Effects of feeding a palatable 'cafeteria' diet on energy balance in young and adult lean (+/?) Zucker rats

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1. The effects of feeding a palatable and varied 'cafeteria' diet on energy balance were studied in young (5·5 week) and adult (5·5 month) lean male Zucker (+/?) rats.

2. Estimates of metabolizable energy (ME) intake derived from food composition tables were almost identical to values obtained from bomb calorimetry of foods, urine and faeces, and ME intake was elevated by approximately 73% in all 'cafeteria' animals compared to stock-fed controls.

3. 'Cafeteria' feeding had no effect on the body-weight of young rats but induced excess weight gains in the older animals and resulted in increased deposition of fat and energy in both groups. Energy expenditure, calculated from ME intake and body-energy gain, was elevated by 77 and 57% in young and adult cafeteria rats respectively. The energy cost of fat deposition could account for only a small proportion of this increased expenditure.

4. The present results confirm previous findings in another strain of rat and show that the increased energy expenditure (i.e. diet-induced thermogenesis, DIT) which occurs in response to hyperphagia is not restricted to young animals but is also seen in older rats. Measurements of resting oxygen consumption after injections of noradrenaline or a β-adrenergic antagonist (propranolol), and changes in brown adipose tissue mass are consistent with the suggestion that the DIT of 'cafeteria'-fed rats results from sympathetic activation of brown fat.

Energy balance studies on rats given a varied and palatable 'cafeteria' diet have revealed that hyperphagia is often accompanied by large, compensatory increases in energy expenditure i.e. diet-induced thermogenesis (DIT) (Rothwell & Stock, 1979*a*, 1980*a*, *b*). Detailed studies on these animals have revealed that DIT results from sympathetic activation of brown adipose tissue (BAT) (Rothwell & Stock, 1979*a*, 1981*a*; Brooks *et al.* 1981; Landsberg *et al.* 1981), and is therefore very similar to, and can substitute for, the non-shivering thermogenesis of cold-adapted animals (Rothwell & Stock, 1980*c*).

Some of these experiments have now been repeated by other workers (e.g. Tulp *et al.* 1980) and are corroborated by the finding that thermogenesis and BAT activity are reduced in genetically-obese mice (Thurlby & Trayhurn, 1979, 1980) and in rats made obese by hypothalamic lesions (Seydoux *et al.* 1981). However, one group (Armitage *et al.* 1981*a*, *b*) have failed to observe large changes in energy expenditure in 'cafeteria'-fed rats and have questioned the importance of DIT (Hervey & Tobin, 1981). It is likely that these discrepancies may be partly due to the age and strain of the animals used by these workers and the low level of hyperphagia achieved, but it has also been suggested (Hervey & Tobin, 1981) that the extent of hyperphagia achieved in the original experiments of Rothwell & Stock (1979*a*) was over-estimated due to the use of food composition tables to assess metabolizable energy (ME) intake.

In the present experiments, attention has been specifically directed to these questions using young and adult rats of a different strain from those in previous studies, and values for energy intake from food composition tables were compared to values estimated from bomb calorimetry of all foods, faeces and urine.

Table 1. *List of food items presented to cafeteria rats during the experiment*

(1) Chopped ham and pork	(15) Battenburg cake
(2) Corned beef	(16) Fruit cake
(3) Liver and bacon paté	(17) Trifle sponges
(4) Luncheon meat	(18) Toblerone
(5) Pork sausages	(19) Chocolate marshmallow
(6) Beef sausages	(20) Plain marshmallow
(7) Lean bacon	(21) Milk chocolate
(8) Shortcake	(22) Mars bars
(9) Chocolate wafers	(23) Crunchie bars
(10) Digestive biscuits	(24) Lasagna
(11) Coconut crunch cake	(25) Popcorn
(12) Chocolate roll	(26) Chocolate rice crisps
(13) Swiss roll	(27) Cheese
(14) Chocolate miniroll	

EXPERIMENTAL AND METHODS

Lean, male Zucker (+/?) rats were maintained in a metabolism room at 24±1° (12 h light–dark cycle), and allowed *ad lib.* access to water and a pelleted stock diet (PRD; Christopher Hill Group Ltd). On the first day of the experiment the body fat content of eighteen young (5·5 weeks) and twenty-two adult (5·5 months) rats was estimated from in vivo measurements of body water by the tritium dilution method (Rothwell & Stock, 1979*b*). From the latter results, rats of the same age were allocated to three groups: control, 'cafeteria', B_O, each with the same mean body-weight and fat content. There were six rats in each B_O group and six young and eight old rats in the control and 'cafeteria' groups. The B_O animals were killed for determination of initial body fat and energy content by direct analysis, and the surviving animals were maintained on stock diet or the 'cafeteria' diet for 24 d.

The technique of 'cafeteria' feeding has been described previously (Rothwell & Stock, 1979*c*) and the food items used in this experiment are listed in Table 1. Rats had continuous access to the stock diet and received four different palatable food items each day. Two foods were presented in the morning and the other two (usually high-fat or high-protein foods) were added in the evening. All animals were housed in pairs in wire-bottomed cages suspended above clean plastic trays. Food intake was measured daily and body-weight recorded on alternate days.

On day 10, resting oxygen consumption ($\dot{V}_{O_2}$) was measured for 2 h before and after injection of propranolol (10 mg/kg body-weight; sub-cutaneously) in a closed-circuit respirometer (Stock, 1975) maintained at 29°, and on day 14 before and after injection of noradrenaline (250 μg/kg body-weight; sub-cutaneously). At the end of the experiment all rats were killed by decapitation and blood collected for determination of plasma glucose (glucose oxidase method; Boehringer kit) and triiodothyronine (T_3) levels (RIA, Radiochemical Centre, Amersham). The interscapular BAT depot was dissected, cleaned of connective and white adipose tissue, and weighed. The carcasses were frozen before determination of water, fat and energy content.

ME intake

Over the course of the experiment 'cafeteria'-fed rats received a total of twenty-seven separate food items (Table 1). Apart from palatability, these foods were selected for high energy content, a reasonable degree of homogeneity and relatively-low water content in

order to minimize errors in collecting split food. The gross energy (GE) content of each food was determined by bomb calorimetry (Miller & Payne, 1959) and, to eliminate errors due to variation between batches, food samples were taken for analysis on each occasion they were presented. Uneaten foods were collected daily, separated and weighed and total ME intake was calculated using energy values from food composition tables (Paul & Southgate, 1978) or manufacturers' values. For stock diet, the value obtained from feeding the control groups was used. The uneaten food was then combined with the faeces and urine and the mixture analysed by bomb calorimetry. A direct estimate of ME intake was then obtained from the GE value of the food presented minus the energy content of all uneaten food, urine and faeces. This procedure was also adopted for stock-fed control rats and, although all the stock diet was taken from one batch, six separate samples were taken for analysis (i.e. each time the food hoppers were refilled). Bomb calorimetry of all items was carried out in quadruplicate on freeze-dried homogenized samples.

Carcass analysis

Frozen carcasses were chopped and freeze-dried to constant weight before homogenizing (Lotfi *et al.* 1976). Six samples of each carcass were taken for bomb calorimetry and four samples for estimation of the fat content by chloroform–methanol extraction. The energy content of the carcasses was also calculated using values of 38·0 and 19·2 kJ/g for the energy value of fat and fat-free dry mass respectively (Djazayery *et al.* 1979).

Calculation of energy expenditure and energetic efficiency

The gain in body energy content over the course of the experiment was calculated from the final energy content minus the average energy content of the B_O group. Expenditure was taken as the difference between ME intake and body-energy gain. Gross energetic efficiency was expressed as the body-energy gain per unit ME intake (kJ/kJ) and net efficiency (body-energy gain per unit ME intake above maintenance) was calculated using values for the energy cost of maintenance for lean Zucker rats obtained by Pullar & Webster (1977). For the adult rats, maintenance requirements were calculated using Pullar & Webster's (1977) value of 422 kJ/kg body-weight$^{0.75}$ per d, which was equivalent to 181 kJ/d for control and 192 kJ/d for 'cafeteria' groups. The energy cost of maintenance for younger rats (average weight, 158 g) was extrapolated from Pullar & Webster's (1977) values for 250 and 350 g rats and a figure of 435 kJ/kg body-weight$^{0.75}$ per d. Since the body-weights of young control and 'cafeteria'-fed rats did not differ throughout the experiment their estimated maintenance requirements were identical (110 kJ/d).

Statistical analysis

Values are presented as means ± SEM. Statistical comparisons between control and 'cafeteria'-fed rats were made using the Student's *t* test for unmatched data. All probabilities are two-tailed.

RESULTS

The mean body-weights of all groups over the course of the experiment are illustrated in Fig. 1 and the initial and final weights and weight gains are shown in Table 2. In young rats 'cafeteria' feeding had no effect on weight gain or final body-weight, but in older animals the 'cafeteria' diet caused significant increases in both. However, it can be seen from Fig. 1 that the rate of weight gain in 'cafeteria'-fed adult rats declined gradually over the course of the experiment, and over the last 5–7 d was almost identical to that of controls.

The values for ME intake of 'cafeteria'-fed rats (Table 2) demonstrate a remarkable agreement between values obtained from food composition tables and those from direct

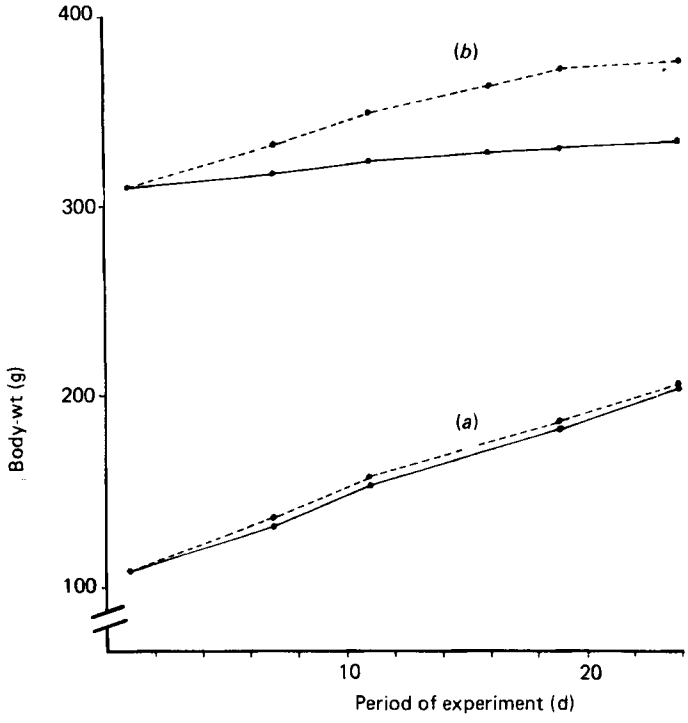

Fig. 1. Body-weights (g) of young (*a*) and adult (*b*) control (——) and 'cafeteria'-fed (- - -) rats during 24 d experiment.

Table 2. *Body-weights and energy intakes of control and 'cafeteria'-fed rats during the 24 d experiment*

(Mean values with their standard errors for six young rats and eight adult rats. Energy intakes were derived from the average food intake of rats housed in pairs)

Group...	Young control		Young cafeteria		Adult control		Adult cafeteria	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Body-weight:								
Initial (g)	111	4	110	13	310	12	311	15
Final (g)	206	12	206	16	337	13	379*	15
Wt gain (g)	94	9	96	8	27	6	69***	7
ME intake:								
Food tables (A) (kJ/rat)	—	—	8472	420	—	—	10392	360
Bomb calorimetry (kJ/rat) (B)	4990	240	8664***	528	5880	380	10152***	330
A/B × 100	—	—	97·8	3·2	—	—	102·4	1·2
ME density of diet (kJ/g as eaten)	12·07		15·14		12·00		15·26	
ME:GE	0·73		0·92		0·69		0·91	

ME, metabolizable energy; GE, gross energy.
Mean values were significantly different from respective stock-fed controls: $*P < 0{\cdot}05$, $***P < 0{\cdot}001$.

analysis of foods, urine and faeces. Intakes determined by these two methods did not differ significantly for either young or adult 'cafeteria'-fed rats (paired *t* test), and values obtained from food tables deviated by only 2% from estimates derived from direct analysis. Furthermore, this small error does not consistently over- or under-estimate intake and when values from all 'cafeteria' groups were combined the two methods agreed to within 0·1%.

Variations in GE content between samples of each human food item were remarkably small, even when separate batches were taken e.g. for three packs of sausages of the same brand, the energy content varied from 12·30–12·45 kJ/g. Similar agreement was obtained on repetitive sampling of the stock diet but in this study all stock diet was taken from the same batch whereas in previous experiments we have observed quite large variations in the ME content of PRD taken from different batches. This was due to differences both in the GE content of the diet (17·10–18·70 kJ/g) and its digestibility (ME:GE, 0·62–0·73).

ME intake over the 24 d of the experiment was elevated by approximately 73% in both 'cafeteria' groups compared to their respective controls. This hyperphagia resulted partly from the greater energy density and higher digestibility of the 'cafeteria' diet (Table 2) but also from a 40–50% increase in the actual weight of food consumed by the 'cafeteria'-fed rats.

'Cafeteria'-fed animals were presented with a wide choice of food items of varied composition (Table 1) and it was therefore surprising to find that the final nutrient composition of the diet selected was very similar for young and old rats. Fat, carbohydrate and protein represented 51, 34 and 15, and 53, 33 and 14% of total energy intake for young and adult 'cafeteria' groups respectively, compared to 9, 63 and 28% for controls, (nutrient intakes derived from food composition tables and manufacturers' values).

The body composition of all animals at the end of the experiment is shown in Table 3. Body fat content of these lean Zucker rats was quite low compared to other laboratory strains of the same age. For example, in control groups aged 9 weeks and 6·5 months at the end of the experiment, fat represented only 5·6 and 8·0% of carcass weight compared to usual values of approximately 10 and 20% for Sprague-Dawley rats at similar ages. In this experiment 'cafeteria'-feeding produced almost a doubling of fat content (% body fat; young $10{\cdot}5 \pm 0{\cdot}7$ adult $15{\cdot}1 \pm 1{\cdot}0$) in both groups, but in view of the previously-mentioned values, these animals might still be considered lean compared to other laboratory strains of rats. No significant differences in water or fat-free dry mass were observed between control and 'cafeteria' groups. In the younger rats 'cafeteria'-feeding did not cause any change in body-weight relative to controls but resulted in the deposition of an extra 9·7 g lipid. Similar values have been reported for other young rats given a 'cafeteria' diet (Stephens, 1980) and it has therefore been assumed that this diet is protein deficient and reduces growth. However, it can be seen (Table 3) that the small deficit in fat-free mass in the young rats was due to a slightly lower water content and fat-free dry mass was almost identical for young control and 'cafeteria'-fed rats.

Body energy content at the end of the experiment was elevated in both groups of 'cafeteria'-fed rats compared to their age-matched controls. The values for energy content presented in Table 3 were determined from bomb calorimetry of the carcasses. However, almost identical results were obtained when energy content was calculated by the method we have previously employed (Rothwell & Stock, 1979*a*) using values of 38·0 and 19·2 kJ/g for the energy density of fat and fat-free dry mass (calculated values: young control 1510 ± 171, young 'cafeteria' 1883 ± 210, adult control 2813 ± 135, adult cafeteria 4160 ± 263). These values were not significantly different from those obtained by bomb calorimetry (paired *t* test). Pullar & Webster's (1977) values for fat and fat-free dry mass (39·3 and 23·5 kJ/g) resulted in a small, non-significant over-estimate compared to bomb calorimetry values.

Table 3. *Body composition, energy expenditure and energetic efficiency of control and 'cafeteria'-fed rats during the 24 d experiment*

(Mean values with their standard errors for six young rats and eight adult rats. Mean body-weights of B_0 groups were 110 ± 6 and 309 ± 12 g and mean body energy contents were 730 ± 116 and 2345 ± 103 kJ for young and adult rate respectively)

	Young control		Young cafeteria		Adult control		Adult cafeteria	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Final body composition								
Body water (g)	141·5	6·3	132·4	10·1	218·1	10·1	225·7	8·2
Body fat	2·1	2·8	21·8*	3·4	27·2	1·5	57·7***	5·3
Fat-free dry mass (g)	53·2	2·7	52·6	4·1	91·8	3·9	96·6	4·8
Body energy content								
Final (kJ)	1522	180	1915	187	2902	120	4150***	290
Gain (kJ)	790	140	1187	190	557	120	1805***	290
Energy expenditure (kJ)	4193	150	7453***	198	5325	250	8334***	187
Gross efficiency	0·156	0·023	0·137	0·11	0·101	0·019	0·177	0·036
Net efficiency	0·336	0·039	0·197**	0·010	0·365	0·102	0·331	0·042

Mean values were significantly different from respective stock-fed controls: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

Table 4. *Resting $\dot{V}_{O_2}$, brown adipose tissue (BAT) mass, blood glucose and plasma T3 levels of control and 'cafeteria'-fed rats*

(Mean values with their standard errors for six young rats and eight adult rats)

	Young control		Young cafeteria		Adult control		Adult cafeteria	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Resting $\dot{V}_{O_2}$ (ml/min per $kg^{0{\cdot}75}$)								
Before noradrenaline	14·18	0·30	18·06***	0·77	10·96	0·25	12·92***	0·28
After noradrenaline	18·00	0·61	25·03**	1·80	14·46	0·61	17·57**	0·54
% preinjection value	128·3	1·6	138·3*	2·9	135·4	6·7	136·5	2·9
Interscapular BAT mass								
(mg)	225	35	468***	61	338	31	604***	52
(mg/100 g)	105·8	11·0	222·8***	14·0	99·8	4·6	159·0***	10·0
Blood glucose (mmol/l)	7·70	0·26	7·86	0·29	6·99	0·30	7·84*	0·20
Plasma T_3 (ng/100 ml)	99	5	131*	6	99	3	94	5

Mean values were significantly different from respective stock-fed controls: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.

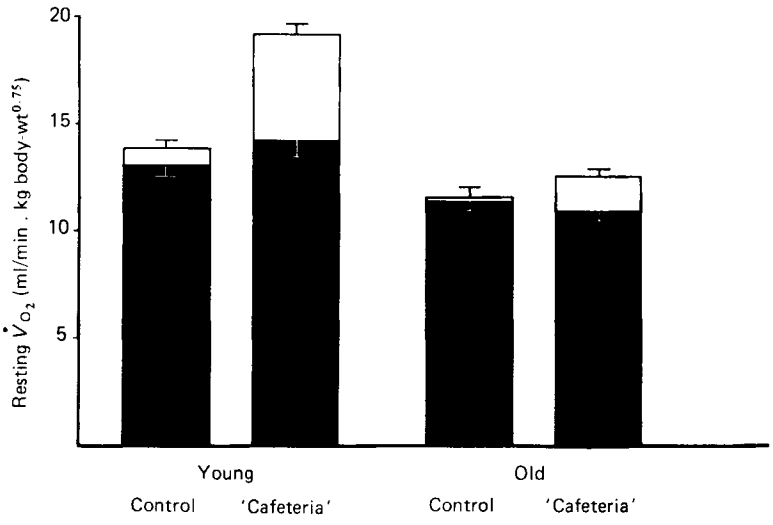

Fig. 2. Resting oxygen consumption (ml/min per kg body-weight$^{0.75}$, $\dot{V}_{O_2}$) of all rats on day 10, before (□) and after (■) injection of propranolol. Values are means with their standard errors represented by vertical bars.

Energy expenditure over the entire course of the experiment was increased by 77 and 57% in young and old 'cafeteria'-fed rats compared to their controls, but gross efficiency was not significantly altered by dietary manipulation. Net efficiency of energy utilization was similar for both older groups but was significantly ($P < 0{\cdot}01$) reduced in young 'cafeteria'-fed rats compared to young controls.

Values for resting $\dot{V}_{O_2}$ (corrected for metabolic body size, kg body-weight$^{0.75}$ and the responses to injection of noradrenaline or propranolol are shown in Table 4 and Fig. 2. On day 14, $\dot{V}_{O_2}$ was significantly elevated in both 'cafeteria' groups before and after injection of noradrenaline. The percentage stimulation of $\dot{V}_{O_2}$ by noradrenaline was similar for all older rats but was greater in young 'cafeteria'-fed animals than their controls. Injection of propranolol (Fig. 2) had no significant effect on resting $\dot{V}_{O_2}$ in any control rats but caused a significant reduction in both 'cafeteria' groups $\dot{V}_{O_2}$ after propranolol, % pre-injection value; young control $95{\cdot}9 \pm 0{\cdot}9$, cafeteria $73{\cdot}0 \pm 4{\cdot}4$, $P < 0{\cdot}001$; adult controls $98{\cdot}4 \pm 1{\cdot}8$, cafeteria $89{\cdot}1 \pm 3{\cdot}4$, $P < 0{\cdot}05$ compared to respective controls).

The mass of interscapular BAT was increased in all cafeteria rats relative to their controls, even when corrected for body size (Table 4), and the greatest level of hypertrophy was observed in younger 'cafeteria'-fed animals. Blood glucose was similar for all young rats and slightly elevated in adult 'cafeteria'-fed animals, while plasma T_3 levels were increased in the young 'cafeteria' group but were unaltered by diet in older rats (Table 4).

DISCUSSION

In earlier publications (Rothwell & Stock, 1979*a*, 1980*a*, *b*) it has been reported that hyperphagic 'cafeteria'-fed rats exhibit large compensatory increases in energy expenditure, or DIT, which serve to reduce weight gain and the development of obesity. The magnitude of these changes in metabolic rate (70–100%) were such that they could not be ascribed to changes in activity or the energy cost of fat deposition.

Armitage *et al.* (1981*a*, *b*), however, have claimed that they were unable to find evidence for DIT in 'cafeteria'-fed rats, and Hervey & Tobin (1981) have suggested that this

phenomenon is either restricted to young rats of a particular strain and is due to the energy cost of growth and fat deposition, or that a large proportion of the increased expenditure is an artefact arising from cumulative errors in intake measurements. However, the results of the present study are consistent with the original observations and conclusions. They demonstrate that errors in the measurement of energy intake are very small, that DIT is not restricted to one strain, and can occur in adult as well as in young rats. Moreover, the energy cost of fat deposition would account for only a small proportion of the increased energy expenditure and it is likely that other thermogenic mechanisms, such as sympathetic activation of BAT, are responsible for the elevated DIT of 'cafeteria'-fed rats.

The results presented in Table 2 demonstrate that the errors associated with calculating energy intake from food tables are remarkably small (less than 1% on average) and unbiased. This close agreement is not surprising since other workers have reported a similar correlation, and in much more difficult circumstances. For example, in a human feeding trial involving three different mixed diets, Southgate & Durnin (1970) found that the determined values for ME agreed with calculated values to within 1·5% on average. Similarly, Norgan & Durnin (1980) observed that direct estimates of ME agreed with food table estimates to within 2–7% during a human over-feeding study. It is not difficult to see how the accuracy of estimates derived from food tables could be improved if human trials used the same degree of dietary and housing control as in animal experiments. It is worth noting that variation between different samples of the human food items used in this study was generally smaller than that often found between different batches of stock diet, where energy sources and raw materials presumably alter depending on availability and cost.

The accuracy of intake measurements in 'cafeteria'-fed rats are at variance with the conclusions of Armitage *et al.* (1981*a*). These workers simultaneously estimated energy expenditure of 'cafeteria'-fed rats from continuous measurements by indirect calorimetry as well as from the difference between ME intake and body-energy gain. The discrepancy between the two methods was entirely ascribed to errors associated with the measurement of intake, but it is likely that there were also errors associated with the other measurements. For example, oven-drying of carcasses has been shown to result in energy losses (Lotfi *et al.* 1976) and, in view of the poor relationship between body-weight and energy content, particularly in older rats, selection of animals on the basis of weight and estimates of energy content from regression analysis may both lead to erroneous results.

Analysis of carcasses at the end of the present experiment indicated that body energy content can be accurately determined from the fat and fat-free dry mass (Rothwell & Stock, 1979*a*) since values for the GE of these constituents yielded results which were almost identical to those obtained from direct determinations using bomb calorimetry. Furthermore, the body composition of 'cafeteria'-fed rats suggests that these animals consumed sufficient protein to allow normal growth. 'Cafeteria'-fed rats were presented with at least one high-protein food each day and also had access to a high-protein (28% of ME) stock diet. It has been observed that the consumption of stock diet by 'cafeteria'-fed animals tends to increase when only low-protein 'cafeteria' foods are presented and it is likely that this could result in some stunting of growth in studies where young 'cafeteria'-fed rats are maintained on stock diets with less than 200 g protein/kg.

The young and adult rats exhibited the same level of hyperphagia when presented with cafeteria diet but, while the former group showed no increase in weight relative to controls, older 'cafeteria'-fed rats became significantly heavier. The age difference in the response to similar levels of hyperphagia apparently results from much larger increases in energy expenditure in the young rats. This confirms our previous suggestion that thermogenic capacity declines with age in the rat (Rothwell & Stock, 1980*b*) and may be related to the decrease in mass and activity of BAT which occurs in many older animals (Bruck, 1970).

Nevertheless, in spite of the lower levels of DIT, 6-month-old 'cafeteria'-fed rats still showed large (57%) increases in energy expenditure when compared to their stock-fed controls, and this tended to compensate for the hyperphagia. In fact, if all the excess energy intake had been deposited these animals would have gained an extra 81 g lipid, rather than the observed 30 g excess (assuming 53 kJ/g for the total energy cost of fat deposition; Pullar & Webster, 1977). It is possible that lower levels of thermogenesis and consequently higher rates of fat deposition, might occur in rats over 6 months of age. However, since most rats can be considered adult by 2 months and the average life-span of wild rats rarely exceeds 6–8 months (C. Richardson, personal communication) experiments on very-old laboratory rats might be considered of doubtful biological significance.

Apart from age, the response to 'cafeteria'-feeding is also dependent on the genetic background of animals and it has been noticed that the level of DIT varies considerably even between Sprague-Dawley rats from two different colonies (Rothwell & Stock, 1980*a*), while another group (Armitage *et al.* 1981*a*, *b*) reported little or no changes in DIT in WAG/C × PVG/C rats. In the latter strain, intake was elevated by only approximately 20–30% in 'cafeteria'-fed rats and control animals showed very low energy gains (5 kJ/d *v.* 33 kJ/d for control Zucker rats of the same age in the present study). Thus, it is possible that in some situations 'cafeteria'-feeding merely results in nutritional improvements that allow the animals to achieve their maximum growth rate. In order to activate DIT under these conditions it is clearly necessary to stimulate voluntary food intake to levels which are unequivocally in excess of the animal's requirements. In this respect it should be noted that the 'cafeteria' diet is not a controlled diet, since it depends as much on the animal's choice of foods as it does on the experimenter's. In order to overcome at least some of these uncertainties, the 'cafeteria' feeding system and the foods offered have been described in some detail in a previous report (Rothwell & Stock, 1979*c*) and again in the present paper.

In the present study, the energy cost of fat deposition could account for only a very small proportion of the increased energy expenditure of 'cafeteria'-fed rats. Assuming all triglyceride was synthesized from carbohydrate (i.e. net energy cost of 14·1 kJ/g; Pullar & Webster, 1977), the cost of excess fat deposition represented 4·2 and 14·3% of the extra energy expenditure of young and adult 'cafeteria'-fed rats respectively. It is however, very unlikely that fat was synthesized mainly from glucose since the 'cafeteria' diet has a very high fat content (50% of ME) and we have observed that in vivo lipogenesis is markedly reduced in 'cafeteria'-fed rats compared to controls (Rothwell, Stock and Trayhurn, unpublished results). The energy cost of depositing dietary lipid is much lower (5·9 kJ/g, ARC/MRC Committee, 1974) and using this value it can be calculated that the energy required for excess fat deposition would represent only 1·8 and 6·0% of the increased expenditure in young and adult 'cafeteria'-fed rats. It should also be remembered that control animals were eating a high-carbohydrate, cereal-based diet and if the high energy cost of *de novo* lipogenesis in these animals were to be allowed for, the differences in DIT between treatments would be even greater.

The values for resting $\dot{V}_{O_2}$ show a similar pattern to the changes in energy expenditure, although the differences between dietary treatments were much smaller. However, there are several reasons why these measurements of $\dot{V}_{O_2}$ cannot be extrapolated to compare with the energy expenditure determined over the 24 d experiment. They were 2 h sample measurements carried out on resting animals during the day-time when food intake is low, at a higher temperature (29 °) than the animal room (24 °) in which these animals were maintained and have also been corrected for body size. $\dot{V}_{O_2}$ has recently been measured for periods of 24 h in 'cafeteria'-fed rats at 24° and found to be elevated by approximately 50% compared to controls (Rothwell & Stock, 1982) while Andrews & Donne (1982) have observed increases in 24 h $\dot{V}_{O_2}$ of up to 60% in 'cafeteria'-fed rats. Nevertheless, short-term

measurements of $\dot{V}_{O_2}$ under controlled conditions are essential for determining the acute responses to drugs which might have a duration of action of less than 2–3 h.

Injection of noradrenaline produced increases in resting $\dot{V}_{O_2}$ in all rats, but the absolute rise was greatest in 'cafeteria'-fed animals ($\Delta\dot{V}_{O_2}$, ml/min per kg body-weight$^{0.75}$; young control 3.82 ± 0.41, cafeteria 6.97 ± 0.71 $P < 0.001$; adult control 3.49 ± 0.43, cafeteria 4.65 ± 0.29, $P < 0.01$). Conversely, β-blockade with propranolol did not affect the metabolic rate of controls but caused a marked reduction in 'cafeteria' groups. These results confirm previous findings (Rothwell & Stock, 1979*a*, 1980*c*) and suggest that all the elevated $\dot{V}_{O_2}$ of the 'cafeteria'-fed rats can be ascribed to an increased sympathetic activity. In separate experiments on 'cafeteria'-fed rats, increases have been observed in BAT noradrenaline turnover in vivo (Landsberg *et al.* 1981) and in the mass and biochemical activity of BAT (Rothwell & Stock, 1979*a*; Brooks *et al.* 1980; Rothwell *et al.* 1981). Measurements of in vivo oxygen consumption of BAT have also demonstrated that all of the enhanced thermogenic capacity of these animals is due to increases in BAT thermogenesis (Rothwell & Stock, 1981*a*).

The Zucker rats maintained on the 'cafeteria' diet also showed hypertrophy of BAT (Table 4) and this was most noticeable in the younger animals, which also exhibited high levels of DIT and large responses to noradrenaline. Protein and DNA content of BAT were not determined in these animals but earlier work has shown large increases in protein content in all 'cafeteria'-fed rats (Brooks *et al.* 1980), whereas DNA content was elevated only in young animals, indicating that hyperplasia can occur during 'cafeteria' feeding in early life (Brooks *et al.* 1981).

Blood glucose levels were slightly elevated in old 'cafeteria'-fed rats compared to their controls but remained unaltered in young animals. Normoglycaemia has also been found in Sprague-Dawley rats fed the 'cafeteria' diet, in spite of reduced insulin levels, which might indicate that insulin sensitivity is increased when this diet is fed to animals with a high capacity for DIT (Rothwell & Stock, 1981*b*). Thyroid hormones have frequently been implicated in the thermogenic responses to diet (Danforth *et al.* 1978; Rothwell & Stock, 1979*a*) and cold (Reichlin *et al.* 1973) since they are potent stimulants of metabolic rate and also potentiate the effects of noradrenaline. The rise in circulating T_3 levels in young 'cafeteria'-fed rats is therefore consistent with these suggestions.

The results presented here support the previous suggestions (Rothwell & Stock, 1979*a*) that hyperphagia in 'cafeteria'-fed rats can induce large compensatory increases in DIT which minimize weight and fat gains. These changes in expenditure did not result from erroneous measurements of energy balance or from the energy cost of excess fat deposition. It could be argued that energy expenditure was not measured directly but was calculated from ME intake and energy gain. However, measurements of ME intake and body energy were each made by two methods which, for both factors, agreed to within 2–4%. Errors of this magnitude are small in comparison to the large differences (57–77%) between intake and gain (i.e. expenditure) of 'cafeteria'-fed rats compared to controls. It is unlikely, therefore, that either the results or their interpretation would be significantly altered by making continuous recordings of $\dot{V}_{O_2}$ or heat production. Furthermore, the comparative slaughter technique used in this study has been tried and tested by animal nutritionists for many years and, as Blaxter (1962) has stated, 'If the change in the heat of combustion of the body, or, alternatively, the change in the amounts of fat and protein in the body, could be determined more simply than with respiration equipment, then an equally valid estimate could be made of over-all energy exchange'.

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