The metabolic response of young women to changes in the frequency of meals

By YOLA E. SWINDELLS, SUSAN A. HOLMES and MARION F. ROBINSON

Department of Nutrition,
University of Otago School of Home Science, Dunedin, New Zealand

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1. Six healthy young women residing in a metabolic unit, but continuing their normal activities, received a uniform diet for 27 days, subdivided into four periods of 6 days and a final collection period of 3 days.

2. The daily food was divided into equal-sized portions; the subjects ate their daily quota as three meals a day in periods 1, 3, and 5 (control), two meals in period 2 (gorging), and nine meals in period 4 (nibbling).

3. The metabolic response of each subject was investigated by measurements of energy expenditure, and by analysis of the food, the urine and the faeces.

4. The subjects showed distinct differences in response, but for most subjects the changes in body-weight and in the metabolism of nitrogen and fat appeared unrelated to the frequency of the meals.

The pattern of ingestion of food has a considerable effect on the metabolism and the body composition of experimental animals (Tepperman, Brobeck & Long, 1943; Cohn, Joseph & Allweiss, 1962; Fisher, 1964; Heggeness, 1965; Leveille & Hanson, 1965). Although the normal feeding pattern of most herbivorous animals is a 'nibbling' one, while carnivorous animals may eat very infrequently ('gorging'), it is uncertain whether omnivorous man fares better on a nibbling, a gorging or a conventional meal pattern. Cohn *et al.* (1962) concluded that a nibbling pattern might be beneficial to man, whereas the modern western tendency is to eat a large proportion of the day's calories at one evening meal. In a study on the effect of frequency of meals, Irwin (1964) emphasized that the source and proportion of the nutrients should be kept constant from meal to meal as well as from day to day. Irwin & Feeley (1967) were unable to demonstrate any metabolic effects of increasing the frequency of meals from three to six per day in fifteen young women on such a uniform diet.

In order to extend the studies of factors which may affect the body-weight of women (Robinson & Watson, 1965), we have now investigated the influence of the meal pattern on the metabolism of protein, fat and carbohydrate. The present report records the metabolic response of six women who consumed a uniform diet while continuing their normal activities. Only the frequency of their meals was varied, from two meals per day (gorging) to three meals (control) to nine meals (nibbling). The subjects showed distinct differences in their metabolic response and in most instances this appeared unrelated to the frequency of their meals.

EXPERIMENTAL

Subjects

The subjects were six young women whose age, height and weight at the time of the experiment are given in Table 1. The subjects are further defined in terms of their mean basal metabolic rate (ml O_2/min), urinary excretion of creatinine (g/24 h) and percentage of body fat, derived from skinfold thickness as described by Durnin & Rahaman (1967). From Table 6 of their paper, the percentage of fat was predicted from the total thickness of skinfolds at four sites (biceps, triceps, subscapular and suprailiac).

The women carried out their normal activities so far as was possible while living at the metabolic unit in our nutrition department. Subject S continued her work as a university lecturer and the graduate student H analysed the gas samples for the measurement of energy expenditure whilst she was a subject. The remaining women were undergraduates; D and W were relaxing after end-of-year examinations but G and M attended regular lectures.

Table 1. *Description of the six women subjects at the time of the experiment*

Subject	Date of expt	Age (years)	Height (cm)	Weight (kg)	Body fat (%)	BMR* (ml O_2/min)	Urinary creatinine* (g/day)
S	July 1964	38	163	65·8	26	210 ± 10	—
H	July 1965	24	177	67·7	24	236 ± 15	—
D	Nov. 1966	21	169	79·6	30	259 ± 33	1·77 ± 0·09
W	Nov. 1966	22	165	61·8	24	236 ± 19	1·51 ± 0·06
G	June 1967	19	154	48·5	24	204 ± 11	1·33 ± 0·03
M	June 1967	20	161	54·2	23	236 ± 20	1·18 ± 0·04

* Mean values and standard deviations.

Procedure

The 1966 study took place in late spring but the remainder were mid-winter experiments, with S and H being studied one at a time and the others in pairs. The women slept at the metabolic unit, prepared meals and did light laboratory work connected with the experiment.

Each experiment lasted for 27 days as indicated in the scheme in Table 2; the subjects were weighed daily on a Shering balance accurate to 1g. On waking the subjects took their morning temperatures; the presumed date of ovulation was estimated from the basal morning temperature graphs as described by Robinson & Watson (1965). The times of menstrual bleeding were recorded. The food for each day was divided into the appropriate number of equal-sized meals; thus the daily quota was eaten as three meals during the control periods 1, 3 and 5, two meals during period 2 and nine meals during period 4. The calorie intake allowed for each subject's daily quota was based on preliminary measurements of her energy expenditure.

Diet

Details of the diet have been described elsewhere (Holmes, Swindells, Sharpe, Wright & Robinson, 1968). It consisted of meat loaf and ice-cream with tea and coffee, and, in 1967, canned orange juice. It contained the kinds of foods normally consumed in New Zealand; bread, butter and savoury foods were combined into the loaves; milk and sugar formed the basis of the ice-cream. The constituents of the meat loaf expressed in g/kg uncooked loaf were: bread, 134 g; butter, 80 g; potatoes, 147 g; carrots, 93 g; onions, 47 g; tomatoes, 73 g; peas, 187 g; minced beef steak, 167 g; eggs, 53 g; salt, 2·3 g; with tomato and soya sauces for flavouring. The day's ration of ice-cream contained evaporated milk, 310 g; gelatin, 4 g; and sugar, 57 g. Each year sufficient food was purchased at one time for the whole experiment; all the meat loaves were cooked in advance, and the ice-cream was prepared daily from one tin of evaporated milk for each subject.

Samples of the meat loaf were taken daily and dried in a vacuum oven at 70°. The pooled dried samples were analysed for nitrogen by the micro-Kjeldahl method, crude fat by Soxhlet extraction, and gross calories with a ballistic bomb calorimeter. Several batches of ice-cream were prepared after each experiment and analysed as above except that fat was determined by a modification of the Gerber method (Davis & Macdonald, 1942). The protein content of the loaf was taken to be 6·25 times and of the ice-cream 6·38 times the N content. Solids and gross calories were determined in the orange juice. The soluble tea and coffee along with the other items of the diet were analysed for sodium, potassium, calcium, magnesium and phosphorus; these findings are to be reported later.

Faeces

Brilliant blue was used as a faecal marker for all except subject S, who used carmine which we have found less satisfactory. Two gelatin capsules containing brilliant blue combined with methyl cellulose (Lutwak & Burton, 1964) were taken during the evening following the last meal of each period; the presence of green faeces marked

Table 2. *Daily routine followed throughout the experiment*

Period ...	1	2	3	4	5
Length of period (days)	6*	6	6	6	3
No. of meals/day	3	2	3	9	3
Time of weighing	08.15 h	08.15 h	08.15 h	08.15 h	08.15 h
Time of eating meals	08.30 h	—	08.30 h	08.30 h	08.30 h
	—	—	—	10.10 h	—
	—	11.50 h	—	11.50 h	—
	13.30 h	—	13.30 h	13.30 h	13.30 h
	—	—	—	15.10 h	—
	—	—	—	16.50 h	—
	18.30 h	18.30 h	18.30 h	18.30 h	18.30 h
	—	—	—	20.10 h	—
	—	—	—	21.50 h	—

* During period 1 the experimental diet was eaten for the whole period but measurements were made on days 4–6 only.

the beginning of the next period. In addition subjects G and M took one capsule containing 0·5 g chromium sesquioxide (Cr_2O_3) three times daily throughout the study (Whitby & Lang, 1960). The capsules were taken with the three meals during periods 1, 3, 5 and at about the same time of day in periods 2 and 4.

Each stool was collected, weighed and dried under an infrared lamp for determination of its solid content. The dried faeces were pooled for each period or for each half period, and determinations of N and gross calories were made as for the dietary analysis. Faecal fat was measured by a wet extraction method (King & Wootton, 1959), and chromium in faeces by atomic absorption spectrophotometry (Williams, David & Iismaa, 1962).

Urine

The urine was collected in 24 h periods with the final collection before each subject was weighed at 08·15 h. The volume of each sample was measured, total N was determined by the micro-Kjeldahl method, urea by colorimetric reaction with *p*-dimethyl-aminobenzaldehyde (Southgate & Barrett, 1966), and creatinine by Folin's method (Hawk, Oser & Summerson, 1947). The calorie value was calculated as 7·9 kcal/g N (Atwater & Bryant, 1900). The specific gravity of the urine was measured daily with a urinometer, and the solids were also determined by drying at 100° samples of each day's urine, slightly acidified with two drops of acetic acid.

Energy expenditure and metabolic mixture

Energy expenditure was calculated from measurements of O_2 consumption, CO_2 output and urinary N. The respiratory exchanges were measured for 7–10 min periods with a Max–Planck respirometer for walking activities and a Douglas bag for resting experiments, using the Lloyd–Haldane apparatus for the analysis of gas samples. Energy expenditure in representative activities was measured five times daily at irregular intervals between 06·30 h and 23·00 h. Although the measurements were made at irregular times, the order in which the activities were studied during the first 6 days of each experiment was repeated in the subsequent 6-day period. For example, if energy expenditure while sitting was measured during the afternoon of day 2 in period 2, a similar measurement was made on day 2 of subsequent periods. In this way the procedure was standardized during the different regimes. The activities were classified as lying, sitting, up-and-about and outdoor walking, and a continuous diary record of the time spent in each of these activities was kept by each subject for the whole experiment. The methods were those described by Passmore, Meiklejohn, Dewar & Thow (1955) and used by Passmore, Strong, Swindells & el Din (1963).

The energy expenditure while sleeping and lying awake was calculated from the basal O_2 consumption taken daily between 06·30 and 08·00 h (Table 1). For the metabolic cost of outdoor walking, subjects S and H used a slightly sloping path around the outside of the metabolic unit. The construction of a treadmill in 1966 enabled subsequent measurements to be made in standardized conditions regulated to the pace of outdoor walking for each student. The up-and-about classification included a combination of standing and indoor walking, since there are many activities where there is a continuous change from one to the other. Both up-and-about and sitting measurements were made with each subject engaged in her usual activities.

Measurements of energy expenditure were made daily except on day 4 of periods 2, 3 and 4 which were taken as rest days. On these days each subject spent an extra 3 h lying down, no measurements of energy expenditure were made but all other aspects of the experiment were carried out as usual. In period 1 the diet was eaten for 6 days but measurements were made on the last 3 days only.

Having measured the utilization of O_2 and the output of CO_2 and of urinary N it is possible to calculate the composition of the metabolic mixture. The equations set out by Consolazio, Johnson & Pecora (1963) were used for the calculations.

RESULTS

Diet

The composition of meat loaf, ice-cream and orange juice is given in Table 3; the minor variations in their solids, N, crude fat and calorie content reflect the difference in composition of the ingredients purchased in different years. Each subject chose her

Table 3. *Composition of meat loaf, ice-cream and orange juice*

Year of expt	Water (g/100 g)	Nitrogen (g/100 g dried food)	Crude fat (g/100 g dried food)	Gross calories (kcal/100 g dried food)
		Meat loaf		
1964	69·5	2·8	23·5	534
1965	63·2	3·5	30·4	594
1966	64·2	3·6	27·4	553
1967	60·2	3·2	27·5	548
		Ice-cream		
1964	63·7	2·8	17·3*	531
1965	63·7	2·8	17·3*	531
1966	63·3	3·2	16·9*	526
1967	64·4	2·9	18·5*	533
		Orange juice		
1967	86·6	—	—	407

* Estimated on melted ice-cream by Gerber method (Davis & Macdonald, 1942).

own level of fluid intake but kept it constant from day to day. The quantity of ice-cream equivalent to 20 oz whole milk was consumed daily, and differences in calorie needs were made up by appropriate amounts of meat loaf. Table 4 gives the mean daily intake of food and beverages for each subject along with the gross calorie value and the intake of water, protein and fat calculated from the analysis of the diet for the whole length of the experiment less days 1, 2 and 3. Reference to food tables (Osmond & Wilson, 1966) and to the recommended dietary allowances (National Research Council: Food and Nutrition Board, 1964) suggests that the subjects had an adequate intake of the major nutrients. The daily addition in 1967 of 100 g orange juice increased the ascorbic acid intake by 40 mg/day. The diet was varied in temperature, texture and flavour, and the subjects enjoyed it even after 27 days.

Faeces

The subjects had different mean daily weights of faeces, smallest for subject S and greatest for subject H (Table 5). Subjects S and H had also the highest and lowest percentages of faecal solids, 38% and 22%, respectively. The mean solid content for 27 days varied between 13 and 26 g/day. The brilliant blue used to mark the faeces for each period was easily seen. Altering the frequency of meals did not produce a uniform change in the amount of faeces. Subjects S, G and M appeared to respond to the nibbling regime with considerable reductions in faecal output. The faeces of subjects G and M had, however, been marked with the 'continuous' faecal marker chromium sesquioxide as well as with the 'intermittent' marker brilliant blue. From the relationship between the chromium ingested and the chromium recovered in the faeces during the periods marked by brilliant blue, it was calculated that the faecal output had in fact changed little during the whole study for these two subjects (see values in parentheses in Table 5). Likewise the faecal loss of N and of fat was almost unchanged

Table 4. *Daily dietary intake of each subject expressed as (a) weight* of food and drink, and (b) water, protein, crude fat and gross calories*

Subject	Meat loaf (g)	Ice-cream (g)	Tea or coffee (g)†	Orange juice (g)	Total water (g)	Protein (g)	Crude fat (g)	Gross calories (kcal)
S	923 ± 3	376 ± 7	1800 ± 0	—	2681	74	75	2230
H	1179 ± 1	365 ± 0	1200 ± 0	—	2178	119	140	3280
D	852 ± 1	375 ± 0	1600 ± 0	—	2384	97	92	2410
W	849 ± 3 750‡ ± 0	375 ± 0	1425 ± 0	—	2154	90	84	2240
G	675 ± 0	375 ± 0	647 ± 0	100 ± 0	1382	78	83	2240
M	720 ± 0	375 ± 0	650 ± 0	100 ± 0	1412	82	88	2340

* Mean values and standard deviations.
† Weight of drink containing soluble tea or coffee.
‡ Intake decreased from 2nd day of period 2.

Table 5. *Daily faecal output of each subject in periods* 2, 3 *and* 4

(Values in parentheses have been corrected for recovery of 6-day ingestion of chromium sesquioxide)

	Weight (g)			Solids (g)			Fat (g)			Nitrogen (g)			Calories (kcal)		
Period... Subject	2	3	4	2	3	4	2	3	4	2	3	4	2	3	4
S	54	37	12	21	14	4	3·4	2·4	0·9	0·7	0·6	0·2	98	63	20
H	153	119	104	31	24	27	2·9	2·1	2·5	2·2	1·5	1·6	164	125	143
D	68	90	46	16	23	12	2·5	2·7	1·7	0·8	1·1	0·6	75	109	57
W	59	91	95	18	27	23	1·5	2·0	1·4	0·9	1·4	1·2	87	131	110
G	71	77	36	19 (17)	21 (19)	10 (17)	1·6 (1·4)	1·9 (1·6)	0·6 (0·9)	1·1 (0·9)	1·2 (0·9)	0·6 (0·9)	84 (72)	90 (75)	45 (68)
M	44	59	19	13 (19)	20 (17)	6 (16)	1·8 (2·4)	2·7 (2·2)	0·6 (1·6)	0·8 (1·1)	1·1 (0·9)	0·3 (0·8)	63 (83)	89 (71)	26 (67)

for these subjects. Moreover the faecal fat accounted for only a small part of the intake of fat, indicating almost complete digestion of fat. The percentage digestibility of fat as derived by the method of Irwin & Feeley (1967) was in fact 98%, a little higher than the 94% these workers had found in their study.

Urine

Table 6 gives the mean daily weights (g) of urine for the duration of the experiment excluding days 1, 2 and 3, and its content of solids, N, urea, and calories; the excretion of urea was not measured for subjects S and H. In spite of the constancy of intake of

Table 6. *Composition* of the daily urine of the six subjects*

Subject	Weight (g)	Solids (g)	Nitrogen (g)	Urea (g)	Calories (kcal)†
S	1905 ± 197	34 ± 2·9	11·2 ± 0·8	—	90
H	1687 ± 180	45 ± 5·9	16·8 ± 0·8	—	130
D	1733 ± 256	38 ± 3·7	14·1 ± 1·1	25·4 ± 2·0	110
W	1501 ± 227	35 ± 4·3	12·3 ± 0·8	21·9 ± 1·6	100
G	1036 ± 126	40 ± 2·8	11·1 ± 0·7	21·0 ± 1·7	90
M	1000 ± 168	44 ± 3·4	11·9 ± 0·7	22·9 ± 1·6	90

* Mean values with standard deviations.
† Calculated as 7·9 kcal/g N (Atwater & Bryant, 1900).

Table 7. *Daily intake and urinary excretion of nitrogen during periods 2, 3 and 4*

Subject	Intake (g)	Urinary nitrogen* (g) in period: 2	3	4
S	11·7	11·5 ± 0·4	11·0 ± 0·6	11·8 ± 0·7
H	18·9	16·2 ± 0·9	17·4 ± 0·3	16·8 ± 0·6
D	15·4	13·1 ± 0·3	14·3 ± 0·8	14·7 ± 0·8
W	14·3	12·9 ± 0·4	12·0 ± 1·2	11·9 ± 0·4
G	12·5	10·7 ± 0·6	11·1 ± 0·5	11·3 ± 0·4
M	13·0	11·9 ± 0·4	11·8 ± 0·9	11·6 ± 0·6

* Mean values with standard deviations.

fluids and food for each subject, the quantity and composition of the urine fluctuated from day to day throughout the experiment. Table 7 compares the N intake with the urinary N excretion during the various periods. When the regime was changed from two to three meals per day, the N excretion increased in subjects H and D and decreased in subject W; there were only small alterations after a change from three to nine meals per day.

The daily excretion of N by our six subjects is illustrated in Fig. 1, together with the times of menstrual bleeding and of ovulation derived from the basal morning temperature (Robinson & Watson, 1965); the vertical lines delimit the periods of gorging (2), nibbling (4) and the control periods (1, 3, 5). The four subjects (S,H,D and W) in whom menstruation occurred in the latter part of the study in periods 4 and 5 showed a rise and a fall in the excretion of N during the postovulatory and premenstrual phases of the menstrual cycle, respectively. Peak values of N excretion occurred about 6 days before menstruation and these happened to coincide with the time of gorging for subject W, with the intermediate control period for subject H and with nibbling for subjects D and S. This tendency for the excretion of N to vary with the phases of the menstrual cycle was not so apparent for subjects G and M, whose menstruation occurred early in the study.

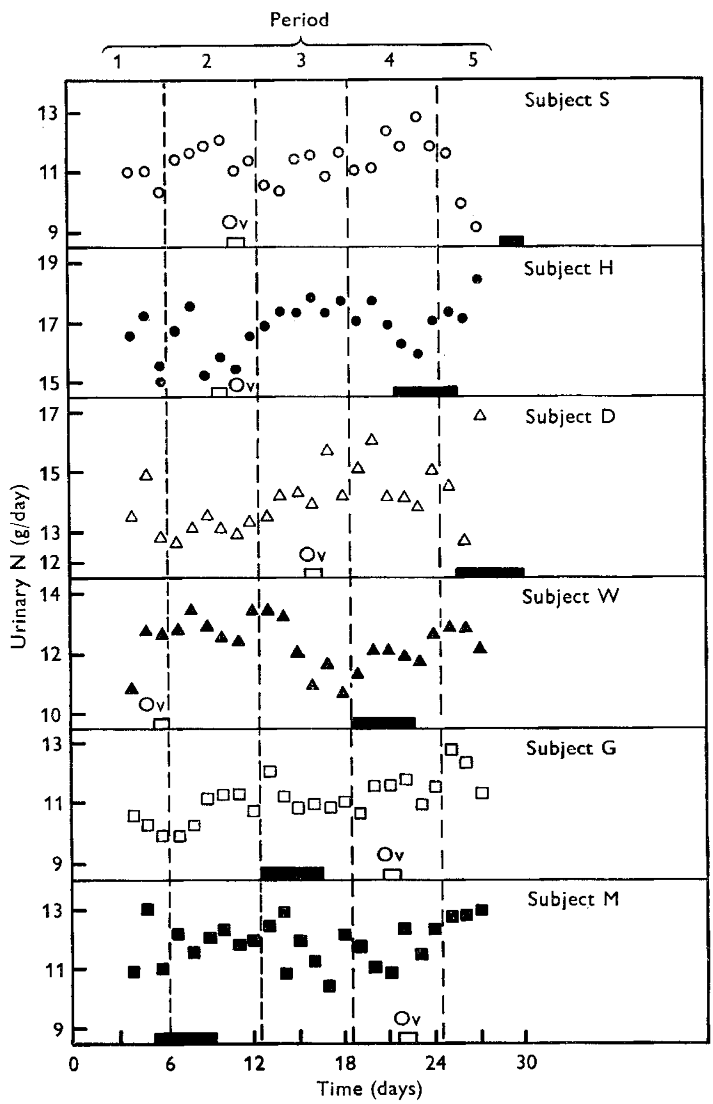

Fig. 1. Daily excretion of urinary nitrogen (g/day) for the six subjects. Vertical lines mark the periods of gorging (2), nibbling (4) and the control periods (1, 3, 5). Occurrence of menstruation (▬) and of ovulation (▭) are shown on the abscissa.

Energy expenditure

Details of the individual variations in energy expenditure and of daily changes in body-weight will be reported in a later paper. Each day the women spent 8 h lying in bed, 1 h walking outside, 5–9 h in light sitting activities and 6–10 h 'up-and-about', excluding the 3 rest days. Subject H, who carried out laboratory work during the experiment, spent approximately 10 h up-and-about each day and consequently only 5 h sitting.

Table 8. *Mean daily values for each subject for oxygen utilization, carbon dioxide production and urinary nitrogen, and for the energy expenditure:** (*a*) *for* 21 *days on which energy expenditure measurements were made,* (*b*) *for* 3 *'rest' days,* (*c*) *for whole experiment*

	O_2 utilization (l.)		CO_2 production (l.)		Urinary N (g)		Energy expenditure (kcal)		
Subject	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*c*)
S	530	498	431	404	10·9	11·8	2470	2320	2450
H	573	537	475	463	16·8	16·6	2670	2520	2650
D	557	515	485	448	14·2	13·7	2620	2430	2600
W	503	457	401	363	12·4	11·8	2330	2110	2300
G	434	410	353	334	11·1	11·3	2020	1900	2000
M	514	470	417	384	11·9	11·9	2390	2190	2360

* Calculated according to Consolazio, Johnson & Pecora (1963).

Table 9. *Percentage of calories in the metabolic mixture derived from protein and fat during period* 2 (*gorging*), *period* 4 (*nibbling*) *and the whole experiment*

	Protein (%)			Fat (%)		
Subject	Period 2	Period 4	24 days	Period 2	Period 4	24 days
S	10·9	13·2	11·6	65·5	52·2	54·5
H	15·5	16·4	16·1	51·0	61·8	46·1
D	12·6	15·1	13·7	37·0	33·1	33·1
W	13·4	13·5	13·5	63·2	55·3	59·1
G	13·7	15·1	14·0	53·6	51·1	52·8
M	12·5	13·2	12·6	53·6	50·6	54·6

Table 8 gives the mean daily utilization of O_2 and production of CO_2 and the calorie expenditure calculated from the gaseous exchange and the urinary N. Section (*a*) of Table 8 gives mean values for the 21 days on which energy expenditure was measured; section (*b*) gives the calculated mean results for the 3 rest days. The diary record and urinary measurements were continued on the rest days, but the metabolic cost of the activities was not measured. In section (*c*) the mean daily calorie expenditure for the whole experiment is shown. This was 2600–2650 kcal/day for subjects D and H, respectively, 2300–2450 kcal/day for M, S and W and only 2000 kcal/day for G, who was the youngest and lightest subject.

The percentage of the calorie expenditure derived from the protein and fat metabolized has been listed for each subject in Table 9 for the whole experiment, as well as for the gorging and nibbling periods. The experimental results forming the basis of Table 9 excluded the rest days. Over the whole experiment five of the subjects derived approximately half of their calories from fat (Table 9*b*) but for D the proportion was only one-third. Subject D exhibited a unique metabolic pattern throughout the experiment: reference to Table 1 suggests that her body composition was also different from that of the other five women.

Meal frequency altered the percentage of calories derived from fat (Table 9*b*), which increased during nibbling for subject H ($t = 3.03$, $P < 0.005$) and during gorging for subject *W* ($t = 4.4$, $P < 0.001$). Subject S also showed an apparent rise during gorging but this was not significant. Changes in the composition of the metabolic mixture are indicated mainly by changes in the relative amounts of carbon dioxide produced and of oxygen utilized, i.e. by changes in the respiratory quotient (RQ). This ratio was uniformly lower for subject H during nibbling and for W during gorging, but for subject S the RQ varied widely in period 2.

Changes in body-weight

The net changes in body-weight during each of the 6-day periods were derived from the basal morning body-weights (Table 10). Although the body-weights of the subjects did tend to fall slightly during most of the periods, nibbling did not appear to be

Table 10. *Changes in body-weight for each experimental period and the mean size of the day-to-day variations in body-weight*

Subject	Weight change (kg) in period: 1	2	3	4	5	24 days	Mean size of day-to-day weight change (kg)
S	+0·12	−0·18	+0·39	−0·46	+0·73	+0·60	0·19
H	+0·24	−0·23	−0·05	+0·26	−0·66	−0·44	0·14
D	+0·04	−0·13	−0·63	−0·40	−0·31	−1·43	0·13
W	+0·55	−1·24*	+0·16	−0·57	+0·03	−1·07	0·19
G	−0·02	−0·05	−0·42	−0·19	+0·34	−0·34	0·11
M	−0·36	−0·49	−0·30	−0·01	−0·24	−1·40	0·11

* Change in intake of food (Table 4).

associated with any appreciable loss in body-weight during this short experimental period. The average sizes of the day-to-day changes in body-weight have been included in Table 10; these changes were considerably less than the mean size of day-to-day variations (0·28 kg) of twenty-eight young women on a regime of unrestricted food, fluids and activity (Robinson & Watson, 1965). Moreover only 4 of the total 144 day-to-day changes in body-weight were greater than 0·5 kg. These were losses and they occurred on the 1st and 3rd days of menstrual bleeding for subjects D and W, respectively, on the day of rest during gorging for subject S, and for subject W on the day after the food intake was reduced. This contributed to the over-all loss of 1·2 kg by subject W during gorging. The importance of these changes will be discussed in a later paper along with values for the metabolic balance of water and electrolytes; body-weight is influenced by the stage reached in the menstrual cycle (Watson & Robinson, 1965).

DISCUSSION

In animals such as young rats and sheep, a regime of gorging was associated with an increased urinary excretion of N (Cohn, 1963; Rakes, Lister & Reid, 1961), but in experiments with human subjects the results have been conflicting. Cuthbertson & Munro (1939) concluded from an experiment on one male subject that meal frequency was only a minor factor in determining urinary N, and that other factors such as the carbohydrate content of each meal were more important. Wu & Wu (1950) reported a small increase in N retention when one male subject on a diet calculated to contain 12·5 g N/day changed from two to four meals a day.

Irwin & Feeley (1967) made a careful study for 60 days on fifteen young women and found no significant difference in N retention when changes were made in the frequency of food intake. Their subjects, like ours and the subject of Cuthbertson & Munro (1939), consumed a uniform diet from meal to meal as well as from day to day. In our study only one subject had a greater urinary N level during gorging while another subject excreted less during this period (Table 7). The N excretion seemed to be linked with the stage reached in the menstrual cycle; this had been reported previously by Pucher, Griffith, Brownell, Klein & Carmer (1934), who had confirmed earlier observations that in women the excretion of N is minimal at about the time of menstruation and maximal in the latter half of the 'intermenstrual period'. Our findings could imply that any effect of frequency of meals upon urinary N excretion was minor and had been overshadowed by cyclic changes because our subjects were young women.

Since we were measuring energy expenditure it was possible to study also the metabolism of carbohydrate and fat. The metabolic response was expressed as a percentage of the energy expenditure because of the wide range in the calorie requirements of the six women (Table 8). The metabolism of carbohydrate and fat showed differences both between individual subjects and between periods in the same subject. Whether such differences would be observed during longer experimental periods needs further investigation.

It is possible that the apparent difference in metabolic response of subjects H and W as shown in the percentage of fat metabolized (Table 9*b*) might have been due either to the small proportion of each day covered by the measurements or to the fact that the experiments were conducted in different years. There were, however, twenty to twenty-five measurements of energy expenditure made on each subject in each of the periods 2, 3, and 4 between 06.30 and 23.30 h. Moreover the order of these measurements was standardized (see p. 670) so that the errors due to timing in the collection of samples of expired air should have been comparable for each period. The validity of the findings is supported by the difference in metabolic response of the two subjects in the 1966 experiment in which the composition of the diet and the timing of the collection of expired air samples were identical.

The introduction of the rest day in each period may have overshadowed the effects of meal frequency. It was introduced to enable the experiment to be continued for almost a month with a small staff and busy subjects, but it was also considered to be typical of the fluctuations in activity and rest which form a common part of the pattern of energy expenditure of sedentary workers in western countries.

There has been considerable interest in the effect that the frequency of feeding might have upon the metabolism of carbohydrates and lipids, in particular upon its possible effect upon the plasma lipids and the tendency to overweight. Hejda & Fábry (1964) and Fábry, Fodor, Hejl, Braun & Zvolankova (1964) studied the normal dietary habits of Czechoslovakian men and found that the incidence of overweight and hypercholesterolaemia was greater in those men eating up to three meals a day than in those eating five or more meals a day; there was presumably considerable variation in the size of the meals eaten during the day. There is a tendency for at least half the day's calories to be consumed at one meal, usually in the evening, and this pattern of eating has been adopted in many studies. Jagannathan, Connell & Beveridge (1964) used a 'gormandizing' regimen (one-third of the food intake being equally divided between breakfast and lunch and the remaining two-thirds eaten in the evening) and compared its effect with 'semicontinuous' feeding of eight meals of equal size; a similar comparison was made by Nunes & Canham (1963). In other studies 'gorging' consisted of eating all the day's food in one meal (Gwinup, Byron, Roush, Kruger & Hamwi, 1963*a*, *b*; Knittle, 1966). In their comprehensive study of the effect of size and of frequency of feeding upon metabolism Irwin & Feeley (1967) served half the calories at the evening meal and compared this three-meal regimen with both three and six equal-sized meals. They found no significant differences among the regimens in the retention of N, in fat digestibility, or in the serum levels of phospholipids, glycerides and total fatty acids; they did find, however, that the serum cholesterol was lower on the regimen of three equal-sized meals per day than on that of two small and one large meal per day.

In the present study the frequency of feeding was the variable primarily being investigated and the daily food intake was divided into two, three or nine equal-sized portions. The nibbling and gorging periods were each of 6 days' duration, and each of these periods was preceded by a 6-day control period of three meals a day. Longer control or experimental periods were not considered necessary because the diet was almost identical with that normally consumed by the subjects. Moreover, when changes were obtained in the percentage of fat metabolized they occurred from the 1st day of the experimental periods, during nibbling for subject H and during gorging for subject W.

On the basis of a limited number of human experiments on gorging and nibbling various suggestions have been made for the therapeutic use of a 'nibbling' meal pattern (Cohn, 1961; Gordon, Goldberg & Chosy, 1963; Norton, 1966). The results presented here suggest that there might be considerable individual variations in the response to a more frequent intake of food; subject D with the highest percentage of body fat metabolized this nutrient with great economy regardless of the frequency of intake. Fábry (1967) pointed out that the metabolic effects of meal frequency are likely to be modified by 'total caloric intake, physical activity and the composition of the diet'. Although every effort was made to keep these factors constant in our experiments, there was considerable day-to-day variation in energy expenditure; this will be discussed in a later communication.

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