Circulating Soluble CD14 Monocyte Receptor Is Associated with Increased Alanine Aminotransferase, José Manuel Fernández-Real,1,2 Abel López-Bermejo,1 Montserrat Broch,2 Joan Vendrell,2 Cristobal Richard,2 and Wifredo Ricart1,2

1 Section of Diabetes, Endocrinology and Nutrition, University Hospital of Girona “Dr Josep Trueta”, Girona, Spain; 2 Unit of Endocrinology and Nutrition, University Hospital of Tarragona “Joan XXIII”, Unit of Advanced Studies, Tarragona, Spain; *address correspondence to this author at: Unitat de Diabetes, Endocrinologia i Nutricio, Hospital de Girona “Dr Josep Trueta”, Carretera de França s/n, 17007 Girona, Spain; fax 34-972-94-02-70, e-mail jmfernandezreal@htrueta.scs.es

Recent studies have suggested that abnormal hepatocellular function is associated with obesity, insulin resistance, and type 2 diabetes (1–3). Nonalcoholic fatty liver disease (NAFLD) is the preferred term to describe a spectrum of liver damage, ranging from steatosis to steatohepatitis, liver fibrosis, and cirrhosis, that can be found in insulin-resistant (obese and type 2 diabetic) and hyperlipidemic patients (4). Insulin sensitivity and liver function exhibit a bidirectional relationship: a normally functioning liver determines, in part, the normal body response to insulin, whereas abnormal insulin sensitivity causes liver damage (5, 6). The insulin-resistant state is characterized by a failure to suppress hepatic glucose production and glycogenolysis (5), usually accompanied by fat accumulation in hepatocytes as a result of increased hepatic uptake and synthesis of triglycerides. The latter is known to impair liver function and, consequently, to worsen insulin resistance (5, 6).

Aminotransferases are considered indicators of hepatocellular health. Alanine aminotransferase (ALT) is found primarily in the liver, whereas aspartate aminotransferase (AST) and γ-glutamyltranspeptidase (γGT) are also found in other tissues. NAFLD is characterized by mild-to-moderate increases in ALT and AST (7). Recent prospective studies have shown that increased ALT and γGT are predictors of the development of type 2 diabetes (1, 2).

CD14, a 55-kDa glycoprotein, is a multifunctional receptor constitutively expressed in considerable amounts on the surface of mature monocytes, macrophages, and neutrophils (8). CD14 has specificity for lipopolysaccharide (LPS) and other bacteria-wall-derived components (9). LPS signaling triggers a cascade that leads to cytokine production and shedding of the extracellular domain of CD14 (sCD14) (10–13). The buffering of LPS is crucial not only during acute inflammatory and infectious processes; in healthy humans, triglyceride-rich lipoproteins contain detectable amounts of endogenous LPS that are presumably scavenged in vivo (11).

LPS is extraordinarily widespread in nature, being present in food and water and in the typical indoor environments as a constituent of house dust (14). Endogenous LPS is continually produced within the gut (15, 16). The neutralization exerted by sCD14 precludes the interaction of LPS with membrane CD14 on phagocytic cells, which would trigger the inflammatory cascade. Importantly, circulating sCD14 appears to antagonize tumor necrosis factor, an adipocyte-derived cytokine known to induce insulin resistance (17, 18).

We have recently described that circulating sCD14 was associated with several insulin-resistant phenotype traits (19). On the other hand, plasma sCD14 concentrations change with acute liver injury (20), but no information is available regarding NAFLD. We hypothesized that sCD14 might play a role in preserving the liver by buffering portal absorbed LPS. Thus, we wished to examine the cross-sectional associations among circulating sCD14 concentrations, insulin sensitivity, and indicators of mild hepatocellular injury in apparently healthy individuals.

We studied 224 consecutive individuals during routine checkup visits between July 1 and September 17, 1997, as part of a population-based study dealing with cardiovascular risk factors in Northern Spain (21). Participation in the study for these individuals was close to 85%. Biochemical analytes, except for insulin and sCD14, were measured immediately after collection of samples, as described below. Insulin and sCD14 were measured in 40-sample batches in frozen serum stored at −80 °C in the months after sampling (insulin) and 3 years after sampling (sCD14).

Inclusion and exclusion criteria were identical to those described previously (19, 21–23). Body mass index (BMI), waist-to-hip ratio, blood pressure, and serum glucose and lipids were measured as reported previously (19). Serum AST, ALT, and γGT were measured colorimetrically by automated tests (Roche Diagnostics GmbH). The intra- and interassay CVs were <4% for all tests. Serum insulin was measured in duplicate by monoclonal IRMA (Medgenix Diagnostics). The intra- and interassay CVs were similar to those reported previously (19, 22, 23).

The homeostasis model of assessment of insulin resistance (HOMA) was calculated (23, 24) as [glucose (mmol/L) × insulin (mIU/L)]/22.5. Serum soluble CD14 was measured by the sCD14-EASIA (Biosource Europe S.A). The intra- and interassay CVs were 5.2% and 7.8%, respectively.

Clinical and laboratory variables for the study participants are summarized in Tables 1 and 2 of the Data Supplement that accompanies the online version of this Technical Brief at http://wwwclinchem.org/content/vol50/issue8/. As described previously, liver enzymes were positively associated with measurements of adiposity, such as BMI and waist-to-hip ratio, and with fasting serum insulin and HOMA values (all P values <0.0001). ALT, but not AST or γGT, was negatively associated with serum sCD14 (r = −0.20; P = 0.004). This relationship was stronger when only obese individuals were considered (BMI >30 kg/m2; r = −0.43; P = 0.006; n = 43).

To further study the relationship between sCD14 and ALT, we divided our population into sCD14 quartiles. Age and BMI were similar across the quartiles of sCD14 (Table 1). The percentage of women in the highest sCD14 quartile was slightly high, but the trend was not statistically significant. Fasting glucose, HOMA value, serum cholesterol, HDL- and LDL-cholesterol, and fasting tri-
glycerides were similar across the quartiles of sCD14. After controlling for age and sex, HDL tended to be higher in the highest sCD14 quartile (P = 0.051). ALT was significantly higher in the lowest quartile compared with the highest quartile (P = 0.02; Table 1) and when compared with the rest of the participants (Fig. 1). GT tended to be higher in the lowest sCD14 quartile in a trend test (P = 0.1).

We constructed multiple regression analysis models to predict serum ALT concentrations (see Tables 3 and 4 in the online Data Supplement). In the first model, BMI, age, sex, and sCD14 contributed to explain 31% of ALT variance (Model I). With HOMA included in the model, only sex and the HOMA value were significant predictors of ALT variance (Model II). These results suggest that low sCD14 contributes to ALT variance through increased insulin resistance.

After controlling for BMI, age, and sex, sCD14 was also significantly associated with γGT (see Table 5, Model III, in the online Data Supplement). sCD14 is produced not only by monocytes but also by hepatocytes, and the liver has been claimed to constitute one of the major sources of sCD14 (25). Here we describe that both insulin resistance and circulating sCD14 concentrations were associated with serum ALT in a population of apparently healthy individuals. Persons in the lowest sCD14 quartile had increased serum ALT concentrations, even within the ALT reference interval. This relationship remained significant even after controlling for known confounders.

ALT could be an indicator of hepatic insulin resistance, in association with decreased sCD14 concentrations. In fact, NAFLD has been consistently linked to insulin resistance (2, 7), and ALT itself is a gluconeogenic enzyme, perhaps a marker for impaired insulin signaling (7).

AST is a less-specific marker of liver injury (AST is found in many other tissues, including cardiac muscle, brain, intestines, fat, skeletal muscle, and kidneys) and thus may play a less important role in the relationship between liver dysfunction and insulin resistance. We also found that γGT tended to be higher in the lowest sCD14 quartile in a trend test (P = 0.1). In fact, γGT was associated with sCD14 after controlling for BMI, age, and sex. Perry et al. (1) reported on the independent predictor role of γGT in the development of type 2 diabetes but did not include ALT in the analysis for comparison. More recently, it was shown that ALT, but neither AST nor γGT, is associated with hepatic insulin resistance and therefore is the only liver enzyme that predicted the development of type 2 diabetes (2). However, the latter study concerned American Indians and was atypical in that γGT

![Fig. 1. 95% confidence interval (CI) distribution of serum ALT according to serum sCD14 concentrations (lowest quartile vs rest of quartiles).](image-url)
values ranged up to 245 U/L, above the clinical laboratory reference interval (which may well be consistent with liver damage). This is in contrast to different studies (26–31) that uniformly indicated that γGT, mostly within the reference interval, is related to several conditions that are themselves related to insulin resistance and the metabolic syndrome.

The decrease in sCD14 could also be a primary event resulting from or leading to liver injury. Circulating sCD14 decreases cellular responses to LPS, such as induction of tumor necrosis factor-α (TNF-α) and interleukin-6 synthesis (12, 13, 32). Serum sCD14 circulates in inverse proportion to the concentration of TNF-α receptors (19). Because TNF-α is a major cytokine contributing to liver damage in NAFLD patients (7), sCD14 might also preserve liver function through down-regulation of the inflammatory cascade.

The opposite possibility (that inflammation leads to decreased sCD14 concentration) seems less plausible because sCD14 concentrations are actually increased in patients with liver injury (20). Decreased efficiency in neutralizing LPS-induced responses in the gut-liver axis would lead to a chronic proinflammatory response, insulin resistance, and fatty liver change, with increased ALT and γGT activity, perhaps leading to NAFLD in the long term. In this sense, sCD14 could be a marker of early damage in NAFLD. It is noteworthy that the etiology of 69% of cases of increased aminotransferase among Americans is unknown and presumably related to adiposity and insulin resistance (3). The identification of new markers of liver dysfunction is therefore imperative in efforts to prevent the development of NAFLD.

In summary, decreased circulating sCD14 is associated with markers of liver injury in healthy individuals, even after obesity is accounted for. Insulin resistance could be involved in this interaction.

This work was supported in part by Grant 00/0024-01 from the Fondo de Investigaciones Sanitarias, National Health Institute of Spain, and by Grants RGDM (G03/212) and RTGO (G03/028) from the Instituto de Salud Carlos III (Madrid, Spain).

References

3. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase among Americans is unknown and presumably related to adiposity and insulin resistance (3). The identification of new markers of liver dysfunction is therefore imperative in efforts to prevent the development of NAFLD.

In summary, decreased circulating sCD14 is associated with markers of liver injury in healthy individuals, even after obesity is accounted for. Insulin resistance could be involved in this interaction.

This work was supported in part by Grant 00/0024-01 from the Fondo de Investigaciones Sanitarias, National Health Institute of Spain, and by Grants RGDM (G03/212) and RTGO (G03/028) from the Instituto de Salud Carlos III (Madrid, Spain).

References

3. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase among Americans is unknown and presumably related to adiposity and insulin resistance (3). The identification of new markers of liver dysfunction is therefore imperative in efforts to prevent the development of NAFLD.

In summary, decreased circulating sCD14 is associated with markers of liver injury in healthy individuals, even after obesity is accounted for. Insulin resistance could be involved in this interaction.

This work was supported in part by Grant 00/0024-01 from the Fondo de Investigaciones Sanitarias, National Health Institute of Spain, and by Grants RGDM (G03/212) and RTGO (G03/028) from the Instituto de Salud Carlos III (Madrid, Spain).