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Effect of fasting on hepatic and renal gluconeogenic enzyme activities in ducklings

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ABSTRACT

Blood glucose concentration, liver and kidney gluconeogenic enzyme activities were investigated during a six-day fasting period in ducklings (English heavy breed Peking Duck). Food deprivation commenced when ducklings reached the age of twenty-eight days. Normally fed ducklings were used as a control group. The ducklings were sacrificed by decapitation after a fasting period of three, four, five and six days, and blood and tissue samples were collected from both groups for analysis. During the experiment the concentration of blood glucose was above 6 mmol/L in both the control and experimental groups, except on experimental day three. Significant rises in glucose-6-phosphatase (EC 3.1.3.9) (G6Pase) activities in the liver were observed on starvation days four, five and six ($P = 0.0012$, $P = 0.0109$, $P = 0.0279$), and in the kidney on starvation days five and six ($P = 0.0373$, $P = 0.0206$, respectively). Hepatic fructose-1.6-bis-phosphatase (EC 3.1.3.11) (FDPase) activity showed a significant difference between the fasted and control group only after a four-day fast ($P = 0.0491$), whereas renal enzyme activity increased after four ($P = 0.0279$) and six ($P = 0.0373$) days of food deprivation. Phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (PEPCK) activity in the liver of fasted ducklings significantly decreased on fasting day five ($P = 0.0012$), whereas in the kidney a significant rise in four-day fasted animals ($P = 0.032$) was observed in comparison with the normally fed controls. The results showed that ducklings are able to maintain blood glucose concentration during a six-day

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fasting period. Changes in gluconeogenic enzyme activities indicate the important role of liver and renal gluconeogenesis for the maintenance of blood glucose concentration in fasted ducklings. These findings agree with earlier data obtained on chicken, thus confirming the similarities between the two avian species regarding the maintenance of blood glucose level with the process of gluconeogenesis during longer fasting.

Key words: carbohydrate metabolism, ducklings, fasting, gluconeogenic enzymes

Introduction

It is a characteristic of all avian species that they maintain a high blood glucose level normally twice that found in most mammals (LANGSLOW, 1978). On the other hand, the glycogen of the avian liver and skeletal muscle is rather lower than that of most mammals, while small glycogen stores are also present in avian kidneys (YAMANO et al., 1988). Even under fasting conditions the birds maintain normoglycemia (HAZELWOOD and LORENZ, 1959), and normal glucose utilization for a long period (BRADY et al., 1978). The maintenance of blood glucose during prolonged starvation in birds is partially attributed to extensive renal gluconeogenesis from numerous precursors (TINKER et al., 1983; WATFORD et al., 1981; YAMANO et al., 1988), and to a very high rate of hepatic gluconeogenesis from lactate (SARKAR, 1971; SOLING et al., 1973; LANGSLOW, 1978). Concomitant with these events, the activities of glucose-6-phosphatase (G6Pase), fructose-1,6-bis-phosphatase (FDPase), pyruvate carboxylase (PC) and phosphoenolpyruvate carboxykinase (PEPCK), the key gluconeogenic enzymes, increase markedly, indicating compensatory gluconeogenesis (STURKIE, 1986). However, in this large group of animals, glucose concentration and glycogen stores vary from one species to another. Ducks in relation to chickens have lower blood glucose levels and a higher quantity of glycogen stored in their liver and skeletal muscles (BARANYIOVA and HOLUB, 1971; SALZANO and RUSSO, 1976).

Most of the research on the influence of fasting on the mentioned parameters in birds was carried out in chickens and pigeons, while there are almost no data on the effect of fasting on the carbohydrate metabolism in ducks. The aim of this study was to define whether ducklings were able to maintain blood glucose level during a six-day fast, and to investigate prospective changes in the key gluconeogenic enzyme activities and their possible involvement in the maintenance of glycemia.

Materials and methods

Animal. One-day-old English heavy breed Peking Ducks were reared under standard management practices and given commercial food and water *ad libitum* until they reached the age of twenty-eight days. After twenty-eight days, ducklings were divided into two groups: control (28 birds) which was fed *ad libitum* until death, and experimental group (32 birds) which was deprived of food on the subsequent six days of experiment. On the 3rd, 4th, 5th and 6th days of fasting, eight ducklings from experimental group and seven control ducklings were sacrificed by decapitation at 8 a.m.

Sample collection and homogenate preparation. Blood samples were collected from the bleeding neck after decapitation and immediately deproteinized with trichloracetic acid, after which they were transported to the laboratory for further procedures.

Livers and kidneys were rapidly removed, blotted, weighed and sliced. The slices were immediately frozen and kept at -20 °C until enzyme activity determination (FRANCETIĆ et al., 1988). For G6Pase assays, livers and kidneys were homogenized in 250 mM saccharose, and for FDPase in 50 mM lactate buffer with glass-homogenizer; enzyme activities were measured in whole homogenate. For PEPCK assay, livers and kidneys were homogenized with ultraturrax homogenizer for 3x5 s at 50 Hz. Homogenates were centrifuged at 100000 g/60 min at 0 °C, and the supernatant was used for determination of cytosolic PEPCK activity.

Analytical methods. G6Pase and FDPase were assayed according to FREEDLAND and HARPER (1959). In both assays, the amount of inorganic phosphorus liberated at 37 °C was the measure of enzyme activity. The amount of inorganic phosphate produced was determined according to FISKE and SUBBAROW (1925). PEPCK activity was determined at 25 °C in the direction of oxaloacetate formation by measuring the oxidation rate of NADH in excess of malate dehydrogenase (CHANG and LANE, 1966). G6Pase and FDPase activities were expressed as μmol of inorganic phosphorus formed per mg protein per minute. PEPCK activity was expressed as nmol of oxaloacetate formed per mg protein per minute.

Proteins were estimated according to LOWRY et al. (1951) using human serum albumin as a standard.

Blood glucose concentration was determined by the orthotoluidine method of HULTMAN (1959) using a commercial diagnostic set produced by Herbos (Sisak, Croatia) and expressed as mmol/L.

Statistical analysis. The results (median, upper and lower quartile) were statistically evaluated using descriptive statistical procedures, and the significance of all between-group differences was checked by Kruskal-Wallis ANOVA by ranks test using commercial "STATISTICA" software.

Results

The effects of fasting on body and liver mass of ducklings and data on blood glucose levels are shown in Table 1. Significant body and liver weight loss was recorded during fasting. During the six-day fasting period, fluctuation of blood glucose was noticed (Table 1).

Table 1. Effect of fasting on body and liver mass and blood glucose level

Days of experiment	Variable	Body mass (g)		Liver mass (g)		Blood glucose (mmol/L)	
		Control	Fasted	Control	Fasted	Control	Fasted
3	median	1000	700 [a]	32.5	16.5 [a]	4,89	4,61
	quartiles	915-1030	600-781	28.2-35.4	15.5-18.4	4.23-4.99	4.10-4.96
4	median	1160	672 [b]	43.5	14.1 [a]	7,32 [A]	6,42 [c,A]
	quartiles	1010-1275	612-732	34.4-48.0	13.0-15.2	6.67-7.33	5.93-6.52
5	median	1160	662 [a]	37.2	15.3 [a]	6,75	6,67
	quartiles	1090-1260	637-700	34.2-43.2	14.5-15.9	6.55-7.15	6.45-6.82
6	median	1280	616 [a]	56.5	13.4 [a]	6,87	6,21 [d,B]
	quartiles	1160-1400	567-642	49.7-69.6	13.0-14.3	6.21-7.23	5.93-6.49

Level of significance between control (n = 7) and fasting group (n = 8): [a]P = 0.0012; [b]P = 0.0026; [c]P = 0.0045; [d]P = 0.0362.
Level of significance according to the preceding control value: [A]P = 0.002.
Level of significance according to the preceding fasting value: [A]P =0.0008; [B]P = 0.02.

In both groups of animals the blood glucose concentration on the 3rd day of the experimental period was lower (4.61 mmol/L in fasted and 4.89 mmol/L in fed ducklings) than on other days of the experiment, when it

ranged from 6.2 to 7.3 mmol/L. In comparison with control ducklings, blood glucose concentration in fasting ducklings was statistically lower on the 4th (P = 0.0045) and 6th (P = 0.0362) experimental days. Analysis of the mentioned parameters in fasted ducklings between consecutive periods showed a statistically significant increase from the 3rd to the 4th (P = 0.0008) day, and a decrease from the 5th to the 6th (P = 0.02) day. Comparison of blood glucose concentrations in the control group yielded a statistically significant difference only between the 3rd and the 4th (P = 0.002) day.

Table 2. Effect of fasting on hepatic and renal glucose-6-phosphatase (G6Pase) activity

Days of experiment	Variable	G6Pase activity (nmolP/mg protein/min)			
		Liver		Kidney	
		Control	Fasted	Control	Fasted
3	median	7.83	12.83	12.64	9.52
	quartiles	6.44-11.59	11.14-15.64	10.07-15.15	7.75-18.61
4	median	7.84	18.02^{a}	8.61	11.54
	quartiles	5.89-8.20	12.91-25.84	3.23-11.51	6.79-17.48
5	median	12.54^{A}	$20.35^{b,A}$	11.44	17.05^{d}
	quartiles	10.48-14.08	16.29-27.34	10.33-12.81	13.45-19.62
6	median	9.15	$18.41^{c,B}$	8.06^{B}	14.67^{e}
	quartiles	6.15-17.05	13.43-22.65	4.05-11.51	10.48-18.52

Level of significance between control (n = 7) and fasting (n = 8) groups: $^{a}P = 0.0012$; $^{b}P = 0.0109$; $^{c}P = 0.0279$; $^{d}P = 0.0373$; $^{e}P = 0.0206$.
Level of significance according to the preceding control value: $^{A}P = 0.02$; $^{B}P = 0.03$.
Level of significance according to the 3rd fasting day: $^{A}P = 0.009$; $^{B}P = 0.046$.

Hepatic G6Pase activity was higher in fasted ducklings than in fed controls throughout the experiment (Table 2), with a significant increase on the 4th (P = 0.0012), 5th (P = 0.019) and 6th (P = 0.0279) fasting days. Renal G6Pase activity was significantly higher in fasted than in control animals on days 5 (P = 0.0373) and 6 (P = 0.0206). In the fasting groups, a significant increase in hepatic G6Pase was recorded from the 3rd to the 5th day (P = 0.009), and from the 3rd to the 6th (P = 0.046) day, whereas in the

control group a statistically significant rise was found between the 4th and 5th (P = 0.02) day. No significant difference was found when daily obtained results of G6Pase in the kidney of fasted ducklings were analysed, whereas in the kidney of control ducklings a statistically significant difference was recorded between the 5th and 6th (P = 0.03) day.

Table 3. Effect of fasting on hepatic and renal fructose-1.6-bis-phosphatase (FDPase) activity

Days of experiment	Variable	FDPase activity (nmolP/mg protein/min)			
		Liver		Kidny	
		Control	Fasted	Control	Fasted
3	median	102.48	95.80	126.09	106.51
	quartiles	69.28-108.11	94.19-117.69	98.08-166.64	96.05-135.79
4	median	63.47	106.98[a]	110.00	181.95[b,A]
	quartiles	54.76-89.76	79.10-143.51	92.17-143.87	114.84-203.98
5	median	70.68	94.05	95.99	168.48[B]
	quartiles	64.43-100.43	70.28-125.73	81.73-168.03	133.68-195.71
6	median	102.05	130.82	135.39	170.09[c,C]
	quartiles	53.94-122.55	92.18-142.32	102.28-147.6	141.61-205.85

Level of significance between control (n = 7) and fasting (n = 8) groups: [a]P = 0.0491; [b]P = 0.0279; [c]P = 0.0373.
Level of significance according to the 3rd fasting day: [A]P = 0.009; [B]P = 0.01; [C]P = 0.01.

During the six days of the experiment, fluctuation of hepatic FDPase activity was observed, with a significant difference between the fed and fasted ducklings on the 4th (P = 0.0491) day (Table 3). Analysis of hepatic FDPase activity showed no statistically significant differences when daily obtained results were compared in the same (experimental or control) group. In fasted animals, renal FDPase activity increased almost 70% between the 3rd and 4th fasting day, and this increased activity persisted up to the end of the fasting period, with significant differences between the fasted and control group on the 4th (P = 0.0279) and 6th (P = 0.0373) days. When the fasting group of ducklings were mutually compared, a statistically significant increase in renal FDPase was recorded between the 3rd and 4th

(P = 0.009), 3rd and 5th (P = 0.01) and 3rd and 6th (P = 0.01) experimental days. There was no statistically significant difference among control group animals.

Table 4. Effect of fasting on hepatic and renal phosphoenolpyruvate carboxykinase (PEPCK) activity

Days of experiment	Variable	PEPCK activity (nmol/mg protein/min)			
		Liver		Kidney	
		Control	Fasted	Control	Fasted
3	median	124.00	136.00	35.00	37.00
	quartiles	108.00-139.00	115.00-148.50	32.00-39.00	31.50-38.50
4	median	82.00^{A}	95.00^{A}	23.00^{C}	31.00^{b}
	quartiles	67.00-91.00	80.50-108.00	19.00-26.00	26.50-34.00
5	median	138.00^{B}	78.00^{a}	30.00	39.00
	quartiles	130.00-157.00	65.00-89.50	27.00-32.00	27.00-52.00
6	median	132.00	109.50^{B}	31.00	32.00
	quartiles	98.00-136.00	84.00-121.00	26.00-35.00	30.00-35.50

Level of significance between control (n = 7) and fasting (n = 8) groups: $^{a}P = 0.0012$; $^{b}P = 0.032$.
Level of significance according to the preceding control value: $^{A}P = 0.008$; $^{B}P = 0.002$; $^{C}P = 0.006$.
Level of significance between fasting days: A = significantly different from the 3rd day of the experiment (P = 0.02); B = significantly different from the 5th day of experiment (P = 0.03).

In comparison with controls, cytosolic PEPCK activity in the liver of fasting ducklings significantly decreased on the 5th (P = 0.0012) fasting day, while on other days of the experimental period it was near the level of control group (Table 4). Renal cytosolic PEPCK activity was significantly higher in four-day fasted animals (P = 0.032) than in fed controls. Analysis of liver PEPCK activity in fasted ducklings between consecutive periods showed a statistically significant decrease from the 3rd to the 4th (P = 0.02) day, and an increase from the 5th to the 6th (P = 0.03) day. Comparison of PEPCK activity in the control group yielded a statistically significant decrease between the 3rd and 4th (P = 0.008) day, and an increase between the 4th and 5th (P = 0.002) experimental day. Renal PEPCK activity of

fasting ducklings showed higher values in all measurements, with a significant difference in control ducklings at the 4th experimental day. When the control group of ducklings were mutually compared, a statistically significant decrease was recorded between the 3rd and 4th ($P = 0.006$) experimental day.

Discussion

The results indicate that the blood glucose concentration in fasting ducklings was well maintained throughout the experiment, showing changes similar to those in control ducklings. On the 3rd day blood glucose level was lower in both fed and fasted animals compared with days 4, 5 and 6, when it was 6-7 mmol/L. Prior to elaborating the issue, the influence of growing on the blood glucose level in birds should be considered. Namely, plasma glucose levels in chicken after hatching tend to increase for several weeks or even months, reaching values that are characteristic for adult birds fed *ad libitum* (STURKIE, 1986). In ducklings, however, a significant decrease in blood glucose occurs after hatching, reaching lowest values on the 21st day of life. This fall is followed by a continuous rise until the 42nd day of life, whereafter an abrupt decrease re-occurs (BARANYIOVA and HOLUB, 1969). Thus, the finding of lower blood glucose in 31- day-old control ducklings than in subsequent days of the experiment is in agreement with the mentioned physiological pattern of blood glucose in ducklings. Furthermore, the blood glucose concentration of three- day fasting ducklings showed no significant changes in relation to controls, indicating that the ducklings maintained their blood glucose at the control group level, i. e. at the age-adjusted physiological level, on the 3rd day of fasting. The concentration of glucose in fasting animals during the next three days of experiment was stable (6-7 mmol/L), although on days 4 and 6 glucose levels measured in these animals were significantly lower than those found in the control group. Again, the fluctuation pattern of glucose was similar between control and fasted animals during the whole experiment, although a lower concentration was measured in fasting ducklings. The question of the contribution of the processes of gluconeogenesis to the stable glycemia of fasting ducklings in the present study now arises. Generally, in birds after hatching dietary composition

switches from high lipid, low carbohydrate to low lipid, high carbohydrate, thereby reducing the demand on the gluconeogenic pathways. It is estimated that G6Pase activity decreases more than 60% from hatch out time to adulthood, and FDPase by at least 50%, indicating that birds lose the ability of glucose recovery from non-carbohydrate sources (STURKIE, 1986). We found homogeneous activities of all gluconeogenic enzymes in the control group throughout the experiment, suggesting that no major decrease occurred in the capacity of glucose recovery in ducklings after four weeks of age.

The measurement of gluconeogenic enzyme activities in the experimental group of ducklings showed an increasing tendency in the activities of G6Pase and FDPase in the liver and kidneys, which reached statistical significance when compared with the control group. The increased activity of these enzymes points to the activation of the process of gluconeogenesis during starvation, which must have contributed considerably to the maintenance of glucose level in the blood of these animals. The measured rise in gluconeogenic enzyme activity become more important in the light of the finding of drastically decreased liver mass to Ľ pre-fasting value. Similar results of measuring G6Pase and FDPase activity were observed in the liver and kidney of starved chicken (SHEN and MISTRY, 1979; DONALDSON, 1973; TANAKA et al., 1984). Gluconeogenesis is responsible not only for the rise of glucose in blood but also partly for the regeneration of liver glycogen (HAZELWOOD and LORENZ, 1959). MILINKOVIĆ-TUR et al. (1996) found a fluctuated level of liver glycogen content during six days of food deprivation in duckling. Since hepatic G6Pase is involved in the processes of glycogenolysis and gluconeogenesis, its higher activity in the liver cannot with certainty be attributed to the process of gluconeogenesis.

The activity of liver PEPCK in the experimental group showed an initially high increase in comparison to the control group, followed by a significant decrease on the 5th day of fasting. The quite high hepatic PEPCK levels without increased activity during fasting resembled the pattern seen in chicks (BRADY et al., 1978; HAMADA and MATSUMOTO, 1984; TANAKA et al., 1984). Renal PEPCK activity of fasting ducklings showed higher values in all measurements compared with control values, reaching the highest point

on the 4th experimental day. But the statistically significant higher activity of renal PEPCK in four-day fasted ducklings, in relation to controls, could be a consequence of extremely low renal PEPCK activity determined in control ducklings at this examination period. Numerous authors have confirmed that during food deprivation in chicken renal PEPCK activity increases (SHEN and MISTRY, 1978; WATFORD, 1985). Intracellular location of PEPCK and utilization of different gluconeogenic precursors in the present study were not examined, but the determined pattern of PEPCK activity in ducklings during food deprivation probably influenced the utilization rate of gluconeogenic precursors. It is assumed, because of the intracellular location of PEPCK in chicken hepatocytes (almost totally mytohondrial), and consequently the lack of cytosolic reducing equivalents, that there is low utilization of the citric acid cycle intermediates for *de novo* glucose formation (BANNISTER and O'NEIL, 1981). Moreover, SOLING et al. (1973), and SARKAR (1971) have confirmed that in chicken the liver functions in gluconeogenesis to recycle lactate carbon (Cori cycle). The kidney, in contrast, possesses a cytosolic form of PEPCK that adapts to dietary stimuli (SHEN and MISTRY, 1978; WATFORD, 1985). In chicks and pigeons, because of the presence of PEPCK in cytosol, kidney is a major organ for net gluconeogenesis from a substrate such as amino acids, pyruvate and glycerol (WATFORD et al., 1981; TINKER et al., 1983). Stated facts of PEPCK intracellular location and their physiological implication could help us to explain the opposite changes in PEPCK activity between liver and kidney of fasting ducklings. Our results of permanently higher PEPCK activity in kidney during food deprivation implied that renal PEPCK in ducklings, like in chicken and pigeon, could adapt to fasting conditions. Contrary to this, liver PEPCK in fasted ducklings, similarly to chicken and pigeon, did not adapt to fasting conditions. Moreover, a significant decrease of PEPCK activity on day 5 was noted. We may assume that the different intracellular location of liver and renal PEPCK in ducklings was similar to that found in chicken and pigeon. Thus, the opposite changes in the activities of PEPCK in the liver and kidney of fasted ducklings might be the consequence of different intracellular PEPCK location and different utilization rate of gluconeogenic precursors. However, we must not exclude the possibility that the noted inconsistent decrease of PEPCK activity in the liver could be a consequence of inaccurate measuring. Further opposite

changes in the enzyme activities of fasting ducklings were found at the 5th experimental day between G6Pase and PEPCK in the liver. As was mentioned earlier, G6Pase is involved in the processes of gluconeogenesis and glycogenolysis, and its high activity in duckling's liver originated from both processes. This fact is concomitant with discussed reducing capabilities of liver for gluconeogenic precursor utilization, resulting in the noted opposite pattern of PEPCK and G6Pase activity in the liver of fasting ducklings.

In conclusion, the results of glucose concentration and gluconeogenic enzyme activities suggest that the ducklings, just like another avian species, e. g. chicken, are able to maintain constant glucose concentration during six days of fast. In addition to the strong efforts of liver to maintain a normal blood glucose concentration, the extensive renal gluconeogenesis also has an important role in the maintenance of blood glucose.

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SAŽETAK

Istraživana je koncentracija glukoze u krvi i aktivnost glukoneogenih enzima u jetri i bubregu pekinških pačića (English heavy breed Peking Duck) tijekom šestodnevnog gladovanja, koje je započeto s navršena četiri tjedna života. Normalno hranjeni pačići služili su kao kontrolna skupina. Pačići su bili žrtvovani dekapitacijom nakon trećeg, četvrtog, petog i šestog dana istraživanja, a krv i uzorci tkiva za analize sakupljani su istovremeno od obje skupine. Tijekom istraživanja, osim trećeg dana, koncentracija glukoze u krvi kontrolne i pokusne skupine bila je veća od 6 mmol/L. Značajan porast aktivnosti glukoze-6-fosfataze (EC 3.1.3.9) (G6Pase) u jetri ustanovljen je četvrtog, petog i šestog dana gladovanja ($P = 0.0012$, $P = 0.019$, $P = 0.0279$), a u bubrezima petog i šestog dana gladovanja ($P = 0.0373$, $P = 0.0206$). Aktivnost fruktoze-1,6-bis-fosfataze (EC 3.1.3.11) (FDPase) u jetri bila je značajno veća u pokusne nego u kontrolne skupine samo četvrtog dana gladovanja ($P = 0.0491$), dok je u bubrezima porasla četvrtog ($P = 0.0279$) i šestog ($P = 0.0373$) dana gladovanja. Aktivnost fosfoenolpiruvat karboksikinaze (EC 4.1.1.32) (PEPCK) u jetri pačića značajno je pala petog dana gladovanja ($P = 0.0012$), dok je u bubrezima značajno porasla četvrtog dana gladovanja ($P = 0.032$). Rezultati su pokazali da pačići s navršena četiri tjedna života mogu održavati koncentraciju glukoze u krvi tijekom šestodnevnog gladovanja. Promjene u aktivnostima glukoneogenih enzima upućuju na važnu ulogu procesa glukoneogeneze u jetri i bubrezima za održavanje normoglikemije pačića koji gladuju. Takvi nalazi slažu se s dobro poznatim podacima za piliće, što pokazuje da postoji sličnost između te dvije ptičje vrste u održavanju razine glukoze u krvi procesom glukoneogeneze tijekom dužeg gladovanja.

Ključne riječi: metabolizam ugljikohidrata, pačići, gladovanje, glukoneogeni enzimi