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A Study on Serum Insulin, 24-hr Urine C-peptide and RBC Insulin Receptor in Patients with Chronic Liver Disease

만성 간질환환자에서의 혈중 인슈린, 24시간 뇨중 C-peptide와 적혈구 인슈린 수용체에 관한 연구

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Impaired glucose tolerance is a consistent finding of chronic liver disease and associated with hyperinsulinemia (Teng et al., 1980; Collins et al., 1970). This finding implies insulin resistance. The exact mechanism of this impaired glucose tolerance and insulin resistance is, however, unclear.

The present study was to understand at least a part of the pathogenic mechanism of this condition. To achieve this aim, we measured fasting and postprandial blood glucose, serum insulin as short-term insulin response, 24-hr urine C-peptide as an index of total daily insulin secretion amount, and total number and binding affinity of RBC insulin receptor as peripheral insulin effect.

SUBJECTS AND METHODS

Eleven patients with chronic liver disease and 8 age- and weight-matched controls were studied, as shown in Table 1.

The diagnosis was confirmed by liver biopsy. 5 patients had chronic active hepatitis with severe tissue injury and 6 patients had cirrhosis of the liver. All patients were hepatitis B surface antigen positive, and had normal renal function and serum electrolytes levels. No patients took any medication before the study that might influence glucose tolerance. All controls had normal liver function tests and no family history of diabetes mellitus.

Table 1. Clinical data of control group and patients with chronic liver disease

	Controls(8)	Patients with chronic liver disease(11)
Mean age	42.9(27-62)	40.2(26-58)
Male/Female	7/1	8/3
% ideal body wt.	103.7±4.7	108.7±3.2

Number in parenthesis are cases studied. 5 chronic active hepatitis and 6 cirrhosis of the liver were studied.

All tests were done after an overnight fast. Blood glucose was measured with autoanalyzer utilizing hexokinase method. Postprandial blood was taken 2-hr after the regular hospital diet. Serum insulin and 24-hr urine C-peptide were measured by radioimmunoassay kits from Daiichi laboratory, Japan. RBC insulin receptor study was performed using the methods of Gambhir et al. (1977) and Kobayashi et al. (1980) with slight modification. RBC insulin receptor study results were analyzed using Scatpack program developed by Rodbard et al. of N.I.H. Statistical

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Table 2. Fasting and postprandial 2-hour blood glucose, serum IRI and 24-hour urine C-peptide in controls and patients with chronic liver disease

	Controls (8)	Chronic liver disease(11)
Fasting blood glucose (mg/dl)	91±2	93±5
PP_2 hour blood glucose (mg/dl)	122±2	165±17*
Fasting serum IRI (uU/ml)	7.0±1.2	20.0±1.53***
PP_2 hour serum IRI (uU/ml)	27.8±1.5	115.3±24.7**
24-hour urine C-peptide (ug/gr. of creatinine)	29.4±4.0	66.2±9.5**

Numbers in parenthesis are number of subjects studied.
*P<0.05 **P<0.025 ***P<0.005 vs controls
Date are shown as Mean±S.E.M.

analysis was done with the Student's t-test and linear regression analysis.

RESULTS

Table 2 shows blood glucose, serum insulin and 24-hr urine C-peptide in controls and patients with chronic liver disease. In chronic liver disease the fasting blood glucose was not different from that of controls, but postprandial blood glucose level was significantly higher(p<0.05). In comparison with controls, the fasting and postprandial insulin levels and 24-hr urine C-peptide were significantly higher in chronic liver disease (p<0.025).

To see further mechanism of impaired glucose tolerance in chronic liver disease, we divided the patients with chronic liver disease into two groups, one with normal glucose tolerance and another with impaired glucose tolerance, with an arbitary criteria of impaired glucose tolerance by fasting blood glucose level above 120 mg/dl and postprandial blood glucose level above 140 mg/dl. Two groups were not different each other in their ages and body weights as shown in Table 3. There was also no difference in the degree of liver derangement as measured by liver function tests (data not shown here).

Table 3. RBC insulin receptor study results in normal controls and patients with chronic liver disease

	Normal controls (n=8)	Patients with chronic liver disease(n=11)
Maximal % bound of^{125} I-insulin	7.9±0.8	5.2±0.6*
$K_1(M^{-1}\times 10^9)$	1.3±0.1	1.3±0.3
$Ke(M^{-1}\times 10^7)$	2.4±0.3	3.1±0.4
Total receptor number per cell	673±104	325±44.4*

K_1 : High affinity association constant
Ke : Average empty site affinity
Data are shown as Mean ± S.E.M.
*P<0.005 vs controls

In both groups, the fasting and postprandial insulin levels and 24-hr urine C-peptide were significantly higher, compared with controls, as shown in table 4. In the impaired glucose tolerance group, the postprandial insulin level was significantly higher than that in the normal glucose tolerance group in the presence of similar 24-hr urine C-peptide (p<0.05).

The RBC insulin receptor study results are shown in Figure 1 and Table 5. RBC from patients with chronic liver disease showed significantly lower binding to ^{125}I-insulin,

Table 4. Clinical and laboratory parameters for patients with chronic liver disease with normal and impaired glucose tolerance*

	With normal glucose tolerance (5)	With impaired glucose tolerance (6)
Mean Age	36.6(26-56)	42.9(36-58)
Male/Female	3/2	6/1
% Ideal body wt.	105.4±2.1	109.4±5.1
Cirrhotic patients	3	3
Chronic hepatitis	2	3

* Normal glucose tolerance; FBS <120mg/dl and PP_2 BS<140mg/dl

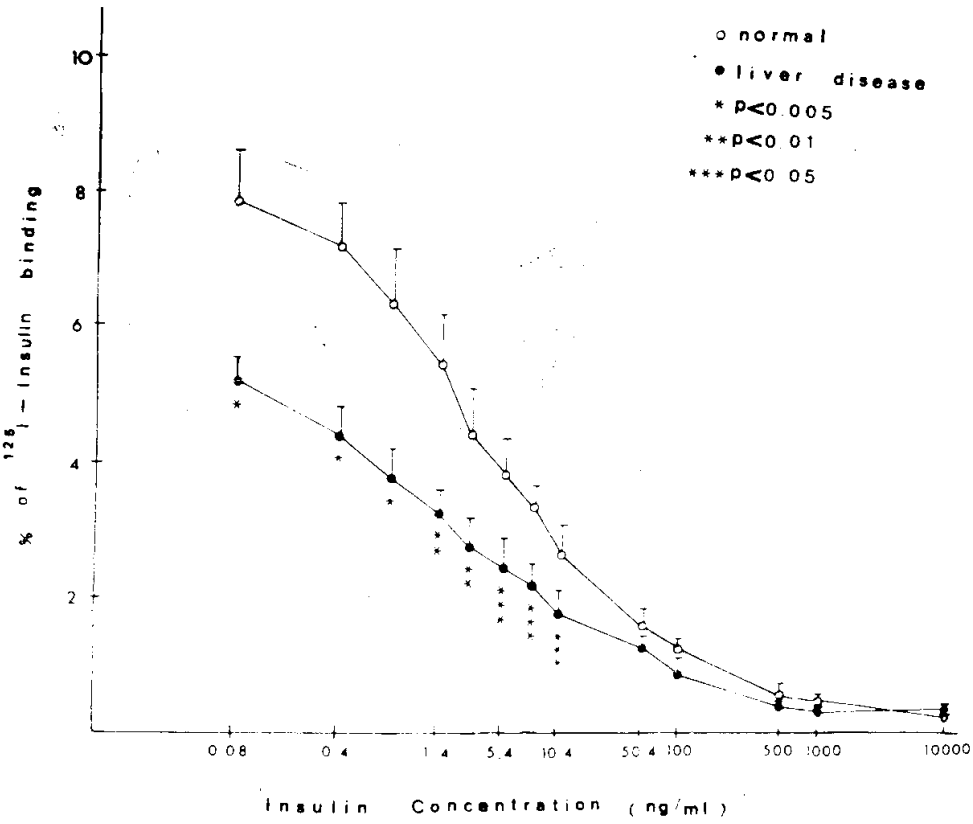

Fig. 1: ^{125}I-insulin binding to RBC insulin receptor in the study subjects

Table 5. Blood glucose, serum IRI, 24-hour urine C-peptide and RBC insulin receptor study results in patients with chronic liver disease; in groups with normal and impaired glucose tolerance

	Controls (8)	With Normal glucose tolerance (5)	With Impaired glucose tolerance (6)
Fasting blood glucose (mg/dl)	91±2	89±6	96±7
PP_2 hour blood glucose (mg/dl)	122±2	118±7	203±19*
Fasting serum IRI (uU/ml)	7.0±1.2	13.2±1.4^{+}	26.7±9.8^{++}
PP_2 hour serum IRI (uU/ml)	27.8±1.5	68.8±14.1^{+}	173.5±37.7**
24-hour urine C-peptide (ug/gr. of creatinine)	29.4±4,0	68.8±9.3^{+}	63.7±17.3^{++}

Data are shown as Mean ± S.E.M.
*P<0.005 **P<0.05 vs with normal glucose tolerance
$^{+}$P<0.005 $^{++}$P<0.05 vs controls

compared with normal controls. The maximal binding was 7.9±0.8% (the mean±S.E.M.) in controls and 5.2±0.6% in chronic liver disease (p<0.005). There was a significant decrease in total receptor number per cell in chronic liver disease (p<0.005), but no difference was detected in the high affinity association constant(K_1) and the average empty

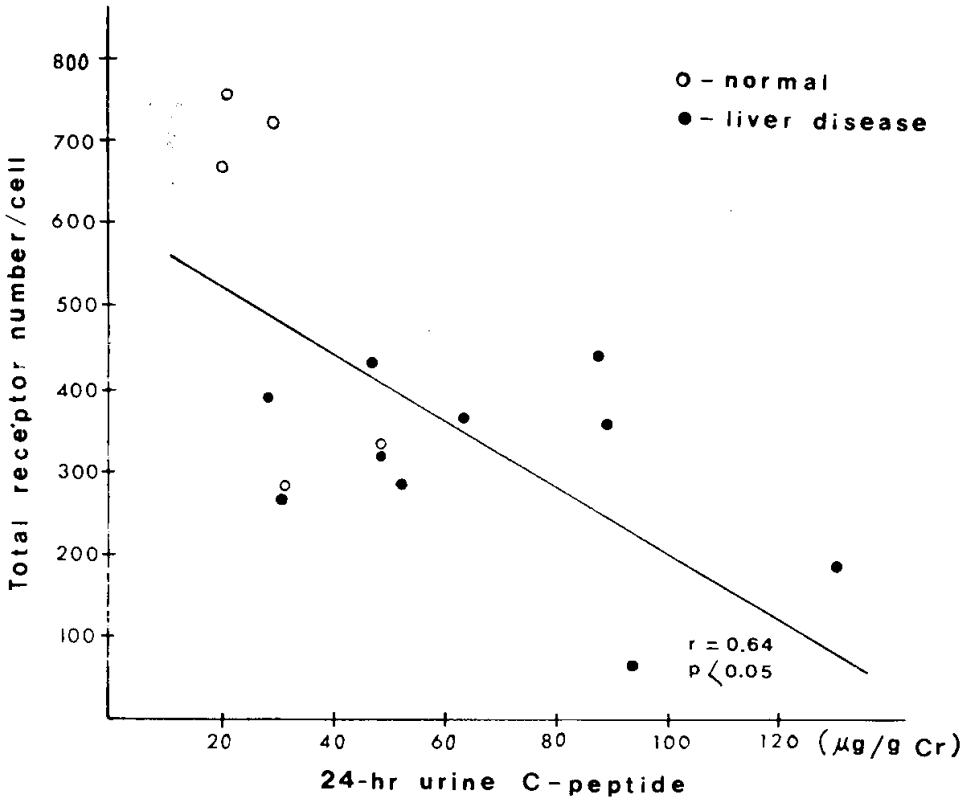

Fig. 2: Correlation between total receptor number per cell and 24-hr urine C-peptide in the study subjects

site affinity(Ke). Total receptor number per cell was negatively correlated with 24-hr urine C-peptide as shown in Figure 2 ($r=0.64$, $p<0.05$) but not with fasting and postprandial insulin levels (data not shown here).

INTERPRETATION AND DISCUSSION

The mechanism of impaired glucose tolerance in chronic liver disease is still not clearly understood. The cause probably varies between different natures of chronic liver disease and may be indeed multifactorial. The most likely causes seem to be a diminished ability of the damaged liver to synthesize and store glycogen (reduced glucose and insulin uptake by the damaged liver) and peripheral insulin resistance (Wright et al., 1979).

In patients with chronic liver disease studied, hyperinsulinemia and impaired glucose tolerance have been found in agreement with other reports (Teng et al., 1982; Proietto et al., 1980; Colliins et al., 1970; Johnston et al., 1978). These results suggest insulin resistance.

In the present study as shown in Table 2, 24-hr urine C-peptide was 2 times as much as that in controls. This doubling of urine C-peptide suggests that the pancreatic B-Cells are responding to hyperglycemia probably to compensate insulin resistance state. Even though 24-hr urine C-peptide was doubled in chronic liver disease, the fasting and postprandial insulin levels were about 3 to 4 times higher than those in controls. This discrepancy between increment rate of 24-hr urine C-peptide and serum insulin levels in chronic liver disease

may be explained by the decreased hepatic insulin degradation. However Johnston et al. (1978) have reported from serum insulin and C-peptide studies that hyperinsulinemia in chronic liver disease is not due to increased insulin secretion, but due to decreased insulin degradation. This discrepancy may be due to different natures of chronic liver disease or different methods used. We measured 24-hr urine C-peptide in the present study whereas they measured serum insulin and C-peptide in their studies. Theoretically, the pancreas in chronic liver disease may be normal and will respond normally to hrperglycemia. In the present study, we divided the patients with chronic liver disease into two groups, one with normal glucose tolerance and another with impaired glucose tolerance (Table 3 and Table 4). In normal glucose tolerance group the blood glucose level may be maintained normal owing to compensation of the insulin resistance by increased insulin secretion and hyperinsuline mia. However in the impaired glucose tolerance group, insulin resistance might not have been fully compensated. The fact that the insulin levels were even higher in this group than those in normal glucose tolerance group in the presence of similiar 24-hr urine C-peptide can be interpreted as evidence of decreased insulin degradation, probably at the liver. Our obser vation that the higher the blood glucose, the higher was the serum insulin levels in the presence of similar 24-hr urine C-peptide in these two groups can only be interpreted as proportionate impairment of the insulin degradation and glucose tolerance in chronic liver disease. However whether this phenomenon is primarily due to liver disease per se or peripheral insulin resistance has not been clarified yet(Wright et al., 1979). Proietto et al.(1980) have found the impaired glucose tolerance of cirrhosis is due to the defect in peripheral glucose utilization.

In the present study, the binding of receptor in RBC was decreased in chronic liver disease, compared with controls. This observation is consistent with other reports (Teng et al., 1982; Piniewski et al., 1980). This decrease in receptor binding was due to a decrease in total receptor number per cell rather than a decrease in the binding affinity(Table 5). Total receptor number per cell was negatively correlated with 24-hr urine C-peptide (Figure 2). Even though in the present study, the correlation between serum insulin level and insulin receptor number is not quite well, the decrease in total number of RBC insulin receptor in the present study may be considered to be the effect of down-regulation of RBC receptor by hyperinsulinemia.

In agreement with our results, Piniewski et al. (1980) have found decreased insulin binding in monocyte in cirrhosis and this was due to a dcrease in receptor number, but binding affinity was unaltered. However, Teng et al. (1982) have found lower insulin binding in cirrhosis and they inferred that binding affinity rather than receptor number was affected in cirrhosis. This discrepancy is difficult to explain. Moreover other workers have reported that such a down-regulation is not present in their studies(Okada et al., 1981; Robinson et al., 1979). If insulin binding of RBC reflects indirectly the insulin binding of other insulin sensitive tissues, down-regulation of insulin receptor in liver cells and peripheral tissue might further contribute to insulin resistance and impaired glucose tolerance in chronic liver disease.

In conclusion, reduced glucose uptake and insulin degradation by the liver (a diminished ability of the damaged liver to retain glycogen) may be primarily responsible for impaired glucose tolerance in chronic liver disease. Hyperinsulinemia caused by compensatory hyper-

secretion from pancreatic B-cells and reduced hepatic degradation leads to down-regulation of insulin receptor in insulin sensitive tissue and insulin resistance in chronic liver disease.

SUMMARY

To elucidate the pathogenic mechanisms of impaired glucose tolerance in chronic liver-disease, we measured serum immunoreactive insulin (IRI), 24-hour urine C-peptide and RBC insulin receptor in 8 normal controls and 11 patients with chronic liver disease.

The fasting and postprandial IRI levels and the 24-hour urine C-peptide concentration were significantly higher in chronic liver disease than normal controls, thus proving the increase in insulin secretion by beta cell in chronic liver disease. RBC from patients with chronic liver disease showed significantly lower ^{125}I insulin binding and there was a significant decrease in the total number of RBC insulin receptor per cell. But no difference was detected in the binding affinity of RBC insulin receptor. The decrease in maximal ^{125}I insulin binding to RBC was not due to a decrease in the binding affinity but to a decrease in the total number of RBC insulin receptor.

=국문초록=

만성 간질환환자에서의 혈중 인슈린, 24시간 뇨중 C-peptide와 적혈구 인슈린 수용체에 관한 연구

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저자들은 만성간질환에서 이상 내당능과 인슈린 저항성의 기전 일부를 밝히고저, 정상인 8례와 만성간질환환자 11례를 대상으로 하여 혈중 인슈린, 24시간 뇨중 C-peptide 및 적혈구 인슈린 수용체를 측정하여 다음과 같은 결과를 얻었다.

1. 만성 간질환에서 고인슈린혈증과 췌장에서 인슈린 분비 증가를 관찰 할수 있었으며 이는 정상 혈당 혹은 고혈당과 동반되어 인슈린 저항상태가 존재함을 보였다.

2. 만성간질환에서 인슈린저항성은 고인슈린혈증에 의해 보상이 되거나 혹은 완전히 보상되지 않는 두가지 형태를 보였다.

3. 표적세포에서 인슈린수용체수의 감소는 인슈린 저항성의 한 원인이 될 수 있다.

4. 간에서 당흡수장애와 인슈린 분해감소가 만성간질환에서 이상 내당능의 가장 중요한 원인으로 생각된다.

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