Contribution of Metabolic Factors to Alanine Aminotransferase Activity in Persons With Other Causes of Liver Disease

GEORGE N. IOANNOU,*,† NOEL S. WEISS,§ EDWARD J. BOYKO,‖,¶ STEVEN E. KAHN,#,** and SUM P. LEE*,†

‖Epidemiologic Research and Information Center, and Divisions of *Gastroenterology, Metabolism, Endocrinology, and Nutrition, Department of Medicine, Veterans Affairs Puget Sound Health Care System, Seattle; Divisions of †Gastroenterology, **Metabolism, Endocrinology, and Nutrition, and ¶General Internal Medicine, Department of Medicine, and §Department of Epidemiology, University of Washington, Seattle, Washington

Background & Aims: **Nonalcoholic fatty liver disease has been defined by the presence of hepatic steatosis in the absence of other chronic liver diseases. We sought to determine whether obesity, insulin resistance, and the metabolic syndrome, which are the main risk factors for nonalcoholic fatty liver disease, are associated with similar elevations in serum alanine aminotransferase activity in persons with and those without other causes of chronic liver disease.** ***Methods:*** **Adult participants of the third National Health and Nutrition Examination Survey were divided into those with causes of chronic liver disease (n = 1037), defined as viral hepatitis, excessive alcohol consumption, or increased transferrin-iron saturation, and those without (n = 8004).** ***Results:*** **Among persons with other causes of chronic liver disease, obesity (adjusted odds ratio, 4.9; 95% confidence interval, 2.5–9.4), insulin resistance (adjusted odds ratio, 6.8; 95% confidence interval, 3.0–15.5, comparing the highest and the lowest quartile), and the metabolic syndrome (adjusted odds ratio, 3.3; 95% confidence interval, 1.4–8.0) were all strongly associated with increased alanine aminotransferase activity (>43 IU/L). Among persons without other causes of chronic liver disease, statistically similar associations were identified.** ***Conclusions:*** **Obesity, insulin resistance, and the metabolic syndrome are strong predictors of increased alanine aminotransferase activity in the US population, both in persons with and in persons without other causes of chronic liver disease. We hypothesize that metabolic fatty liver disease related to these conditions is the cause of the increased alanine aminotransferase activity and may be underrecognized in persons with other causes of chronic liver disease.**

It is currently believed that the clinical diagnosis of nonalcoholic fatty liver disease (NAFLD) "requires the exclusion of alcohol abuse and viral, genetic, autoimmune, and drug-induced liver disease"[1] together with ultrasonographic or, even better, histological evidence of hepatic steatosis. Similarly, for the purposes of epidemiological studies, it has been suggested that NAFLD may be defined by the presence of increased aminotransferases in the absence of other known liver diseases, such as viral hepatitis, alcoholic liver disease, or iron overload.[2,3] Thus, the current clinical and epidemiological definitions of NAFLD both emphasize the exclusion of other liver diseases before a diagnosis of NAFLD can be entertained.

However, this concept of NAFLD, and in fact the very term nonalcoholic fatty liver disease, may be misleading: there is no reason to assume that fatty liver disease cannot be caused by insulin resistance or the metabolic syndrome in persons who consume alcohol to excess or who have viral hepatitis. In fact, the presence of fatty liver disease may have even more important consequences in persons who have an additional risk factor for liver disease than in persons who have no other risk factors.

Obesity, insulin resistance, and the metabolic syndrome are major predisposing conditions to NAFLD.[4–17] These conditions are very common in the US population and are rapidly increasing in prevalence. This raises the suspicion that fatty liver related to these conditions may be a relatively common coexisting condition in patients who already have other additional liver diseases, such as alcoholic or viral hepatitis.

On the basis of these observations, we aimed to determine whether known risk factors for NAFLD, such as obesity, insulin resistance, and the metabolic syndrome,

Abbreviations used in this paper: BMI, body mass index; HOMA, homeostasis model assessment; NAFLD, nonalcoholic fatty liver disease; NHANES III, Third National Health and Nutrition Examination Survey; TS, transferrin-iron saturation.

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are associated with similar, or even greater, increases in serum alanine aminotransferase (ALT) activity in persons with vs. persons without other causes of chronic liver disease. If that is true, it would argue that NAFLD should be suspected in patients with the appropriate risk factors whether they have additional known chronic liver diseases or not.

Methods

Survey Design

Data were derived from the Third National Health and Nutrition Examination Survey (NHANES III), a cross-sectional study conducted by the National Center for Health Statistics from 1988 to 1994 to assess the health and nutritional status of the noninstitutionalized US population.[18] Data included laboratory investigation results, physical examination findings, and structured questionnaires conducted in mobile examination centers at 89 locations throughout the United States.

Study Sample

Of 18,827 participants aged 20 years or older, we excluded participants with a positive pregnancy test (n = 288); without available serum tests for ALT, transferrin-iron saturation (TS), hepatitis B virus (HBV), or hepatitis C virus (HCV) (n = 3443); and without data on alcohol consumption (n = 627), body mass index (BMI; n = 27), or educational attainment (n = 89). An additional 1703 participants were excluded because they consumed food or beverages other than water within 6 hours of venipuncture (because fasting serum glucose and insulin levels were required for the determination of insulin resistance and the metabolic syndrome), and 3609 were excluded because data were incomplete for determination of the metabolic syndrome. This left 9041 in this analysis. The study sample was divided into persons with (n = 1037) and those without (n = 8004) known causes of chronic liver disease, as described below.

Potential Causes of Chronic Liver Disease

In accordance with previous studies, we considered chronic viral hepatitis, excessive alcohol consumption, and increased serum markers of iron stores as potential causes of chronic liver disease.[3,14] Although there is an association between NAFLD/nonalcoholic steatohepatitis and increased serum markers of iron stores,[19] we included the presence of increased TS in the potential causes of chronic liver disease so that our results could be directly compared with other recent studies based on NHANES III data.[3,14] Data are not available in NHANES III to evaluate for the presence of other less common liver diseases, such as autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and Wilson's disease. HBV infection was defined by the presence of hepatitis B surface antigen measured by using a sandwich radioimmunoassay (Abbott Laboratories, North Chicago, IL).

A second-generation enzyme immunoassay and a supplemental test (EIA 2.0 and HCV MATRIX; Abbott Laboratories) were used to test for anti-HCV antibodies. Samples found to be anti-HCV positive were subsequently tested for HCV RNA by reverse-transcriptase polymerase chain reaction.[20,21] Samples found to be negative by reverse-transcriptase polymerase chain reaction were extracted a second time by the same procedure, with an additional incubation at 50°C for 45 minutes with 25 U of reverse transcriptase (Boehringer Mannheim, Indianapolis, IN) and 10 U of RNAsin (Boehringer Mannheim). For this study, only participants with positive HCV RNA were considered chronically infected with HCV.

Participants reported the number of days during which alcohol was consumed over the preceding year and the average number of alcoholic drinks that were consumed on those days. An alcoholic drink was defined as a 12-oz beer, a 4-oz glass of wine, or 1 oz of liquor. Using this information, we calculated the average number of alcoholic drinks consumed per day during the preceding year. Alcohol was considered a potential cause of chronic liver disease in women who consumed $\geq$1 and in men who consumed $\geq$2 alcoholic drinks per day, consistent with prior studies.[3,22,23]

Serum iron and total iron binding capacity were measured colorimetrically (RFA analyzer; Alpkem, Clackamas, OR), and 1% thiourea was added to complex copper and prevent copper interference. TS was calculated as the ratio of serum iron to total iron binding capacity; a value >50% was considered as suggestive of hemochromatosis or iron overload.[3]

For the purposes of sensitivity analyses, we divided the potential causes of chronic liver disease into (1) "viral hepatitis" in persons with positive HBV surface antigen or HCV RNA; (2) "excessive alcohol consumption" in men who consumed $\geq$2 drinks per day and women who consumed $\geq$1 alcoholic drink per day in the absence of viral hepatitis, as defined previously; and (3) "increased TS" in persons with serum TS >50% in the absence of viral hepatitis or excessive alcohol consumption.

Increased Serum Alanine Aminotransferase Activity

Serum ALT activity was considered increased if it exceeded 43 IU/L in both men and women, in accordance with the NHANES III plan and operations manual[18] and previous studies.[14] After collection of venous blood, samples were immediately centrifuged. Specimens were then frozen and shipped weekly to a central laboratory where they were initially stored at −20°C and then at −70°C. Serum ALT assay was performed with a Hitachi model 737 multichannel analyzer (Boehringer Manheim) by using the a-ketoglutarate reaction.

Predictors of Increased Activity

The following variables, which have been established as very strong risk factors for NAFLD,[4–17] were evaluated as predictors of increased ALT activity in persons with or without other causes of chronic liver disease: