Physiol. Res. 45: 379–383, 1996

Effect of Intermittent Feeding With High-Fat Diet on Changes of Glycogen, Protein and Fat Content in Liver and Skeletal Muscle in the Laboratory Mouse

E. KŘÍŽOVÁ, V. ŠIMEK

Department of Comparative Animal Physiology and General Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic

Received October 26, 1995
Accepted April 29, 1996

Summary

After 8 weeks of intermittent fasting, mice fed both a standard laboratory diet and a high-fat diet became hyperphagic and showed an increased amount of glycogen storage in the liver. An important effect of the adaptation to intermittent feeding with a high-fat diet seems to be an activation of the oxidation of lipids. Lipid oxidation prevails over lipogenesis so that the protein levels in the liver and skeletal muscle are preserved and maintained constant.

Key words
Intermittent feeding – High-fat diet – Metabolism – Liver – Skeletal muscle

Introduction

Organisms are able to adapt their macronutrient utilization capacity to be optimally convenient to new nutrient conditions if the feeding pattern is changed. The macronutrient composition of the diet can influence hunger, satiety, food intake, body weight and body composition (Rolls 1995), postprandial utilization (McGregor and Lee 1995) and it is an important determinant of lipolysis (Callesescandon and Driscoll 1995).

Excessive intake of a high-fat diet leads to changes in lipid metabolism. Cumulative errors in the fat balance lead eventually to changes in adipose tissue mass which can substantially alter plasma fatty acid concentration, insulin sensitivity and lipid oxidation. Thus, the adjustment of fat oxidation, which is closely related to carbohydrate economy, to fat intake occurs. Fat intake and habitual glycogen concentrations are important in determining of lipid oxidation activity (Flatt 1995).

The food restriction by intermittent fasting provokes hyperphagia and hypertrophy of the alimentary tract (Fábry and Kujalová 1958, Holečková and Fábry 1959). The intake of an unusually large amount of food on feeding days leads to marked changes in the metabolic functions of the organism and in the biochemical composition of tissues, particularly in carbohydrate metabolism – an increase in liver (Fábry 1955) and muscle glycogen (Vrbová and Gutmann 1958), and also in protein (Fábry and Hrůza 1956, Hrůza and Fábry 1955) and lipid metabolism (Petrásek *et al.* 1964, Petrásek 1966).

The purpose of this study was to examine the changes in the amount of glycogen, proteins and lipids in the mouse liver and skeletal muscle after a high-fat diet and intermittent fasting.

Methods

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-hour-period of light (0700–1900 h) was followed by a 12-hour period of darkness (1900–0700 h). The duration of experimental treatment was 8 weeks (September–October).

The mice of the control group were allowed a standard laboratory diet (for composition of the diet see Fábry 1959) *ad libitum* 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: palmitic acid (8–11 %), oleic acid and stearic acid (75–82 %). linoleic acid (6.5–10 %), linolenic acid (0.5–2.5 %), (for composition of the diet see Fábry 1959) during the subsequent 4 weeks of the experiment. The fat content in the high-fat diet was 70 Cal %.

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.

Feeding started 3 h before light was turned off. Water was available *ad libitum* throughout the whole experiment. The animals were weighed periodically. Each of the groups contained 10 animals at the end of the experimental treatment.

The animals were sacrificed by decapitation at 0800 h, five in the state of satiation and five after 16 h of total starvation. This 16-hour period of total fasting was followed by a 24-hour phase during which the animals were adapted to suffer from hunger by an intermittent feeding schedule.

The fresh weight of the stomach content and the area of both individual sections of the stomach (the forestomach and the glandular part of the stomach) were assessed in animals sacrificed in the state of satiation. A planimeter REISS was used for measuring the stomach area.

The right lobe of the liver and a piece of vastus lateralis muscle were removed and partly used for the determination of glycogen and lipid content, partly kept at $-20\ ^{o}C$ until the assessment of protein content. Glycogen and fat were separated from tissues by a modification of the method of Van Handel (1965) and evaluated according to the methods of Carrol *et al.* (1956) and Folch *et al.* (1957), respectively. Tissue proteins were measured according to the method of Lowry *et al.* (1951). The differences among experimental groups were evaluated by one-way analysis of variance and Kruscal-Wallis analysis. The evaluation was carried out for the level of significance $a = 0.05$.

Table 1
Effects of high-fat diet and intermittent starvation on mouse liver and skeletal muscle composition and morphological changes of the stomach

	Standard laboratory diet		High-fat diet	
	Ad libitum	Intermittently	Ad libitum	Intermittently
Body weight gain (g)	9.000 ± 2.080	7.400 ± 0.485	9.600 ± 1.478	7.700 ± 1.136
Content of the stomach (g)	0.124 ± 0.025	1.134 ± 0.227*	0.223 ± 0.031	1.009 ± 0.253*
Area of the forestomach (cm^2)	1.228 ± 0.232	4.624 ± 0.491*	1.568 ± 0.097	3.452 ± 0.103*
Area of the glandular part of the stomach (cm^2)	2.032 ± 0.314	2.894 ± 0.098	3.360 ± 0.236*	3.822 ± 0.226*
Liver (g)	1.581 ± 0.084	2.086 ± 0.099*	1.551 ± 0.065	2.021 ± 0.043*
Liver/B. W. (%)	4.721 ± 0.122	6.389 ± 0.318*	4.353 ± 0.306	6.195 ± 0.203*
Liver composition:				
Proteins (%)	12.353 ± 0.419	9.738 ± 0.479	12.327 ± 0.733	10.499 ± 0.335
Lipids (%)	5.797 ± 0.277	5.923 ± 0.488	6.936 ± 0.308	8.965 ± 0.593
Glycogen (%)	4.284 ± 0.294	9.129 ± 0.672*	3.668 ± 0.284	10.262 ± 0.361*
Vastus lateralis composition:				
Proteins (mg/g)	112.150 ± 11.299	62.714 ± 11.498*	78.511 ± 7.087	121.032 ± 11.596
Lipids (mg/g)	42.951 ± 1.316	6.242 ± 1.779*	12.740 ± 4.391*	7.899 ± 1.966*
Glycogen (mg/g)	0.310 ± 0.015	0.509 ± 0.091*	0.270 ± 0.025	0.356 ± 0.038

*Data are means ± S.E.M. * significantly different from the controls. $n = 5$, $P < 0.05$.*

Results

The animals adapted to intermittent fasting fed both the standard laboratory diet and the high-fat diet become hyperphagic after 8 weeks of experimental feeding (Table 1). The area of the forestomach is increased significantly in both adapted groups (standard laboratory diet, high-fat diet). The enlargement of the glandular part of the stomach was found in animals fed the high-fat diet not only in adapted mice but also in mice fed *ad libitum* (Table 1).

The increased fresh weight of the liver was present in both groups adapted to intermittent fasting, the body weight gain is, at the same time, mildly lower than that in the controls. Nevertheless, this reduction is not significant (Table 1).

The liver glycogen content is elevated in both groups of animals adapted to intermittent fasting (standard laboratory diet, high-fat diet). The amount of proteins and lipids in the liver does not differ from that of the controls (Table 1).

Significant differences in protein and lipid content were found in the skeletal muscle. The animals adapted to intermittent fasting and fed standard laboratory diet had a lower protein content in the skeletal muscle than the controls. The lipid content was also decreased (Table 1). A lower lipid content in the skeletal muscle was revealed in animals fed a high-fat diet both in those adapted to intermittent fasting and those fed *ad libitum* (Table 1).

Glycogen content in the skeletal muscle is increased in adapted animals fed the standard laboratory diet, whereas this does not differ from controls in adapted animals fed a high-fat diet (Table 1). The fresh weight of the liver, liver glycogen content and proteins in the liver and skeletal muscle are decreased after 16 h of total starvation in the controls.

The fresh weight of liver is decreased in both experimental groups adapted to intermittent fasting (Table 2). Liver glycogen content declined significantly in all groups (Table 2). After 16 h of total fasting liver steatosis develops in all groups except the group adapted to intermittent feeding on a high-fat diet. The protein content in the skeletal muscle was decreased (Table 2).

Table 2
Total starvation effect on mouse liver and the skeletal muscle composition after intermittent feeding with a high-fat diet

	Standard laboratory diet		High-fat diet	
	Ad libitum	Intermittently	Ad libitum	Intermittently
Liver (g)	1.268 ± 0.053*	1.100 ± 0.040*	1.358 ± 0.055	0.995 ± 0.052*
Liver/B. W. (%)	3.961 ± 0.094*	3.785 ± 0.256*	3.985 ± 0.223	4.030 ± 0.264*
Liver composition:				
Proteins (%)	9.627 ± 0.534*	10.661 ± 0.553	12.143 ± 0.999	10.677 ± 0.216
Lipids (%)	15.806 ± 1.809*	15.646 ± 0.931*	16.763 ± 0.590*	9.877 ± 2.515
Glycogen (%)	0.078 ± 0.006*	0.139 ± 0.024*	0.104 ± 0.010*	0.134 ± 0.016*
Vastus lateralis composition:				
Proteins (mg/g)	62.324 ± 2.703*	70.354 ± 4.450	81.329 ± 2.678	74.886 ± 3.089*
Lipids (mg/g)	31.593 ± 7.257	20.486 ± 4.854	25.557 ± 2.381	18.107 ± 4.650
Glycogen (mg/g)	0.237 ± 0.009	0.381 ± 0.038	0.205 ± 0.018	0.281 ± 0.008

*Data are means ± S.E.M. * significantly different from corresponding groups in satiety. $n = 5$, $P < 0.05$.*

Discussion

The mice fed both the standard laboratory diet and the high-fat diet become hyperphagic after 8 weeks of adaptation to intermittent fasting as Lawrence and Mason (1955) and Fábry (1969) already described for the standard laboratory diet. Especially, the area of the forestomach is enlarged (Fábry 1969, Šimek *et al.* 1973). Nevertheless, the enlargement of the glandular part of the stomach was found in animals fed a high-fat diet not only in adapted mice but also in mice fed *ad libitum*. It is apparent from our results and from our previous work (Křížová, Šimek 1996) that the effect of a high-fat diet on morphological changes of the digestive system cannot be neglected. A high-fat diet obviously supports the hypertrophy of the glandular part of the stomach, which had not previously been described.

The ability to elevate the formation of glycogen storage in the liver (Fábry 1955, Tepperman and Tepperman 1958) and in skeletal muscles (Vrbová and Gutmann 1958) was revealed in animals adapted to intermittent fasting. Similar conclusions were obtained in our animals intermittently fed the standard laboratory diet. The amount of liver proteins does not differ from that of the controls which corresponds to the findings of Hrůza and Fábry (1955) and Holečková and Fábry (1959). Nevertheless, the protein and lipid content in the skeletal muscle of intermittently fasting animals fed the standard laboratory diet decreased significantly.

The fall of proteins in the skeletal muscle is apparently connected in part with a restriction of physical activity, in part with changes in carbohydrate metabolism.

An accentuated formation of glycogen reserves in both the liver and the skeletal muscle is consequently the result of an 8-week adaptation to intermittent feeding. In view of the fact that 70 % of soluble proteins in skeletal muscles are glycolytic enzymes (Karlson 1981), accentuated glycogen synthesis not only causes the increased glycogen content in skeletal muscle but even reduces its ability to release it.

This situation corresponds to the state of total starvation where the glycogen reserves are completed mainly by gluconeogenesis. CBAxC17/Bl 10 strain of mice seems to be very sensitive to changes in feeding pattern, which is proved by their response to total fasting. The protein content losses in both the liver and the skeletal muscle were caused in *ad libitum* fed controls by total fasting lasting 16 h.

The ability of adaptation to a high-fat diet seems to be more developed. The intensity of fat oxidation is not activated as much as it is if intermittent fasting is involved. The energy demands of the organism are covered by glycogen supplies without protein degradation after 16 h of total fasting.

The animals fed intermittently a high-fat diet had large supplies of liver glycogen. The glycogen content in the skeletal muscle does not differ from that of the controls. Lipids in the skeletal muscle are reduced in animals fed a high-fat diet both intermittently and *ad libitum*. The diet rich in fat elevates fatty acids and the glycerol supply in the liver (Karlson *et al.* 1987) and the periodic consumption of a large amount of food during short time period leads to an enhanced lipogenesis in the liver (Beaton *et al.* 1964, Cohn 1963, Hollifield and Parson 1962a,b, Tepperman and Tepperman 1958, 1964). Nevertheless, our results revealed neither fat deposition in the depots (Křížová and Šimek 1996) nor fat accumulation in the liver and the fat content in the skeletal muscle significantly depressed.

Thus, an important effect of adaptation to intermittent feeding with the high-fat diet seems to be the activation of lipid oxidation. Lipid oxidation prevails over lipogenesis, similarly as was observed in *ad libitum* feeding with a high-fat diet by Kimura and Ashida (1969) or Romsos and Leveille (1974), by which proteins in the liver and muscle are preserved and maintained at the same level as in the controls.

The increase in muscle proteolysis during fasting seems to be attributable to an enhancement of the energy-requiring process (Kettelhut *et al.* 1994). After total fasting lasting for 16 h, rapid protein loss occurs in the skeletal muscle because body fat reserves of the intermittently fasted animals are depleted and the liver glycogen supply is significantly reduced.

Acknowledgements

The authors wish to acknowledge Prof. Dr. R. Petrásek, CSc from the IKEM in Prague for his advice and stimulating suggestions. The publication was partially supported by the research grant No. Z 153-8 (Grant Agency of the Czech Republic).

References

BEATON J.R., FELEKI V., SZLAVKO A.J., STEVENSON J.A.F.: Meal-eating and lipogenesis in vitro of rats fed a low protein diet. *Can. J. Physiol.* **42:** 665–667, 1964.

CALLESESCANDON J., DRISCOLL P.: Diet and body composition as determinants of basal lipolysis in humans. *Am. J. Clin. Nutr.* **61:** 543–548, 1995.

CARROL N.V., LONGLEY R.W., ROE J.H.: The determination of glycogen in liver and muscle by use of anthron reagent. *J. Biol. Chem.* **2220:** 583–587, 1956.

COHN C.: Feeding frequency and body composition. *Ann. N.Y. Acad. Sci.* **110:** 395–400, 1963.

FÁBRY P.: Studies on the adaptation of metabolism. I. On the glycogen reserves of rats accustomed to interrupted starvation. *Physiol. Bohemoslov.* **4:** 33–41, 1955.

FÁBRY P.: A simple system of standard laboratory diets with various proportions of main nutrients. (in Czech), *Čs. fysiol.* **6:** 529-533, 1959.

FÁBRY P.: Morphological changes of the stomach and small intestine. In: *Feeding Pattern and Nutritional Adaptations.* POUPA O. (ed.), Academia, Prague, 1969, pp. 36–50.

FÁBRY P., HRŮZA Z.: Studies on the adaptation of metabolism IV. Adaptation of glucogenesis in animals accustomed to intermittent starvation. *Physiol. Bohemoslov.* **5:** 136–142, 1956.

FÁBRY P., KUJALOVÁ J.: Wachstum des Dündarmes bei intermittierend hungernden Ratten. *Naturwissenschaften* **45:** 373–379, 1958.

FLATT J.P.: Use and storage of carbohydrate and fat. *Am. J. Clin. Nutr.* **61:** S952–S956, 1995.

FOLCH J. M., LEES M., SLOANE-STANLEY G.M.S.: A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509, 1957.

HOLEČKOVÁ E., FÁBRY P:: Hyperphagia and gastric hypertrophy in rats adapted to intermittent starvation. *Br. J. Nutr.* **13:** 260–265, 1959.

HOLLIFIELD G., PARSON W.: Metabolic adaptations to a "stuff and starve" feeding program. I. Studies of adipose tissue and liver glycogen in rats limited to a short daily feeding period. *J. Clin. Invest.* **41:** 245–249, 1962a.

HOLLIFIELD G., PARSON W.: Metabolic adaptations to a "stuff and starve" feeding program. II. Obesity and the persistence of adaptive changes in adipose tissue and liver occurring in rats limited to a short daily feeding period. *J. Clin. Invest.* **41:** 250–255, 1962b.

HRŮZA Z., FÁBRY P.: Studies on the adaptation of metabolism. II. Adaptation of protein metabolism under varying conditions of nutrition. *Physiol. Bohemoslov.* **4:** 152–155, 1955.

KARLSON P.: Biochemistry of muscles. In: *Biochemistry.* (In Czech). Academia, Prague, 1981, pp. 453–457.

KARLSON P., GEROK W., GROSS W.: XVI. Liver. In: *Pathobiochemie.* Academia, Prague, 1987, pp. 294–317.

KETTELHUT I.C., PEPATO H.T., MIGLIORINI R.H., MEDINA R., GOLDBERG A.L.: Regulation of different proteolytic pathways in skeletal muscle in fasting and diabetes-mellitus. *Braz. J. Med. Res.* **27:** 981–993, 1994.

KIMURA T., ASHIDA K.: Influence of dietary carbohydrate, fat and protein on lipogenesis in rats. *Agr. Biol. Chem.* **7:** 1001–1006, 1969.

KŘÍZOVÁ E., ŠIMEK V.: Influence of intermittent fasting and high-fat diet on morphological changes of the digestive system and on the changes of lipid metabolism in the laboratory mouse. *Physiol. Res.* **45:** *145–151, 1996.*

LAWRENCE D. H., MASON W. A.: Intake and weight adjustment in rats to changes in feeding schedules. *J. Comp. Physiol.* **48:** 43–46, 1955.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–273, 1951.

McGREGOR I. S., LEE A. M.: Metabolic changes associated with ingestion of different macronutrients and different meal sizes in rats. *Physiol. Behav.* **57:** 277–286, 1995.

PETRÁSEK R.: Tissue adaptation to changes of time distribution of the food. *Vop. Pit.* **25:** 18–21, 1966.

PETRÁSEK R., FÁBRY P., POLEDNE R.: Effect of feeding pattern on fatty acid oxidation by rat liver slices. *Experientia* **20:** 434–439, 1964.

ROLLS B.J.: Carbohydrates, fats and satiety. *Am. J. Clin. Nutr.* **61** (Suppl. 4): S960–S967, 1995.

ROMSOS D. R., LEVEILLE G. A.: Effect of meal frequency and diet composition on glucose tolerance in the rat. *J. Nutr.* **11:** 1503–1512, 1974.

ŠIMEK V., TUYET H.M., PETRÁSEK R.: Morphological changes of digestive tract and level of liver glycogen as demonstration of intermittent fasting in laboratory white mouse. *Věstník Čs. spol. zool.* **37:** 212–221, 1973.

TEPPERMAN J., TEPPERMAN H.M.: Effects of antecedent food intake on hepatic lipogenesis. *Am. J. Physiol.* **193:** 55–59, 1958.

TEPPERMAN H.M., TEPPERMAN J.: Adaptive hyperlipogenesis. *Fed. Proc.* **23:** 73–77, 1964.

VAN HANDEL E.: Microseparation of glycogen, sugars and lipids. *Anal. Biochem.* **11:** 266–271, 1965.

VRBOVÁ G., GUTMANN E.: Adaptation of carbohydrate metabolism in striated muscle to intermittent starvation. *Physiol. Bohemoslov.* **7:** 221–224, 1958.

Reprint Requests

E. Křížová, Department of Comparative Animal Physiology and General Zoology, Faculty of Science, Masaryk University, 611 37 Brno, Kotlářská 2, Czech Republic.