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Regulation of Energy Balance in Two Models of Reversible Obesity in the Rat

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Adult male rats were made obese either by tube feeding varying fractions (34%, 47%, 68% or 75%) of their normal food intake or by offering them a varied and palatable diet (cafeteria diet). After 17–30 days of these regimens, the treatments were withdrawn, and the animals were allowed free access to the normal stock diet. Tube-fed animals precisely adjusted voluntary food intake to compensate for the energy delivered by tube but nevertheless became obese as a result of an increased metabolic efficiency. Cafeteria-fed rats were hyperphagic and became obese without any apparent change in metabolic efficiency. Recovery from obesity was more rapid in the cafeteria animals and was due to a pronounced increase in heat production as well as concomitant hypophagia. Animals previously made obese by tube feeding exhibited hypophagia and returned to normal weight without any change in heat production. The relevance of these results to the concept of lipostasis and the relative roles of energy intake and expenditure in the regulation of energy balance are discussed.

The regulation of energy balance by the adult rat is generally considered to result from a precise control of energy intake which serves to maintain body energy stores constant despite fluctuations in energy expenditure. This view, which is best exemplified in the "lipostatic" theory of appetite control (Kennedy, 1953) and its later variant, the "glucolipostatic" theory (Le Magnen, Devos, Gaudilliere, Louis-Sylvestre, & Tallon, 1973), constitutes the physiological basis for many investigations into the influence of afferent metabolic signals on the hypothalamic control of feeding behavior. The concept of a lipostatic control of energy balance is mainly based on the observation that body fat remains relatively constant in adult rats and also on the grounds that fat is a quantitatively important labile component of body mass that could function in the long run to integrate transient fluctuations in energy balance (Kennedy, 1953). While these arguments provide circumstantial support for lipostasis, they are not as satisfactory as direct experimental evidence.

A feature of any regulated system is that disturbance of the regulated variable results in compensatory responses that tend to restore the system to its previous value. Applying this principle to lipostasis suggests that experimentally induced deviations in body fat should provoke an appropriate change in energy balance in order to restore fat content to its original value. Previous investigators have utilized partial lipectomy (Chlouverakis & Hojniki, 1974; Liebelt, Ichinoe, & Nicholson, 1965) or insulin-induced hyperphagia (Hausberger & Hausberger, 1958; Hoebel & Teitelbaum, 1966; Macdonald, Rothwell, & Stock, 1976) to produce such changes in body fat and have noted that recovery of body weight occurs when sufficient time has elapsed from treatment. However, both methods involve quite severe trauma (surgical or metabolic) and produce rather variable results. We therefore attempted to investigate the extent to which adult rats exhibit lipostasis, using

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two different dietary regimens to induce reversible obesity. The two dietary treatments we used involved tube feeding a semisynthetic diet or feeding a varied and palatable diet, the so-called "cafeteria" diet (Sclafani & Springer, 1976). On both regimens rats deposited excessive amounts of fat in a relatively short period, and when treatment was ceased, body fat rapidly returned to normal, which suggests the existence of a lipostatic control of body weight.

When describing the regulation of energy balance, most workers have ascribed a dominant role to intake control, but there is now evidence to suggest that variations in dietary-induced thermogenesis (or its reciprocal, metabolic efficiency) can exert a significant influence on energy balance. This evidence has arisen from studies on overfeeding (Apfelbaum, Botscarron, & Lacatis, 1971; Miller, Mumford, & Stock, 1967; Sims, Danforth, Horton, Bray, Glennon, & Salans, 1973), meal frequency (Fabry, 1969), and genetic obesity (Cox & Powley, 1977). In the experiments described here, an attempt was made to assess the contributions of both energy intake and expenditure to the overall regulation of energy balance, and the results provide further evidence for the involvement of dietary-induced thermogenesis. The overall view that emerges from this study of reversible obesity is that the rat attempts to regulate energy balance by reference to its fat stores and utilizes controls that operate on both energy intake and energy dissipation.

General Method

Food Intake

The semisynthetic stock diet used for all experiments was obtained from Unilever Research Laboratory (Bedford, England) and was composed of 48% carbohydrate, 25% protein, and 27% fat, with a metabolizable energy density of 17.3 kJ/g (value determined in separate rat feeding trials). The daily intake of stock diet was determined from changes in food-pot weights and the weight of spillage collected from under each cage. In tube-feeding experiments, animals received intragastric loads of a proprietary formula diet (Complan, Glaxo Ltd.) of similar nutrient composition to the stock diet. The metabolizable energy density of this diet was 18.6 kJ/g (manufacturer's analysis). In cafeteria experiments, the proportion of energy intake derived from cafeteria items was assessed by the weighed inventory method used by human nutritionists and dieticians; that is, from the weight of each food consumed, metabolizable energy intake was calculated by food composition tables (McCance & Widdowson, 1960). Because all animals were housed in pairs, intakes were measured as an average.

Body Fat

Body fat was estimated from an in vivo determination of total body water by tritium dilution. A dose of 10 μCi of tritiated water in 1 ml of saline was injected ip, and after a 2-hr equilibration period a sample of blood was taken from the tail, and the specific activity of plasma water was determined in a liquid scintillation counter. The value of total body water obtained by this method was used to calculate body fat by using a value of 73.1% for the water content of fat-free mass; hence the percentage of body fat equals 100 minus (percentage of body water divided by .731).

This value for the water content of fat-free mass was determined by direct analysis in a separate group of adult male rats, and we have shown that body fat determined by this indirect method correlates well with fat determined by analysis ($r = .87, p < .001, n = 32$). However, the values obtained from the tritium method tend to underestimate body fat, so all results were corrected for this by using the following equation derived from the regression between the two methods: body $\text{fat}_{\text{analysis}} = .53$ body $\text{fat}_{\text{tritium}} + 8.86$.

A detailed validation of the tritium method for estimating carcass composition in vivo is the subject of another article (Rothwell & Stock, 1979).

Energy Expenditure

Balance method. This method involves calculating heat production (E_{OUT}) from energy intake (E_{IN}) and the changes in body energy content (ΔE_{B}) from the equation for energy balance, namely, $E_{\text{OUT}} = E_{\text{IN}} - \Delta E_{\text{B}}$.

For the purpose of these experiments, the changes in body energy content are assumed to be entirely due to variations in body fat content and were calculated by using a value of 39 kJ/g for the energy density of fat.

Oxygen consumption. The resting oxygen consumption of rats was measured during the day in a closed-circuit respirometer previously described (Stock, 1975). After an initial equilibration period of 30–60 min, oxygen consumption was recorded for 1 hr, and any measurements associated with activity were discarded. Measurements were made at a temperature of 24 ± .5 °C, which corresponded to the temperature of the animal house, and animals had access to food up to the time when oxygen consumption measurements started.

Animal Housing

Rats were housed in pairs in a metabolism room maintained at 24 ± 1 °C with a 12:12 hr light/dark cycle.

Statistical Method

Values are given as means (± *SE*), and statistical differences were assessed by Student's *t* test for unmatched data. All probabilities are two-tailed.

Experiment 1: Tube Feeding

Experiment 1A

In the initial experiment, an attempt was made to induce obesity in adult rats by feeding a portion of their normal daily food intake by stomach tube while allowing free access to stock diet.

Method

Twelve adult male Sprague-Dawley rats were divided into two groups, each with a mean body weight of 440 g. One group was tube-fed Complan as a slurry (30% dry weight) delivered daily at 1000 hours. Over the 30 days of the experiment, the total energy delivered by stomach tube corresponded to 34% of normal energy intake (% control intake). Both the control and experimental groups had free access to the stock diet and water throughout. Body weight was recorded daily at 0900, and daily intake of stock diet was also determined.

Results

Table 1 presents the energy intake, body weight gain, and food efficiency (grams of weight gained/megajoule eaten) of control and tube-fed rats for the total duration of the experiment. Control and experimental animals consumed almost exactly the same amount of energy, which indicates that tube-fed rats reduced voluntary intake such as to compensate for the energy delivered by stomach tube. However, in spite of the similar intakes, tube-fed animals gained significantly more weight than controls and thus showed a marked increase in food efficiency.

Table 1
Mean (± SE) Weight Gain, Energy Intake, and Efficiency of Weight Gain in Rats Tube-Fed 34% of Normal Intake

Group	Body weight gain (in g)	Total energy intake (in kJ)	g gain/MJ eaten
Control	44 ± 5	11,340 ± 110	3.67 ± .35
Tube fed	71 ± 10*	11,650 ± 140	5.92 ± .59**

*$p < .05$, significantly different from controls. **$p < .01$.

This experiment established that tube feeding offers a successful method of inducing obesity in the rat, although some time was required to achieve a significant increase in body weight. In the next experiment, two levels of tube feeding were studied, and the effects of withdrawing treatment were also followed.

Experiment 1B

Method

Twenty-four adult male Sprague-Dawley rats were divided into four groups of six, and two of these groups acted as free-feeding controls for the other two groups which were tube-fed either 47% or 68% of normal energy intake. Control and experimental animals were allowed free access to stock diet, and food intake was determined throughout the experiment.

The group receiving 47% of intake by stomach tube were fed Complan as a slurry (40% dry weight) twice daily at 1000 and 1800 hours. After 21 days, treatment was ceased, and body composition was determined in control and experimental rats by the in vivo tritium dilution method. These animals continued on stock diet for a further 10 days after removal of tube feeding.

The second tube-fed group received three equal gastric loads per day (Complan, 50% dry weight) at 0800, 1400, and 1900 hours. To avoid excessive stomach loading, we increased the amount of food delivered intragastrically stepwise over the initial phase of the experiment. Tube feeding was continued for 24 days, and at the end of this period animals had received an average of 68% of normal energy intake by stomach tube.

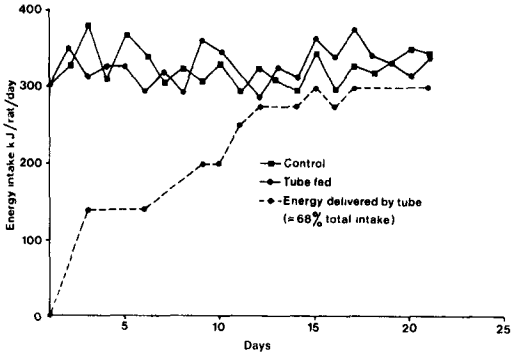

Figure 1. Mean daily intake of control rats and rats receiving a total of 68% of their intake by stomach tube (Experiment 1B).

Table 2
Mean (± SE) Body Weight, Energy Intake, and Efficiency of Weight Gain Before and After Withdrawal of Tube Feeding 47% of Normal Intake

Group	Body weight (in g)		Energy intake (Days 0–21)		g gained/ MJ eaten (Days 0–21)	Body weight (in g) (Day 31)	Energy intake (Days 21–31)	
	Day 0	Day 21	kJ/day	% controls			kJ/day	% controls
Control	320 ± 6	390 ± 5	400 ± 10	—	7.75 ± .30	415 ± 8	410 ± 10	—
Tube fed	320 ± 6	420 ± 10*	400 ± 10	100	11.63 ± .81**	417 ± 10	370 ± 10**	90

*$p < .05$. **$p < .01$.

Measurements were continued for a further 8 days in these rats after removal of treatment.

Body weight was recorded daily in all groups.

Results

As in the previous experiment, tube-fed animals reduced voluntary food intake to compensate for the energy delivered by stomach tube, and total energy intake for the period of treatment was almost identical for tube-fed rats and their respective controls (Tables 2 and 3). Furthermore, it is apparent from Figure 1 that this compensation is evident within the first 2 days of treatment and persists throughout the experiment in spite of the fact that the energy delivered by stomach tube was progressively increasing in those rats tube-fed 68% of intake (Table 3).

Body weight gain and feed efficiency were significantly increased in all tube-fed rats. Measurements of body composition in the 47% tube-fed group and their controls reveal that almost all (87%) of the excess weight gain of experimental rats was due to an increase in body fat content (body fat on Day 21: control, 71 ± 7 g; tube fed, 97 ± 5 g; $p < .05$).

When the treatment was withdrawn, obese rats rapidly lost weight and exhibited marked hypophagia. Body composition was not determined during this period, so it is not possible to determine whether the weight loss was entirely due to a reduction in body fat content.

Experiment 1C

In this experiment, the level of tube feeding was increased to 75% of normal intake. In addition to the measurements made in previous experiments, body composition and energy expenditure were estimated during the period of weight loss.

Method

Twenty-four adult male Sprague-Dawley rats were fed ad lib amounts of the semisynthetic stock diet for a period of 37 days. Half of these animals also received three daily intragastic meals of Complan in the form of a slurry (60% dry weight) at 0800, 1400, and 1900 hours. Tube feeding continued for 22 days, and the total energy delivered intragastrically represented 75% of normal

Table 3
Mean (± SE) Body Weight, Energy Intake, and Efficiency of Weight Gain Before and After Withdrawal of Tube Feeding 68% of Normal Intake

Group	Body weight (in g)		Energy intake (Days 0–24)		g gained/ MJ eaten (Days 0–24)	Body weight (in g) (Day 32)	Energy intake (Days 24–32)	
	Day 0	Day 24	kJ/day	% controls			kJ/day	% controls
Control	300 ± 8	358 ± 10	380 ± 10	—	6.54 ± .22	387 ± 10	390 ± 10	—
Tube fed	290 ± 2	385 ± 5*	370 ± 10	97	11.28 ± .77*	384 ± 6	320 ± 10**	82

*$p < .01$. **$p < .001$, significantly different from controls.

Table 4
Mean (± SE) Body Weight, Body Fat, Energy Intake, and Efficiency of Weight Gain of Rats Tube-Fed 75% of Normal Intake

Group	Energy intake		Body weight		Body fat (Day 22)	g gained/MJ eaten (Days 0–22)
	kJ/day	% controls	Day 0	Day 22		
Control	7,700 ± 30	—	402 ± 10	440 ± 13	109 ± 12	4.52 ± .14
Tube fed	7,710 ± 50	100	400 ± 11	498 ± 14*	158 ± 12*	10.53 ± .67*

Note. Data for energy intake are for Days 0–22. Weights are expressed in grams.
*$p < .01$, significantly different from controls.

energy intake. Food intake and body weight were recorded daily, and body fat was estimated by the tritium dilution method in all rats on Days 22 and 26 of the experiment. Resting oxygen consumption ($\dot{V}O_2$) was measured in control and experimental animals on Days 24 and 25 of the experiment.

Results

The results presented in Table 4 demonstrate that rats tube-fed 75% of normal intake behave similarly to those tube-fed at lower levels (Experiments 1A and 1B). Experimental animals compensated for the energy delivered intragastrically by reducing voluntary intake yet gained significantly more weight than controls during the period of treatment. This increase in body weight was accompanied by a similar increase in body fat such that fat represented 83% of the excess weight gain.

Fifteen days after withdrawal of tube feeding (Day 37), the experimental group had lost 28 g of body weight and was not significantly heavier than the control group (Table 5). The most rapid loss of weight occurred in the first 4 days following withdrawal of treatment when experimental animals lost 15 g of body weight; 14 g (90%) of this loss was due to fat (Table 5). Also shown in Table 5 are the energy intakes and calculated energy expenditures of the two groups during this initial period of rapid weight loss. The experimental rats consumed significantly less food than controls, but their energy expenditure (calculated by the balance method) was similar to control values. These results therefore suggest that the loss of body fat was due to hypophagia, with changes in metabolic efficiency apparently making no contribution. This assumption is supported by the observation that resting $\dot{V}O_2$ was similar for control and experimental groups on the second and third days of recovery (Day 24: control, 11.21 ± .20; tube fed, 11.36 ± .32; Day 25: control, 11.36 ± .41; tube fed, 11.30 ± .42, ml of O_2/min/$W^{.75}$). The values for oxygen consumption were corrected for differences in body weight by using metabolic body size ($kg^{.75}$) and showed no significant differences between the two groups.

Table 5
Mean (± SE) Body Weight, Body Fat, Energy Intake, and Energy Expenditure After Withdrawal of Tube Feeding

Variable	Control	Tube fed
Body weight (Day 26)	440 ± 14	483 ± 20
Body fat (Day 26)	105 ± 9	144 ± 12
Energy intake (Days 22–26)	1,600 ± 70	990 ± 30*
Energy expenditure (Days 22–26)	1,640 ± 280	1,540 ± 300
Body weight (Day 37)	455 ± 20	470 ± 38

Note. Weights are expressed in grams; energy in kilojoules.
*$p < .01$, significantly different from controls.

Discussion of Experiment 1

Tube feeding at levels below ad lib intake offers a successful method of inducing reversible obesity; the excess weight is rapidly gained and is not associated with marked alterations in fat-free mass.

The development of obesity in tube-fed rats is predictable, since previous workers (for review see Fabry, 1969) have reported that a reduction in meal frequency results in a greater metabolic efficiency and enhanced fat deposition. The increased lipogenic ca-

pacity seen in meal-fed and tube-fed animals has been ascribed to changes in a variety of key enzymes involved in nutrient assimilation (Fabry, 1969), although this does not necessarily explain the greater efficiency of these animals in energetic terms and questions the fate of ingested energy in the less efficient nibbling animal.

In the present study, the degree of obesity induced by tube feeding was more related to the gastric load than to meal frequency, i.e., the greater the fraction tube-fed the greater the excess weight gain. Other workers have reported an increased body fat content with no change in body weight when rats were fed a portion of their daily food intake by stomach tube; this has been called "nonobese obesity" (Cohn & Joseph, 1959). In the present experiments though, the increased fat deposition was accompanied by increases in body weight of a similar magnitude. There was evidence of small increases in fat-free mass, which might explain why, following withdrawal of treatment, body weight did not return to exactly the same level as control weights. This residual excess weight, however, was small and not statistically significant.

In spite of the stress and the disruptions to normal meal pattern imposed by tube feeding, a precise compensation of voluntary intake was exhibited in all groups of tube-fed animals receiving anything from 34% to 75% of their intake intragastrically. This compensation was apparent throughout each experiment and was not affected by variations in the bulk food delivered by tube or the number of daily intragastric meals. Although these results would support the contention that the rat exerts precise control over its energy intake, it has to be reconciled with the fact that tube-fed animals become obese. Thus a paradoxical situation exists in which food intake is voluntarily matched to that of free-feeding controls but is not controlled in order to maintain energy balance. This dissociation between appetite and energy balance raises serious questions about some current theories of intake control which we have discussed elsewhere (Rothwell & Stock, 1978).

The control animals in these experiments were not sham-fed by stomach tube since it was considered essential to compare the experimental animals with normal free-feeding controls. This view is justified by the fact that the observed compensation of voluntary intake of tube-fed animals was unaffected by factors such as the size of the intragastric loadings (34%–75% of daily intake), the bulk of the loads (varying from 30% to 60% dry weight), and the frequency of loading (1–3 meals/day), and it suggests that the dissociation between intake and energy balance is not a result of gastric intubation per se. It remains to be explained what is responsible for the precise compensation of voluntary intake in tube-fed animals, and it is possible that the rats were not controlling their energy intake but were eating for a specific nutrient or nutrients (e.g., protein, vitamins).

The recovery from obesity observed when tube feeding is terminated appears to be entirely due to hypophagia, since calculated daily energy expenditure and measured resting oxygen consumption were no different from control values during the period of rapid weight loss. However, it should be noted that energy expenditure was depressed during the development of obesity (this is deduced from the observed increases in metabolic efficiency) and must therefore have risen to control values on cessation of tube feeding. Another feature of the recovery phase worth noting is the hypophagia of tube-fed animals. This suggests that these rats, having previously matched their energy intake to that of the free-feeding rats, now relax this control to favor a restoration of normal body weight, although the abrupt change in feeding procedure may partly contribute to the hypophagia.

Experiment 2: Cafeteria Feeding

Tube feeding, as a method of inducing reversible obesity, was obviously successful, but nevertheless it involves deliberate disruption of normal meal patterns and some inevitable stress to the animal. Cafeteria feeding of rats was therefore tested as an additional method of inducing reversible obesity that would avoid these problems. The cafeteria-feeding system involves offering rats a choice of palatable food items

and was first described by Sclafani and Springer (1976).

Experiment 2A

Method

Twelve adult male Sprague-Dawley rats were maintained on ad lib amounts of stock diet, and half of these animals were offered, in addition, four other food items, the choice of which changed daily. The foods offered were conventional human foods, and over 40 food items were tested for preference. The most favored food items were found to be fruitcake, banana, liver, ham, cornflakes, chocolate, cookies, popcorn, and peanuts.

After 17 days of this feeding schedule, the mixed diet was removed, and animals were fed on stock diet alone for a further 12 days. Food intake was measured on Days 17–29, and body weight was recorded daily.

Results

The mean body weights of cafeteria rats and their controls are displayed in Figure 2. Animals offered the mixed diet gained weight rapidly such that by Day 17 they had achieved a body weight more than 60 g greater than control (mean body weights on Day 17 were 419 ± 16 g and 483 ± 16 g for control and experimental groups, respectively, $p < .001$). On removal of the mixed diet, experimental rats rapidly lost weight and completely returned to control weight by Day 30 (450 ± 20 and 451 ± 17 g for control and experimental groups, respectively), and thereafter the two groups continued to grow at the same rate. The intake (in kilojoules/rat/day) on Days 17–29 was slightly greater for cafeteria animals, and even when corrected for body size (kJ/W$^{.75}$/day), there was no significant difference between experimental and control rats (cafeteria: 824 ± 83; control: 735 ± 38). This finding suggests that the weight loss of cafeteria rats was brought about by an increase in energy expenditure.

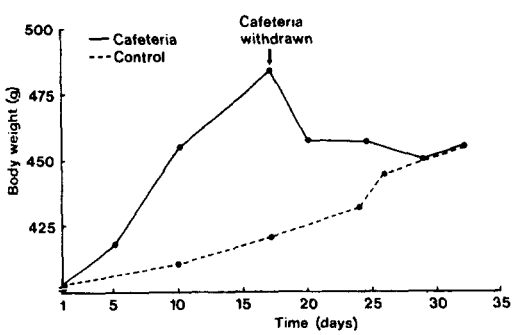

Figure 2. Mean body weight of control and cafeteria rats (Experiment 2A).

Experiment 2B

Having found that cafeteria feeding induces very rapid increases in body weight, we performed the present experiment to determine to what extent these gains were due to fat deposition.

Method

Adult male Sprague-Dawley rats were divided into two groups of 12, each with the same mean body weight (405 ± 10 g), and were allowed either stock diet alone (control group) or stock diet plus choice of four other food items (cafeteria group). After 22 days the mixed diet was removed, and all animals were allowed free access to stock diet for a further 15 days.

Body fat was estimated by the tritium dilution method in all rats on Days 22, 26, and 37, and body weight was recorded daily. Food intake was measured from Day 22 to Day 37 after the mixed diet had been withdrawn.

Results

During the period of mixed diet feeding, cafeteria rats gained significantly more weight than controls such that by Day 22 the body weights were 441 ± 13 and 490 ± 10 g for control and cafeteria rats, respectively ($p < .01$). When the mixed diet was withdrawn, obese rats rapidly lost weight and completely returned to control body weight by Day 37 (452 ± 40 g for controls and 450 ± 23 g for cafeteria rats).

These changes in body weight were accompanied by changes in body fat content of the same magnitude and direction (Table 6).

Table 6
Mean (± SE) Body Fat Content (in g) of Control and Cafeteria Rats (Experiment 2B)

Day	Control	Cafeteria
22	109 ± 7	161 ± 6*
26	105 ± 9	129 ± 8
37	98 ± 4	99 ± 5

*$p < .001$, significantly different from controls.

Table 7
Initial Recovery From Cafeteria Feeding (Experiment 2B)

Variable	Control	Cafeteria
Energy intake (Days 22–26)	1,600 ± 70	1,170 ± 50
Energy intake (Days 22–37)	5,670 ± 30	3,920 ± 210**
Energy expenditure (Days 22–26)	1,760 ± 280	2,460 ± 290*
Energy expenditure (Days 27–37)	4,350 ± 300	3,950 ± 370

Note. Data are means ± *SE*. Energy intake is expressed in kilojoules. Each group $n = 12$.
*$p < .05$. **$p < .01$, significantly different from controls.

On Day 22, cafeteria rats had gained 49 g of excess weight and 52 g of excess fat, which indicates that no significant alterations in lean body mass had occurred. Weight loss was also accompanied by changes in body fat, and on Day 37, when the body weights of the two groups were the same, body fat content was also identical.

Unlike in the first experiment, the rapid weight loss of cafeteria rats was accompanied by a marked hypophagia relative to controls (Table 7), and to determine whether this reduced intake was sufficient to account for all of the weight loss, we estimated energy expenditure by the balance method (see General Method). This calculation (Table 7) revealed that the expenditure of cafeteria rats was approximately 40% greater than that of controls during the first 4 days after removal of the mixed diet but that it was similar to the expenditure of the controls during the last 10 days of the experiment (Days 27–37).

Experiment 2C

The results of Experiments 2A and 2B suggest that recovery from obesity induced by cafeteria feeding is partly due to an increase in energy expenditure. However, the evidence for this relies on indirect measurements of expenditure. In the present experiment, therefore, measurements of food intake and oxygen consumption were carried out both during and after the period of cafeteria feeding.

Table 8
Mean (± SE) Body Weights (in g) of Control and Cafeteria Rats (Experiment 2C)

Day	Control	Cafeteria
1	433 ± 4	425 ± 3
22	505 ± 9	545 ± 6*
26	500 ± 13	524 ± 7

*$p < .01$, significantly different from controls.

Method

Adult male rats were divided into two groups of nine and maintained on stock diet or stock diet plus cafeteria foods for a period of 22 days, after which all rats continued on stock diet for a further 10 days.

Energy intake and body weight were measured daily throughout the experiment, and resting oxygen consumption was determined on Days 20, 21, 23, 24, 25, 26, 28, and 29.

Results

Cafeteria rats gained 40 g of excess weight and lost half of this excess weight within 4 days of returning to stock diet alone (Table 8).

Measurements of energy intake (Table 9) revealed that cafeteria rats overate by approximately 75%, and this increased intake is still apparent when corrected for body size. The hyperphagia is partly due to the greater energy density of the cafeteria foods (average 21 kJ/g) compared with stock diet (17 kJ/g), but cafeteria rats also consumed a greater

Table 9
Mean (± SE) Daily Food Intake and Energy Cost of Weight Gain for Control and Cafeteria Rats

Variable	Control	Cafeteria
Food intake (Days 1–22)	22 ± 2	32 ± 1**
Energy intake (Days 1–22)	320 ± 10	480 ± 10**
Energy intake[a] (Days 1–22)	590 ± 10	830 ± 10***
g gain/MJ eaten (Days 1–22)	10.5 ± 1.5	11.0 ± .6
Energy intake (Days 22–26)	534 ± 25	349 ± 22*

Note. Food intake is expressed in grams; energy, in kilojoules.
[a]Corrected for body weight (in kilojoules/$W^{.75}$/day).
*$p < .05$. **$p < .01$. ***$p < .001$, significantly different from controls.

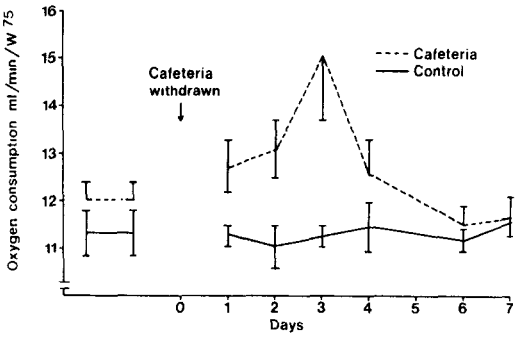

Figure 3. Resting oxygen consumption, corrected for body size, of cafeteria and control animals during the period of cafeteria feeding and following its withdrawal (Experiment 2C).

weight of food. It is interesting to note that when rats were presented with the mixed diet, they continued to eat significant amounts of the stock diet, which accounted for about 30% of their total energy intake. The effect of offering cafeteria foods on nutrient intake was to increase carbohydrate and fat intake at the expense of protein. The percentage of energy derived from fat, carbohydrate, and protein was, respectively, 36, 53, and 11 for cafeteria rats and 25, 48, and 27 for controls.

The energy cost of weight gain (Table 9) and the resting oxygen consumption (Figure 3) were similar for control and experimental rats during cafeteria feeding, but it can be seen from Figure 3 that cafeteria rats displayed a marked increase in resting oxygen consumption (ml/min/$W^{.75}$) during the first 3 days of recovery when weight loss was most rapid. The average increase in $\dot{V}O_2$ over these 3 days was equal to 24% of control rate, and the peak rise was equal to 39%; 7 days after withdrawal of the cafeteria diet, $\dot{V}O_2$ had returned to normal. These measurements were made only when the animals were at rest, and all values were corrected for differences in body size. The observed increase in expenditure of cafeteria rats is therefore not due to variations in physical activity and apparently results from an enhanced thermogenesis.

Discussion of Experiment 2

It seems that, unlike tube feeding, cafeteria feeding produces an obesity that is entirely due to an excessive energy intake since no changes in either metabolic efficiency or resting $\dot{V}O_2$ were observed. Thus, when the rat is offered a diet varying in flavor, appearance, texture, and composition, it will exhibit hyperphagia and become obese. It is interesting that these rats not only consume more energy but also eat a greater bulk of food, which demonstrates that any control of food intake in the rat can easily be overridden by psychological factors such as palatability and variety.

The elevated body weight of cafeteria rats is due to a greater fat deposition, with the excess fat accounting for almost exactly 100% of the excess weight. Furthermore, the spontaneous loss of body weight following removal of cafeteria foods is accompanied by an equal loss of fat such that by the end of the experiment the average fat content of cafeteria rats was within 5 g of that of controls. Although the loss of body weight and fat was often accompanied by a reduction of food intake, this was never sufficient to account for all of the lost body energy, and in each experiment there is evidence for a concomitant increase in energy expenditure.

In Experiment 2A, weight loss was apparently achieved entirely by changes in metabolic efficiency, since the energy intake of control and experimental animals was identical. In the other experiments, estimates of heat production by the balance method and measurements of oxygen consumption indicate that elevations in metabolic rate can make a significant contribution to body fat loss. Unfortunately, it is not possible to equate directly the observed increases in oxygen consumption with heat production estimated from the energy balance. The former were made in resting animals during the day and exclude the effects of activity and nighttime metabolic rate, which would include a large fraction of the metabolic response to feeding. Nevertheless, at least half of the increased heat production observed over the first 4 days of weight loss could be ascribed to changes in daytime resting oxygen consumption.

These findings illustrate the importance of thermogenesis in the regulation of energy balance. Previous demonstrations of thermogenic control have mainly arisen from overfeeding studies (Apfelbaum et al., 1971;

Miller et al., 1967; Sims et al., 1973), and it might be argued that an elevated heat production is simply the consequence of an increased substrate supply and the energy cost of fat and protein synthesis. It is difficult to concede to such explanations in the present study since the rats with the greater heat production were not only eating the same as, or less than, controls (and hence substrate supply was no greater) but were also in negative energy balance and so there was no net synthesis.

General Discussion

Compared with other methods of producing temporary changes in body fat content (e.g., high-fat feeding, insulin injection, lipectomy, and fat grafting), the cafeteria and tube-feeding systems offer obvious advantages. Both methods produce a significant increase in body weight and fat content within a short period of time, and these gains are rapidly and spontaneously reversed as soon as treatment is withdrawn. Of the two, the cafeteria system probably involves the lesser amount of stress to animals and experimenter alike, and it appears to be the method of choice. However, the mechanisms by which fat gains and losses are achieved differ considerably between the two methods, so that both provide equally useful models for the study of energy balance regulation.

A summary of the results from cafeteria and tube-feeding experiments described in this article is presented in Table 10 in order to compare these two types of reversible obesity. Cafeteria feeding produces a greater rate of weight gain than tube feeding, and this is due to an elevated energy intake without an apparent change in metabolic efficiency (g gain/MJ eaten). Tube-fed animals, however, achieve their more moderate degree of obesity on exactly the same intake as control animals, which confirms the potent effects of meal feeding on metabolic efficiency. Given that the means by which obesity develops are different in the two systems, it is perhaps not surprising that the reversal of obesity is also achieved by essentially different means. Tube-fed animals take longer to lose their excess weight and do so by a reduction in intake without a noticeable change in expenditure during the period of weight loss. Cafeteria rats, however, return more rapidly to normal weight; this is due to both a reduced energy intake and an elevated expenditure, which, although relatively short-lived (about 4 days), appears to be of primary importance since weight loss can be achieved even when energy intake is normal. In more succinct but less accurate terms, this comparison shows that obesity in tube-fed rats follows an increase in metabolic efficiency and recovery is due to hypophagia whereas obesity in cafeteria rats is due to hyperphagia and recovery is due to a decrease in metabolic efficiency. Thus, the mechanisms of weight gain and loss are apparently dependent on the method of inducing obesity, and to assume that the metabolic and behavioral responses to an increase in body weight should be similar and independent of antecedent nutrition is not justified in these examples.

Table 10
Comparison of the Two Types of Reversible Obesity

Variable	Tube feeding	Cafeteria
	Development	
Weight gain (g/day above control)	.50–2.33	1.41–3.90
Energy intake (% control)	100	170
g gain/MJ eaten (% control)	161–232	100
	Recovery	
Days to reach control weight	15–20+	9–15
Energy intake (% control)	69–89	73–100
Energy expenditure for first 4 days (% control)	100	130
VO_2 for first 4 days (% control)	100	124

Both techniques demonstrate that adult animals are able to spontaneously recover from experimentally induced deviations in body fat content and therefore provide experimental support for the concept of body fat regulation (lipostasis). In addition, it has generally been presumed that this lipostatic regulation is achieved by controlling energy intake (Kennedy, 1953; Le Magnen et al.,

1973), but the results of the present study indicate that control of thermogenesis may be just as important as food intake in the regulation of energy balance. It must be said, though, that both types of control fail to operate during the period of treatment when obesity is developing. Apart from indicating that any type of lipostatic control is absent during treatment, this also poses the question of why cafeteria rats fail to increase energy expenditure to reduce fat gains when overeating but immediately increase expenditure when returned to the stock diet. Similarly, tube-fed rats failed to reduce energy intake to compensate for excessive fat gains until tube feeding was withdrawn. If other factors, e.g., nutrient intake, were responsible for the precise control of a constant food intake during tube feeding, it is surprising that these parameters then take a lower priority during the recovery phase when hypophagia develops. It is possible that the control of energy intake and expenditure are so closely related that disruption of one parameter results in a simultaneous disruption of the other. Thus in cafeteria rats hyperphagia interferes with the control of thermogenesis, whereas it could be argued that in tube-fed rats variations in metabolic efficiency induced by meal feeding might also be responsible for the dissociation between food intake and energy balance.

These contrasting models of reversible obesity provide a cogent demonstration of the need to identify changes in both intake and expenditure if a complete understanding of energy-balance regulation is desired and suggest that the dominant role generally ascribed to appetite control is no longer justified. Finally, we would point out that in the search for a better understanding of energy balance, much attention has been focused on the development of obesity in humans and experimental animals, and we would like to suggest that investigations into the recovery from temporary obesity provide an alternative approach that could be equally instructive.

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