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PANCREATIC α- AND β-CELL FUNCTION AND METABOLIC CHANGES DURING ORAL L-ALANINE AND GLUCOSE ADMINISTRATION: COMPARATIVE STUDIES BETWEEN NORMAL, DIABETIC AND CIRRHOTIC SUBJECTS

TADAKAZU HATTORI[1], NIGISHI HOTTA[2], KIYOJI OHARA[2], HIROSHI SHINODA[2], TAKEHIDE KUNIEDA[2], TAKAHIDE NOMURA[3], HIRONOBU KAKUTA[2], TATSUO TAMAGAWA[2] and NOBUO SAKAMOTO[2]

[1]*Kakegawa City Hospital, Sugitani, Kakegawa, Shizuoka 436, Japan*
[2]*Third Department of Internal Medicine, Nagoya University School of Medicine, 65 Tsuruma-cho, Showa-ku, Nagoya 466, Japan*
[3]*Department of Pharmacology, Fujita-Gakuen Health University, School of Medicine, Kukkake-cho, Toyoake, Aichi 470-11, Japan*

ABSTRACT

The present study investigated whether or not, in addition to the oral glucose tolerance test, oral alanine loading was a useful diagnostic tool for hormonal and metabolic diseases. Fifty g of L-alanine was administered orally in 14 normal, 12 diabetic, and 8 liver cirrhotic subjects. The influence of oral alanine loading on hormones and metabolites was compared with the results of 100 g oral glucose loading. The results obtained were as follows: 1) In the normal subjects and cirrhotics, lactate and pyruvate concentrations gradually increased with time and reached their peak levels at 60 min, whereas they remained unchanged throughout the course in the diabetic group at glucose loading. 2) Alanine administration accelerated ureogenesis but did not affect blood glucose levels. 3) In both glucose and L-alanine administration, free fatty acid, glycerol and ketone body levels declined nonspecifically in all groups. 4) Serum glucagon levels during L-alanine loading increased in all groups, especially in liver cirrhotics. 5) L-alanine was a potent stimulus for insulin secretion in diabetics, while no insulin release during glucose loading was observed. 6) The molar ratio of insulin levels (during glucose loading)/glucagon levels (during L-alanine loading) was a good indicator of systemic glucose homeostasis from the hormonal aspect.

It is suggested that, in addition to the oral glucose tolerance test, the oral administration of L-alanine can be a useful tool for the diagnosis of the status in diabetes mellitus and cirrhosis.

Key Words: L-Alanine oral loading, Lactate, Glucagon, Molar ratio of insulin/glucagon
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INTRODUCTION

Caloric homeostasis in the body, particularly glucose homeostasis, is properly maintained by the mutual regulation of glucose, protein, and fat metabolism. It is well known that insulin and its antagonizing hormones, such as glucagon, are closely associated with this metabolic regulation.[1)] First, the "metabolic fuel" is taken in orally and then passes to the liver through the portal vein.[2)] In diabetes mellitus, which is characterized by the abnormal function of the pancreatic α- and β-cells, and in liver cirrhosis, where there is a marked impairment of hepatocyte function, the capacity to process these "metabolic fuels" in the liver is considered to be impaired, and thereby various

著者名：
服部忠和・堀田　饒・大原清仁・篠田　広・国枝武英・野村隆英・角田博信・玉川達雄・坂本信夫
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metabolic and endocrine disorders may ensue. Glucose is a substrate of the glycolytic system and alanine is a glycogenic amino acid that is selectively released from muscle and extracted by the liver in significant quantities during fasting and exercise. Both are quite specific metabolic fuels, and both also stimulate the pancreatic α- and β-cells.[3] Thus, we can expect that the analysis of the metabolic and endocrine patterns following the oral administration of both substances may provide useful information to understand the pathogenesis of diabetes mellitus and liver cirrhosis, in addition to the maintenance mechanisms of caloric or glucose homeostasis.

Therefore, in normal subjects, and in patients with diabetes mellitus and liver cirrhosis, we comparatively studied the endocrine and metabolic changes following oral glucose and L-alanine administration and evaluated the specific reactions in these diseases. From the findings obtained here, it was also discussed whether or not oral L-alanine administration was a useful diagnostic tool for hormonal and metabolic diseases.

SUBJECTS AND METHODS

The normal subjects examined were 14 men, who were 27 ± 2 years of age. There were 12 patients with diabetes mellitus, seven men and five women, who were 42 ± 2 years of age. They were insulin-dependent and had no response of insulin or C-peptide to oral glucose loading. There were eight patients with cirrhosis, five men and three women, who were 51 ± 3 years of age. The patients examined were in a compensated state and the diagnosis was confirmed by laparoscopy and liver biopsy. After 12 hr fasting a 100 g oral glucose tolerance test (OGTT) and a 50 g L-alanine tolerance test (OATT) were performed early in the morning, at intervals of one week in each subject. One hundred grams of glucose and 50 g L-alanine are equal in molarity. Blood samples were taken 0, 30, 60, 120, and 180 minutes after loading, and the measurement of the following metabolites and hormones was performed.

The blood glucose concentrations were measured with the glucose-oxidase method, free fatty acids[4] (FFA) and α-amino acid[5] levels in serum by colorimetric assay, and the glycerol[6] and urea nitrogen levels in serum by enzymatic assay (Yatron® Test Kit, Yatron Co., Tokyo, Japan) using the serum samples. Also, after a part of the blood sample was deproteinized with 70 % perchloric acid and neutralized with 30 % KOH, the supernatant obtained was used for the determination of lactate,[7] pyruvate[8] and ketone bodies (acetoacetate[9] and β-hydroxybutyrate[10]) by enzymatic assay. Insulin (IRI), C-peptide (CPR), glucagon (IRG) and growth hormone (HGH) in serum were determined by immunological assay using antibody. Here, the 30 K antibody was used for the determination of IRG.

The significance was calculated using Student's t test for paired data within groups. Analysis between the two groups was done by Scheffe's general linear models.

RESULTS

1. The endocrine and metabolic changes of normal subjects, diabetics and cirrhotics in the OGTT and OATT.

In Tables 1, 2, and 3, the endocrine and metabolic changes of normal subjects, diabetics and cirrhotics in the OGTT and OATT are shown for the duration of 180 min.

1) Endocrine and metabolic changes in the OGTT

Hyperglycemia naturally occurred in the diabetic group, but slight disturbances in the glucose tolerance test were seen even in the cirrhotic group. The changes of lactate and pyruvate concentrations were almost parallel among the three groups; however, in the normal subjects and

cirrhotics, they gradually increased with time and reached their peak levels at 60 min, whereas they remained unchanged throughout the course in the diabetic group. These changes of lactate and pyruvate concentrations were well reflected in those of IRI and CPR as well. IRG gradually increased in the diabetic group and reached its peak at 60 min, but it remained unchanged or tended to gradually decrease in the other two groups. However, FFA, glycerol, and ketone body concentrations were reduced in each group and there was no significant difference among the three groups.

2) Endocrine and metabolic changes in the OATT.

Blood glucose concentrations were unchanged in all groups. Lactate levels were increased in each group, and markedly raised in the diabetic group in particular, showing peak levels at 120 min of about twice as much as the pre-loading values. An increase of α-amino acid concentrations was observed in each group. Possibly due to the direct effect of alanine loading, there was a gradual increase of urea nitrogen concentration, and the increase was particularly large in the diabetic group, but it was only slight in the cirrhotic group. Also, FFA, glycerol, and ketone body concentrations gradually decreased in all groups. As for the endocrine changes, even in the diabetic group, unlike the results obtained in the OGTT, IRI and CPR secretory reactions were seen. Hypersecretory reactions of IRI and CPR were observed in the cirrhotic group. Secretory reactions of IRG and HGH were observed in all groups but they were particularly marked in the cirrhotic group.

On the basis of the results shown in Tables 1, 2 and 3, the integrated areas under the blood or serum concentration time curves for 180-min duration of the OGTT and OATT were calculated in terms of the characteristic changes, and comparatively evaluated among the groups (Figs. 1 and 2).

2. Disease specificity of the insulin/glucagon molar ratio.

Since the amounts of glucose and L-alanine given in this study were the same molar concentrations, the molar ratios were calculated on the basis of the IRI and IRG values obtained (IRI during OGTT/IRG during OATT), and they were compared over time among the normal subjects, diabetics and cirrhotics (Fig. 3). This IRI/IRG molar ratio in normal subjects reached its peak at 30 min and almost returned to the pre-determination value by 180 min. In the cirrhotics, the peak value was at 60 min and was 2/3 of the value seen in normal subjects. It was slightly higher than that of normal subjects at 120 min, thus reflecting the excess-insulin-reaction in the OGTT. Whereas, in the diabetic group, the pre-loading values were almost the same as those of the other two groups, and they remained low and unchanged throughout the entire 180 min.

DISCUSSION

It was reported by Felig et al.[11] that an increase of lactate concentration, which peaked after 30 min of glucose loading, was seen, and it is also known that in the in vitro rat liver perfusion experiments the lactate concentrations in the perfusate are elevated with time by the addition of insulin.[12] It is suggested that the balance of glucose metabolism in the livers of normal subjects is altered from gluconeogenesis to glycolysis by the administration of either glucose or insulin, and our results support these findings. The reason the increase of lactate concentrations in the cirrhotic group was maintained until after 180 min was considered to be due to the fact that lactate utilization in the liver was reduced, unlike that in normal subjects. In the diabetic group, lactate and pyruvate concentrations remained unchanged throughout the entire course, and this was considered to be due to a defect in the inhibition of hepatic gluconeogenesis accompanying the functional disorder of the pancreatic β-cells.

On the other hand, the finding that the blood lactate and pyruvate concentrations in the diabetic group increased with time in the OATT was extremely characteristic. In short-term fasting the

Table 1. Blood or serum levels of metabolites and hormones during the 3-hour OGTT and the 3-hour OATT in 14 normal subjects.

Oral Glucose Tolerance Test		Time (min)				
		0	30	60	120	180
Blood glucose	(mg/dl)	92 ± 1.8	136 ± 5.4**	113 ± 4.9**	92 ± 4.1	83 ± 3.8
Lactate	(mM/L)	1.00 ± 0.08	1.22 ± 0.09	1.44 ± 0.08**	1.12 ± 0.05	0.96 ± 0.07
Pyruvate	(mM/L)	0.14 ± 0.02	0.16 ± 0.01	0.17 ± 0.02	0.06 ± 0.02	0.13 ± 0.01
α-Amino nitrogen	(mg/dl)	19.2 ± 1.2	18.4 ± 1.3	18.1 ± 1.4	16.4 ± 1.2	15.1 ± 1.0 *
Urea nitrogen	(mg/dl)	11.1 ± 0.5	10.9 ± 0.7	10.9 ± 0.5	10.9 ± 0.4	10.5 ± 0.5
Free fatty acid	(μEq/L)	359 ± 37	255 ± 21*	210 ± 18**	192 ± 16**	192 ± 19**
Glycerol	(μM/ml)	0.25 ± 0.04	0.14 ± 0.02*	0.11 ± 0.02*	0.12 ± 0.02*	0.14 ± 0.02*
Total ketone bodies	(mM/L)	0.08 ± 0.01	0.09 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Insulin	(μU/ml)	7.4 ± 0.9	54.0 ± 7.2**	54.6 ± 6.5**	35.5 ± 5.3**	24.8 ± 4.0**
C-peptide	(ng/ml)	1.8 ± 0.1	5.1 ± 0.6**	5.6 ± 0.7**	5.1 ± 0.7**	4.1 ± 0.6*
Growth hormone	(ng/ml)	1.5 ± 0.2	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.5 ± 0.4
Glucagon	(pg/ml)	103 ± 13	95 ± 15	88 ± 11	102 ± 21	97 ± 15
Oral Alanine Tolerance Test		**0**	**30**	**60**	**120**	**180**
Blood glucose	(mg/dl)	86 ± 3	85 ± 3	86 ± 4	83 ± 3	84 ± 3
Lactate	(mM/L)	0.88 ± 0.08	1.05 ± 0.08	1.16 ± 0.09*	1.01 ± 0,10	1.16 ± 0.08*
Pyruvate	(mM/L)	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
α-Amino nitrogen	(mg/dl)	17.2 ± 0.9	34.0 ± 1.5**	38.7 ± 1.8**	37.8 ± 1.6**	33.1 ± 2.6**
Urea nitrogen	(mg/dl)	10.7 ± 0.8	10.7 ± 0.8	11.6 ± 0.8	13.9 ± 0.9*	16.0 ± 1.2**
Free fatty acid	(μEq/L)	393 ± 29	323 ± 25	237 ± 24**	208 ± 18 **	265 ± 28**
Glycerol	(μM/ml)	0.26 ± 0.05	0.17 ± 0.03	0.20 ± 0.04	0.16 ± 0.03	0.17 ± 0.03
Total ketone bodies	(mM/L)	0.10 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
Insulin	(μU/ml)	16.2 ± 2.0	25.0 ± 2.9**	29.1 ± 3.6**	22.4 ± 2.0*	17.7 ± 1.9
C-peptide	(ng/ml)	1.9 ± 0.2	2.6 ± 0.2*	2.9 ± 0.3**	2.6 ± 0.3*	2.2 ± 0.2
Growth hormone	(ng/ml)	2.9 ± 0.7	1.6 ± 0.3	1.6 ± 0.3	6.1 ± 2.1	11.0 ± 4.0
Glucagon	(pg/ml)	85 ± 10	174 ± 19**	205 ± 21**	260 ± 26**	235 ± 27**

Mean values ± SEM of 14 normal subjects.
** $p<0.005$ as compared with 0 min basal value.
* $p<0.05$ as compared with 0 min basal value.
Total ketone bodies : acetoacetate+β-hydroxybutyrate.

nitrogen release from muscles is accelerated and, particularly, a marked release of alanine, a glycogenic amino acid, is seen.[13] Although the conversion from alanine incorporated into the liver to glucose rapidly takes place, only 10 % to 30 % of the administered alanine is converted to glucose whose splanchnic production rate is reported to be only 5 to 8 g per hour.[14,15] Thus, it is unlikely that gluconeogenesis occurs at a higher rate than the glucose utilization in peripheral tissues. In addition, even in the diabetic state with augmented hepatic gluconeogenesis, the blood glucose derived from the administered alanine only accounts for 8 % to 11 % of total blood glucose.[14,15] Accordingly, a slight increase of blood sugar levels in the OATT was seen in the diabetic group. Our results, which showed no significant changes among all the groups, including the normal subjects and cirrhotic patients, when compared to the pre-loading levels, were compatible with those of other investigators.[14,15] When a large quantity of alanine is infused at one time in the diabetic state, it is considered that the pyruvate formed is converted lactate and the release of lactate from the liver is augmented, thus causing the increased lactate concentrations in the blood. Thus, it seems that the

Table 2. Blood or serum levels of metabolites and hormones during the 3-hour OGTT and the 3-hour OATT in 12 diabetic subjects.

Oral Glucose Tolerance Test		Time (min)				
		0	30	60	120	180
Blood glucose	(mg/dl)	213±12	313±18**	408±25**	454±20**	416±22**
Lactate	(mM/L)	0.89±0.07	0.99±0.08	1.00±0.07	1.04±0.08	1.08±0.07
Pyruvate	(mM/L)	0.12±0.01	0.12±0.01	0.13±0.01	0.13±0.01	0.14±0.01
α-Amino nitrogen	(mg/dl)	14.6±0.9	14.2±0.9	13.2±0.8	13.3±0.9	12.9±0.7
Urea nitrogen	(mg/dl)	13.0±0.8	13.7±0.8	13.5±0.9	12.3±0.9	12.3±1.0
Free fatty acid	(μEq/L)	632±110	501±86	424±68	353±75*	342±83*
Glycerol	(μM/ml)	0.56±0.09	0.41±0.06	0.37±0.08	0.42±0.08	0.37±0.07
Total ketone bodies	(mM/L)	0.45±0.14	0.46±0.14	0.35±0.11	0.26±0.08	0.28±0.08
Insulin☆	(μU/ml)	5.7±1.7	6.8±1.6	9.2±1.7	8.7±1.1	8.1±2.0
C-peptide	(ng/ml)	2.2±0.4	2.6±0.5	2.6±0.5	2.8±0.5	2.7±0.5
Growth hormone	(ng/ml)	3.3±0.8	2.7±0.7	3.5±0.7	3.3±0.8	3.1±0.6
Glucagon	(pg/ml)	110±8	135±12	144±13*	123±13	120±9
Oral Alanine Tolerance Test		**0**	**30**	**60**	**120**	**180**
Blood glucose	(mg/dl)	210±13	219±14	229±14	225±17	215±17
Lactate	(mM/L)	1.00±0.07	1.44±0.11**	1.87±0.14**	1.91±0.16**	1.87±0.21**
Pyruvate	(mM/L)	0.11±0.01	0.16±0.01*	0.21±0.02**	0.21±0.01**	0.22±0.02**
α-Amino nitrogen	(mg/dl)	15.1±0.7	28.4±2.6**	33.8±2.1**	32.3±2.5**	29.4±2.7**
Urea nitrogen	(mg/dl)	15.1±1.0	14.0±1.2	16.6±1.1	20.4±1.3*	23.5±1.8**
Free fatty acid	(μEq/L)	575±69	403±38*	317±25*	221±28**	249±56**
Glycerol	(μM/ml)	0.62±0.08	0.58±0.12	0.52±0.08	0.40±0.09	0.49±0.09
Total ketone bodies	(mM/L)	0.45±0.13	0.37±0.12	0.22±0.05	0.12±0.03*	0.10±0.02*
Insulin☆	(μU/ml)	9.5±1.4	16.7±2.8*	17.7±3.6	16.1±3.4	15.5±3.4
C-peptide	(ng/ml)	1.7±0.3	2.2±0.5	2.2±0.4	2.4±0.4	2.2±0.4
Growth hormone	(ng/ml)	3.5±0.7	4.8±1.4	7.8±1.8*	13.4±3.2*	8.6±2.2*
Glucagon	(pg/ml)	134±18	253±30**	314±41**	345±23**	298±25**

Mean values ± SEM of 12 diabetic subjects.
** $p<0.005$ as compared with 0 min basal value.
* $p<0.05$ as compared with 0 min basal value.
☆ The values of 6 patients before insulin treatment.
Total ketone bodies : acetoacetate+β-hydroxybutyrate.

specific changes of the blood metabolites in the OATT and OGTT may be influenced by the activities of the glycolytic and gluconeogenetic systems dependent on the hormonal action of insulin.

In the OATT, the urea nitrogen concentrations gradually increased with time in all cases and a marked increase was particularly seen in the diabetic group, but the trend was slight in the cirrhotic group. Since the urinary and blood creatinine concentrations remained unchanged during the OATT, the urinary output of urea nitrogen was constant.[16] Since urea nitrogen is formed by the deamination of alanine in the liver through the process in which pyruvate is produced, the increase of the blood urea nitrogen concentration in the OATT signified the accelerated conversion of alanine to pyruvate.[16,17] Therefore, the findings that in the cirrhotic group the α-amino acid concentrations following alanine administration were higher than those of the other two groups and that the urea nitrogen levels were lower suggest that the conversion mechanism from alanine to pyruvate is impaired in this condition. And also, when we consider the coupling of gluconeogenesis

Table 3. Blood or serum levels of metabolites and hormones during the 3-hour OGTT and the 3-hour OATT in 8 cirrhotic subjects.

Oral Glucose Tolerance Test		Time (min)				
		0	30	60	120	180
Blood glucose	(mg/dl)	108 ± 2	200 ± 12**	228 ± 17**	214 ± 19**	145 ± 19
Lactate	(mM/L)	1.14 ± 0.12	1.26 ± 0.10	1.51 ± 0.07*	1.51 ± 0.11*	1.43 ± 0.18
Pyruvate	(mM/L)	0.12 ± 0.02	0.15 ± 0.02	0.18 ± 0.02*	0.16 ± 0.01*	0.13 ± 0.01
α-Amino nitrogen	(mg/dl)	15.2 ± 1.0	13.9 ± 0.8	13.1 ± 0.9	12.8 ± 0.9	12.2 ± 0.6*
Urea nitrogen	(mg/dl)	11.6 ± 0.8	11.1 ± 1.1	11.2 ± 0.9	10.8 ± 1.0	10.8 ± 1.0
Free fatty acid	(μEq/L)	569 ± 67	414 ± 66	235 ± 27**	234 ± 51**	267 ± 84*
Glycerol	(μM/ml)	0.64 ± 0.15	0.46 ± 0.11	0.36 ± 0.07	0.25 ± 0.07	0.29 ± 0.11
Total ketone bodies	(mM/L)	0.10 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.06 ± 0.02
Insulin	(μU/ml)	11.3 ± 1.7	66.6 ± 11.7**	92.6 ± 19.1**	119.6 ± 18.3**	67.9 ± 12.5**
C-peptide	(ng/ml)	2.4 ± 0.4	4.6 ± 0.9*	5.6 ± 0.8**	7.7 ± 1.6*	7.1 ± 1.7*
Growth hormone	(ng/ml)	5.8 ± 1.2	3.7 ± 1.1	3.0 ± 1.1	1.5 ± 0.4*	1.9 ± 0.2**
Glucagon	(pg/ml)	185 ± 39	139 ± 24	138 ± 15	122 ± 18	130 ± 20
Oral Alanine Tolerance Test		**0**	**30**	**60**	**120**	**180**
Blood glucose	(mg/dl)	104 ± 5	109 ± 3	109 ± 4	110 ± 4	108 ± 3
Lactate	(mM/L)	1.14 ± 0.05	1.43 ± 0.09*	1.42 ± 0.14	1.19 ± 0.10	1.46 ± 0.08*
Pyruvate	(mM/L)	0.15 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.20 ± 0.02
α-Amino nitrogen	(mg/dl)	18.7 ± 1.5	36.9 ± 2.0**	43.7 ± 2.3**	43.1 ± 1.6**	44.7 ± 1.7**
Urea nitrogen	(mg/dl)	8.6 ± 1.2	8.7 ± 1.3	9.5 ± 1.4	10.5 ± 1.5	13.4 ± 2.3
Free fatty acid	(μEq/L)	770 ± 119	646 ± 74	337 ± 37*	329 ± 51*	513 ± 124
Glycerol	(μM/ml)	0.67 ± 0.08	0.46 ± 0.07	0.37 ± 0.05*	0.38 ± 0.05*	0.46 ± 0.06
Total ketone bodies	(mM/L)	0.17 ± 0.03	0.11 ± 0.01	0.09 ± 0.01*	0.09 ± 0.01*	0.09 ± 0.01*
Insulin	(μU/ml)	13.1 ± 2.1	29.6 ± 8.2	30.4 ± 9.4	24.9 ± 6.5	20.8 ± 4.6
C-peptide	(ng/ml)	3.1 ± 0.3	3.8 ± 0.4	3.8 ± 0.2	3.5 ± 0.4	3.7 ± 0.6
Growth hormone	(ng/ml)	5.5 ± 1.4	8.8 ± 2.4	11.4 ± 5.1	16.9 ± 3.3*	11.5 ± 4.1
Glucagon	(pg/ml)	246 ± 90	575 ± 196	611 ± 192	796 ± 99*	759 ± 138*

Mean values ± SEM of 8 cirrhotic subjects.
** $p<0.005$ as compared with 0 min basal value.
* $p<0.05$ as compared with 0 min basal value.
Total ketone bodies : acetoacetate +β-hydroxybutyrate.

and ureogenesis, ureogenesis is accelerated in the diabetic state along with accelerated gluconeogenesis. This ureogenesis is further augmented by the influx of a large quantity of alanine, which is a precursor to these metabolic processes, into the liver. It is well known that urea production is inhibited by insulin and accelerated by glucagon.[18] However, it is unclear whether the changes observed in this study were due to glucagon secreted in response to the alanine loading.

It is well known that glucose inhibits pancreatic α-cell function and accelerates β-cell activity.[19] On the other hand, amino acids such as arginine and leucine are widely used in vivo or in vitro to stimulate production of various hormones, and alanine has a stimulating action on the pancreatic α- and β-cells as well.[3] The hormonal stimulatory effects of amino acids are not always similar in nature, and it is reported that the secretory response to alanine stimulation[3] is not disturbed despite decreased insulin secretion due to glucose and arginine stimulation in the long-term fasting state in both humans[20] and dogs.[3] This accorded with our findings that in diabetic patients the insulin secretory reaction was only slight in the OGTT, whereas in the OATT it was almost comparable

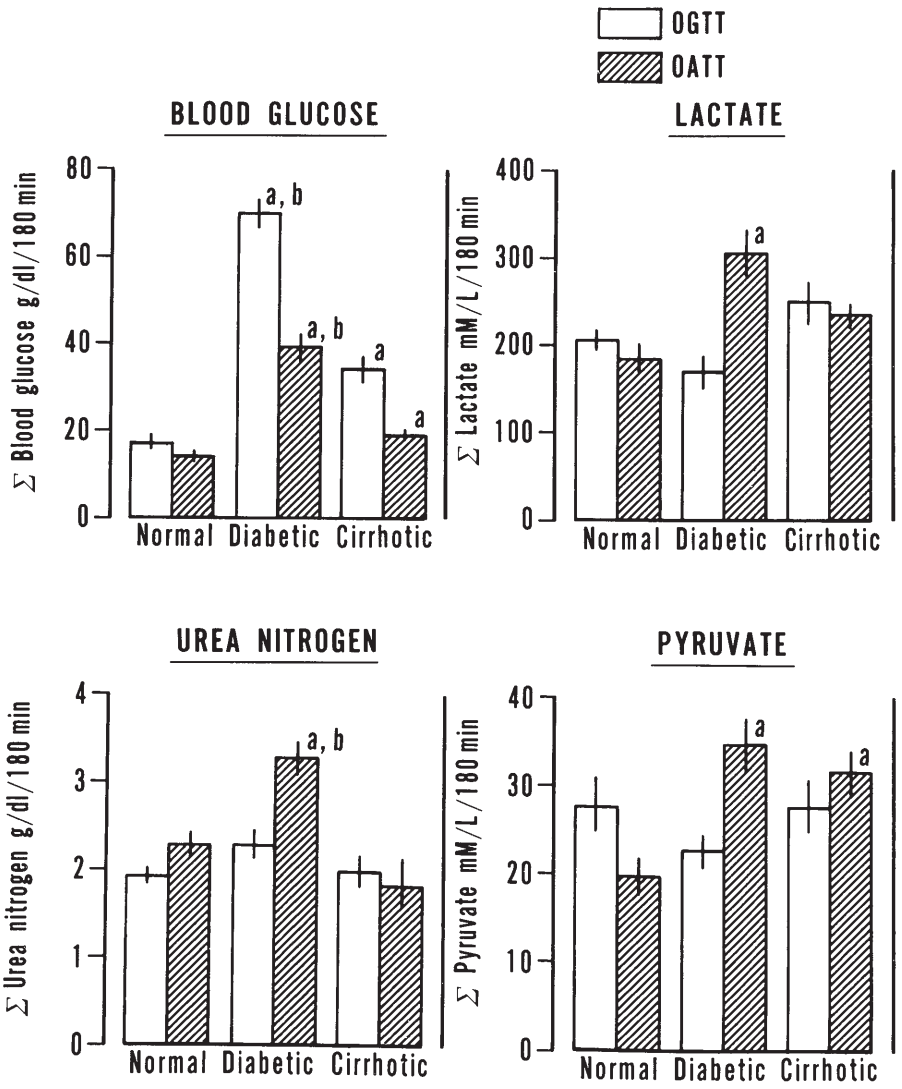

Fig. 1. Integrated areas of blood glucose, lactate, pyruvate and serum urea nitrogen during OGTT and OATT in normal, diabetic, and cirrhotic subjects.
Mean values ± SEM of 14 normal, 12 diabetic and 8 cirrhotic subjects. [a]$p<0.01$ compared with normal subjects; [b]$p<0.01$ compared with cirrhotic subjects, respectively.

with that of normal subjects.

The potency of the glucagon secretory reactions in the OATT ranked in the order of cirrhotic group, diabetic group, and normal subjects. The glucagon secretory reaction in the cirrhotic group was extremely potent and even its pre-loading values were approximately twice as high as those of normal subjects and diabetic patients. It has been reported that the values in cirrhotic patients who have had a portocaval shunt operation were about three times as high as those of normal subjects,[21] but patients with a past history of shunt operation were not included in this study. Since the high

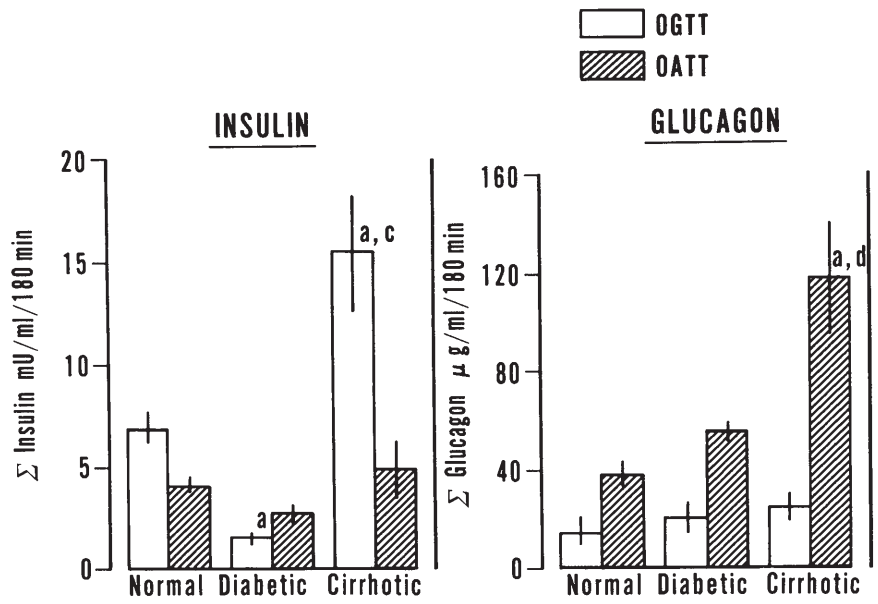

Fig. 2. Integrated areas of serum insulin and glucagon during OGTT and OATT in normal, diabetic, and cirrhotic subjects.
Mean values ± SEM of 14 normal, 6–12 diabetic and 8 cirrhotic subjects. Insulin area for the diabetic subjects is calculated from the values of 6 patients before insulin treatment. $^{a,b}p<0.01$, 0.05 compared with normal subjects; $^{c,d}p<0.01$, 0.05 compared with diabetic subjects, respectively. Integrated area means the calculated value under the serum response curve during the 3-hour OGTT or OATT.

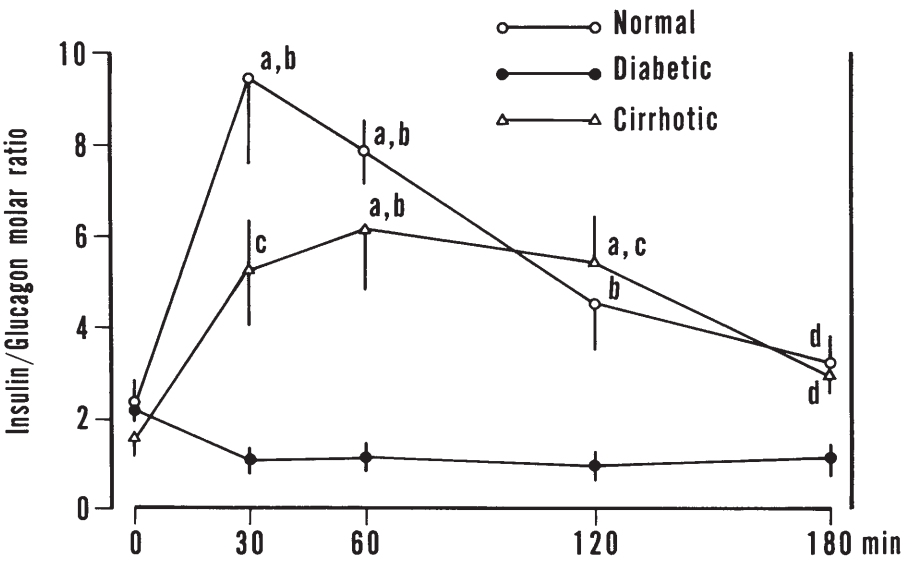

Fig. 3. Insulin/glucagon molar ratio calculated with insulin during OGTT vs glucagon during OATT in normal, diabetic, and cirrhotic subjects.
$^{a}p<0.01$ compared with 0 min basal value in each group; $^{b,c,d}p<0.01$, 0.02, 0.05 compared with diabetic subjects, respectively.

glucagon values observed in the subjects in this study were recorded in the presence of esophageal varices, it is quite clear that some kind of shunt may be involved. Also, it was considered that these values may have resulted from reduced glucagon degradation in the liver. The reason why no noticeable rise of the blood glucose was observed in the diabetic and cirrhotic groups, in which hyperglucagonemia was seen in the OATT, may be due to the fact that peripheral glucose utilization is greater than the gluconeogenesis from alanine.

In recent years, the effects of insulin-stimulating anabolism and of glucagon-stimulating catabolism in metabolic regulation have been discussed in terms of the insulin/glucagon molar ratio, whose significance has received special emphasis.[19,22] When both hormones are simultaneously added to the perfusate to steadily maintain the insulin/glucagon molar ratio constant in isolated rat liver perfusion experiments, the liver's metabolic efficiency remains unchanged irrespective of the concentrations used.[23] As shown in Fig. 3, the changes of the insulin (during OGTT)/glucagon (during OATT) molar ratio with time may be considered to be a good indicator of the capacity for utilization of glucose in the whole body, and characteristic features were seen in the patients with diabetes mellitus and cirrhosis. These findings of characteristic metabolic patterns during glucose and L-alanine loading in each condition may be applicable to clinical use.

CONCLUSION

Glucose and L-alanine were orally administered to normal subjects and to patients with diabetes mellitus and cirrhosis of the liver, and observations of the main metabolic and hormonal changes were made over 180 min. Each metabolic product and hormone underwent changes according to the mutual regulation mechanisms, and the changes in blood glucose, lactate, pyruvate, urea nitrogen, insulin and glucagon levels were particularly noted. From the results it appeared that, in addition to the OGTT, the oral administration of L-alanine can be a useful tool for the diagnosis of the status in diabetes mellitus and cirrhosis

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