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Influence of Intermittent Fasting and High-Fat Diet on Morphological Changes of the Digestive System and on Changes of Lipid Metabolism in the Laboratory Mouse

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Summary

The simultaneous effect of intermittent starvation and a high-fat diet were investigated in mice after several weeks of experimental feeding. The animals adapted to intermittent fasting fed a high-fat diet showed a lower degree of hyperphagia than animals adapted to intermittent fasting fed a standard laboratory diet. The weight of both individual portions of the stomach was elevated in adapted animals fed both a standard laboratory diet and the high-fat diet. The weight of the small intestine was increased in adapted animals fed a high-fat diet. The length of the small intestine was not changed after 8 weeks of intermittent starvation in both adapted groups (standard laboratory diet, high-fat diet). A higher amount of body fat was found in both groups of animals adapted to intermittent fasting (standard laboratory diet, high-fat diet) but adapted animals fed a high-fat diet showed less body fat than adapted animals fed a standard laboratory diet. Lower levels of serum lipids were found in adapted animals fed a high-fat diet. These results suggest that both lipogenesis and lipid oxidation are accentuated by intermittent starvation and a high-fat diet act concomitantly.

Key words

Intermittent fasting – High-fat diet – Total body fat – Lipogenesis – Digestive system

Introduction

Altered feeding frequency provokes a number of adaptive changes in the digestive systems which are manifested by the activity of some enzymatic systems, in the rate of intestinal absorption and also by the anatomical configuration of individual segments of the digestive system (Fábry and Kujalová 1958, 1960, Holečková and Fábry 1959, Lojda and Fábry 1959).

Marked hypertrophy of the gastric mucosa and gastric muscle especially in the forestomach and the prolongation of the intestine in adapted animals corresponding to an even greater increment of the functional tissue of the organ were demonstrated by histological analysis (Holečková and Fábry 1959, Lojda and Fábry 1959). However, changes of digestive system function were much more rapid than the changes in the morphological appearance of this organ (Fábry 1969a,b).

The changes in carbohydrate and lipid metabolism are caused by adaptation to intermittent fasting. Intestinal glucose and fat absorption are enhanced and the ability of the tissue to oxidize glucose as well as fatty acids (Petrásek *et al.* 1964) and to convert glucose into fat stores are markedly elevated (Petrásek and Fábry 1958, Petrásek *et al.* 1964, Petrásek 1965). Consequently, the final amount of total body fat is dependent on the prevalence of lipogenesis or fat oxidation. Another factor which can modify the character of adaptation to intermittent fasting is the composition of the diet. In particular, the changes in systems involved in fat conversion, i.e. the activation of

lipid oxidation and inhibition of lipogenesis, are assumed to be induced by a high-fat diet (Kimura and Ashida 1969, Romsos and Leveille 1974, Zaragoza-Hermans and Felber 1972).

The purpose of this study was to examine the effect of intermittent starvation and a high-fat diet on lipid metabolism and morphological changes of the digestive system in mice.

Methods

Animals and diets

Male CBA x C17/Bl-10 mice aged 6–8 weeks were used (supplied by the Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno). The initial weight of the animals was approximately 20 g. The animals were housed in plastic cages in a climatized room at 23±1 °C. A 12-h-period of light (0700–1900) was followed by a 12-h-period of darkness (1900–0700). The experimental treatment lasted 8 weeks (April–May).

Mice of the control group were given a standard laboratory diet *ad libitum* (for composition of the diet see Fábry 1959) throughout the whole experiment.

The experimental group I had access to a standard laboratory diet every other day, i.e. free access to food and the day of total fasting were alternated regularly.

The animals of experimental group II were fed a standard laboratory diet *ad libitum* during the first 4 weeks of the experiment and a high-fat diet *ad libitum* (margarine, composition see Fábry 1959) during the subsequent 4 weeks of the experiment. The fat content in the high-fat diet was 40 %.

The experimental group III had access to a standard laboratory diet every other day during the first 4 weeks of the experiment and intermittently to a high-fat diet during the subsequent 4 weeks.

Each of the groups contained 10 animals at the end of the experimental treatment. Water was available *ad libitum* throughout the whole experiment. The animals were weighed periodically. The changes of fresh weight of the gastric and intestinal content were assessed to demonstrate the periodic hyperphagia in groups adapted to intermittent fasting.

Estimation of morphological changes in the digestive system

The animals were sacrificed in the state of satiation at the end of the experimental period. The content of the stomach and intestine was removed and the weight of both individual sections of stomach (the forestomach and the glandular part of stomach) and the weight of the small intestine were assessed. The length of the small intestine was measured using a standard load of 3 g.

Carcass lipid analysis

The carcass analysis was achieved by a modification of the method of Mickelson and Anderson (1959), by hydrolysis of the whole bodies of animals in an alcoholic solution of potassium hydroxide. The aliquote of hydrolyzate was extracted by chloroform and the total amount of extracted carcass lipids was assessed by weighing.

Lipaemia estimation

Serum lipid levels were assessed using standard sets manufactured by Lachema (Brno, Czech Republic).

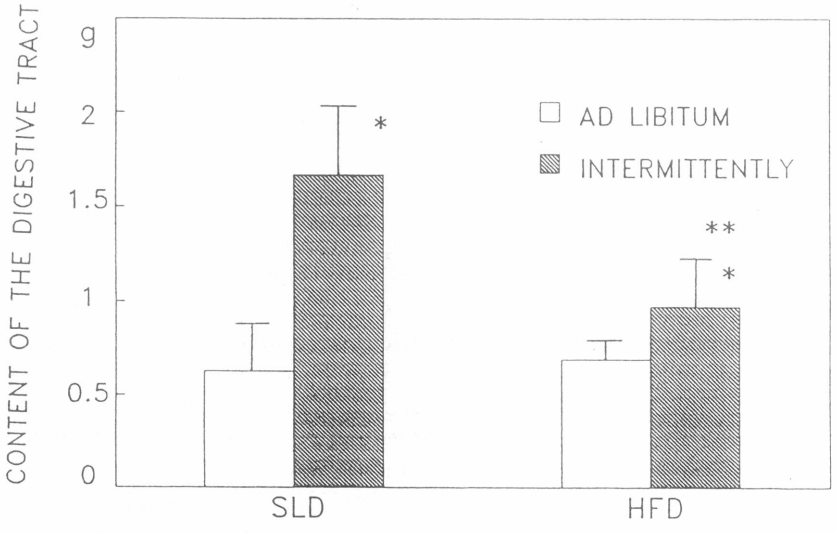

Fig. 1
*The fresh weight of the content of stomach and intestine in grammes (means ± S.D.). SLD – standard laboratory diet, HFD – high-fat diet. *Significant difference as compared with the control group (SLD ad libitum). **Significant difference as compared with experimental group I (SLD intermittently), $P<0.05$.*

Statistic analysis

Data are expressed as means±S.D. The differences among experimental groups were evaluated by the one-way analysis of variance. The effect of intermittent starvation and a high-fat diet was evaluated by the multifactorial analysis of variance for two factors. The authenticity of results obtained by the analysis of variance was verified by Kruskal-Wallis analysis in cases when the condition of homogeneity of variance was not attained. $P<0.05$ value was considered to be significant.

Results and Discussion

Morphological changes of the digestive system

Periodic hyperphagia is the primary and most important change in adaptation to intermittent fasting (Holečková and Fábry 1959). In our experiment, periodic hyperphagia in animals adapted to intermittent fasting fed both the standard laboratory diet and the high-fat diet was clearly demonstrated by a significant increase of the gastric and intestinal content (Fig. 1).

Richard *et al.* (1988) reported periodic hyperphagia in mice fed *ad libitum* a high-fat diet with a certain degree of palatability. In our experiment, the high-fat diet without a palatability component was used and periodic hyperphagia did not occur in animals fed this diet *ad libitum* (Fig. 1). Consequently, if the diet lacks a sensory stimulus of a palatable attractive component the animals consume only such an amount of food by which hypoglycaemia is avoided (Larue-Achagiotis and Le Magnen 1982).

The occurrence of hyperphagia is connected with the changes in stomach size. Deutsch and Gonzalez (1980) noted that the inhibition of oral intake is not controlled by the volume of ingested food in the stomach but by the caloric content of food. We can thus explain why the degree of hyperphagia of mice fed the high-fat diet is significantly lower than that of mice fed a standard laboratory diet intermittently (Fig. 1)

Most studies on the influence of infrequent feeding on the morphology of various organs, especially the digestive tract, were carried out on rats (e.g Petrásek 1965, Fábry 1969a,b, Petrásek *et al.* 1969, and others). Similar experiments were also done on the golden hamster (*Mesocricetus auratus*) (Šimek 1968, 1969) and mice (Šimek *et al.* 1973).

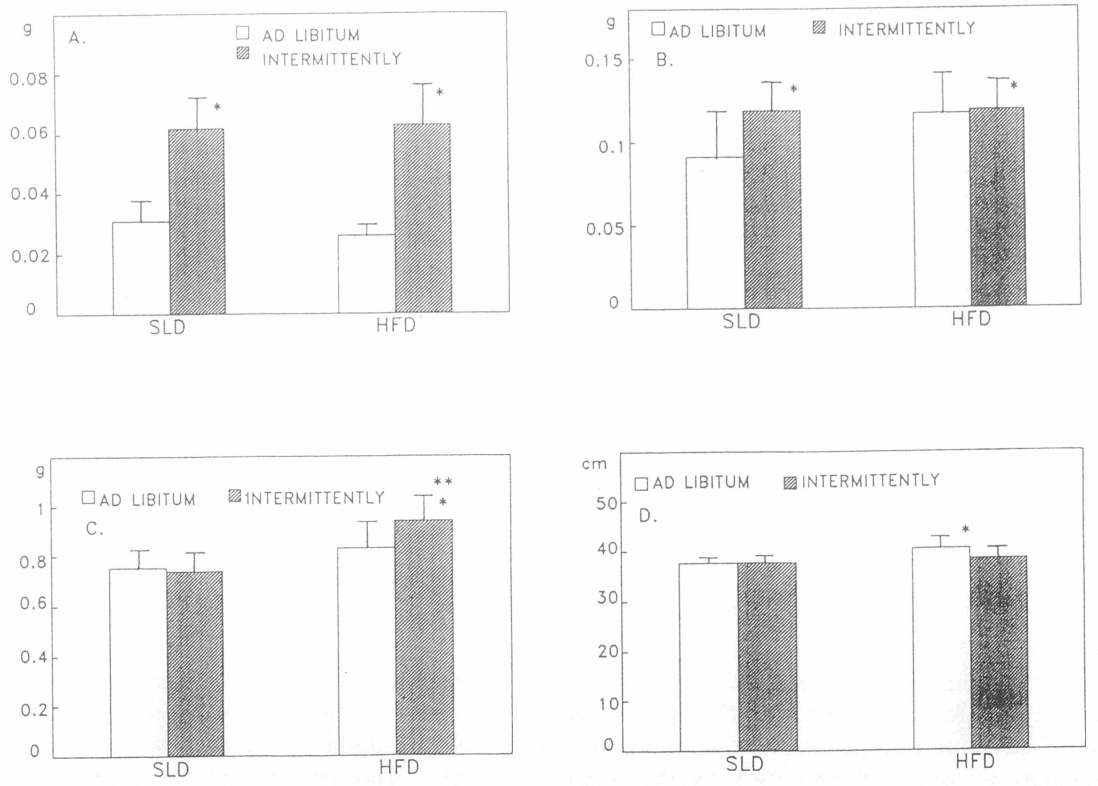

Fig. 2

The fresh weight of forestomach (A), the glandular part of the stomach (B), and small intestine (C) in grammes, the length of small intestine in centimeters (D). For other legend see Fig. 1.

The differences in the adaptive reaction of some species are not surprising. Nevertheless, our results demonstrate that the morphological changes of the digestive system produced by infrequent feeding may be different in individual strains of mice.

In the strain of mice used in our experiment, significant hypertrophy of both the forestomach and the glandular part of the stomach was found after 8 weeks of adaptation in both groups (I,III) adapted to intermittent fasting (Fig. 2). The absolute weight of the forestomach is by 50 % greater and the weight of the glandular part of the stomach is by 30 % greater than that of the controls. In male mice of strain H a marked hypertrophy of the forestomach only was found after 8 weeks of adaptation (Šimek *et al.* 1973).

A significant increase of the absolute weight and the length of the small intestine after 8 weeks of adaptation was reported by Šimek *et al.* (1973) in mice, although the body weight of these animals declined. In our experiment, the body weight of mice was decreased significantly in both adapted groups (I, III), (Fig. 3). The length of the small intestine did not change after 8 weeks of intermittent fasting in both adapted groups (I,III) (Fig. 2).

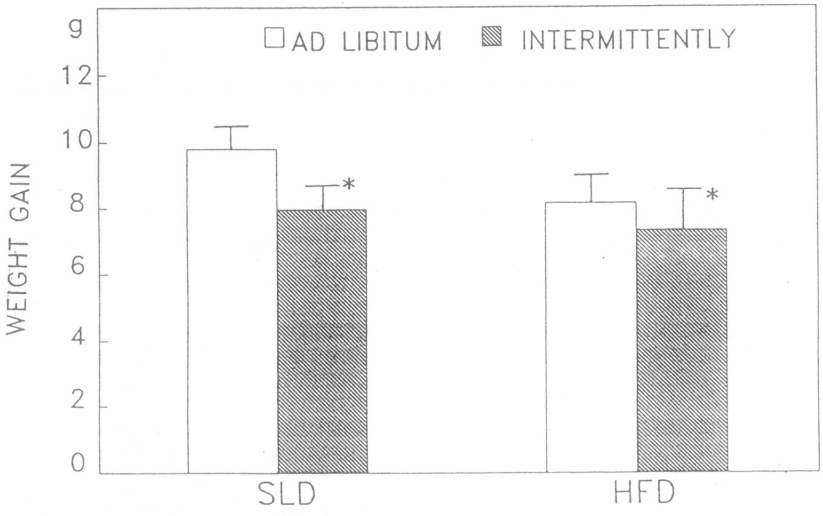

Fig. 3
The weight gain of animals in grammes. For other legend see Fig. 1.

A significant increase of the absolute weight and the length of the small intestine after 8 weeks of adaptation was reported by Šimek *et al.* (1973) in mice, although the body weight of these animals declined. In our experiment, the body weight of mice was decreased significantly in both adapted groups (I, III), (Fig. 3). The length of the small intestine did not change after 8 weeks of intermittent fasting in both adapted groups (I,III) (Fig. 2).

A predominance of some of the main nutrients in the diet increases both the release of digestive enzymes which are involved in the utilization of the nutrient and activates the systems responsible for the active transport of the nutrient in the intestinal wall. In the same way, intermittent fasting produces many changes in the activity of enzymatic systems and in intestinal absorption (Fábry 1969a,b). Our results indicate that a high-fat diet supports the hypertrophy of the digestive system. The weight of the small intestine was elevated in mice fed a high-fat diet intermittently. The weight of the glandular part of the stomach (insignificantly) and the length of the small intestine were elevated in mice fed a high-fat diet *ad libitum* (Fig. 2).

A simultaneous effect of intermittent starvation and a high-fat diet leads to the hypertrophy of the small intestine, whereas a high-fat diet fed *ad libitum* causes the prolongation of this organ (Slabochová and Placer 1962).

It is apparent from our results that the degree of periodic hyperphagia is relatively small in animals fed the high-fat diet intermittently. On the other hand, the hypertrophy of both parts of the stomach seems to be identical in both groups adapted to intermittent fasting (I, III). In case of the intermittent feeding with a high-fat diet the wall hypertrophy of the stomach developed whereas the size of the area of the stomach did not seem to be affected, similarly as was found in the case of the small intestine.

Changes of lipid metabolism

The excess of fat in high-fat diets does not always enhance the formation of fat reserves and the total body weight as has been reported in a number of previous studies (Mickelsen *et al.* 1955, Schemmel *et al.* 1972, Salmon and Flatt 1985, Boozer and Atkinson 1990). The amount and the type of fatty acids in a specific dietary fat affect fat deposition in depots and/or lipid oxidation (see review Pan *et al.* 1994). Furthermore, it is known that extensive fluctuations of both body fat and body weight occur in various strains of rats and mice especially in response to a "cafeteria

diet" or diets rich in fats (Rothwell *et al.* 1982, Ismail *et al.* 1986, Fisler *et al.* 1987). The body weight of animals in both groups adapted to intermittent fasting (I, III) was significantly lower compared with that of the controls (Fig. 3). A decline of total body fat was found in both groups adapted to intermittent fasting (I, III). Animals fed intermittently a high-fat diet exhibited a significantly lower content of body fat than animals intermittently fed a standard laboratory diet (Fig. 4). It is probable that the weight gain of adapted animals fed a high-fat diet was due to the gain in active lean body mass, because the body weight gain of both adapted groups which received food intermittently (I, III) almost did not differ. The situation was the same in the group fed the high-fat diet *ad libitum* (Figs 3 and 4). An additive effect of the intermittent fasting and a high-fat diet can be involved. From the statistic analysis, the concomitant influence of both these factors caused an increase in fat utilization and oxidation which were more intensive in animals fed a high-fat diet. The significant decline of total serum lipid levels was demonstrated in animals adapted to the intermittent feeding with the high-fat diet (Fig. 5). The intensity of lipogenesis became greater as a result of the simultaneous effect of both a high-fat diet and intermittent fasting.

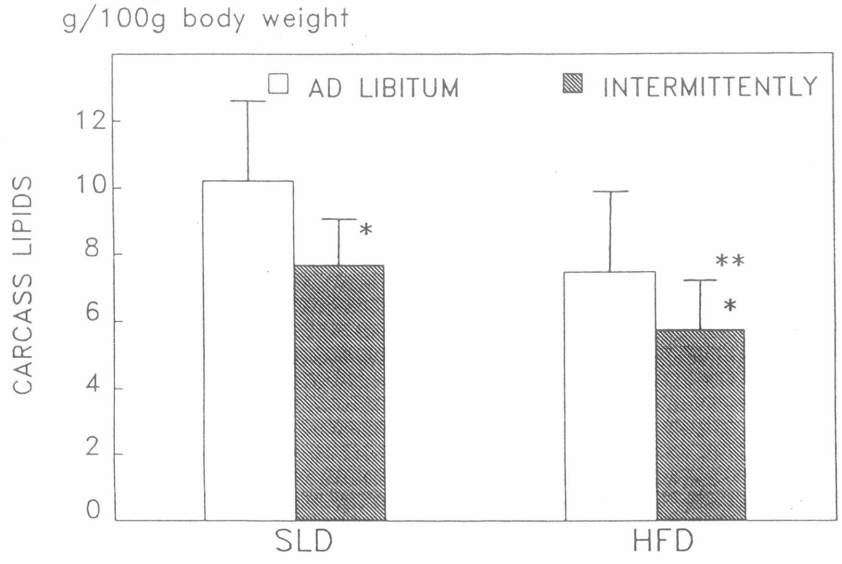

Fig. 4
The total carcass lipids in g/100 g b.w. For other legend see Fig. 1.

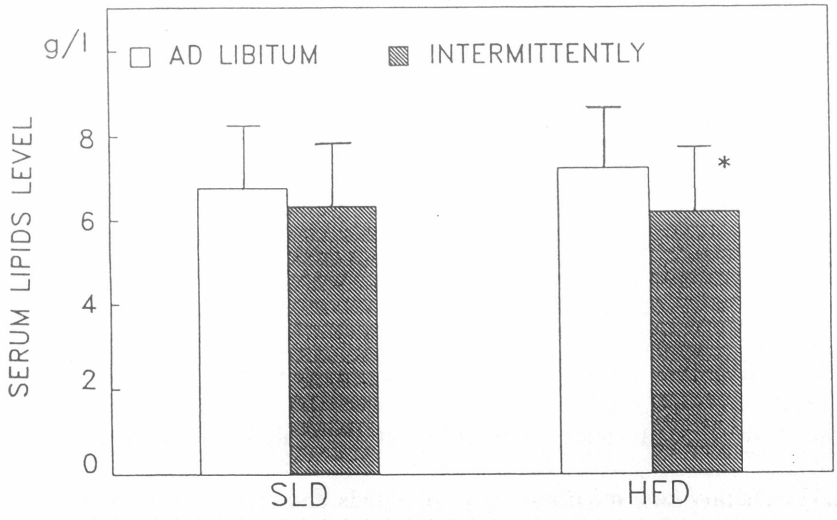

Fig. 5
The total serum lipids level in g/l blood. For other legend see Fig. 1.

It is known that intermittently fasted animals showed accentuated conversion of dietary carbohydrates to fat, without enhancing the body fat reserves. The limiting factors are the total amount of ingested calories and the activity of the systems which control the contrary process, i.e. fat mobilization and

oxidation (Fábry 1969a,b). In our experiment, the body fat content of animals adapted to intermittent fasting was significantly decreased in both those fed a standard laboratory diet and those fed the high-fat diet. These findings provide evidence of enhanced lipid oxidation and mobilization of fat reserves. On the other hand, the assessment of serum lipid levels serves as evidence of accentuated lipogenesis and the formation and deposition of fat reserves in animals adapted to intermittent feeding with a high-fat diet because the level of serum lipids in the blood dropped significantly. This decline was not significant in animals adapted to intermittent feeding with a standard laboratory diet. As far as the loss of body fat deposits is concerned, the dominance of lipolytic processes can be presumed.

Consequently, if the feeding with a high-fat diet and intermittent fasting act simultaneously, both lipogenesis and lipid oxidation are accentuated, which suggests an elevation of total lipid metabolism to a high level corresponding to the specific nutritional regimen. Regarding the loss of body fat, we presume that lipid oxidation prevails over lipogenesis.

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