

Changes in Body Composition and Efficiency of Food Utilization for Growth in Young Adult Female Rats Before, During and After a Period of Food Restriction

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Abstract

Changes in body composition were studied in three groups of young adult female rats; the treatments were (1) *ad libitum* food intake to obtain normal growth, (2) restricted food intake to cause body weight loss, and (3) restricted followed by *ad libitum* food intake to obtain recovery of lost body weight.

In each of the three groups of rats the percentage body water was linearly and negatively correlated with the percentage body fat, the weight of body water was linearly and positively correlated with the weight of body protein, and the ratio of the weight of body protein to water was relatively constant at $1.3.20 \pm 0.02$ (mean $\pm$ standard error).

The percentage body water in the fat-free body was linearly and negatively correlated with fat-free body weight during normal growth between 109 and 334 g body weight but positively correlated during body weight loss and recovery.

During recovery of body weight rats laid down more fat and less protein than during normal growth through the same body weight range and the percentage of digestible energy retained as body tissue was increased.

Introduction

Since Pace and Rathbun (1945) proposed the use of a constant for the percentage body water in the fat-free body mass of mature mammals to indirectly calculate the amount of body fat, a number of authors have criticized its use (e.g. Keys and Brožek 1953; Clawson *et al.* 1955). There is also conflicting evidence (Burton *et al.* 1974) as to whether a period of restricted food intake followed by *ad libitum* food alters the proportion of body fat to protein in the body weight gain, compared to that laid down during normal growth through the same body weight range. These two matters have been studied in young adult female rats. Estimations have also been made of the relative efficiency of utilization of digestible energy for body weight gain during normal growth and during recovery of lost body weight after food restriction.

Materials and Methods

Animals, Housing and Environment

Young virgin female rats derived from the Sprague-Dawley strain were housed separately in wire mesh metabolism cages. The temperature was maintained at 20-22°C and lighting was provided from 0600 to 1800 h daily. No attempt was made to control or record humidity.

Food

A ground laboratory chow was used. It was composed of 82.4% grains and grain derivatives, 13% meat and fish meals, 4% lucerne meal, 0.3% sodium chloride and 0.3% vitamin supplement.

Analysis showed the gross chemical composition to be 7.1% ash, 21.3% crude protein and 4.3% lipid. 'Carbohydrate', calculated by difference, was therefore 67.3%. The total energy content as determined by bomb calorimetry was 18.3 kJ/g. More complete data on this food have been published elsewhere (Williams *et al.* 1976).

Feeding and Weighing

All animals were fed *ad libitum* at the start of experimentation to determine the normal intake for each individual. The animals selected for restricted feeding were then offered 50% of this amount for 7 days. Daily quantities of food were then individually adjusted at 7-day intervals to obtain steady and similar losses within each group. Recovery of body weight was obtained by offering food *ad libitum*. Distilled water was always available. Feeding was between 0900 and 0930 h. The rats were weighed once a week before feeding.

Analyses of Body Composition

Deprived animals were not fed on the morning they were killed; animals fed *ad libitum* were without food for 24 h before being killed.

The rats were killed with an overdose of sodium pentobarbitone and the volume of the injection accounted for in the measurement of body water.

After deep anaesthesia had been achieved the animals were weighed and placed in tared 14.5-cm evaporating bowls. A midline incision was made into the body cavity from the anus to the base of the neck. The caecum was incised and the contents removed.

(i) Body water

The whole animal was dried in an oven at 96°C for 4 days and body water calculated as weight loss.

(ii) Light petroleum-extractable lipid

The whole dried bodies were coarsely ground in a mortar, transferred to tared 8 by 2 cm Soxhlet thimbles and extracted with light petroleum (b.p. 40–70°C) for 21 h. Lipid was calculated as loss in dry weight.

(iii) Crude protein

Each carcass, after light petroleum extraction, was finely ground. Nitrogen content was measured on two samples of 200 mg digested by the Kjeldahl technique with selenium as the catalyst; an aliquot was distilled. Crude protein was calculated as $N \times 6.25$.

(iv) Ash

Three samples which had been ground and extracted with light petroleum were ashed in a muffle furnace at 400°C for 1 h and then at 600°C for 20 h.

Faeces Collection

Faeces were collected each morning at 0900–0930 h, dried for 24 h at 96°C and bulked for 5 consecutive days. This was followed by 2 days without collections to equilibrate the alimentary tract to any change in the amount of food offered in the following period.

Chemical Composition of Faeces and Food

The methods used for ash, crude protein and lipid were the same as for rat bodies except that the nitrogen and ash contents were determined on non-petroleum extracted samples. 'Carbohydrate' was calculated by difference.

Energy Contents of Food, Faeces and Bodies

The energy of the food and faeces was determined either by bomb calorimetry, or calculated from their chemical components using the following values in kilojoules per gram: 'carbohydrate', 17.5; lipid, 39.6; protein, 23.9. Both methods were used on several random samples and virtually identical results were obtained.

The energy of the rat carcasses was calculated using the above values for lipid and protein.

Experimental

Forty-seven rats were allowed to grow normally on *ad libitum* food intake. They were killed for body analysis at weights ranging from 109 to 334 g. Other rats were made to lose weight by restricting food intake. Of these, some were killed for analysis while losing weight, and some were killed while

regaining weight on *ad libitum* food intake after a weight loss of 40%. Another group of rats was made to lose 40% body weight and then fed *ad libitum* until the weight of age peers had been reached. The numbers of animals made to lose body weight, and the stages at which they were killed are summarized in Table 1.

Table 1. Percentage difference from initial body weight of food-deprived rats when killed for body chemical analysis

Number of rats	% Difference from initial body weight	
	Mean	s.e.
(a) During body weight loss		
4	-9.0	0.9
4	-21.5	1.2
4	-30.2	0.3
9	-44.7	3.6
(b) During recovery of body weight		
3	-27.6	0.2
4	-18.3	1.5
11	+0.9	1.1
12	+9.3	0.5
4 ^A	+22.7	2.2
4 ^A	+36.6	2.4

^A To match body weight of age controls.

Ad libitum food intake and body weight gain were measured in 53 normally growing rats ranging in body weight from 70 to 337 g. Other rats were made to lose 40% body weight and they were then fed *ad libitum* and their food intake, faeces output and body weight gain were measured during recovery to their initial body weights. These data were used in the calculations of the percentage of the digestible energy retained as body tissue in the two groups of rats.

Results

All means are presented with their standard errors.

Body Composition

In the normally growing animals, of body weights within the range 109–334 g, the percentage of water plus lipid was relatively constant at 75.3 ± 0.14 . Consequently, the percentage lipid-free dry matter was also reasonably constant (24.7 ± 0.15). Ash and protein were not correlated with one another.

After animals had lost about 20% body weight the percentage water plus lipid had decreased to about 71% (Table 2) and it then remained relatively constant with further weight loss. During body weight recovery the percentage water plus lipid increased and reached that in normal growing animals when initial body weight was re-attained; there was no change in rats taken to higher body weights (Table 2).

The percentage body water ($y_1\%$) was negatively correlated with percentage body fat ($x_1\%$) in all treatments:

normal growth:

$$y_1 = 74.7 - 0.94x_1, \quad n = 47, \quad r = 0.96, \quad P < 0.001; \quad (1)$$

losing body weight:

$$y_1 = 71.6 - 0.69x_1, \quad n = 16, \quad r = 0.69, \quad P < 0.01; \quad (2)$$

recovering body weight:

$$y_1 = 70.4 - 0.51x_1, \quad n = 37, \quad r = 0.88, \quad P < 0.001. \quad (3)$$

The weight of body water (y_2 g) was positively correlated with the weight of body protein (x_2 g):
normal growth:

$$y_2 = 27.7 + 2.58x_2, \quad n = 47, \quad r = 0.97, \quad P < 0.001; \quad (4)$$

losing body weight:

$$y_2 = -22.4 + 3.87x_2, \quad n = 26, \quad r = 0.96, \quad P < 0.001; \quad (5)$$

recovering body weight:

$$y_2 = -2.8 + 3.27x_2, \quad n = 37, \quad r = 0.98, \quad P < 0.001. \quad (6)$$

The ratio of the weight of protein to the weight of water in the body was relatively constant, being $1.3.28 \pm 0.03$ during normal growth, $1.3.10 \pm 0.04$ during body weight loss, and $1.3.18 \pm 0.03$ during body weight recovery. The value was $1.3.20 \pm 0.02$ for all results combined.

Table 2. Percentage water plus lipid in the body during body weight loss and during recovery of body weight

Values given are means $\pm$ s.e.

% Predeprivation body weight	Number of animals	Percentage water + lipid ^A	% Predeprivation body weight	Number of animals	Percentage water + lipid ^A
(a) During body weight loss			(b) During recovery of body weight		
88-93	4	74.9 $\pm$ 0.5	72-86	7	73.9 $\pm$ 0.2 ^a
48-81	18	71.0 $\pm$ 0.3 ^b	96-109	19	75.1 $\pm$ 0.2
			110-140	13	75.5 $\pm$ 0.2 ^B

^A Superscripts *a* and *b* indicate that the means are significantly different ($t = 5.22$, $P < 0.001$).

^B In normally growing rats $75.3 \pm 0.14\%$.

The percentage body water in the fat-free body (y_3 %) was negatively correlated with the fat-free body weight (x_3 g) during normal growth, but positively correlated during body weight loss and recovery:
normal growth:

$$y_3 = 78.1 - 0.03x_3, \quad n = 47, \quad r = 0.82, \quad P < 0.001; \quad (7)$$

losing body weight:

$$y_3 = 62.9 + 0.07x_3, \quad n = 26, \quad r = 0.81, \quad P < 0.001; \quad (8)$$

recovering body weight:

$$y_3 = 68.3 + 0.02x_3, \quad n = 37, \quad r = 0.55, \quad P < 0.001. \quad (9)$$

Therefore during normal growth the percentage body water in the fat-free body mass decreased; it decreased in rats losing weight, and rose again as body weight was recovered.

Regression equations relating the weight of body constituents to body weight during normal growth and during recovery of body weight are shown in Table 3. Comparisons of the regression coefficients showed that during recovery animals laid down protein ($P < 0.001$) and ash ($P < 0.001$) at a slower rate than during normal growth but they laid down fat at a much faster rate ($P < 0.001$).

Food Utilization

Rats during normal growth, measured as increase in body weight, have an early fast rate followed by a much reduced growth rate (Williams 1976). Normal growth was related to age by two regression equations.

Table 3. Regression equations relating the weight of body constituents (y , grams) to body weight (x , grams) in normally growing female rats and in rats during recovery of body weight

17 pairs of observations were made. a is the y intercept, b is the regression coefficient, and r is the correlation coefficient

	a	b	r	P
(a) During normal growth				
Water	8.6	+0.64	0.99	<0.001
Protein	-4.9	+0.24	0.98	<0.001
Ash	0.9	+0.04	0.98	<0.001
Lipid	-3.3	+0.08	0.69	<0.01
(b) During recovery after food deprivation				
Water	12.5	+0.59	0.98	<0.001
Protein	7.5	+0.16	0.96	<0.001
Ash	4.7	+0.02	0.72	<0.01
Lipid	-24.2	+0.23	0.85	<0.001

For age range 35–103 days, and body weight range 70–220 g, the expression was

$$\log y_4 = 0.62 + 0.86 \log x_4, \quad n = 72, \quad r = 0.94, \quad P < 0.001, \quad (10)$$

and for age range 138–232 days and body weight range 205–305 g, it was

$$y_4 = 134.6 + 0.79x_4, \quad n = 97, \quad r = 0.65, \quad P < 0.001, \quad (11)$$

where y_4 is body weight (grams) and x_4 is age (days). The change in rate of body weight gain occurred at 210 g body weight and 95 days of age.

From these relationships the number of days required for an increase of 10 g body weight was calculated starting at ages within the range 35–232 days.

Also in normally growing animals dry matter intake per day was regressed against body weight. The expression was

$$y_5 = 9.6 + 0.03x_5, \quad n = 186, \quad r = 0.79, \quad P < 0.001, \quad (12)$$

where y_5 is food intake (grams) and x_5 is body weight (grams). The 95% confidence limits were ± 3 g.

Table 4. Comparisons of digested energy intakes and energy retained in rats recovering body weight after food restriction and those anticipated for rats growing normally through the same body weight range

Rat	Body weight (g)		During recovery after food restriction:				During normal growth:			
	Initial	Final	Time ^b (days)	Digested energy (MJ)	Body energy gain ^a ^b (MJ)	% Digested energy retained	Time ^b (days)	Digested energy (MJ)	Body energy gain ^a ^c (MJ)	% Digested energy retained
305	142	254	14	5.0	1.2	24	92	17.6	0.8	4.5
308	134	252	14	4.7	1.2	26	92	17.7	0.8	4.5
316	124	251	14	4.4	1.1	25	96	18.3	0.9	4.9
318	127	272	14	4.8	1.2	25	121	23.6	0.9	3.8
312	139	271	21	6.4	1.3	20	114	22.3	0.9	4.1
314	116	255	28	8.0	1.3	16	105	20.0	0.9	4.5
507	106	188	18	3.8	0.9	24	41	7.4	0.6	8.1
508	110	181	18	3.6	0.9	25	35	6.4	0.5	7.8
504	109	185	14	3.1	0.7	23	38	6.8	0.5	7.4
506	111	193	21	4.0	1.1	28	41	7.5	0.6	8.0

^a Initial energy in body calculated from Table 3. ^b Final energy in body calculated from chemical analysis.

^c Final energy in body calculated from Table 3. ^d Time to grow from initial to final body weight.

From the above relationships the value for the minimum dry matter intake, based on 95% confidence limits, for a normal female rat to gain 10 g body weight from any previous weight was calculated. The values obtained were used in the estimates of the digestible energy intakes shown in Table 4.

The digestible energy coefficient, 76.7%, was determined experimentally in rats during recovery after deprivation; it has been shown that this value can be applied to normally growing animals (Williams and Senior 1978).

The body compositions upon which the estimates of the energy in the body weight gain were based (Table 4) were obtained as follows: estimates of gain in protein and fat during recovery were based on actual body analysis at the end of the experiment and the calculated initial composition using equations from Table 3; estimates of theoretical gain in these constituents during normal growth were calculated using equations from the same table. Estimates of energy gained were then made from the calculated gains of protein and fat.

Table 4 shows that the percentage of the digested energy retained was 3–6.6 times greater during body weight recovery than during normal growth and that the proportion of the digestible energy retained during body weight recovery was little affected by the amount of weight gain. However, during normal growth, increase in the lower weight ranges resulted in greater calculated energy retention.

Discussion

The body composition data were obtained from young adult female rats and therefore the relationships which have been derived apply strictly only to such animals.

The percentage body water was negatively correlated with the percentage body fat (equations 1, 2 and 3). This has been found by other investigators studying body composition in farm animals, e.g. Garrett *et al.* (1959) studying sheep, and Kay and Jones (1962) studying swine. The relationship is the result of adipose tissue having a very low water content compared to other soft tissues (Babineau and Pagé 1955).

Pace and Rathbun (1945) analysed the eviscerated hairless bodies of 50 guinea pigs within the body weight range of 304–1000 g and obtained a value of 72.42 ± 2.11 (mean $\pm$ s.d.) for the percentage of water in the fat-free body mass. Keys and Brožek (1953) pointed out that this meant that 90% of the animals fell within the range 68.2–76.7%. Pace and Rathbun (1945) also calculated values for rats, rabbits, cats, dogs and monkeys from published data and obtained a mean of 73.2% with a range of 71.8–76.3%. The value 73.2% has been used to calculate indirectly the fat content of the body, e.g. Barnard *et al.* (1969) for humans, and Meyer *et al.* (1956) for rats.

In the present experiment the weight of body water was positively correlated with the weight of body protein (equations 4, 5 and 6) and the ratio of protein to water was relatively constant at $1:3.20 \pm 0.02$ (mean $\pm$ s.e.) irrespective of treatment. Thus 90 out of 100 rats during normal growth add between 2.8 and 3.6 g of water to each 1 g of protein and lose water and protein in the same ratio during body weight loss. As a young adult animal losing body weight due to food restriction retains its body ash while losing protein (Williams *et al.* 1976), the percentage water in the fat-free body mass must inevitably decrease during body weight loss. Our results showed that the percentage body water in the fat-free body mass during body weight loss and recovery was linearly and positively correlated with fat-free body mass (equations 8 and 9), and it was linearly and negatively correlated in rats during normal growth

(body weight range 109–334 g; equation 7). It is therefore not valid to use a constant for rats in these experimental conditions. Meyer and Clawson (1964) used 72.5% and estimated a high value of 5.6% fat in the bodies of young rats even after 32% body weight loss whereas Williams *et al.* (1976) found by complete chemical analysis 1% or less fat after only 20% body weight loss; this is a more probable explanation for the discrepancy than the difference in initial body weights.

The percentage of fat plus water in the bodies of rats growing normally was relatively constant (Table 2). This confirms the observations of Babineau and Pagé (1955) and Rérat *et al.* (1964). When rats had recovered after a loss of body weight the same constant applied as during normal growth (Table 2) because of the reciprocal relationship between the percentage body water and the percentage body fat. However, while weight was being lost or recovered the percentage of fat plus water was significantly less than normal. This was due to the relatively very rapid loss of body fat during food restriction observed previously (Williams *et al.* 1976).

The regression equations in Table 3 show that animals during recovery in body weight laid down more fat but less protein than animals growing normally through the same body weight range. The result for fat confirms the results of Szepesi and Vojnik (1975) and Szepesi and Epstein (1976) in this species, but they did not measure protein. Meyer and Clawson (1964) came to the same conclusion but it is not possible to say how they determined body protein and their method for calculating body fat is subject to criticism. On the contrary, Barnard *et al.* (1969) studying obese humans, Robinson and Lambourne (1970) studying mice, and McManus *et al.* (1972) and Burton *et al.* (1974) studying sheep, produced data showing that these species laid down more protein and less fat during recovery of body weight. Thus there is conflicting evidence on the statement by Wilson and Osbourn (1960, p. 341), that 'the effect of compensatory growth appears to be to increase the fat content of the body compared to unrestricted controls'. Black (1974) predicted from a computer model that the differences between results from various studies can be largely explained by variations in the rates of body weight loss and the times spent on treatment before rehabilitation.

The calculated digestible energy retained during normal growth was low, but the animals were held at 22°C and Terroine [cited by Brody (1945, fig. 11.13)] has shown that fasting metabolism is increased at this temperature by 50% above that at 30°C, substantially increasing maintenance requirements.

However, rats retain a greater proportion of their digestible energy intake during recovery of body weight compared with normal growth under the same conditions (Table 4). The increased efficiency of food utilization was not due to an enhanced ability to digest any food component (Williams and Senior 1978). The effect was partly due to the relatively rapid rate of weight gain during recovery decreasing the overall maintenance requirement, and possibly partly due to the reduction in fasting metabolic rate which is induced in rats during body weight loss (Westterterp 1977). Keys *et al.* (1950) showed that a depressed basal metabolic rate in adult humans induced by food restriction was still 30% below normal 8 weeks after commencement of rehabilitation. Graham and Searle (1975) in an experiment on weaner sheep showed that basal metabolic rate per kg^{0.75} tended to fall during body weight stasis. It rose in the first month of resumed growth but remained below that of control animals growing through the same body weight range, thus indicating some economy in the rehabilitation period.

In conclusion it can be said that these studies in the young adult female rat showed that recovered body weight resulting from *ad libitum* food intake after a period of food restriction was higher in fat and lower in protein than the composition of weight gain in normally growing animals over the same body weight range. Rats growing normally, losing weight, or recovering lost body weight, had different relationships between the percentage water in the fat-free body and the fat-free body mass. The digestible energy requirement per unit of recovered tissue energy was very low compared with the needs for tissue synthesis during normal growth, and a part of this better utilization was due to the rapid rate of synthesis decreasing the overall maintenance requirement.

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