NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) – A NOVEL COMMON ASPECT OF THE METABOLIC SYNDROME

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Non-alcoholic fatty liver disease (NAFLD) is emerging as one of the most common liver disorders claiming the urgent attention of both medical professionals and the public sphere because of the imminent epidemic of advanced liver injury that appendages epidemic of obesity. Recent research reveals simple triglyceride accumulation in hepatocytes (i.e., liver steatosis) frequently becoming complicated by inflammation (i.e., non-alcoholic steatohepatitis, or NASH) that may progress into more advanced stages of the disease including cirrhosis or, eventually, hepatocellular carcinoma. The exact mechanisms of the progression of NAFLD into overt NASH and advanced disease stages are largely unknown. There is urgent need in terms of both intensive research pursuits and effective practical measures to deal with this common threat.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a nosological unit first introduced by Ludwig and his colleagues at the Mayo clinic in 1980¹. It is emerging as the most common liver disorder recognized in Europe and North America. It includes a wide spectrum of hepatic alterations of metabolic origin. According to current estimates, the prevalence of NAFLD in the general population ranges from 5 % to 33 %. Up to more than 75 % of obese adult subjects can be affected by NAFLD^{2–4}. Formerly a disease of adults, NAFLD now seriously extends to lower-age population segments, the true prevalence remaining unknown. Data obtained by Franzese et al.⁵ have indicated that this condition can be prevalent in as much as 53 % of obese children, and NAFLD is now recognized as an important childhood liver disease, especially since childhood obesity is much more common.^{6–8} According to estimates, NAFLD is likely to reach epidemic proportions in children worldwide in the next decade^{9,10}.

NAFLD IS A NOVEL COMMON FEATURE OF THE METABOLIC SYNDROME

In most cases, NAFLD is associated with obesity, hyper- or dyslipidemia, and insulin resistance. This close association suggests possible causal links between the disorders. Based on recent research, the condition most

closely associated with NAFLD is obesity and central obesity, in particular^{10–12}. Widespread hyper- or dyslipidemia supported by inappropriate dietary and lifestyle habits are considered as the most common denominators and key determinants of this undesirable trend^{13,14}. On this basis, NAFLD has been included as part of the metabolic syndrome, or syndrome X^{15–17}. An available data support this association. A large portion of cases with metabolic syndrome suffer from NAFLD that can be now envisaged as a specific hepatic manifestation of this systemic disorder, both conditions probably sharing common pathogenic mechanism(s)^{18–21}. Although representing a special problem among obese people, NAFLD can also occur in non-obese/non-diabetic subjects as well as in patients with lipodystrophy^{3,13,14,22}.

OUTLINE OF THE CLINICAL COURSE

In pure fatty liver, the disease may have a benign clinical course without excess mortality²³. However, contrary to what has been thought previously, fatty liver is not a trivial condition. Simple forms of NAFLD are characterized by triglyceride accumulation in hepatocytes (i.e., liver steatosis). This frequently becomes complicated by inflammation (i.e., non-alcoholic steatohepatitis, or NASH)¹. Unfortunately, the mode and exact rate of progression of NAFLD into more advanced stages of the disease have been characterized only partially. NAFLD may progress

to hepatitis, cirrhosis and, eventually, to hepatocellular carcinoma. Several histological stages have been delineated in the process of progression of NAFLD to cirrhosis. These progressively include: fatty liver alone, steatohepatitis, steatohepatitis with fibrosis, and cirrhosis^{18,24}. According to the recent review, steatosis may develop into NASH in one third of cases², and NASH can be envisaged as a turning point in the development of the disorder^{1,10}. Patients with NASH have a significantly increased risk of cirrhosis. Reportedly, a considerable subset (up to 50 %) of NASH patients can develop fibrosis and about 15 % to 19 % develop cirrhosis. Of note, the 7- to 10-year liver-related mortality due to cirrhosis is 12 % to 25 %. A fraction of NAFLD patients develop hepatocellular carcinoma. The risk in this malignant complication is increased more than 4-times in such patients. About 3 % of cases may progress to terminal liver failure, requiring liver transplantation^{2,10,17,25,26}. At present, up to 4 % to 10 % of liver transplants can probably be accounted for by the liver failure caused by end-stage NASH; this estimate is very likely to rise.² Furthermore, in patients who have undergone liver transplantation, NAFLD frequently recurs^{3,15}.

SYMPTOMS AND DIAGNOSIS

NAFLD is essentially an asymptomatic condition. Non-specific symptoms are present in approximately 30 % of the patients prior to diagnosis. The diagnosis is usually made when liver alanin aminotransferase (ALT) and/or aspartate aminotransferase (AST) enzymes are elevated or when there is a fortuitous ultrasonographic or radiological evidence of fatty liver. Mild to moderate elevation of ALT or AST or both are the most common findings (with the AST:ALT ratio <1) and, according to recent estimates, NAFLD is the most frequent cause of abnormal liver function tests and abnormal liver enzymes on routine blood testing that should be considered as a first presentation of NAFLD or NASH^{1,3,15,27}. However, it has been shown that the degree of ALT elevation does not correlate with liver histology^{24,28}. In patients with NAFLD without manifestation of a more advanced liver disease, the common finding on physical examination may be hepatomegaly^{1,29}. Classification of NAFLD progress into phases 1 to 4 has been proposed based on the morphological biopsy patterns³⁰, and more detailed staging and grading systems have been elaborated.^{18,31} Furthermore, primary and secondary forms of NAFLD have been delineated¹⁰.

No specific laboratory tests or noninvasive investigation to diagnose NASH are available to date. The sensitivity of noninvasive imaging techniques is not adequate since these fail to distinguish NASH from simple steatosis^{10,32,33}. The definite diagnosis of the disease is based on histological criteria. While not every patient with mild elevation of liver enzymes can be referred for a liver biopsy, it is often difficult to make accurate diagnostic decisions^{3,33,34}. Recently, Palekar et al.³⁵ have proposed a diagnostic model aimed at distinguishing patients with simple steatosis from those with NASH. The character-

istics considered by the authors³⁵ are as follows: female gender, age ≥ 50 years, BMI ≥ 30 mg/m², serum AST ≥ 45 IU/l, AST/ALT ratio ≥ 0.8 , and plasma hyaluronic acid concentration ≥ 55 μ g/l. Patients with three or more of these characteristics are more likely to have NASH than simple steatosis³⁵.

PATHOBIOCHEMISTRY AND PATHOPHYSIOLOGY

The liver has been shown to have a high capacity for accumulating triglycerides. For instance, short term fat feeding may lead to an about 3-fold increase in rat liver triglyceride content without increase in visceral or skeletal muscle fat¹⁴. Moreover, the content of intra-abdominal fat has been reported to directly correlate with liver fat¹². According to Thomas et al.³⁶, intrahepatic lipids increase by 22 % for any 1 % increase in total adipose tissue, by 21 % for any 1 % increase in subcutaneous adipose tissue, and by 104 % for 1 % increase in intra-abdominal adipose tissue. Liver fat does not simply reflect fat stores, but is markedly controlled by dietary fat³⁷. Changes in dietary fat can change liver fat independently of any change in body weight, free fatty acid (FA) concentration, intra-abdominal or subcutaneous fat mass, or rate of carbohydrate, lipid or protein oxidation^{12,38}. Up to 20 % of dietary FA are secreted as VLDL triglycerides in humans within 6 hours after a meal^{39,40}. Weight loss enhances insulin sensitivity and subjects with high liver fat submitted to a weight loss showed a larger decrease in hepatic fat and a more marked decrease in insulin concentration than control subjects with low liver fat^{41,42}. Thus, diet may influence steatosis markedly.

Plasma lipid overflow and resulting tissue overload, decreased FA oxidation, increased hepatic synthesis of triglycerides and/or altered secretion of very-low-density lipoproteins (VLDLs) from the liver are probably the most critical contributors to developing NAFLD¹⁰. At the same time, NAFLD is intimately associated with progressive manifestation of insulin resistance in the liver. Importantly, in over-fed animals, insulin resistance in liver and adipose tissue precedes the impairment of insulin sensitivity in skeletal muscle⁴³. Simple (i.e., early) hepatic steatosis alone is insufficient to increase endogenous glucose production in the liver but it is linked to insulin resistance so that higher insulin concentrations are required to control liver metabolism and gluconeogenesis, in particular^{14,44}. Suppression of both direct and indirect (extrahepatic) effect of insulin may be responsible for enhanced liver gluconeogenesis^{45,46}. Transcription factors sterol regulatory element-binding protein-1c (SREBP-1c), peroxisome proliferator activated receptors (PPARs), and carbohydrate response element-binding protein (ChREBP) have been described as the main physiologic molecular regulators of lipogenesis; they are also implicated in the abnormal accumulation of triglycerides in NAFLD. A number of regulatory peptides, particularly those released from adipocytes, such as TNF α , leptin, adiponectin, resistin, visfatin, etc., may be involved in all

phases of liver damage in NAFLD and, subsequently, NASH^{10, 13, 47–51}.

The exact mechanism of the progression of simple steatosis into overt NASH remains unclear. Hepatic lipid accumulation is not the sole factor responsible for hepatocellular injury. Once developed, oxidative stress and diminished antioxidants within the liver can initiate the progression from steatosis alone to NASH and ultimately to cirrhosis^{20, 52}. Increased hepatic FA oxidation can generate reactive oxygen radicals (ROS) that may promote mitochondrial dysfunction, lipid peroxidation, and/or cytokine secretion⁴⁷. A two-hit model has been proposed⁵³ involving a cellular event or events that, in steatotic liver, promote(s) inflammation, fibrosis, cell death, and cirrhosis. Abnormal cytokine secretion, particularly that of inflammatory cytokines (e.g., TNF α , IL-6, etc.), and ROS as well appear as the major players implemented in the conversion of relatively benign steatosis into NASH. Activation of specific redox-sensitive kinases and inflammatory signalling pathways, e.g., inhibitor kappa-B kinase (IKK- β) and nuclear factor- κ B (NF- κ B) cascade, can initiate a self-perpetuating and self-reinforcing positive feedbacks leading to irreversible liver injury that characterizes NASH histopathologically. FA themselves may cause hepatocyte apoptosis, i.e., the final mechanism of cellular injury in NAFLD^{3, 10, 13, 54–56}.

THERAPY AND PREVENTIVE MEASURES

Collectively, the recent research reveals that a large part of human population is susceptible to the serious liver damage brought on by their lifestyle. This fact calls for acceptance of urgent practical measures to tackle the imminent epidemic threat. There is no specific drug treatment available for NAFLD at present. Therefore, treatment strategies lay stress on modifying risk factors of the disease. The importance of calorie restriction, physical activity, and the presence of antioxidants in the diet in NAFLD prevention have been emphasized repeatedly^{9, 20, 37, 47}.

Several pilot clinical studies have convincingly shown that changes in lifestyle may halt NAFLD progression. Ueno et al.⁵⁷ have reported that restricted diet and exercise therapy, such as walking and jogging, are useful means for improving blood biochemical data and histological findings in NAFLD. Investigating NASH patients who successfully completed one year of intense dietary intervention, Huang et al.⁵⁸ have found that these do display histological improvement, thus confirming the effectiveness of dietary intervention in biopsy-proven NASH-affected individuals. Finally, in a group of subjects followed for one year and receiving regular counselling, weight loss and regular exercise have been shown to improve blood ALT levels and increase the clinical odds of normalization of this liver enzyme. Reducing weight by at least 5 % together with subsequent weight control and regular exercising has been revealed as a reasonable target in NAFLD treatment⁵⁹.

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REFERENCES

1. Ludwig J, Viggiano TR, McGill DB, Oh BJ. (1980) Non-alcoholic steatohepatitis. Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 55, 434–438.
2. Farrell GC, Larter CZ. (2006) Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 43, S99–S112.
3. Malnick SDH, Beergabel M, Knobler H. (2003) Non-alcoholic fatty liver: A common manifestation of a metabolic disorder. *Q J Med* 96, 699–709.
4. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. (2003) Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 37, 917–923.
5. Franzese A, Vajro P, Argenziano A, Puzziello A, Iannucci MP, Saviano MC, Brunetti M, Rubino A. (1997) liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosing and during follow-up in an Italian population. *Dig Dis Sci* 42, 1438–42.
6. Roberts EA. (2002) Steatohepatitis in children. *Best Pract Res Clin Gastroenterol* 16, 749–765.
7. Roberts EA. (2003) Nonalcoholic steatohepatitis in children. *Curr Gastroenterol* 5, 253–259.
8. Roberts EA. (2005) Non-alcoholic fatty liver disease (NAFLD) in children. *Front Biosci* 1, 2306–2318.
9. Mager DR, Roberts EA. (2006) Nonalcoholic fatty liver disease in children. *Clin Liver Dis* 10, 109–131, vi–vii.
10. Festi D, Colecchia A, Sacco T, Bondi M, Roda E, Marchesini G. (2004) Hepatic steatosis in obese patients: clinical aspects and prognostic significance. *Obes Rev* 5, 27–42.
11. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. (1999) Association of nonalcoholic fatty liver with insulin resistance. *Am Med* 107, 450–455.
12. Westerbacka J, Conner A, Tiikkainen M, Tamminen M, Vehkavaara S, Hakkinen AM, Fredriksson J, Yki-Jarvinen H. (2004) Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia* 47, 1360–1369.
13. Browning JD, Horton JD. (2004) Molecular mediators of hepatic steatosis and liver injury. *J Clin Invest* 114, 147–152.
14. Samuel VT, Liu ZX, Qu X, Elder BJ, Bilz S, Befroy D, Romanelli AJ, Shulman GI. (2004) Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem* 279, 32345–32353.
15. Angulo P. (2002) Nonalcoholic fatty liver disease. *N Engl J Med* 346, 1221–31.
16. Chitturi S, Farrell GC. (2001) Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 21, 27–41.
17. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. (2001) Nonalcoholic fatty liver disease. A feature of the metabolic syndrome. *Diabetes* 50, 1844–50.
18. Akbar DA, Kawther AH. (2006) Non-alcoholic fatty liver disease and metabolic syndrome: what we know and what we don't know. *Med Sci Monit* 12, 23–26.
19. Eckel RH, Grundy SM, Zimmet PZ. (2005) The metabolic syndrome. *Lancet* 365, 1415–1428.
20. Fehér J, Németh E, Lengyel G. (2005) Non-alcoholic steatohepatitis (NASH) – is it a part of the metabolic syndrome? *AMS* 1, 1–11.
21. Song S. (2002) The role of increased liver triglyceride content: a

- culprit of diabetic hyperglycaemia? *Diabetes Metab Res Rev* 18, 5–12.
22. Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, Ponti V, Pagano G, Ferrannini E, Rizzetto M. (2005) Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* 48, 634–642.
 23. Dam-Larsen S, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sorensen TI, Becker U, Bendsten F. (2004) Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut* 53, 750–755.
 24. James OF, Day CP. (1998) Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. *J Hepatol* 29, 495–501.
 25. Fassio E, Alvarez E, Dominguez N, Landeira G, Longo C. (2004) Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. *Hepatology* 40, 820–826.
 26. Sheth SG, Gordon FD, Chopra S. (1997) Nonalcoholic steatohepatitis. *Ann Intern Med* 126, 137–145.
 27. Sorbi D, Boynton J, Lindor KD. (1999) The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. *Am J Gastroenterol* 94, 1018–1022.
 28. Van Ness MM, Diah AM. (1989) Is liver biopsy useful in the evaluation of patients with chronically elevated liver enzymes? *Ann Intern Med* 11, 473–478.
 29. Bacon BR, Farahvash MJ, JanneyCG, Neuschwander-Tetri BA. (1994) Nonalcoholic steatosis: an expanded clinical entity. *Gastroenterology* 107, 1103–1109.
 30. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. (1999) Nonalcoholic fatty liver disease: A spectrum of clinical and pathological severity. *Gastroenterology* 116, 1413–1419.
 31. Brunt EM, Janney CG, Di Bisceglie AM, Szczepaniak BA, Bacon BR. (1999) Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 94, 2467–2474.
 32. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. (2002) The utility of radiological imaging in non-alcoholic fatty liver disease. *Gastroenterology* 123, 745–750.
 33. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. (2005) Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 288, E462–E468.
 34. Szczepaniak BA, Caldwell SH. (2003) Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. *Hepatology* 37, 1202–1219.
 35. Palekar NA, Naus R, Larson SP, Ward J, Harrison SA. (2006) Clinical model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. *Liver Int* 26, 151–156.
 36. Thomas EL, Hamilton G, Patel N, O'Dwyer R, Dore CJ, Goldin RD, Bell JD, Taylor-Robinson SD. (2005) Hepatic triglyceride content and its relation to body adiposity: a magnetic resonance imaging and proton magnetic resonance spectroscopy study. *Gut* 54, 122–127.
 37. Musso G, Gambino R, De Micheli F, Cassader M, Rizzetto M, Durazzo M, Faga E, Silli B, Pagano G. (2003) Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis. *Hepatology* 37, 909–916.
 38. Westerbacka J, Lammi K, Hakkinen AM, Rissanen A, Salminen I, Aro A, Yki-Järvinen H. (2005) Dietary fat content modifies liver fat in overweight non-diabetic subjects. *J Clin Endocrinol Metab* 90, 2804–2809.
 39. Heath RB, Karpe F, Milne RW, Burdge GC, Wootton SA, Frayn KN. (2003) Selective partitioning of dietary fatty acids into the VLDL TG pool in the early postprandial period. *J Lipid Res* 44, 2065–2072.
 40. Cassader M, Gambino R, Rui G, Marena S, Bodoni P, Pagano G. (1996) Postprandial triglyceride-rich lipoprotein changes in elderly and young subjects. *Aging (Milano)* 8, 421–428.
 41. Tiikkainen M, Bergholm R, Rissanen A, Aro A, Salminen I, Tamminen M, Teramo K, Yki-Järvinen H. (2004) Effects of equal weight loss with orlistat and placebo on body fat and serum fatty acid composition and insulin resistance in obese women. *Am J Clinical Nutrition* 79, 22–30.
 42. Tiikkainen M, Bergholm R, Vehkavaara S, Rissanen A, Hakkinen AM, Tamminen M, Teramo K, Yki-Järvinen H. (2003) Effects of identical weight loss on body composition and features of insulin resistance in obese women with high and low liver fat content. *Diabetes* 52, 701–707.
 43. Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH. (1991) Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes* 40, 1397–1403.
 44. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Järvinen H. (2002) Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 87, 3023–3028.
 45. Rebrin K, Steil GM, Getty L, Bergman RN. (1995) Free fatty acid as a link in the regulation of hepatic glucose output by peripheral insulin. *Diabetes* 44, 1038–1045.
 46. Fisher SJ, Kahn CR. (2003) Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. *J Clin Invest* 111, 463–468.
 47. Feher J, Lengyel G. (2003) A new approach to drug therapy in non-alcoholic steatohepatitis (NASH). *J Int Med Res* 31, 537–551.
 48. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T, Murakami H, Watanabe E, Takagi T, Akiyoshi M, Ohtsubo T, Kihara S, Yamashita S, Makishima M, Funahashi T, Yamanaka S, Hiramatsu R, Matsuzawa Y, Shimomura I. (2005) Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 307, 426–430.
 49. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. (2004) Beyond Insulin Resistance in NASH: TNF- α or adiponectin? *Hepatology* 40, 46–54.
 50. Kaser S, Moschen A., Cayon A., Kaser A, Crespo J, Pons-Romero F, Ebenbichler CF, Patsch JR, Tilg H. (2005) Adiponectin and its receptors in non-alcoholic steatohepatitis. *Gut* 54, 117–121.
 51. Vuppalanchi R, Marri S, Kolwankar D, Considine RV, Chalasani N. (2005) Is adiponectin involved in the pathogenesis of nonalcoholic steatohepatitis? A preliminary human study. *J Clin Gastroenterol* 39, 237–242.
 52. McCullough AJ. (2006) Pathophysiology of nonalcoholic steatohepatitis. *J Clin Gastroenterol* 40, Suppl 1, S17–29.
 53. Day CP, James OFW. (1998) Steatohepatitis: a tale of two "hits"? *Gastroenterology* 114, 842–845.
 54. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. (2002) Oxidative stress and stress-activated signaling pathways: A unifying hypothesis of type 2 diabetes. *Endocrin Rev* 23, 599–622.
 55. Fromenty B, Robin MA, Igoudjil A, Mansouri A, Pessayre D. (2004) The ins and outs of mitochondrial dysfunction in NASH. *Diabetes Metab* 30, 121–38.
 56. Savage DB, Petersen KF, Shulman GI. (2005) Mechanisms of insulin resistance in humans and possible links with inflammation. *Hypertension* 45, 828–833.
 57. Ueno T, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, Torimura T, Inuzuka S, Sata M, Tanikawa K. (1997) Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J Hepatol* 27, 103–107.
 58. Huang MA, Greenon JK, Chao C, Anderson L, Peterman D, Jacobson J, Emick D, Lok AS, Conjeevaram HS. (2005) One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: a pilot study. *Am J Gastroenterol* 100, 1072–1081.
 59. Suzuki A, Lindor K, St Sauver J, Lymp J, Mendes F, Muto A, Okada T, Angulo P. (2005) Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. *J Hepatol* 43, 1060–1066.