# Increased Serum Soluble Tumor Necrosis Factor Receptor Levels Are Associated With Insulin Resistance in Liver Cirrhosis

S.-Y. Lin, Y.-Y. Wang, and W.-H.H. Sheu

**Insulin resistance is present in nearly all patients with liver cirrhosis, but its etiology remains unclear. Recent studies have shown that tumor necrosis factor-$\alpha$ (TNF-$\alpha$) system is involved in the insulin resistance of human obesity. Serum concentrations of TNF-$\alpha$, and 2 soluble TNF receptors (sTNF-RI and sTNF-RII) are increased in cirrhotic patients. This study explored whether TNF-$\alpha$ system activity was associated with insulin resistance in liver cirrhosis. A total of 26 male nondiabetic patients with liver cirrhosis (mean age, 59 ± 3 years; body mass index, 23.7 ± 0.4 kg/m$^2$) and 25 male control subjects (age, 65 ± 2 years; body mass index, 24.4 ± 0.5 kg/m$^2$) were studied. Serum insulin, c-peptide, TNF-$\alpha$, sTNF-RI, and sTNF-RII concentrations were determined by immunoassay. The insulin resistance was estimated by homeostasis assessment model (HOMA IR). In cirrhotic patients, serum levels of TNF-$\alpha$, sTNF-RI, and sTNF-RII were all higher than those in the controls, and correlated with disease severity. Also, the serum c-peptide, insulin concentrations, and the HOMA IR were higher in liver cirrhosis with comparable blood glucose to control subjects, indicating a degree of insulin insensitivity. In the whole population, there was a moderate, but statistically significant, correlation between serum sTNF-RI or sTNF-RII, and HOMA IR. Also, body mass index was associated with HOMA IR, but not related to serum TNF-$\alpha$, and sTNF-Rs levels. In multiple regression analysis, both sTNF-RII and body mass index jointly contributed to 30% variance of HOMA IR. Our study demonstrated that elevated sTNF-RII levels were associated with insulin resistance in liver cirrhosis. The data indicated that TNF-$\alpha$ system might play a role in modulating insulin action in patients with liver cirrhosis.**


ALTERATIONS OF GLUCOSE metabolism are frequently encountered in patients with liver cirrhosis. It is reported that 60% to 80% of cirrhotic patients are glucose-intolerant, and 10% to 15% develop overt diabetes mellitus during the natural history of the illness.[1-3] Several studies have reported that glucose intolerance in chronic liver disease mainly arises from impaired insulin-mediated glucose utilization in muscles (ie, decreased glucose transport and glycogen synthesis), despite high circulating insulin levels.[4-6] It is proposed that this hyperinsulinemia in liver cirrhosis can downregulate receptor or postreceptor function, thus impairing insulin action.[7-9] Additionally, other factors, such as elevated blood levels of cortisol, glucagon, growth hormone, and free fatty acid or decreased circulating insulin-like growth factor, may also contribute to insulin resistance in liver cirrhosis.[10] However, the exact mechanisms for insulin resistance in liver cirrhosis still remain unclear.

Tumor necrosis factor-$\alpha$ (TNF-$\alpha$) is a pleiotropic cytokine with numerous immunoregulatory and metabolic activities.[11-12] TNF-$\alpha$ signals through at least 2 distinct cell-surface receptors, termed TNF-RI and TNF-RII.[13,14] Both TNF-$\alpha$ receptors can also exist in soluble form after their extracellular domains have been proteolytically cleaved from the cell surface. These soluble receptors (sTNF-R) can either block TNF-$\alpha$ activity by competing with cell-surface forms, or prolong its biological effect as a buffer system. As circulating sTNF-R levels remain elevated for a longer time than TNF-$\alpha$, it is proposed that sTNF-R levels may be more valuable to monitor the degree of TNF-$\alpha$ system activity.

Several studies have demonstrated that TNF-$\alpha$ plays an important role in the development of insulin resistance in obesity state and a variety of catabolic disorders, such as cancer and infection.[15-18] Administration of TNF-$\alpha$ can impair the whole-body glucose and lipid metabolism. Conversely, neutralization of TNF-$\alpha$ activities improves insulin action.[15,19,20] In cirrhotic patients, the circulating levels of TNF-$\alpha$ and its soluble receptors are elevated, and generally thought to correlate with the severity of liver damage.[21-23] However, their clinical relevance to cirrhotic insulin resistance has been little studied. This study was conducted to examine whether increased TNF-$\alpha$ system activity is associated with altered insulin sensitivity in cirrhotic patients. Also, another elevated cytokine in liver cirrhosis, interleukin-6 (IL-6), was evaluated as this cytokine was demonstrated recently to be associated with insulin resistance in some human studies.[24,25]

## MATERIALS AND METHODS

### *Subjects*

Twenty-six male patients with a previous history of cirrhosis were consecutively enrolled from the clinic of the Veterans General Hospital, Taiwan. The initial diagnosis of cirrhosis was based on the typical findings of hepatic cirrhotic appearance, splenomegaly, esophageal varices, and/or ascites (by ultrasonography and upper gastrointestinal endoscope examinations), with supporting biochemical data.[26] At enrollment, all cirrhotic patients were in stable condition, and those with (1) concomitant acute complications, such as gastrointestinal hemorrhage, hepatic encephalopathy, or clinical signs of infection; (2) ascites or peripheral edema; (3) renal insufficiency; and (4) diabetes mellitus

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or endocrine diseases were excluded from entry into the study. The causes of cirrhosis were hepatitis B–related in 13 patients, hepatitis C–related in 9 patients, and alcohol abuse in 4 patients. The severity of liver impairment was scored according to the Pugh-Child classification, which is composed of 5 variables: serum albumin, serum bilirubin, prothrombin time, and degrees of encephalopathy and ascites.[27] Each variable was given a value of 1 to 3 for increasing abnormality, and then the values were summed for each patient with 14 being classified as Pugh-Child A (score 5 to 6), and the other 12 patients as Pugh-Child B (score 7 to 9) or C (score 10 to 15). In addition, 25 control subjects were also enrolled from our hospital and considered normal on the basis of history, physical examination, and biochemical screening. The Hospital Ethics Committee approved the study and all patients and control subjects gave their informed consent.

### *Laboratory Investigation and Immunoassays*

All blood samples were drawn after overnight fasting. Serum was separated from blood cells immediately by centrifugation at 1,000 × g (4°C) and then stored at −70°C until subsequent analysis. The serum albumin (reference range, 37 to 53 g/L), total bilirubin (3.42 to 28.4 μmol/L), aspartate aminotransferase (5 to 35 U/L), alanine aminotransferase (0 to 40 U/L), and glucose were measured at the central laboratory in our hospital. Serum TNF-α, sTNF-RI, sTNF-RII, IL-6, and IL-6 receptor (IL-6R) levels were all measured in duplicate with enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). The lowest limits of TNF-α, sTNF-RI, sTNF-RII, IL-6, and sIL-6R assays sensitivity were 0.18 ng/L, 25 ng/L, 50 ng/L, 0.7 ng/L, and 3.5 ng/L, respectively. The intra- and interassay coefficients of variation (CVs) were 5.5% and 7.5% for TNF-α, less than 5% and 7 % for sTNF-RI and sTNF-RII, 3.7% and 4.4% for IL-6, and 3% and 5% for IL-6R, respectively. The serum insulin and c-peptide levels were determined by a chemiluminescent enzyme immunoassay (Diagnostic Products Corp, Los Angeles, CA); the lowest detection limits were 14.4 pmol/L and 0.1 nmol/L, and the intra- and interassay CVs were 4.2% and 5%, and 6.1%, and 8.3%, respectively.

### *Evaluation of Insulin Resistance*

A recent report describes that homeostasis model assessment (HOMA) can be used reliably to estimate insulin sensitivity in patients with liver cirrhosis.[28] Therefore, in our study, a quantitative evaluation of insulin resistance was calculated according to HOMA as described by Matthews et al.[29] The HOMA insulin resistance (HOMA IR) index = [(fasting insulin (μIU/mL) · fasting glycemia (μmol/L)]/22.5. In addition, fasting blood C-peptide levels were measured to determine β-cell secretion.

### *Statistics*

All data were expressed as mean value ± SEM. Comparisons between subject groups were analyzed using Mann-Whitney *U* nonparametric test. The relationship between HOMA IR index and body mass index, serum cytokines, serum cytokine soluble receptors, or the other variables was determined by Spearman's correlation. Further, a multiple regression analysis was performed with HOMA IR index as the dependent variable and body mass index, age, and the other inflammatory cytokines and disease status as independent variables. Results are considered statistically significant at $P < .05$. All data were analyzed by SPSS software (Statistical Package for the Social Science, version 6.0 for Windows; SPSS Inc, Chicago, IL)

## RESULTS

Table 1 summarizes the biochemical and anthropometric characteristics of the study subjects. Among the 26 patients with liver cirrhosis, 22 patients had a normal fasting glucose level, whereas the other 4 patients had impaired fasting glucose according to the criteria by American Diabetes Association.[30] All 25 control subjects had a normal fasting blood glucose range. Overall, the mean serum concentrations of cytokines, their soluble receptors, insulin, and c-peptide were all significantly higher in cirrhotic patients than in controls in the presence of comparable fasting glucose concentrations (Table 1). In parallel with these findings, the HOMA IR in cirrhotic patients was also higher than in controls. In cirrhotic patients, the serum sTNF-RI, sTNF-RII, and IL-6 levels correlated significantly to Pugh-Child scores ($r = 0.515$, $r = 0.468$, $r = 0.390$, respectively; $P < .05$). However, there was no correlation between serum insulin levels or HOMA IR, and Pugh-Child scores.

**Table 1. Baseline Demographic, Body Composition, Biochemical, and Hormonal Data in Cirrhotic Patients and Control Subjects**

| | Cirrhosis (n = 26) | Controls (n = 25) | *P* Value |
|---|---|---|---|
| Age (yr) | 59 ± 3 | 65 ± 2 | <.05 |
| BMI (kg/m$^2$) | 23.7 ± 0.4 | 24.4 ± 0.5 | NS |
| Glucose (mmol/L) | 6.0 ± 0.7 | 5.2 ± 0.1 | NS |
| C-peptide (nmol/L) | 1.1 ± 0.2 | 0.6 ± 0.1 | <.05 |
| Insulin (μIU/mL) | 18.8 ± 3.0 | 11.8 ± 2.4 | <.01 |
| HOMA IR | 4.5 ± 0.7 | 2.8 ± 0.6 | <.05 |
| ALT (u/L) | 65.1 ± 9.9 | 24.4 ± 1.8 | <.01 |
| Biliurbin (μmol/L) | 28.9 ± 1.7 | 17.1 ± 1.7 | <.01 |
| Albumin (g/L) | 36 ± 1 | 42 ± 1 | <.01 |
| Creatinine (μmol/L) | 88.4 ± 8.8 | 106.1 ± 8.8 | <.05 |
| TNF-α (ng/L) | 11.3 ± 1.2 | 5.8 ± 0.7 | <.01 |
| sTNF-RI (ng/L) | 1,820.1 ± 84.5 | 1,374.0 ± 71.1 | <.01 |
| sTNF-RII (ng/L) | 4,510.3 ± 153.2 | 3,369.7 ± 189.8 | <.01 |
| IL-6 (ng/L) | 3.6 ± 0.9 | 1.5 ± 0.2 | <.01 |
| sIL-6R (ng/L) | 36,611.6 ± 1,785.6 | 31,675.9 ± 2,261.9 | <.05 |

Abbreviations: BMI, body mass index; HOMA IR, homeostasis model assessment insulin resistance; ALT, alanine transferase; NS, not significant.

In the whole population, fasting insulin, c-peptide, and HOMA IR correlated with body mass index, respectively ($r = 0.38$,$r = 0.45$, $r = 0.33$,respectively; $P < .05$). Also, all of circulating insulin, c-peptide, and HOMA IR showed a significant correlation with serum sTNF-RI ($r = 0.31$, $r = 0.44$, $r = 0.31$,respectively; $P < .05$), and sTNF-RII ($r = 0.30$, $r = 0.40$, $r = 0.30$; $P < .05$) (Fig 1). However, serum TNF-α and sTNF-Rs did not correlate with body mass index. There was no relationship found between serum IL-6, or sIL-6R, and fasting insulin, c-peptide, or HOMA IR. Finally, a multiple regression analysis was constructed in the whole population to predict HOMA IR. This model, with HOMA IR as dependent variable and body mass index, age, serum cytokines, cytokine receptors, and categorized groups (cirrhotic patients and controls) as independent variables, showed that mean HOMA IR was significantly associated with both body mass index and serum sTNF-RII level (joint $r^2 = 0.30$; $F = 0.03$) (Table 2).

## DISCUSSION

Although in this study the standard insulin clamp method was not used to measure insulin sensitivity, the higher HOMA IR values in patients with liver cirrhosis would suggest the presence of an insulin-resistant state, as a recent study has validated HOMA IR as a reliable tool to assess insulin sensi-

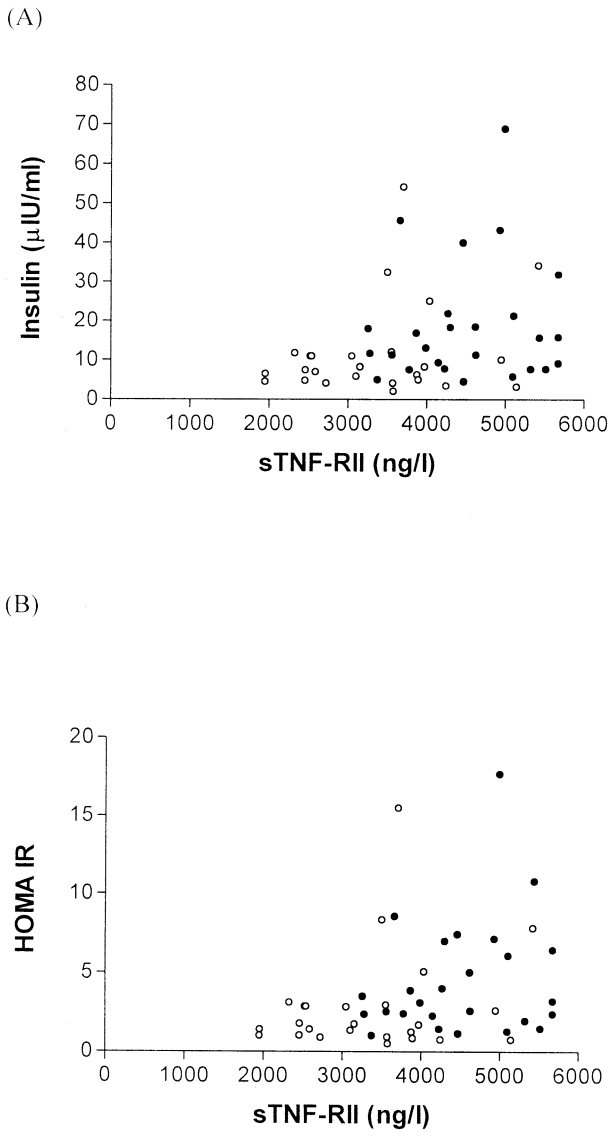


**Fig 1. (A) Correlation between serum sTNF-RII and insulin levels in cirrhotic patients (●) and control subjects (○); $r = 0.30$, $P < .05$. (B) Correlation between serum sTNF-RII levels and HOMA IR in cirrhotic patients and control subjects; $r = 0.30$, $P < .05$.**

tivity in chronic liver disease.[28] Moreover, they also had elevated fasting C-peptide levels in the face of comparable fasting blood glucose to control subjects, indicating a compensatory response of pancreatic $\beta$ cells, which was compatible with the results of HOMA IR. In liver cirrhosis, the glucose intolerance mainly comes from decreased insulin-mediated glucose transport and glycogen synthesis, both of which are comparable to the in vitro effects of TNF-$\alpha$ on glucose metabolism in skeletal muscle and adipose tissues.[1-3,16,31] Accordingly, we examined a potential role of TNF-$\alpha$ system in cirrhotic insulin resistance,[16,31] and for the first time showed that circulating sTNF-RII levels correlated positively with the HOMA IR index, suggesting that the activated TNF-$\alpha$ system may play a role in the mechanism of insulin resistance in liver cirrhosis. Several studies have demonstrated that obese subjects overexpress TNF-$\alpha$ and TNF-RII in adipose tissue with elevated serum sTNF-RII levels in relation to lean controls.[32,33] In addition, the adipose tissue TNF-$\alpha$ and TNF-RII gene expression strongly correlates with the levels of insulinemia, and circulating sTNF-RII values are inversely associated with insulin sensitivity.[16,32,33] All these data indicate that adipose TNF-$\alpha$ may, in association with TNF-RII, play an important role in obesity-related insulin resistance in a local autocrine/paracrine way. In liver cirrhosis, the activated TNF-$\alpha$ system is generally thought to be involved in hepatic damages.[21-23] However, it was not clear whether this association between the TNF-$\alpha$ system activity and insulin resistance would represent an increased biological effect of TNF-$\alpha$ upon insulin-mediated target organs in patients with liver cirrhosis. In animal studies, it has been shown that administration of endotoxin can increase TNF-$\alpha$ expression in adipose and skeletal muscle tissues.[34,35] As endotoxinemia is common in patients with liver cirrhosis,[21] we speculated that the TNF-$\alpha$ expression in adipose and skeletal muscle tissues could be upregulated, and thus through a local manner contribute to insulin resistance as in obesity states. Our recent studies in cirrhotic rodents found that TNF-$\alpha$ levels were increased in adipose and skeletal muscle tissues (unpublished observation), which seemed to support the hypothesis. However, it was also possible that TNF-$\alpha$ might mediate, through other factors, to cause cirrhotic insulin resistance. A recent study by Picardi et al showed that serum TNF-$\alpha$ levels were associated with elevated growth hormone levels in liver cirrhosis, and suggested that TNF-$\alpha$ might play a role in the pathogenesis of cirrhotic growth hormone resistance.[36] As growth hormone counteracts insulin action, this TNF-$\alpha$–growth hormone link would support the potential role of TNF-$\alpha$ for insulin resistance in cirrhosis.[10] Finally, serum sTNF-RII levels correlated with hepatic functional status. It is still unclear whether the disease severity rather than TNF-$\alpha$ contributed to the insulin resistance in liver cirrhosis.

The reasons for the discrepancy between the sTNF-RI or sTNF-RII relationship to insulin sensitivity in liver cirrhosis are not clear. Generally, TNF-RI is thought to be the major receptor for TNF-$\alpha$ action.[13,14] In rodents, it has been demonstrated that TNF-$\alpha$ mainly via TNF-RI mediates insulin resistance.[37] While in human adipocytes, it is shown that TNF-$\alpha$ can upregulate TNF-RII expression and through this receptor inhibit

**Table 2. Multiple Linear Regression Analysis in Patients With Liver Cirrhosis and Control Subjects, Considering HOMA IR as a Dependent Variable**

| Independent Variable | Multiple Regression Coefficient | | 2-Tailed *P* Value |
|---|---|---|---|
| | $\beta$ | SE ($\beta$) | |
| BMI | 0.630 | 0.211 | .004 |
| Group | −0.496 | 1.616 | .760 |
| TNF-$\alpha$ | −0.024 | 0.110 | .822 |
| TNF-RI | −0.003 | 0.002 | .055 |
| TNF-RII | 0.003 | 0.001 | .04 |
| IL-6 | 0.122 | 0.147 | .410 |
| IL-6R | −0.00003 | 0.00005 | .532 |
| Age | −0.070 | 0.046 | .138 |

insulin signaling.[32,38] These data might indicate underlying species-specific properties of these 2 receptors in regard to their metabolic functions. Further experiments will still be necessary to elucidate the relative role of each receptor, as well as their respective action in regard to insulin resistance.

In this study, serum IL-6 and its soluble receptor levels, similar to the TNF-$\alpha$ system, increased progressively with the advancement of hepatic disease, suggesting that increased activity of the IL-6 system might originate in macrophages stimulated by endotoxinemia or from decreased IL-6 clearance in cirrhotic livers.[39,40] It has been shown in several human studies that circulating IL-6 can act as an endocrine cytokine and participate in obesity-associated insulin resistance.[24,25] However, in patients with liver cirrhosis, neither IL-6 nor sIL-6R concentrations correlated with insulin resistance index. It seemed that IL-6 alone might less contribute to cirrhotic insulin resistance.

In summary, the TNF-$\alpha$ system activity as well as insulin resistance was elevated in patients with liver cirrhosis. A negative correlation was observed between sTNF-RII levels and HOMA IR. This finding indicated that activation of the TNF-$\alpha$ system might play a role in the pathogenesis of insulin resistance in liver cirrhosis. However, whether TNF-$\alpha$ was only a simple marker of underlying disease activity causing cirrhotic insulin resistance should be further studied.

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