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Changes in circulating glycerol, free fatty acids and glucose levels following liver transplant in the pig

BY

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(2 figures)

Female pigs, fasted overnight, received an orthotopic liver transplant. During the unhepatic phase, both blood glycerol and plasma free fatty acid concentrations increased, returning to basal values after the transplant, indicating that the liver is the main receptor of these products released in the blood from the glyceride breakdown in peripheral fat deposits. Blood glucose level rose during the unhepatic phase, probably due in part to the perfusion of glucosated saline received by the animals during this phase. After liver transplant, blood glucose levels progressively decreased and this effect was greatly reduced by administering L-alanine. Our data indicate that metabolic changes in the donor's liver diminish the availability of gluconeogenetic substrates immediately following transplant, while administration of exogenous alanine permits faster restoration of gluconeogenetic function in the transplanted liver.

Introduction

The liver plays an important role in both glucose- and fat metabolism (LUNDQUIST & TYGSTRUP, 1974), being the main organ involved in glucose synthesis and glycerol- and fatty acid utilization. It has been shown that evisceration produces a rapid increase in free fatty acids (FFA) concentration (PENHOS *et al.*, 1976; CARMANIU & HERRERA, 1979) and glycerol (CARMANIU & HERRERA, 1980) and a decrease in that of glucose (RUSSELL, 1942; CARMANIU & HERRERA, 1980). Most studies of reduced hepatic function have been performed in the rat (RUSSELL, 1942; LUNDQUIST & TYGSTRUP, 1974; PENHOS *et al.*, 1976; CARMANIU & HERRERA, 1979 & 1980; KATZ *et al.*, 1979) but the pig has been used as a model for orthotopic liver transplant in humans (CAMPRODÓN *et al.*, 1973 & 1974 *a & b*). The present investigation was performed in female pigs to study the circulation changes in glucose, FFA and glycerol during the unhepatic phase and after liver transplant. Because hypoglycemia occurred after liver transplant, our work was extended to determine the effect on blood glucose levels of perfusion with alanine, a main precursor of glucose synthesis in the liver (GOLDBERG *et al.*, 1978; SNELL & DUFF, 1980).

Materials and Methods

Female pigs weighing 20 to 28 kg were anaesthetized with halophane (CAMPRODÓN *et al.*, 1974*a*) after an overnight fast, to receive an orthotopic liver transplant from sex and age matched donors according to the technique of CAMPRODÓN *et al.* (1973 & 1974*a* & *b*). During the unhepatic phase the receptor animal was perfused with a total of 700 ml of glucosated serum (3.3 g glucose and 0.3 g NaCl/dl) through a jugular vein. The liver was preserved for up to 60 min in ice after infusion through the portal vein of 2.0 litres of cold (4 °C) heparinized saline (5 000 IU heparine/litre). After 15 min of end to end portal vein anasthomosis (following the liver transplant), some of the animals were intravenously perfused with 5 g/min of L-alanine (Sigma, St Louis, Mo.). Intact control animals were studied in parallel, undergoing the same anaesthesia, laparotomy and organ handling. Blood samples were collected from the external jugular vein into heparinized tubes at different times beginning 10 min before the unhepatic phase. Aliquots of whole blood were rapidly deproteinized with Ba $(OH)_2$-$ZnSO_4$ (SOMOGYI, 1945) and after centrifugation, supernatants were kept frozen

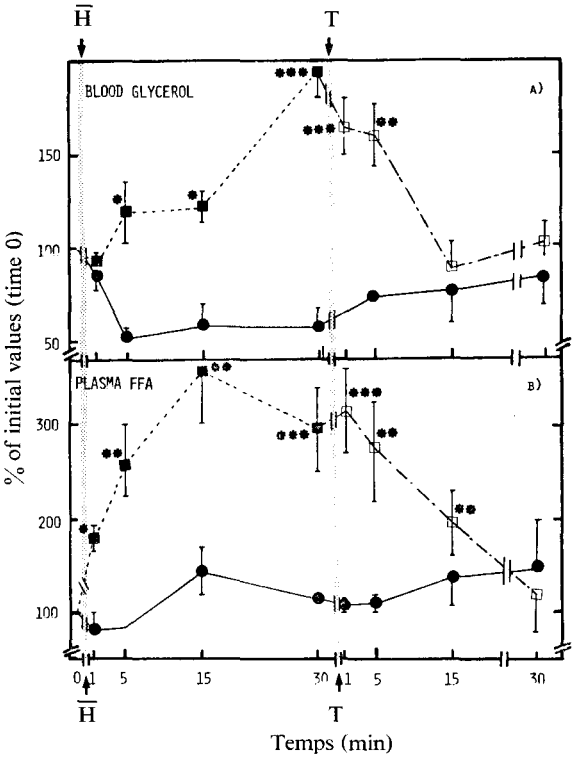

FIG. 1. *Blood glycerol (Fig. 1A) and plasma FFA levels (Fig. 1B) following liver transplant in the pig.*
Means ± SEM of 8 animals in each group.
Basal absolute values (time 0) : 10 ± 2 μmol/dl, for glycerol
360 ± 94 μEq/litre, for FFA.
●——● : Sham-operated controls.
■- - - - - - ■ : Unhepatic phase (H̄ = hepatectomy).
□ .-.-.-.□ : Post-transplant (T = transplant).
P vs. time 0 : * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

until processing for glucose (HUGGETT & NIXON, 1957) and glycerol (GARLAND & RANDLE, 1962) evaluation by enzymatic procedures. Other blood aliquots were centrifuged immediately at 3 000 rev./min for 10 min at 4 °C and plasma aliquots were used for the analysis of FFA concentration (FALHOLT *et al.*, 1973). Calculations were performed by considering the basal values as the mean of the two last blood samples collected prior to the unhepatic phase. Other values were expressed as percentages of the basals for each animal. Statistical comparisons were made with the Student's *t* test.

Results

Basal values of glycerol and FFA in the pig (Figs. 1A and 1B) were similar to those found in the normal rat (CARMANIU & HERRERA, 1979 & 1980). During the unhepatic phase in the pig, there was a rapid and significant increase in concentrations of both blood glycerol (Fig. 1A) and plasma FFA (Fig. 1B), and 5 to 15 min after the liver transplant, glycerol- and FFA levels returned to basal values. During the first 5 min of the unhepatic phase (Fig. 2), blood glucose levels rose, returning to basal values at the 15 min time. Blood glucose levels decreased significantly and progressively to below the basal level from the 5 min after the end to end portal vein anasthomosis. This hypoglycemia after liver transplant disappeared by perfusing the transplanted pigs with L-alanine (Fig. 2). None of the described modifications

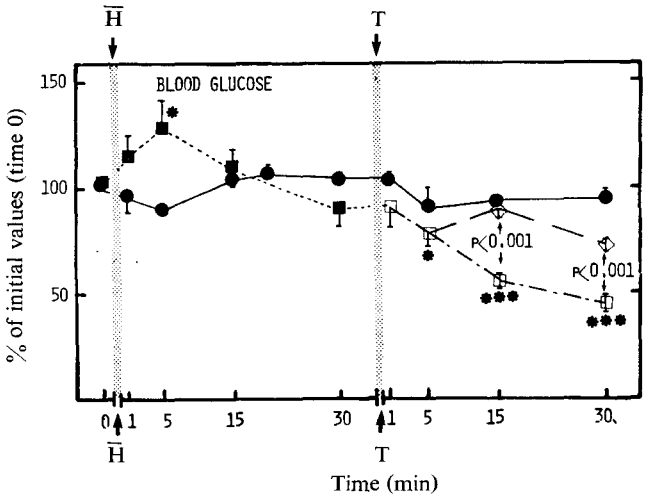

FIG. 2. *Blood glucose levels following liver transplant in the pig. Effect of alanine perfusion after liver transplant.*

Means ± SEM of 4-8 animals in each group.
Basal values (time 0) : 57 ± 8 mg/dl.
●——● : Sham-operated controls.
■- - - - - - ■ : Unhepatic phase ($\bar{H}$ = hepatectomy).
□ .-.-.-.□ : Post-transplant no receiving alanine (T = transplant).
◇- - - - - ◇ : Post-transplant, perfused with L-alanine from min 15 to min 30 after transplant (5 g/min).
P vs. time 0 : * = $P < 0.05$, *** = $P < 0.001$.
Comparison between animals receiving alanine and those no receiving it is shown by the *P* values in the Figure.

could be attributed to the anaesthesia or to surgical interventions because they were not observed in the sham-operated control pigs (Figs 1A, 1B, & 2).

Discussion

Present results show that, in the pig, the liver constitutes the main receptor of circulating FFA and glycerol, presumably originating mainly from glyceride release in the peripheral fat deposits. Similar results were previously found in eviscerated rats (CARMANIU & HERRERA, 1979 & 1980). Increased lipolysis from adipose tissue during reduced hepatic function (PENHOS *et al.*, 1976; PECTOR *et al.*, 1978) may contribute to the rise in circulating glycerol and FFA. This interpretation would be valid for results of kinetic studies in eviscerated rats (CARMANIU & HERRERA, 1979) but the fact that liver transplant in the pig reduced the circulating levels of both substances indicates the important role of the liver in the continuous handling of these products of peripheral glyceride breakdown. This reducing action of transplanted liver appears after a short delay (about 5 min) during which both glycerol- and FFA blood levels remain high. The lag period may be caused by metabolic damage to the liver during preservation time. Liver uptake of both glycerol and FFA requires an active metabolic state favouring their activation (BREMER *et al.*, 1974; LIN, 1976) for subsequent utilization.

The observed changes in blood glucose levels during the unhepatic phase are difficult to interpret because at this time the animals need to receive 700 ml of glucosated serum to avoid circulatory problems (CAMPRODÓN *et al.*, 1974*b*). This glucose perfusion, together with the compensatory production of glucose by the kidney cortex which is known to be active during reduced hepatic function (RUSSELL, 1942; KATZ *et al.*, 1979) may be responsible for the hyperglycemia during the unhepatic phase. The progressive reduction of blood glucose levels observed after liver transplant suggests that the transplanted liver initially behaves more as a glucose consumer than as an exporter. The preservation time apparently alters the endogenous pool of amino acids in the donor's liver, producing important changes in the circulating levels of amino acids after its transplant (HERRERA *et al.*, 1979). These changes could limit the availability of gluconeogenetic substrates in the recently transplanted liver, as indicated by the rise in glycemia following alanine perfusion.

Our findings demonstrate the importance of alanine as a main gluconeogenetic substrate, in agreement with recent proposals (GOLDBERG *et al.*, 1978; SNELL & DUFF, 1980) and indicate a more efficient method of postoperative treatment following liver transplant to achieve faster recuperation of liver function.

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