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Influence of environmental temperature on energy balance, diet-induced thermogenesis and brown fat activity in 'cafeteria'-fed rats

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1. Young male rats were fed on a pelleted stock diet or a variety of palatable food items ('cafeteria' diet) and housed at 24° or 29°.

2. 'Cafeteria' feeding at the lower temperature stimulated energy intake, gain and expenditure, but reduced energetic efficiency such that over 70% of the excess intake was expended.

3. Housing at 29° suppressed intake and expenditure in animals on both diets, but to a greater extent in 'cafeteria'-fed rats and energetic efficiency was greater than control values at this higher temperature.

4. The thermogenic capacity of brown fat (mitochondrial purine nucleotide binding) was increased by 'cafeteria' feeding, but was suppressed in animals kept at 29°.

5. The results demonstrate that diet-induced thermogenesis is inhibited by high environmental temperatures.

Voluntary food intake and body-weight regulation are highly dependent on environmental temperature and closely related to thermoregulation, particularly in homeotherms (Brobeck, 1948; Hamilton, 1967). Thus Brobeck (1948) was led to the suggestion that food intake was largely determined by the requirement to produce heat for the maintenance of body temperature. It is now obvious that intake control is subject to many diverse influences, but Brobeck's (1948) thermostatic hypothesis does suggest that various relations could exist between energy-balance regulation and thermoregulation. For example, apart from the obligatory changes in metabolic rate following ingestion of food, exposure to cold or hyperphagia also stimulates adaptive increases in heat production known respectively as non-shivering thermogenesis (NST) and diet-induced thermogenesis (DIT). Conversely, it is likely that high environmental temperatures could affect both hyperphagia and the heat produced via DIT, as well as inhibiting NST.

DIT can be readily demonstrated by presenting young rodents with a choice of palatable food items (known as the 'cafeteria' diet) to induce hyperphagia (Rothwell & Stock, 1979, 1982*a*, *b*; Trayhurn *et al.* 1982). 'Cafeteria'-fed rats can show increases in heat production of up to 100%, which apparently result from the same processes as those involved in non-shivering thermogenesis, i.e. sympathetic activation of heat production in brown adipose tissue (BAT) via the mitochondrial proton conductance pathway. These similarities and close relations between NST and DIT are now well described (for reviews see Rothwell & Stock, 1983; Himms-Hagen, 1985) but, as an example, it has been shown that, compared with normophagic controls, 'cafeteria'-fed rats maintained in a warm environment (24°) show improved cold tolerance when acutely exposed to 5° (Rothwell & Stock, 1980). Conversely, cold-adaptation enhances the acute postprandial thermic response to food (Rothwell *et al.* 1982), and feeding rats on a 'cafeteria' diet in the cold produces larger increases in metabolic rate and BAT activity than seen in stock-fed animals at the same temperature (Rothwell & Stock, 1980).

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Most 'cafeteria'-feeding studies have been performed at temperatures of 21–25°, but the findings described previously indicate that while cool environments enhance DIT, warmer temperatures may suppress thermogenesis and promote obesity. We have tested this possibility in the present experiments by studying the effects of 'cafeteria' feeding on energy balance and BAT activity in rats maintained at either 24° or their thermoneutral temperature, 29°.

EXPERIMENTAL

Thirty-eight male, Sprague–Dawley rats (Charles River, Kent), aged 35 d, were divided into four groups of eight and one of six with the same mean body-weight, and the latter group (Bo) was killed on day 1 for determination of initial body energy content. The remaining animals were split between two adjacent rooms maintained at either $24 \pm 1°$ or $29 \pm 1°$ (12 h light–12 h dark cycle) and housed for 14 d with free access to water and a pelleted stock diet (PRD, Christopher Hill Group Ltd, Dorset; metabolizable energy (ME) density 12·0 kJ/g, protein content 27% ME). Half the rats at each temperature were also fed on a 'cafeteria' diet, comprising four different food items each day. The food items were selected from a list of twenty-five foods, comprising various biscuits, chocolate, cakes, pasta, crisps, meats and pastries. The selection changed every day, but always included a meat or meat product which was fed in the evening at the beginning of the dark cycle (for further details of 'cafeteria'-feeding, see Rothwell & Stock, 1982*a*). ME intake was measured as described previously (Rothwell & Stock, 1982*a*) from the weight of each food presented (including the stock diet) and its gross energy density minus the energy losses in any spilt food, urine and faeces. Gross energy was determined by ballistic bomb calorimetry of replicate freeze-dried samples.

After 14 d, all rats were killed and the interscapular BAT depot dissected, weighed and homogenized in sucrose before isolation of mitochondria. The binding of [^{3}H]guanosine diphosphate (10 Ci/mmol, Amersham International plc, Amersham, Bucks; GDP) to isolated mitochondria was measured (for details, see Brooks *et al.* 1982) to assess the activity of the mitochondrial proton conductance pathway (Nicholls & Locke, 1983). The protein content of whole-tissue homogenates and mitochondrial samples was measured using a coomassie blue dye reagent method (Bio-Rad, Watford).

The carcasses were frozen and gross energy content subsequently determined by ballistic bomb calorimetry (Gallenkamp, Loughborough) of multiple (usually 5–6) homogenized, freeze-dried samples. The coefficient of variation of these determinations ranged from 0·5 to 4·5%. Body energy gain was estimated by subtracting initial body energy content (average of Bo values) from final energy content. Energy expenditure over the experiment was calculated as ME intake minus body energy gain, and gross and net efficiencies expressed as the body energy gain per unit energy, or per unit intake above maintenance (assumed to be 420 kJ/kg body-weight$^{0·75}$ per d) respectively.

Values are presented as means with their standard errors, and differences between groups were tested by analysis of variance and Student's *t* test for unmatched data.

RESULTS

'Cafeteria'-fed rats gained slightly more weight than their respective controls at both temperatures, but these small differences in body-weight and weight gain were not statistically significant (Table 1). ME intake was increased by 32% in 'cafeteria'-fed rats compared with controls at 24°, and exposure to 29° suppressed intake in both groups, but to a greater extent in the 'cafeteria'-fed group. Thus the level of hyperphagia was reduced in 'cafeteria'-fed rats at 29° (14% above controls), and was further diminished when values

Table 1. *Body-weights and energy balance of control and 'cafeteria'-fed rats maintained at 24° or 29°*

(Mean values with their standard errors for eight rats)

Environmental temperature (°) ...	24				29				
Feeding regimen ...	Control		'Cafeteria'		Control		'Cafeteria'		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*F* value
Final body-wt (g)	232	3	244	7	255	7	250	8	4·04
Wt gain (g)	100	3	113	6	95	7	120	7	3·98
ME intake (kJ)	3755	65	4955***	30	3470††	15	3960*†††	170	12·90
(kJ/kg body-wt$^{0·75}$ per d)	925	10	1205***	25	870	25	945†††	20	44·00
Body-energy gain (kJ)	1075	10	1405**	65	1165	70	1615***†	65	46·80
Energy expenditure (kJ)	2685	35	3550***	65	2305†††	65	2350†††	140	15·70
(kJ/kg body-wt$^{0·75}$ per d)	660	10	865***	30	575††	20	555†††	20	41·90
Gross efficiency (%)	28·5	0·9	28·4	1·3	33·5††	1·1	41·1**†††	1·7	22·00
Net efficiency (%)	50·6	1·2	42·8**	2·1	63·7††	2·8	72·8†††	3·6	26·90

ME, metabolizable energy.

Mean values were significantly different from control values: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

Mean values were significantly different from those for 24° group: † $P < 0·05$, †† $P < 0·01$, ††† $P < 0·001$.

Table 2. *Interscapular brown adipose tissue mass and activity of control and 'cafeteria'-fed rats maintained at 24° or 29°*

(Mean values with their standard errors for eight rats)

Environmental temperature (°)...	24				29				
Feeding regimen...	Control		'Cafeteria'		Control		'Cafeteria'		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*F* value
BAT									
Mass (mg)	397	22	607***	35	300†	38	419*††	27	42·3
(mg/kg)	1730	90	2500***	10	1390	160	1720†††	90	42·1
Protein content (mg)	32·5	1·2	26·6	1·9	15·8†††	1·4	23·9***†††	1·5	33·4
(%)	8·2	0·2	6·1**	0·4	5·4†††	0·3	5·7	0·4	15·8
Mitochondrial protein (mg)	3·3	0·2	3·6	0·2	2·0†††	0·1	2·7*††	0·2	13·0
Specific GDP-binding (pmol/mg protein)	70	3	123***	9	29†††	1	40***†††	2	47·3

BAT, brown adipose tissue; GDP, [^{3}H]guanosine diphosphate.
Mean values were significantly different from control values: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
Mean values were significantly different from those for 24° group: † $P < 0·05$, †† $P < 0·01$, ††† $P < 0·001$.

were corrected for body size. Casual observations indicated that food selection and behaviour were unaffected by environmental temperature, although rats housed at 29° were more often observed to be licking and grooming themselves, possibly indicating some thermal stress.

Body-energy gain (Table 1) was greater in the 'cafeteria'-fed groups, and this was increased further by housing at 29°. Energy expenditure was increased in 'cafeteria'-fed rats kept at the lower temperature (32% above control) but was reduced in all animals kept at the high temperature and was unaffected by diet. Gross energetic efficiency was similar for control and 'cafeteria'-fed rats at 24°, but net efficiency was significantly lower in the latter group. At 29°, gross and net energetic efficiencies on both diets were greater than the values obtained at 24°. Thus, unlike the effects seen at 24°, 'cafeteria' feeding at 29° caused a 23% increase in gross efficiency and a 14% (but non-significant) increase in net efficiency.

'Cafeteria' feeding at both temperatures caused hypertrophy of the interscapular BAT depot, i.e. increases in mass and protein content (Table 2), but the absolute values were all very much lower in animals maintained at 29°. The thermogenic activity of BAT, assessed from the binding of GDP to isolated mitochondria, was increased in 'cafeteria'-fed rats at 24° (76% above control) and at 29° (38% above control), but reduced by over twofold in animals fed on either of the two diets at the higher temperature.

DISCUSSION

The methods employed in the present study to assess energy balance have been used extensively in previous experiments on 'cafeteria'-fed rats. For example, the three components of energy balance (intake, carcass gain and expenditure) have each been measured by two different methods and shown to agree to within a few per cent (Rothwell & Stock 1982*a*, *b*) in both stock- and 'cafeteria'-fed rats. The results obtained in the present study in rats maintained at 24° are in general agreement with our own previous work (e.g. Rothwell & Stock, 1979, 1982*a*, *b*; Rothwell *et al.* 1985) and those of other groups (Stephens *et al.* 1981; Tulp, 1981; Trayhurn *et al.* 1982); i.e. 'cafeteria' feeding stimulated energy intake and expenditure and suppressed energetic efficiency. The level of hyperphagia was slightly lower than that usually obtained, possibly because some foods preferred by the rats (e.g. fresh liver) were not used in order to avoid deterioration at the higher temperature.

In spite of the lower level of hyperphagia, over 72% (870 kJ) of the excess intake (1200 kJ) of the 'cafeteria'-fed rats housed at 24° was expended as heat and less than 30% was retained in the carcass. This means that the extra energy consumed by the 'cafeteria'-fed rats was utilized with an efficiency of only 28%, which is considerably lower than their overall net energetic efficiency (43%) and suggests that when extra energy is consumed it is utilized by different metabolic processes. The increased energy expenditure of these animals was accompanied by a 76% increase in BAT mitochondrial GDP-binding capacity, and in earlier experiments we have confirmed that these changes are associated with increases in BAT noradrenaline turnover (Young *et al.* 1982) and greater in vivo oxygen consumption of the tissue (Rothwell & Stock, 1981).

At 29°, rats fed on both diets showed lower levels of energy intake and expenditure, but due to an overall increase in energetic efficiency the rates of body-energy gain were slightly (control) or significantly ('cafeteria') greater than in those housed at 24°. In addition to the general effects of the higher ambient temperature on the level of metabolism, exposure to 29° also affected the magnitude of the response to 'cafeteria' feeding by attenuating the increases in intake and expenditure. Also, the higher temperature produced an increase, rather than a decrease in energetic efficiency in 'cafeteria'-fed rats. Unlike the 'cafeteria'-fed rats housed at 24°, only 8% (40 kJ) of the excess intake (490 kJ) was dissipated as heat in

those maintained at the higher temperature. Thus the net efficiency of utilization of this extra energy was 92% (cf. 28% at 24°). As might be expected, housing the animals at thermoneutrality markedly suppressed the activity of the proton conductance pathway (i.e. GDP-binding) in BAT mitochondria and, although 'cafeteria' feeding produced an increase, the absolute level was considerably lower than that seen in control and 'cafeteria'-fed animals at 24°.

Theoretically, the efficiency of energy utilization for body-energy (i.e. fat) gain should increase when animals are fed on the 'cafeteria' diet, which is typically high-fat (over 40% of ME) compared with the stock diet (less than 10% of ME). However, it is apparent from the present and previous studies conducted at 24° that the energy spared on the cost of lipogenesis as a result of the greater intake of dietary lipid is more than offset by adaptive increases in heat production. This results in either no change or even a decrease in energetic efficiency. Inhibition of DIT (to avoid hyperthermia, for example) would prevent any adaptive decrease in energetic efficiency, thus unmasking the predictable effects of feeding high-fat diets on the cost of carcass energy gain and producing an increase in energetic efficiency. It could be argued that the failure to observe adaptive DIT in 'cafeteria'-fed rats at 29° was simply due to the absence of hyperphagia. However, it should be noted that, whereas energy expenditure was practically identical in control and 'cafeteria'-fed rats at 29°, voluntary food intake was still elevated in the 'cafeteria'-fed group. This suggests that heat production was strictly determined by thermoregulatory requirements, whereas the hedonistic properties of the 'cafeteria' diet prevailed, to produce the most damaging combination for the regulation of energy balance: hyperphagia plus hyper-efficiency.

We have previously reported a close relation between low environmental temperatures and DIT in 'cafeteria'-fed rats, and demonstrated that 'cafeteria'-fed rats show improved cold tolerance and a greater capacity for DIT during chronic exposure to the cold (Rothwell & Stock, 1980). These interactions between the two stimuli are to be expected in view of the common mechanisms shared by NST and DIT, but they may operate at several levels. For example, environmental temperature may influence thermogenesis via effects on peripheral thermoreceptors and the hypothalamic pre-optic thermoregulatory centres, with secondary changes in energy intake compensating for alterations in the level of expenditure. Alternatively, some of the increased heat production during cold exposure could be secondary to cold-induced increases in food intake, thus producing DIT. The involvement of the ventromedial hypothalamus (VMH) in both the control of food intake and BAT thermogenesis (see Rothwell & Stock, 1983) and the influence of pre-optic cooling on VMH activity and BAT thermogenesis (Imai-Matsumura *et al.* 1984) suggests that central mechanisms exist to allow a complex interaction between dietary and thermal stimuli.

The present results have not only demonstrated that DIT in 'cafeteria'-fed rats can be inhibited at high environmental temperatures, but may also explain some apparent anomalies between our own studies and those of other workers. Barr & McCracken (1984), for example, reported a high efficiency of energy utilization in 'cafeteria'-fed rats kept at 29° and suggested that this conflicted with our previous observations of increased DIT and reduced efficiency during 'cafeteria' feeding. It is now obvious that conducting experiments at high environmental temperatures is likely to suppress DIT and should not be used to assess its quantitative importance in animals housed at lower ambient temperatures. It would also seem that the thermoneutral zone and the lower critical temperature of hyperphagic rats exhibiting DIT will be considerably lower than in normophagic stock-fed rats. This effect of the plane of nutrition on critical temperature has been described previously in the pig (Close & Mount, 1978).

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