

# GLUCAGON INSENSITIVITY OF GLYCOGEN DEPLETED LIVER IN BILE DUCT LIGATED GUINEA PIGS

Atilla ENGİN, M.D., Ercüment Hayri TEKİN, M.D., Emin ERSOY, M.D.,  
Burhanettin Şükrü BOZKURT, M.D.

Gazi University, Faculty of Medicine, Department of General Surgery, Ankara, Turkey  
Gazi Medical Journal 6 : 165-167, 1995

**SUMMARY :** Plasma glucose, glucagon and insulin concentrations and liver glycogen levels were determined in guinea pigs with common bile duct obstruction.

Fasting blood glucose concentrations were within the normal range in both test and control groups. Significant hyperglucagonaemia was documented ( $p < 0.001$ ) despite euglycaemia in animals with extrahepatic cholestasis. However, a considerable decrease in basal glycogen values of hepatocytes was observed in these animals ( $p < 0.001$ ). Our data regarding the level of immunoreactive insulin and the rate of glucose utilization revealed that peripheral glucose consumption increased in guinea pigs with bile duct ligation.

Thus, it is apparent that the well-accepted feed-back mechanism between plasma glucose and glucagon secretion is broken down, but there is no alteration in hormone-receptor interaction in hepatocytes of these animals.

**Key Words :** Bile Duct Obstruction (Extrahepatic), Cholestasis, Blood Glucose.

## INTRODUCTION

Cessation of the bile flow by mechanical obstruction of the extrahepatic biliary tree results in regurgitation of bile from the canalicular system into the systemic circulation (1, 4). In vitro studies on cholestatic liver cells suggested that reabsorption of bile salts changes membrane fluidity in the submembraneal zone, and a loss of hormonal control of glucagon receptors in the liver could contribute to disturbed glucose homeostasis (6, 11). In vivo studies in rabbits revealed that bile duct obstruction causes four fold elevation of plasma immunoreactive glucagon levels, but has little influence on plasma immunoreactive insulin and blood glucose (9). Ho-

wever, there is very limited data regarding the hormonal sensitivity of receptors and changes in hormone levels related to the glucose-glycogen metabolism. In this study, the effect of bile duct obstruction on plasma immunoreactive glucagon and insulin levels and its relationship between peripheral glucose consumption and liver glycogen was investigated in guinea pigs.

## MATERIALS AND METHODS

The experiment was conducted on 69 male guinea pigs weighing 400-450 g. They were fed on a standard supplemented cereal diet fortified with vitamin C and housed in individual cages in a heat controlled environment. All animals were starved

for 18 hours before the experiments. Pentobarbital sodium, 4 mg/100 g, was administered i.p. to induce general anaesthesia. Laparotomy was performed through an upper midline incision in sterile conditions. In the bile duct ligated group (BDL) the common bile duct was identified at its entrance to the duodenum and ligated at that junction with a 5-0 silk suture. cholecystectomy was performed synchronously. In the SHAM operated group only laparotomy was performed and the guinea pigs were included in the experiments after a postoperative period of 72 hours.

This experiment was carried out on three groups of guinea pigs. In the first group seventy-two hours after surgical intervention, BDL (n : 13) and SHAM (n : 10) animals were sacrificed by cervical dislocation. Liver samples were removed and immersed immediately in liquid nitrogen and the liver glycogen levels were determined by the method of Orrego (7). The histopathological evidence of bile regurgitation was observed in the postoperative 72 hours. For histological examination, liver specimens were fixed and stored in neutral buffered 10 % formalin and embedded in paraffin. Paraffin sections were stained with haematoxylin and eosin.

A histological scoring system was applied based on the presence, extent and severity of necrosis. Blind quantitation of liver cell necrosis was performed using the scale of Orrego et al : 0, no necrosis; +1, focal necrosis of one or two lesions; +2, focal necrosis of more than two lesions; +3, massive centrilobular necrosis; +4, massive centrilobular necrosis plus necrotic bridging between central veins (8). In the second group, BDL (n : 12) and SHAM (n : 12) guinea pigs were used for detection of pancreatic insulin and glucagon levels by radioimmunoassay. In this study, DPC Coat-A-Count TKIN 1 was used for insulin, Glucagon Kit (10904) Biodata was used for glucagon measurements. In the third group, basal plasma glucose levels were determined and intravenous glucose tolerance tests (IVGTT) were done on BDL (n : 10) and SHAM

(n : 10) animals (7). Blood glucose levels were determined by the Somogy-Nelson micromethod (2) and liver glycogen levels by the glucose oxydase method (2).

All data are expressed as mean  $\pm$  SEM and for the statistical analysis Student's t test was used.

## RESULTS

As shown in Table 1, plasma glucose was not significantly different in BDL animals and SHAM guinea pigs irrespective of exhausted liver glycogen.

Despite the normal levels of blood glucose in the BDL group, average liver glycogen was markedly depleted. There was no significant relationship between plasma glucose and the degree the hepatic parenchymal cell damage. However a definite relationship was observed between plasma glucagon and the severity of liver necrosis in BDL animals. In BDL group +3, +4 necrosis was established using the cell necrosis scale. Glucose utilization rates were calculated as 1.24 mg/dl/min and 0.63 mg/dl/min in BDL and SHAM subjects respectively (Table 2). In the BDL group mean plasma insulin level was  $11.9 \pm 1.2$  micro IU/ml (Table 1). This high value was in accordance with the high glucose utilization rate in these cholestatic animals.

It has been shown that bile salts alter hormone-receptor interaction, thus leading to the impairment of hormonal control in isolated liver cells from bile duct ligated animals (10, 11). We found normal blood glucose levels in the presence of extremely low liver glycogen levels in BDL guinea pigs ( $p < 0.001$ ). Moreover, plasma immunoreactive glucagon was found to be 6.8 times higher in comparison with the controls ( $p < 0.001$ ). It is known that glucagon primarily binds to the specific receptors of liver cells and causes glycogenolysis (3). In this case, decreased liver glycogen could be due to postoperative stress leading to glycogen depletion. Thus, glucagon addition did not result in an increase of glucose release in cells from cholestatic livers

Group	SHAM	BDL
Blood Glucose (mg/dl)	$114.5 \pm 3.6$	$112.7 \pm 3.9$
Blood Glucagon (pg/ml)	$147.8 \pm 38.4$	$1014 \pm 119.6$
Liver Glycogen (mg glucose/g wet weight)	$19.28 \pm 1.46$	$0.96 \pm 0.61$
Blood Insulin (IU/ml)	$5.6 \pm 0.3$	$11.9 \pm 1.2$

Table 1: Average blood glucose, glucagon and insulin levels and mean liver glycogen values in SHAM and BDL animals (Data are mean  $\pm$  SEM).

Group	t2	t10	t20	t40	t60
SHAM k : 0.63	320.2 ± 9.9	264.4 ± 8.6	239.2 ± 8.4	204.2 ± 9.5	170.8 ± 9.4
BDL k : 1.24	319.8 ± 9.5	268.4 ± 10.4	232.9 ± 6.4	178.3 ± 5.1	146.6 ± 6.2

Table 2 : Average blood glucose (mg/dl) levels and glucose utilization rates (k : mg/dl/min) in SHAM and BDL guinea pigs (Data are mean ± SEM).

regardless of the duration of cholestasis (11).

In previous studies, expected glucagon response could not be demonstrated because of the considerable fall in liver glycogen level in BDL animals (3, 5, 9). Decreased insulin/glucagon ratio may represent the stimulation of gluconeogenic activity that is most probably responsible for maintaining the basal blood glucose in normal ranges. The glucose utilization rate was approximately two times higher in BDL guinea pigs than controls. Also, plasma insulin levels were relevant to this increased rate ( $p < 0.001$ ).

The pathogenesis of hyperglucagonaemia in BDL animals is not clearly defined. These data suggested that the lack of feedback between glucose and glucagon may be a result of the failure of hepatic response to circulating glucagon, a state similar to a state of insulin resistance. However we believe that the insensitivity to glucagon in the BDL group may be secondary to depleted hepatic glycogen stores.

In conclusion, the present study elucidated that 72 hours after common bile duct obstruction, the feed-back control between plasma glucose and glucagon secretion is broken down without any alteration in the sensitivity to glucagon.

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Correspondence to : Dr. Atilla ENGİN  
Gazi Üniversitesi Tıp Fakültesi  
Genel Cerrahi Anabilim Dalı  
06500 ANKARA - TÜRKİYE  
Phone : 312 - 214 10 00 / 5707