Alterations in Liver ATP Homeostasis in Human Nonalcoholic Steatohepatitis: A Pilot Study

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Fatty liver affects about 25% of the general population. Although many individuals with fatty liver are obese and/or consume alcohol regularly, others have no obvious predisposing condition. Most of the time, fatty liver (also called hepatic steatosis) appears to have a benign prognosis. However, some individuals with fatty liver develop steatohepatitis, a condition in which steatosis is accompanied by hepatic inflammation and liver cell death. The true prevalence of steatohepatitis is unknown because neither alcohol-related steatohepatitis nor nonalcoholic steatohepatitis (NASH) is predictably accompanied by obvious symptoms or signs of liver disease. However, a recent literature review reports that NASH has been detected in 1.2% to 9% of patients undergoing routine diagnostic liver biopsy. Five 10-year follow-up studies of patients with biopsy-proven NASH suggest that progressive liver fibrosis and cirrhosis may develop in as many as 20% to 40% of these individuals. Such observations indicate that NASH may be a relatively common cause of cryptogenic cirrhosis and liver-related mortality. Caldwell and colleagues suggest that NASH is the major cause of cryptogenic cirrhosis in their population of liver transplant candidates.

Animal models might be helpful in clarifying the mechanisms that mediate the progression from fatty liver to NASH. Animal models of fatty liver show that hepatic adenosine triphosphate (ATP) depletion and necrosis occur in obese mice and in mice with fatty liver, suggesting that altered hepatic energy homeostasis may be involved.

Objective To determine if patients with fatty liver disease exhibit impaired recovery from hepatic ATP depletion.

Design Laboratory analysis of liver ATP stores monitored by nuclear magnetic resonance spectroscopy before and after transient hepatic ATP depletion was induced by fructose injection. The study was conducted between July 15 and August 30, 1998.

Setting University hospital.

Patients Eight consecutive adults with biopsy-proven nonalcoholic steatohepatitis and 7 healthy age- and sex-matched controls.

Main Outcome Measure Level of ATP 1 hour after fructose infusion in patients vs controls.

Results In patients, serum aminotransferase levels were increased (P = .02 vs controls); albumin and bilirubin values were normal and clinical evidence of portal hypertension was absent in both groups. However, 2 patients had moderate fibrosis and 1 had cirrhosis on liver biopsy. Mean serum glucose, cholesterol, and triglyceride levels were similar between groups but patients weighed significantly more than controls (P = .02). Liver ATP levels were similar in the 2 groups before fructose infusion and decreased similarly in both after fructose infusion (P = .01 vs initial ATP levels). However, controls replenished their hepatic ATP stores during the 1-hour follow-up period (P < .02 vs minimum ATP) but patients did not. Hence, patients’ hepatic ATP levels were lower than those of controls at the end of the study (P = .04). Body mass index correlated inversely with ATP recovery, even in controls (R = −0.768; P = .07). Although BMI was greater in patients than controls (P = .02) and correlated strongly with fatty liver and serum aminotransferase elevations, neither of the latter 2 parameters nor the histologic severity of fibrosis strongly predicted hepatic ATP recovery.

Conclusions These data suggest that recovery from hepatic ATP depletion becomes progressively less efficient as body mass increases in healthy controls and is severely impaired in patients with obesity-related nonalcoholic steatohepatitis.

JAMA. 1999;282:1659-1664 www.jama.com

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LIVER ATP HOMEOSTASIS IN NONALCOHOLIC STEATOHEPATITIS

Genetically obese ob/ob mice have several characteristics that occur in patients who are at high risk for NASH, including obesity, hyperinsulinemia, insulin resistance, hypertriglyceridemia, and fatty liver. We recently demonstrated that these mice are very likely to develop steatohepatitis when exposed to low doses of endotoxin. Furthermore, when ob/ob mice are subjected to minor surgical stress or transient hepatic hypoxia, significant and sustained depletion of hepatic adenosine triphosphate (ATP) stores results because the obese mice are less efficient than lean mice in replenishing their liver ATP stores. Subsequent work has identified a potential molecular mechanism for the inefficient ATP production of ob/ob mouse liver. Hepatocytes from ob/ob mice have upregulated the expression of the mitochondrial uncoupling protein UCP2. UCP2 uncouples oxidative phosphorylation from mitochondrial respiration, reducing the efficiency of mitochondrial ATP synthesis. Therefore, we have proposed that the transition from fatty liver to NASH may be a result of impaired energy homeostasis.

The purpose of this small pilot project was to evaluate the possibility that, similar to ob/ob mice with fatty liver, patients with fatty liver disease might have a diminished ability to replenish hepatic ATP stores after an acute ATP-depleting challenge. To minimize the risk of precipitating serious liver damage, clinically compensated patients with biopsy-proven NASH and a group of healthy age- and sex-matched control subjects were given an intravenous bolus of fructose, which produces a transient decrease in hepatocyte ATP stores that is well tolerated by healthy adults. Hepatic ATP stores were monitored continuously before, during, and for 60 minutes after fructose administration to determine if the patients and controls differed in their responses to fructose-induced ATP depletion.

METHODS

Subjects

Funding to perform nuclear magnetic resonance (NMR) spectroscopy in 15 subjects was provided by a grant from the Johns Hopkins University School of Medicine General Clinical Research Unit, Baltimore, Md. Based on our earlier studies in ob/ob mice, we estimated that an evaluation of 8 patients with fatty liver and 7 controls should be sufficient to detect a fatty liver–related trend toward decreased ATP recovery if the latter occurred in patients with fatty liver disease. Because ethical constraints prohibit liver biopsy in individuals with ultrasonographic evidence of fatty liver and normal serum liver enzymes, the study population was selected from a total of 16 patients with biopsy-proven NASH who had minimally increased serum liver enzymes but no clinical evidence of liver insufficiency. All these patients had been referred to 1 of us (A.M.D.) during the previous 12 months. The first 8 patients who were invited to participate consented and were studied within 6 weeks of enrollment. Once the patient group had been identified, a control group of 7 healthy individuals was selected to match the age and sex distribution of the patients. All controls were unpaid volunteers selected from among colleagues who were aware of our work and interested in learning more about NASH pathogenesis. The controls were also studied within the same 6-week study period.

None of the patients with NASH had serologic evidence of viral hepatitis, autoimmune liver disease, hemochromatosis, alpha-antitrypsin deficiency or Wilson disease. All denied alcohol consumption, and this history was confirmed by at least 1 close relative. The diagnosis of NASH had been previously established by liver biopsy in all patients using established histological criteria. These biopsies had been performed a mean (SD) of 17 (10) months (range, 0.5-44 months) before enrollment.

Although body mass was not used as an enrollment criterion for either patients or controls, on the day of the study, the body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Immediately before NMR evaluation and immediately thereafter, blood was obtained to determine serum glucose, albumin, and total and direct-reacting bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, cholesterol (high-density lipoprotein and low-density lipoprotein), and triglyceride levels. All of these assays were performed in the routine hospital laboratory.

The study was approved by the Johns Hopkins Hospital Joint Committee on Clinical Investigation. All patients and controls gave written informed consent.

Study Design

All studies were performed in the morning, between 6:30 AM and 9:30 AM, after an overnight fast. For all subjects, a vein was catheterized and a venous blood sample was drawn for laboratory evaluation. A slow infusion of isotonic sodium chloride solution was started to maintain catheter patency. After 2 basal NMR spectra were obtained, fructose (250 mg/kg of body weight) dissolved in 25 to 50 mL of isotonic sodium chloride solution was infused rapidly (for 30-60 seconds) through the indwelling venous catheter; further spectra were then collected every 5 minutes for 1 hour. The slow saline infusion was continued until the end of the study. At that time, another venous blood sample was collected for laboratory evaluation.

NMR Spectroscopy

Studies were carried out on a General Electric (Milwaukee, Wis) Signa 1.5-T whole-body magnetic resonance imaging system. Subjects were placed in a supine position on top of a standard transmit/receive (6.5-cm diameter) surface coil assembly. A plastic vial containing approximately 2 mL of 0.1 mol/L phenylphosphonic acid and 5 mmol/L chromium acetylacetone in a water-ethanol mixture was placed in the center of the surface coil. This provided a reference signal for the phosphorus P 31 NMR spectra as well as a visible marker at the center of the coil in the hydrogen H 1 image. The patients were positioned such that the center of the coil, identified by the vial, was under the center of the liver.

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Multislice 1H-magnetic resonance images were collected without breath-holding using a gradient recall acquisition in the steady state imaging sequence to confirm the position of the surface coil with respect to the liver. A 1H-NMR spectrum from a single voxel (approximate dimensions 4 × 4 × 4 cm³) of the liver was collected using a standard stimulated echo acquisition mode sequence with a 3-second interpulse delay, to determine the fat-water ratio of the liver. 31P-NMR spectra were collected using 1-dimensional chemical shift imaging sequence as described in detail elsewhere. Spectra were collected with a 1-second interpulse delay, 32 phase-encode steps with 8 averages per phase-encode, and a field of view of 32 cm. This resulted in a total acquisition time of 4.3 minutes per spectrum and a slice thickness of 1 cm. Two 1-dimensional chemical shift imaging data sets were collected prior to the fructose challenge; fructose was administered at the start of the third chemical shift imaging data acquisition and spectra were collected continuously for the next 60 minutes. Spectra were processed with a combination of 30-Hz exponential and 20-Hz gaussian filter. Peak areas were determined using a gaussian line-fitting routine following baseline correction, and intensities were normalized to the intensity of the phosphate standard.

**Statistical Methods**

Results are expressed as mean (SD). Because of the small sample sizes, data were analyzed by both parametric (unpaired t test) and nonparametric (Mann-Whitney) tests to assure that subject heterogeneity within groups did not bias our interpretation of the results. Correlations were evaluated with linear regression analysis. Probability values less than .05 were considered significant.

**RESULTS**

The demographic and anthropometric characteristics of the study population are summarized in the TABLE. The age, sex distribution, and height of patients and controls were similar. However, the patient group weighed significantly more and had a higher BMI than the control group. Of interest, although type 2 diabetes and hyperlipidemia have been associated with NASH, the mean serum glucose, cholesterol, and triglyceride levels in our patients were not significantly greater than in our control group. Nevertheless, at least half of the patient group had evidence of insulin resistance because 4 of 8 patients (compared with 0 of 7 controls) had a fasting blood glucose level higher than 7.9 mmol/L (140 mg/dL). Hence, if a larger number of subjects had been studied, it is likely that the differences in blood glucose between patients and controls would have achieved statistical significance. No patient was taking lipid-lowering drugs and only 1 of 8 patients was being treated with oral medications to control hyperglycemia. The latter individual did not take his medication on the morning of the study.

As expected, 1H spectroscopy confirmed that the patient group had a significantly higher liver fat-water ratio than the control group (0.12 [0.03] vs 0.02 [0.01]; P = .009 by both parametric and nonparametric tests). Of interest, there was some overlap between the 2 groups with regard to this parameter; 3 of 8 patients had fat-water ratios that were within the normal range on the study day. This observation is consistent with anecdotal reports that document the resolution of hepatic steatosis in some patients with NASH.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Illustrates representative 31P spectra obtained from the liver of a healthy control subject before, at 12 minutes after, and at 60 minutes after fructose infusion. Notice that shortly after fructose infusion, the amplitude of the β-ATP peak decreased in association with a marked increase in the amplitude of the phosphomonoester peak, principally due to the accumulation of

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fructose-1-phosphate. This is consistent with data that have been reported by other groups who have studied the effects of fructose administration on liver ATP stores. This early decrease in hepatic ATP content and increase in phosphomonoesters reflect the use of hepatic ATP during fructose metabolism. However, eventually, fructose metabolites enter glycolytic pathways and ATP is recovered. This restoration of hepatic ATP stores is illustrated by the return of the β-ATP peak and inorganic phosphate peak amplitudes toward their respective pretreatment values by 60 minutes after fructose injection (Figure 1).

As shown in Figure 2, NASH patients and healthy controls had a different response to fructose infusion. Liver ATP levels were similar in the 2 groups before fructose administration and, in both groups, fructose infusion resulted in a significant decline in liver ATP stores. There was no evidence that liver ATP levels were more reduced by fructose injection in patients than in the control group. However, unlike the control subjects, who replenished their hepatic ATP stores during the 1-hour follow-up period, patients barely recovered their ATP levels during this period. Hence, at the end of the study, hepatic ATP levels in the NASH patients were significantly lower than those of healthy controls. Despite these differences in liver ATP stores, patients and controls had similar blood glucose levels at the end of the study. Furthermore, the changes in hepatic ATP content did not increase serum values of the liver-associated enzymes from their pretreatment values in either study group.

Regression analysis identified BMI as the best predictor of ATP recovery (Figure 3). Higher BMIs were associated with lower recoveries of hepatic ATP stores. Interestingly, this relationship was stronger in healthy controls ($r^2 = 0.59$), whose BMIs ranged from lean to overweight, than in NASH patients ($r^2 = 0.003$), all but 1 of whom were overweight or obese. Furthermore, although increases in BMI were positively correlated with high liver fat-water ratios ($r^2 = 0.678; P = .001$) and increased serum aspartate aminotransferase ($r^2 = 0.268; P = .048$) and alanine aminotransferase ($r^2 = 0.446; P = .009$) values, neither liver fat ($r^2 = 0.268; P = .07$) nor liver enzymes ($r^2 = 0.278; P = .13$) was as strong a predictor of persistent hepatic ATP depletion as the BMI ($r^2 = 0.544; P = .004$).

**COMMENT**

This pilot study demonstrates that patients with NASH have impaired hepatic ATP homeostasis. The patients that were evaluated had well-compensated liver disease and minimally elevated liver enzymes. Their baseline liver ATP stores were no lower than those of healthy age- and sex-matched controls. However, unlike these control subjects, the NASH patients were unable to recover from a modest ATP-depleting challenge—they exhibited sustained hepatic ATP depletion after an intravenous bolus injection of fructose, which produced only a transient fall in the liver ATP stores of healthy subjects.

The design of our study does not permit us to identify the mechanism(s) that mediates the defective ATP response in patients with NASH; however, a number of possibilities merit consideration.
First, it is conceivable that fructose metabolism may have been different in the NASH patients and the controls. This concern is reasonable because the patients were generally heavier and, thus, received a larger absolute amount of fructose than controls. In addition, because of their obesity, the patient group (while not overtly diabetic) may have been relatively more insulin-resistant than the leaner controls. Indeed, the latter possibility is supported by evidence that half of the NASH patients had fasting blood glucose levels higher than 6.7 mmol/L (120 mg/dL). Either of these issues might influence hepatic fructose metabolism. However, the data do not support these possibilities because the magnitude of the initial drop in liver ATP, caused by the early events in fructose metabolism, was no greater in patients than controls. Furthermore, blood glucose levels at the end of the study period, an indicator of insulin actions, were unchanged by fructose administration in either patients or controls. Thus, it seems unlikely that the patients’ impaired hepatic ATP recovery was primarily due to inadvertent differences in their exposure to or metabolism of the test substance.

Because liver disease was a criterion for patient enrollment, it is also possible that the impaired hepatic ATP homeostatic responses in the NASH patients were a consequence (rather than a cause) of their liver damage. A larger study involving more controls with different types of liver diseases will be required to resolve this important issue. However, if liver damage was the main reason for persistent liver ATP depletion, then one would expect that liver enzyme abnormalities would have been the best predictor of the ATP response. They were not. More importantly, the presence of severe (grade 3+ or 4+) fibrosis was not associated with a poorer recovery of liver ATP than the presence of less severe fibrosis on liver biopsy. Finally, ATP recovery also varied among the control subjects, who had no clinical or biochemical evidence of liver disease.

An additional interesting (and entirely unexpected) result of this study was the finding that hepatic ATP recovery is inversely correlated with BMI (Figure 3). Surprisingly, this relationship is particularly robust in apparently healthy control subjects who are not grossly overweight. Evidence that increased body mass might negatively affect hepatic ATP homeostasis in humans is particularly intriguing in light of our recent studies in genetically obese ob/ob mice. Unlike the human subjects evaluated in the current study, these mice were morbidly obese, weighing almost 3 times as much as their lean littermates. However, similar to many patients with NASH, adult ob/ob mice are hyperinsulinemic, insulin-resistant, and have hypertriglyceridemia. Evaluation of these mice by 31P-NMR spectroscopy demonstrated that they also have profound abnormalities in hepatic ATP homeostasis. Thus, adaptive responses to obesity might create a state of hepatic vulnerability that predisposes obese subjects to liver damage. This possibility is further supported by reports that serum alanine aminotransferase values, a marker of liver injury, increase with increasing BMI in humans. However, because the inverse correlation between hepatic ATP recovery and BMI emerged during our retrospective analysis of data that were accrued in this small pilot study that was designed to assess hepatic energy homeostasis in NASH, additional larger studies that include more obese subjects without liver disease are necessary to reach firm conclusions.
about the relationship between obe-
sity and hepatic energy homeostasis.

Nevertheless, the present results in hu-
man subjects complement results from
previous human and animal studies and,
together, suggest that the liver is an im-
portant target tissue for obesity-related
damage. While fatty liver has long been
associated with obesity, the current NMR
data in a small number of apparently
healthy control subjects suggest that as
body mass increases, important metabo-
litic adaptations occur even before steatosis
becomes evident. Some of these responses
apparently delay hepatic recovery from
ATP-depleting interferences. Replenish-
ment of liver ATP stores seems to become
progressively less efficient with increas-
ing body mass and significantly compro-
mises liver ATP homeostasis in over-
weight and obese individuals.

It is important to emphasize that in the
current study, BMI was not used as a cri-
terion for patient selection. Yet, all but 1
of our NASH patients were overweight
and most were obese. The association of
NASH with obesity in our group of
patients is not surprising. Indeed, it is
consistent with the findings of many
other investigators. However, as a result,
the experimental design of the current
study does not permit us to determine if
obesity-related defects in energy homeo-
stats actually cause overt liver damage.
Nor do our results imply that obesity is
the only factor that can impair hepatic
ATP recovery. However, regardless of its
cause, compromised hepatic ATP
homeostasis might increase the risk of
liver injury from other insults. For
example, morbibly obese mice, which
also have difficulties with hepatic ATP
balance, typically have fatty livers but
develop hepatic necrosis only when mito-
ochondrial electron transport is inhib-
ited acutely. If, as suggested by our
NMR data, overweight and obese humans
are also more vulnerable to hepatic ATP
depletion, this might help explain why
obesity has been identified as a risk fac-
tor for liver disease due to jejunal-ileal
bypass surgery, alcohol, and various other
toxins. Furthermore, because sev-
eral of the patients in the current study
had unsuspected hepatic fibrosis, it seems
likely that overweight individuals expe-
rience recurrent episodes of subclinical
hepatic necrosis. The latter suggests a
driving mechanism for clinical observa-
tions that indicate that obesity is a major
risk factor for cryptogenic cirrhosis.11

Because the results of our small pilot
study suggest a novel mechanism that
might be involved in the pathogenesis of
obesity-related liver disease, a common
but poorly understood problem, they are
being reported to stimulate investiga-
tion in this area. Additional work will be
required to validate whether or not obe-
sity per se increases vulnerability to
hepatic ATP depletion and to clarify the
molecular basis for this response. Poten-
tially important targets for obesity-
related dysregulation, including the mito-
ochondrial uncoupling protein, UCPC1
have been identified by studying animal
models. However, direct evaluation of
the expression of this and other mito-
ochondrial proteins in human subjects will
always be limited by ethical concerns that
constrain the use of invasive proce-
dures such as liver biopsy. In this regard,
the current results encourage further
efforts to use noninvasive NMR tech-
niques to determine if mitochondrial dys-
function plays an important permissive
role in the genesis of NASH.

Funding/Support: This work was partially
supported by grants to Dr Cortez-Pinto from the Fundacao Luso-
Americana Para o Desenvolvimento, Lisbon, Portugal, and to Dr Diehl
from the Johns Hopkins General Clini-
cal Research Center and the Women’s Health Initiative.

Acknowledgment: We thank Peter Barker, MD, for his assistance and use of software for data analysis.

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