestimate protein requirements for even short-term balance if the presently-accepted correction factors are used (Garza *et al.* 1977*a*, *b*). It is of interest that our N balance values from similar subjects while consuming graded levels of egg protein in the sub-maintenance range gave a mean requirement of 0·62 g protein/kg per d (Yáñez *et al.* 1981) *v.* a factorially-derived value of 0·45 g (Joint FAO/WHO ad hoc Expert Committee on Energy and Protein Requirements, 1973). These results confirm the inadequacy of the 30% correction for less efficient use of protein at higher intakes.

It is remarkable that the obligatory urinary-N losses we observed are virtually identical to those reported by Scrimshaw *et al.* (1972) and similar to those of Huang *et al.* (1972). But faecal-N losses in our subjects are much higher than those observed previously. This observation suggests that even endogenous N digestibility may be reduced in subjects from developing regions with poor environmental sanitation and chronic sub-clinical intestinal mucosal damage. Continuing the N-free diet showed an important decrease in faecal N to nearly half the value obtained in the first pool, which is comparable to the N loss reported by Scrimshaw *et al.* (1972). This finding could possibly be attributed to the additional decline in labile N after stability had been reached or to changes in gut flora which contribute to faecal-N losses.

Almost all subjects reached stability in urinary N loss by day 7. Subject SL did not reach stability until day 14. Therefore values obtained were excluded from the pooled data analysis. It was decided that this individual would be re-evaluated in a future study. Subject ER was noticed to have a sudden rise in urinary N in day 6 in relation to unauthorized heavy exercise resulting in a probable energy deficit for that day. Most subjects steadily lost weight during the initial 10 d and lost less during the last 8 d.

Our subjects received slightly higher energy intakes than was customarily used in this type of study. This approach was based on a careful evaluation of the habitual intake, activity pattern and a prospective observation of weight change and food intake. It is also noteworthy that for our subjects as for others previously studied with comparable methodology those with low W/H indices had higher energy intakes if expressed on a unit body-weight basis. Since our subjects had a slightly lower weight relative to ideal weight for height we would expect their energy intakes to be higher. The weight loss was commensurate with cumulative N loss and can be accounted for by a loss in lean tissue, mainly muscle, as evidenced by a drop in mean daily creatinine excretion. This finding can probably be explained by the excess energy provided during this latter period.

The effect of excess energy given in the original studies on obligatory N losses has been suggested as a possible explanation of the inadequacy of current factorially-derived protein recomendations. Our results cannot exclude an effect determined by the prolongation of the protein-free period but the excess energy did decrease significantly the urinary-N loss especially during the last 4 d. Monitoring energy expenditure was not feasible in our study but subjects did maintain a stable activity pattern which was recorded daily. The effect of energy balance deficit can be anticipated to raise obligatory N losses. Nevertheless the biological significance of excess energy appears to be minor.

All subjects were closely monitored during the experiment and none reported subjective symptoms related to the protein-free diet or showed decreased work performance. It is indeed remarkable that these subjects were able to tolerate without untoward effects protein deprivation. The functional significance of short-term dietary protein deficits seems to be minor for young adults.

The biochemical changes induced by the N depletion can be documented by the significant decrease in serum urea-N and a slight decrease in albumin. The rise in SGOT observed on day 18 confirms the sensitivity of this measurement in determining depletion of essential amino-N. Previous studies have shown significant rises in serum non-essential amino acids (NEAA) level and declines in essential amino acids (EAA) including histidine. Our results on serum amino acids and hormone profiles are pending analysis and will be reported elsewhere.

If we accept the presently-used correction factor for utilization and use the coefficient of variation from our results the factorially-derived daily safe recommended allowance is 0·62 g protein/kg in terms of egg or milk protein. In the light of our N balance results this amount was enough for only half the subjects studied (Yáñez *et al.* 1981).

The need for further research on the changes in protein utilization and digestibility at maintenance range of intakes in subjects living under conditions prevailing in developing nations is established, based on this study.

We can conclude that for low-income Chilean subjects factorially-derived requirements are slightly higher than previously reported. This difference is entirely due to higher faecal-N loss. Excess energy produces a slight decline in obligatory urinary-N losses.

The authors gratefully acknowledge Laboratorios Pfizer de Chile for kindly supplying the vitamin–mineral supplement (Polyterra) used in this experiment. The authors also acknowledge the excellent secretarial assistance of Ms Viola Lyon. The collaboration of the subjects involved in the study is also highly appreciated.

REFERENCES

Association of Official Analytical Chemists (1970). *Official Methods of Analysis*. 11th ed., Washington, DC: Association of Official Analytical Chemists.

Calloway, D. H., Odell, A. C. & Margen, S. (1971). *J. Nutr.* **101**, 775.

FAO/WHO (1965). *Protein requirements*. Report of a Joint FAO/WHO Expert Group. *FAO Nutr. Mtgs. Rep. Ser.* no. 37, *Tech. Rep. Ser. Wld Hlth Org.* no. 301.

FAO/WHO (1973). *Energy and protein requirements*. Report of a Joint FAO/WHO Ad Hoc Expert Committee. *FAO Nutr. Mtgs. Rep. Ser.* no. 52, *Tech. Rep. Ser. WHO* no. 522.

Garza, C., Scrimshaw, N. S. & Young, V. R. (1977*a*). *Br. J. Nutr.* **37**, 403.

Garza, C., Scrimshaw, N. S. & Young, V. R. (1977*b*). *J. Nutr.* **107**, 335.

Huang, P. C., Chong, H. E. & Rand, W. M. (1972). *J. Nutr.* **102**, 1605.

Lutwak, L. & Burton, B. T. (1964). *Am. J. clin. Nutr.* **14**, 109.

National Research Council, Food and Nutrition Board (1968). *Recommended Dietary Allowances*. Washington, DC: National Research Council/National Academy of Sciences.

National Academy of Sciences (1974). *Recommended Dietary Allowances*. 8th ed. Washington, DC: National Academy of Sciences.

Nicol, B. M. & Phillips, P. G. (1976). *Br. J. Nutr.* **35**, 181.

Rand, W. M., Young, V. R. & Scrimshaw, N. S. (1976). *Am. J. clin. Nutr.* **29**, 639.

Scrimshaw, N. S., Hussein, M. A., Murray, E., Rand, W. M. & Young, V. R. (1972). *J. Nutr.* **102**, 1595.

Snedecor, G. W. & Cochran, W. G. (1967). *Statistical Methods*, 6th ed. Ames, Iowa: The Iowa State University Press.

Tietz, N. W. (1970). In *Fundamentals of Clinical Chemistry*. p. 722 [W. B. Saunders, editor]. Philadelphia Pa.

Yáñez, E., Uauy, R., Ballester, D., Barrera, G., Chávez, N., Guzmán, E., Saitúa, M. T. & Zacarías, I. (1981). *Br. J. Nutr.* **47**, 1.

Printed in Great Britain