Associations Between Liver Histology and Severity of the Metabolic Syndrome in Subjects With Nonalcoholic Fatty Liver Disease

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Nonalcoholic fatty liver disease (NAFLD) is associated with a histopathological picture resembling alcohol-induced liver injury occurring in subjects who consume insignificant amounts of alcohol. In NAFLD, steatosis alone is associated with good prognosis, whereas nonalcoholic steatohepatitis (NASH) can progress to fibrosis and cirrhosis in up to 30% of cases, potentially leading to liver failure and hepatocellular carcinoma. The prevalence of NAFLD and NASH in the U.S. is estimated at 10–20 and 2–3%, respectively (1–3).

The metabolic syndrome, as defined by Adult Treatment Panel (ATP) III criteria, incorporates features known to predict cardiovascular disease and type 2 diabetes (4). NAFLD has been demonstrated to be associated with features of the metabolic syndrome, including hyperglycemia (5,6), dyslipidemia (5,6), hypertension (7), and central obesity (7,8). In addition, insulin resistance, even without these other features, is almost universal in subjects with NAFLD (6). However, the factors specifically associated with hepatic inflammation, fibrosis, and thus liver disease progression are still being elucidated.

In this study of human subjects with NAFLD, we explored specific relationships between hepatic histology and markers of the metabolic syndrome.

RESEARCH DESIGN AND METHODS — Forty-six subjects with NAFLD were recruited from clinics. The diagnosis was based on the histological presence of macrovesicular steatosis, with or without lobular inflammation, hepatocellular degeneration, or fibrosis (8,9). All subjects were negative for viral hepatitis, anti-nuclear antibody, anti–smooth muscle antibody, and anti-mitochondrial antibody and had normal iron and copper studies. All subjects consumed <14 standard drinks of alcohol per week (9). Nine male subjects and eight female subjects had preexisting type 2 diabetes, five managed their diabetes with diet alone, and 12 were taking metformin. Approval for the study was obtained from the institutional research ethics committee, and written informed consent was obtained.

Adiposity was assessed by BMI and dual-energy X-ray absorptiometry (DEXA) (Lunar DPX-L; Lunar, Madison, WI). Metabolic syndrome was defined by ATP III criteria (10). Each subject and their respective control was given a score of 1 for each feature of the metabolic syndrome, for a maximum score of 5, with a score of ≥3 being diagnostic of the metabolic syndrome (10). Insulin resistance was estimated using the homeostasis model assessment (HOMA) for insulin resistance with fasting insulin and glucose levels (11). A pathologist blinded to subject details scored liver biopsies, allotting a score from 0 to 4 for inflammation, steatosis, and fibrosis as previously described (12). For additional fibrosis assessment, all biopsies were stained with Masson’s Trichrome, percent fibrosis was calculated in triplicate by microscopy and image analysis (AIS, Toronto, ON, Canada), and data were expressed as mean percentages. Continuous variables were log\textsubscript{10} transformed, and correlations were assessed by Pearson’s correlations and stepwise regression. Correlations with categorical variables were analyzed using Spearman correlations. A P value <0.05 was considered statistically significant.

RESULTS — Liver histopathology results were steatosis alone (10 subjects), NASH with fibrosis score of 0 (12 subjects), NASH/fibrosis score 1 (14 subjects), NASH/fibrosis score 2 (5 subjects), and NASH/fibrosis score 3 (5 subjects). None had cirrhosis (a score of 4 for fibrosis).

Hepatic steatosis was associated with BMI ($r = 0.36, P = 0.02$) and percentage trunk fat measured by DEXA ($r = 0.3, P = 0.05$). Hepatic inflammation was only significantly associated with BMI ($r = 0.35, P = 0.02$), and hepatic fibrosis was not correlated with any measure of adiposity.

However, there were significant associations seen between hepatic inflammation, fibrosis, and features of the metabolic syndrome. Both inflammation and fibrosis correlated significantly with serum insulin, HOMA for insulin resistance, and ATP III score. Other measures of the metabolic syndrome analyzed individually did not correlate with hepatic fibrosis. The two measures of fibrosis...
correlated with fibrosis (46.1%). Total fat mass was most highly correlated with fibrosis, independent of age, and serum HDL were all associated with DEXA, serum alanine aminotransferase, aspartate aminotransferase, but not serum alanine aminotransferase, correlated significantly with hepatic inflammation and fibrosis.

A total of 30 of 46 subjects (65%) had three or more features of the metabolic syndrome, 9 had two criteria, 6 had one criterion, and 1 had none. Subjects with the metabolic syndrome had a higher hepatic fibrosis score (3.3 vs. 1.6, P = 0.01) and a higher percentage fibrosis (0.40 ± 0.10 vs. 0.18 ± 0.03%, P = 0.02) than those without the metabolic syndrome. There was a significant increase in fibrosis as the number of features of the metabolic syndrome increased (P = 0.014, ANOVA) (Fig. 1).

By stepwise regression, sex, presence of diabetes, HOMA score, total fat by DEXA, serum alanine aminotransferase, and serum HDL were all associated with fibrosis, independent of age (R² = 46.1%). Total fat mass was most highly correlated with fibrosis (P = 0.001).

**CONCLUSIONS** — In this cross-sectional study of 46 patients with biopsy-proven NAFLD, a relationship between the severity of the metabolic syndrome and NAFLD was observed.

While measures of adiposity correlated with hepatic steatosis, hepatic inflammation and fibrosis were associated with the presence and severity of the metabolic syndrome. This finding has clinical implications, since hepatic ultrasound and serum transaminases have limited utility in predicting hepatic inflammation and fibrosis (13), and there is current reliance on liver biopsies to confirm the diagnosis and indicate prognosis. We suggest that features of the metabolic syndrome would potentially be a better guide in determining which patients should be considered for biopsy and/or potential specific therapy.

In this study, markers of insulin resistance correlated independently with hepatic fibrosis. Insulin has been demonstrated to have fibrogenic actions on cultured mesangial cells, playing a permissive role on extracellular matrix production via the release of transforming growth factor β1 and stimulating accumulation of type 1 collagen (14) and connective tissue growth factor (15) in hepatic stellate cells. Whether the hyperinsulinemia seen in human insulin resistance or other individual features associated with the metabolic syndrome contribute directly to hepatic fibrosis is unknown, and strategies to target insulin resistance in NAFLD are of particular interest.

This study suggests that use of ATP III–related guidelines in clinical practice might be of use both in screening for liver disease in those with the metabolic syndrome and in the selection of NAFLD patients at risk of progression of liver disease. These patients could be targeted for close observation, definitive histologic investigation, and in the future, potential therapies such as insulin sensitizers. Assessment of the validity of such an approach warrants prospective study in a larger group.

**References**


