

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 hour period after graft liver reperfusion in 29 recipients of living related liver transplantation (LRLT). Insulin levels did not change significantly throughout this study, while glucagon levels decreased immediately after reperfusion, indicating rapid degradation of glucagon by the graft liver. Insulin/glucagon ratio (I/G ratio) increased after reperfusion concomitantly with AKBR. In addition, I/G ratio was significantly correlated with AKBR after reperfusion. It is concluded that the increase in I/G ratio was closely related to the recovery of graft liver function reflected by the AKBR in LRLT.

Key words

Living related liver transplantation; graft function; energy metabolism, insulin/glucagon ratio, arterial ketone body ratio.

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 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 the changes in insulin and glucagon levels in relation to glucose metabolism in human liver transplantation. In their report, serum insulin levels decreased 30 minutes after graft liver reperfusion and increased 24 hours after reperfusion, while glucagon levels did not change significantly for 24 hours after reperfusion [2]. 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 with 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 failure [10]. On the other hand, in our previous study on pediatric patients who underwent living related liver transplantation (LRLT) between June 1990 and January 1992 at our hospital, it has been shown that, immediately after operation,

some patients showed a transient decrease in blood glucose levels below 150 mg/dl and concomitant decrease in AKBR, despite well-functioning graft livers. This indicated that glucose metabolism was closely associated with the recovery process of AKBR after liver transplantation, and that 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 the graft liver function reflected by AKBR during and immediately after LRLT in pediatric patients.

Materials and Methods

One hundred pediatric cases of LRLT have been performed 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 with informed consent obtained from each patient and approval of the institutional human research committee. The present analysis was completed on 29 of the recipients. Recipient and graft profiles are shown in the table 1. No recipients were diabetic. The graft liver was harvested and preserved as previously described using Belzer UW solution (ViaSpan™, DuPont Pharmaceuticals, USA) without steroids, insulin nor antibiotics [11, 17]. Anesthesia of recipients was induced with sodium thiopental, fentanyl, midazolam and alcuronium chloride, and maintained with the latter three agents. Recipient operation was performed as reported [11, 13]. Exogenous glucose sources other than blood products were acetated Ringer's solution containing 5% glucose which was intravenously administered during operation and a solution containing 10-15% glucose which was administered after operation. Glucose administration was gradually increased to 20% (12g of glucose/kg of body weight/day) unless severe hyperglycemia over 300mg/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 (10mg/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), 1, 4, 6, 12, 18

and 30 hours after portal vein reperfusion of the graft liver. Blood glucose levels were measured with the o-toluidine method; serum insulin (IRI) and plasma pancreatic glucagon (IRG) levels with radioimmunoassay; and plasma ketone body levels (acetoacetate and 3-hydroxybutyrate) with Ketorex Kit (Sanwa Chemical Co., Nagoya, Japan) and Keto-340 system (Ihara Electric Co., Kasugai, Japan) [15]. AKBR was expressed as acetoacetate/3-hydroxybutyrate and it was accepted when both acetoacetate and 3-hydroxybutyrate levels were $\geq 10 \mu$ mol/l.

Results are expressed as means $\pm$ SEM. Statistical analysis was made with one factor ANOVA for repeated measurements. Correlation was analyzed by simple regression analysis. Statistical significance was defined as P value < 0.05 .

Results

Among the 30 recipients, a ten-year-old girl with biliary atresia was excluded from the present analysis since we could not complete the protocol in that case. The analysis was thus made on the remaining 29 recipients. In these recipients, a two-year-old girl with biliary atresia died on 3 POD of thrombosis of both the portal vein and the hepatic artery which 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 2 POD.

Table 3 shows the changes in blood glucose (BG), serum insulin (IRI), plasma glucagon (IRG) levels, insulin/glucagon ratio (I/G ratio) and AKBR. Blood glucose levels increased after AHP. IRI levels did not change throughout this study. IRG levels decreased after reperfusion. I/G ratio and AKBR increased after 18 hours, that is, during the final 12 hours of the 30 hour period examined.

Figure 1 shows the correlation between 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, 3]. In liver transplantation, glucose is released from graft livers and supplied via blood products, supplemental infusions and some preservative 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 [3]. 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 of 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 which are later described tended to be lower than previously reported [2]. These factors may explain the lower glucose levels seen in this study.

IRI levels did not significantly change throughout this study, although Mallett et al. reported an increase in IRI levels at 24 hours after reperfusion. This may be due to the lower glucose levels in this study. On the other hand, 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 while it degrades only half of the insulin [4], it is reasonable to assume that IRG levels may decrease after reperfusion rather than IRI levels 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 O₂ saturation levels recover to normal range by the end of operation in LRLT [12]. Thus it is thought that these two factors would not contribute notably to changes in AKBR during the 30 hours after reperfusion. In this study, AKBR elevated to over 1.0 at 18 hours after reperfusion, indicating successful recovery of graft liver function. Decreased bilirubin levels also confirms 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 survival rate after major hepatectomy in insulin-dependent diabetic patients [5]. By contrast, glucagon facilitates hepatic energy consumption [9]. Thus, insulin/glucagon ratio (I/G ratio) has been defined so as to view their combined effects. In this study, I/G ratio increased at 18 and 30 hours after reperfusion. AKBR also increased concomitantly with the I/G ratio. Furthermore, I/G ratio showed the most significant correlation with AKBR after reperfusion. Regarding the previous reports and the present results, we concluded that the increase in I/G ratio after reperfusion was closely related to the recovery of graft liver function reflected by the AKBR.

It remains to be clarified whether the same phenomena would obtain in brain dead donor liver transplantation. It would be especially interesting to determine how the I/G ratio of recipients behaves in primary non-functioning 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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Table 1. Profiles of recipients and grafts of 30 LRLT cases^a.

1. Recipient	Sex	22 girls and 8 boys
	Age	4.5 ± 0.9 ^b years old
	Body weight	17.1 ± 2.67 kg
2. Indication	Biliary atresia	23
	Wilson's disease	2
	Fulminant hepatitis	1
	Liver cirrhosis	1
	Tyrosinemia	1
	Progressive intrahepatic cholestasis	1
	Glycogen storage disease	1
3. Donor	18 maternal and 12 paternal	
4. Graft	19 lateral segments and 11 left lobes	
	Graft weight	273 ± 11.6 g
5. Graft/body weight ratio ^c	2.42 ± 0.23%	
6. Graft ischemic time ^d	124 ± 10.2 minute	

^aAmong 30 cases, a ten-year-old girl with biliary atresia was excluded from the analysis.

^bValues are means ± SEM.

^cPercent weight of graft liver to recipient body weight.

^dTotal ischemic time (sum of cold and warm ischemic time of graft liver).

Table 2. Changes in total bilirubin (T-Bil), AST and ALT levels.

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

^aPreoperation.

^bValues are mean ± SEM.

*P<0.05 versus preoperation.

Table 3. Changes in blood glucose (BG), serum insulin (IRI), plasma glucagon (IRG) levels, insulin/glucagon ratio (I/G ratio) and AKBR.

	BG(mg/dl)	IRI(μ U/ml)	IRG(pg/ml)	I/G ratio ^a	AKBR
Pre ^b	103 $\pm$ 8.5 ^c	25.3 $\pm$ 14.9	368 $\pm$ 101	2.57 $\pm$ 1.04	0.62 $\pm$ 0.12
AHP	164 $\pm$ 14.3*	22.4 $\pm$ 5.5	379 $\pm$ 95.7	2.52 $\pm$ 0.70	0.61 $\pm$ 0.19
1hr ^d	240 $\pm$ 24.7*	19.7 $\pm$ 3.7	169 $\pm$ 34.3*	4.08 $\pm$ 0.91	0.57 $\pm$ 0.07
4hr	212 $\pm$ 13.8*	24.4 $\pm$ 14.3	133 $\pm$ 26.4*	3.69 $\pm$ 1.07	0.67 $\pm$ 0.05
6hr	194 $\pm$ 12.8*	16.0 $\pm$ 6.0	128 $\pm$ 31.7*	3.67 $\pm$ 0.86	0.77 $\pm$ 0.06
12hr	214 $\pm$ 14.4*	27.1 $\pm$ 7.5	95.7 $\pm$ 18.3*	7.51 $\pm$ 1.25	0.91 $\pm$ 0.06
18hr	221 $\pm$ 12.5*	41.0 $\pm$ 14.0	111 $\pm$ 30.0*	13.1 $\pm$ 4.55*	1.31 $\pm$ 0.12*
30hr	208 $\pm$ 11.9*	30.6 $\pm$ 10.2	104 $\pm$ 20.3*	11.7 $\pm$ 5.46*	1.55 $\pm$ 0.13*

^aMolar ratio. Insulin: 1 μ U/ml=7.46 $\times 10^{-12}$ mol/l. Glucagon: 1pg/ml=2.87 $\times 10^{-13}$ mol/l.

^bPreoperation.

^cValues are mean $\pm$ SEM.

^d1hr 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 exists between them.

