

Effects of Semi-starvation on the Total Body Composition and Absorptive Function of the Small Intestine of the Young Adult Female Rat

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Abstract

Sixteen female rats aged about 80 days and with a mean body weight of 175 g were fed 40% of their *ad libitum* intake of a laboratory chow. They were killed and analysed for water, protein, lipid and ash after 9, 21.5, 30.2 and 38.8% of body weight had been lost. Compared to a control group of four animals, the 38.8% group lost 13 g or 34% of their protein. The animals in the 21.5, 30.2 and 38.8% groups lost 7.5 g or 87% of their lipid leaving only 1.1 g of lipid. The percentage protein in the body was little affected by body weight loss but lipid decreased from 5 to 1%.

In another experiment with 26 rats of 205 g mean body weight and aged about 115 days, absorption rates by the small intestine were measured *in vivo* after variable weight losses between 0 and 39%. D(+)-Glucose uptake was increased by about 70% in those animals which had lost only 5% of body weight and this increased uptake was retained in those rats which had lost up to 39% of body weight. The absorption of L-leucine was not affected by the decline in body weight compared to the controls but relative to body weight, the ability of the intestine to absorb increased.

In the same animals, the wet and dry weights of the small intestine declined slightly faster than body weight and the length of the small intestine tended to decrease slightly with increasing loss of body weight.

Introduction

Experimental studies in the rat have shown enhanced absorptive capacity of the small intestine for some nutrients after dietary restriction had resulted in up to 23% body weight loss (Kershaw *et al.* 1960; Hindmarsh *et al.* 1967).

Jackson (1925) reported that young adult mammals, such as the rats used in the above experiments, are capable of losing about 50% of their body weight before death. The present studies of the rat were therefore undertaken to determine whether the reported enhanced absorptive capacity of the small intestine is retained after body weight losses near to the probable maximum. The body composition of rats subjected to identical dietary restriction and body weight losses was also determined in order to estimate the nutritional severity of the treatment.

A preliminary report of this work has already been published (Williams *et al.* 1975).

Materials and Methods

Animals

All animals were young adult female albino rats derived from the Sprague-Dawley strain. In the experiment measuring body composition, the mean body weight was 175 g and age was 80 days; in the study of small intestine absorption, mean body weight was 205 g and age was 115 days. No animals were used for both experiments.

Housing and Environmental Temperature

All the animals were housed singly in wire mesh cages. The environmental temperature ranged from 15 to 30°C and it was uncontrolled except for some supplementary heating.

Diet

The following laboratory diet (Fielders Pty.Ltd, Tamworth, N.S.W., Australia) was given in powdered form.

Constituent	Amount (g/kg)	Constituent	Amount (g/kg)
Linseed meal	44	Lucerne meal	40
Maize grain	150	Fish meal	50
Sorghum grain	160	Meat meal	80
Wheat grain	200	Sodium chloride	3
Pollard	250	Vitamin supplement	3
Bran	20		

The vitamin supplement consisted of the following: vitamin A, 1814 i.u./kg; vitamin D₃, 454 i.u./kg; vitamin E, 5 i.u./kg. The diet had a crude protein content of 21.5%, a light petroleum-extractable lipid content of 4%, and ash content of 7% of which 35.7% was calcium and 18.6% was phosphorus.

Feeding and Weighing of Animals

All animals were fed individually. Tap water for drinking was always available. Control animals were fed *ad libitum* until 24 h before a perfusion experiment or before being killed for studies on body composition. Experimental animals subjected to a restricted diet were fed 6.65 g dry matter per day, i.e. approximately 40% of the *ad libitum* intake. Feeding was usually at 0900 h but was at about 0930 h on weighing days. The restricted diet was always eaten within 45 min. All animals were weighed three times a week, but individuals were weighed more often when approaching a required weight.

Analyses of Body Composition

All animals were killed by intraperitoneal injection of 25 mg of sodium pentobarbitone in 0.5 ml of water, and they were then weighed and placed in tared 14.5-cm porcelain evaporating dishes. A midline incision was made along the whole ventral surface from the anus to the base of the neck. The contents of the caecum were removed.

(i) Body water

The whole animal was dried in an oven at 96°C for 4 days and the body water was calculated as weight loss. It was considered unnecessary to correct for the 0.5-ml injection.

(ii) Light petroleum-extractable lipid

The whole dried bodies were coarsely ground with a mortar and pestle. Each body was divided between six dried, tared 8- by 2-cm Soxhlet thimbles and extracted with light petroleum of b.p. 40–60°C for 21 h. Lipid was calculated as loss in dry weight.

(iii) Crude Protein

Each carcass which had been totally extracted with light petroleum was finely ground. The 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 of each carcass that had been extracted with light petroleum and then finely ground were ashed in a muffle furnace at 400°C for 1 h and then at 600°C for 20 h.

Perfusion of the Small Intestine to Measure Absorption of Glucose and Leucine

The method used has been described in detail by Cripps and Williams (1975). The animals were anaesthetized by intraperitoneal injection of a 50 mg/kg body weight dose of sodium pentobarbitone. A midline abdominal laparotomy was performed and the small intestinal lumen was washed with normal saline (9 g/l) and cannulated at the pylorus and ileo-caecal valve.

The lumen was filled with an isotonic sodium phosphate-sodium chloride buffer, pH 6.5 (Table 1), and perfused by pumping at 1 ml/min against a 7.5-cm head at the point of outflow. The outflow was collected for 20 min to measure any efflux of glucose or non-protein amino nitrogen. The lumen was then flushed with the test solution (Table 1). After pumping for 15 min to equilibrate the system, two

consecutive 20-min collections of perfusion solution outflow were made. After correcting for net water flux and efflux of glucose and amino nitrogen, absorption was measured as the difference between the inflow and outflow of leucine and glucose. All solutions were at 39°C. At the completion of the last perfusion the animals were killed with an overdose of sodium pentobarbitone.

The test solution had a pH value of 6.5, which is close to the optimum for glucose absorption (Iida *et al.* 1968). The transport of valine, a branched-chain neutral amino acid which has similar pK_{a1} and pK_{a2} to leucine (Hodgman 1950), is not affected by pH in the range 5–8, and a much simpler solution than Krebs–Ringer bicarbonate may be used without detriment (Reiser and Christiansen 1967).

Leucine was measured in tungstic acid filtrates by a modification of the trinitrobenzenesulphonic acid technique of Satake *et al.* (1960) for determination of amino nitrogen, and glucose by *o*-toluidine (Dubowski 1962).

Table 1. Constituents of phosphate buffer, pH 6.5, and test solution infused into rat intestines

Solution	Constituent	Concn (g/l)	Composition			Osmolarity (m-osmol/l)
			Na (mmol/l)	P (mmol/l)	Cl (mmol/l)	
Phosphate buffer	NaH ₂ PO ₄ ·2H ₂ O	6.52	41.78	41.78	86.04	} 290
	Na ₂ HPO ₄ ·12H ₂ O	8.91	49.74	24.87		
	NaCl	5.03	86.04			
Test solution	Phosphate buffer (as above)			(g/l)	(mmol/l)	} 310
	L-Leucine			1.31	10.00	
	D(+)-Glucose			1.80	10.00	
	[⁵¹ Cr]EDTA, 2 × 10 ³ counts min ⁻¹ ml ⁻¹					

Anatomical Measurements of the Small Intestine

The small intestine was removed from the body as soon as possible after perfusion. The organ was stripped from its mesentery and its length was measured on a metre rule laid on a bench. The intestine was then weighed and dried at 70°C for 48 h to obtain the dry weight.

Statistical Measurements

Significance of differences between means was determined by Student's *t* test and linear regression was calculated by the method of least squares.

Experimental Procedure

(i) *Controls*

In both body composition and absorption experiments, the control animals were subjected to study at the same age and body weight as the experimental groups at the start of restricted feeding.

(ii) *Body composition*

Twenty rats with body weights ranging from 161 to 186 g were allocated to five groups of four animals at random. One group was the control and the others were subjected to dietary restriction which led to weight losses of 9.0 ± 0.9, 21.5 ± 1.2, 30.2 ± 0.3 and 38.8 ± 1.2% (mean ± s.e.). Results were obtained on only 19 animals owing to the death of one member of the group with the greatest weight loss. The initial body weights of the groups were (mean ± s.e.): control, 171.4 ± 5.3 g; experimental, 177.0 ± 2.1, 177.8 ± 3.1, 175 ± 4.3 and 173.7 ± 5.3 g in order of increasing weight loss.

(iii) *Absorption of D(+)-glucose and L-leucine from the small intestine*

Twenty-six animals were allocated at random to two groups. The control group of nine had a body weight (mean ± s.e.) of 193 ± 4.6 g and the experimental group of 17 had a body weight of 210.8 ± 4.4 g. Later analysis showed that the two groups had a significant weight difference ($t = 2.55$, $P < 0.02$).

Individuals of the experimental group showed between 5.3 and 38.8% weight loss by dietary restriction and it took an average of 43 days to attain the greatest decrease. When the allocated body weight loss had been attained a small intestine absorption experiment was performed and gross anatomical measurements were made on the small intestine.

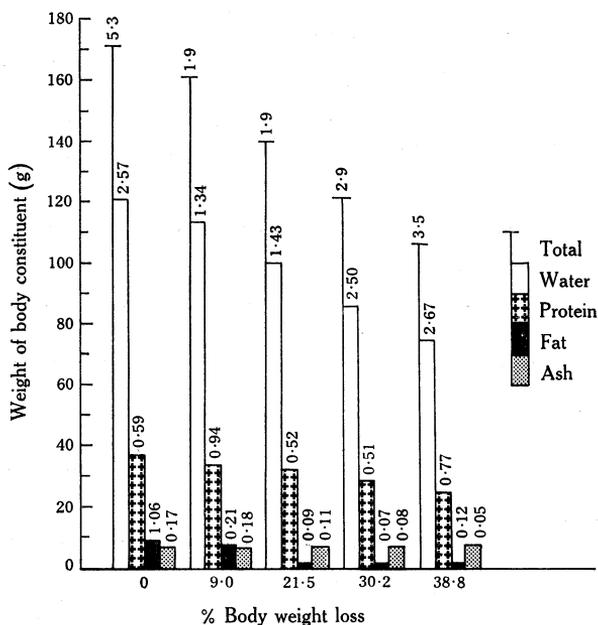


Fig. 1. Quantity of body constituents after loss of body weight.

Results

Analyses of Body Composition

The weights of body constituents presented in Fig. 1 show that the bodies had declined in light petroleum-extractable lipid from 8.6 to 1.1 g when 21.5% of the body weight had been lost and that there was no further decline with greater weight loss. Protein declined by 5 g with a 21.5% decrease in body weight but a greater amount, 8 g, was lost with the next 17% decrease in body weight. Water declined most: 20 g was lost with a 21.5% decrease in body weight and a further 25 g with the next 17% decrease in body weight. Compared with the controls, the 38.8% weight loss group had lost 34% protein and 38% water. Unlike the other constituents, ash did not decrease in weight.

Fig. 2 shows that the percentages of water and protein in the total carcass were little affected by body weight loss but light petroleum-extractable lipid declined from 5 to 1% at 21.5% body weight loss and ash increased gradually from 3.85 to 6.81%, an increase of 77%.

However, when water was expressed as a percentage of the fat-free body matter, y , there was a correlation with percentage body weight loss, x . The equation is

$$y = 74.22 - 0.089x, \quad r = -0.78, \quad P < 0.001,$$

i.e. the proportion of water constantly decreased.

This study thus shows that the animals maintained their ash content when on the restricted diet but they lost most of their useable lipid and one third of their protein.

Anatomical Measurements of the Small Intestine

A regression analysis of the length of the small intestine per gram initial body weight, y , against percentage body weight loss, x , showed a trend indicating that semi-starvation had decreased the length of the small intestine. The equation is

$$y = 5.35 - 0.013 x, \quad r = 0.4, \quad P < 0.05.$$

The mean percentage water content of the small intestine was not affected by semi-starvation. The correlation between percentage water content and percentage body weight loss was not significant ($r = 0.33$, d.f. 26).

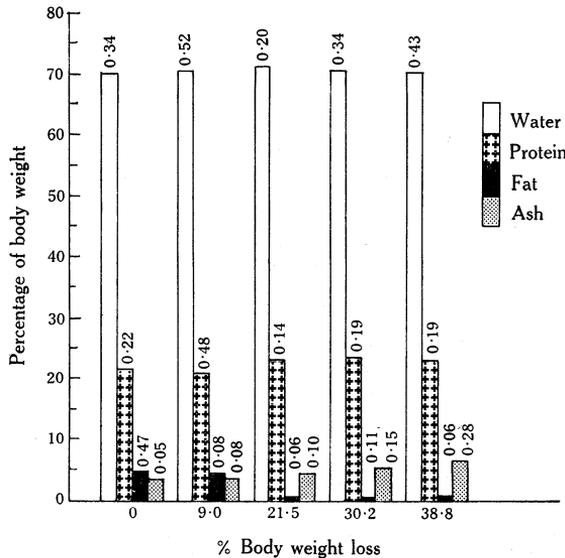


Fig. 2. Percentage of body constituents after loss of body weight.

A regression analysis of the dry weight of the small intestine (in grams) y , against percentage body weight loss, x , gave the equation

$$y = 1.081 - 0.122 x, \quad r = 0.81, \quad P < 0.001.$$

Thus the small intestine lost dry matter as body weight decreased.

The dry weight of the small intestine decreased at a faster rate than the body weight decreased. This was shown by the regression of the dry weight (in milligrams) of small intestine per gram of killed body weight, y , against percentage body weight loss, x . The equation is

$$y = 5.5 - 0.026 x, \quad r = 0.59, \quad P < 0.001.$$

This did not apply to the wet weight ($r = 0.27$, d.f. 26).

A regression analysis of the dry weight of the small intestine per unit length (in milligrams per centimetre), y , against percentage body weight loss, x , gave the equation

$$y = 10.37 - 0.122 x, \quad r = 0.83, \quad P < 0.001.$$

Thus dry weight per unit length decreased with increasing body weight loss. The same was true for wet weight, and the equation is

$$y = 57.83 - 0.594 x, \quad r = 0.78, \quad P < 0.001.$$

Absorption of D(+)-Glucose

Glucose absorption per centimetre per hour (Fig. 3a) increased about 70% by the time only 5% of body weight had been lost. The data from the experimental animals in Fig. 3a were not linearly related to percentage body weight loss ($r = 0.32$, d.f. 14). However, the difference between the means of the control and experimental groups was highly significant ($t = 5.61$, $P < 0.001$).

Absorption of glucose expressed as micromoles per gram initial body weight per hour showed the same pattern as absorption per unit length (in centimetres) ($t = 6.39$, $P < 0.001$).

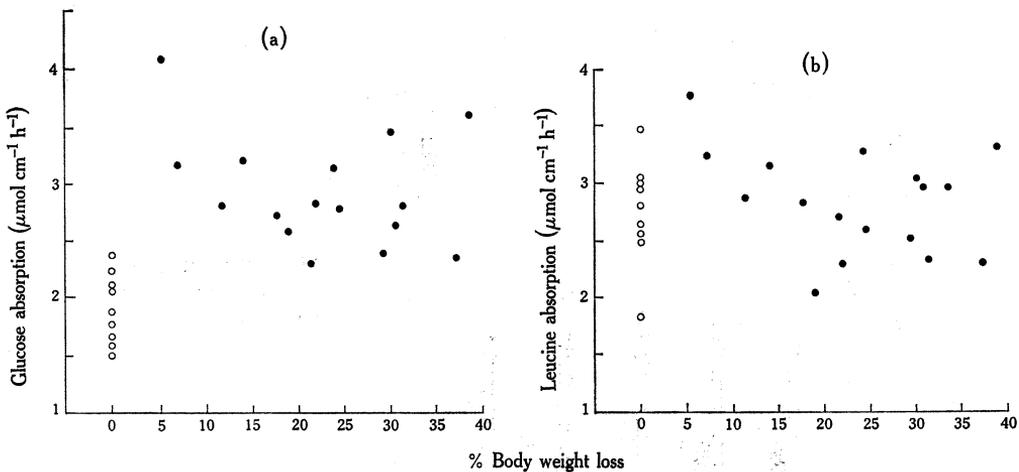


Fig. 3. (a) Glucose absorption and (b) leucine absorption (measured per centimetre of small intestine per hour) after loss of body weight. ○ Control individuals. ● Experimental individuals.

Absorption of L-Leucine

Body weight loss caused no effect on leucine absorption per centimetre per hour ($r = 0.07$, d.f. = 22) but the variation in the data was great (Fig. 3b). When absorption was expressed as micromoles per gram initial body weight per hour, again no effect was apparent. However, when weight was lost the small intestine lost length only slightly, and so absorption expressed as micromoles per gram killed body weight per hour, y , increased linearly with body weight loss, x (see Fig. 4). The equation is

$$y = 1.488 + 0.0167x, \quad r = 0.65, \quad P < 0.001.$$

Discussion

In both the absorption and body composition studies the control rats were fed *ad libitum* until the day before experimentation and deprived rats were not fed on the morning of experimentation. Rats are night feeders so the control group were effectively without food only overnight or for about 9 h. This treatment of the control group was necessary to decrease gut fill and so to determine more accurately true body weight and composition; it also allowed the small intestine to be washed free of digesta without damage before a perfusion experiment.

Cizek (1954) made a study of the effect of various times of fasting on the amount of water present in the gut lumen of female rats. Unfortunately the composition of the diet was not mentioned; presumably it was a laboratory chow. After the rats had been without food for 24 h but had water available *ad libitum*, the gut water content was $3.1 \pm 1.6\%$ of body weight. The error in the present experiment would have been less as the caecum was emptied of digesta. However, there can be no doubt that the body water values would have been about 2% less if Cizek's (1954) data are applicable.

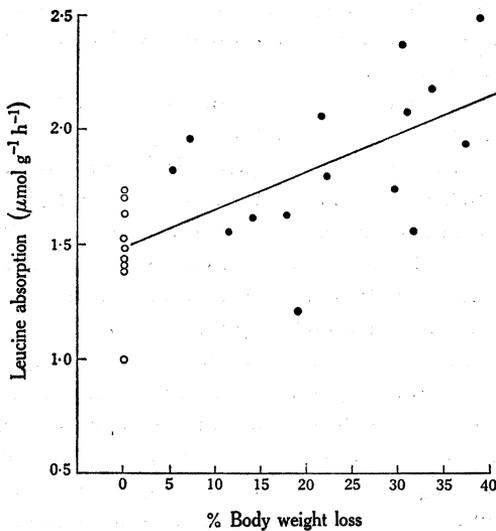


Fig. 4. Leucine absorption (measured per gram of killed weight per hour) after loss of body weight. ○ Control individuals. ● Experimental individuals.

By the time the animals had lost 21.5% of body weight, lipid had decreased from 5 to 1%. Probably most of the lipid retained was that required for the integrity of the tissues, such as phospholipids.

Hair was included in the determination of body protein. No marked increase in hair loss was noted in the deprived animals. With the progressive loss of body weight, hair could therefore have been providing an increasing proportion of the measured body protein and, together with the ash component (which maintained its original weight), would have contributed to the steady decrease found in the percentage water in fat-free body matter. The constancy of the ratio of water to fat-free dry matter is emphasized in a number of reports (Pace and Rathbun 1945; Babineau and Pagé 1955).

The effect of semi-starvation of the rat (Hindmarsh *et al.* 1967) and mouse (Madge 1970), and marasmus in children (Brunser *et al.* 1968) is to decrease the weight of the small intestine. The present study confirms these observations.

Steiner *et al.* (1968) starved rats for 6 days and showed that the small intestine lost weight faster than the body as a whole; the present experiment indicated that this was true when the dietary restriction imposed was less severe but was of longer duration.

James (1971) and Viteri *et al.* (1973) both present comprehensive data on intestinal malabsorption in children with protein-calorie malnutrition. However, such studies are complicated by the variable extent to which individuals have been deprived of dietary needs such as vitamins as well as protein and calories, the presence of pathogens in the gut, and the incidence of diarrhoea of unknown origin. Consequently animal

studies in which many of these variables can be controlled may be useful in indicating their relative importance in affecting intestinal absorption in man. It is of interest, therefore, that the absorptive capacity of the small intestine of the rat and mouse is enhanced, rather than decreased, for some nutrients such as D(+)-glucose after the imposition of a severe decrease in intake of a balanced food (Kershaw *et al.* 1960; Hindmarsh *et al.* 1967; Madge 1970) and the present work shows that this response is retained even when the animals have lost 40% of body weight. Although there will always be difficulties in extrapolating data from one species to another, it seems valuable to pursue the influence of variable dietary deprivation at different ages and for different periods of time on digestive function in the rat because some principles may emerge which are applicable across species.

It is particularly interesting that the greatest measured increase in glucose absorption was apparent in rats which had lost only 5% of body weight (Fig. 3a). If this observation is coupled with that of Levinson and Englert (1972) that D-galactose absorption was enhanced in rats which were totally without food for only 24 h (i.e. the treatment applied to the control animals in the present study), it seems that the enhanced absorptive capacity is stimulated in a very short time with food deprivation.

Rats which had lost 30% or more of body weight were very sensitive to stress. On one occasion feeding was delayed for 1 h after a brief appearance in the room where the rats were kept of the person who fed the rats, and two animals with this high loss of body weight were then found in a coma apparently caused by excitement and increased physical activity. They revived with warmth and lavage with glucose solution. In such animals the enhanced absorption of glucose from the small intestine, apparent in the group with 5% body weight loss, was substantially retained (Fig. 3a).

The effect of semi-starvation on amino acid absorption rate from the small intestine has been studied previously. The active transport of histidine has been shown to increase (Kershaw *et al.* 1960; Hindmarsh *et al.* 1967; Madge 1970). Lis *et al.* (1972) showed for rats that after 10 days of food restriction to prevent growth but to maintain body weight there was increased L-methionine absorption from the small intestine, but the effect was no longer apparent after 51 days.

Two possible reasons are suggested to explain why enhanced absorption of L-leucine after dietary restriction was not shown in this study. First, the measurement of L-leucine was by a non-specific method. Secondly, the control animals had no access to food for 24 h before absorption was measured and, during this time, a mechanism for enhanced absorption of neutral amino acids, similar to that known to occur for galactose (Levinson and Englert 1972), may have been invoked which was not exceeded in the semi-starved animals.

In summary, therefore, it can be stated that after 40% body weight loss in the young adult rat the enhanced absorptive capacity of the small intestine for glucose, apparent after only 5% body weight loss compared with *ad libitum* controls, was maintained, and that throughout the entire range of body weight loss absorption of L-leucine was not impaired although it was not increased.

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