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Thermic effect of a meal
3. Effect of dietary supplementation in chronically undernourished human subjects*

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Five apparently healthy, chronically undernourished (UN) male volunteers aged between 18 and 30 years were studied before and after 12 weeks of dietary supplementation. The thermic effect of a meal (TEM) was measured over a period of 6 h using a ventilated-hood system. Results indicated a significant increase in body-weight after supplementation due to increases in body fat and fat-free mass (FFM) in the proportion of 69% and 31% respectively. The basal metabolic rates (BMR) measured post supplementation were significantly higher in absolute terms, with a trend towards higher values when adjusted for the changes in FFM. TEM responses measured after 12 weeks of supplementation were significantly lower when expressed either in absolute terms (presupplementation 227·0 kJ *v.* post supplementation 193·5 kJ), or as a percentage of the energy density of the meal (9·1% *v.* 7·7%). This lower TEM was reciprocal to the changes in the BMR (*r* −0·86). The post-meal total energy output (PMTEO) was, however, not significantly different after 12 weeks of dietary supplementation. The unchanged PMTEO would indicate an unaltered 'thermogenic capacity', following supplementation, in these chronically undernourished subjects. These results confirm our earlier conclusion that, in chronic undernutrition, the thermic response to a meal may not contribute to any energy saving.

Chronic undernutrition: Dietary supplementation: Basal metabolic rates: Thermic effect: Substrate oxidation rates

We have shown, earlier, that the thermic effect of a meal (TEM) responses to a standard meal are higher in chronically undernourished (UN) subjects compared with well-nourished subjects. However, the post-meal total energy output (PMTEO) was not different when corrected for differences in body size. This suggested that the TEM response in the UN subjects did not contribute to any energy saving, since thermogenic capacities were comparable with those of the well nourished (Piers *et al.* 1992*b*). One of the characteristics of a physiological adaptive response is its reversal when the underlying cause is corrected (Waterlow, 1984). We therefore measured the thermic response in the UN, before and after a period of controlled dietary supplementation, based on the hypothesis that, in the UN subject, the PMTEO would remain unchanged following dietary supplementation. This, in effect, would confirm our earlier conclusion that the thermic response to a meal, in the UN subject, may not contribute to any energy saving.

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MATERIALS AND METHODS

Subjects

Five apparently healthy, chronically undernourished male volunteers aged 18–30 years were investigated. They were selected on the basis of body mass index (BMI; weight/height^2 (kg/m^2)) < 18·5 (James *et al.* 1988), from a lower socio-economic class (Class IV; Kuppuswamy, 1984). Subjects were unskilled manual labourers on a daily wage, resident in a neighbouring slum. All subjects underwent a complete clinical assessment before recruitment. Nutritional status was assessed by anthropometry i.e. body-weight (kg) and height (m). Body fat was estimated from the sum of four skinfold thicknesses (biceps, triceps, subscapular and supra-iliac) measured with a Holtain skinfold calliper (Crymmych, UK), and the use of the formula of Durnin & Womersley (1974). Fat-free mass (FFM) was calculated from the difference of body fat and body-weight.

Study design

The study was conducted as a paired comparison of TEM responses before and during the last week of supervised dietary supplementation. Following recruitment, the TEM response to a standard liquid test meal was measured. The subjects were then given a dietary supplement of 3·35 MJ/d for 12 weeks. Their TEM responses to the same test meal were measured once again during the 12th week of supplementation.

Dietary intakes

Dietary recalls (24 h) of the preceding day's energy and protein intakes were obtained for at least two consecutive days in the week before and for three consecutive days in the week following the 12 weeks of supplementation. Subjects were instructed to maintain their normal dietary intakes and eating habits for the duration of the dietary supplementation. During the intervening weeks of supplementation, a single 24 h recall of the preceding day's intake was obtained every 10 d (i.e. eight recalls over 12 weeks). This was carried out to monitor the dietary energy and protein intakes and to uncover any 'substitution' during the period of supplementation. All recalls were conducted by an experienced dietician using standardized household measures and utensils.

During the supplementation period, 24 h dietary intakes were assessed by self-weighing in four of the five subjects on three separate occasions. Dietary recalls (24 h) were also obtained within 3 d of each weighed intake in order to validate intakes estimated by the recall method.

Dietary supplement

The dietary supplement provided 3·35 MJ/d and consisted of maize, soya-bean meal, sugar and maize oil (protein 15 g, fat 34·5 g, carbohydrate 105 g). The nutrient content was calculated from standard food tables (Gopalan *et al.* 1985). The supplement was served as two isoenergetic snacks, once each in the morning and evening, and eaten under supervision. The subjects did not receive the supplement on the morning of the TEM measurement, during the 12th week of supplementation.

Test meal

The test meal for TEM responses was the same as that used in the previous studies (Piers *et al.* 1992*a*, *b*), and consisted of tinned milk powder, rice cereal and sugar made up to 350 ml with water and served at room temperature. It provided 2·5 MJ energy with a nutrient composition of (g/kg) protein 100, fat 150 and carbohydrate 750. Energy and nutrient compositions were derived from the manufacturers' product information. All tinned products were purchased at the same time, belonged to the same batch of manufacture and were used well before their date of expiry.

Measurement of the TEM response

An indirect calorimetry protocol (protocol 2) described earlier (Piers *et al.* 1992*a*), was used for the measurement of the basal metabolic rate (BMR), TEM, PMTEO and substrate oxidation rates (SOR). It consisted of an intermittent measurement of the oxygen consumption over a 6 h period.

Calculation of BMR, TEM, PMTEO and SOR

BMR, TEM, PMTEO and SOR were calculated as described earlier (Piers *et al.* 1992*a*). No corrections were made to the protein oxidation rates for possible changes in the plasma urea pool from the fasted to the fed state.

Statistical analysis

Dietary intakes obtained before, during and following supplementation, by the recall method, were compared by means of a two-way analysis of variance (ANOVA; Sokal & Rohlf, 1969). Dietary intakes obtained by recall during the period of supplementation were also compared with weighed intakes using a two-way ANOVA. Anthropometric and TEM data were analysed by means of paired t tests at the 5% significance level. BMR and PMTEO were compared by an analysis of covariance (ANACOVA; Dowdy & Wearden, 1983) with FFM as the covariate.

Ethical approval

Ethical approval was obtained for the study from a duly constituted Human Investigation Committee of the medical school and all subjects gave fully informed written consent.

RESULTS

During the supplementation period there were no significant differences in the dietary energy or protein intakes estimated by recall and the weighed-intake method on an ANOVA (Table 1). However, there was a significant ($P < 0.001$) subject × method interaction in the estimation of energy intakes. There were no significant differences in the unsupplemented intakes of energy or protein between the week before, during the 12 weeks of supplementation, and during the week after cessation of supplementation. There was, however, a significant subject × period interaction in the estimation of energy intakes (Table 2).

There were significant increases ($P < 0.05$) in body-weight, fat mass and BMI. However, the increases in FFM did not attain statistical significance (Table 3). BMR expressed in absolute terms was significantly higher post supplementation. This difference was not apparent (F ratio 1·39 at 1,7 df; $P > 0.05$) when BMR was adjusted by ANACOVA for the change in FFM, although the values tended to be higher (Table 4). The TEM response lasted for 6 h during the post-supplementation measurement, therefore all responses (i.e. presupplementation and post supplementation) were compared over this duration of time. The TEM response was significantly higher during the presupplementation measurement, compared with the post-supplementation measurement. These differences persisted even when the TEM responses were expressed as a percentage of the energy content of the meal (Table 4). However, the PMTEO measured over 6 h was not significantly different (F ratio 0·83 at 1,7 df; $P > 0.05$) adjusted for changes in FFM using an ANACOVA (Table 4). There was a significant inverse correlation between the BMR and TEM (r −0·862, $P < 0.01$).

Table 1. *ANOVA of dietary energy and protein intake as estimated by the 24 h recall method and the 24 h weighed intake method for chronically undernourished subjects**

Source	Sum of squares	df	Mean square	*F*-ratio	Statistical significance: *P*
Energy					
Subjects (*n* 4)	1317747·1	3	439249·0	4·702	0·015
Method of estimation	8550·4	1	8550·4†	0·01	> 0·050
Subject × method of estimation	2441623·1	3	813874·4	8·713	0·001
Residual	1494571·3	16	93410·7		
Protein					
Subjects (*n* 4)	1409·1	3	469·7	4·376	0·020
Method of estimation	25·0	1	25·0	0·233	> 0·050
Subject × method of estimation	866·0	3	288·8	2·690	> 0·050
Residual	1717·3	16	107·3		

* For details of subjects and procedures, see pp. 188–189.
† Tested over interaction term (i.e. subject × method of estimation).

Table 2. *ANOVA of dietary energy and protein intake as estimated by the 24 h recall method before, during and following the period of dietary supplementation for chronically undernourished subjects**

Source	Sum of squares	df	Mean square	*F*-ratio	Statistical significance: *P*
Energy					
Between subjects (*n* 5)	4718072·1	4	1179518·0	15·986	0·000
Between periods	18822·3	2	9411·1†	0·046	> 0·050
Subject × period	1651915·2	8	206489·4	2·798	0·012
Residual	3615512·5	49‡	73785·9		
Protein					
Between subjects (*n* 5)	4620·8	4	1155·2	11·200	0·000
Between periods	103·9	2	51·9	0·504	> 0·050
Subject × period	1219·0	8	152·3	1·477	> 0·050
Residual	5054·0	49‡	103·1		

* For details of subjects and procedures, see pp. 188–189.
† Tested over interaction term (i.e. subject × period).
‡ 1 df subtracted because of missing value.

The SOR of the five UN subjects over the duration of the TEM response are given in Table 5. Mean non-protein respiratory quotients (NPRQ) obtained over the duration of the BMR and TEM measurements were not significantly higher post supplementation than the presupplementation values. There were no differences in the oxidation rates of carbohydrate, fat or protein between the measurements made in the pre- and post-supplementation periods in the five subjects studied (Table 5).

Energy intakes and nutrient utilization rates corrected for differences in body-weight are presented in Table 6. The percentage of the test meal oxidized post supplementation was

Table 3. *Anthropometric indices pre- and post supplementation in chronically undernourished subjects*†

(Mean values with their standard errors)

Subject no. ...	1	2	3	4	5	Mean	SE
Presupplementation							
Height (m)	1·672	1·658	1·625	1·600	1·595	1·635	0·0002
Body-wt (kg)	46·1	40·5	41·0	45·1	42·3	43·0	1·11
FFM (kg)	39·9	37·0	36·9	40·8	38·1	38·5	0·78
Fat mass (kg)	6·2	3·5	4·1	4·3	4·2	4·5	0·45
BMI (kg/m²)	16·5	14·7	15·5	17·6	16·6	16·2	0·50
Post supplementation							
Body-wt (kg)	48·2	41·6	43·3	47·3	43·5	44·8*	1·27
FFM (kg)	41·1	37·0	37·3	41·6	38·6	39·1	0·96
Fat mass (kg)	7·1	3·7	5·9	5·7	4·9	5·7*	0·44
BMI (kg/m²)	17·3	14·9	16·3	18·5	17·0	16·8*	0·59

FFM, fat-free mass; BMI, body mass index (weight/height²).
Mean values were significantly different from presupplementation values (paired *t* test): * $P < 0·05$.
† For details of subjects and procedures, see pp. 188–189.

Table 4. *Metabolic indices measured pre- and post supplementation for chronically undernourished subjects*†

(Mean values with their standard errors)

		Presupplementation		Post supplementation	
		Mean	SE	Mean	SE
BMR:	kJ/h	213·7	6·8	225·8*	5·9
	Adjusted for FFM‡	214·4	6·3	225·0	6·3
TEM:	kJ/6 h	227·0	16·7	193·5*	17·6
	% energy in meal	9·1	0·7	7·7*	0·7
PMTEO:	kJ/6 h	1509·1	30·1	1548·1	20·0
	Adjusted for FFM‡	1511·1	26·1	1545·55	26·1

Mean values were significantly different from those presupplementation (paired *t* test): * $P < 0·05$.
† For details of subjects and procedures, see pp. 188–189.
‡ Using analysis of covariance (ANACOVA).

not significantly different from the presupplementation values, although subjects showed a trend towards net fat storage and lower protein oxidation rates.

DISCUSSION

In an earlier study we had demonstrated that chronically undernourished subjects had thermogenic capacities similar to age- and sex-matched controls, despite having higher TEM responses (Piers *et al.* 1992*b*). We concluded that the response to a meal in the UN subjects was unlikely to result in any energy saving. The present study was aimed at evaluating this hypothesis by looking for changes in the thermogenic capacities of these UN subjects following dietary supplementation.

The 24 h recall method indicates the actual food consumption of population groups (Rasanen, 1982). We attempted to estimate the relative accuracy of the recall method by

Table 5. *Non-protein respiratory quotients (NPRQ) and substrate oxidation rates during the basal metabolic rate (BMR) (g/h) and post-prandial (g/6 h) periods pre- (Pre) and post supplementation (Post) for chronically undernourished subjects**

(Mean values with their standard errors)

	NPRQ		Carbohydrate		Fat		Protein	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
BMR (post-absorptive)								
Mean	0·943	1·035	8·82	10·97	0·80	−0·45†	2·18	2·09
SE	0·028	0·030	1·35	0·57	0·31	0·44	0·20	0·19
Statistical significance: *P*	NS		NS		NS		NS	
Post-prandial								
Mean	0·994	1·089	72·00	79·36	0·88	−8·92†	11·88	10·41
SE	0·013	0·039	3·71	1·38	2·04	4·04	0·68	0·85
Statistical significance: *P*	NS		NS		NS		NS	

* For details of subjects and procedures, see pp. 188–189.
† A negative fat oxidation rate is indicative of a net lipogenesis from glucose.

Table 6. *Energy intakes and substrate oxidation rates, per kg body-weight pre- and post-prandially for chronically undernourished subjects*†

(Mean values with their standard errors)

	Pre-prandial		Post-prandial					
	Oxidized (kJ/h)		Intake (kJ)		Oxidized (kJ/6 h)		Intake oxidized (%)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Presupplementation								
Carbohydrate	3·41	0·48	43·72	1·12	28·09	1·58	64·27	3·31
Fat	0·72	0·29	8·74	0·22	0·83	1·78	8·85	20·49
Protein	0·86	0·10	5·83	0·15	4·65	0·35	79·54	4·58
Post supplementation								
Carbohydrate	4·10	0·18	42·02**	1·18	29·75	0·88	70·84	1·23
Fat	0		8·40**	0·23	0		0	
Protein	0·78	0·07	5·61**	0·16	3·89	0·29	69·62	5·69

Mean values were significantly different from those presupplementation (paired *t* test): ** $P < 0·01$.
† For details of subjects and procedures, see pp. 188–189.

comparing this with weighed intakes in the same subjects. Although overall there were no significant differences in energy and protein intakes by the two methods, the significant subject × method interaction would suggest that use of each method varied considerably from one subject to another. The 24 h recall method was used to reveal any 'substitution' in the unsupplemented intakes during the period of supplementation. The results (Table 2) show no differences in intakes of energy and protein; however, there was a significant subject × period interaction for energy intakes, indicating variations in energy intakes in each subject over time.

Despite the limitations of dietary recalls in the present study, the significant increases in body-weight are suggestive of successful dietary augmentation in spite of possible substitution. The greater proportion of fat, compared with fat-free tissue, gained during this period (Table 3) may be related to net lipogenesis, following supplementation, both in the fasted (BMR) and the fed state. There were significant increases in the absolute BMR following supplementation, with this trend persisting even after adjusting for FFM using an ANACOVA, indicative of an increase in the metabolic activity of FFM. Similar results have been obtained in this laboratory from serial measurements of BMR in these UN subjects over 12 weeks (Soares *et al.* 1992), and were also observed following refeeding of semi-starved individuals (Keys *et al.* 1950; Grande *et al.* 1958).

The TEM responses measured post supplementation were significantly lower both in absolute terms and when expressed as a percentage of the energy content of the meal. Although altered SOR could contribute to this lowering of the TEM response, we found no statistically significant differences, only trends. This may due to the small sample size or the fact that each individual may respond in different ways to the dietary supplementation. The interesting inverse correlation of BMR to TEM (r −0·86) may in fact be the major contributor to this 'apparent' lowering of the TEM response. We believe that for differences in TEM to have physiological meaning, they must be accompanied by differences in the PMTEO in the same direction as the TEM (Soares *et al.* 1989).

Garrow (1985) has earlier argued that the apparently smaller thermic response in obese compared with lean subjects, would not in itself provide an explanation for their obesity, since lean controls have a low resting metabolic rate (before the meal is given); following a meal they demonstrate a larger increase in metabolic rate, compared with the obese. However, the total energy expenditure (analogous to the PMTEO in the present study) is less for lean controls than for the obese at every stage of the measurement. The changes in the TEM response following supplementation would therefore have little biological significance, unless accompanied by a concomitant change in PMTEO. The unchanged PMTEO in the present study, in absolute terms or even corrected for the small changes in body-weights or FFM, indicate an unaltered 'thermogenic capacity' following dietary supplementation.

In conclusion, UN individuals show a lowering of the TEM response following dietary supplementation. However, this lowering appears to be reciprocal (r −0·86) to the raised BMR, since PMTEO were unaltered following supplementation. These results validate our earlier conclusions that in the chronically undernourished there may be no energy saving associated with modulations in TEM (Piers *et al.* 1992*b*).

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