

## Energy metabolism and growth in rats adapted to intermittent starvation\*

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The nutritive value of a diet depends not only upon its amount and composition but also upon the frequency with which it is taken, for the way in which food intake is distributed over a given period of time influences metabolic processes. This fact was neglected for a long time, until the work of Tepperman and his colleagues (Tepperman, Brobeck & Long, 1943; Tepperman & Tepperman, 1958) and recently that of Cohn and his group (Cohn & Joseph, 1960) focused more attention on this problem.

We have been concerned for several years with the problem of adaptation to intermittent starvation. Rats that were fed only two or three times a week were shown to adapt in various ways to this dietary pattern after 6 or more weeks (Fábry, Kujalová & Petrásek, 1959; Fábry, Petrásek, Kujalová & Holečková, 1962*a, b*). The stomach and small intestine hypertrophied (Holečková & Fábry, 1959; Fábry & Kujalová, 1958, 1960), intestinal absorption was enhanced (Kujalová & Fábry, 1960) and there were changes of carbohydrate metabolism and its regulation (Fábry, 1955; Mosinger, Kujalová & Lojda, 1961). The main stimulus precipitating the adaptive changes was shown to be periodically increased nutrient supply to tissues, resulting from hyperphagia on days of free access to food. It must be emphasized, however, that the total calorie intake was only about 50–70% that of control animals fed *ad lib*.

One of the most surprising findings was that intermittently starving rats had an increased basal oxygen consumption, not only compared with continuously underfed animals, but also with controls given an unrestricted diet (Fábry, Petrásek & Krulich, 1961). The thyroid activity was reduced in intermittently starving rats, as measured by radioactive iodine uptake (Fábry *et al.* 1961). On the other hand, *in vitro* experiments indicated a 60–100% increase in endogenous respiration and a similar increase in the activity of a number of enzymes in various tissues (Fábry, Petrásek, Krulich, Hoeschl, Šonka & Waelsch, 1960; Petrásek, 1960, 1961; Petrásek, Vavrečka, Vavřínková, Komárková & Fábry, 1962), which supports the hypothesis that the increased basal metabolism reflects increased tissue oxidation resulting from enzyme adaptation on the tissue level.

An increase of oxidative processes in tissues and a raised basal metabolism are at variance with generally accepted views as to the consequences of calorie restriction.

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The aim of the study now described was to find out how this raised metabolism comes about. The food intake and growth of intermittently starving rats were compared with those of pair-fed and pair-gaining continuously underfed rats. By pair-gaining rats we mean rats with the quantity of food so adjusted within pairs that the weight gains of the two rats were very nearly the same. In addition, the total oxygen consumption of intermittently starving rats and of well-nourished controls was measured during the 24 h period.

#### EXPERIMENTAL

##### *Experimental animals and feeding pattern*

Albino rats from a random-bred colony of Wistar origin, kept under standard conditions (room temperature  $22 \pm 1^\circ$ ), were used. The animals were fed on a standard laboratory diet (Larsen mixture; for composition see Fábry, 1959) and had free access to water. In the pair-feeding and pair-gaining experiment male rats kept in separate cages were used. In the experiment in which the oxygen consumption was assessed female rats were used.

For the pair-feeding experiment the growth of ninety rats aged 6 weeks was followed for 4 weeks. Fifty-two of these animals were then selected, in sets of three whenever possible, on the basis of similarity of initial body-weight and growth rate over the 4 weeks. These animals formed the three experimental groups.

Group 1, the continuously underfed rats, were given daily a measured amount (c. 12–14 g/animal) of food, corresponding to about half that of the controls. Group 2, the intermittently starving rats, were fed for the first 2 weeks on alternate days and for the rest of the experiment three times a week. After 1 or 2 days of starvation they were given an amount of food corresponding to two or three daily rations of continuously underfed animals respectively, i.e. the amount of food consumed by those animals during the same period. Group 3, the control rats, were fed to appetite.

All animals were weighed twice a week in the same state of satiety (23–24 h after feeding). After 11 weeks of experimental feeding the animals were killed and the length of the femur was measured.

The pair-gaining experiment was arranged in the same way as the previous experiment with the difference that for a period of 8 weeks the daily ration of the continuously underfed animals was adjusted in such a way that their body-weight corresponded to that of the parallel group of intermittently starving rats. In all, 126 rats were used in this study.

##### *Total oxygen consumption*

The dietary treatment of the animals in the intermittently starved and well-nourished groups was the same during the last 24 h before the oxygen consumption was determined. After eating a measured ration of laboratory diet (10 g/100 g body-weight), all animals fasted for 18 h; the oxygen consumption was then measured for 24 h.

For the estimation a closed-circuit respirometer, modified as described by Luštinec (1956), was used. The values obtained were related to the body surface of the animals by the formula:

$$\text{Surface (cm}^2\text{)} = 9.1 \times \text{weight}^{2/3} \text{ (g)}.$$

## RESULTS

*Pair-feeding experiment.* The results are summarized in Table 1. The controls fed *ad lib.* grew more rapidly than the animals on the restricted dietary intake. The mean weight of the intermittently starving rats was throughout the entire period almost the same as that of the continuously underfed animals receiving the same amount of food divided into regular daily rations (Fig. 1). Similarly the weight change, calculated from the difference between the weights at the outset and at the end of the experiment, as well as the length of the femur, taken as an index of skeletal growth, were equal in both underfed groups.

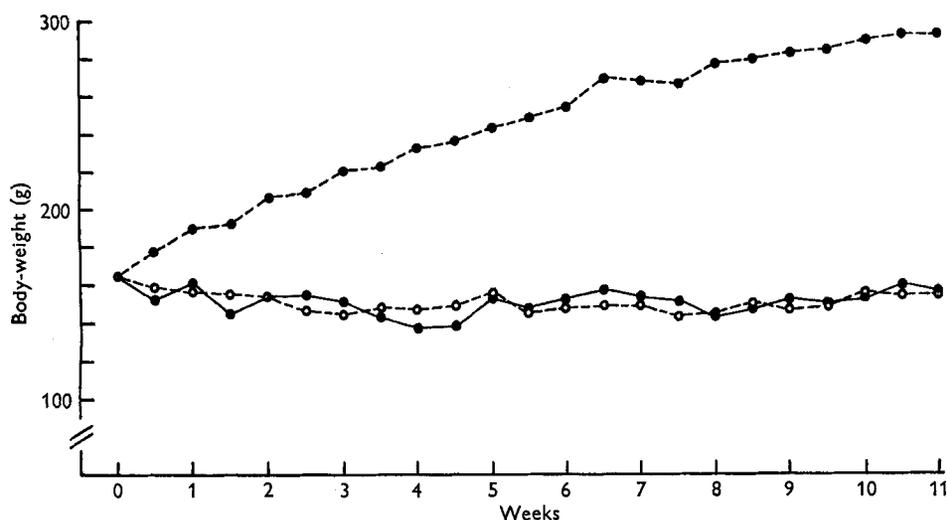


Fig. 1. Mean body-weight of intermittently starving rats (○---○), pair-fed, continuously underfed rats (●—●) and control rats fed *ad lib.* (●---●). Mean values for groups of seventeen or eighteen rats.

Table 1. Mean values with their standard errors for body-weight, length of femur and food intake of rats subjected to intermittent starvation or continuous underfeeding and of controls fed *ad lib.*

Treatment	No. of rats	Body-weight (g)		Weight change (g)	Length of femur (mm)	Mean daily food intake (g/rat)
		Initial	Final			
11-week period						
1. Intermittent starvation	18	164 ± 3.3	157 ± 3.5	-7 ± 1.61	272 ± 1.5	11.6
2. Continuous underfeeding (pair-fed with 1)	17	164 ± 3.6	156 ± 3.1	-8 ± 2.08	275 ± 3.1	11.6
3. Feeding <i>ad lib.</i> (controls)	17	164 ± 4.2	291 ± 9.3	127 ± 9.41	317 ± 3.5	27.6
8-week period						
4. Intermittent starvation	36	145 ± 3.0	150 ± 5.9	5 ± 0.9	272 ± 1.5	12.1
5. Continuous underfeeding (pair-gaining with 4)	45	143 ± 2.7	149 ± 4.8	6 ± 0.2	277 ± 1.5	11.9
6. Feeding <i>ad lib.</i> (controls)	45	142 ± 2.9	260 ± 14.8	118 ± 3.4	308 ± 5.3	23.0

*Pair-gaining experiment.* The results are summarized in Table 1. Again, the controls grew more rapidly than the animals on the restricted intake. During the first 10 days differences between the mean weights of the two underfed groups occurred, before

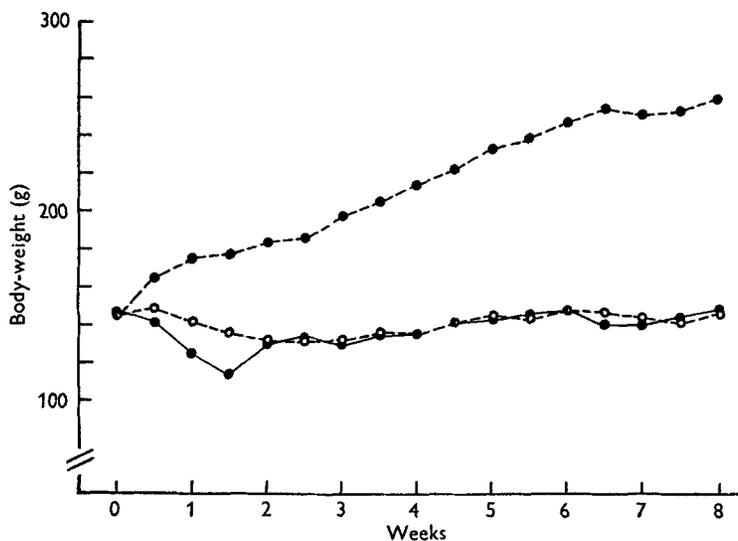


Fig. 2. Mean body-weight of intermittently starving rats (○---○), pair-gaining, continuously underfed rats (●—●) and control rats fed *ad lib.* (●---●). Mean values for groups of thirty-six to forty-five rats.

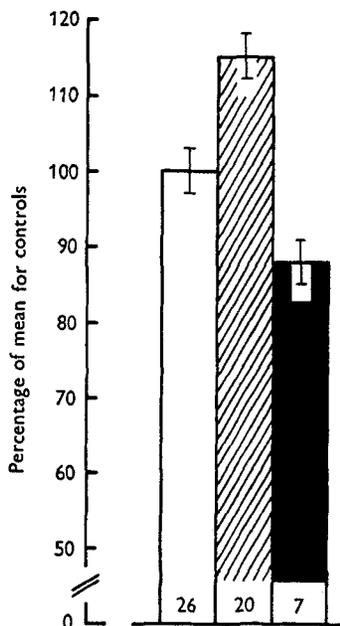


Fig. 3. Basal oxygen consumption (per surface area) of control rats fed *ad lib.* (□), intermittently starving rats (▨) and rats subjected to continuous underfeeding (■). Mean values with their standard errors for twenty-six, twenty, and seven rats, respectively. Summarized data from Fábry *et al.* (1961).

it was possible to find the suitable ration that maintained the continuously underfed animals at the same weight as the intermittently starving group (Fig. 2). However, the mean final body-weight and the length of femur did not differ significantly between the two underfed groups. It should be noted that the daily food intake/rat, calculated from the total amount of food ingested during the experiment, was also equal in the intermittently starved and continuously underfed groups, and amounted to 52% of that of the controls (Table 1).

*Oxygen consumption.* The results of the pair-feeding and pair-gaining experiments are remarkable if it is true that the basal metabolism of intermittently starving rats is raised as compared with the control group, whereas the basal metabolism of the continuously underfed rats is reduced, as illustrated by the results of our previous experiments (Fig. 3).

Table 2. Mean values with their standard errors for oxygen consumption during 24 h of intermittently starving rats and of controls fed *ad lib.*, expressed as ml O<sub>2</sub> per m<sup>2</sup> surface area per h

Treatment	No. of rats	Period of relative rest (7 a.m.-7 p.m.)	Period of relative activity (7 p.m.-7 a.m.)	Mean during 24 h
Intermittent starvation for 8 weeks	6	7661 ± 165*	9538 ± 262**	8646 ± 198**
Feeding <i>ad lib.</i> (controls)	6	6891 ± 195	12524 ± 347	9787 ± 332

Difference from value for control group statistically significant at: \*,  $P < 0.05$ ; \*\*,  $P < 0.02$ .

The answer to this apparent discrepancy between energy expenditure on the one hand and food intake or growth on the other was provided by the values for total oxygen consumption of intermittently starving rats over the course of 24 h. From Table 2 it is apparent that during the period of relative rest (in daytime), i.e. when the previous oxygen consumption measurements were made, the oxygen consumption of intermittently starving rats was higher than of controls, in keeping with the results of our previous experiments in which we investigated the resting metabolism of intermittently starving rats after feeding and after different periods of fasting (Fábry *et al.* 1961). On the other hand, during the night, when the physical activity of the rat is greatest, the oxygen consumption was substantially lower than that of controls. This reduction was sufficient to compensate for the period of rest, as apparent from the significantly lower mean values of oxygen consumption during the entire 24 h period.

#### DISCUSSION

From these results it is apparent that, whereas under conditions of physical rest the oxygen consumption is higher in intermittently starving rats than in controls fed to appetite, the reverse is true during the period of maximum physical activity. Intermittently starving rats thus compensate for the high rate of tissue oxidation by a reduction of physical activity or by lower energy cost per unit of physical work or by a combination of the two mechanisms. The former seems to predominate.

Preliminary experiments with Dr Fraňková in our laboratory, in which the physical activity of rats subjected to various feeding patterns was measured, revealed that intermittent starvation leads to a greater reduction of physical activity than a comparable continuous calorie restriction. Moreover, the results of the Minnesota experiment (Keys, Brožek, Henschel, Mickelsen & Taylor, 1950) indicate that in man the decrease of physical activity is the most effective energy-sparing mechanism in an organism adapted to calorie restriction (Taylor & Keys, 1950).

The efficiency of energy adaptation of intermittently starving animals is apparent also from the results of carcass analysis, published in detail elsewhere (Fábry, Petrásek, Braun, Bednárek, Horáková & Konopásek, 1962). The carcasses of intermittently starved animals contain a significantly higher percentage of fat, not only as compared with those of continuously underfed animals, but also with those of controls given unrestricted food. It is so because of enhanced lipogenesis (Petrásek & Fábry, 1958; Fábry, Petrásek, Braun *et al.* 1962). At the end of the pair-feeding experiment the mean sum of calories derived from protein and fat in the carcasses was  $232 \pm 8.4$  kcal for the intermittently starved rats and  $226 \pm 8.4$  kcal for continuously underfed animals.

The carcass analysis revealed, moreover, that previous results for energy expenditure (Fábry *et al.* 1961) could not have been influenced by the body composition of the animals, for an increased basal and resting oxygen consumption, reflecting tissue metabolism, was found in intermittently starving rats, though their lean body mass was relatively smaller because their bodies contained relatively more fat.

The metabolic adaptation to intermittent starvation obviously takes place along two main lines, both of which are probably moderated by the central nervous system. The experimental animal learns gradually to compensate for the periods of starvation by an increased food intake during the feeding periods. This periodic hyperphagia leads then, by adaptation of the gastro-intestinal tract, to a chain of metabolic changes, the result of which is a raised level of tissue metabolism. The energy balance corresponding to the chronically reduced calorie intake is then achieved by a reduced energy output during periods of maximum physical activity.

It would be interesting to know whether and to what extent a nutritionally induced adaptation of similar type may occur in man. The experience of Dr J. Gilman and Dr Margaret W. Stanier (personal communications) with population groups in economically underdeveloped parts of Africa, where calorie deficiency is combined with occasional hyperphagia, seems to support this possibility.

#### SUMMARY

1. Food intake, weight gain and femur length were measured in each of two experiments for three groups of male albino rats. One group was intermittently fasted (for periods of 1–3 days) and given free access to food (for 1 day), one group was given daily a restricted food ration, and the third was fed to appetite. In addition, total oxygen consumption of a group of female intermittently starved rats and of a group of well-nourished controls was measured for 24 h.

2. In a pair-feeding experiment lasting 11 weeks the loss of weight and the length

of femur were equal in the groups subjected to either intermittent or continuous underfeeding; the food intake was reduced to 46 % of that of the controls. In a pair-gaining experiment the intermittently starving rats consumed the same amount of food as the continuously underfed group.

3. Assessment of the total oxygen consumption during 24 h revealed that the rats intermittently starved for 8 weeks had, as compared with non-fasted controls, a higher oxygen consumption, per unit of surface area, during the period of relative rest, i.e. in daytime, whereas during the period of maximum physical activity (at night) the consumption was substantially reduced. Mean values for oxygen consumption for the whole 24 h period were significantly lower for intermittently starving animals than for controls.

4. From the results it appears that rats adapted to intermittent starvation compensate by a reduced energy output during the period of maximum physical activity for the increase in tissue oxidation activity, which manifests itself also by a higher resting and basal metabolism.

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