Non-alcoholic Fatty Liver Disease and Type 2 Diabetes

a report by
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Non-alcoholic fatty liver disease (NAFLD) was practically unheard of even 30 years ago, but is now considered one of the most common liver disorders in the US.[1–3] It may be the most common cause of liver enzyme elevation in US adults as well as a leading cause of cirrhosis.[4,5] The prevalence of NAFLD has increased in parallel with the epidemics of obesity and type 2 diabetes, which are risk factors for NAFLD.[3,6] Whereas the association of type 2 diabetes with microvascular complications and macrovascular disease is well established, the association of type 2 diabetes with NAFLD is more recently recognised and probably less well-known to physicians. Furthermore, because patients are usually asymptomatic and routine blood tests are often normal, it may be a diagnosis that is overlooked in patients with type 2 diabetes.[7,8]

There is evidence that patients with NAFLD who have type 2 diabetes are particularly at risk of progressive forms of the disease and that they are at higher risk of developing cirrhosis compared with those who do not have diabetes.[9,10] Although cardiovascular disease is the major cause of excess morbidity and mortality in type 2 diabetes, liver failure may also be a threat to patients with type 2 diabetes NAFLD.[3,10] Therefore, it is important for physicians to be aware of the high likelihood that their patients with type 2 diabetes have NAFLD, as this is another potential complication that requires attention.

Definition

NAFLD is characterised by fatty infiltration of the liver, mostly in the form of triglycerides, which exceeds 5% of the liver weight.[11] NAFLD is histologically similar to alcoholic liver disease, but by definition it occurs in the absence of excessive alcohol consumption and is not due to other identifiable causes of fatty liver such as hepatitis C and certain medications.[11] NAFLD represents a spectrum of clinical–pathological features ranging from simple steatosis, which is characterised by fatty infiltration only, to non-alcoholic steatohepatitis (NASH), which is characterised by inflammation and hepatocellular injury with or without fibrosis and cirrhosis.[11,12] Most with NAFLD have an increase in liver fat content alone, which is apparently benign; others develop NASH that can progress to cirrhosis.[10–12]

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Prevalence

Fatty liver is a common problem in the US.[3] Data from the Dallas Heart Study suggested that about one-third of the population of Dallas County, Texas, had hepatic steatosis.[7] This study used proton magnetic resonance spectroscopy (MRS) to measure liver fat and defined steatosis as hepatic triglyceride content >5.5%.[7] Having an abnormal fasting glucose carries an even higher risk. Sixty-two per cent of subjects in the Dallas Heart Study who had either known diabetes or a fasting glucose >110mg/dl had hepatic steatosis.[7] In a Japanese population, 27% with normal fasting glucose had NAFLD; the prevalence rose to 43% among those with fasting hyperglycaemia, and 62% among those with type 2 diabetes.[13] Others have reported a similarly high prevalence of NAFLD in patients with type 2 diabetes. The prevalence by clinical history and liver ultrasound (US) was 69.5% in a large outpatient clinic in Italy and as high as 75% in the Valpolicella Heart Study.[8,14] Morbid obesity places patients with type 2 diabetes at particularly high risk. Virtually 100% of these patients were found to have at least mild steatosis on liver biopsy.[15]

As most who have NAFLD have no specific signs or symptoms, the prevalence is probably underappreciated.[6,16] In clinical practice, elevated aminotransferase levels, especially alanine transaminase (ALT), are considered a marker for liver disease. However, many patients who have NAFLD do not have elevated levels. In the Dallas Heart Study, 79% of those with hepatic steatosis had normal ALT levels.[7] Others reported that 86% of those with NAFLD had normal ALT levels.[8] Therefore, relying on the ALT level as a surrogate marker for NAFLD may overlook a lot of patients who do have it. Making the matter of establishing the diagnosis of NAFLD even more complicated is that aminotransferase levels do not necessarily correlate with the severity of NAFLD.[16] ALT levels may be normal in the presence of advanced fibrosis or cirrhosis.[17] Thus, a normal ALT does not exclude steatosis and does not ensure the absence of underlying advanced liver disease.[16,17] Therefore, since non-invasive methods of detection were used in these epidemiological studies, the prevalence of pure steatosis versus more advanced stages of disease such as steatohepatitis, fibrosis or cirrhosis is unknown.

Diagnosis of Non-alcoholic Fatty Liver Disease

The diagnosis of NAFLD is typically suspected in asymptomatic patients who are found to have elevated aminotransferase levels and/or radiological evidence of fatty liver.[16] The most common laboratory abnormality is a mild to moderate increase in aspartate transaminase (AST) and/or the ALT level.[11] Of the liver enzymes, ALT is most closely related to liver fat accumulation and has been reported to correlate with liver fat independent of obesity.[18] Patients with NAFLD usually have a ratio of AST to ALT of <1. However, if fibrosis develops, this

ratio can reverse.[11] Other liver enzymes such as alkaline phophatase and gamma-glutamyl transferase (GGT) may be abnormal.[6] A low albumin, prolonged prothrombintime and elevated bilirubin are less common and suggest advanced disease.[6,16]

Liver US, computed tomography (CT) scan and MR imaging (MRI) are able to detect fatty infiltration of the liver. However, their sensitivity is limited, and they cannot quantify the amount of fat present. The sensitivities of US and CT scan for detecting steatosis were reported as 100 and 93%, respectively, if liver fat exceeded 33% on liver biopsy.[19] As the amount of fat dropped below this, the sensitivity of these tests diminished.[19] Liver fat can be more reliably detected and quantified by proton MRS.[20] However, this technique is not currently available for routine clinical practice.

While all of these imaging methods can detect a significant accumulation of fat, none can differentiate steatosis from more advanced stages of NAFLD. Histological examination of the liver is the only way to make this distinction. However, recommending liver biopsy for everyone suspected of having NAFLD would not be practical. Arguments against performing a biopsy in everyone include the high prevalence of NAFLD, cost, potential risks with the procedure and the relatively good prognosis of steatosis alone.[6] Liver biopsy is important for confirming the diagnosis, evaluating disease severity and determining its prognosis.[6,16] Detecting and grading fibrosis in patients with NASH is considered one of the most important reasons to perform a biopsy, as the presence of fibrosis is a predictor of subsequent progression to cirrhosis.[21]

Certain characteristics such as older age, obesity and type 2 diabetes and laboratory measures such as an AST to ALT ratio of >1, elevated triglycerides and low platelets are predictors for hepatic fibrosis.[6,16,21] Scoring systems that take these features into account have been devised to help decide by non-invasive means which patients are likely to have simple steatosis versus those who are more likely to have steatohepatitis and liver fibrosis.[21] In general, a biopsy should be considered in those patients at high risk of fibrosis.[16] It has been recommended that patients undergo liver biopsy if they are at high risk of having advance disease and if liver enzymes remain chronically elevated despite lifestyle changes.[6]

Pathogenesis of Non-alcoholic Fatty Liver Disease

There is a strong relationship between hepatic triglyceride content and insulin resistance.[22] NAFLD is associated with insulin resistance.[23,24] It appears to be both a cause and a consequence of insulin resistance. A mechanism by which hepatic steatosis causes insulin resistance involves an inhibition of insulin signalling at the level of the insulin receptor.[25] The central mechanism by which insulin resistance causes hepatic steatosis appears to be via its effect on peripheral free fatty acid levels.[26]

A net change in the amount of lipid in the liver will occur if there is a change in the balance between the liver's uptake or synthesis of fatty acids and the liver's disposal of fatty acids by oxidation or export.[26,27] Insulin resistance impairs the suppression of lipolysis, and this leads to an increased release of free fatty acids from adipose tissue so that more are delivered to and taken up by the liver.[6,26] This excess amount of free fatty acids can overload the hepatic mitochondrial beta oxidation system, the major pathway of fatty acid oxidation in the liver, leading to the accumulation of fatty acids in the liver.[28]

In the majority of patients, simple steatosis – the accumulation of fat in the liver – follows a relatively benign course.[29] It can be present for decades without leading to more serious liver damage.[27] However, it may evolve into NASH, which is a more aggressive liver disease that tends to be progressive and may lead to cirrhosis.[16,29] What determines whether steatosis advances to steatohepatitis is not fully understood.

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However, overload of the hepatic mitochondrial beta oxidation system appears to be important. The resulting increase in oxidative processes promotes oxidative stress by leading to the generation of reactive oxygen species (ROS), free electrons and hydrogen peroxide (H_2O_2), which can directly damage mitochondrial DNA and impair mitochondrial function.[16,29] ROS can also induce hepatic injury through lipid peroxidation within the hepatocytes and stimulation of cytokine synthesis, causing activation of inflammatory pathways.[16,29] These pro-inflammatory cytokines in particular are believed to mediate the shift in pathology from steatosis to steatohepatitis.[27] Increased production of pro-inflammatory cytokines causes a migration of inflammatory cells into the liver and activates stellate cells, which are responsible for collagen synthesis and the development of fibrosis.[16,28,29] A 'two-hit' hypothesis has been proposed to explain the pathogenesis of NASH.[30] Events that promote hepatic steatosis are the first liver insult or hit;[27,30] events that lead to hepatic inflammation such as the exacerbation of oxidative stress and exposure of the hepatocytes to ROS, free radicals and pro-inflammatory cytokines are a superimposed insult or second hit.[27,30]

Patients who develop liver steatosis are likely to be obese, have type 2 diabetes and/or meet the criteria for the metabolic syndrome.[4,7] Patients who are most likely to progress from simple steatosis to steatohepatitis are those who are obese and have type 2 diabetes.[31] Type 2 diabetes is also a prominent risk factor for developing fibrosis and cirrhosis.[10] That type 2 diabetes and the metabolic syndrome are risk factors for more serious forms of NAFLD may suggest that insulin resistance itself also has a role in promoting disease progression. Insulin resistance is associated with oxidative stress and dysfunctional adipocytes that produce increased amounts of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukin (IL-6) and decreased amounts of adiponectin.[32,33] Adiponectin has antilipogenic qualities and increases fatty-acid oxidation, thereby perhaps protecting the liver from lipid accumulation and inflammation.[35`] Based on such observations, some have characterised NAFLD as the hepatic component of the metabolic syndrome.[35]

Treatment of Non-alcoholic Fatty Liver Disease

Since evidence supports the role of insulin resistance in the development of NAFLD, treatment has focused on measures that could reverse insulin resistance such as weight loss and pharmacological therapy with metformin and thiazolidinediones (TZDs). Weight loss, which improves insulin sensitivity, has been shown to lead to reduction in liver enzymes and hepatic steatosis.[36–38] Few studies, generally with small numbers of patients, have involved liver biopsy so that the impact of weight loss on histological changes could be assessed. However, these have reported histological improvement in patients who lost weight.[39,40] While weight loss has beneficial effects in the early stages of NAFLD, its effect on patients with advanced fibrosis or cirrhosis has not been as well studied.

The association between liver fat and insulin resistance also has relevance from the standpoint of managing hyperglycaemia in patients with type 2 diabetes.

However, rapid weight loss has been associated with worsening liver histology.[41,42] This may be related to increased free-fatty-acid levels that occur as a result of increased lipolysis witnessed with fasting.[34] Weight reduction not exceeding 2lb per week has been recommended, with an initial target of weight loss of 10% of baseline.[16,43] Furthermore, physical activity should be emphasised as this may help to achieve and maintain weight loss as well as improve insulin sensitivity. The ideal meal composition of a diet is not known. Some have suggested the Mediterranean diet for patients with type 2 diabetes and NAFLD.[34] This is a diet that includes eating generous amounts of fruits and vegetables, using fats such as olive and canola oil and consuming little red meat. Alcohol should be eliminated because of its potential harmful effect on the liver.

There are few clinical studies that have evaluated the effects of pharmacological therapy in patients with type 2 diabetes and NAFLD. Metformin, which is thought to improve insulin sensitivity, has been studied as a treatment for NAFLD. Controlled studies that have excluded patients with type 2 diabetes have shown that metformin is an effective treatment.[44,45] However, in the only randomised trial that involved metformin as a treatment option in patients with type 2 diabetes, metformin was found to have no effect on hepatic triglyceride content.[46]

Initiation of metformin together with insulin in patients newly diagnosed with type 2 diabetes was shown to normalise aminotransferase levels and reduce hepatic steatosis.[47] Hepatic triglyceride content decreased by 45% ($p<0.001$) and normalised in 75% of the subjects after three months of treatment. These patients were poorly controlled at the outset (average glycated hemoglobin [HbA_{1c}] 11.2%) and therefore were likely to have been insulin-deficient. The authors proposed that correction of this deficiency with exogenous insulin may have activated peripheral lipogenic pathways that redirected fat deposition to the peripheral adipose tissues instead of the liver.

TZDs have been shown to improve insulin sensitivity and reduce liver fat in patients with type 2 diabetes.[46,48,49] In a double-blind, randomised study of patients with type 2 diabetes, rosiglitazone reduced liver fat content whereas metformin did not.[46] In a six-month randomised trial of patients with biopsy-proven NASH and either impaired glucose tolerance or type 2 diabetes, pioglitazone was shown to improve not only ALT levels and hepatic fat content but also liver histology.[48] Subjects in the pioglitazone group had a significantly greater reduction ($p=0.001$) in necroinflammation than the placebo group. Treatment with pioglitazone was also associated with improvement in markers of systemic inflammation. Levels of TNF-α and transforming growth factor-β (TGF-β) decreased significantly ($p=0.02$ and 0.03, respectively) and levels of adiponectin increased significantly ($p<0.001$) in the pioglitazone group, whereas none of these changed in the placebo group. The improvement in the hepatic fat content occurred despite an average weight gain of 2.5kg in the pioglitazone group. These observations might suggest that pioglitazone's beneficial effects involved a redistribution of fatty acids from the liver (visceral tissue) to subcutaneous adipose tissue and an improvement in adipose tissue function, decreasing hepatic inflammation.

Insulin Requirements in Patients with Type 2 Diabetes and Non-alcoholic Fatty Liver Disease

The association between liver fat and insulin resistance also has relevance from the standpoint of managing hyperglycaemia in patients with type 2 diabetes. First, the degree of fat accumulation in the liver is an important determinant of the amount of insulin required to lower glucose levels in patients with type 2 diabetes.[50,51] The insulin requirement correlates closely with hepatic fat content,[50,51] so a patient who has NAFLD would be expected to need more insulin to achieve glycaemic control than if he or she did not have NAFLD. Second, a decrease in the hepatic fat content by dieting has been shown to decrease insulin resistance.[52] Obese patients with type 2 diabetes who lost 8kg with a diet had a significant ($p=0.0009$) reduction in intrahepatic lipid.[52] This was associated with a reversal of their hepatic insulin resistance and normalisation of their basal glucose production and fasting hyperglycaemia. Therefore, a modest weight loss of <10% in obese patients with a fatty liver could improve their sensitivity to insulin and consequently make it easier to achieve glycaemic control.

Cardiovascular Risk in Patients with Type 2 Diabetes and Non-alcoholic Fatty Liver Disease

Among patients with type 2 diabetes, those who have NAFLD are at higher risk of cardiovascular disease than those who do not.[53] Significantly higher ($p<0.001$) age- and sex-adjusted prevalences of cardiovascular, cerebrovascular and peripheral vascular disease have been reported in patients with type 2 diabetes and NAFLD than those without NAFLD.[54] The Valpolicella Heart Study prospectively followed patients with type 2 diabetes for the development of cardiovascular disease.[14] The prevalence of NAFLD at baseline was associated with a significantly increased risk of cardiovascular disease during follow-up (odds ratio [OR] 1.84; $p<0.001$).[14] In both of these studies, the increased cardiovascular risk was independent of classic risk factors and components of the metabolic syndrome.

The increased cardiovascular risk for patients with type 2 diabetes and NAFLD emphasises the importance of recognisng NAFLD in these

patients. Targeting these patients in particular with aggressive low-density lipoprotein (LDL)-lowering and other cardiovascular risk factor interventions will hopefully prevent the occurrence of any cardiovascular event. Although NAFLD in itself is a potentially serious life-threatening disease, many with type 2 diabetes and NAFLD will experience cardiovascular morbidity and mortality long before the development of clinically significant liver disease. Cardiovascular disease is still the major killer of these patients.

Summary

Given the high prevalence of NAFLD in patients with type 2 diabetes, the possibility of NAFLD should be considered in all of them. This is especially true if they are obese or have abnormal liver enzymes. Furthermore, having type 2 diabetes places these patients at risk of having progression of the liver disease to NASH and cirrhosis. Studies have documented the benefit of weight loss on reducing hepatic steatosis. Although long-term studies have not been performed to prove this, it seems reasonable to expect that early diagnosis of NAFLD and early intervention with weight loss would prevent progression to more serious stages. Therefore, lifestyle changes that promote weight loss should be strenuously emphasised to these patients.

Metformin has been shown to be beneficial in patients with NAFLD who do not have type 2 diabetes, but more studies are needed to determine its effect in patients who have type 2 diabetes. TZDs are promising as pharmacological therapy for NAFLD in patients with type 2 diabetes. Studies are needed to determine their long-term safety and effectiveness. Treatment should also focus on optimising glucose control and minimising cardiovascular risk factors, as patients with type 2 diabetes and NAFLD are particularly at cardiovascular risk. ■

1. Ludwig J, Viggiano TR, McGill DB, Oh BJ, Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease, *Mayo Clin Proc*, 1980;55:434–8.
2. Malnick SDH, Beergabel M, Knobler H, Non-alcoholic fatty liver: a common manifestation of a metabolic disorder, *Q J Med*, 2003; 96:699–709.
3. Bugianesi E, Vanni E, Marchesini G, NASH and the risk of cirrhosis and hepatocellular carcinoma in type 2 diabetes, *Current Diabetes Reports*, 2007;7:175–80.
4. Clark JM, Brancati FL, Diehl AM, The prevalence and etiology of elevated aminotransferase levels in the United States, *Am J Gastroenterol*, 2003;98:960–67.
5. Caldwell SH, Oelsner DH, Iezzoni JC, et al., Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease, *Hepatology*, 1999;29:664–9.
6. Collantes R, Ong JP, Younossi ZM, Nonalcoholic fatty liver disease and the epidemic of obesity, *Cleveland Clin J Med*, 2004;71: 657–64.
7. Browning JD, Szczepniak LS, Dobbins R, et al., Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity, *Hepatology*, 2004;40:1387–95.
8. Targher G, Bertolini L, Padovani R, et al., Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients, *Diabetes Care*, 2007;30:1212–18.
9. Angulo P, Keach JC, Batts KP, Lindor KD, Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis, *Hepatology*, 1999;30:1356–62.
10. Younossi ZM, Gramlich T, Matteoni CA, et al., Nonalcoholic fatty liver disease in patients with type 2 diabetes, *Clin Gastroenterol Hepatol*, 2004;2:262–5.
11. Angulo P, Obesity and nonalcoholic fatty liver disease, *Nutrition Reviews*, 2007;65:S57–63.
12. Fassio E, Alvarez E, Dominguez N, et al., Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies, *Hepatology*, 2004;40:820–26.
13. Jimba S, Nakagami T, Takahashi M, et al., Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults, *Diabet Med*, 2005;22: 1141–5.
14. Targher G, Bertolini L, Poli F, Rodella S, et al., Nonalcoholic fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients, *Diabetes*, 2005;54:3541–6.
15. Silverman JF, O'Brien KF, Long S, et al. Liver pathology in morbidly obese patients with and without diabetes, *Am J Gastroent*, 1990; 85:1349–55.
16. Perlemuter G, Bigorgne A, Cassard-Doulcier A-M, Naveau S, Nonalcoholic fatty liver disease: from pathogenesis to patient care, *Nature Clinical Practice*, 2007;3:458–69.
17. Mofrad P, Clinical and histologic spectrum of nonalcolholic fatty liver disease associated with normal ALT levels, *Hepatology*, 2003;37:1286–92.
18. Kotronen A, Westerbacka J, Bergholm R, et al., Liver fat in the metabolic syndrome, *J Clin Endocrinol Metab*, 2007;92:3490–97.
19. Saadeh S, Younossi ZM, Remer EM, et al., The utility of radiologic imaging in nonalcoholic fatty liver disease, *Gastroenterology*, 2002;123:745–50.
20. Longo R, Pollesello P, Ricci C, et al., Proton mr spectroscopy in quantitative *in vivo* determination of fat content in human liver steatosis, *J Magn Reson Imaging*, 1995;5:281–5.
21. Farrell GC, Larter CZ, Nonalcoholic fatty liver disease: from steatosis to cirrhosis, *Hepatology*, 2006;43:S99–112.
22. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, et al., 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*, 2002;87:3023–8.
23. Marchesini G, Avagnina S, Barantani EG, et al., Aminotransferase and gamma-glutamyltranspeptidase levels in obesity are associated with insulin resistance and the metabolic syndrome, *J Endocrinol Invest*, 2005;28:333–9.
24. Utzschneider KM, Kahn SE, The role of insulin resistance in non-alcoholic fatty liver disease, *J Clin Endocrinol Metab*, 2006;91: 4753–61.
25. Shulman GI, Cellular mechanisms of insulin resistance, *J Clin Invest*, 2000;106:171–6.
26. Marchesini G, Brizi M, Morselli-Labate AM, et al., Association of nonalcoholic fatty liver disease with insulin resistance, *Am J Med*, 1999;107:450–55.
27. Li Z, Clark J, Diehl AM, The liver in obesity and type 2 diabetes mellitus, *Clin Liver Dis*, 2002;6:867–77.
28. Angulo P, Nonalcoholic fatty liver disease, *N Engl J Med*, 2002; 346:1221–31.
29. McCullough AJ, Update on nonalcoholic fatty liver disease, *J Clin Gastroenterol*, 2002;34:255–62.
30. Day CP, James OF, Steatohepatitis: a tale of two 'hits'?, *Gastroenterol*, 1998;114:842–5.
31. Angulo P, Keach JC, Batts KP, Lindor KD, Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis, *Hepatology*, 1999;30:1356–62.
32. Paolisso G, D'Amore A, Volpe C, et al., Evidence for a relationship between oxidative stress and insulin action in non-insulin dependent (type II) diabetic patients, *Metabolism*, 1994;43: 1426–9.
33. Evans JL, Goldfine ID, Maddux BA, Grodsky GM, Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes, *Endocr Rev*, 2002;23:599–622.
34. Tolman KG, Fonseca V, Dalpiaz A, Tan MH, Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease, *Diabetes Care*, 2007;30:734–43.
35. Willner IR, Waters B, Patil SR, et al, Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease, *Am J Gastroenterol*, 2001;96:2957–61.
36. Nomura F, Ohnishi K, Ochiai T, Okuda K, Obesity-related nonalcoholic fatty liver: CT features and follow-up studies after low-calorie diet, *Radiology* 1987;162:845–7.
37. Luyckx FH, Lefebvre PJ, Scheen AJ, Non-alcoholic steatohepatitis: association with obesity and insulin resistance, and influence of weight loss, *Diabetes Metab*, 2000;26:98–106.
38. Suzuki A, Lindor K, St Saver J, et al., Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease, *J Hepatol*, 2005;43:1060–66.
39. Eriksson S, Erikkson KF, Bondesson L, Nonalcoholic steatohepatitis in obesity: a reversible condition, *Acta Med Scand*, 1986;220:83–8.
40. Ueno T, Sugawara H, Sujaku K, et al., Therapeutic effects of restricted diet and exercise in obese patients with fatty liver, *J Hepatol*, 1997;27:103–7.
41. Andersen T, Gluud C, Franzmann MB, Christoffersen P, Hepatic effects of dietary weight loss in morbidly obese subjects, *J Hepatol*, 1991;12:224–9.
42. Luyckx FH, Desaive C, Thiry A, et al., Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty, *Int J Obes Relat Metab Disord*, 1998;22:222–6.
43. Farrell GC, Larter CZ, Nonalcoholic fatty liver disease: from steatosis to cirrhosis, *Hepatology*, 2006;43:S99–112.
44. Uygun A, Kadayifci A, Isik AT, et al., Metformin in the treatment of patients with non-alcoholic steatohepatitis, *Aliment Pharmacol Ther*, 2004;19:537–44.
45. Bugianesi E, Gentilcore E, Manini R, et al., A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease, *Am J Gastroenterol*, 2005;100: 1082–90.
46. Tiikkaineen M, Hakkinen A-M, Korsheninnikova E, et al., Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes, *Diabetes*, 2004;53:2169–76.
47. Lingvay I, Raskin P, Szczepaniak LS, Effect of insulin-metformin combination on hepatic steatosis in patients with type 2 diabetes, *J Diabetes Complications*, 2007;21:137–42.
48. Belfort R, Harrison SA, Brown K, et al., A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis, *N Engl J Med*, 2006;355:2297–307.
49. Juurinen L, Kotronen A, Graner M, Yki-Jarvinen H, Rosiglitazone reduces liver fat and insulin requirements and improves hepatic insulin sensitivity and glycemic control in patients with type 2 diabetes requiring high insulin doses, *J Clin Endocrinol Metab*, 2008;93:118–24.
50. Ryysy L, Hakkinen AM, Goto T, et al., Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients, *Diabetes*, 2000;49:749–58.
51. Juurinen L, Tiikkainen M, Hakkinen AM, Yki-Jarvinen H, Effects of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes, *Am J Physiol Endocrinol Metab*, 2007;292:E829–35.
52. Petersen KF, Dufour S, Befroy D, et al., Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes, *Diabetes*, 2005;54:603–8.
53. Targher G, Bertolini L, Padovani R, et al., Increased prevalence of cardiovascular disease in type 2 diabetic patients with non-alcoholic fatty liver disease, *Diabetic Medicine*, 2006;23:403–9.
54. Targher G, Bertolini L, Rodella S, et al., Nonalcoholic fatty liver disease is independently associated with as increased incidence of cardiovascular evens in type 2 diabetic patients, *Diabetes Care*, 2007;30:2119–21.