

Tetrazoline  
Depot

## Review Article

# Association between non-alcoholic fatty liver disease and metabolic syndrome

Rasheeta Sivapackianathan<sup>1</sup>, Arthur J. Asivatham<sup>2</sup>, Mamun-Al-Mahtab<sup>3</sup>, \*Tahseen A. Chowdhury<sup>1</sup>

<sup>1</sup>Department of Diabetes and Metabolism, The Royal London Hospital, London, UK;

<sup>2</sup>Department of Diabetology, Madurai Medical College, Madurai, India;

<sup>3</sup>Department of Hepatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

## \*Correspondence to

Department of Diabetes and Metabolism, 7<sup>th</sup> Floor, John Harrison House, The Royal London Hospital, Whitechapel, London E1 1BB, UK, Tel: +44 020 8223 8384, Fax: +44 020 8223 8806

E-mail: tahseen.chowdhury@bartsandthelondon.nhs.uk

## Abstract

Metabolic syndrome describes the co-occurrence of central adiposity, dysglycaemia, hypertension, lipid abnormalities and a number of other metabolic changes that increase risk of cardiovascular disease. This multi-system condition has adverse effects on many organs, the liver being one of them. Non-alcoholic fatty liver disease appears to be the hepatic manifestation of metabolic syndrome, and is increasingly recognised as a major contributor to the burden of chronic liver disease world-wide. Metabolic syndrome and non-alcoholic fatty liver disease appear to have a common pathogenesis, arising from insulin resistance, central adiposity and chronic low grade inflammation. Treatment of metabolic syndrome may have a significant impact on progression of non-alcoholic fatty liver disease, and therapeutic options treating the underlying cause of metabolic syndrome (weight loss and insulin sensitising drug therapy) appear to be valid options in treating liver disease to prevent progression to fibrosis and cirrhosis. Recent studies suggest a possible role for vitamin E. Prevention of obesity is extremely important to reduce the risk of this condition leading to a growing cause of liver morbidity in the future.

## Keywords

Non-alcoholic steatohepatitis, non-alcoholic fatty liver disease, metabolic syndrome

## Introduction

Metabolic syndrome (MetS) describes the co-occurrence

of a constellation of metabolic disorders, which increase the risk of developing atherosclerotic vascular disease and Type 2 diabetes (T2D). The syndrome affects around one in five people worldwide, with the prevalence mirroring the rapid rise in obesity [1]. Non-alcoholic fatty liver disease (NAFLD) is common, and increasingly recognised as a major cause of hepatic morbidity in developed and developing countries. Epidemiological evidence suggests a close link between the prevalence of MetS and NAFLD. This brief review discusses the evidence suggesting a potential link between MetS and NAFLD, and the potential pathogenic mechanisms behind this link.

## Definition of metabolic syndrome

In 1947 Jean Vague, observed that upper body obesity predisposed to diabetes, atherosclerosis and gout [2]. Some thirty years later, Haller and colleagues used the term “metabolic syndrome” for the association of diabetes mellitus, obesity and hepatic steatosis when describing the additive effects of risk factors on atherosclerosis [3]. Gerald Reaven subsequently proposed that insulin resistance was an underlying factor linking the metabolic abnormalities associated with his “syndrome X” [4]. MetS has been variously named as metabolic syndrome X, syndrome X, insulin resistance syndrome, Reaven’s syndrome and CHAOS (coronary artery disease, hypertension, adult onset diabetes, obesity, stroke).

There has been some debate over the clinical utility of the metabolic syndrome to delineate increased risk of cardiovascular disease, and whether the label of metabolic syndrome offers any benefit over and above the individual risk. A very recent position of the World Health Organization,

is that the MetS has limited practical utility as a diagnostic or management tool [5], and some authors suggest that the use of term metabolic syndrome may divert focus away from simpler and more precise risk models [6].

Nevertheless, a number of major international organisations have developed definitions for MetS, and in recent years, a single unified definition has been agreed, based on the presence of abdominal obesity and a number of other cardiovascular factors (Table 1) [7].

### Epidemiology of metabolic syndrome

Prevalence of MetS in Europe varies from 12-26% depending on geographical area, urbanisation and ethnic mix [8]. Studies in Asia, suggest the prevalence is 5-20%, with an overall global prevalence of around 16% of the adult population [8,9]. Prevalence in India appears to be highest, at around 26% of the adult urban population [10,11], and prevalence appears to be increasing as obesity rates and urbanisation increase. Data from the USA National Health and Nutritional Examination Survey (NHANES) show an age adjusted prevalence increase by 23.5% in women and 22.2% in men between 1994 and 2000 [12].

Overweight and obesity appears to be increasing in children and adolescents, suggesting that the prevalence of metabolic syndrome is also increasing, and likely to go on increasing for the foreseeable future. A recent study comprising of 105 obese adolescent subjects undergoing laparoscopic obesity surgery, reported a 25% incidence of Non-alcoholic steatohepatitis (NASH), and also confirms that the presence of metabolic syndrome in obese adolescents predicts impaired glucose tolerance and NAFLD [13].

### Risk factors for the development of metabolic syndrome and NAFLD

Non-alcoholic fatty liver disease is common, and may contribute significantly to the burden of chronic liver disease. It is important to note, however, that selection bias may be a factor in the study of MetS in patients with NAFLD and NASH. Liver biopsy is generally only performed in selected patients, and the natural history of the disease may be completely different in subjects from the general population. A recent study of long term follow up of NAFLD patients suggests that overall prognosis of NAFLD is good, and only a minority of patients develop NASH and cirrhosis [14]. Nevertheless, the number of people with NAFLD is large, and hence even a small number of them progressing to NASH is likely to lead to a significant

burden of chronic liver disease (figure 1).

Risk factors for the development of MetS and NAFLD include:

- Increasing age - around 44% of the US population above the age of 50 years have MetS, possibly due to weight gain, reduced physical activity & hormonal effects [12].
- Obesity - increased waist circumference and central adiposity is strongly linked with MetS, with an increase of 1 cm in waist circumference increasing the risk of MetS by around 7.4% [15].
- Physical inactivity - is a potent predictor of cardiovascular mortality and morbidity, probably mediated via central adiposity, reduced high density lipoprotein (HDL) cholesterol levels and hypertension [16].
- Female sex - women are affected by MetS more commonly than men, particularly post menopause [17].
- Hormonal changes – low levels of testosterone and sex hormone binding globulin (SHBG) [18], growth hormone (GH) [19], and high levels of glucocorticoids [20] are all associated with increased risk of MetS.
- Stress - physiological, emotional or psychological stress can be an underlying cause of MetS, possibly due to imbalance of the hypothalamic-pituitary-adrenal (HPA) axis [21].
- Ethnicity - South Asians appear to be the highest risk for development of MetS [10,11,22].
- Polycystic Ovarian Syndrome (PCOS) - Peripheral insulin resistance with a compensatory hyperinsulinaemia is frequently seen in PCOS, and the insulin excess leads to ovarian and adrenal androgen production. PCOS is frequently, although not always, associated with obesity and glucose intolerance [23]. Treatment with metformin can improve insulin sensitivity and lead to ovulatory cycles.

### Common pathogenesis of metabolic syndrome and NAFLD

A number of pathogenic mechanisms underlying MetS and NAFLD have been postulated, all of which are likely to be

interlinked:

- Insulin resistance - fasting hyperinsulinaemia at baseline is related to MetS and subsequent development of T2D. Elevated insulin levels as well as fasting proinsulin levels both are associated with a number of metabolic disorders related to insulin resistance syndrome [24]. Insulin resistance is characteristic in patients with NAFLD and NASH [25]
- Systemic Inflammation - markers of systemic inflammation such as C-reactive protein (CRP), fibrinogen, interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF- $\alpha$ ), are all increased in MetS [26]. Raised levels of high sensitivity CRP is a potent predictor for development non-alcoholic steatohepatitis (NASH) [27], with degree of insulin resistance directly proportional to the degree of liver fibrosis.
- Visceral adiposity - adipocytes are highly metabolically active tissue, with autocrine, paracrine and endocrine functions. Visceral fat surrounds the internal organs and is composed of the mesenteric and omental depots, and higher levels of visceral adiposity are associated with increase in plasma TNF-  $\alpha$ , as well as altered levels of adiponectin, resistin and PAI-1 [28]. TNF-  $\alpha$  triggers production of inflammatory cytokines, leading to inflammation.
- Lipotoxicity – It has been proposed that obesity and hyperleptinemia protect lipid-intolerant nonadipose organs against lipotoxic lipid spillover during sustained caloric surplus [29]. It is suggested that metabolic syndrome may be due to lipotoxicity caused by age-related resistance to antilipotoxic protection by leptin.
- Liver-gut axis - recent evidence in animals suggests that changes in gut microbial flora may predispose to metabolic disorders, with a high-fat diet being associated with higher endotoxaemia and lower caecal Bifidobacterium in mice [30]. It has been hypothesized that this endotoxaemia may contribute to diabetes, obesity and NASH.

Despite NAFLD and NASH being commoner amongst obese subjects, some studies suggest that hepatic steatosis is prevalent amongst lean patients. In an unselected autopsy series of 351 nonalcoholic patients, steatosis was noted in 70% of obese and 35% of lean patients, and steatohepatitis

was found in 18.5% of obese and 2.7% of lean patients. Advanced fibrosis was greater in obese (13.8%) than in lean (6.6%) patients, and the difference was associated with the concomitant increased prevalence of diabetes [31].

### Association between hepatic steatosis and insulin resistance

There is firm epidemiological evidence that NASH is strongly associated with MetS. Most cases of NAFLD occur in patients with obesity (60-95%), T2D (28-55%) and hyperlipidaemia (27-92%) [32], and liver biopsy in patients with T2D shows hepatic steatosis at histological examination in around 50% of cases. Measures of insulin resistance are increased in patients with NAFLD compared to matched controls, and patients with NASH have more MetS compared to age, sex and severity of fibrosis matched patients with hepatitis [32]. Reduced insulin sensitivity is shown in many studies of patients with NAFLD or NASH, including studies with hyperinsulinaemic, euglycaemic clamps, and oral / intravenous glucose tolerance tests [25]. There appears to be a direct correlation with the degree of insulin resistance with severity of liver disease from mild NAFLD to severe NASH and cirrhosis.

Both peripheral and hepatic insulin resistance is present in patients with NAFLD, irrespective of the coexistence of impaired glucose tolerance or obesity. This observation, together with the frequent presence of hypertension, hypertriglyceridemia, central adiposity and family history of diabetes, has led to NAFLD disease being considered as the “hepatic manifestation of the metabolic syndrome”.

Insulin resistance and increased non-esterified fatty acid (NEFA) are associated with increased intra-hepatic production of free fatty acids (FFA) from glucose not taken up by peripheral adipocytes and myocytes [33]. Excess hepatic fatty acids are not oxidised and are converted to diacyl- and triacylglycerols, and are stored in the hepatocyte cytoplasm, leading to steatosis. There are a combination of genetic and acquired factors that are responsible for insulin resistance leading to the development of steatosis, through increased lipolysis and delivery of free fatty acids to the liver.

The development of progressive liver disease in people with NAFLD may be mediated via oxidative stress [34]. Examination of liver biopsies shows increase lipid peroxidation (a marker of oxidative stress) in patients with NASH compared to control subjects, suggesting that the hepatocytes in NASH are been subject to increased oxidative



stress. In animal models of NASH, increased reactive oxygen species (ROS) formation from the mitochondria, leading to increased hepatic supply of free fatty acids (FFA) has been demonstrated, arising from insulin resistance and visceral obesity. Humans with NASH exhibit ultrastructural mitochondrial lesions and have decreased activity of respiratory chain complexes. Other potential sources of oxidative stress that have been suggested to play a role in NASH include the cytochrome P450 enzymes, CYP2E1 and CYP3A4, and an increased in liver iron observed in some patients.

A report of NASH and cryptogenic cirrhosis occurring within kindreds suggested that genetic factors may be important [35]. Genes involved in the development of insulin resistance and free fatty acid formation may play a pertinent role. Similarly, genetic factors that play a role in determining body mass and distribution may be important in the development of NASH, as illustrated by a study demonstrating an association between the gene encoding a microsomal triglyceride transfer factor and raised transaminases in patients with T2D [36], which has been replicated in a Brazilian study of patients with NASH [37]. Other genetic loci that have been associated with NASH are the angiotensin II type 1 receptor [38], apolipoprotein E [39], methylenetetrahydrofolate [40], and the CD14 genes [41].

### Treatment of NAFLD in patients with metabolic syndrome (Table 2)

Currently there is no established therapy for NAFLD or NASH to prevent progression to fibrosis, cirrhosis and liver failure. Lifestyle change and weight loss remain the mainstay of therapy, and are effective in improving liver function tests and histology [42]. In view of the association between insulin resistance and NAFLD/NASH, therapeutic agents improving insulin resistance have been considered for use in patients with such hepatic problems. Supplementation with vitamin E has been examined in patients with NASH in small studies, with some positive results.

### Weight loss

The Diabetes Prevention Programme (DPP) studied 3234 study pre-diabetic obese individuals from 27 clinical centres around US, who were randomised to lifestyle intervention, metformin 850 mg twice a day, and placebo [43]. In the lifestyle intervention group, a mean 5.6 kg weight loss led to a 58% reduction in progression to diabetes. Metformin therapy led to a 31% reduction in progression to diabetes.

Similar results have been found from studies in China and Finland, suggesting that improved lifestyle leading to a modest weight loss improves metabolic indices.

Small randomized trials suggest that weight loss programmes with a hypocaloric diet and exercise can improve fibrosis scores in adults and children with NAFLD [44, 45]. Several studies also suggest an emerging role for bariatric surgery, resulting in both chemical improvement and histologic improvement of NASH [46]. Roux-en-Y gastric bypass has shown improvement in NASH in 100% of patients [47].

### Pharmacological therapy in the treatment of NAFLD/NASH

Drugs used in treatment of T2D may be useful in treatment of NAFLD or NASH.

- Metformin - in open label studies, metformin is effective in improving liver biochemistry, but did not result in improvement of fibrosis in a small study of patients with NASH [48]. A large scale RCT is needed to establish whether metformin has any protective role in patients with NAFLD or NASH.
- Glitazones - Glitazones improve insulin sensitivity by acting as selective agonists of the nuclear peroxisome proliferator-activated receptor (PPAR  $\gamma$ ). Small clinical trials involving glitazones in the management of NASH have shown a beneficial effect on liver biochemistry and histology [49-51]. A more recent study of larger numbers of patients has suggested that pioglitazone does not have a significant modulatory effect on liver fibrosis in patients with NASH, but did lead to reduction in steatosis and inflammation [52].
- Orlistat - Orlistat inhibits pancreatic lipase, thus reducing fat absorption. The drug is effective in reducing weight to a modest degree (5-10% weight loss), and also improves lipid profiles. Use of orlistat in patients with obesity and IGT leads to a reduction in the incidence of newly detected diabetes by 37% compared with placebo. Weight loss is associated with a reduction in hepatic steatosis. Small case series studies of liver biopsies in patients with NASH who lose weight has shown histo-pathological improvements following treatment for 6-12 months with orlistat [53].
- Vitamin E - Recently published data suggests an

*Vit E 800 IU*  
 important role for vitamin E in reducing progression of NASH [52]. Vitamin E is a potent anti-oxidant, and in a placebo controlled randomised trial of 247 subjects with NASH, 43% of patients on vitamin E 800 IU daily had a significant improvement in liver histology, with few adverse effects. Importantly, however, fibrosis scores did not change, and vitamin E.

- Lipid lowering agents – statins have been used in small clinical trials to determine efficacy in NASH. A small Japanese study of 31 patients treated with atorvastatin showed an improvement in liver steatosis and nonalcoholic fatty liver disease activity score [54]. A prospective open labeled study of atorvastatin versus fenofibrate or both in 186 patients with NAFLD showed significant regression of NAFLD in patients treated with the statin [55].

## Conclusion

The link between MetS and NAFLD is well established, but there are many questions unanswered. Whilst pathophysiological mechanisms are beginning to be unravelled, underlying genetic factors need to be established. Why are some people more susceptible to the adverse effects of fat in the liver compared to others? The role of inflammatory substances in the development and progression of fatty liver is clearly important. Further elucidation of this area may enable effective treatments to prevent development or progression of the condition, and may also be useful in identifying at risk subjects.

Much greater study is required into the use of drug therapy in the treatment of established NAFLD and NASH. Large RCTs are urgently needed to address whether older and newer drugs may prevent the progression to end stage liver disease.

Rapid development, urbanisation and consequent change in diet and physical activity levels has led to a rapid growth in obesity and prevalence of MetS. The commensurate rise in NAFLD worldwide is consequent on these changes. Whilst fatty liver disease is less of a burden of total liver disease worldwide, compared to viral hepatitis and alcohol, it is likely to grow in prevalence, especially in the developing world, unless major improvements in the prevention and management of MetS are developed.

## References

1. Ford ES, Giles WH, Dietz WH (2002). Prevalence of metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA* 287: 356-9.
2. Vague J. La différenciation sexuelle, facteur déterminant des formes de l'obésité. *Presse Med* 1947; 30: 339-40.
3. Haller H. Epidemiology and associated risk factors of hyperlipoproteinemia (German). *Z Gesamte Inn Med* 1977; 32: 124-8.
4. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; 37: 1595-607.
5. Simmons RK, Alberti KGMM, Gale EAM, Colagui S, Tuomilehto J, Qiao Q, Ramachandran A, Tajima N, Brajkovich Mirchov I, Ben-Nakhi N, Reaven G, Hama-Sambo B, Mendis R, Roglic G. The metabolic syndrome: useful concept or clinical tool? Report of a WHO Expert Consultation. *Diabetologia* 2010; 50: 600-5.
6. Borch-Johnsen K, Wareham N. The rise and fall of the metabolic syndrome. *Diabetologia* 2010; 53: 597-9.
7. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JJ, Donato KA, Fruchart J-C, James WPT, Loria CM, Smith SC. Harmonizing the Metabolic Syndrome. A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity Circulation 2009; 120: 1640-5.
8. IDF Diabetes Atlas accessed at <http://www.diabetesatlas.org/map> accessed on 19.06.10
9. Viswanathan M, Deepa M. The Metabolic Syndrome in Developing Countries. *Diabetes Voice*. 2006; 5: 15-17.
10. Deepa M, Farooq S, Datta M, et al. Prevalence of metabolic syndrome using WHO, ATPIII and IDF definitions in Asian Indians: The Chennai Urban Rural Epidemiology Study (CURES-34). *Diabetes Metab Res Rev* 2006
11. Mohan V, Shanthirani S, Deepa R, et al. Intra-urban differences in the prevalence of metabolic syndrome in southern India: The Chennai Urban Population Study (CUPS No 4). *Diab Med* 2001; 18:280-7
12. Jacobson TA, Case CC, Roberts S, Buckley A, Murtaugh KM, Sung JC, Gause D, Varas C, Ballantyne CM. Characteristics of US adults with the metabolic syndrome and therapeutic implications. *Diabetes Obes Metab*. 2004; 6: 353-62.
13. Love-Osborne K, Nadeau K, Sheeder J et al. Presence of the Metabolic Syndrome in Obese Adolescents Predicts Impaired Glucose Tolerance and Nonalcoholic Fatty Liver Disease. *Journal of Adolescent Health* 2008; 42: 543-8.
14. Dam-Larsen S, Becker U, Franzmann MB, Larsen K, Christoffersen P, Bendtsen F. Final results of a long-term, clinical follow-up in fatty liver patients. *Scandinavian Journal of Gastroenterology* 2009; 44: 1236-43.

15. Fox C, Massaro J et al. Abdominal Visceral and Subcutaneous Adipose Tissue Compartments. *Circulation*. 2007;116:39-48
16. Weinstein A, Sesso H et al. Relationship of Physical Activity vs Body Mass Index With Type 2 Diabetes in Women. *JAMA*. 2004;292:1188-94.
17. Petri Nahas E, Padoani N, et al. Metabolic syndrome in postmenopausal women: higher prevalence in the Northeastern Region of Brazil than in other Latin American countries and the influence of obesity and socioeconomic factor. *Climacteric* 2009; 12: 431-8.
18. Laaksonen D, Niskanen L, et al. Sex hormones, inflammation and the metabolic syndrome: a population-based study. *European Journal of Endocrinology* 2003; 149: 601-8.
19. Johannsson G, Bengtsson B, et al. Growth hormone and the metabolic syndrome. *J Endocrinol Invest*. 1999; 22 (Suppl 5): 41-6.
20. Syed A and Weaver J. Glucocorticoid Sensitivity: The Hypothalamic-Pituitary-Adrenal-Tissue Axis. *Obesity Research* 2005; 13: 1131-3.
21. Tsigos, C; Chrousos, G. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res*. 2002; 53: 865-71.
22. McKeigue P, Shah B, Marmot M. Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* 1991; 337: 382-6.
23. Talbott E, Guzick D, Sutton-Tyrrell K et al. Evidence for association between polycystic ovary syndrome and premature carotid atherosclerosis in middle-aged women. *Arterioscler Thromb Vasc Biol* 2000 20: 2414-21.
24. Fernández-Real J, Ricart W. Insulin Resistance and Chronic Cardiovascular Inflammatory Syndrome. *Endocrine Reviews* 2003; 24 (3): 278-301
25. Comert B, Mas M, et al. Insulin resistance in non-alcoholic steatohepatitis. *Digestive and Liver disease*. 2001; 33: 353-8.
26. Haffner S. Insulin resistance, inflammation, and the prediabetic state. *Am J Cardiol*. 2003; 92 (4A): 18J-26J.
27. Targher G, Bertolini L, et al. NASH predicts plasma inflammatory biomarkers independently of visceral fat in men. *Obesity* 2008;16 6, 1394-1399
28. Festa A, D'Agostino et al. Relative Contribution of Insulin and Its Precursors to Fibrinogen and PAI-1 in a Large Population With Different States Of Glucose Tolerance. The Insulin Resistance Atherosclerosis Study (IRAS) Arteriosclerosis, Thrombosis, and Vascular Biology. 1999;19:562-8.
29. Unger RH, Scherer PE. Gluttony, sloth and the metabolic syndrome: a roadmap to lipotoxicity. *Trends in Endocrinology & Metabolism* 2010; 21; 345-52.
30. Siebler J, Galle PR, Weber MM. The gut-liver-axis: endotoxemia, inflammation, insulin resistance and NASH. *J Hepatol*. 2008; 48: 1032-4.
31. Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 1990; 12:1106-10.
32. Marchesini, G., Brizi, M., Bianchi, G. et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; 50: 1844-50.
33. Day C. Non-alcoholic steatohepatitis (NASH): where are we now and where are we going? *Gut* 2002; 50: 585-8.
34. Pessayre D, Fromenty B. NASH: a mitochondrial disease. *J Hepatol* 2005; 42:928-40.
35. Struben V, Hespeneide E, Caldwell S et al. Non-alcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. *Am J Med* 2000; 108: 9-13. *Genetics*
36. Bernard S, Personne I, Lapras V et al. Association between microsomal triglyceride transfer protein gene polymorphism and the biological features of liver steatosis in patients with type 2 diabetes. *Diabetologia* 2000; 43: 995-9.
37. Oliveira CP, Stefano JT, Cavaleiro AM, Zanella Fortes MA, Vieira SM, Rodrigues Lima VM, Santos TE, Santos VN, de Azevedo Salgado AL, Parise ER, Ferreira Alves VA, Carrilho FJ, Corrêa-Giannella ML. Association of polymorphisms of glutamate-cystein ligase and microsomal triglyceride transfer protein genes in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol*. 2010; 25: 357-61.
38. Yoneda M, Hotta K, Nozaki Y, Endo H, Uchiyama T, Mawatari H, Iida H, Kato S, Fujita K, Takahashi H, Kirikoshi H, Kobayashi N, Inamori M, Abe Y, Kubota K, Saito S, Maeyama S, Wada K, Nakajima A. Association between angiotensin II type 1 receptor polymorphisms and the occurrence of nonalcoholic fatty liver disease. *Liver Int*. 2009; 29: 1078-85.
39. Sazci A, Akpinar G, Aygun C, Ergul E, Senturk O, Hulagu S. Association of apolipoprotein E polymorphisms in patients with non-alcoholic steatohepatitis. *Dig Dis Sci*. 2008; 53: 3218-24.
40. Sazci A, Ergul E, Aygun C, Akpinar G, Senturk O, Hulagu S. Methylenetetrahydrofolate reductase gene polymorphisms in patients with nonalcoholic steatohepatitis (NASH). *Cell Biochem Funct*. 2008; 26: 291-6.
41. Brun P, Castagliuolo I, Floreani AR, Buda A, Blasone L, Palù G, Martinez D. Increased risk of NASH in patients carrying the C(-159)T polymorphism in the CD14 gene promoter region. *Gut*. 2006; 55: 1212.
42. Park H, Sim S, Park J. Effect of weight reduction on metabolic syndrome in Korean obese patients. *J Korean Med Sci*. 2004; 19: 202-8.
43. Bo-abbas Y, Brousseau V, Louri D et al. Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin – Diabetes Prevention Program Research Group. *N Eng J Med*. 2002; 346:393-403.
44. Vilar Gomez E, Rodriguez De Miranda A, Gra Oramas B, Arus Soler E, Llanio Navarro R, Calzadilla Bertot L, Yasells Garcia A, Del Rosario Abreu Vazquez M. Clinical trial: a nutritional supplement Viucid, in combination with diet and exercise, in patients with



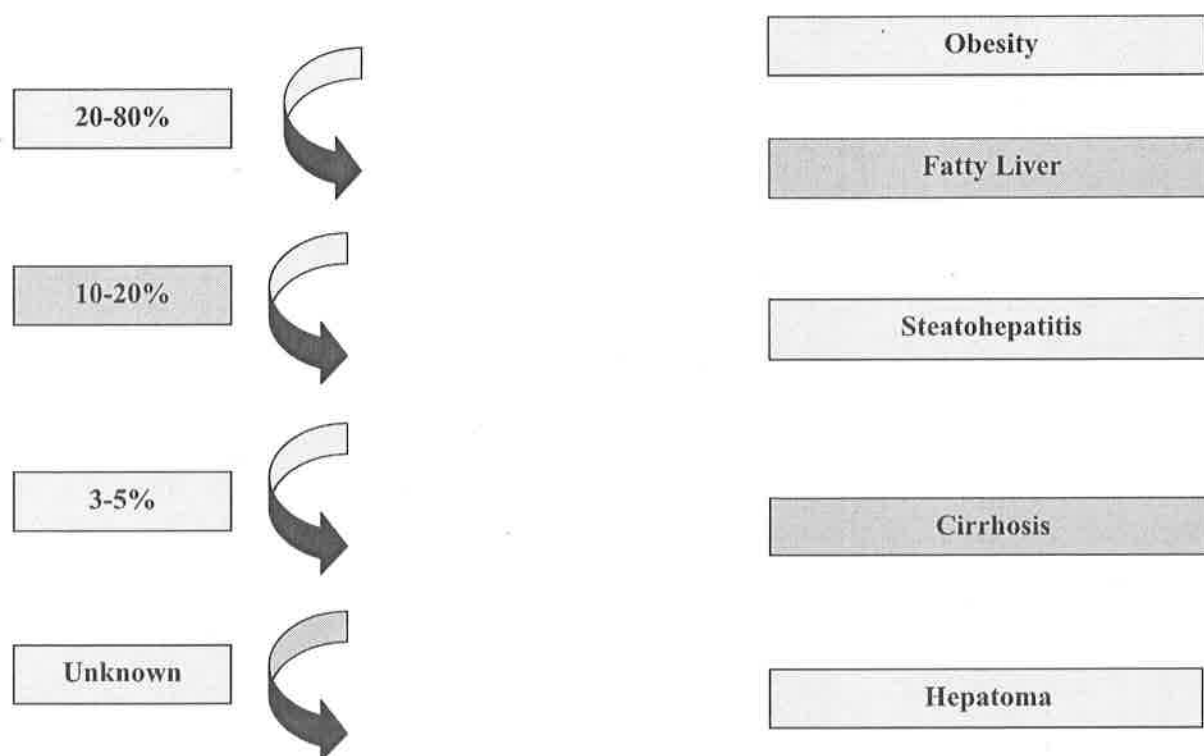
- nonalcoholic fatty liver disease. *Aliment Pharmacol Ther.* 2009; 30: 999-1009
45. Nobili V, Manco M, Devito R, Di Ciommo V, Comparcola D, Sartorelli MR, Picmonte F, Marcellini M, Angulo P. Lifestyle intervention and antioxidant therapy in children with nonalcoholic fatty liver disease: a randomized, controlled trial. *Hepatology.* 2008; 48: 119-28.
  46. Furuya CK Jr, de Oliveira CP, de Mello ES, et al. Effects of bariatric surgery on nonalcoholic fatty liver disease: preliminary findings after 2 years. *J Gastroenterol Hepatol.* 2007; 22: 510-4.
  47. Liu, Lazenby, Clements, et al. Resolution of NASH after Gastric Bypass Surgery. *Obesity Surgery* 2007; 17: 486-92.
  48. Loomba R, Lutchman G, Kleiner DE, Ricks M, Feld JJ, Borg BB, Modi A, Nagabhyru P, Sumner AE, Liang TJ, Hoofnagle JH. Clinical trial: Pilot study of metformin for the treatment of nonalcoholic steatohepatitis. *Aliment Pharmacol Ther.* 2008 Oct 9 [Epub ahead of print]
  49. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, et al. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology* 2003; 38: 1008-17
  50. Promrat K, Lutchman G, Uwaifo GI, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 2004; 39: 188-96
  51. Belfort R, Harrison SA, Bro of pioglitazone in subjects *Engl J Med.* 2006; 355: 229
  52. Sanyal A, Chalasani N, F E or placebo for nonalco 10.1056/NEJMoa0907929.
  53. Harrison S, Fincke C, Helinski D, et al. A Pilot Study of Treatment in Obese, Non-Alcoholic Steatohepatitis Patients. *Alimentary Pharmacology & Therapeutics.* 2004; 20: 623-8.
  54. Hyogo H, Tazuma S, Arihiro K, Iwamoto K, Nabeshima Y, Inoue M, Ishitobi T, Nonaka M, Chayama K. Efficacy of atorvastatin for the treatment of nonalcoholic steatohepatitis with dyslipidemia. *Metabolism.* 2008; 57: 1711-8.
  55. Athyros VG, Mikhailidis DP, Didangelos TP, Giouleme OI, Liberopoulos EN, Karagiannis A, Kakafika AI, Tziomalos K, Burroughs AK, Elisaf MS. Effect of multifactorial treatment on non-alcoholic fatty liver disease in metabolic syndrome: a randomised study. *Curr Med Res Opin.* 2006; 22: 873-83.
  56. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology.* 2007; 45: 846-54.

**Table 1. Definition of metabolic syndrome**

<b>Glucose</b>	5.6mmol/L (>100mg/dL) or previously diagnosed Type 2 diabetes
<b>Blood Pressure</b>	>130/85 mmHg
<b>Triglycerides</b>	>1.7mmol/l (150mg/dL) or specific treatment for this
<b>High Density Lipoprotein (HDL) Cholesterol</b>	Men: 1.03mmol/L (<40mg/dL) Women: 1.29mmol/L (<50mg/dL)
<b>Obesity</b>	BMI>30kg/m <sup>2</sup> or Abdominal Waist Circumference – population specific: Europid men: >102cm (40") Europid women >88cm (34.5") South Asian men: >90cm (35") South Asian women: >80cm (31.5")

**Table 2. Tests used in diagnosis of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis**

Test	Abnormality
Liver Function Test	Elevated aspartate aminotransferase [AST] and alanine aminotransferase [ALT]. AST:ALT ratio > 1 may indicate more severe disease
NAFLD Fibrosis Score	Age, hyperglycemia, BMI, platelet count, albumin and AST/ALT ratio has been suggested as predictive of degree of fibrosis [56]
Ultrasound	Liver may be hyperechogenic or bright. Steatosis is detected only when substantial (30% or more) fatty change is present
Ultrasound Elastography	Non invasive scan, as yet not fully validated for routine diagnostic and follow up use.
Liver biopsy	Gold standard. Histologic findings of NASH include: <ul style="list-style-type: none"> <li>• Steatosis - usually macrovesicular</li> <li>• Inflammatory infiltrates</li> <li>• Ballooning degeneration</li> <li>• Fibrosis.</li> <li>• First 3 findings are used to calculate the NAFLD activity score (0-8).</li> </ul>

**Figure 1. Progression of non-alcoholic fatty liver disease**



## Non-alcoholic fatty liver disease and the metabolic syndrome: Effects of weight loss and a review of popular diets. Are low carbohydrate diets the answer?

Harjot K Gill, George Y Wu

Harjot K Gill, George Y Wu, Division of Gastroenterology-Hepatology, Department of Medicine, University of Connecticut Health Center, Farmington, Connecticut, United States  
Correspondence to: George Y Wu, MD, PhD, Division of Gastroenterology-Hepatology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, Connecticut, 06030-1845, United States, wu@nso.uchc.edu  
Telephone: +860-679-3158 Fax: +860-679-3159  
Received: 2005-05-09 Accepted: 2005-06-24

### Abstract

Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of fat-induced liver injury, ranging from relatively benign steatosis to cirrhosis and liver failure. The presence of obesity and insulin resistance is strongly associated with non-alcoholic fatty liver and confers on it a greater risk of histologically advanced disease. There is a growing concern in the medical profession as the prevalence of this disease continues to rise in parallel with the rise in obesity and the metabolic syndrome. Treatment options are limited and dietary weight loss is often advised. Low fat diets are difficult to adhere to and recent studies have shown the potential of low carbohydrate diets for weight loss and improving insulin resistance. Thus far, no study has evaluated the effect of low carbohydrate diets on NAFLD. Future studies will be required to address this question and others with regards to the nutritional adequacy and long-term side effects of these diets.

© 2006 The WJG Press. All rights reserved.

**Key words:** Non-alcoholic fatty liver disease; Obesity; Metabolic syndrome; Diet management

Gill HK, Wu GY. Non-alcoholic fatty liver disease and the metabolic syndrome: Effects of weight loss and a review of popular diets. Are low carbohydrate diets the answer? *World J Gastroenterol* 2006; 12(3): 345-353

<http://www.wjgnet.com/1007-9327/12/345.asp>

### INTRODUCTION

In the 1970s, patients undergoing jejunioileal bypass surgery

for morbid obesity were noted to develop steatohepatitis and even liver failure following rapid weight loss<sup>[1]</sup>. Their liver histology was similar to that seen in alcoholics, with macrovesicular steatosis, Mallory hyaline, focal hepatocyte necrosis, mixed lobular inflammation and fibrosis<sup>[2]</sup>. Similar findings were later described in obese patients without significant alcohol consumption. In 1980, Ludwig *et al*<sup>[3]</sup> coined the term 'non-alcoholic steatohepatitis' (NASH) to describe these findings. Since then, interest in the disease has grown exponentially in keeping with its rising prevalence. NASH was initially thought to be a benign condition largely limited to middle-aged obese women with diabetes. However, recent studies have shown it to be a far more complex disease that is found in men, women and even children. The spectrum of disease ranges from pure steatosis alone to NASH with hepatic fibrosis, cirrhosis, hepatocellular carcinoma, liver failure and even death<sup>[4,6]</sup>. Estimates suggest that 20-30% of adults in Western countries may have NAFLD and about 10% of these individuals meet criteria for diagnosis of NASH<sup>[7]</sup>. NAFLD is now recognized as the most common cause of chronically elevated liver transaminases<sup>[8,9]</sup> and may be the most common liver disorder<sup>[10]</sup>. Obesity is the single most common condition found in association with NAFLD. Other features of the metabolic syndrome, such as hyperinsulinemia, hypertriglyceridemia and hypertension, also play a significant pathophysiologic role in its development.

In general, NAFLD in the absence of NASH is an indolent disease with a benign course. However, as noted, end stage liver disease may occur as a consequence of NASH. The seriousness of this condition is demonstrated by the fact that approximately 50% of patients develop fibrosis, 15% develop cirrhosis and 3% may advance to liver failure requiring transplantation<sup>[11]</sup>. NASH is now being recognized as the underlying cause of most cases of cryptogenic cirrhosis<sup>[12,13]</sup>. The natural history of NAFLD is poorly understood, and it is not known why some patients progress to cirrhosis, while others do not. However, obesity and insulin resistance have been shown to be associated with more histological advanced disease.

The aim of this article is to review the role of the metabolic syndrome, especially insulin resistance and obesity in the development of NAFLD, to discuss the effect of weight loss on NAFLD and, finally, to evaluate popular diets and compare them with regard to their effects on the metabolic syndrome and NAFLD.

## NASH AND METABOLIC SYNDROME

Fatty liver is commonly associated with obesity and insulin resistance. The increasing incidence of NAFLD closely parallels these conditions. There is abundant data showing a relationship between obesity and NAFLD. Wanless *et al*<sup>[14]</sup> in an autopsy study of 351 patients found that 70% of obese patients had liver steatosis, and the degree of steatosis was proportional to the degree of obesity. The authors also found steatohepatitis in 18.5% and severe fibrosis in 13.8% of markedly obese patients, compared to steatohepatitis in 2.7% and severe fibrosis in 6.6% of lean people. A prospective study performed by Klain *et al*<sup>[15]</sup> evaluated liver biopsies from 100 consecutive morbidly obese patients undergoing Roux-en-Y gastric bypass. Histological abnormalities were found in 98% of biopsies, and ranged from mild fatty infiltration through inflammatory change to fibrosis and cirrhosis<sup>[15]</sup>. Data from 90 patients with NASH demonstrated insulin resistance in 85% of them<sup>[16]</sup>. An Italian study evaluated the risk factors associated with hepatic steatosis. A total of 257 participants were assigned to one of four categories: Controls, teetotalers with normal body mass index (BMI); obese teetotalers; heavy drinkers (> 60 g of alcohol per day) with normal BMI; and heavy drinkers with obesity. The prevalence of steatosis on ultrasound increased from 16% in controls to 46% in heavy drinkers, 76% in obese individuals and 95% in patients with both obesity and heavy alcohol intake. Compared with controls, steatosis was more common by 2.8-fold in heavy drinkers, 4.6-fold in obese persons and 5.8 fold in obese heavy drinkers. In heavy drinkers, obesity increased the risk of steatosis 2.0-fold, while heavy drinking was associated with only a 1.0-fold increased risk in obese subjects<sup>[17]</sup>. The authors concluded that steatosis was more strongly associated with obesity than with heavy drinking.

Evidence of an etiologic association between NAFLD and metabolic syndrome (hyperglycemia, central obesity, hypertension, hypertriglyceridemia and low HDL-cholesterol) has been shown in both obese and non-obese patients<sup>[18]</sup>. Studies also have shown that patients with NASH are more insulin resistant than patients with fatty liver alone<sup>[19]</sup>. Given the wealth of data supporting it, many researchers now consider NAFLD to be a hepatic manifestation of the metabolic syndrome, instead of a primary liver disease<sup>[20,21]</sup>. Chitturi *et al*<sup>[22]</sup> tested the hypothesis that insulin resistance is an essential requirement for the development of NASH, and that a high association between insulin resistance and liver disease is relatively specific for NASH. Sixty-six patients with NASH were studied. Insulin resistance was found in virtually all patients (98%) and was seen in both lean and overweight patients. A subset of 36 patients with less severe NASH were compared to 36 age- and sex-matched patients with chronic hepatitis C. The prevalence of insulin resistance was significantly higher in those with NASH than in comparable cases of HCV (75% *vs* 8.3%)<sup>[22]</sup>. Marchesini *et al*<sup>[19]</sup> studied liver biopsies in patients with NAFLD. Based on histology, these were classified as having NASH *vs* pure fatty liver. The investigators found that 88% of patients with NASH had metabolic syndrome

compared with 53% in patients with pure fatty liver<sup>[19]</sup>. Marceau *et al*<sup>[23]</sup> investigated the relationship between liver pathology and the metabolic syndrome. Five hundred fifty one severely obese patients undergoing anti-obesity surgery were studied. Steatosis was found in 86%, fibrosis in 74%, steatohepatitis in 24% and unexpected cirrhosis in 2%. With each addition of the components of metabolic syndrome, the risk of steatosis increased exponentially from 1- to 99-fold<sup>[23]</sup>. In a series of 505 severely obese patients evaluated before gastropasty, prevalence of steatosis was significantly higher in patients with impaired glucose tolerance or type II diabetes as compared with non-diabetics. The severity of steatosis was positively correlated with BMI, fasting plasma glucose, insulin and triglyceride concentrations, as well as serum ALT, AST and GGT levels<sup>[24]</sup>.

Issues regarding the nature of hyperinsulinemia in NASH have been raised. It has been questioned as to whether hyperinsulinemia and insulin resistance occur as part of the metabolic syndrome or whether liver damage itself leads to chronic hyperinsulinemia and insulin resistance from impaired insulin degradation, as is seen in cirrhosis. Chitturi *et al*<sup>[22]</sup> compared the patients with NASH and mild or absent fibrosis with age- and sex-matched patients with HCV, and found that the patients with NASH showed more attributes of insulin resistance than the controls. They had much higher levels of insulin resistance, serum insulin and C-peptide levels. However, the serum C-peptide/insulin ratio was similar in both groups<sup>[22]</sup>. Pagano *et al*<sup>[25]</sup> addressed the same question, comparing 19 patients with histologically mild NASH, who had functionally competent livers with 19 normal subjects. Patients with NASH showed marked hyperinsulinemia and insulin resistance as compared with controls, however, the hepatic insulin extraction was similar in both groups<sup>[25]</sup>. These two studies showed that insulin hypersecretion, and not just impaired insulin degradation, was the basis for hyperinsulinemia in NASH.

The overall incidence of NASH in the severely obese is reported to range from 25-36.4%<sup>[26,28]</sup>. The prevalence of obesity in the Western world has shown a large increase in the last 20 years. The data of the National Health and Nutrition Examination Survey (NHANES II, 1976-1980) showed a prevalence of 14.5%. By NHANES III (1988-1994), this number had increased to 22.5%, and the data of NHANES 1999-2000 showed a prevalence of 30.5%<sup>[29]</sup>. Significantly, this number could reach 40% by the year 2025<sup>[30]</sup>. A similar increase in the number of patients with type 2 diabetes is expected. By some estimates, 29 million people or 7.2% of the population will have type 2 diabetes by the year 2050<sup>[31]</sup>. Of grave concern is the increasing incidence of obesity in children and adolescents. Given these statistics, the incidence of NASH will rise significantly in the coming years and so will hepatic complications arising from it.

Factors responsible for the development of NAFLD in obese patients are not clear, and the exact mechanism of its progression to fibrosis and cirrhosis has yet to be elucidated. However, our understanding of disease pathogenesis has advanced significantly. Insulin resistance is thought to be a primary pathophysiologic mechanism

in development of fatty liver. Current understanding of the pathogenesis is as follows: Insulin resistance and visceral obesity lead to a hepatic influx of free fatty acids, resulting in increased triglyceride synthesis and decreased triglyceride export. This leads to hepatic steatosis. At this stage, patients have the relatively benign condition of NAFLD. Some of these patients will go on to steatohepatitis. It is unclear why only a small fraction will advance to NASH and what is the exact impetus for this advance. One proposal is that these lipid-laden hepatocytes are susceptible to a "second-hit"<sup>[32]</sup>. The exact mechanism of this second-hit is unknown. In NASH, as in alcoholic hepatitis, oxidative stress and lipid peroxidation have emerged as the most likely candidates. This "hit" occurs via increased mitochondrial beta-oxidation of the free fatty acids, production of reactive oxygen species and depletion of antioxidants glutathione and vitamin E. This depletion of anti-oxidants hampers reactive oxygen species inactivation, and increases the deleterious effects on the mitochondria. Oxidative stress also results in abnormal cytokine production, especially TNF- $\alpha$  through up-regulation of nuclear translocation of transcription factor nuclear factor  $\kappa$ B. This combination of lipid peroxidation and cytokine production results in hepatocyte death.

Another proposed mechanism of development of NASH includes a primary mitochondrial abnormality, as proposed by Sanyal *et al.*<sup>[33]</sup>. This defect, otherwise clinically silent, leads to increased mitochondrial beta oxidation and production of reactive oxygen species in the presence of insulin resistance.

Yang *et al.*<sup>[34]</sup> have demonstrated that obesity itself may cause progression to steatohepatitis by causing Kupffer cell dysfunction and sensitizing the hepatocytes to endotoxin, suggesting that the progression of liver disease may depend on the extent of fatty infiltration<sup>[34]</sup>.

Iron, a strong oxidative agent, has also been proposed as a factor causing the second-hit. Elevated serum ferritin and insulin resistance on those levels have been noted in patients with NASH, as well as increased prevalence of C282Y and H63D mutations in the HFE gene<sup>[35,36]</sup>. However, evidence that hepatic insulin resistance plays a significant role in fibrosis was found in only one study, and recent studies suggested that increased ferritin levels were likely markers of severe histologic damage and not iron overload<sup>[37]</sup>. Leptin production by activated hepatic stellate cells has also been considered an important factor in the progression of fatty liver disease and development of fibrosis<sup>[38]</sup>. Supporting evidence is furnished by genetically leptin-deficient ob/ob mice, which do not develop fibrosis even when fed a methionine-choline-deficient diet.

## OBESITY AND INSULIN RESISTANCE AS PREDICTORS OF FIBROSIS

The natural history of NAFLD is not well known, but it is known that prognosis varies according to histologic type. Matteoni *et al.*<sup>[39]</sup> conducted a retrospective study to compare clinical characteristics and outcomes of patients with different types of NAFLD. Patients were separated into four histologic types: Simple fatty liver; steatohepatitis;

steatonecrosis; and steatonecrosis plus either Mallory hyaline or fibrosis. Cirrhosis and liver-related death were seen almost exclusively in patients with steatonecrosis with or without Mallory hyaline or fibrosis<sup>[39]</sup>. The study also confirmed that the prognosis of simple steatosis is favorable.

A number of risk factors for more histologically advanced disease have been identified. These include central weight distribution and metabolic syndrome. Dixon *et al.*<sup>[40]</sup> studied 105 severely obese individuals undergoing bariatric surgery, and showed that hyperinsulinemia and increased insulin resistance were associated with adverse histologic findings. The study found that C-peptide was the best predictor of advanced fibrosis (stage 3-4) and that patients with advanced fibrosis had significantly higher C-peptide levels. The insulin resistance index and systemic hypertension were independently associated with advanced NAFLD. Insulin resistance was found to be the best predictor of zone 3-centric steatosis, inflammation and fibrosis. Interestingly, this study found that moderate alcohol consumption reduced the risk of NAFLD by decreasing insulin resistance. Angulo *et al.*<sup>[41]</sup> studied liver biopsies from 144 patients with NASH. In multivariate analysis, older age (>45 years), obesity, diabetes mellitus and an AST/ALT ratio >1 were significant predictors of severe fibrosis (bridging/cirrhosis). The investigators concluded that this subgroup would benefit most from liver biopsy and investigational therapies<sup>[41]</sup>. Researchers in France investigated 93 obese patients, and found that age  $\geq 50$  years, BMI >28 kg/m<sup>2</sup>, triglycerides >1.7 mmol/L and ALT >2 times normal value were independently associated with septal fibrosis<sup>[42]</sup>. A univariate analysis showed that diabetes and impaired glucose tolerance were significantly associated with fibrosis. Another recent study evaluating steatosis in chronic hepatitis C found that an increased BMI had a role in pathogenesis of steatosis in chronic hepatitis C, and that this may contribute to fibrosis<sup>[43]</sup>. Studies by both Marceau *et al.*<sup>[23]</sup> and Willner *et al.*<sup>[16]</sup> showed that patients with cirrhosis were more obese than those without cirrhosis. In addition, Marceau *et al.*<sup>[23]</sup> found the presence of diabetes to be the strongest predictor of cirrhosis. Finally, a recent investigation of NAFLD and the metabolic syndrome showed that the presence of metabolic syndrome carried a high risk for NASH among NAFLD patients, and was also associated with a high risk of severe fibrosis<sup>[19]</sup>.

Thus, features of the metabolic syndrome like obesity, insulin resistance and hypertriglyceridemia are not only predisposing factors for NASH, but are also risk factors for more severe fibrosis and advanced disease.

## TREATMENT OPTIONS

In light of the increasing incidence of NAFLD-associated comorbid conditions and NAFLD itself, as well as increased awareness of adverse outcomes associated with steatohepatitis, a number of treatment options are being explored. These combine specific therapies for NAFLD as well as the management of comorbid conditions. Therapies that have been evaluated include lifestyle changes such as

diet and exercise, antioxidants like vitamin E and betaine, cytoprotective agents such as ursodeoxycholic acid, lipid-lowering agents, anti-diabetics, weight-loss agents like orlistat and iron reduction therapy, i.e. phlebotomy. The management of associated conditions, such as diabetes, obesity and hyperlipidemia, is especially important, given their association with more advanced liver disease. This may be achieved by optimizing medical treatment of these conditions, as well as through weight loss strategies.

## EFFECT OF WEIGHT LOSS ON NAFLD

Because obesity is the most commonly associated condition with NAFLD, weight loss has traditionally been the most commonly suggested intervention. Patients are encouraged to lose weight through exercise and dietary fat restriction. Exercise is of great value as it reduces weight by preferentially decreasing visceral obesity while preventing the loss of muscle mass. It also enhances muscle insulin sensitivity even in the absence of weight loss. A number of studies suggest that NAFLD may improve after weight loss. Improvement in liver biochemistry and ultrasonographic appearance is a consistent finding with moderate weight reduction. However, serum aminotransferases are unreliable markers for follow-up, and do not provide accurate data on prognosis. Worsening of fibrosis can occur even as the levels of transaminases decline<sup>[44]</sup>. A few studies have evaluated and shown histologic improvement.

The effects of weight reduction on hepatic tests and physical findings were studied in a retrospective review of thirty-nine obese patients without primary liver disease. A weight loss of >10% corrected abnormal liver tests, decreased hepatosplenomegaly and resolved some stigmata of liver disease<sup>[45]</sup>. Early case series showed that improved liver chemistry and histology was evident with even a modest reduction in weight, as considerable extra weight persisted in the subjects under evaluation<sup>[46,47]</sup>.

Drenick *et al*<sup>[48]</sup> studied liver biopsies of 41 obese patients undergoing massive weight reduction (>100 lb). Biopsies were obtained at various stages before, during and after weight loss. Based on the method of weight loss, patients were divided into three groups, including prolonged fasting, low-calorie dieting and intestinal bypass surgery. In the non-surgical groups, a transient increase in hepatocellular degeneration and focal necrosis was noted along with progressive diminution of fatty infiltration during weight loss. However, late biopsies revealed normal histology. In patients who underwent intestinal bypass, biopsies variously revealed massive fatty changes, cholestasis, polynuclear inflammatory infiltrates, diffuse fibrosis, bile-duct proliferation and fatal hepatic necrosis<sup>[48]</sup>. These findings relating to jejunoileal bypass have been demonstrated in several studies<sup>[49-51]</sup>. This surgical procedure has been abandoned in favor of safer weight-loss surgery.

In a study of twenty-five obese Japanese subjects<sup>[52]</sup>, fifteen underwent a program of restricted diet and exercise for a period of three months, while ten did not. Patients in the intervention group showed a significant decrease in BMI, aminotransferases, total protein, cholinesterase, total

cholesterol and fasting plasma glucose levels. In addition, steatosis was significantly improved on liver biopsy in these patients. The ten patients in the control group did not show any change in biochemical parameters or liver histology.

Regular body weight and biological measurements were obtained from 505 severely obese patients before and after undergoing gastroplasty<sup>[53]</sup>. There was a high prevalence of biologic abnormalities associated with the metabolic syndrome at baseline. After a mean follow-up of  $21 \pm 14$  mo post-surgery, significant reductions in biological markers of metabolic syndrome, such as blood glucose, insulin, and triglycerides, were noted. Also, total cholesterol, uric acid and fibrinogen and ALT levels were reduced, along with an increase in HDL cholesterol levels. Data on children with NASH, who underwent weight reduction, also showed improvement in biochemical and ultrasonographic findings<sup>[54,55]</sup>.

The effect of weight loss on NAFLD was studied in 36 obese patients. Paired liver biopsies were obtained, the first at the time of laparoscopic adjustable gastric band placement and the second after weight reduction. Initial biopsies showed steatosis alone in 12 patients and NASH in 23 patients. Initial fibrosis score of 2 or more was noted in 18 patients. Follow-up biopsies were obtained at  $25.6 \pm 10$  mo after surgery. Weight loss resulted in a significant improvement in liver histology, with repeat biopsies showing NASH in only 4 patients and a fibrosis score of 2 or more in only 3 patients. Greater improvement was seen in patients who had been diagnosed with metabolic syndrome prior to surgery<sup>[56]</sup>.

Knobler *et al*<sup>[57]</sup> suggested that NAFLD is not only associated with biochemical abnormalities of the metabolic syndrome, but it responds to their amelioration. Forty-eight patients with chronically elevated liver enzymes with clinical, ultrasound, and histologic findings consistent with fatty liver were evaluated. Most of the patients were overweight or obese (64%), 44% had diabetes mellitus, 29% had impaired glucose tolerance, and 17% were hyperinsulinemic. Dietary intervention was the primary mode of weight loss. This was supplemented by oral hypoglycemic or lipid-lowering drugs as needed. The results showed moderate weight loss (3.7 kg), improvement in fasting plasma glucose and lipid levels. An improvement in liver enzymes was noted in 96% patients with normalization in 50% patients.

In a recent pilot study, ten obese patients with NASH were treated with orlistat in addition to diet for weight reduction over a period of 6 mo<sup>[58]</sup>. BMI, liver enzymes, hemoglobin A1c, fasting lipids, glucose and liver histology were assessed at baseline and at completion of the study. Mean weight loss was 22.7 lb. There was a significant decrease in the BMI, and levels of hemoglobin A1c, ALT, AST. Steatosis improved in six patients and fibrosis in three patients. Hickman *et al*<sup>[59]</sup> found that modest weight loss through exercise and dietary intervention resulted in sustained improvements not only of ALT and fasting insulin levels, but also of health-related quality of life.

The above data demonstrates that NAFLD and NASH improve significantly with weight reduction. However, this must be done in a controlled manner over a period



of time, as rapid weight loss may lead to exacerbation of liver disease. This has been shown following drastic weight reduction through diet as well as through bariatric surgery. Forty-one patients who had weight loss on a very-low-calorie diet showed improvement in fatty change on liver biopsy. However, 24% developed portal inflammation or fibrosis. These were patients who underwent a very rapid weight reduction ( $>1.6$  kg per week)<sup>[60]</sup>. Liver biopsies obtained from patients undergoing gastroplasty for morbid obesity were compared before surgery and after weight loss (mean  $32 \pm 19$  kg). There was a significant decrease in the prevalence of steatosis (38% of patients after weight loss *vs* 83% before). However, an increase in the prevalence of hepatitis was observed after significant weight reduction (26% of biopsies after weight loss *versus* 14% before)<sup>[61]</sup>. The pathogenesis appears to be massive mobilization of fatty acids from visceral stores, which reach the liver via the portal vein and may be toxic to the liver. Therefore, initial target weight reduction should be 10% of baseline weight and should not exceed 1.6 kg/wk.

## POPULAR DIETS

The effects of many popular diets on fatty liver are not known. However, metabolic improvements related to dietary weight reduction may favorably influence NASH. If dietary intervention can positively affect insulin resistance and other features of the metabolic syndrome, it would be important to know which particular diet is most beneficial. Conventional diets usually fall into two main categories. Those that alter the macronutrient composition of the diet and those that limit overall energy intake.

### Alterations in macronutrient composition

Diets that promote weight loss by emphasizing their macronutrient make-up, as opposed to caloric intake, include low-fat and low-carbohydrate diets. Low-fat diets are traditionally the most recommended by medical professionals. Over time, these have been shown to be safe, cardio-protective and effective in weight loss. Adherence, however, has been a problem. Energy density of food is an important consideration. This refers to the energy (calories) in a given weight of food. Weight loss from a low-fat diet may be due to the low energy density of the diet. Both carbohydrates and proteins have an energy density of 4 kcal/kg, compared to 9 kcal/kg for fats. Researchers for the National Weight Control Registry found that members who maintained successful weight loss, consumed less energy and a lower percentage of energy from fat when compared to the general population<sup>[62]</sup>. The low-fat (30%) diet is advocated by the National Cholesterol Education Program and by the American Heart Association.

Low carbohydrate diets have been popular periodically over the last several decades, and are currently undergoing a resurgence. An NPD survey on diet trends in the United States showed that in early 2004 about 9% of the population was on a low carbohydrate diet. These diets limit the composition and/or amount ( $<100$  g/d) of carbohydrates, with an increase in dietary protein and

fat. They are marketed as low carbohydrate, high protein diets, although they could also be called low carbohydrate, high fat diets. A diet high in carbohydrates results in an increase in blood glucose, insulin and triglycerides, all of which are risk factors for the development of NAFLD. Carbohydrate restriction leads to ketosis resulting not only in weight loss, but also a decrease in blood glucose, insulin and triglyceride levels. Studies have shown these diets to be effective in short-term weight loss<sup>[63,64]</sup>. Early weight loss is a result of diuresis associated with ketone and urea nitrogen excretion<sup>[65]</sup>. However, over time, weight loss is a result of loss of body fat. Proponents believe that these diets have a high satiety level, which make them easier to adhere to. This is very important, as dietary adherence is one of the main challenges faced by dieters. Questions with regard to their nutritional adequacy and long-term effects have been raised. In the short-term, these diets have been found to be safe.

Popular low carbohydrate diets include the Atkins, South Beach and the Zone diets. Originally published by Dr. Robert Atkins in 1972 (Dr. Atkins diet revolution), the Atkins diet is the most popular low carbohydrate diet in the United States. Weight reduction is achieved in four phases. The first phase is an induction diet which limits carbohydrates to 20 g/d, but allows unlimited amounts of fat. In phase two, there is ongoing weight loss with easing of the carbohydrate restriction. Through phases three and four, pre-maintenance and maintenance, individuals determine the amount of carbohydrate they can consume while maintaining their weight loss<sup>[66]</sup>. The South Beach diet consists of 3 phases, gradually increasing in proportion of carbohydrates and emphasizes good carbohydrates and fats. The Zone diet recommends a low carbohydrate, high protein diet, with macronutrient intake in the 40:30:30 ratio, i.e. 40% calories from carbohydrates, 30% from protein and 30% from fat.

### Reduction in energy intake

Studies have found that weight loss on a caloric-restricted diet is due to decreased energy intake and not nutrient composition. Golay *et al*<sup>[67]</sup> evaluated the effect of diets that were equally low in energy, but widely different in amounts of fat and carbohydrate over a 6-wk period. No significant difference in amount of weight loss was noted<sup>[67]</sup>. Alford *et al*<sup>[68]</sup> studied the effects of three different diets with a fixed caloric intake of 1 200 kcal/d. They found no significant difference in weight loss among diets with 25%, 45%, 75% carbohydrate. The authors concluded that weight loss is the result of reduction in caloric intake in proportion to caloric requirements. Schlundt *et al*<sup>[69]</sup> found greater weight loss with a low-fat, calorie-restricted diet when compared to a low-fat, *ad libitum* carbohydrate intake diet. A systematic review of low carbohydrate diets by Freedman *et al*<sup>[70]</sup> found weight loss to be associated with energy restriction and not carbohydrate restriction. It is likely that individuals on diets that alter macronutrient composition (low fat or low carbohydrate) actually lose weight because of a concurrent reduction in energy intake.

## LOW-CARBOHYDRATE VERSUS LOW-FAT DIETS: EFFECTS ON BIOCHEMICAL MARKERS OF METABOLIC SYNDROME AND NAFLD

As mentioned previously, dietary weight reduction has been shown to have a positive effect on biochemical markers of the metabolic syndrome. This may translate into a positive effect on NAFLD. Encouraging histologic findings have been noted in these patients. Therefore, it is important to compare the various diets and determine which, if any, is most beneficial in NAFLD.

Low-carbohydrate diets have long been considered fad diets by the medical profession. Several questions have been raised regarding their weight loss potential and possible adverse effects. Public interest continues unabated, and books detailing low carbohydrate lifestyles are regulars on best seller lists. A number of studies have been published in recent years evaluating the effects of low-carbohydrate diets on weight loss as well as on metabolic markers, comparing these diets to traditional low-fat diets. However, none has compared their effects on liver histology and NAFLD. Effects on obesity and biomarkers of the metabolic syndrome will be reviewed, given their important etiologic association with NAFLD.

In an uncontrolled study, Westman *et al*<sup>[71]</sup> showed that in mildly obese, motivated persons, a very low-carbohydrate diet led to sustained weight loss during a 6-mo period. Positive effects were also noted on the serum lipid profile, with a decrease in triglycerides, total and low-density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol levels. More recently, several randomized controlled trials comparing various diets have been published. Brehm *et al*<sup>[72]</sup> studied the effects of a very low-carbohydrate diet on body composition and cardiovascular risk factors. Fifty three healthy, obese women were randomized to 6 mo of an *ad libitum* very low-carbohydrate diet versus a calorie-restricted low-fat diet. The very low carbohydrate group had more weight loss ( $8.5 \pm 1.0$  vs  $3.9 \pm 1.0$  kg,  $P < 0.001$ ) without any deleterious effect on cardiovascular risk factors. Foster *et al* randomized 63 non-diabetic obese subjects to a low carbohydrate (Atkins) versus a conventional diet (low fat, high carbohydrate, calorie-restricted diet). The Atkins group had more weight loss at 3 mo ( $-6.8 \pm 5.0$  % vs  $-2.7 \pm 3.7$  %) and 6 mo ( $-7.0 \pm 6.5$  % vs  $-3.2 \pm 5.6$  %), but at 12 mo the difference between the 2 groups was not significant ( $-4.4 \pm 6.7$  % vs  $-2.5 \pm 6.3$  %). The low carbohydrate group was associated with a greater decrease in triglycerides and a greater increase in high-density lipoprotein cholesterol levels. No difference in the total of low-density lipoprotein cholesterol levels was seen between the both groups. Insulin sensitivity increased in both groups without any significant difference between groups<sup>[64]</sup>. In a study by Samaha *et al*<sup>[63]</sup>, 132 severely obese subjects with a mean body mass index (BMI) of 43 (diabetes mellitus in 39% and metabolic syndrome in 43%) were randomized to a low-carbohydrate or a low-fat, calorie-restricted diet. At 6 mo, the low carbohydrate group had more weight loss ( $-5.8 \pm 8.6$  kg vs  $-1.9 \pm 4.2$  kg)

and a greater decrease in triglyceride levels ( $-20 \pm 43$  % vs  $-4 \pm 31$  %). In non-diabetics, the insulin sensitivity improved more, and in diabetics, fasting glucose levels decreased more in the low carbohydrate group<sup>[63]</sup>. No adverse effects on the serum lipid levels were observed. The 12-mo data from this study was published in 2004. Weight loss between the two groups at this point was no longer significant. The favorable metabolic response to the low-carbohydrate diet, however, persisted<sup>[73]</sup>. A recently published study of 120 overweight, hyperlipidemic subjects showed similar findings. At 24 wk, the low carbohydrate (Atkins) group had greater weight loss (mean  $-12.9$  % vs  $-6.7$  %,  $P < 0.001$ ). Effects on triglyceride and high-density lipoprotein cholesterol levels were also more favorable with the low-carbohydrate diet<sup>[74]</sup>. Comparison of the National Cholesterol Education Program (NCEP) diet with a modified low-carbohydrate diet (low in total carbohydrates but higher in complex carbohydrates, protein and monounsaturated fat) showed a significantly greater weight loss in the modified low-carbohydrate diet over a period of 12 wk. This study did not show any significant difference between groups in blood lipid levels<sup>[75]</sup>. A low-carbohydrate diet over 12 wk was also found to be more effective than a low-fat diet for weight loss in overweight adolescents without adversely affecting their lipid profile<sup>[76]</sup>. In comparing 4 popular diets (Atkins, Zone, Weight Watcher's and Ornish) over one year, all were found to reduce weight modestly, and to have a significant reduction in low-density lipoprotein/high-density lipoprotein cholesterol ratio. No significant difference was noted between diets, and no diet-related adverse effects were noted<sup>[77]</sup>. Evaluation of 3 different diets in overweight, insulin-resistant women showed greater weight reduction from the Atkins and Zone diets when compared to a conventional low-fat diet. A greater reduction in triglycerides and waist circumference was also noted with these diets. A significant increase in low-density lipoprotein levels was noted in 25% of subjects on the Atkins diet, whereas this was seen in only 13% of subjects on the low-fat diet and 3% of those on the Zone diet<sup>[78]</sup>. Improvement in characteristics of the metabolic syndrome as demonstrated by a decrease in triglycerides, triglyceride/high-density lipoprotein ratio, postprandial lipemia and increase in low-density lipoprotein particle size was shown in overweight men on a very low-carbohydrate diet<sup>[79]</sup>. Similarly, improved insulin sensitivity and prevention of HDL cholesterol decline were noted in overweight men<sup>[80]</sup>.

Results from these studies suggest that a low-carbohydrate diet results in weight loss and may even be more effective than a conventional, low-fat diet in the short-term period. Greater weight loss may be the result of the monotony and simplicity of the diet inhibiting appetite and food intake<sup>[81]</sup>. Enhanced satiety, palatability and novelty of the diet may also play a role<sup>[63,73]</sup>. There has been great concern regarding negative effects on renal function, bone health and cancer risk. Some studies showed that low-carbohydrate diets had a higher incidence of minor side-effects, such as constipation, headache, halitosis, muscle cramps, diarrhea, rash and general weakness<sup>[74]</sup>. The major concern of an adverse effect on serum lipids, renal and cardiovascular health was not realized in these

studies. Again, these studies have relatively short follow-up periods, and the effect on low-density lipoprotein cholesterol levels remains uncertain, and requires further study. Caution must be exercised in subjects with baseline abnormal low-density lipoprotein cholesterol levels. Effects on biochemical markers associated with the metabolic syndrome appear to be more favorable with low-carbohydrate diets. In general, these diets show greater improvements in insulin sensitivity, triglyceride and high-density cholesterol levels. It is possible that for patients with the metabolic syndrome, a low-carbohydrate diet may be more advantageous. This, in turn, may positively affect NAFLD.

## CONCLUSION

As the prevalence of obesity increases, so will that of the metabolic syndrome and NAFLD. It is now recognized that the consequences of NAFLD are not always benign. While pure steatosis alone is generally an indolent disease, steatohepatitis can be a progressive disease leading to cirrhosis and even liver failure. The etiologic association between NAFLD and the metabolic syndrome is so well established that NAFLD is considered a hepatic manifestation of the disease. Obesity and insulin resistance are associated with more histologically advanced disease. Given this scenario, it is important to develop new strategies to treat and prevent NASH. While it is known that dietary weight loss improves markers of the metabolic syndrome and data also suggest that judicious weight loss affects the liver favorably in NAFLD, the best dietary approach is yet unknown. Traditionally, a low-fat diet has been recommended, but recent studies, show greater short-term weight loss and greater improvement in markers of the metabolic syndrome without significant adverse effects with low-carbohydrate diets. This raises the question of whether low-carbohydrate diets should be recommended as part of a weight loss strategy for our patients. At this point, questions regarding the nutritional adequacy and long-term safety remain. While studies have evaluated the effect of these diets on weight loss, cardiovascular and metabolic marker studies are needed to evaluate the effect of these diets specifically on NAFLD.

## REFERENCES

- McGill DB, Humpherys SR, Baggenstoss AH, Dickson ER. Cirrhosis and death after jejunoileal shunt. *Gastroenterology* 1972; **63**: 872-877
- Peters RL, Gay T, Reynolds TB. Post-jejunoileal-bypass hepatic disease. Its similarity to alcoholic hepatic disease. *Am J Clin Pathol* 1975; **63**: 318-331
- Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438
- Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology* 1994; **107**: 1103-1109
- Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419
- Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140
- Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219
- Clark J, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003; **98**: 960-967
- Daniel S, Ben-Menachem T, Vasudevan G, Ma CK, Blumenkehl M. Prospective evaluation of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients. *Am J Gastroenterol* 1999; **94**: 3010-3014
- Mathiesen UL, Franzen LE, Fryden A, Foberg U, Bodemar G. The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. *Scand J Gastroenterol* 1999; **34**: 85-91
- Sheth SG, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. *Ann Intern Med* 1997; **126**: 137-145
- Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669
- Poonawala A, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. *Hepatology* 2000; **32**: 689-692
- Wanless IR, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 1990; **12**: 1106-1110
- Klain J, Fraser D, Goldstein J, Peiser J, Avinoah E, Ovnat A, Charuzi I. Liver histology abnormalities in the morbidly obese. *Hepatology* 1989; **10**: 873-876
- Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001; **96**: 2957-2961
- Bellentani S, Saccoccio G, Masutti F, Croce LS, Brandi G, Sasso F, Cristanini G, Tiribelli C. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000; **132**: 112-117
- Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999; **107**: 450-455
- Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923
- Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Non alcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850
- Willner IR, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001; **96**: 2957-2961
- Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; **35**: 373-379
- Marceau P, Biron S, Hould FS, Marceau S, Simard S, Thung SN, Kral JG. Liver pathology and the metabolic syndrome X in severe obesity. *J Clin Endocrinol Metab* 1999; **84**: 1513-1517
- Luyckx FH, Scheen AJ, Desai C, Dewe W, Gielen JE, Lefebvre PJ. Effects of gastroplasty on body weight and related biological abnormalities in morbid obesity. *Diabetes Metab* 1998; **24**: 355-361
- Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, Cassader M, David E, Cavallo-Perin P, Rizzetto M. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *Hepatology* 2002; **35**: 367-372

- 26 Braillon A, Capron JP, Herve MA, Degott C, Quenum C. Liver in obesity. *Gut* 1985; 26: 133-139
- 27 Silverman JF, O'Brien KF, Long S, Leggett N, Khazanie PG, Pories WJ, Norris HT, Caro JF. Liver pathology in morbidly obese patients with and without diabetes. *Am J Gastroenterol* 1990; 85: 1349-1355
- 28 Abrams G, Kunde S, Lazenby A, Clements R. Portal fibrosis and hepatic steatosis in morbidly obese subjects: a spectrum of nonalcoholic fatty liver disease. *Hepatology* 2004; 40: 475-483
- 29 Flegal K, Carroll M, Ogden C, Johnson C. Prevalence and trends in obesity among US adults 1999-2000. *JAMA* 2002; 288: 1723-1727
- 30 Kopelman PG. Obesity as a medical problem. *Nature* 2000; 404: 635-643
- 31 Boyle JP, Honeycutt AA, Narayan KM, Hoerger TJ, Geiss LS, Chen H, Thompson TJ. Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the U.S. *Diabetes Care* 2001; 24: 1936-1940
- 32 Day CP, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; 114: 842-845
- 33 Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; 120: 1183-1192
- 34 Yang SQ, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 1997; 94: 2557-2562
- 35 George DK, Goldwurm S, MacDonald GA, Cowley LL, Walker NI, Ward PJ, Jazwinska EC, Powell LW. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology* 1998; 114: 311-318
- 36 Bonkovsky H, Jawaid Q, Tortelli K, LeClair P, Cobb J, Lambrecht R, Banner B. Nonalcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in nonalcoholic steatohepatitis. *J Hepatol* 1999; 31: 421-429
- 37 Bugianesi E, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, Massarenti P, Piga A, Marchesini G, Rizzetto M. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; 39: 179-187
- 38 Saxena NK, Ikeda K, Rockey DC, Friedman SL, Anania FA. Leptin in hepatic fibrosis: evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. *Hepatology* 2002; 35: 762-771
- 39 Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough A. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; 116: 1413-1419
- 40 Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; 121: 91-100
- 41 Angulo P, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; 30: 1356-1362
- 42 Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000; 118: 1117-1123
- 43 Hourigan LF, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, Powell EE. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999; 29: 1215-1219
- 44 Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; 42: 132-138
- 45 Palmer M, Schaffner F. Effect of weight reduction on hepatic abnormalities in overweight patients. *Gastroenterology* 1990; 99: 1408-1413
- 46 Keefe EB, Adesman PW, Stenzel P, Palmer RM. Steatosis and cirrhosis in an obese diabetic. Resolution of fatty liver by fasting. *Dig Dis Sci* 1987; 32: 441-445
- 47 Eriksson S, Eriksson KF, Bondesson L. Nonalcoholic steatohepatitis in obesity: a reversible condition. *Acta Med Scand* 1986; 220: 83-88
- 48 Drenick EJ, Simmons F, Murphy JF. Effect on hepatic morphology of treatment of obesity by fasting, reducing diets and small-bowel bypass. *N Engl J Med* 1970; 282: 829-834
- 49 Ames FC, Copeland EM, Leeb DC, Moore DL, Dudrick SJ. Liver dysfunction following small-bowel bypass for obesity. Nonoperative treatment of fatty metamorphosis with parenteral hyperalimentation. *JAMA* 1976; 235: 1249-1252
- 50 Kroyer JM, Talbert WM Jr. Morphologic liver changes in intestinal bypass patients. *Am J Surg* 1980; 139: 855-859
- 51 Kaminski DL, Herrmann VM, Martin S. Late effects of jejunoileal bypass operations on hepatic inflammation, fibrosis and lipid content. *Hepatogastroenterology* 1985; 32: 159-162
- 52 Ueno T, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, Torimura T, Inuzuka S, Sata M, Tanikawa K. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J Hepatol* 1997; 27: 103-107
- 53 Luyckx FH, Scheen AJ, Desai C, Dewe W, Gielen JE, Lefebvre PJ. Effects of gastroplasty on body weight and related biological abnormalities in morbid obesity. *Diabetes Metab* 1998; 24: 355-361
- 54 Vajro P, Fontanella A, Perna C, Orso G, Tedesco M, De Vincenzo A. Persistent hyperamino-transferasemia resolving after weight reduction in obese children. *J Pediatr* 1994; 125: 239-241
- 55 Franzese A, Vajro P, Argenziano A, Puziello A, Iannucci MP, Saviano MC, Brunetti F, Rubino A. Liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. *Dig Dis Sci* 1997; 42: 1428-1432
- 56 Dixon JB, Bhathal PS, Hughes NR, O'Brien PE. Nonalcoholic fatty liver disease: Improvement in liver histological analysis with weight loss. *Hepatology* 2004; 39: 1647-1654
- 57 Knobler H, Schattner A, Zhornicki T, Malnick SD, Keter D, Sokolovskaya N, Lurie Y, Bass DD. Fatty liver—an additional and treatable feature of the insulin resistance syndrome. *QJM* 1999; 92: 73-79
- 58 Harrison SA, Fincke C, Helinski D, Torgerson S, Hayashi P. A pilot study of orlistat treatment in obese, non-alcoholic steatohepatitis patients. *Aliment Pharmacol Ther* 2004; 20: 623-628
- 59 Hickman JJ, Jonsson JR, Prins JB, Ash S, Purdie DM, Clouston AD, Powell EE. Modest weight loss and physical activity in overweight patients with chronic liver disease results in sustained improvements in alanine aminotransferase, fasting insulin, and quality of life. *Gut* 2004; 53: 413-419
- 60 Andersen T, Glud C, Franzmann MB, Christoffersen P. Hepatic effects of dietary weight loss in morbidly obese subjects. *J Hepatol* 1991; 12: 224-229
- 61 Luyckx FH, Desai C, Thiry A, Dewe W, Scheen AJ, Gielen JE, Lefebvre PJ. Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. *Int J Obes Relat Metab Disord* 1998; 22: 222-226
- 62 Shick SM, Wing RR, Klem ML, McGuire MT, Hill JO, Seagle H. Persons successful at long-term weight loss and maintenance continue to consume a low-energy, low-fat diet. *J Am Diet Assoc* 1998; 98: 408-413
- 63 Samaha FF, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams T, Williams M, Gracely EJ, Stern L. A low-carbohydrate as compared with a low-fat diet in severe obesity. *N Engl J Med* 2003; 348: 2074-2081
- 64 Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, Szapary PO, Rader DJ, Edman JS, Klein S. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med* 2003; 348: 2082-2090
- 65 Yang MU, Van Itallie TB. Composition of weight lost during short-term weight reduction. Metabolic responses of obese subjects to starvation and low-calorie ketogenic and nonketogenic diets. *J Clin Invest* 1976; 58: 722-730
- 66 Atkins RC. Dr. Atkins New Diet Revolution. New York: Avon Books, 2002: 123-226
- 67 Golay A, Allaz AF, Morel Y, de Tonnac N, Tankova S, Reaven



- G. Similar weight loss with low- or high-carbohydrate diets. *Am J Clin Nutr* 1996; 63: 174-178
- 68 Alford BB, Blankenship AC, Hagen RD. The effects of variations in carbohydrate, protein, and fat content of the diet upon weight loss, blood values, and nutrient intake of adult obese women. *J Am Diet Assoc* 1990; 90: 534-540
- 69 Schlundt DG, Hill JO, Pope-Cordle J, Arnold D, Virts KL, Kattahn M. Randomized evaluation of a low fat ad libitum carbohydrate diet for weight reduction. *Int J Obes Relat Metab Disord* 1993; 17: 623-629
- 70 Freedman MR, King J, Kennedy E. Popular diets: a scientific review. *Obes Res* 2001; 9 Suppl 1: 1S-40S
- 71 Westman EC, Yancy WS, Edman JS, Tomlin KF, Perkins CE. Effect of 6-month adherence to a very low carbohydrate diet program. *Am J Med* 2002; 113: 30-36
- 72 Brehm BJ, Seeley RJ, Daniels SR, D'Alessio DA. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *J Clin Endocrinol Metab* 2003; 88: 1617-1623
- 73 Stern L, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams M, Gracely EJ, Samaha FF. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Ann Intern Med* 2004; 140: 778-785
- 74 Yancy WS Jr, Olsen MK, Guyton JR, Bakst RP, Westman EC. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. *Ann Intern Med* 2004; 140: 769-777
- 75 Aude YW, Agatston AS, Lopez-Jimenez F, Lieberman EH, Marie Almon, Hansen M, Rojas G, Lamas GA, Hennekens CH. The national cholesterol education program diet vs a diet lower in carbohydrates and higher in protein and monounsaturated fat: a randomized trial. *Arch Intern Med* 2004; 164: 2141-2146
- 76 Sondike SB, Copperman N, Jacobson MS. Effects of a low-carbohydrate diet on weight loss and cardiovascular risk factor in overweight adolescents. *J Pediatr* 2003; 142: 253-258
- 77 Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. *JAMA* 2005; 293: 43-53
- 78 McAuley KA, Hopkins CM, Smith KJ, McLay RT, Williams SM, Taylor RW, Mann JI. Comparison of high fat and high protein diets with a high carbohydrate diet in insulin-resistant obese women. *Diabetologia* 2005; 48: 8-16
- 79 Sharman MJ, Gomez AL, Kraemer WJ, Volek JS. Very low carbohydrate and low fat diets affect fasting lipids and postprandial lipemia differently in overweight men. *J Nutr* 2004; 134: 880-885
- 80 Volek JS, Sharman MJ, Gomez AL, DiPasquale C, Roti M, Pomerantz A, Kraemer WJ. Comparison of a very low-carbohydrate and low-fat diet on fasting lipids, LDL subclasses, insulin resistance, and postprandial lipemic responses in overweight women. *J Am Coll Nutr* 2004; 23: 177-184
- 81 Astrup A, Meinert Larsen T, Harper A. Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? *Lancet* 2004; 364: 897-899

S- Editor Kumar M L- Editor Elsevier HK E- Editor Liu WF