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Liver News Elsewhere

Experimental evidence of obstructive sleep apnea syndrome as a second hit accomplice in nonalcoholic steatohepatitis pathogenesis.*

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Abstract

Obstructive sleep apnea (OSA) causes chronic intermittent hypoxia (CIH) during sleep. OSA is associated with nonalcoholic steatohepatitis (NASH) in obese individuals and may contribute to progression of nonalcoholic fatty liver disease from steatosis to NASH. The purpose of this study was to examine whether CIH induces inflammatory changes in the liver in mice with diet-induced hepatic steatosis. C57BL/6J mice (n = 8) on a high-fat, high-cholesterol diet were exposed to CIH for 6 mo and were compared with mice on the same diet exposed to intermittent air (control; n = 8). CIH caused liver injury with an increase in serum ALT (461 ± 58 U/l *vs*. 103 ± 16 U/l in the control group; $p < 0.01$) and AST (637 ± 37 U/l *vs*. 175 ± 13 U/l in the control group; $p < 0.001$), whereas alkaline phosphatase and total bilirubin levels were unchanged. Histology revealed hepatic steatosis in both groups, with mild accentuation of fat staining in the zone 3 hepatocytes in mice exposed to CIH. Animals exposed to CIH exhibited lobular inflammation and fibrosis in the liver, which were not evident in control mice. CIH caused significant increases in lipid peroxidation in serum and liver tissue; significant increases in hepatic levels of myeloperoxidase and proinflammatory cytokines IL-1β, IL-6, and CXC chemokine MIP-2; a trend toward an increase in TNF-α; and an increase in α1(I)-collagen mRNA. We conclude that CIH induces lipid peroxidation and inflammation in the livers of mice on a high-fat, high-cholesterol diet.

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Key words: Obstructive sleep apnea, nonalcoholic steatohepatitis, obesity, liver.

Comment

Nonalcoholic fatty liver disease (NAFLD) is a wide pathological complex ranging from steatosis to cirrhosis and hepatocellular carcinoma.[1] Nonalcoholic steatohepatitis (NASH) is considered to be the cornerstone of NAFLD, showing abnormal triglyceride deposition in hepatocytes along with acute and chronic lobular and portal inflammation.[2] The presence and severity of NAFLD risk factors (older age, central adiposity, obesity, insulin resistance, hypertriglyceridemia, hypoalphalipoproteinemia, metabolic syndrome and type 2 diabetes mellitus) are not decisive for a diagnosis of NASH, and a few patients do not even exhibit these risk factors.[3] Progression of hepatic steatosis into NASH has been linked to oxidative damage through lipid peroxidation, however, accurate understanding of the trigger of inflammation in the so-called «second hit» remains elusive.[4]

Experimental studies have shown that in a model of sleep-disordered breathing in mice, which reproduces obstructive sleep apnea syndrome (OSAS),[5] chronic intermittent hypoxia (CIH) leads to increased expression of genes involved in fatty acid synthesis and hepatic oxidative stress without NASH in lean wild-type mice.[6,7]

Savransky *et al*. used wild-type mice with diet-induced fatty liver to study the effect of CIH in this setting. They observed a 4-fold increase in serum ALT levels and lipid peroxidation (serum and hepatic malondialdehyde levels) compared with controls; histopathological analysis revealed hepatocyte swelling, macrovesicular steatosis, glycogen deposition, lobular inflammation and mild fibrosis in mice exposed to CIH, compatible with both NASH and glycogenic hepatopathy, without evidence of

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ischemic hepatitis or cardiac hepatopathy.

Mice undergoing CIH showed decreased weight and fasting glucose serum levels without changes in insulin levels. These findings suggest increased hepatic insulin sensitivity in a glucose-overloaded environment, which induce increased glucose uptake and glycogen synthesis.

A leptin-deficient obese mice model was previously found to have decreased insulin sensitivity when exposed to CIH.[8] These findings suggest a role for leptin in the induction of hepatic insulin sensitivity during CIH. Leptin has been found to be an important determinant of hepatic triglyceride deposition due to its role on hepatic fatty acid oxidation and the deleterious effect of leptin hepatic resistance.[9,10]

Hypoxia-inducible factor (HIF)-1α has been shown to regulate several genes during hypoxia, such as visfatin, a novel adipocytokine with insulin-mimetic functions. Along with leptin actions, visfatin-induced insulin receptor activation might help to explain the improved hepatic insulin sensitivity observed in this model.[11-13]

Insulin resistance is thought to play a major role in NAFLD and NASH, which have leaded some authors to suggest that they are a feature of the metabolic syndrome.[14] However, increased hepatic insulin sensitivity has been shown to be deleterious in abnormal conditions such as those observed in PTEN$^{-/-}$ mice and diacylglycerol acyltransferase 2 (DGAT2) antisense oligonucleotide-treated mice with methionine and choline-deficient diet-induced NAFLD.[15,16]

How can this apparently contradictory phenomenon occur? Insulin decreases glucose oxidation and induces energy deposition, activating fatty acid synthesis through SREBP-1c.[17] Insulin also decreases apo-B100 and microsomal triglyceride transfer protein (MTTP) expression, reducing triglyceride exportation in VLDL form.[18] This insulin-mediated gene regulation causes increased hepatic triglyceride content and steatosis under conditions with abnormally high available lipids. Under stressful, metabolically-demanding situations (such as CIH), the protective effect of triglyceride deposition may be lost by massive induction of fatty acid oxidation, due to leptin and other factors, provoking oxidative damage and lipid peroxidation.

These experimental findings point at OSAS as a probable factor in NASH progression, which has already been associated with features of the metabolic syndrome, abnormal liver enzymes and lipid peroxidation.[19-22] However, consistent clinical data linking OSAS to NASH is lacking as most studies have used either questionnaires testing OSAS symptoms or non-invasive NASH diagnostic tests to evaluate this association.[19-23] A small case series (n = 18) documented an association between OSAS and NASH. In this study, 66.6% of patients with moderate-to-severe OSAS and abnormal liver enzymes were found to have NASH.[24] Unfortunately, Tanne et al. used the presence of abnormal liver enzymes to decide whether to biopsy or not the liver of a patient with suspected NAFLD, which has been shown to be a poor diagnostic tool for NASH screening.

In conclusion, further clinical evidence is eagerly awaited to evaluate the role of OSAS as a factor in the progression of hepatic steatosis to NASH. In the meantime, OSAS screening and treatment in NASH patients seems to be appropriated.

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