Human protein requirements: evaluation of the 1973 FAO/WHO safe level of protein intake for young men at high energy intakes

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1. Six Caucasian male Massachusetts Institute of Technology students participated in a 77–87 d metabolic balance study to determine the adequacy of the 1973 FAO/WHO egg protein allowance for men (0·57 g/kg body-weight per d). Each subject was given an initial energy allowance calculated to meet his particular requirements, and these intakes were raised by increments of 10% approximately every 2 weeks until a slightly positive balance was achieved. Each individual's energy intake was maintained at this final level for the remainder of the study.

2. At energy intakes sufficient to meet their estimated requirements, five of six subjects were in negative nitrogen balance. In five subjects, N balance improved with increased energy intake until N balance was achieved. The mean change in N balance was 0·335 mg N/additional kJ consumed. All of the subjects gained weight at the higher energy intakes.

3. Serum aspartate aminotransferase and alanine aminotransferase activities increased with continued intake of the experimental diet and reached abnormal levels in five of the six subjects. In the two subjects showing the earliest increases in serum transferase activity, the levels returned towards normal when protein intake was raised to 0·73–1·0 g/kg body-weight per d. All subjects showed normal serum transferase values on an *ad lib.* diet 2–3 weeks after termination of the study.

4. The findings indicate that at energy intakes necessary to bring subjects into slightly positive N balance at the level of 0·57 g egg protein/kg per d, a significant proportion of young adults continue to gain weight for as long as 70 d.

5. These findings suggest that the 1973 FAO/WHO safe allowance of 0·57 g egg protein/kg per d is not sufficient for most healthy young men receiving dietary energy intakes appropriate for long-term maintenance of body-weight.

We have previously reported (Garza, Scrimshaw & Young, 1976, 1977) the results of two metabolic balance studies designed to assess the adequacy of the FAO/WHO safe level of egg protein intake for healthy young men (FAO/WHO, 1973). In the first study, we concluded that the safe level of egg protein would maintain nitrogen balance only when excess energy was provided by the experimental diet. The second paper reported that under conditions of a long-term metabolic balance study and at energy intakes 10% above estimated requirements, the FAO/WHO level of egg protein was not sufficient to maintain an adequate nutritional status, as judged by negative cumulative N balances, decreases in total body potassium (TBK), and/or alterations in the serum protein profile. In addition, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities rose to abnormal levels in two of six subjects. The TBK and urinary creatinine results indicated that the N balance method may significantly underestimate N losses, as others have also reported (Holmes, Jones, Lyle & Stainer, 1956; Walker, 1962; Mitchell & Edelman, 1962; Consolazio, Nelson, Matoush, Harding & Canham, 1963; Isaksson & Sjögren, 1967).

Because of the interaction between energy intake and protein utilization, most studies designed to estimate the 'minimum protein requirement' have utilized generous energy intakes to ensure that a lack of dietary energy does not adversely affect protein utilization. Findings from such studies have served as the basis for dietary recommendations, which, as a result, have generally ignored both the effects of the apparently high energy levels necessary to maintain N equilibrium at low levels of protein intake and the relationship of these high energy intakes to actual energy requirements. The 1973 FAO/WHO 'safe level' of protein intake must now be re-evaluated at energy levels sufficient for at least temporary positive N balance, in order (a) to determine whether N balance can be maintained at these high energy intakes, and (b) to measure physiological parameters other than N balance under these conditions.

Six young men participated in this metabolic balance study lasting 77–87 d, and the results support the conclusion of our previous experiments (Garza *et al.* 1976, 1977), i.e. the 1973 FAO/WHO 'safe level' of egg protein is inadequate for young men. Our findings also indicate that a 10% excess energy intake does not raise the efficiency of N utilization to a level sufficient for maintenance when this amount of egg protein is consumed for extended periods.

EXPERIMENTAL

Subjects and experimental design

The six Caucasian male subjects (Table 1) were free of any abnormalities detectable by medical history, physical examination, blood haematocrit, haemoglobin, total and differential white blood cell determinations, urinalysis, and measurement of AST and ALT activities. Subjects continued their normal daily routines, which included their full academic schedules, but refrained from any unusual physical activity.

The initial dietary period provided 0·57 g egg protein/kg body-weight per d and the individual energy requirements, as estimated by a dietary history and appropriate nomograms (Wilder & Russel, 1950). Vitamin and mineral supplements were provided as previously described (Garza *et al.* 1976). The composition of the four equal daily meals has been described (Garza *et al.* 1976). Carbohydrate and fat each supplied approximately 40–50% of the energy intake during each period.

The first week of the initial 16–17 d period was allowed for adaptation to the experimental diet, and unless a positive N balance was achieved during the subsequent 10 d period, the subjects' energy intakes were increased by 10%. N balance was determined by subtracting daily urinary N, average daily faecal N (as calculated from the 10 d pools), and daily integumental and miscellaneous losses (5 mg N/kg body-weight) from the total N intake. The incremental increases in energy intake were repeated until each individual came into positive N balance within approximately 2 weeks. After achieving positive N balance, each subject continued at the corresponding energy intake for the remainder of the study.

In a previous study using 0·59 g egg protein/kg (Garza *et al.* 1977), increased AST and ALT activities fell quickly when protein was increased to 1·5 g/kg body-weight.

Table 1. *Characteristics of subjects*

Subject	Age (years)	Body-weight (kg)	Height (m)
D.C.	22	67·8	1·73
T.C.	21	82·9	1·80
S.L.$_1$	21	63·4	1·78
S.L.$_2$	18	72·2	1·70
M.R.	20	55·4	1·66
R.W.	21	72·9	1·79

Table 2. *Summary of individual energy intakes, nitrogen balance and weight changes for each dietary period*

Subject	Diet period	Duration* (d)	Energy (kJ(kcal)/kg)	Protein level (g/kg)	Average daily N balance (g)	Wt change (kg)
D.C.	1	16	184 (44)	0·57	−0·87†	—
	2	10	205 (49)	0·57	−0·30	—
	3	51	226 (54)	0·57	+0·14	+1·0‡
T.C.	1	32	196 (47)	0·57	−0·34†	0·0§
	2	13	213 (51)	0·57	0·00	—
	3	28	215 (51)	0·73	+0·26†	+1·2‡
	4	14	205 (49)	1·00	+0·81	—
S.L.$_1$	1	17	180 (43)	0·57	−0·53†	—
	2	15	196 (47)	0·57	−0·15	—
	3	34	196 (47)	0·73	0·00†	+0·7‡
	4	12	196 (47)	1·00	+0·90	—
	5	9	180 (43)	1·00	0·00	—
S.L.$_2$	1	17	196 (47)	0·57	−0·65†	—
	2	15	213 (51)	0·57	−0·10	—
	3	55	234 (56)	0·57	+0·10	+2·6§
M.R.	1	17	209 (50)	0·57	0·00†	—
	2	70	230 (55)	0·57	+0·26	+3·1‡
R.W.	1	17	176 (42)	0·57	−1·30†	—
	2	15	192 (46)	0·57	−0·67	—
	3	18	213 (51)	0·57	−0·70	—
	4	24	234 (56)	0·57	+0·33	+2·4‡
	5	5	234 (56)	0·73	+1·74	—
	6	8	213 (51)	0·73	+0·58	—

* Each subject was studied for a total of 87 d, with the exception of subject D.C., who was studied 77 d.

† The first 7 d were not included in estimating average N balance and wt change.

‡ Wt change is based on the slope of a regression equation relating weight to the time on the diet.

§ Wt change is based on the difference between the mean of the first 3 and last 3 d of the dietary period.

In the present study, we wished to determine whether increased enzyme activities would respond to smaller increments in protein. Therefore, for subjects who developed sustained abnormal AST and ALT activity levels while consuming 0·57 g egg protein/kg, protein intakes were increased by 30% to 0·73 g egg protein/kg. This level of protein intake was increased further to 1·0 g/kg if the transferase levels did not decrease to normal levels after 1 month at the 0·73 g/kg intake.

One subject (T.C.) followed a different protocol because an earlier study (Garza *et al.* 1976) had shown that he required 196 kJ (47 kcal)/kg body-weight to achieve N balance at 0·57 g/kg protein intake. This energy level was used during his initial 32 d period even though his usual energy requirement (estimated by diet history and nomograms) was 180 kJ (43 kcal)/kg.

Individual energy intakes, dietary protein levels, and duration of the diet periods are summarized in Table 2.

Samples and measurements

Complete daily urine and faecal collections were made throughout the study, and the faecal samples were pooled for 10 d periods. Total N, urea, urinary creatinine, faecal and dietary N and TBK were measured as previously described (Young & Scrimshaw, 1968). Nude body-weights were obtained daily after each subject had voided the first morning urine, but before he had eaten breakfast.

Venous blood samples were drawn before breakfast from the antecubital fossa at approximately two-weekly intervals. Serum glucose, urea N, creatinine, AST, ALT, total protein, and albumin were measured in addition to haematocrit, haemoglobin, and total and differential white blood cell counts (Young, Taylor, Rand & Scrimshaw, 1973). Total serum proteins were separated by electrophoresis (Chin, 1970), and lactate dehydrogenase (LDH) isoenzymes were also determined (Elejitch, Aronson, Fleichtmeir & Enterline, 1966).

Statistical analyses

During each diet period, body-weight and urinary N excretion were nested in 3 d groups to determine the linearity of changes by analysis of variance (Afifi & Azen, 1972). A 5% confidence level was chosen for assessing linearity and, when appropriate, for determining the significance of slopes. If significantly different from zero, the mean of the slope of equations relating body-weight to time was accepted as the best estimate of daily weight change.

Whenever body-weight changes proved to be nonlinear, one-way analysis of variance was used to test the significance of the differences among the 3 d groups. If significant differences were found, the *t*-statistic was used as described by Dixon & Massey (1969) to estimate the difference between the means of appropriate 3 d periods. If the 95% confidence interval did not include zero, the difference between means was accepted as the best estimate of net change. If this interval included zero, weight was judged to have remained stable. This method treats day-to-day variability objectively, and uniform criteria were used to estimate net weight changes. Body-weight changes for periods shorter than 20 d were not estimated.

Estimation of energy requirements

The difficulties in estimating individual energy requirements are well known (Durnin, Edholm, Miller & Waterlow, 1973; Hegsted, 1974). Problems arise in attempting to measure changes in body composition, in physical activity in response to alterations in energy intake, and in the efficiency of energy utilization at various energy levels. Individual energy requirements were estimated by two methods: method A was based

on nomograms and diet interviews conducted by our staff nutritionist; method B was based on weight changes and estimates of N balance. A value of 30 g N/kg lean body-mass (Widdowson & Dickerson, 1964) was used to calculate the expected weight change from N balance values. The result was compared with the actual weight change, and the difference between observed and expected values was assumed to represent a loss or gain of body fat. Using an estimate of 29·26 MJ (7000 kcal) for the energy value of 1 kg of body fat (Hegsted, 1974; Garrow, 1974), we either added or subtracted the energy equivalent of the calculated daily change in body fat from the actual energy intake in order to estimate individual energy requirements. The additional energy cost of the synthesis of fat tissues was not considered.

To minimize potential sources of error, subjects maintained a diary-record of any deviations from normal routines and were interviewed daily concerning their physical activities.

Ethical considerations

The experimental protocol received the administrative approval of the Massachusetts Institute of Technology Committee on the Use of Humans as Experimental Subjects and the Executive and Policy Committees of the Massachusetts Institute of Technology Clinical Research Center. The purposes and nature of the experiments, and possible risks and hazards involved, were discussed with the subjects and they were required to sign consent forms. They were free to terminate their participation in the study at any time.

RESULTS

Body-weight

Weight changes over diet periods longer than 20 d are summarized in Table 2. Figs 1 and 2 graphically illustrate the body-weights during the diet periods providing 0·57 and 0·73 g protein/kg per d. Of the diet periods longer than 20 d, six were characterized by weight gain and one by weight stability. All weight gains exceeded the predicted changes based on the N balance values. There were no clear indications that weight had stabilized during the six periods characterized by weight gain.

Urinary N

Three of the six subjects remained on the same energy and protein intakes for periods longer than 50 d. Two of the other subjects (T.C. and $S.L._1$) developed abnormal serum transferase activity levels early in the study, and the sixth subject (R.W.) did not achieve positive balance until day 50, after three increments in energy intake. He developed abnormally high transferase levels during the final period when N balance was positive.

Figs 3 and 4 show that among the three subjects who maintained a slightly positive N balance for more than 50 d at 0·57 g protein/kg, no consistent change occurred in the urinary N (U_N) output. Subject $S.L._2$ had a statistically significant linear decrease in U_N, which amounted to 10 mg N/day. U_N remained stable for subject M.R. throughout the 70 d diet period; for subject D.C., it remained stable during the first

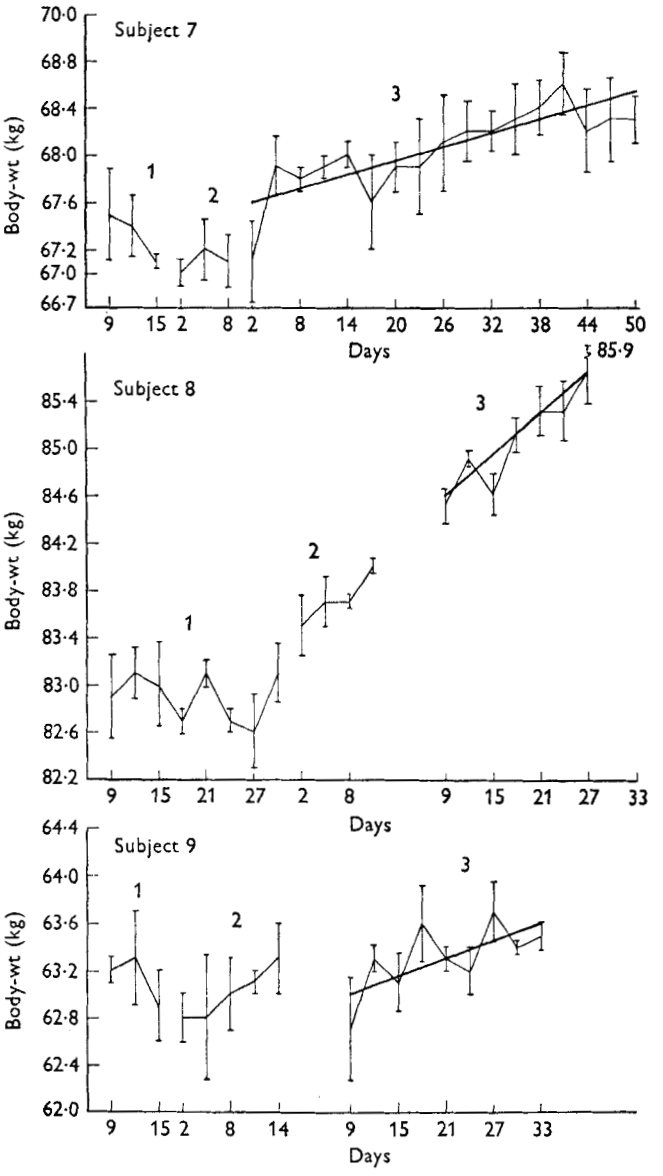

Fig. 1. Daily weight (kg, mean ± SD) *v.* time (d) on a specific protein and energy intake. Straight lines indicate the periods with statistically significant linear changes in weight; the absence of such a line indicates a nonlinear change. Methods of analysis are described in the text. The specific diet periods of each subject are referred to by numbers (1, 2, and 3) and the vertical lines give the mean ± 1 SD. Subject key: 7 D.C.; 8 T.C.; 9 S.L.$_1$.

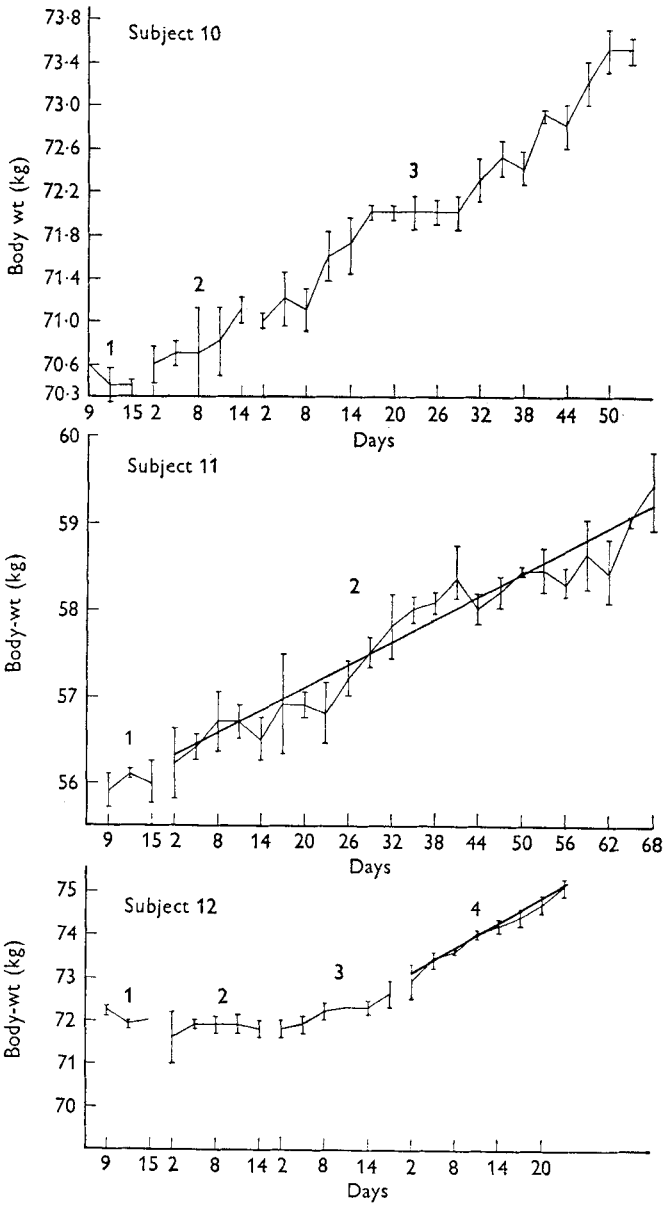

Fig. 2. Daily weight (kg, mean ± SD) *v.* time (d) on a specific protein and energy intake. Straight lines indicate the periods with statistically significant linear changes in weight; the absence of such a line indicates a nonlinear change. Methods of analysis are described in the text (see p. 406). The specific diet periods of each subject are referred to by numbers 1, 2, 3 and 4) and the vertical lines give the mean ± 1 SD. Subject key: 10 S.L.$_2$; 11 M.R.; 12 R.W.

third of his 51 d period, declined during the second third, and increased during the final third. Despite these differences in individual U_N responses, U_N was negatively correlated with time ($P < 0.05$). For subjects D.C., S.L.$_2$, and M.R., the correlation coefficients were 0·24, 0·36, and 0·22, respectively.

Two subjects developed infections during the study. Subject M.R. complained of symptoms of an upper respiratory tract infection on day 24. A throat culture yielded scant growth of coagulase-positive *Staphylococcus aureus*, and he was treated with oxacillin for a week. He did not develop a fever, and his symptoms were mild. Fig. 4 indicates the duration of his symptoms. No unusual fluctuations in U_N excretion were observed during this period. Subject T.C. reported symptoms of a urinary tract infection during the first few days of the final dietary period, at 1·0 g protein/kg body-weight per d. He was treated with tetracycline and all signs and symptoms returned to normal by the end of the study.

Faecal N

Analysis of variance indicated no time-related differences in faecal N among the individuals studied. Mean values were 0.87 ± 0.18 g N/d or 12.8 ± 2.50 mg/kg per d. The average apparent digestibility was 86 %. Assuming 12 mg N/kg per d for endogenous faecal losses (FAO/WHO, 1973), we estimated the true digestibility to be 99 %. While losses of faecal N at 1·0 g egg protein/kg tended to be greater than at 0·57 g/kg, the difference was not statistically significant (*t*-test: $P < 0.05$).

Total body potassium (TBK)

Results for TBK are summarized in Table 3. The marked fluctuations among successive measurements limit the usefulness of these findings. Changes equal to or greater than 10 g of K characterized more than 20 % of the measurements, and almost 40 % of the successive measurements differed by more than two standard deviations. Moreover, these changes seldom suggested any directional consistency.

Energy intakes necessary for N balance

Subjects M.R. and R.W. showed the most positive N balances, which were obtained by increasing their energy intakes (Table 2). Subject M.R.'s average N balance was 0·26 g N/d during a 70 d period. During this period, he consumed 230 kJ (55 kcal)/kg and his weight increased at an average rate of 44 g/d. His N balance was negative only during the initial week of the study. Subject R.W.'s average N balance was 0·33 g N/d during a 24 d period. For 50 d preceding this period, he was in negative N balance. While in positive balance, he consumed 234 kJ (56 kcal)/kg and gained weight at an average rate of 98 g/d. All of the subjects experienced a constant feeling of fullness and sensed they could not increase their intake further and still function efficiently.

The striking linear relationship between N balance and energy intake, which we observed in our previous study (Garza *et al.* 1976*a*), was not apparent in this study. The best approximations of the energy intake necessary to achieve N balance were (kJ(kcal)/kg): subject D.C., between 205 (49) and 226 (54); subject T.C., about 213 (51); subject S.L.$_2$, between 213 (51) and 234 (56); subject M.R., about

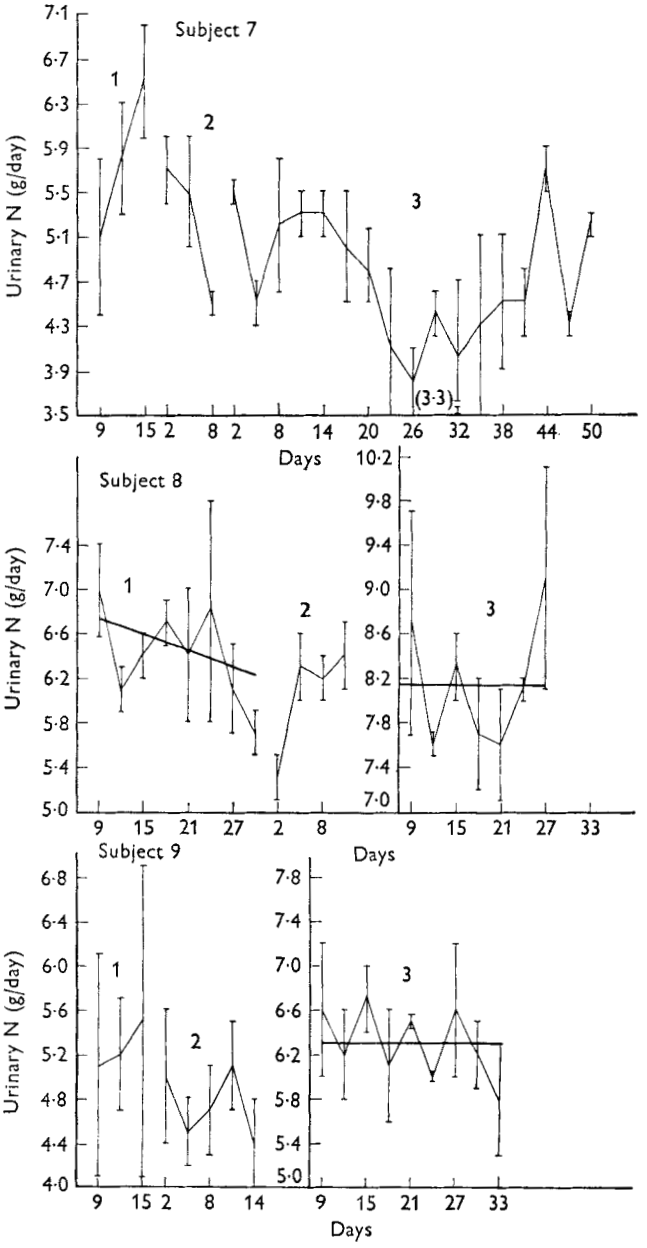

Fig. 3. Daily urinary N excretion (g, mean ± 1 SD) *v.* time (d) on a specific protein and energy intake. Straight lines indicate periods with statistically significant linear changes in daily total urinary N excretion; the absence of such a line indicates a nonlinear change. Methods of analysis are described in the text (see p. 404). The specific diet periods of each subject are referred to by numbers (1, 2 and 3) and the vertical lines give the mean ± 1 SD. Subject key: 7 D.C.; 8 T.C.; 9 S.L.$_1$.

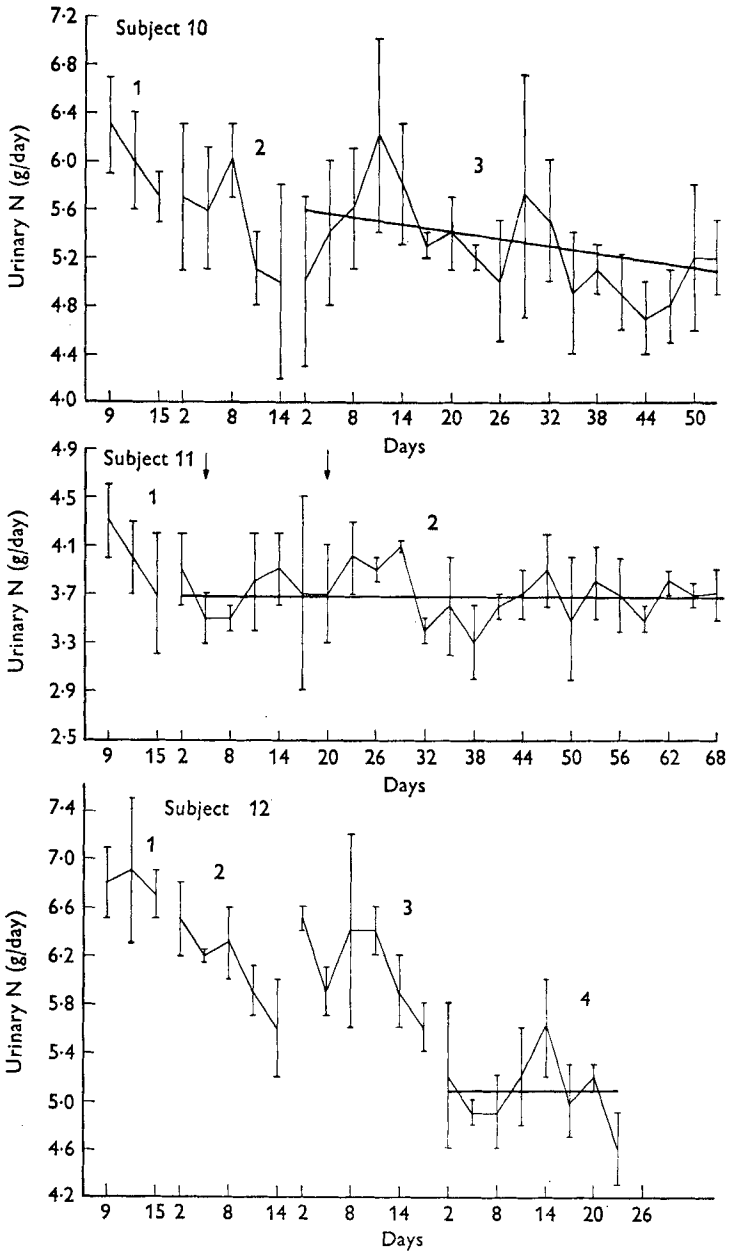

Fig. 4. Daily urinary N excretion (g, mean ±SD) *v.* time (d) on a specific protein and energy intake. Straight lines indicate periods with statistically significant linear changes in daily total urinary N excretion; the absence of such a line indicates a nonlinear change. Methods of analysis are described in the text. The specific diet periods of each subject are referred to by numbers (1, 2, 3 and 4) and the vertical lines give the mean ±1 SD. Subject key: 10 S.L.$_2$; 11 M.R.; 12 R.W.

209 (50); and subject R.W., between 213 (51) and 234 (56). Subject S.L.$_1$, at 196 kJ(47 kcal)/kg, had the highest energy intake during the 0·57 g protein/kg period; however, his N balance was slightly negative at this level of energy intake.

Table 3. *Summary of total body potassium (TBK) results during dietary period*

Subject	TBK measurements* (g)					
	Day 4	Day 32	Day 46	Day 60	Day 74	Day 87
D.C.	159±4†	146±4	161±4	156±4	155±4	—
T.C.	—	181±4	173±4‡	172±4‡	178±4§	165±4§
S.L.$_1$	143±4	142±4	133±4‡	140±4‡	135±4§	131±4§
S.L.$_2$	149±4	141±3	144±4	141±3	143±4	143±4
M.R.	128±3	129±3	131±4	134±3	128±3	138±4
R.W.	177±4	163±4	170±4	160±4	169±4	

* Mean ± 1 SD.
† Measurement obtained on day 12.
‡ Subject intake 0·73 g egg protein/kg body-weight.
§ Subject intake 1·0 g egg protein/kg body-weight.

Table 4. *Comparison of energy requirements estimated by methods A and B**

Subject	Estimated energy requirement†		Actual intake (kJ(kcal)/kg)
	Method A (kJ(kcal)/kg)	Method B (kJ(kcal)/kg)	
D.C.	184 (44)	217 (52)	226 (54)
T.C.	180 (43)	291 (48)	196 (47)
	180 (43)	196 (47)	213 (51)
S.L.$_1$	180 (43)	184 (44)	196 (47)
S.L.$_2$	196 (47)	222 (53)	234 (56)
M.R.	209 (50)	213 (51)	230 (55)
R.W.	176 (42)	201 (48)	234 (56)

* See text for details, p. 406.
† The estimated energy requirements derived by method B are based on the following diet periods of each subject: subject D.C., diet period 3; subject T.C., diet periods 1 and 3, respectively; subject S.L.$_1$, diet period 3; subject S.L.$_2$, diet period 3; subject M.R., diet period 2; and subject R.W., diet period 4.

Estimated energy requirements

Table 4 compares the energy requirement estimates based on methods A and B. Estimates obtained by method B were 15, 12, 13 and 14% greater than those obtained by method A for subjects D.C., T.C., S.L.$_2$ and R.W., respectively. The two estimates were in agreement for subjects S.L.$_1$ and M.R. Subjects T.C. and S.L.$_1$ received 0·73 g egg protein/kg during their third dietary period, a fact that should be noted when comparing estimates obtained during these periods (Table 4).

Biochemical measures in serum

Tables 5 and 6 summarize changes in serum total proteins, albumin, globulin levels (as determined by the difference between total protein and albumin), and various globulin fractions separated by electrophoresis. No general response or relationship to N balance was evident for total serum proteins. Albumin increased or remained stable in all of the individuals studied. Values for the separated globulin fractions of subjects D.C., S.L.$_2$, M.R. and R.W. (Table 6) represent the initial and final measure-

Table 5. *Serum biochemical measurements*

Subject	Day	Total proteins (g/l)	Albumin (g/l)	Globulin (g/l)	Alanine amino-transferase (ALT)* (i.u./l)	Aspartate amino-transferase (AST)† (i.u./l)
D.C.	0	67	47	20	12	14
	15	72	50	22	10	22
	29‡	77	52	25	12	31
	43	76	57	15	5	22
	59	—	50	—	29	27
	73	75	55	20	45	29
	77	76	54	22	34	23
T.C.	0	74	45	29	17	17
	15	76	50	26	56	34
	29	71	49	22	31	27
	43‡	69	52	17	104	53
	59§	73	42	31	60	34
	73	77	54	23	54	31
	77	—	—	—	35	15
	85	74	—	—	—	—
	87	—	—	—	56	39
S.L.$_1$	0	75	46	29	12	17
	15	74	49	25	56	39
	29	74	50	24	48	31
	43‡	75	52	23	28	18
	59	73	42	31	27	29
	73	75	51	24	24	19
	77	75	51	24	17	15
	85	70	49	19	15	10
	87	—	51	—	12	22
S.L.$_2$	0	75	50	25	14	10
	15	74	51	23	22	19
	29	71	50	21	19	17
	43‡	73	50	23	12	17
	59	—	41	—	22	27
	73	73	52	21	17	17
	77	—	—	—	—	—
	85	68	51	17	34	34
	87	69	52	17	27	31
M.R.	0	68	45	23	7	14
	15‡	72	50	22	15	15
	29	62	50	12	15	15
	43	71	52	19	17	14
	59	—	40	—	12	19
	73	74	54	20	17	12
	77	—	—	—	—	—
	85	67	50	17	22	27
	87	69	52	17	19	24
R.W.	0	71	49	22	3	14
	15	72	50	22	7	19
	29	69	53	16	7	17
	43	71	53	18	14	19
	59‡	—	48	—	27	24
	73	70	52	18	62	42
	77	—	—	—	—	—
	85§	70	50	26	87	56
	87	68	53	15	92	46

* Normal range for ALT: 5–19 i.u./l. † Normal range for AST: 4–25 i.u./l.

‡ Subject was in zero or positive N balance on this day and throughout the rest of the study. Intake was 0·57 g egg protein unless otherwise indicated.

§ Subject's intake was greater than 0·57 g egg protein on this day and throughout the rest of the study.

Table 6. *Final and initial measurements of globulin fractions*

Subject	Day	Globulin fraction (g/l) α_1	α_2	β	γ	Globulin fraction (% total protein) α_1	α_2	β	γ
D.C.	0	1·7	6·2	6·6	6·8	2·5	9·3	9·9	10·1
	77	1·7	6·0	8·1	7·3	2·5	8·0	10·8	9·7
T.C.	0	1·7	7·9	7·9	9·9	2·3	10·7	10·7	13·4
	87	1·6	5·4	7·8	7·2	2·1	7·4	10·5	9·7
S.L.$_1$	0	1·8	6·8	6·8	12·0	2·3	9·0	9·1	16·0
	87	1·3	5·6	7·3	11·8	1·8	8·1	10·4	16·9
S.L.$_2$	0	1·6	8·6	7·9	11·9	2·1	11·4	10·6	15·9
	87	0·3	5·5	7·4	6·8	0·5	8·2	10·9	9·9
M.R.	0	1·9	6·9	7·5	7·6	2·8	10·1	11·0	11·1
	87	0·9	4·9	4·8	6·5	1·3	7·3	7·2	9·6
R.W.	0	1·6	6·1	7·5	7·2	2·2	8·6	10·5	10·1
	87	1·0	5·0	6·6	5·3	1·4	7·4	9·7	7·8

ments while the subjects were on the FAO/WHO safe level of protein intake. Three of these subjects showed decreases in globulin levels as determined by electrophoresis. The average initial level in these subjects was 25 g/l and the average final level was 18 g/l, a decrease of 28 % (normal level 20–30 g/l). Values for subjects T.C. and S.L$_1$. represent the initial values at 0·57 g protein/kg and final ones obtained at the study's conclusion, when they were consuming 1·0 g egg protein/kg (Table 2).

Table 5 summarizes changes in serum transferase values. Increases above initial levels were observed in all cases, and the resulting levels were above normal for four of the six subjects. The magnitude of the increases and the time at which they occurred differed among individuals. No changes in either direct or indirect serum bilirubin values were observed, nor were any clinical abnormalities detected in subjects with abnormally increased transferase levels. When we noted sustained increases in transferase levels, we increased the subjects' dietary protein intake to 0·73 g protein/kg to determine whether abnormal transferase levels would fall as quickly as they did when protein intake was increased from 0·59 to 1·5 g protein/kg in subjects with similar abnormal transferase elevations (Garza *et al.* 1977).

Transferase levels in subject T.C. increased to above normal by day 15. Periodic checks during the following 14 d showed that ALT level fell from a high of 56 i.u./l to 31 i.u./l during this period. In response to subject T.C.'s persistent negative N balance, we increased his energy intake by 10 % and re-examined his transferase levels after 5 d. ALT level had increased to 57 i.u./l, and 5 d later it had risen to 104 i.u./l. His protein intake was then increased to 0·73 g egg protein/kg body-weight. After 12 d on this diet, the subject's ALT level fell from 104 to 60 and remained at this level during the subsequent 2 weeks. During the final 2 weeks of the study, subject T.C. was placed on a diet providing 1·0 g egg protein/kg. Although his transferase level remained unchanged at about 60 i.u./l, the subject's intercurrent infection at this time was a complicating factor. His transferase level returned to normal 12 d after the study had ended.

Table 7. *Comparison of lactate dehydrogenase (LDH) isoenzyme activity with serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity levels*

Subject	Day	LDH activity (i.u./l)	Activity associated with liver LDH band (%)	ALT activity (i.u./l)	AST activity (i.u./l)
D.C.	5	48	1·1	12	12
	73	88	4·4	45	29
T.C.	1	61	1·4	17	17
	21	69	6·2	48	34
	43	70	15·3	104	53
	50	69	9·9	85	39
	87	32	0·0	56	39
S.L.$_1$	1	52	0·0	12	17
	24	74	13·0	53	31
	53	70	9·3	29	19
	85	40	0·9	12	22
S.L.$_2$	1	57	0·0	14	10
	85	80	1·7	34	34
M.R.	1	61	0·0	7	14
	85	50	1·6	22	27
R.W.	1	53	2·6	3	14
	59	45	2·0	27	24
	73	30	1·6	62	42
	75	70	12·2	77	39
	87	32	6·4	92	46

Subject S.L.$_1$ also had transferase levels above normal limits by day 15. ALT level remained at about 50 i.u./l for the next 2 weeks, after which this subject's protein intake was increased to 0·73 g egg protein/kg. Two weeks later, his ALT activity had dropped to 30 i.u./l, which was still above our normal limits. After a total of 34 d on 0·73 mg protein/kg, subject S.L.$_1$'s ALT level continued at about 30. His protein intake was then increased to 1·0 g egg protein/kg body-weight; after 2 weeks his transferase activity fell within normal limits and returned to initial values by the end of the study.

The transferases of subject R.W. were noted to be elevated on day 73. Transferase levels were checked 3 d later, and a 25 % increase in ALT activity was observed. The diet was changed then to 0·73 g egg protein/kg body-weight and, after about 2 weeks at this level of protein intake, his transferase levels had risen by 16 %. Subject D.C.'s ALT activity was 45 i.u./l on day 73. Transferases were re-examined 4 d later at the conclusion of the study and his ALT activity was 34 i.u./l.

Because the planned study period had come to an end, these subjects (D.C., T.C., S.L.$_1$ and R.W.) could not be followed further on the experimental diet. However, after 2 or 3 weeks on a free-choice diet, they all had normal transferase levels.

Table 7 summarizes the LDH isoenzyme measurements. We observed increases in the percentage of total LDH activity associated with the liver LDH band and also noted increases in serum transferase activity.

Haematological findings are summarized in Table 8. Three of the four subjects

Table 8. *Haematological measurements*

Subject	Haemaglobin (g/l)		Haematocrit (%)		Reticulocyte (%)		White blood cells ($\times 10^3$/mm^3)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
D.C.	160	152	48	45	—	1·6	6·90	8·55
T.C.	160	160	48	48	1·3	1·8	5·75	7·20
S.L.$_1$	156	144	47	43	1·6	1·9	5·90	4·60
S.L.$_2$	150	140	44	40	1·4	1·1	6·30	5·90
M.R.	136	140	41	40	0·8	0·8	6·00	7·20
R.W.	154	142	45	42	0·7	1·6	4·90	4·90

maintained at 0·57 g egg protein/kg for more than 50 d (D.C., S.L.$_2$ and R.W.) had decreases in haemoglobin and haematocrit. One of the two subjects with early transferase changes (S.L.$_1$) had a decline in haemoglobin and haematocrit along with a decrease in total serum proteins and globulin levels.

DISCUSSION

In this study five of six individuals were in negative N balance while consuming the FAO/WHO safe level of egg protein at energy intakes that equalled their requirements, as estimated by diet histories and nomograms (method A); three of the six were in negative N balance at energy levels greater than their requirements, as estimated by method B. Increasing the dietary energy was associated with improvements in the efficiency of N utilization great enough to bring these subjects into N equilibrium or slightly positive N balance.

The small magnitude of positive N balances at the high energy intakes leaves little room for the probable cumulative errors favouring apparent cumulative N retention that can occur when skin and miscellaneous N losses are underestimated (Wallace, 1959; Walker, 1962; Williams, Harper, Hegsted, Arroyave & Holt, 1974). Furthermore, we have used 5 mg N/kg for skin and miscellaneous losses, as proposed by FAO/WHO (1973). The study used by the FAO/WHO committee to reach their recommendation (Calloway, Odell & Margen, 1971) actually suggested that an allowance of 0·5 g N/day should be made for unmeasured N losses in calculating N balance in male adults. If this allowance had been used, two of the three subjects studied at 0·57 g protein/kg for 50 d or more would have been in negative N balance, in spite of high energy intakes. Our prior 81 d study at 0·59 g protein/kg (Garza *et al.* 1976*b*) suggested that the integumental and other minor N losses are approximately 15 mg N/kg, a value similar to those obtained by Mitchell & Edelman (1962) and by Isaksson & Sjögren (1967). If this value is accepted, all of the subjects were in negative N balance.

Even though the final TBK measurements were lower than the initial measurements in five of the six individuals studied, the fluctuations between successive measurements rendered the TBK results in this experiment of limited significance. Nevertheless, no discernible differences existed in the counting procedures, and the

standard deviations of the means of each individual's measurements fell within the range to be expected from counting statistics alone. Decreases in creatinine excretion were also observed in all subjects, but their interpretation is confounded by the effects of creatine- and creatinine-free diets on urinary creatinine excretion (Bleiler & Schedl, 1962; Crim, Calloway & Margen, 1975).

We have previously reported (Garza *et al.* 1977) that N balance improved by 0·47–0·95 mg N/kg for each kJ/kg added to the diet of three out of four subjects. However, as we pointed out, the experimental design did not allow a differentation between the effects caused by changes in efficiency of N utilization (resulting from changes in energy intake) or the effects caused by prior N depletion at lower energy levels. In the current study the greatest improvement in balance with increasing energy intakes occurred at 0·67 mg N/additional kJ, the average change in N balance being 0·33 mg N/kJ (range, 0–0·67). This value is similar to that reported by Calloway (1975) for the effect of a change in energy intake on N retention.

With these findings, the changes in the efficiency of N utilization resulting from changes in energy intake can be compared with those resulting from changes in N intake. Our studies (Young *et al.* 1973) and those of Inoue, Fujita, Kishi, Yamamoto & Niiyama (1974) have demonstrated a curvilinear relationship between the level of N intake and the efficiency of N utilization, although for practical purposes it can be taken as linear over a narrow protein intake range. Differences in efficiency between two levels of N intake will, therefore, approximate the true rate of change. Inoue *et al.* (1974) observed balance to improve by 2·39 mg N/protein kJ per kg when intake was increased from 0·4 to 0·6 g egg protein/kg. Our findings indicate that, under the experimental conditions of this study, the energy changes averaged only 10–15% as effective as changes in protein intake in bringing about alterations in N balance. In our previous study (Garza *et al.* 1976*a*), we estimated that energy was 20–40% as effective as protein in improving N balance. The most likely factor accounting for the difference between these two studies is the longer duration of the negative N balance periods in the former study. In this study, the greatest improvement in N balance following an increase in energy intake was observed in the subject with the most negative cumulative N balance.

The decreased protein-sparing action of energy is significant because it indicates that even higher energy intakes than those previously estimated (Garza *et al.* 1976) may be needed by a significant proportion of the young adult population to maintain N equilibrium at the 1973 FAO/WHO 'safe level' of egg protein intake. The history of the one subject common to both studies (subject T.C.) supports this conclusion. Linear regression of N balance *v.* energy intake predicted that he would need 196 kJ (47 kcal)/kg to maintain N balance at the FAO/WHO level; however, when placed on this energy intake for 32 d, he remained in negative N balance.

Energy requirement estimates obtained by diet history and appropriate nomograms (method A) agreed with estimates based on weight and N balance results (method B) in only two subjects (Table 4). In the other four, estimates obtained by method B were higher by about 10–20%. Although one factor causing this discrepancy may be that energy requirements are underestimated by method A, a decrease in

the efficiency of energy utilization at the high energy levels studied (mean, 222 kJ (53 kcal)/kg, with only one subject below 209 kJ (50 kcal)/kg) is also a likely explanation. Other published results, showing reduced efficiency of energy utilization with increasing energy intakes, support the second possibility (Miller & Mumford, 1967; Blaxter, 1971; Simms, Danforth, Horton, Bray, Glennon & Salans, 1973). If the energy cost of depositing 1 kg of fat is greater than 29·3 MJ (7000 kcal), then the amount of excess energy would have been underestimated.

Transferase levels rose above their initial values in all six subjects and reached abnormal levels in five. LDH isoenzymes were separated by electrophoresis, and the band associated with the liver isozyme accounted for an increasing percentage of total LDH activity as transferases increased. This finding, coupled with the greater increases in ALT than in AST, suggests that the liver was the source of increased serum enzymatic activity.

Similar abnormal increases in serum transferase activity were observed in two of six subjects studied at the 0·59 g level of egg protein intake in a previous experiment (Garza *et al.* 1977); an increase in their protein intake to 1·5 g/kg for 2 weeks lowered their transferase levels. A return to an intake of 0·59 g protein/kg was associated with a second increase in transferase activity in one of two subjects. In the current study, we increased the dietary protein to 0·73 g/kg in three subjects with abnormal transferase levels. After 2 weeks at this higher protein intake, transferase values showed small changes in two young men and fell by 42% in the third. Hence, the transferase response appears to be dependent upon the level of protein intake, and 1·5 g protein/kg is more effective than 0·73 g/kg in reducing elevated serum transferase activity levels.

These changes, together with the general decrease in all of the globulin fractions identified by electrophoresis, suggest that the liver may be adversely affected by the combination of high energy intakes and a marginal intake of dietary protein. Our findings clearly fail to support the adequacy of the 1973 FAO/WHO recommendation of 0·57 g egg protein/kg as a safe allowance for healthy young men.

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