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Insulin and glucagon levels in living related liver transplantation: their interaction with the recovery of graft liver function

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Abstract Insulin and glucagon have opposite effects on various hepatic functions, including energy metabolism, which is essential for hepatic viability. To evaluate the effects of insulin and glucagon on the recovery of graft liver function, changes in these levels were investigated in relation to arterial ketone body ratio (AKBR) during a 30-h period after graft liver reperfusion in 29 recipients of living related liver transplants. Insulin levels did not change significantly throughout this study, while glucagon levels decreased immediately after reperfusion, indicating a rapid degradation of glucagon by the graft liver. The insulin/glucagon (I/G) ratio increased after reperfusion concomitantly with AKBR. In addition, the I/G ratio was significantly correlated with AKBR after reperfusion. It is concluded that the increase in the I/G ratio was closely related to the recovery of graft liver function as reflected by the AKBR in living related liver transplantation.

Key words Insulin, glucagon, liver transplantation · Liver transplantation, insulin, glucagon

Introduction

It is widely accepted that insulin has a typical hepatotrophic activity [8]. Insulin is necessary to boost energy production in hepatic mitochondria in order to maintain the hepatic energy charge against energy-consuming reactions. For example, it has been shown that the hepatic energy charge decreases greatly after hepatectomy and that the remnant liver cannot easily regenerate in alloxan-diabetic rats [16]. By contrast, since it has been shown that glucagon administration decreases the hepatic energy charge transiently in normal rabbits [9], glucagon can facilitate hepatic energy-consuming reactions such as bile or urea production. Thus, it is conceivable that insulin and glucagon greatly influence hepatic energy metabolism in various kinds of surgery, including liver transplantation. Mallett et al. reported changes in insulin and glucagon levels in relation to glucose metabolism in human liver transplantation. In their report, serum insulin levels decreased 30 min after graft liver reperfusion and increased 24 h after reperfusion, while glucagon levels did not change significantly for 24 h after reperfusion [3]. However, it has yet to be determined how these changes in hormonal levels are related to the recovery of graft liver function.

Arterial ketone body ratio (AKBR), which reflects hepatic mitochondrial redox potential [7], can indicate graft liver viability since it is closely related to graft outcome in human liver transplantation. That is, elevation of AKBR to above 1.0 within 2 days is essential for successful liver transplantation, whereas suppression of AKBR below 0.7 for 2 days is an indication of graft fail-

ure [10]. However, in our previous study on pediatric patients who underwent living related liver transplantation (LRLT) between June 1990 and January 1992 at our hospital, it was shown that, immediately after surgery, some patients showed a transient decrease in blood glucose levels below 150 mg/dl and a concomitant decrease in AKBR, despite well-functioning graft livers. This indicates that glucose metabolism is closely associated with the recovery process of AKBR after liver transplantation and that a sufficient glucose load is necessary to correctly evaluate graft liver viability with AKBR [11].

In this study, we investigated (1) changes in insulin and glucagon levels in relation to glucose metabolism and (2) the interaction of these hormones with the recovery of graft liver function as reflected by AKBR during and immediately after LRLT in pediatric patients.

Materials and methods

One hundred LRLTs were performed on pediatric patients at the Second Department of Surgery, Kyoto University Hospital, between June 1990 and May 1994. This study involved 30 consecutive pediatric patients who underwent LRLT between March 1992 and March 1993. Informed consent was obtained for each patient, as was approval from the institutional human research committee. The present analysis was completed on 29 of the recipients. Recipient and graft profiles are shown in Table 1. None of the recipients was diabetic.

The graft liver was harvested and preserved, as previously described, using Belzer UW solution (ViaSpan, DuPont Pharmaceuticals, USA) without steroids, insulin, or antibiotics [11, 17]. Anesthesia in recipients was induced with sodium thiopental, fentanyl, midazolam, and alcuronium chloride, and was maintained with the latter three agents. The recipient operation was performed as reported elsewhere [11, 13]. Exogenous glucose sources, other than blood products, included acetated Ringer's solution containing 5% glucose, which was intravenously administered during the operation, and a solution containing 10%–15% glucose, which was administered after the operation. Glucose administration was gradually increased to 20% (12 g glucose per kilogram body weight per day) unless severe hyperglycemia over 300 mg/dl occurred. Neither exogenous insulin nor glucagon was administered. Blood loss was replaced with an equal volume of washed red blood cells and 5% plasma protein fraction. Methylprednisolone was administered as an intraoperative immunosuppressant just before reperfusion (10 mg/kg of body weight). Postoperative immunosuppressive therapy consisted of steroids and FK 506 [14].

Arterial blood was obtained from the recipients preoperatively after induction of anesthesia (Pre), during the anhepatic phase (AHP), and 1, 4, 6, 12, 18, and 30 h after portal vein reperfusion of the graft liver. Blood glucose levels were measured using the o-toluidine method; serum insulin (IRI) and plasma pancreatic glucagon (IRG) levels using radioimmunoassay; and plasma ketone body levels (acetoacetate and 3-hydroxybutyrate) using a Ketorex Kit (Sanwa Chemical, Nagoya, Japan) and a Keto-340 system (Ihara Electric, Kasugai, Japan) [15]. AKBR was expressed as acetoacetate/3-hydroxybutyrate and it was accepted when both acetoacetate and 3-hydroxybutyrate levels were greater than or equal to 10 μmol/l.

Table 1 Profiles of recipients and grafts of 30 LRLT cases[a]. Values are expressed as mean ± SEM

Recipient	Sex	22 females and 8 males
	Age (years)	4.5 ± 0.9
	Body weight (kg)	17.1 ± 2.67
Indication	Biliary atresia	23
	Wilson's disease	2
	Fulminant hepatitis	1
	Liver cirrhosis	1
	Tyrosinemia	1
	Progressive intrahepatic cholestasis	1
	Glycogen storage disease	1
Donor	18 maternal and 12 paternal	
Graft	19 lateral segments and 11 left lobes	
	Graft weight (g)	273 ± 11.6
Graft/body weight ratio[b]		2.42 ± 0.23
Graft ischemic time (min)[c]		124 ± 10.2

[a] 10-year-old girl with biliary atresia was excluded from the analysis
[b] Percent weight of graft liver to recipient body weight
[c] Total ischemic time (sum of cold and warm ischemic time of graft liver)

Results are expressed as means ± SEM. Statistical analysis was made using a one-factor ANOVA for repeated measurements. Correlation was analyzed by simple regression analysis. Statistical significance was defined as a P value below 0.05.

Results

Among the 30 recipients was a 10-year-old girl with biliary atresia. She was excluded from the present analysis since we could not complete the protocol in her case. The analysis was thus made on the remaining 29 recipients. Among these recipients was a 3-year-old girl with biliary atresia who died on postoperative day (POD) 3 of thrombosis of both the portal vein and the hepatic artery, this occurred after the last sampling. All of the other recipients left the intensive care unit within a week in good postoperative condition.

Table 2 shows the changes in serum levels of total bilirubin, AST, and ALT. Total bilirubin levels decreased after transplantation. AST levels did not change, while ALT levels increased on POD 2.

Table 3 shows the changes in blood glucose (BG), serum insulin (IRI), and plasma glucagon (IRG) levels, the insulin/glucagon (I/G) ratio, and AKBR. Blood glucose levels increased after AHP. IRI levels did not change throughout this study. IRG levels decreased after reperfusion. The I/G ratio and AKBR increased

Table 2 Changes in total bilirubin (T bil), AST, and ALT levels. Value are expressed as mean ± SEM (*Pre* preoperation, *POD* postoperative day)

	T bil (mg/dl)	AST (IU/l)	ALT (IU/l)
Pre	19.5 ± 2.8	183 ± 16	97 ± 9
POD 1	6.7 ± 1.2*	215 ± 38	218 ± 34
POD 2	5.3 ± 0.7*	240 ± 79	368 ± 120*

* $P < 0.05$ versus preoperation

after 18 h, i.e., during the final 12 h of the 30-h period examined.

Figure 1 shows the correlation between the I/G ratio and AKBR after graft liver reperfusion ($r = 0.551$, $P < 0.001$). There was also a significant correlation between IRI levels and AKBR ($r = 0.522$, $P < 0.001$), but no significant correlation between IRG levels and AKBR after reperfusion (data not shown).

Discussion

In this study, blood glucose levels increased after the anhepatic phase but tended to be lower than those previously reported [1, 2]. In liver transplantation, glucose is released from graft livers and supplied via blood products, supplemental infusions, and some preservation solutions. In addition, steroids, some anesthetic agents, and endogenous hormones may influence blood glucose levels. This makes it difficult to explain the differences in blood glucose levels. In general, however, hypoglycemia can more easily occur in children as ketoacidosis than in adults. Moreover, it has been shown that glucose levels after reperfusion are lower with successful grafts than with failed grafts [2]. It has also been reported that graft livers from living donors are generally more viable than those from brain-dead donors [18], a finding that would lead one to expect lower glucose levels in recipients with living related grafts. In this study, all recipients were children and all grafts were from living donors. In addition, glucagon levels in this study tended to be lower than those previously reported [3]. These factors may explain the lower glucose levels seen in this study.

IRI levels did not change significantly throughout this study, a finding unlike that of Mallett et al., who reported an increase in IRI levels at 24 h after reperfusion. This may be due to the lower glucose levels in this study. In contrast, the decrease in IRG levels after reperfusion was not seen in their report. Indeed, surgical stress might increase glucagon secretion. However, since the normal liver degrades most of the glucagon but only half of the insulin [4], it is reasonable to assume that IRG levels, rather than IRI levels, may decrease after reperfusion when the graft liver has sufficient viability. Consequently, the expected high viability of graft livers in LRLT can account for the immediate decrease in IRG levels after reperfusion.

To evaluate graft viability correctly with AKBR, it is important to note that hypoglycemia or hepatic hypoxia decreases AKBR. In this study, glucose was administered in sufficient amounts to avoid hypoglycemia. Moreover, it has been demonstrated that hepatic O_2 saturation levels recover to within the normal range by the end of the operation in LRLT [12]. Thus, it is thought that these two factors would not contribute notably to changes in AKBR during the 30 h after reperfusion. In this study, AKBR was elevated to over 1.0 at 18 h after reperfusion, indicating successful recovery of graft liver function. Decreased bilirubin levels also confirmed recovery of graft function.

Insulin is essential to improve hepatic mitochondrial energy production [6, 16]. In clinical cases, we have shown that intraportal insulin administration elevates AKBR and improves the survival rate after major hepatectomy in insulin-dependent diabetic patients [5]. By contrast, glucagon facilitates hepatic energy consumption [9]. Thus, the I/G ratio has been defined so as to view their combined effects. In this study, the I/G ratio increased at 18 and 30 h after reperfusion. AKBR also increased concomitantly with the I/G ratio. Further-

Table 3 Changes in blood glucose (BG), serum insulin (IRI), and plasma glucagon (IRG) levels, as well as in the insulin/glucagon (I/G) ratio, and arterial ketone body ratio (AKBR). Value are expressed as mean ± SEM (*Pre* preoperation, *AHP* anhepatic phase)

	BG (mg/dl)	IRI (μU/ml)	IRG (pg/ml)	I/G ratio[a]	AKBR
Pre	103 ± 8.5	25.3 ± 14.9	368 ± 101	2.48 ± 1.00	0.62 ± 0.12
AHP	164 ± 14.3*	22.4 ± 5.5	379 ± 95.7	2.43 ± 0.68	0.61 ± 0.19
1 h[b]	240 ± 24.7*	19.7 ± 3.7	169 ± 34.3*	3.93 ± 0.88	0.57 ± 0.07
4 h	212 ± 13.8*	24.4 ± 14.3	133 ± 26.4*	3.55 ± 1.03	0.67 ± 0.05
6 h	194 ± 12.8*	16.0 ± 6.0	128 ± 31.7*	3.53 ± 0.83	0.77 ± 0.06
12 h	214 ± 14.4*	27.1 ± 7.5	95.7 ± 18.3*	7.23 ± 1.20	0.91 ± 0.06
18 h	221 ± 12.5*	41.0 ± 14.0	111 ± 30.0*	12.6 ± 4.38*	1.31 ± 0.12*
30 h	208 ± 11.9*	30.6 ± 10.2	104 ± 20.3*	11.3 ± 5.26*	1.55 ± 0.13*

[a] Molar ratio. Insulin: 1 μU/ml = 7.18×10^{-12} mol/l; glucagon: 1 pg/ml = 2.87×10^{-13} mol/l

[b] Time after reperfusion of the graft liver

* $P < 0.05$ versus preoperation

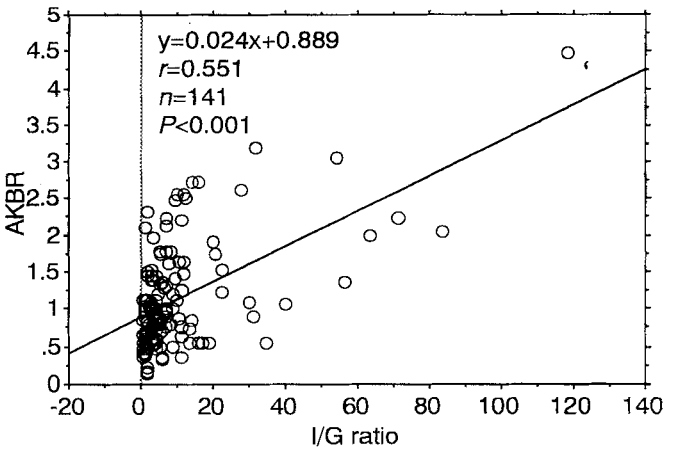

Fig. 1 Relationship between I/G ratio (molar ratio) and AKBR after graft liver reperfusion. A significant correlation was found to exist between the two

more, the I/G ratio showed the most significant correlation with AKBR after reperfusion. Given the previous reports and the present results, we conclude that the increase in the I/G ratio after reperfusion was closely related to the recovery of graft liver function as reflected by the AKBR.

Whether the same phenomena would be obtained in brain-dead donor liver transplantation has yet to be investigated. It would be especially interesting to determine how the I/G ratio of recipients behaves in primary nonfunctioning grafts, in which the AKBR remains at low levels without recovering to above 1.0. Further studies should be undertaken to clarify these aspects.

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