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Intra-strain Differences in Young Rats Fed on Cafeteria Diet as Determined by Calorimeter and Comparative Carcass Techniques

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ABSTRAK

Kajian telah dijalankan untuk mengesan perbezaan komposisi badan, pengambilan makanan dan penggunaan tenaga terhadap tikus Sprague Dawley daripada dua koloni (SDQEC dan SDCR) yang diberi makanan kafeteria. Pengambilan makanan meningkat daripada 21% bagi tikus SDQEC hingga 31% bagi tikus SDCR yang diberi makanan kafeteria. Perbezaan yang mempunyai keertian ($P < 0.05$) kepada berat badan dan kandungan lemak hanya terdapat pada tikus SDCR sahaja. Peningkatan pengambilan makanan menyebabkan kenaikan kadar penggunaan tenaga ($P < 0.01$) bagi tikus yang diberi makanan kafeteria jika dibandingkan dengan tikus yang diberi makanan piawai. Keputusan yang diperolehi menunjukkan kehadiran variasi dalam strain dan mengesahkan kaedah penilaian karkas untuk mendapatkan anggaran penggunaan tenaga bagi tikus.

ABSTRACT

The effects of cafeteria feeding on body composition, metabolizable energy (ME) intake and energy expenditure of young Sprague Dawley rats obtained from two different colonies (SDQEC and SDCR) were studied. The ME intake increased significantly ($P < 0.01$) from 21% in SDQEC to 31% in SDCR cafeteria-fed rats, with a significant change ($P < 0.05$) in body weight and body fat in the latter but not the former. This mild hyperphagia induced a significant increase ($P < 0.01$) in energy expenditure in cafeteria-fed rats as compared to their respective controls. These results confirm the presence of intra-strain variations and reaffirm the validity of the carcass comparative technique for estimating energy expenditure in rats.

INTRODUCTION

Rodents are generally believed to have a precise control of energy intake and this is shown from the classical work of Adolph (1947) and later by Hervey (1969) where rats, in a constant environment "eat for energy". In these experiments when the concentration of energy in the animal diet was changed, they accurately adjusted the amount they ate; thus keeping the body energy store fairly constant despite fluctuations in energy expenditure. These workers however only fed monotonous stock diet and did not tempt their animals with a variety of different foods.

However, over the years, various techniques such as forced-feeding, chronic injections of insulin and feeding energy-densed diets have successfully been employed to induce hyperphagia in experimental animals. Strangely enough, it was not before Sclafani and Springer

(1976) found that food on the supermarket shelves could produce the desired effect did the realization of 'cafeteria' feeding system came to be known. Providing rodents with a varied and highly palatable diet produces two distinct effects. It promotes hyperphagia and provokes caloric output as has been consistently reported by Rothwell and Stock (1979a, b, 1980, 1982a, b).

The efficiency with which an animal can utilize the metabolizable energy (ME) of food consumed largely depends on the amount of heat it produces in metabolism. It has been widely accepted that an increase in ME intake is usually accompanied by an increase in heat production. The ruminant, or Kellner, school of nutritionists call this the heat increment of feeding (HIF) while the monogastric, or Rubner school called it specific dynamic effect (SDE), and now such a phenomena is largely referred to as diet-induced thermogenesis (DIT) (Webster, 1981). Recent interest in DIT derived largely from work of Rothwell and Stock (1979b) who report that the cafeteria-fed rats increased their energy intake by 80% and dissipated 90% of the extra intake as heat. They also suggested that brown adipose tissue (BAT) plays an important role in the regulation of body weight, by disposing thermogenically excess energy. However, in recent years, several conflicting pieces of evidence have been reported with regards to the presence of hyperphagia and DIT in cafeteria-fed rats (Armitage *et al.*, 1981; Bestley *et al.*, 1982; Mc Cracken and Barr, 1982; Barr and Mc Cracken, 1983). The objective of this study was to determine intra-strain differences in young rats fed cafeteria diet using calorimeter and comparative carcass techniques.

MATERIALS AND METHODS

The animals used in this study were male Sprague Dawley rats (Charles River, Kent, UK) and male Sprague Dawley rats (Queen Elizabeth College, UK) aged 4 to 5 weeks. These groups (n = 8) are abbreviated to SDCR and SDQEC rats in the text. These animals were maintained in all animal room kept at 25 ± 1°C. Food and water were given *ad libitum* for 28 days. Since the animals were housed in pairs, results were expressed as an average of two. The cafeteria-fed group was offered a total of 54 different food items out of which 36 items were preferred (Table 1). The CRM breeding diet (Christopher

TABLE 1
List of food items presented to cafeteria-fed rats during the experiment

All bran*	Minced beef*	Chocolate wafers	Butter shortbread*
Shredded wheat*	Luncheon meat*	Cadbury milk chocolate*	Butter madiera*
Cornflakes*	Corned beef*	Marathon*	Dig. biscuit (plain)*
Pasta	Pork sausages*	Plain chocolate	Dig. biscuit (chocolate)*
Cream crackers*	Liver pate*	Mars bars*	Butter crumbles
Bread (toasted)*	Liver sausages*	Milky ways	Muesli cookies
Wholegrain roll*	Pork & duck meat pate*	Swiss milk chocolate	Chocolate chip cookies
Frosties*	Beef sausages*	Wholenut chocolate*	Coconut mallows*
Potato crisps*	Chicken liver pate*	Kit-kat	Sultana cake*
Potato sticks	Beef burgers*	Bounty chocolate*	Spotted dick
Corn snack	Steak & kidney pie*	Honeycomb crunch	Fig rolls
Cheese snaps	Cheese & bacon pizza*	Galaxy chocolate	Swiss rolls*
Cheddar cheese*		Plain chocolate waffles	Chocolate sponge rolls*
Marzipan		Groundnuts*	Pop corn*

*Preferred food items

Hill Group Ltd. Dorset, UK) was fed to the control group.

Food Intake

For the control group, stock diet was fed *ad libitum*. The cafeteria-fed group were given stock diet and in addition, they were presented with four different food items daily in which two were given in the morning (9.00 am) and the other two (usually high fat and high protein foods) were added in the evening (5.00 pm). A grace of three days was given before similar food items were re-fed to the animals. All food items were dried to a constant weight at 105°C in an oven, homogenized and the gross energy (GE) contents were determined in triplicate using ballistie bomb calorimeter. To eliminate erros due to variations between batches, energy content of food samples was analysed on each occasion they were purchased.

The ME intake was obtained from the determined GE content of the food provided minus the GE content of uneaten food plus faeces and urine, hence:

ME intake = Energy IN − Energy OUT

The weight of food items presented was carefully recorded daily with any left-over food being removed as new food items were introduced. The ME intake was determined on a weekly basis.

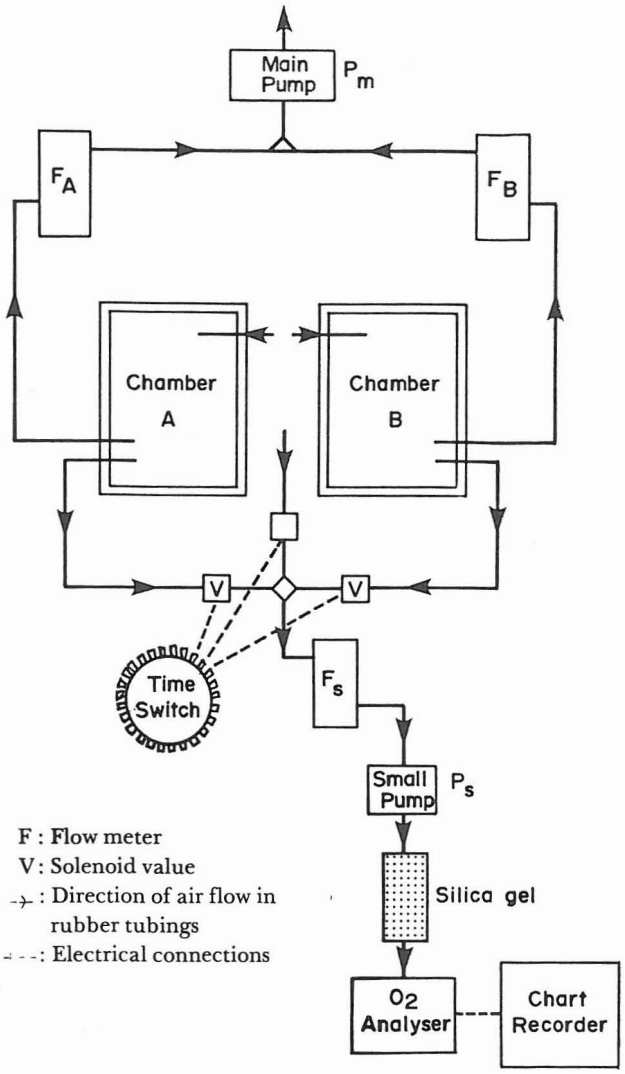

Fig. 1. Diagrammatic representation of open-circuit indirect animal calorimeter.

Measurement of Oxygen Consumption

The calorimeter used for the 24 hour measurement is based on an open circuit system designed by Boroumand (1977) and later modified by Dulloo (1982). The set-up enables the animals to remain in their habitual cages thus enabling measurements to be made with minimal disturbance to the animals. A diagrammatic representation of the animal calorimeter is shown in *Fig. 1*. The change of oxygen concentration between the exhaust air and the room air is measured and energy expenditure was calculated using the Weir (1949) formula.

Carcass Analysis

After recording the final body weight, the animals were sacrificed by ether anaesthesia. Incisions were made exposing much of its content and left to dry to a constant weight in an oven kept at 105°C for 24 to 48 hours. The dry matter and water contents were calculated by the difference in weight. The dried carcass was then chopped to facilitate grinding process and the homogenised product was placed in a sealed

bottle and kept in a dessicator until required for the following estimations:

Fat Content Duplicate samples from the homogenised carcass were extracted with petroleum ether (60°C – 80°C) as a solvent (Golowick and Kaplan method, 1957).

Energy Content The gross energy values were determined in triplicates by bomb calorimeter technique (Miller and Payne, 1959). Dried sucrose (16.5 KJ/g) was used as a standard for all energy values obtained in this experiment.

Protein Content Carcass protein was calculated by using a general formula relating to energy derived from the fat with the total energetic value of the carcass and the energy derived from protein (Djazayery, Miller and Stock, 1979). This formula is based on the fact that fat and protein are the only energy yielding components and that contribution from carbohydrate is negligible (less than 1%). Hence:

$$GE = F \times f + (N \times 6.25)\, p$$

where,

GE = the gross energy content (KJ/g)
F = energetic value of fat (KJ/g)
N = nitrogen content (g)
p = energetic value of protein (KJ/g)

The energetic value for fat and protein used in the equation were 38.6 KJ/g and 22.7 KJ/g, respectively (Boroumand, 1977). Thus:

$$P = 0.44\, GE - 1.7\, F$$

Estimation of Energy Expenditure by Comparative Carcass Technique

This technique involves measurement of ME intake and the gain in carcass energy and by the process of substraction the value of energy expenditure is obtained. At the start of each experiment, one group of animals (initial control group) with a closely matched weight to the other experimental groups was sacrificed and retained for analysis of its energy content. At the end of the experiment, the experimental groups were sacrificed and their energy content also analysed by bomb calorimetry. The standard formula is given below:

$$\text{Energy Expenditure} = I - B - B_o$$

where,

I = ME intake
B = Final carcass energy content
B_o = Initial carcass energy content

Methods of Expression

Method used for expressing both ME intake and energy expenditure involves value judgement. Some workers prefer values per animal while others express them as per unit of body weight (W) or per metabolic body size ($KgW^{3/4}$). For purposes of comparison, results presented in this paper are expressed in both ways where appropriate. Statistical comparisons were done using student's t-test.

RESULTS

The body weight and carcass composition are shown in Table 2. Cafeteria feeding induced a significant increase ($P < 0.05$) in body weight and body fat content of SDCR rats as compared to their controls. Although there were increases in SDQEC rats, these were not statistically significant. Reduction in protein content observed in all cafeteria-fed rats was minimal while body water content in both control and cafeteria-fed rats remained virtually unchanged. The overall body weight gain and ME intake is shown in Table 3. While cafeteria diet failed to produce significant weight gain in SDQEC rats, a significant ($P < 0.01$) change was observed in SDCR rats. The ME intake KJ/rat/day produced a significant increase ($P < 0.01$) while the effect was more pronounced when expressed per metabolic body size ($P < 0.001$) compared to their respective controls. Despite increases in ME intake, changes in carcass energy content were only significant ($P < 0.01$) in the SDCR rats while energy expenditure of cafeteria-fed rats as measured by the comparative carcass technique

TABLE 2
Body weight and carcass composition of young rats fed on stock diet and cafeteria diet

Group	Diet	Body weight (g)	Fat (g)	Fat (%)	Protein (g)	Protein (%)	Water (g)	Water (%)
SDQEC	Control	269 ± 10	29.5 ± 4	10.9 ± 1.1	46.6 ± 0.7	17.7 ± 0.7	180 ± 4.5	67.1 ± 0.9
	Cafeteria	288 ± 8	48.5 ± 4	16.8 ± 1.0b	41.5 ± 2.5	14.4 ± 0.6	179 ± 5.0	62.3 ± 1.1
	Initial	79 ± 1	6.6 ± 0.2	8.5 ± 0.2	11.8 ± 0.6	15.0 ± 0.6	57 ± 0.9	72.7 ± 0.1
SDCR	Control	319 ± 8	34.2 ± 2.5	10.1 ± 0.5	54.2 ± 0.8	17.0 ± 0.4	215 ± 4.4	67.5 ± 0.5
	Cafeteria	378 ± 16c	82.3 ± 14c	21.4 ± 2.8b	50.3 ± 2.5	13.3 ± 0.7	215 ± 1.0	57.5 ± 4.0
	Initial	118 ± 1	9.5 ± 0.4	8.0 ± 0.2	15.1 ± 0.5	12.7 ± 0.3	86 ± 1.0	73.0 ± 0.4

[1]Mean values ± SEM; n = 8, trial duration: 28 days

[a]$P < 0.001$, [b]$P < 0.01$, [c] $P < 0.05$ compared to respective controls

TABLE 3
Effect of cafeteria feeding on body weight gain and food intake of young rats from two colonies[1]

Strain	Diet	Body weight (g)			ME intake	
		Initial	Final	Gain	KJ/day	KJ/KgW$^{3/4}$ day
SDQEC	Control	79 ± 1	269 ± 10	190 ± 9	246 ± 10	914 ± 18
	Cafeteria	79 ± 1	288 ± 10	209 ± 8	298 ± 8b	1065 ± 14a
SDCR	Control	118 ± 1	319 ± 8	201 ± 6	292 ± 7	914 ± 6
	Cafeteria	118 ± 1	378 ± 16c	260 ± 14b	382 ± 15b	1086 ± 13a

[1]Mean values ± SEM; n = 8, trial duration: 28 days

[a]$P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ as compared to respective controls

revealed a significant increase ($P < 0.01$) as compared to their respective controls (Table 4). These results support the findings of the 24 hour measurement of energy expenditure where cafeteria-fed rats maintained a significantly higher metabolic rate as expressed per rat or as per metabolic body size ($Kg_w^{0.75}$) as compared to their respective controls (Table 5). Comparison between the two techniques used to measure energy expenditure showed very good agreement with increases in metabolic rate of cafeteria-fed rats, irrespective of the techniques used (Table 6).

DISCUSSION

The results of this study demonstrated that differences in energy utilization occur among rats of the same strain. Of the two groups the SDCR rats appeared to be more efficient in laying down fat when overfed (Ismail, 1983b). Sprague Dawley rats have been shown to be one of the more efficient laboratory rodents (Schemmel *et al.*, 1970) and this was clearly evident in this experiment in the SEQEC rats (less efficient of the two groups) where the body weight gained by cafeteria-fed rats were virtually all deposited as fat (Table 2). One may argue that the differences observed were due to the greater body weight of the SDCR rats (Table 3). However, the fact that a marginal difference of 2% in ME intake $KJ/kg_w^{0.75}$/day could produce a 24% difference in weight gained (Table 3) or an excess of 33.8 g fat accumulated (Table 2) clearly indicate a considerable intra-strain variation between the two cafeteria-fed groups. The degree of overeating showed an increase of 21% in SDQEC and 31% in SDCR rats, respectively (Table 4). While these figures do not match the levels of 80 – 90% as reported by Rothwell and Stock (1980), using a similar experimental design, several other workers (Bestley *et al.*, 1982; Mc Cracken and Barr, 1982; Barr and Mc Cracken, 1982) have reported similar low levels of hyperphagia in cafeteria-fed rats. The hyperphagia exhibited by the cafeteria-fed rats during the 24 hour period in the calorimeter were 39 and 59% in SDQEC and SDCR rats, respectively as compared to their controls (Table 5).

The intra-strain differences in cafeteria-fed rats as measured in the calorimeter showed that SDQEC rats gained 1.2 g in weight, significantly increased their ME intake by 100 KJ/rat/day and energy expenditure by 40 KJ/rat/day than their controls while the cafeteria-fed SDCR rats gained 2.5 g in weight, ate significantly more by 178 KJ/rat/day and elevated their energy expenditure by only 42 KJ/rat/day (Table 5). Based on this one day measurement, the differences between these two groups of the same strain of rats was quite clearly evident. This findings also suggested that if reliance is placed on one or two 24 hour ME intake and energy expenditure measurements in the calorimeter, the possibility of over-estimating both these parameters over a longer experimental period could be real (Ismail, 1983a).

TABLE 4
Effect of cafeteria feeding on energy balance of young rats[1,2]

Strain	Diet	ME intake KJ	Final body energy KJ	Body energy gain KJ	Energy expenditure KJ
SDQEC	Control	6888 ± 278	2114 ± 163	1600 ± 165	5289 ± 115
	Cafeteria	8351 ± 219	2670 ± 164	2157 ± 165	6194 ± 114b
SDCR	Control	8190 ± 192	2480 ± 107	1773 ± 107	6417 ± 100
	Cafeteria	10703 ± 425b	3679 ± 207b	2972 ± 208b	7733 ± 223b

[1]Mean values ± SME; n = 8, trial duration: 28 days

[2]Energy content of initial group (B_0) SDQEC – 514 KJ, SDCR – 707 KJ

[a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ as compared to respective controls.

TABLE 5
Body weight gain, ME intake and energy expenditure of young rats as measured in calorimeter for 24 hours[1]

Strain	Diet	Weight gain (g)	ME intake /rat	ME intake /$Kg_w^{6.75}$	Energy expenditure /rat	Energy expenditure /$KgW^{3/4}$
SDEC	Control	8.3 ± 1.2	257 ± 17	1031 ± 63	187 ± 10	732 ± 11
	Cafeteria	9.5 ± 2.2	357 ± 19b	1361 ± 71c	227 ± 11c	850 ± 30c
SDCR	Control	7.5 ± 1.7	304 ± 21	979 ± 43	215 ± 9	694 ± 6
	Cafeteria	10.0 ± 1.4	482 ± 36b	1460 ± 78b	257 ± 6c	780 ± 20b

[1]Mean values ± SEM: n = 8: recorded between 1st and 2nd week of experiment.

SDQEC – Sprague Dawley, Queen Elizabeth College Colony, London, England.

SDCR – Sprague Dawley, Charles River, Kent, England.

[a] $P < 0.001$, [b] $P < 0.01$, [c] $P < 0.05$ as compared to respective controls.

This present study also revealed differences in ME intake in Sprague Dawley rats obtained from the same supplier (Charles River). Rothwell and Stock (1982c) were able to induce a 53% increase in ME intake, Mc Cracken and Barr (1982) could only achieve a 25% increase while the present study showed a 31% increase in ME intake. One could speculate that such differences in ME intake could only be influenced by any of the following factors: environmental temperature, duration of experiment, selection of palatable diet and whether animals were fed in pairs or caged singly and so on. Recent controversy in regulation of energy balance has led to some critical review of basic measurement of the three vital components, that is, food intake, energy expenditure and energy storage (Hervey and Tobin, 1983). The trend seems clear, that unless a concerted effort to standardize the methods of measuring the various components of energy balance is made, there will always be variations in findings.

TABLE 6
Energy expenditure[1] of young rats as measured by calorimetery technique (A) and comparative carcass technique (B) (KJ/day)

Strain	Method	Control		Cafeteria	
		/rat	/KgW$^{3/4}$	/rat	/KgW$^{3/4}$
SDQEC	A	187 ± 10	732 ± 11	227 ± 11c	850 ± 30c
	B	189 ± 4	702 ± 6	222 ± 4b	792 ± 15b
SDCR	A	215 ± 9	694 ± 6	257 ± 6c	780 ± 20b
	B	229 ± 4	717 ± 9	276 ± 8b	785 ± 3a

[1]Mean value ± SEM; n = 8

[A]24 hours energy expenditure measurement between 1st and 2nd week of experiment

[B]TME intake − Carcass energy gain = Energy expenditure: for a 28 days duration

[a]$P < 0.01$, [b] $P < 0.01$, [c]$P < 0.05$ as compared to respective controls

*Differences between the two methods were not statistically significant

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