Arch. Biol. Sci., Belgrade, 60 (1), 49-58, 2008 DOI:10.2298/ABS0801049M

THE EFFECT OF FASTING ON THE GLYCOGEN METABOLISM IN HEAT-ACCLIMATED RATS

BILJANA MIOVA, SUZANA DINEVSKA-KJOVKAROVSKA, S. MITEV, and MIRSADA DERVIŠEVIĆ

Department of Physiology and Biochemistry, Faculty of Natural Sciences and Mathematics, 1000 Skopje, R. Macedonia

Abstract — We investigated the influence of successive fasting for 24, 48, 72, and 96 h on some key enzymes and substrates of liver, kidney, and muscle in control and heat-acclimated (30 days at 35 ± 1°C) rats. Short-term fasting (for 24 and 48 h) resulted in decrease of liver glycogen content, blood glucose level, and concentration of glucose-6-phosphate, as well as increase of glucose-6-phosphatase activity, regardless of the previous temperature of acclimation. During a period of prolonged fasting (for 72 and 96 h), there was a rebound of liver glycogen content only in animals kept at room temperature. Fasting induced increase of renal glycogen content in animals kept at room temperature and increase of renal glucose-6-phosphatase activity in both experimental groups. As for muscle metabolism, endogenous nutrition resulted in decrease of muscle glycogen content in heat-acclimated animals. Activity of muscle glycogen phosphorylase (a+b) was decreased in the control and increased in heat-acclimated animals. The obtained results indicate that the examined carbohydrate-related parameters show time-dependent changes during 4 days of fasting. Twenty-four- and 48-h fasting intensifies glycogenolytic processes, while 72- and 96-h fasting intensifies gluconeogenic processes, doing so to a lesser extent in heat-acclimated animals. The changes caused by the fasting were modified by acclimation to moderate heat, primarily in the liver and to a lesser extent in the kidney and muscle.

Key words: Fasting, acclimation, glycogen, glucose-6-phosphatase, glycogen phosphorylase, liver, kidney, muscle, rats

UDC 612.83:59:612.82

INTRODUCTION

Fasting is a specific metabolic state of the organism accompanied by a number of hormonal and enzymatic disturbances which cause changes in the direction of metabolic processes (Buyse et al., 2000). The effects of fasting, i.e., of endogenous nutrition, are mostly manifested in changes of carbohydrate metabolism.

In humans, total endogenous production of glucose is known to decrease during prolonged fasting (Owen, 1969). This decrease is essentially due to decrease of hepatic gluconeogenesis, which is partially balanced by increase of gluconeogenesis in the kidney. Glucose production by the human kidney in the postapsorptive state accounts for about 5% of total endogenous glucose production (Owen et al., 1969), whereas during fasting for 60 h the net renal glucose output was reported to be 22% of total glucose production (Eckberg et al., 1999). As for muscle carbohydrate metabolism, 18 to 36% contributions of glucose production through the Cory cycle were reported during 12- and 40-h fasting, respectively (Katz and Tayek, 1998).

Carbohydrate metabolism is also affected by acclimation of the organism to moderate environmental heat. Acclimation to moderate heat alters metabolic pathways in the direction of rebound of hepatic glycogen stores (Mitev, 1983; Mitev and Buzalkov, 1985; Mitev et al., 1991; Dinevska, 1992; Dinevska-Kjovkarovska, 1998), increased gluconeogenesis (Dinevska-Kjovkarovska, 1998), and decrease in the rate of metabolism (Katsumata et al., 1990; Horowitz 1994).

Since both fasting and acclimation to heat have specific effects on the effect of fasting on carbohydrate metabolism in animals acclimated to moderate heat, there is a need for further research on the common effect of both factors. Such research would contribute to clarification of the metabolic changes caused by high environmental temperature during endogenous nutrition.

To our knowledge, there are no published data on the effect of fasting on carbohydrate metabolism in animals acclimated to moderate heat. Our goal was to estimate the effect of long-term heat acclimation on changes of some glycogen-metabolizing enzymes and substrates in the liver, kidney, and muscle during four days of endogenous nutrition, with special emphasis on distinguishing changes caused by short-term (24- and 48-h) as opposed to long-term (72- and 96-h) fasting.

MATERIALS AND METHODS

Experimental animals. Experiments were performed on 110 female Wistar laboratory rats, weighing 170-210 g. The animals were kept under conditions of a 12-h light regime (light from 6:00 to 18:00 h).

Experimental conditions. The effects of 24-, 48-, 72-, and 96-h fasting were estimated in control rats (kept at room temperature of 20 ± 2°C) and heat-acclimated animals. Heat acclimation for 30 days was performed in a special heated chamber at a temperature of 35±1°C and relative air humidity of 30-40%. The fasting animals received water *ad libitum* during the whole experimental period and were sacrificed after fasting for 24, 48, 72, and 96 h. Comparisons were drawn between fed animals from room temperature and ones heat-acclimated for 30 days, as well as between the respective groups at different temperatures.

Isolation and preparation of material for analysis. The experimental animals were anesthetized with ether. After laparatomy, blood was taken from the v. cava posterior. Pieces of the liver, right kidney, and right m. gastrocnemius were isolated and immediately frozen in liquid nitrogen. The frozen tissues were kept at -80°C until the analyses. Sacrifice of the animals was performed always in the period between 8:00 and 9:00 h. Homogenization of the material for analysis was performed with Cole-Parmer 4710 ultrasonic homogenizator for a period of 10-15 s. The whole procedure was performed at a temperature of 0 - 4°C (on ice).

Analytical methods. The activity of glucose-6-phosphatase in the liver and kidney (Hers, 1959) and that of muscle glycogen phosphorylase (*a* + *b*) (Morgan and Parmeggiani, 1964) were determined in tissue homogenates. Enzyme activity was determined indirectly through the quantity of inorganic phosphate produced (Fiske and Subbarow, 1925). Protein content was determined by the Lowry method (1951). The concentration of glucose-6-phosphate was indirectly calculated through changes in the concentration of NADPH (Lang and Michal, 1974). Glycogen content was determined by the anthrone method (Seifter et al., 1951), the blood glucose level by the enzyme-colorimetric method with a GOD-PAP instrument (Chronolab).

*Statistics***.** Results are presented as means ± SD. To examine statistical differences between each group and the control, we used one way ANOVA analysis with the Newman-Keuls post-hoc test. Linear regression and correlation analyses between the parameters were performed and only significant coefficients are presented. In all tests, differences with a probability level of $p<0.05$ were considered significant.

RESULTS

Blood glucose level. The obtained results (Fig. 1) showed that fasting for 24 to 96 h causes a continuous and significant decrease of the blood glucose level in animalskept at room temperature (from -13.7 to -31.1%, $p<0.05$), whereas in heat-acclimated animals significant changes occur only during prolonged fasting for 72 and 96 h (from -12.5% to -17.7%, $p<0.05$).

As for the effects of high environmental temperature, we found that only the control-fed and 24-h fasted heat-acclimated animals have a significantly

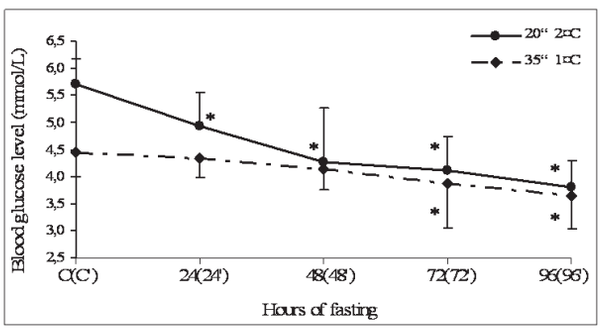


Fig. 1. Blood glucose level during fasting in control and heat-acclimated rats. Control (C) and 24, 48, 72, and 96-h fasted rats from room temperature (20 ± 2°C); and control (C') and 24', 48', 72', and 96'-h fasted heat-acclimated animals (35 ± 1°C). *p<0.05 in comparison with control fed animals from the corresponding temperature.

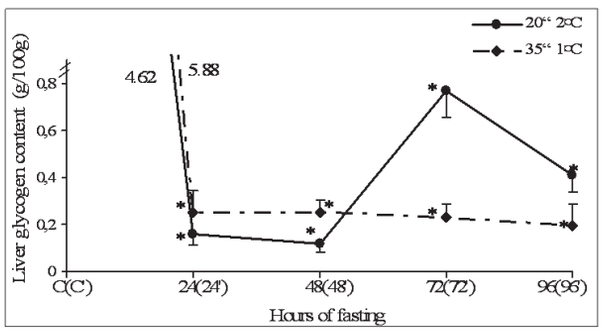


Fig. 2. Liver glycogen content during fasting in control and heat-acclimated rats. Legend as in Fig. 1.

Table 1. Statistical analysis of differences between experimental groups with respect to blood glucose level.

20±2°C / 35±1°C		
Ratio	%	p<
C:C'	-22,1	0,05
24:24'	-12,1	0,05
48:48'	-2,8	n.s.
72:72'	-5,6	n.s.
96:96'	-4,2	n.s.

Table 2. Statistical analysis of differences between experimental groups with respect to liver glycogen content.

20±2°C / 35±1°C			Long-term/short term fasting		
Ratio	%	p<	Ratio	%	p<
C:C'	27,2	0,05	24.72	380,7	0.05
24:24'	58,6	0,05	24.96	155,2	0.05
48:48'	110	0,05			
72:72'	-70,2	0,05	24'.72'	-9,8	n.s.
96:96'	-52,1	0,05	24'.96'	-23.0	n.s.

lower glucose level compared to the corresponding groups from room temperature (C:C', p<0.05; 24 : 24', p<0.05).

Liver

Glycogen content. Our results showed that 4-day fasting leads to a significant decrease of hepatic glycogen content (Fig. 2) regardless of previous thermal acclimation (from -83.4% to -97.4%, p<0.05 for animals from room temperature and from - 95.7% to -96.7%, p<0.05 for heat acclimated animals).

In comparison with short-term fasting, during prolonged fasting (for 72 and 96 h) there was a significant increase of liver glycogen content in the animals from room temperature (24:72, +380,7%, p<0,05 and 24:96, +155,2%, p<0,05), even though significantly less marked than in the control-fed animals. It is important to note that in heat-acclimated rats subjected to prolonged fasting there were no significant changes of liver glycogen content in comparison with the short-term fasted animals (24':72' and 24':96', n.s).

With respect to heat acclimation, we found higher hepatic glycogen content in fed heat-acclimated animals compared to those from room temperature (C:C', p<0.05). Short-term fasted (for 24 and 48 h) heat-acclimated animals have a significantly higher hepatic glycogen content (+56.8 to +110.2%, p <0.05), while ones fasted for 72 and 96 h have significantly lower glycogen content compared to animals kept at room temperature (from -52.1% to -70.2%, p <0.05).

Glucose-6-phosphatase activity. It can be seen from the obtained results (Fig. 3) that fasting caused increase in the activity of glucose-6-phosphatase in animals from both thermal environments. As for animals from room temperature, there was a less intensive increase during the first 24 and 48 hours (from +43.4 to +58.2%, p<0.05) and a more intensive one during prolonged fasting (from +109.5 to

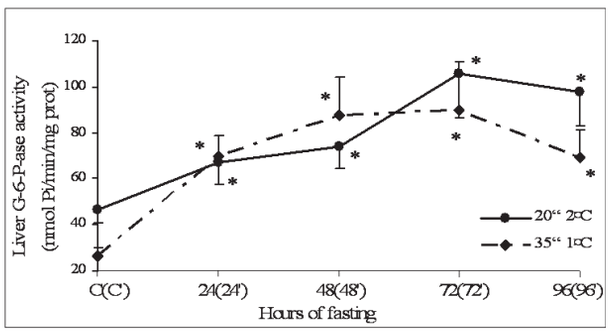


Fig. 3. Liver glucose-6-phosphatase activity during fasting in control and heat-acclimated rats. Legend as in Fig. 1.

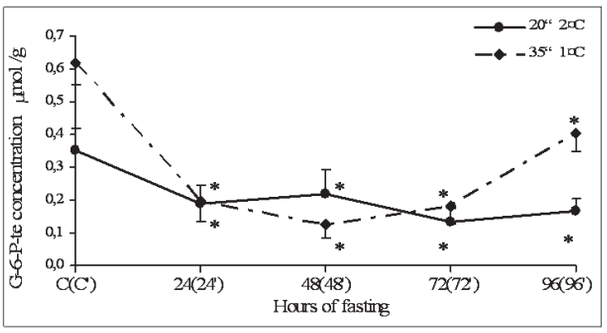


Fig. 4. Glucose-6-phosphate concentration during fasting in control and heat-acclimated rats. Legend as in Fig. 1.

Table 3. Statistical analysis of differences between experimental groups with respect to liver glucose-6-phosphatase activity.

20±2°C / 35±1°C		
Ratio	%	p<
C:C'	-43,2	0,05
24:24'	4,4	n.s.
48:48'	19,0	0,05
72:72'	-15,0	0,05
96:96'	-29,1	0,05

Table 4. Statistical analysis of differences between experimental groups with respect to glucose-6-phosphate concentration.

20±2°C / 35±1°C		
Ratio	%	p<
C:C'	74,6	0,05
24:24'	4,1	n.s.
48:48'	-42,9	0,05
72:72'	33,3	n.s.
96:96'	140,0	0,05

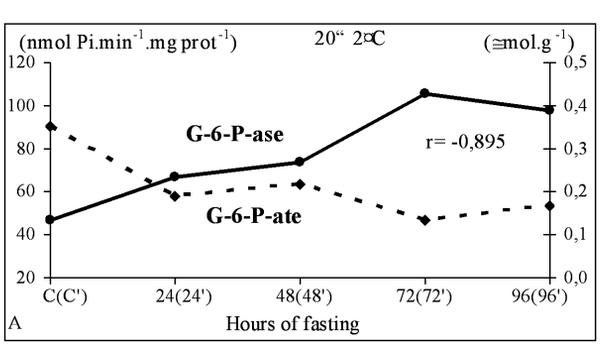
+126.2%, p<0.05) compared to the control fed rats.

In heat acclimated rats, a continuous increase of the enzyme activity was observed over the whole period of fasting, with higher activity in the group fasted for 72 h and a tendency toward normalization in the 96-h fasted group.

It can be seen that all groups of heat-acclimated rats have lower enzyme activity compared to those from room temperature (C:C', 24:24', 72:72', 96:96' from -15.0 to - 43.2%, p<0.05), except for the 48-h fasted group (48 : 48', +19.0%, p<0.05).

Glucose-6-phosphate concentration. Four days of fasting caused a significant decrease in the concentration of glucose-6-phosphate in both experimental groups (from -38.0 to -61.5%, p<0.05 for the animals from room temperature and from -34.8 to -79.7%, p<0.05 for heat-acclimated ones).

Acclimation to a hyperthermic environment provoked some changes which differ depending on the duration of endogenous nutrition [higher concentration in control-fed and 96-h fasted animals (C:C', +74.6%; 96:96', +140.0%, p<0.05) and lower concentration in 48-h fasted ones (48:48', -42.9%,



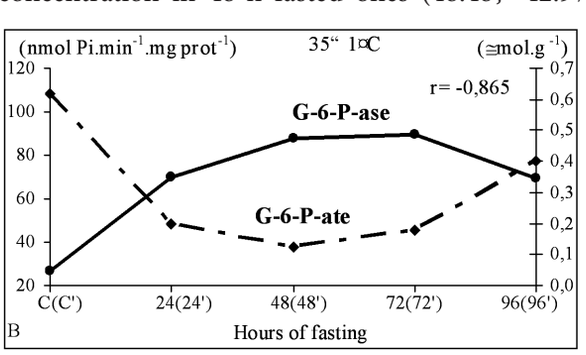


Fig. 5. Liver glucose-6-phosphatase activity during fasting in control and heat-acclimated rats. Legend as in Fig.1

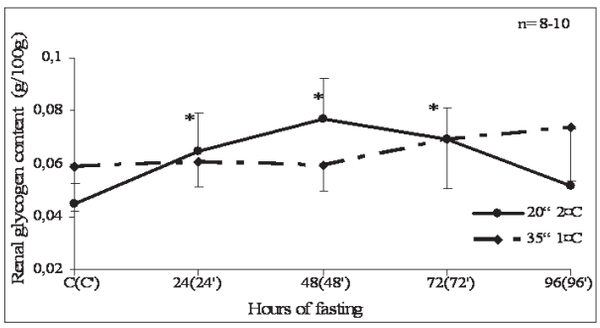


Fig. 6. Renal glycogen content during fasting in control and heat-acclimated rats. Legend as in Fig. 1.

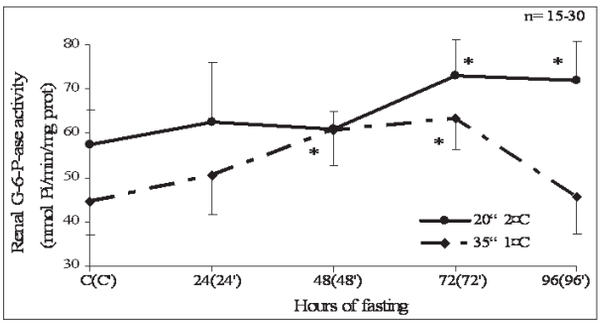


Fig. 7. Renal glucose-6-phosphatase activity during fasting in control and heat-acclimated rats. Legend as in Fig. 1.

Table 5. Statistical analysis of differences between experimental groups with respect to renal glycogen content.

20±2°C / 35±1°C		
Ratio	%	p<
C:C'	32,4	0,05
24:24'	-6,9	n.s.
48:48'	-23,1	0,05
72:72'	0,2	n.s.
96:96'	43,0	0,05

Table 6. Statistical analysis of differences between experimental groups with respect to renal glucose-6-phosphatase activity.

20±2°C / 35±1°C		
Ratio	%	p<
C:C'	-22,3	0,05
24:24'	-19,5	0,05
48:48'	-0,3	n.s.
72:72'	-13,4	0.05
96:96'	-36,7	0.05

p<0.05].

It can be seen that a very strong negative correlation exist between glucose-6-phosphatase activity and glucose-6-phosphate concentration (Fig. 5) in control (r_1=0,-895) and heat-acclimated animals (r_1=0,-865).

Kidney

Glycogen content. We recorded a significant increase of renal glycogen in animals kept at room temperature and fasted for up to 72 h (from +45.2 to +71.9%, p<0.05) and a non-significant increase after fasting for 96 h (C: 96, n.s) There were no significant changes of kidney glycogen content in heat-acclimated rats throughout the whole fasting period.

Comparing the groups acclimated to 35±1°C with the corresponding groups kept at room temperature, we recorded higher glycogen content in the control animals and ones fasted for 96 h (C:C' and 96:96', p<0.05), whereas glycogen was significantly lower content in heat-acclimatized animals fasted for 48 h (24:24', p<0.05).

Glucose-6-phosphatase activity. Our results showed that there was a continuous increase of enzyme activity in rats kept at room temperature, especially during a period of prolonged fasting (C:72, +27.4% and C:96, +25.4%, r<0.05). In heat-acclimated animals, enzyme activity increased up (C':72', +41.9%. r<0.05) with a tendency toward normalization discernible at 96 h of fasting (C':96', n.s).

Acclimation to moderate heat significantly reduces enzyme activity in comparison with the corresponding groups at room temperature, i.e., all of the heat-acclimated groups have lower enzyme activities compared to those from room temperature (from -13.4 to -36.7%, p<0.05).

Muscle

Glycogen content. Fasting causes a decrease of muscle glycogen content regardless of prior thermal acclimation (Fig. 8). A more intensive decrease of glycogen content was recorded in heat-acclimated rats (from -30.4 to -43.6%, p<0.05) than in those kept at room temperature (from -13.6 to -19.2%, p<0.05). Heat-acclimated animals have significantly

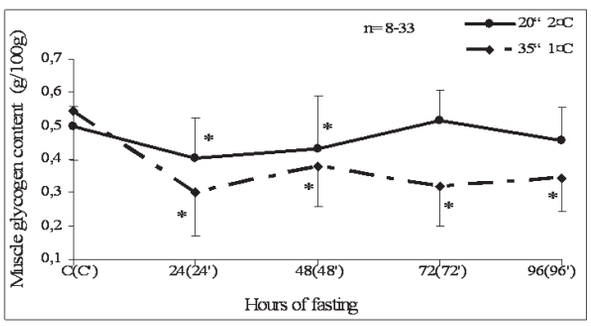


Fig. 8. Muscle glycogen content during fasting in control and heat-acclimated rats. Legend as in Fig. 1.

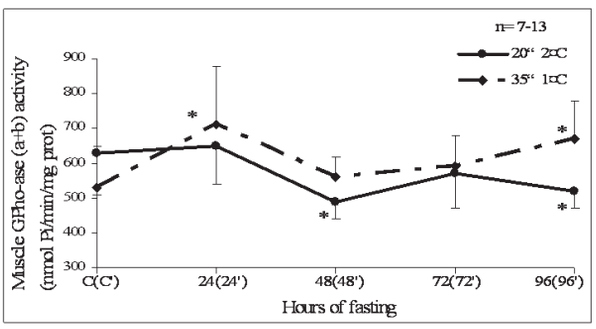


Fig. 9. Muscle glycogen phosphorylase (a+b) activity during fasting in control and heat-acclimated rats. Legend as in Fig. 1.

Table 7. Statistical analysis of differences between experimental groups with respect to muscle glycogen content.

20±2°C / 35±1°C		
Ratio	%	p<
C:C'	9.2	n.s.
24:24'	-23.8	0.05
48:48'	-12.0	0.05
72:72'	-38.5	0.05
96:96'	-25.2	0.05

Table 8. Statistical analysis of differences between experimental groups with respect to muscle glycogen phosphorylase (a+b) activity.

20±2°C / 35±1		
Ratio	%	p<
C:C'	-14,5	n.s.
24:24'	8,8	n.s.
48:48'	14,2	n.s.
72:72'	3,4	n.s.
96:96'	28,6	0.05

lower muscle glycogen content compared to animals from room temperature (from -12.3 to -38.5%, p<0.05).

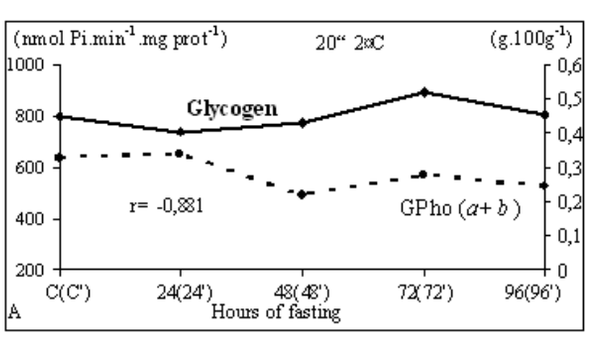
Glycogen phosphorylase (a+b) activity. The obtained results showed significant decrease of enzyme activity during four days of fasting in animals kept at room temperature and increase in heat-acclimated ones.

Acclimation to high environmental temperature did not cause any significant changes except in the 96-h fasted group (96:96', p<0.05).

There is a negative correlation (Fig. 10) between muscle glycogen phosphorylase *a+b* and glycogen in control (r_1 = -0,881) and heat-acclimated (r_1 = -0,820) rats.

DISCUSSION

The liver and fasting. The obtained results point to significant changes in all examined parameters in the liver, which vary depending on previous thermal acclimation and the duration of fasting. The observed changes occurred in two phases – during short-term fasting (for 24 to 48 h) and during long-



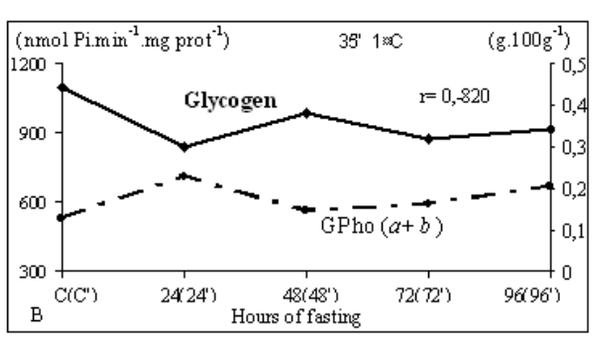


Fig. 10. Correlation between glycogen phosphorylase and glycogen in muscle. A - control rats; B - heat-acclimatized rats; r - linear coefficient of correlation. Legend as Fig. 1.

term (prolonged) fasting (for 72 to 96 h).

It can be seen that intensive mobilization of liver glycogen content occurs in the first 48 h of fasting. This decrease of glycogen reserves in the liver is almost the same in animals from both thermal environments (about 95%, Fig. 2). However, it is very important to note that at this time (24 and 48 h) heat-acclimated fasted animals have a higher glycogen content compared to the corresponding groups from room temperature (24:24', +58,6% and 48:48', +110%, $p<0,050$). This might be a result of acclimation changes in animals before the period of fasting, i.e., higher glycogen content was recorded in heat-acclimated fed rats in comparison with the fed rats kept at the room temperature.

Decrease of liver glycogen content in the first 48 h of fasting is accompanied by increase of glucose-6-phosphatase activity in animals of both experimental groups (Fig. 3), but this increase is more pronounced in heat-acclimated ones. According to some previous results obtained on fasted rats from room temperature (Minassian and Mithieux, 1994; Mithieux, 1996; Minassian et al., 1999), glucose-6-phosphatase activity increases in the first 48 h of endogenous nutrition, which is a result of induction of the mRNA responsible for synthesis of glucose-6-phosphatase (Dhahbi et al., 2001). In the same experimental period, we recorded decrease in the concentration of hepatic glucose-6-phosphate, regardless of previous thermal acclimation (Fig. 4). Our results show that changes in the concentration of glucose-6-phosphate are more intensive in heat-acclimated animals, which might be a consequence of the higher glucose-6-phosphatase activity observed in these animals. This, together with decreased glycogen content in the liver, points to increased glycogenolysis in the first 48 h of the fasting period.

As for glucose levels in the blood, during short-term fasting we found significant decrease only in rats kept at room temperature, non-significant changes being recorded in heat-acclimated ones. This might be a consequence of the fact that heat acclimation by itself probably causes decrease of the blood glucose level in fed animals (C:C', -22,1%, $p<0,05$).

In contrast to short-term fasting, during prolonged fasting (for 72 and 96 h) we observed a rebound of hepatic glycogen, but only in the animals kept at room temperature (Fig. 2), which probably is a result of increased intensity of gluconeogenesis (Jahoor et al., 1990; Landau et al., 1996; Chandramauli et al.. 1997). According to Van de Werve and Jeanrenaud (1987), increased re-synthesis of glycogen in the liver does not involve activation of glycogen synthesis or inactivation of glycogen phosphorylation, but is regulated through the concentration of glucose-6-phosphate as a substrate precursor for synthesis of glycogen. However, contrary to the findings of Minassian and Mithieux, (1994), instead of increased concentration of glucose-6-phosphate and decreased activity of glucose-6-phosphatase after 3-4 days of fasting, we found decrease of the substrate and increase of the enzyme in the same experimental period. We assume that the process of gluconeogenesis is accelerated and glucose-6-phosphate is intensively consumed by the increased glucose-6-phosphatase activity. Moreover, it might be that not only gluconeogenesis to glucose, but also resynthesis of glycogen from gluconeogenic substrates and glucose-6-phosphate is intensified, while glycogenolysis is decreased.

It is important to note that there is no rebound of hepatic glycogen in heat-acclimated rats during a period of prolonged fasting (Fig. 2). According to Chayoth et al. (1982), 24-h fasting caused sixfold elevation in hepatic gluconeogenesis in the control, whereas only fourfold enhancement of this pathway was found in heat-acclimated animals, which suggests that a decrease of hepatic gluconeogenesis in heat acclimation occurs in fasted animals. Also, lowered levels of branched-chain amino acids and arginine were found in heat-acclimated hamsters, which implies that this lower level of gluconeogenic substrates causes decrease in the intensity of the gluconeogenesis (Chayoth et al., 1984).

In the same experimental period, heat-acclimated rats showed a tendency toward normalization (decrease) of glucose-6-phosphatase activity that resulted in normalization (increase) of glucose-6-

phosphate. We found that heat-acclimated animals (fasted for 72- and 96 h) have a lower activity of glucose-6-phosphatase and higher concentration of glucose-6-phosphate compared to the same animals from room temperature. This indicates a high negative correlation between the enzyme and the substrate regardless of thermal acclimation (Fig. 5).

From the above data, it can be assumed that endogenous glucose production during short-term fasting is due to intensive liver glycogenolysis, whereas during prolonged fasting the process of gluconeogenesis is favored. The intensity of glycogenolysis and gluconeogenesis depends on previous thermal acclimation.

The kidney and fasting. As one of the gluconeogenic organs, the kidney plays a significant part in the maintenance of glucose homeostasis in fasting conditions. It is well known that in the fed condition kidney gluconeogenesis provides about 5-10% of total glucose (Owen et al., 1969) and about 20-25% during 60-h fasting (Ekberg et al., 1999).

We investigated the biochemical pathways involved in the kidney carbohydrate metabolism of fasted heat-acclimated rats through glycogen content (Fig. 6) and the activity of glucose-6-phosphatase (Fig. 7). During 4 days of fasting, glycogen content shows a tendency to increase in animals of both experimental groups (but with significantly lower values in heat-acclimated ones). Thus, as in the liver, the increase of kidney glycogen in heat-acclimated rats is less marked than in animals kept at room temperature.

With respect to renal glucose-6-phosphatase, we observed a continuous increase in activity of the enzyme throughout the whole period of fasting (Fig. 7) in both thermal groups, but lower enzyme activity was recorded in the heat-acclimated ones.

There are also time-dependent differences in the contributions of hepatic and renal gluconeogenesis during fasting. To be specific, hepatic glucose-6-phosphatase is dominant in the first 48 h of the fasting, the renal enzyme only during 72- and 96-h fasting (Minassian and Mithieux, 1994). It was previously reported that contributions of these two organs during prolonged fasting are almost the same (Owen et al., 1969).

According to Clutter and Cryer (1994), increased renal gluconeogenesis during endogenous nutrition is a mechanism for combating the production of ammonia and metabolic acidosis characteristic of fasting. To be specific, it is known that production of ammonia is associated with intensive gluconeogenesis from glutamine (Pogson et al., 1976). Metabolic acidosis causes induction of the *PEPCK* gene and increases the activity of this enzyme (Kaiser and Curthoys, 1991).

The above-mentioned findings emphasise the importance of kidney gluconeogenesis in the condition of prolonged fasting. The obtained results – lower glycogen content and lower activity of glucose-6-phosphatase in heat-acclimated fasted rats – indicate reduced intensity of kidney gluconeogenesis in these animals.

Muscle tissue and fasting. Since muscle has a glycogenolytic enzyme profile, we studied changes caused by fasting and heat acclimation through its glycogen content and the activity of total glycogen phosphorylase ($a+b$) .

It can be seen that fasting causes decrease of muscle glycogen content and glycogen phosphorylase activity (Figs. 8 and 9). According to Sakada et al. (1987), muscle glycogen participates in the maintenance of glycemia in fasting rats (through the Cory cycle), but only during prolonged fasting, after consumption of hepatic glycogen reserves. It is important to note that in humans, the major part of glucose production after overnight fasting derives from lactate and alanine, the rest consisting of the glutamine flux emerging from muscle proteins (Sakada et al., 1987)

Other investigators found a progressive decrease of muscle glycogen in fasting rats (Hiroshe et al., 1986; Oi et al., 1997), the indicated decrease being accompanied by an increase in the activity of glycogen phosphorylase (Hiroshe et al., 1986).

Changes in the examined parameters caused by exposure to high environmental temperature are of

special interest. In animals previously acclimated to moderate heat, we found decrease of muscle glycogen content (Fig. 8) and increase in the activity of glycogen phosphorylase *a+b* (Fig. 9). Compared to the animals exposed to room temperature, heat-acclimated animals have lower glycogen content in muscle tissue and somewhat higher activity of muscle glycogen phosphorylase. Increased activity of glycogen phosphorylase in these animals probably results in additional decrease of glycogen content in the muscles.

On the basis of the obtained results, we can conclude that in the condition of endogenous nutrition, carbohydrate-metabolizing enzymes and substrates in the liver, kidney, and muscle are highly influenced by temperature as one of the most important ecological factors of the environment.

REFERENCES

Buyse, J., Decuypere, E., Darrs, V. M., Vleurick, L. M., Kuhn, E. R., and *J. D. Veldhuis* (2000). Food deprivation and feeding of broiler chickens is associated with rapid and interdependent changes in the somatotrophic and thyrotrophic axes. *Br. Poultry Sci.* **41**.107-116.

Chandramouli, V., Ekberg, K., Schumann, V., Kalhan, S., Wahren, J., and *B. Landau* (1997). Quantifying gluconeogenesis during fasting. *Am. J. Physiol. Endocrinol. Metab*., **273**, 6, E1209-E1215.

Chayoth, R., Aharon, M., and *Y. Graziani* (1982). Protein kinase activity in liver of heat-acclimated hamsters. *J. Appl. Physiol.* **53**, 3, 716-8.

Chayoth, R., Nakhooda, F. A., Poussier, P., and *B. E. Marliss* (1984). Glucoregulatory and metabolic responses to heat exposure in rats. *Am. J. Physiol. Endocrinol. Metab*., **246**, E465-E470.

Clutter, W., and *P. Cryer* (1994). Hypoglycemia, In: *Internal Medicine* (Ed. J. Stein), 1424-1430. Mosby, St. Louis, MO.

Dhahbi, M. J., Mote, L. P., Wingo, J., Rowley, C. B., Cao, X. S., Walford, L. R., and *R. S. Spindler* (2001). Caloric restriction alters the feeding response of key metabolic enzyme genes. *Mech. of Age. Develop.* **122**, 1033-1048.

Dinevska, S. (1992). *The Influence of High Environmental Temperature and Thyroid Hormones on Liver Glycogen content*. Master's thesis. Zagreb (In Croatian).

Dinevska-Kjovkarovska, S. (1998). *The Influence of High Environmental Temperature on Some Key Enzymes of Carbohydrate Metabolism in Rats*. Doctoral Thesis. Skopje (In Macedonian).

Ekberg, K., Landau, B., Wajngot, A., Chandramauli, V., Efendic, S., Brunengraber, H., and *J. Wahren* (1999). Contributions by kidney and liver glucose production in the postabsorptive state and after 60 h of fasting. *Diabetes* **48**, 292-297.

Fiske, C. A., and *Y. Subbarow* (1925). The colorimetric determination of phosphorus. *J. Biol. Chem.* **66**, 357-400.

Hers, H. G. (1959). Glycogen storage disease, In: *Advances in Metabolic Disorders* (Eds. R. Levine and R. Luft), 36-85. Academic Press, New York-London.

Hiroshe, K., Watanabe, J., Kanamura, S., Tokunaga, H., and *R. Ogewa* (1986). Significance of the increase of glucose-6-phosphatase activity in skeletal muscle cells of mouse by starvation. *Anat. Rec*. **216**, 2, 133-8.

Horowitz, M. (1994). Heat stress and heat acclimation: the cellular response modifier of autonomic control, In: *Integrative and Cellular Aspects of Autonomic Function: Temperature and Osmoregulation*, 87-95. Libbey Eurotext, Paris.

Jahoor, F., Peters, E. J., and *R. R. Wolfe* (1990). The relationship between gluconeogenic substrate supply and glucose production in humans. *Am. J. Physiol. Endocrinol. Metab*., **21**, E288-E296.

Kaiser, S., and *N. P. Curtoys* (1991). Effect of pH and bicarbonate on phosphoenol-pyruvate carboxykinase and glutaminase mRNA levels in cultured renal epitelial cells. *J. Biol. Chem*. **266**, 9397-9402.

Katsumata, M., Yano, H., Ishida, N., and *A. Miyazaki* (1990). Influence of a high ambient temperature and administration of clenbuterol on body composition in rats. *J. Nutr. Sci. Vitaminol*., **36**, 569-578.

Katz, J., and *J. A. Tayek* (1998). Gluconeogenesis and Corycycle in 12-, 20- and 40-h-fasted humans. *Am. J. Physiol. Endocrinol. Metab*. **38**, E537-E542.

Landau, B. R., Wahren, J., Chandramouli, V., Schumann, W., Ekberg, K., and *S. Kalhan* (1996). Contributions of gluconeogenesis to glucose production in the fasted state. *J. Clin. Invest*. **98**, 2, 378-385

Lang, G., and *G. Michal* (1974). *Methods of Enzymatic Analysis* (Ed. H. U. Bermeyer), 1283-1287. Verlag Chemie.

Lowry, O. H., Rosebrough, J. N., Farr, L. A., and J. R. Randall (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*. **193**, 265-275.

Minassian C., and *G. Mithieux* (1994). Differential time course of liver and kidney G-6-P-ase during fast. *J. Biol. Chem*., **269**, 24, 16585-16588.

Minassian C., Montano S., and *G. Mithieux* (1999). Regulatory

role of glucose-6-phosphatase in the repletion of liver glycogen during refeeding in fasted rats. *Biochim. Biophys. Acta* **1452**, 172-178.

Mitev, S. (1983). *Influence of High Environmental Temperature and Some Endocrine Factors on Liver Glycogen Content and Other Parameters in white rats*. Doctoral Thesis. Skopje (in Macedonian).

Mitev, S., and *R. Buzalkov* (1985). The influence of thyroidectomy and treatment with Novothyral on liver glycogen content and some other parameters in rats acclimated to different environmental temperature. *Ann. Rev. Med. Fac*. **31**, 1, 3-8.

Mitev, S., Buzalkov, R. T., Dinevska, S., and *J. Dimovska* (1991). The effect of thyroidectomy and thyroid hormone administration on liver glycogen content in rats previously acclimated to various environmental temperatures. *Yugoslav. Physiol. Pharmacol. Acta* **27**, 53-59.

Mithieux, G. (1996). Role of glucokinase and glucose-6-phosphatase in the nutritional regulation of endogenous glucose production. *Reprod. Nutr. Dev*. **36**, 357-362.

Morgan, H. E., and *A. Permeggiani* (1964). Regulation of glycogen in muscle. II. Control of glycogen phosphorylase reaction in isolated perfused heart. *J. Biol. Chem*. **239**, 2435-2439.

Oi, K., Komori, H., and *H. Kajinuma* (1997). Changes in plasma glucose, insulin, glucagon, cateaholamine and glycogen contents in tissues during development of alloxan diabetes mellitus in rats. *Biochem. Molec. Med*. **62**, 70-75.

Owen O. E. Feling, P., Morgan, A. P., Wahren, J., and *G. F. Cahill Jr.* (1969). Liver and kidney metabolism during prolonged starvation. *J. Clin. Invest*. **48**, 574-583.

Pogson, C. I., Longshew, I. D., Roobol, A., Smith, S. A., and G. A. O. Alleyne (1976). Phosphoenolpyruvate carboxykinase and renal gluconeogenesis, In: *Gluconeogenesis. Its Regulation in Mammalian Species* (Eds. R.W. Hanson and M. A. Mehlman). 335-368. John Wiley and Sons, New York.

Sakada, M., Watanabe, J., Kanamura, S., Tokunaga, H., and *R. Ogewa* (1987). Physiological role of skeletal muscle glycogen in starved rats. *Anat. Rec*. **218**, 3, 267-74.

Seifter, S., Dayton, S., Novic, B., and *M. Muntwyler* (1951). The estimation of glycogen with the anthrone reagent. *Arch. Biochem*. **25**, 191-200.

Van de Werve, G., and *B. Jeanrenaud* (1987). Liver glycogen metabolism: an overview. *Diabetes Metab. Rev*. **3**. 47-78.

ЕФЕКАТ ГЛАДОВАЊА НА МЕТАБОЛИЗАМ ГЛИКОГЕНА КОД ТОПЛО-АКЛИМОВАНИХ ПАЦОВА

БИЉАНА МИОВА, СУЗАНА ДИНЕВСКА-КЈОВКАРЕВСКА, С. МИТЕВ и МИРСАДА ДЕРВИШЕВИЋ

Одсек за физиологију и биохемију, Природно-математички факултет, 1000 Скопље, Р. Македонија

Добијени резултати указују да испитивани параметри угљених хидрата показују временом условљене промене током четири дана гладовања. Гладовање у трајању од 24 и 48 часова интензификује гликогенолитичке процесе, док гладовање током 72 и 96 часова интензификује глуконеогене процесе, не у мањој мери код топло аклимованих пацова. Промене узроковане гладовањем мењају се током аклимације на умерену топлоту, првенствено у јетри, а у мањем обиму и у бубрезима и мишићима.