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Liver glycogen in fasted rat livers does not improve outcome of liver transplantation

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Abstract Controversy exists over how the nutritional condition of the donor liver affects transplant outcome. Some studies suggest that livers from fasted animals (liver glycogen-depleted) are more readily injured than livers from fed animals. Our previous study suggested the opposite, i.e., livers from donors fasted for 4 days were significantly more viable on orthotopic liver transplantation. Fasting may decrease the sensitivity of the liver to an inflammatory response or block Kupffer cell activation following transplantation. Thus, long-term fasting may be beneficial for reasons unrelated to liver glycogen content. In this study we attempted to separate out the roles of fasting and liver glycogen in liver transplant outcome by fasting donors for 2 days and then feeding them only glucose to elevate liver glycogen. Rats (Brown Norway) were fed (standard diet), fasted (4 days), or fasted 2 days and then fed glucose (in water) for 2 days. Livers were preserved for either 30 or 44 h in UW solution and transplanted. Four-day fasting of the donor improved the survival rate in liver transplantation (50 %–100 % in 30-h cold storage, 29 %–83 % in 44-h cold storage). However, feeding glucose for 2 days to fasted animals caused a decrease in survival in this series of transplants (40 % in 30-h cold storage, 0 % in 44-h cold storage). In the glucose-fed group, liver glycogen was 240 % of that in the control group. This suggests that the presence of a high concentration of liver glycogen is not beneficial to the preserved and transplanted rat liver.

Key words Liver transplantation, rat, fasting · Glucose, liver transplantation, rat · Liver glycogen, rat, transplantation

Introduction

Controversy exists over the effect of the nutritional condition of the donor liver on transplant outcome [3, 9, 12]. Some studies suggest that livers from fasted animals (liver-glycogen depleted) are more readily injured than livers from fed animals [1, 6, 10]. Our previous study suggested the opposite [14] i.e., that livers from donors fasted for 4 days were significantly more viable on orthotopic transplantation. Fasting may decrease the sensitivity of the liver to an inflammatory response or block Kupffer cell activation following transplantation. Thus, long-term fasting may be beneficial for reasons unrelated to liver glycogen content. In this study, we attempted to distinguish the roles of fasting and liver glycogen in liver transplant outcome by fasting donor rats for 2 days, then feeding them only glucose to elevate liver glycogen.

Materials and methods

Inbred Brown Norway rats weighing 150–250 g were used as donors and recipients. Donor rats were divided into three groups: (1) a fed group, in which donor rats were fed with standard laboratory chow, (2) a fasted group, where food was withheld for 4 days

and water given ad libitum, and (3) a glucose-supplemented group, in which food was withheld for 4 days and water was given for the first 2 days, after which water containing 40 % dextrose was given for another 2 days. Recipient rats were fed standard laboratory chow.

The methods of orthotopic liver transplantation and liver preservation were identical to those described previously [5]. For the animal experiments, the 'Principles of laboratory animal care' (NIH Publication No. 86–23, revised 1985) were followed, as well as the regulations of the Animal Research Laboratory of Hiroshima University School of Medicine. Donor livers were skeletonized and flushed in situ with 3–5 ml of cold University of Wisconsin (UW) solution via the portal vein. After cuff preparation, livers were stored for 30–44 h. At the end of storage, the livers were reflushed with 3–5 ml of cold Ringer's lactate solution and transplanted orthotopically. The 1-week survival rate of recipients was compared with respect to the three groups of donors. Recipient rats were bled 6 h after transplantation for measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) by methods used in the clinics of the University of Wisconsin Hospital.

Serum glucose levels and free fatty acid were examined during 4 days of donor fasting and glucose supplementation. Hepatic glycogen content was measured in livers from the fed, fasted, and glucose-supplemented groups before and after preservation (24 and 48 h), by the enzyme hydrolysis method using amyloglucosidase, and the amount of glycogen was expressed as mg glycogen per g liver tissue. Liver adenosine triphosphate (ATP) content was measured in livers from the three groups before and after 24- and 48-h preservation using high-performance liquid chromotography (HPLC) [13]. Liver lactate content was also measured in livers from the three groups before and after cold storage.

Results

Hepatic glycogen and ATP content of rats in the three donor groups before and after liver preservation are shown in Figs. 1 and 2, respectively. Livers from normal, fed rats contained 177 ± 25 mg glycogen per g liver tissue. This value decreased to 48.2 ± 25.8 and 26.6 ± 4.8 mg/g liver tissue following 24 and 48 h of preservation, respectively. Livers from rats fasted for 4 days included only 31.8 ± 18 mg/g liver glycogen, and this decreased to 1.7 ± 0.8 mg/g after 24-h preservation and to 3.9 ± 0.6 mg/g after 48-h preservation.

Glucose supplementation in fasted rats elevated the hepatic glycogen content to 426 ± 52 mg/g liver, which was almost 2.5 times the concentration in livers from normal, fed rats. The level in this group also decreased to 275 ± 28.5 mg/g liver after 24-h preservation and to 261 ± 58.6 mg/g liver after 48-h preservation.

Hepatic ATP content in fed rats was 1740 ± 230 nmols/g liver, and this level decreased to 120 and 67.5 ± 40 nmols/g liver after 24-h and 48-h preservation, respectively. Livers from rats fasted for 4 days contained 630 ± 100 nmol/g liver of ATP. This further decreased to 74 and 13 ± 0.7 nmol/g liver following 24 and 48 h of preservation, respectively. Glucose supplementation in fasted rats improved the hepatic ATP content to a level similar to that in fed rats, namely, to 1890 ± 250 nmols/g liver. This decreased to 127 ± 150 nmols/g liver and 24 ± 14 nmols/g liver after 24 and 48 h of liver preservation, respectively.

Hepatic lactate content in fed rats was 750 ± 400 nmols/g liver (Fig. 3). This increased to 4220 ± 220 nmol/g liver after 24-h liver preservation and to 4040 ± 1160 nmol/g liver after 48-h liver preservation, suggesting enhancement of the anaerobic glycolysis pathway. Livers from fasted rats contained 1200 ± 180 nmol of lactic acid per g liver, and this level increased to 2100 ± 680 and 2330 ± 160 nmol/g liver after 24 and 48 h of preservation, respectively. Livers from fasted rats given glucose for 2 days contained 3200 ± 170 nmol of lactate per g liver. This level reached a maximum of 7660 ± 1030 nmol/g liver after 24-h preservation and decreased to 4910 ± 520 nmol/g liver after 48-h preservation.

Serum glucose levels were measured in the fed rats and in rats fasted for 1–4 days (Fig. 4). Serum glucose maintained almost constant values throughout the fasting period. Although the serum glucose level was slightly higher after 2 days of glucose supplementation, there was no significant difference. Serum free fatty acid was measured in the three groups and results are shown in Fig. 5. Serum free fatty acid increased from 586 ± 67 to 856 ± 5.6 nmol/ml after 1 day of fasting and thereafter remained fairly stable throughout the fasting period.

This all suggests that catabolism persists during starvation. However, glucose supplementation after 2 days of fasting lowered the serum free fatty acid level to 255 ± 16 from 867 ± 2.8 nmols/ml, indicating that glucose supplementation halted the catabolism.

Survival (Fig. 6)

In our hands, more than 96 % of the recipient rats survived for over 1 week following fresh liver transplantation. With livers removed from fed donors and preserved for 30 h in cold UW solution, 50 % of the recipient rats survived for 7 days. However, livers from rats fasted for 4 days were all viable after 30 h of preservation, and nine out of nine recipient rats survived for over 1 week. In contrast, when glucose was given to the donor animals for 2 days after 2 days of fasting, only 40 % of the recipient rats survived. Following 44 h of preservation, 29 % of recipient rats survived when the donor livers were obtained from fed animals. However, survival increased to 83 % when the donors had fasted for 4 days. None of the recipient rats survived when glucose was included in the drinking water of the donor rats for 2 days after fasting.

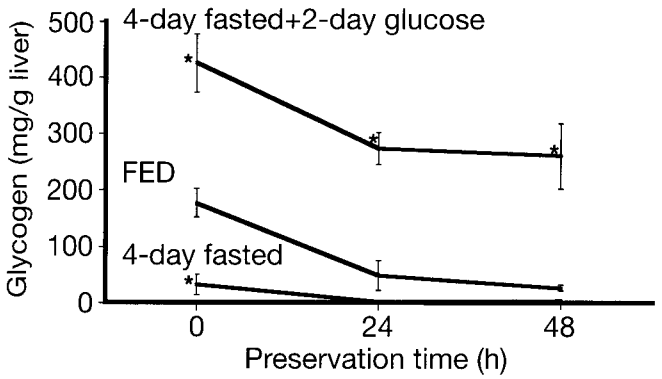

Fig. 1 Hepatic glycogen content before and during liver preservation. Glycogen content was higher in the glucose-supplemented donor group and lower in the fasted group both before and during liver preservation. * $P < 0.01$ vs fed donors

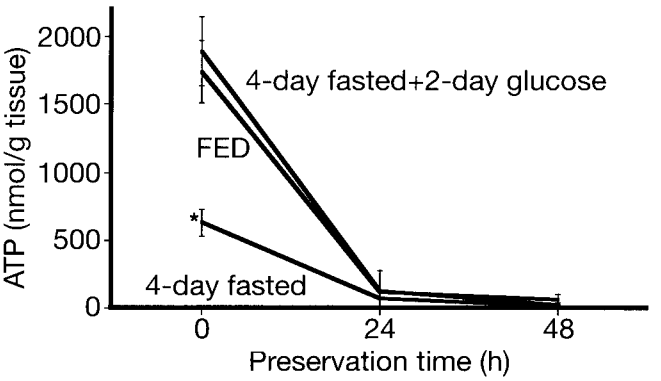

Fig. 2 Adenosine triphosphate (ATP) content in the liver before and during liver preservation. ATP content in the liver was lower in the fasted donor group before preservation. * $P < 0.01$ vs fed donors

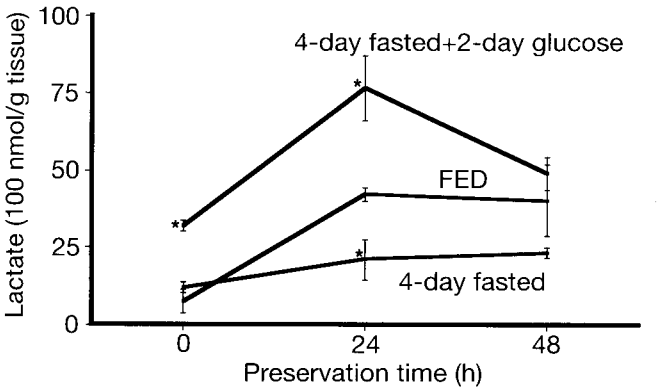

Fig. 3 Hepatic lactate content before and during liver preservation. Lactate content in the liver was higher in the glucose-supplemented donor group and lower in the fasted group during liver preservation. * $P < 0.01$ vs fed donors

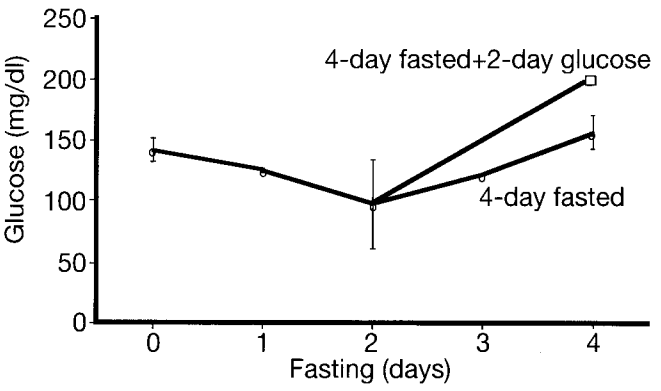

Fig. 4 Serum glucose level during fasting and glucose supplementation. There was no significant difference in serum glucose value between the fasted and glucose-supplemented donor groups

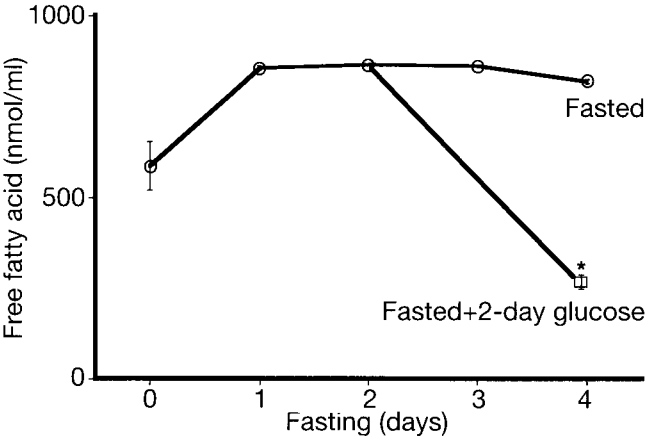

Fig. 5 Serum free fatty acid concentration during fasting and glucose supplementation. Glucose supplementation to fasted donor animals decreased the serum free fatty acid concentration, suggesting the cessation of catabolism.

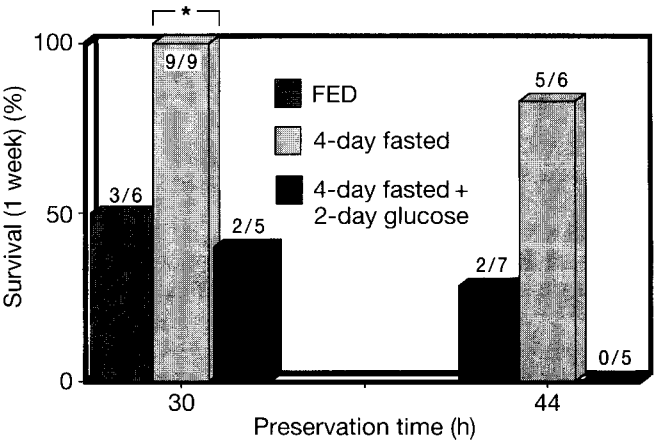

Fig. 6 The 1-week recipient survival rate after 30–44 h of cold liver preservation. Survival was higher in livers from the fasted donor group and lower in those from the glucose-supplemented donor group. * $P < 0.05$ (Fisher's exact test)

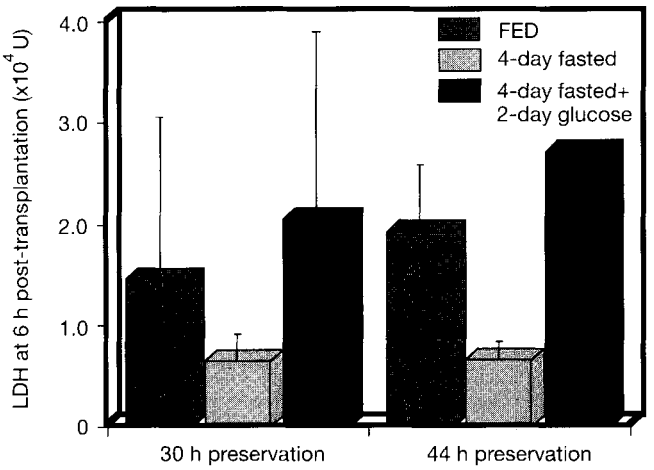

Fig. 7 Serum lactate dehydrogenase (LDH) levels at 6 h after transplantation. LDH serum levels in recipient rats at 6 h after transplantation were higher in those with livers from the glucose-supplemented donor group and lower in those with livers from the fasted group

Liver enzymes (Fig. 7)

As with the survival rates, there were significant differences among the three donor groups in liver enzyme values in recipient rats at 6 h after transplantation. The LDH value at 6 h post-transplant was 14603 ± 16087 U/l in recipient rats that had received 30-h preserved livers from fed donors. However, LDH was markedly suppressed in rats that received livers from 4-day fasted rats following 30 h of liver preservation (6492 ± 2678 U/l). LDH was highly elevated (20587 ± 18455 U/l) in transplanted livers obtained from glucose-supplemented rats. AST and ALT values showed patterns similar to that of LDH (data not shown).

Discussion

In this study, donor fasting depleted the hepatic glycogen and ATP content of the liver to 17 % and 36 % of those in fed donor rats, respectively. In contrast, glucose supplementation elevated hepatic glycogen and ATP levels to 240 % and 109 % of those in normal, fed animals, respectively. The 1-week survival rate of rats that received livers from fed donors was 50 % after 30-h liver preservation and 29 % after 44-h preservation. Livers from rats fasted for 4 days were all viable (9/9, 100 %) after 30-h preservation and 83 % were viable after 44-h preservation. However, survival decreased to 40 % after 30-h preservation and 0 % after 44-h preservation with livers obtained from glucose-supplemented rats. Enzyme levels (LDH, ALT, AST) 6 h after liver transplantation were highest in livers from the glucose supplemented donor group, lowest in livers from the fasted group, and intermediate in those from the fed group. These results show that so-called nutritionally depleted livers were more tolerant of preservation injury than nutritionally replete livers.

It is generally believed that livers from nutritionally deprived animals are more susceptible to ischemic damage than those from nutritionally enriched animals [1, 3, 6, 9, 10, 12]. This is based on the physiological observations that livers from fed animals maintain higher tissue glycogen concentrations during cold preservation than livers from fasted animals, and that cell damage more readily recovers with a rapid supply of energy from glycogen reserves. Many studies [1, 3, 6, 9, 10, 12] have suggested that fasting may sensitize the liver to various forms of injury and could be a cause of primary nonfunction or hepatocellular injury after cold preservation and transplantation. Cywes et al. have shown that intraportal infusion of glucose results in rapid glycogenation and increased glycogen degradation during human donor hepatectomy and improves outcome following liver transplantation [4]. The reason for the discrepancy between our results and those of other studies is not known.

We have recently shown that not only livers, but also hearts and pancreases from rats fasted for 3–4 days are more tolerant of ischemic injury (warm and cold) than those from fed animals [11]. More recently, the beneficial effect of donor fasting on liver preservation has also been confirmed by Lindell et al. using the same experimental model [8]. Neverthless, the mechanisms of the effects of donor fasting on multiple organs are not yet known. Thus, there may be no organ specificity with respect to the effects of donor fasting, and this suggests that fasting may be related to functions of nonparenchymal cells such as endothelial cells or macrophages rather than to those of parenchymal cells. Alternatively, the suppression of cell acidosis may lead to better preservation due to the fact that fasting decreases tissue lactate content during cold preservation, as shown in Fig. 3. At this time, our results at least suggest that the presence of a high concentration of glycogen is not necessarily beneficial to the preserved liver in terms of the suppression of preservation injury.

Recent evidence suggests that nonparenchymal cells may be important mediators of cold ischemic preservation-reperfusion injury [2, 7]. It has been shown recently that sinusoidal lining cell damage following Kupffer cell activation is a major cause of reperfusion injury in the liver. Activated Kupffer cells can cause preservation/reperfusion injury and, conversely, the suppression of Kupffer cells ameliorates this injury. It was also demonstrated that fasting causes macrophage suppression in terms of decreased phagocytosis and division by macrophages. Thus, parenchymal cell function is not crucial and may in fact be masked by the influence of nonparenchymal cell function.

In conclusion, 4-day fasting of donor rats improved the outcome of liver transplantation, while glucose supplementation for 2 days in fasted donors decreased recipient survival in this series of transplants. In the glucose-supplemented donor group, liver glycogen was 240 % of that in the control group. This suggests that the presence of a high concentration of liver glycogen is not beneficial to the preserved and transplanted rat liver. The reason for this is unclear, but our data confirm that manipulation of the nutritional status of the liver donor can have dramatic effects on post-transplant liver function.

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