Effect of varying energy and protein intake on nitrogen balance in adults engaged in heavy manual labour

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1. The effect of varying energy and protein intake on nitrogen balance in adult men engaged in heavy manual labour was investigated.

2. Low protein and energy levels resulted in negative N balance and urea index (Waterlow, 1963) was decreased.

3. Urinary excretion of hydroxyproline was found to be sensitive to dietary protein level, but not to energy.

It has been recognized for a long time that there is an intimate relationship between nitrogen balance and energy intake. Munro (1951) has critically reviewed the quantitative aspects of this relationship between energy and protein. Calloway & Spector (1954) have discussed N balance in relation to energy and protein intakes in active young men. Nageswara Rao *et al.* (1975) have reported recently the effect of changing energy intake at two levels of protein intake on N balance, in well-nourished young adult men engaged in sedentary work.

The present investigation was undertaken to obtain information on the interaction between energy and protein on N balance, in subjects belonging to the low socio-economic group, engaged in manual labour involving energy expenditure, roughly corresponding to moderate work. In addition, the effect of varying protein and energy intakes on other factors such as urinary excretion of urea and hydroxyproline and plasma amino acid levels was also studied.

MATERIALS AND METHODS

Four subjects whose habitual occupation was gardening were investigated. Relevant details of the subjects are given in Table 1. Heights and weights of all subjects were recorded before the commencement of the experiment. Weights were also recorded at the beginning and at the end of each dietary regimen.

The basal metabolic rate (BMR) of the subjects was determined at the beginning of the study using a Benedict-Roth apparatus.

An oral-questionnaire dietary survey was carried out on the subjects to determine the pattern of food and nutrient intake, to enable the formulations of the experimental diet. It was observed that their diets comprized mainly of cereals (rice) with small quantities of pulses, vegetables, milk, sugar and fat. The average energy and protein intake of the subjects on their habitual diets were $232 \pm 10\cdot9$ kJ ($55\cdot5 \pm 2\cdot65$ Kcals) and $1\cdot0 \pm 0\cdot06$ g/kg body-weight respectively.

Experimental diet. The subjects were fed at two energy levels, 186 and 232 kJ/kg (44·4 and 55·5 Kcals/kg), and two protein levels, 1·0 and 1·2 g/kg. The composition of the diets are given in Table 2.

Experimental design. Each of the subjects received all four diets in a Latin-square design.

Table 1. *Details of subjects studied*

Subject	Age (years)	Body surface area (m^2)	Height (m)	Wt (kg)	Basal metabolism (J/h)
KR	18·5	1·48	1·65	48·5	232·4
PR	23·5	1·44	1·57	45·5	218·8
AK	25·5	1·38	1·59	42·5	205·1
KM	22·0	1·46	1·61	47·8	222·4

Table 2. *Composition (g/kg body-weight) of diets fed to adult male Indians engaged in heavy manual labour**

Dietary level energy-protein	232:1·2	186:1·0	186:1·2	232:1·0
Rice	10·0	9·0	10·0	9·0
Red gram (*Cajanus cajan*)	0·9	0·8	0·8	0·8
Green gram (*Phaseolus radiatus*)	0·4	0·3	0·5	0·3
Milk (ml)	2·0	1·5	2·0	1·5
Potatoes	2·5	2·5	1·0	2·5
Onions	2·0	2·0	1·0	2·0
Brinjal (*Solanum melongena*)	1·0	1·0	1·0	1·0
Sugar	0·75	0·41	0·2	0·75
Peanut oil	0·3	0·25	0·12	0·37
Cassava starch (*Manihot esculenta*)	1·2	0·2	—	2·4

* For details, see Table 1.

Each diet was given for a period of 11 d, the urine and faeces being collected on the last 4 d of each period.

Collection procedure. Urine and faeces were collected for 24 h periods during the last 4 d of each 11 d period. Urine was collected in toluene and acetic acid, while faeces were collected in suitable plastic containers. Fasting blood samples was taken from each subject on the last day of each 11 d period, centrifuged, plasma separated and stored frozen until analysed.

Urine and faeces were analysed for total N. Urine was also analysed for ammonia-N, urea N, creatinine and hydroxyproline. Plasma was analysed for free amino acids.

Analytical methods. Faeces collected in each of the 24 h periods were mixed in a Waring blender and portions analysed in duplicate for N. Urine collected in each 24 h period was mixed well, the volume measured and portions taken for various analyses. Total N in urine and faeces was determined by the macro-kjeldahl procedure.

Urinary ammonia-N was estimated directly by Nesselerization after diluting the urine twenty-fold and the intensity of colour produced was measured at 500 nm. Nesseler's reagent was prepared according to Bock & Benedict as described by Oser (1965). Urinary urea-N was determined by treatment with buffered urease (*EC* 3.5.1.5) as described by Chaney & Marbach (1962), followed by Nesselerization. Urinary creatinine was estimated as described by Clark & Thompson (1949), after diluting urine 100-fold. Urinary hydroxyproline was estimated by the method of Prockop & Udenfriend (1960) as modified by Jasin *et al.* (1962).

Plasma amino acids were determined using an automatic amino acid analyser (designed and assembled by Dr Kenneth Woods of New York Blood Centre, New York) employing conditions similar to those described by Spackman *et al.* (1958) but using Beckman special resins PA 25 and PA 38.

Table 3. *Daily nitrogen excretion (g) and balance (g) at different levels of energy and protein intake* for adult male Indians engaged in heavy manual labour†*

(Mean values with their standard errors for four determinations)

Dietry level energy-protein	Subject	Total N intake	Mean Faecal N		Mean urinary N		Total N excreted	Mean creatinine		Balance
			Mean	SE	Mean	SE		Mean	SE	
232:1·2	KR	9·3	2·4	0·40	4·7	0·13	7·1	1·09	0·030	+2·2
	PR	8·9	2·4	0·38	4·9	0·14	7·3	1·00	0·043	+1·6
	AK	8·3	2·5	0·41	5·3	0·20	7·8	1·18	0·035	+0·5
	KM	8·8	2·5	1·06	5·2	0·20	7·7	1·08	0·073	+1·1
	Mean	8·8	2·5	0·029	5·0	0·14	7·5	1·09	0·037	+1·3
186:1·0	KR	7·6	2·4	0·23	5·1	0·10‡	7·5	1·04	0·027	+0·1
	PR	7·3	3·0	0·45	5·1	0·02‡	8·1	0·98	0·057	−0·8
	AK	6·6	2·8	0·50	4·0	0·14	6·8	0·85	0·049	−0·2
	KM	7·5	3·0	0·86	4·7	0·08	7·7	1·07	0·093	−0·2
	Mean	7·2	2·8	0·14	4·7	0·26	7·5	0·98	0·049	−0·3
186:1·2	KR	8·6	2·6	0·60	3·6	0·07	6·2	0·94	0·033	+2·4
	PR	8·5	2·1	0·23	4·8	0·12	6·9	1·01	0·094‡	+1·6
	AK	8·2	2·6	0·47‡	5·1	0·12	7·7	0·91	0·026	+0·5
	KM	9·0	1·3	0·19	5·9	0·08	7·2	1·15	0·024§	+1·8
	Mean	8·6	2·1	0·31	4·9	0·48	7·0	1·00	0·053	+1·6
232:1·0	KR	7·2	2·1	0·37	2·7	0·15	4·8	0·86	0·012‡	+2·4
	PR	7·2	2·0	0·09‡	4·6	0·19	6·6	1·13	0·039	+0·6
	AK	6·7	2·3	0·37	3·7	0·12	6·0	0·89	0·034	+0·7
	KM	7·6	3·8	0·48	3·8	0·16	7·6	0·97	0·028	0
	Mean	7·2	2·5	0·42	3·7	0·39	6·2	0·96	0·060	+1·0

* For details, see Table 2. † For details, see Table 1. ‡ Mean for 3 d. § Mean for 2 d.

Table 4. *Effect of varying energy and protein intakes* on urea-nitrogen excretion (g) in adult male Indians engaged in heavy manual labour*†

(Mean values with their standard errors for four determinations)

Dietary level energy-protein	Subject	Ammonia-N		Urea-N		Urea index % Urea-N / total urinary N
		Mean	SE	Mean	SE	
232:1·2	KR	0·22	0·055	3·5	0·13	73·6
	PR	0·49	0·075	3·6	0·19	73·9
	AK	0·62	0·102	3·7	0·24	70·4
	KM	0·60	0·027	4·1	0·26	79·1
	Mean	0·48	0·092	3·7	0·13	74·3
	SE					1·80
186:1·0	KR	0·37	0·038	3·2	0·15	65·3
	PR	0·37	0·076	3·2	0·18	66·8
	AK	0·42	0·028	2·5	0·15	62·1
	KM	0·57	0·056	2·8	0·03	60·1
	Mean	0·42	0·047	2·9	0·17	63·6
	SE					1·52
186:1·2	KR	0·30	0·029	2·5	0·05	70·9
	PR	0·40	0·063	3·6	0·27	74·7
	AK	0·47	0·107	3·6	0·13	73·1
	KM	0·51	0·109	4·0	0·12	67·7
	Mean	0·42	0·046	3·4	0·32	71·6
	SE					1·51
232:1·0	KR	0·26	0·044	1·9	0·11	67·1
	PR	0·46	0·062	3·1	0·21	67·2
	AK	0·37	0·052	2·8	0·03	74·7
	KM	0·40	0·102	2·8	0·13	73·1
	Mean	0·37	0·041	2·7	0·26	70·5
	SE					1·98

* For details, see Table 2. † For details, see Table 1.

RESULTS

In Table 3 are reported the individual values for N intake, faecal-N, urinary-N, creatinine and N balance.

Analysis of variance carried out for faecal N and creatinine-N excretion did not show any significant difference between diets suggesting that the excretion of these two constituents was not influenced by the level of either protein or energy in the diet.

N balance was found to be positive in all subjects fed on diets at energy-protein levels (KJ/g per kg) 232:1·2, 186:1·2 and 232:1·0. Three of the four subjects went into negative balance when fed at dietary level 186:1·0, while the other subject (KR) showed a marginal positive balance. Analysis of variance on N balance values revealed that diets with energy-protein of 232:1·2, 186:1·2 and 232:1·0 were not significantly different among themselves but diets 232:1·2 and 232:1·0 were significantly different from diet 186:1·0 ($P < 0·01$).

These changes in N balance were reflected in urinary N excretion. A decrease in energy intake at a protein level of 1·2 g/kg body-weight was not accompanied by any change in urinary N, while a similar decrease in energy intake at a lower protein level of 1·0 g/kg body-weight resulted in an increased urinary N excretion leading to a negative balance.

Results for ammonia-N and urea-N expressed as a percentage of total urinary N are presented in Table 4. Excretion of ammonia-N was essentially similar at all levels of dietary energy and protein. The values for urea N:total urinary N on diets 232:1·2, 232:1·0 and 186:1·2 were not significantly different among themselves, but those for diets 232:1·2, 186:1·2 and 232:1·0 were different when compared to diet 186:1·0 ($P < 0·05$). The mean

Table 5. *Effect of varying energy and protein intakes* on urinary hydroxyproline excretion in adult male Indians engaged in heavy manual labour* †

(Mean values with their standard errors for four determinations)

Dietary level energy-protein...	232:1·2		232:1·0		186:1·2		186:1·0	
	Urinary hydroxyproline (mg/24 h per m^2 body surface area)							
Subject	Mean	SE	Mean	SE	Mean	SE	Mean	SE
KR	21·5	0·64	18·1	0·27	20·4	0·44	18·1	0·41
PR	17·6	0·32	14·9	0·51	16·2	0·45	12·8	0·63
AK	18·9	0·52	16·4	0·32	17·6	0·53	15·7	0·52
KM	15·7	0·56	14·1	0·27	15·4	0·89	12·8	0·54
Mean	18·4	1·22	15·9	0·88	17·4	1·10	14·8	1·28

* For details, see Table 2. † For details, see Table 1.

Table 6. *Effect of varying energy and protein intakes* on plasma essential: nonessential amino acids in adult male Indians engaged in heavy manual labour*†

(Mean values with their standard errors for four determinations)

Dietary level energy-protein...	232:1·2	186:1·0	186:1·2	232:1·0
Subject				
KR	2·13	1·88	1·73	1·43
PR	1·53	1·49	1·66	1·50
AK	1·38	1·29	1·54	1·27
KM	1·66	1·48	1·99	1·62
Mean	1·67	1·53	1·73	1·45
SE	0·162	0·124	0·095	0·073

* For details, see Table 2. † For details, see Table 1.

value was highest for diet 232:1·2 (74·3%) followed by diets 186:1·2 (71·6%), 232:1·0 (70·5%) and 186:1·0 (63·6%). The lowest value was seen on the diet 186:1·0 for which a negative N balance was observed.

Values for total hydroxyproline peptides in urine are presented in Table 5. The excretion of hydroxyproline at any energy level decreased when there was a reduction in the protein level ($P <$ 0·05). However, no significant difference was found when the energy level was reduced at a particular protein intake.

Table 6 gives values for non-essential:essential amino acids. The values were generally lower at a protein intake of 1·0 kg/kg as compared to intakes of 1·2 g/kg at both the energy levels. These were however not statistically significant.

DISCUSSION

The results clearly indicated that a reduction of energy by 20% at the habitual energy-protein level of 232:1·0 produced a change to a negative N balance, an observation similar to that of Werner (1948), who reported that a reduction of energy at constant protein intake results in negative N balance. The relationship between energy intake and protein utilization is well known and studies in man and animals (Munro, 1951; Calloway & Spector, 1954; Rosenthal & Allison, 1956; Fuller *et al.* 1973) reveal that protein utilization is impaired by energy restriction.

On an energy intake of 232 kJ/kg body-weight all the subjects were in positive N balance at both levels of protein intake. Results of earlier studies in Indian subjects on a similar type of diet had indicated that protein intake for N equilibrium at adequate energy intakes of 13·6 MJ (251 kJ/kg body-weight) was 0·55 g/kg body-weight (Phansalkar & Patwardhan, 1956). The minimum protein requirement at lower energy intake of 232 kJ/kg may be expected to be slightly higher than this value. The subjects investigated in the present study were gardeners with moderate level of activity whose energy requirement was approximately 232 kJ/kg body-weight. When their energy intake was reduced by 20%, i.e. to 186 kJ/kg body-weight they were in positive balance only when the protein intake was 1·2 g/kg body-weight while at an intake of 1·0 g/kg body-weight they were in negative N balance. Thus the protein requirement for N equilibrium at 186 kJ intake in these subjects can be considered to lie between 1·0 and 1·2 g/kg body-weight as compared to a value of 0·55 g/kg body-weight at adequate energy intake (Phansalkar & Patwardhan, 1956). Studies in sedentary subjects by Nageswara Rao *et al.* (1975) revealed that the energy intake for N equilibrium at a protein intake of 1·11 g/kg body-weight per d was 161 kJ/kg body-weight and when the protein intake was reduced to 0·75 g/kg body-weight per d the corresponding energy requirement increased to 176 kJ/kg body-weight. A comparison of the two studies indicates that the reduction in protein intakes for both types of subjects is compensated by similar magnitude of increases in energy intakes irrespective of their activities. Thus the results of the present study indicate that protein levels are inadequate when energy intake is below requirement and that a higher level of protein intake is necessary to keep the subjects in N equilibrium. It is not clear whether protein requirement for growth is similarly affected by energy intake in growing children in whom the problem of inadequate energy intake is much more widespread. This aspect is being currently studied.

Urea in relation to urinary N excretion is related to dietary protein intake and its adequacy. Urea index (Waterlow, 1963) in subjects on diets 232:1·2, 232:1·0 and 186:1·2 were within the normal range (Phansalkar & Patwardhan, 1954; Ramamurti, 1955), but showed a significant reduction on diet 186:1·0. A low urea index on low protein intakes is attributed to an adaptive decrease in urea-cycle enzymes (Schimke, 1962). It would appear from the present study that a reduction of 20% in either energy or protein, which leads to negative N balance, can bring about a similar adaptive change.

Urinary excretion of hydroxyproline was found to be sensitive to dietary protein level, but not to energy. It was reported earlier from this Institute that hydroxyproline excretion in adults was reduced on a protein-free diet (Anasuya, 1968). Results presented in this paper show that even moderate changes in protein intake can cause alterations in hydroxyproline excretion suggesting an observation disimilar to that reported by Prockop & Sjoerdsma (1961). It must be pointed out however that hydroxyproline excretion was measured in their study within 2–3 d of changing the protein intake (a period that may be too short for adaptation of collagen metabolism to changes in protein intake).

It is well known that changes in concentration of free amino acids in blood is related to protein nutrition (Swendseid *et al.* 1966; Young & Scrimshaw, 1968). The moderate and inconsistent changes in the value for nonessential : essential amino acids seen here suggest that this factor may be insensitive to small changes in protein intakes over short periods of time.

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