Altered Basal C-peptide/insulin Molar Ratios in Obese Patients with Fatty Liver

Toshiki Inokuchi, Keiko Watanabe, Hisako Kameyama and Miyuki Orita

To elucidate if the presence of fatty liver in obesity influences hepatic insulin extraction under basal conditions, serum immunoreactive insulin (IRI) and C-peptide immunoreactivity (CPR) were measured in 20 obese patients with normal glucose tolerance and in 8 normal subjects. The obese patients were subdivided into two groups matched for age and body weight according to the presence or absence of fatty liver: 8 obese patients without fatty liver (OBN) and 14 with fatty liver (OBF). Basal levels of IRI and CPR were significantly greater in the obese patients than in the normals, but were similar in the two obese groups. In the OBF group, the CPR/IRI molar ratios, a relative measure of hepatic insulin uptake, were significantly lower than in the other two groups, while the ratios of the normal and OBN groups were similar. The CPR/IRI molar ratios in all obese patients correlated well with the degree of fatty liver (r=0.785, p<0.001). These results suggest that hepatic insulin extraction in a subgroup of obese patients is either reduced or indistinguishable from that of non-obese subjects, and that basal CPR/IRI molar ratio may serve as a useful indicator of the presence of fatty liver in simple obesity.

Key Words: Insulin, C-peptide, CPR/IRI molar ratio, Fatty liver, Simple obesity

Hyperinsulinemia in the basal state is a well-recognized feature of simple obesity. The circulating insulin level is theoretically a result of the rate of insulin secretion and of its metabolic clearance rate. However, previous results of human obesity studies on insulin metabolism at the hepatic level underlying this hyperinsulinemia have been conflicting1-4. Although interpretation of plasma insulin values is hampered by uncertainty as to the extraction of hepatic insulin, simultaneous determinations of insulin and C-peptide in peripheral blood are a better reflection of endogenous insulin secretion as well as of the extent of hepatic uptake of insulin, as hepatic extraction of C-peptide is negligible5,6. Several investigators, however, have recently emphasized certain limitations in interpreting the C-peptide/insulin molar ratios7, especially under non-steady state conditions, such as those after various loading and stimulation procedures8-10.

Obese patients are also associated with an increased incidence of fatty liver11,12, which may be partly related to the presence of hyperinsulinemia, because the liver is the major site of insulin metabolism. The effect of the occurrence of fatty liver on insulin metabolism has not yet been investigated in moderate obesity.

Therefore, we evaluated the basal C-peptide/insulin molar ratio in weight-matched, middle-aged, moderately obese patients with normal glucose tolerance to determine whether the existence of fatty liver in obesity may or may not contribute to hepatic insulin extraction.

MATERIALS AND METHODS

Twenty obese patients were selected for this study on the basis of having a normal glucose tolerance and body mass index of greater than 30.
The normal-weight group consisted of 8 healthy subjects, each having a body mass index of less than 25. The obese patients were otherwise healthy and had maintained a constant body weight for at least 3 months before testing; none of the subjects was taking medication known to affect glucose and insulin metabolism, nor drinking alcohol. In all subjects, renal function was normal.

Glucose tolerance was estimated according to the 1980 WHO criteria and degree of obesity by the body mass index (BMI=Kg/m²). The degree of hepatic steatosis was assessed by computed tomography (CT) using a Toshiba TCT 80-A whole-body scanner. A liver: spleen ratio in CT numbers (Hounsfield units) of less than 0.9 was taken to represent significant hepatic steatosis.

All subjects received a diet containing more than 250g of carbohydrates daily before and during the study. After an overnight fast, a standard oral glucose tolerance test (75g OGTT) was performed. Blood samples were taken for 2 hours and analyzed for plasma glucose (PG), serum immunoreactive insulin (IRI) and C-peptide immunoreactivity (CPR). Furthermore, blood samples were taken for estimation of IRI and CPR in the fasting state on at least two separate days, and the means of the measurements of the respective variables were used as basal values. All examinations undertaken in this study were performed randomly within one week.

Plasma glucose concentration was measured by the glucose oxidase method. Serum IRI was determined using an INSULIN RIA BEAD kit (Dainabott Co., Ltd. Tokyo). Serum CPR was measured by radioimmunoassay using a commercially-available kit (Daichi Radioisotope Co., Tokyo).

The CPR/IRI molar ratio was calculated on the basis of a molecular weight of 6,000 for insulin and a molecular weight of 3,000 for C-peptide. The PG/IRI and PG/CPR values were also calculated as a measure of insulin resistance and of the pancreatic B cell reactivity to glucose, respectively.

Statistical analysis was made using Student’s t-test. A P value of less than 0.05 was considered statistically significant. All data in the text and figures are expressed as mean ± SD.

RESULTS

On the basis of the liver: spleen ratio in CT numbers, twenty moderately obese patients were divided into two groups: one of 8 obese patients without fatty liver (OBN) and one of 12 with fatty liver (OBF). The ratios were 1.15 ± 0.05, 1.07 ± 0.09 and 0.61 ± 0.13 in normal, OBN and OBF groups, respectively, indicating a significantly (p<0.001) lower value in OBF, compared with the other groups.

Table 1 shows the clinical characteristics of the subjects. Age was similar in each group and the degree of obesity was also similar in the two obese subject groups, the BMI range being 30.0 – 40.8 and 30.0 – 40.2 in the OBN and OBF groups, respectively. Significant differences were noted in fasting PG, serum IRI and CPR concentrations between normal subjects and obese patients, although all of the obese patients had fasting PG levels of less than 6.05 m mol/L. All obese patients showed hyperinsulinemia in comparison to basal IRI levels found in normals. However, among obese patients, it was found that the mean fasting PG and IRI levels were slightly higher, but not significantly different in the OBG group, whereas CPR basal levels tended to be somewhat higher in the OBN group. BMI correlated significantly with basal IRI (r=0.572, p<0.009, n=20) as well as with CPR levels (r=0.777, p<0.001) in the obese patients. A relationship between basal IRI and CPR values was also noted in the obese patients (r=0.702, p<0.001).

Figure 1 shows the CPR/IRI molar ratios, as

<table>
<thead>
<tr>
<th>Subjects (Number)</th>
<th>Age (yr)</th>
<th>BMI (kg/m²)</th>
<th>PG (mmol/L)</th>
<th>IRI (pmol/mL)</th>
<th>CPR (pmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects (8)</td>
<td>31.4±7.0</td>
<td>22.8±1.3</td>
<td>5.0±0.3</td>
<td>0.05±0.01</td>
<td>0.50±0.10</td>
</tr>
<tr>
<td>Obese patients (20)</td>
<td>29.6±8.8</td>
<td>32.7±3.1</td>
<td>5.5±0.4</td>
<td>0.14±0.06</td>
<td>1.04±0.38</td>
</tr>
<tr>
<td>OBN (8)</td>
<td>32.4±8.7</td>
<td>32.5±1.4</td>
<td>5.4±0.5</td>
<td>0.11±0.03</td>
<td>1.10±0.40</td>
</tr>
<tr>
<td>OBF (12)</td>
<td>27.0±8.2</td>
<td>32.2±2.8</td>
<td>5.5±0.3</td>
<td>0.15±0.07</td>
<td>1.09±0.37</td>
</tr>
</tbody>
</table>

OBN: Obese patients without fatty liver, OBF: Obese patients with fatty liver. Values are mean ± SD.
* p<0.01, ** p<0.05, compared with normal subjects.
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Fig. 1. Basal CPR/IRI molar ratio, PG/IRI and PG/CPR values in the normal, OBN and OBF groups.

OBN: Obese patients without fatty liver, OBF: Obese patients with fatty liver. Numbers in parenthesis. Values are mean ±SD. *p<0.01, **p<0.05, compared with values between the corresponding groups.

Fig. 2. Relationship between basal CPR/IRI molar ratios and degree of fatty liver in obese patients.

○: Obese patients with fatty liver (n=12), ◆: Obese patients without fatty liver (n=8).

well as the PG/IRI and PG/CPR values for each group, under basal conditions. The calculated molar ratios of CPR/IRI were 11.62 ± 1.53, 10.53 ± 2.50 and 6.73 ± 1.60 in normal subjects, OBN, and OBF patients, respectively. The ratio in the OBF group was significantly lower than those in the other two groups, whereas no differences were found between normal and OBN groups. Both the PG/IRI and PG/CPR values were significantly (p<0.01) lower in obese patients (PG/IRI: 45.49 ± 14.01, PG/CPR: 5.80 ± 1.68) than in normals (PG/IRI: 115.40 ± 37.18, PG/CPR: 10.25 ± 2.30; p<0.01, p<0.01, respectively). On the other hand, when compared between the two obese patients groups, the OBF group was seen to have a significantly (p<0.049) lower PG/IRI (OBF: 40.52 ± 11.79, OBN: 52.96 ± 14.43), whereas the PG/CPR value in the OBF group was somewhat higher than that of the OBN group (OBF: 6.01 ± 1.60, OBN: 5.49 ± 1.85). BMI correlated significantly with PG/IRI (r=-0.529, p<0.017, n=20) as well as with PG/CPR values (r=-0.735, p<0.001) in all obese patients, whereas the CPR/IRI molar ratios showed no significant correlation with BMI (r=0.142, p=NS). The CPR/IRI ratios in turn correlated well with basal IRI (r=-0.497, p<0.026), but poorly with CPR levels (r=0.239).

Figure 2 shows the relationship between the degree of fatty liver and basal CPR/IRI molar ratios in obese patients. A strong correlation was noted between the liver: spleen ratios in CT numbers and basal CPR/IRI molar ratios (r=0.785, p<0.001). A relationship between the liver: spleen ratios in CT numbers and basal IRI or PG/IRI values was also found in all obese patients (r=-0.593; p<0.006, r=0.532; p<0.016, respectively), whereas neither basal CPR nor PG/CPR values correlated significantly with the degree of fatty liver (r=-0.038, r=-0.043, respectively).
DISCUSSION

The present study demonstrated that obese patients with hyperinsulinemia can be separated into two subgroups based on differences in insulin metabolism at the liver. Our data also confirmed previous observations of basal hyperinsulinemia and insulin resistance in the obese patients\(^1\,{}^5\,{}^6\).

Our results demonstrate that reduced hepatic extraction, as assessed by CPR/IRI molar ratios, is the primary cause of hyperinsulinemia in obese patients with fatty liver, while pancreatic hypersecretion plays a lesser role. In assessing molar ratio data, the contribution of the circulating proinsulin to these hormone immunoassays should be considered. In our patients, this contribution seemed to be negligible in obese individuals with impaired IRI/CPR ratios\(^4\). Thus, impairment at the hepatic level, as is seen in such patients with mild insulin resistance, may be due solely to reductions in the hepatic insulin receptors\(^1\,{}^6\,{}^7\). In contrast, the CPR/IRI ratio in obese patients without fatty liver may reflect the fact that the extent of metabolic clearance at the liver is almost the same as that of normals. However, a definitive explanation for hyperinsulinemia in these patients remains unclear from this study.

Moreover, we found a strong correlation between the CPR/IRI molar ratio and the degree of fatty liver. This result supports our hypothesis that this ratio could be a useful parameter in detecting the presence of fatty liver among obese patients. In addition, this ratio in obese patients was correlated with the basal IRI, but not with the CPR level. This would agree with the report published by Rossell et al\(^4\); although in their study the presence or absence of fatty liver was not evaluated, the possibility exists that most of their obese patients had fatty liver.

With regard to the relationship between liver disease and hepatic insulin metabolism, decreased hepatic insulin extraction has been well demonstrated in cirrhosis of the liver with absolute hyperinsulinemia\(^1\,{}^8\,{}^9\). In these patients, fasting CPR/IRI molar ratios were decreased in the presence of normal\(^1\,{}^8\) or slightly elevated\(^9\) peripheral C-peptide concentrations. On the contrary, in our obese patients with fatty liver, this ratio was reduced in the presence of markedly increased C-peptide levels. Thus, it should be noted that there is an apparently pathophysiological difference in insulin metabolism between functional defect of the liver parenchyma, including a relative reduction in effective hepatic blood flow, as is the case in cirrhosis, and a functional alteration in the hepatocytes, as is the case in fatty liver of obese patients. From the above data, it is reasonable to suggest that fatty liver itself, observed in moderately obese patients, could result in a decreased insulin degradation, which may, to some extent, influence the insulin resistance of obese patients.

In summary, these results indicate that hepatic insulin extraction in a subgroup of obese patients with normal glucose tolerance, matched for age and body weight, is either lower than or indistinguishable from that of non-obese subjects, and that the fatty liver in moderate obesity may play an important role in differentiating between normal and diminished insulin removal from the blood. It could also be concluded that basal CPR/IRI molar ratio can serve as a useful indicator of the presence or absence of fatty liver in simple obesity.

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