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NON – ALCOHOLIC FATTY LIVER DISEASE: PATHOGENESIS AND ITS CONNECTION WITH CARDIOVASCULAR RISK

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Summary. Non-alcoholic fatty liver disease is marked by hepatic fat accumulation not due to alcohol abuse. Several studies have demonstrated that NAFLD is associated with insulin resistance leading to a resistance in the antilipolytic effect of insulin in the adipose tissue with an increase of free fatty acids. In this review we analyzed the current understanding of NAFLD pathogenesis, discussed the mechanisms that relate NAFLD with metabolic syndrome and atherosclerosis and its association with the development and progression of cardiovascular disease.

Key words: Non-alcoholic fatty liver disease, pathogenesis, cardiovascular risk.

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of disease ranging from hepatocellular steatosis through steatohepatitis to fibrosis and irreversible cirrhosis. The prevalence of NAFLD has risen rapidly in parallel with the dramatic rise in obesity and diabetes [11,32] and is rapidly becoming the most common cause of liver disease in Western countries [18] and the USA. It has been estimated that as many as 30% of adults in the USA and other Western countries have NAFLD [10], rising up to 90% in morbidly obese individuals [26]. The more severe, and clinically significant form of NAFLD, non-alcoholic steatohepatitis (NASH) is less common, affecting an estimated 2–3% of the general population [27] and up to 37% of the morbidly obese [26]. Nonetheless, the real prevalence is unknown as NAFLD is often undiagnosed and most subjects with NAFLD, even those with diabetes, can have normal liver aminotransferases and clinicians do not suspect the potential presence of NAFLD [21,22,24]. In addition to hepatic complications, patients with NAFLD are at increased risk for cardiovascular complications [10,8].

Non-alcoholic fatty liver disease is the most common cause of chronic liver disease in the general population and is present when fatty infiltration affects 5% of hepatocytes, in the presence of 20 g (2.5 U) of alcohol consumption per day, without evidence of other causes of liver disease [28]. Insulin resistance and obesity, both key features of the metabolic syndrome (MetS), are strongly associated with NAFLD progression [25]. The prevalence of NAFLD in subjects with MetS is increased fourfold compared with those without the disease and 30% of NAFLD subjects have MetS [29].

Pathogenesis

The '2-hit hypothesis'

Initial theories for the pathogenesis of NASH were based on a '2-hit hypothesis'. The 'first hit', hepatic triglyceride accumulation, or steatosis, increases susceptibility of the liver to injury mediated by 'second hits', such as inflammatory cytokines/adipokines, mitochondrial dysfunction and oxidative stress, which in turn lead to steatohepatitis and/or fibrosis [16,17]. However, there is increasing recognition of the role that free fatty acids (FFA) play in directly promoting liver injury, which has led to modification of this theory. In obesity and IR there is an increased influx of FFA to the liver. These FFA either undergo b-oxidation or are esterified with glycerol to form triglycerides, leading to hepatic fat accumulation. There is now substantial evidence that FFA can directly cause toxicity by increasing oxidative stress and by activation of inflammatory pathways [19] therefore hepatic triglyceride accumulation may be a protective mechanism by preventing the toxic effects of unesterified FFA [33]. Additionally, a further component, or 'third-hit' has been added to reflect inadequate hepatocyte proliferation . In the healthy liver, cell death stimulates replication of mature hepatocytes which replace the dead cells and reconstitute normal tissue function [23]. However oxidative stress, a central feature of NAFLD pathogenesis, inhibits the replication of mature hepatocytes which results in expansion of the hepatic progenitor cell (oval cell) population [31]. These cells can differentiate into hepatocyte-like cells, and both oval cell and intermediate hepatocyte-like cell numbers are strongly correlated with fibrosis stage, suggesting that cumulative hepatocyte loss promotes both accumulation of progenitor cells and their differentiation towards hepatocytes. Activation of these cells has also been implicated in hepatocellular carcinogenesis [31]. In chronic liver injury, the development of fibrosis/cirrhosis is dependent on the efficacy of hepatocyte regeneration, and therefore cell death with impaired proliferation of hepatocyte progenitors represents the proposed 'third hit' in NAFLD pathogenesis [23]. Current laboratory and radiological methods to diagnose NAFLD are either too insensitive or not specific enough to grade disease presence and severity. As the early stages of NAFLD are often asymptomatic, mildly abnormal liver enzymes are usually the only clue pointing to the disease. However, up to 70% of NAFLD pa-

tients may have normal liver enzymes [5] and although alanineaminotransferase (ALT) levels have shown to be the best single biochemical correlate of hepatic steatosis[28] they do not distinguish between varying stages of NASH and can be normal in histologically severe disease[29]. Furthermore, ultrasound imaging can only detect steatosis when 30% of the liver is affected, but is still recommended as the first-line investigation to 'confirm' the presence of fatty liver due to its widespread availability and low cost[15]. Although magnetic resonance spectroscopy (MRS) has excellent sensitivity in detecting and accurately quantifying hepatic steatosis, none of the non-invasive modalities can detect inflammation and/or fibrosis, i.e. NASH. Consequently, liver biopsy is at present the 'gold-standard' (taking into account potential inaccuracies of sampling variability) for diagnosing NAFLD and staging the degree of NASH and fibrosis by histological assessment, as well as monitoring disease progression [20]. Because of the highly invasive and potentially risky nature of liver biopsy, various algorithms of combined clinical and specialized blood biomarkers, along with advanced imaging methods (e.g. MR/ultrasound elastography) are being developed to allow improved non-invasive detection of disease stage and activity[15].

Metabolic syndrome, insulin resistance and NAFLD

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 they define an individual with the metabolic syndrome with three of the five criteria—including elevated waist circumference, elevated triglycerides, and reduced high-density lipoprotein (HDL)—cholesterol levels, elevated blood pressure, and elevated fasting-glucose levels. In this new definition, waist circumference is just one of five criteria that physicians can use when diagnosing the metabolic syndrome (Population—and country—specific definitions)[3]. Insulin resistance which is a landmark of metabolic syndrome has been shown to be associated with 95% of individuals with NAFLD. Importantly, both metabolic syndrome and NAFLD are associated with high incidence of CVD and most features of metabolic syndrome present in subjects with NAFLD [1]. There is now general agreement in the literature that NAFLD is another clinical feature of the metabolic syndrome [2, 7, 8, 6]. A marked increase in body mass index (BMI) and waist/hip ratio has been shown in NAFLD patients [30]. Insulin resistance is not dependent on BMI but is related to central obesity, which is a feature of NAFLD. Obesity is associated with a higher incidence of a wide spectrum of liver diseases associated with NAFLD from steatosis to fibrosis and cirrhosis [12]. In individuals with diabetes and no diabetes, NAFLD is associated with increased risk of CVD [14, 4].

Atherosclerosis

Non-alcoholic fatty liver disease is characterized by an atherogenic lipid profile, consisting of high TG levels, low high-density lipoprotein (HDL) cholesterol, an increase in small, dense low-density lipoprotein (LDL) particles, increased very low-density lipoprotein(VLDL) cholesterol levels and elevated apolipoprotein B100 concentration [13]. Interestingly, the histological severity of NAFLD and inflammation is strongly associated with increased risk of CVD and atherogenic lipid profile [23].

Conclusions

To summarize, screening for diabetes is an essential part of the assessment of risk of CVD in individuals with NAFLD, although there is currently no established pharmacological treatment for NAFLD, and lifestyle interventions such as increasing exercise, reducing dietary fat intake, and encouraging weight loss are the only recommended therapeutic strategies with proven benefit. From a cardiologist's perspective, lipid-lowering drugs (e.g. statins), insulin-sensitizers (e.g. thiazolidinediones, metformin) and anti-hypertensive agents have not as yet shown adequate added risk/benefit value in NAFLD over and above already established evidence-based guidelines for the individual treatment of dyslipidaemia, diabetes and hypertension. Given the increased CV risk associated with NAFLD attributed to its pro-atherogenic and pro-inflammatory states, it is perhaps surprising that statins, with their antiatherosclerotic and pleiotropic (anti-oxidant, anti-inflammatory) effects, have thus far not shown a consistent benefit in NAFLD outcomes. It is noteworthy that patients with hepatic steatosis have not been shown to be at increased risk for statin hepatotoxicity [9] and the Liver Expert Panel stated in a report in 2006 that statins can indeed be safely used in NAFLD and NASH, without the need for routine liver enzyme monitoring [13].

References

1. Ahmed M.H. and Byrne C. D., "Metabolic syndrome, diabetes &CHDrisk," in *The Year in Lipid Disorders*, C. J. Packard, Ed., pp. 3–26, Clinical Publishing, Oxford, UK, 2007.
2. Ahmed M. H. and Byrne C. D., "Non alcoholic steatatohepatitis and metabolic syndrome," in*Metabolic Syndrome*, C. Byrne and S.Wild, Eds., pp. 279–305, JohnWiley & Sons, Chichester,UK, 2005.
3. Alberti K. G. M. M., Eckel R. H., Grundy S. M. et al.,"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*, vol. 120, no. 16, pp. 1640–1645, 2009.
4. Alkhouri N., Tamimi T. A. R., Yerian L., Lopez R., Zein N. N., and Feldstein A. E., "The inflamed liver and atherosclerosis: a Link between histologic severity of nonalcoholic fatty liver disease and increased cardiovascular risk,"

Digestive Diseases and Sciences, vol. 55, no. 9, pp. 2644–2650, 2010.
5. Balkau B, Charles MA. Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). Diabet Med 1999; 16:442–3.
6. Bedogni G., Miglioli L., Masutti F., Tiribelli C., Marchesini G., and Bellentani S., "Prevalence of and risk factors for nonalcoholic fatty liver disease: the dionysos nutrition and liver study," *Hepatology*, vol. 42, no. 1, pp. 44–52, 2005.
7. Bellentani S., Saccoccio G., Masutti F.et al., "Prevalence of and risk factors for hepatic steatosis in Northern Italy," *Annals of Internal Medicine*, vol. 132, no. 2, pp. 112–117, 2000.
8. Bhatia, L.S.; Curzen, N.P.; Calder, P.C.; Byrne, C.D. Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? *Eur Heart J.* **2012**, *33*, 1190–1200.
9. Browning JD. Statins and hepatic steatosis: perspectives from the Dallas Heart Study. Hepatology 2006;44:466–471.
10. Chalasani, N.; Younossi, Z.; Lavine, J.E.; Diehl, A.M.; Brunt, E.M.; Cusi, K.; Charlton, M.; Sanyal, A.J. The diagnosis and management of non-alcoholic fatty liver disease: Practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* **2012**, *142*, 1592–1609.
11. Charlton M. Nonalcoholic fatty liver disease: a review of current understanding and future impact. Clin Gastroenterol Hepatol 2004; 2:1048–58.
12. Chitturi S., Abeygunasekera S., Farrell G. C. et al., "NASH and insulin resistance: insulinhypersecretion and specific association with the insulin resistance syndrome," *Hepatology*, vol. 35, no. 2, pp. 373–379, 2002.
13. Cohen DE, Anania FA, Chalasani N. An assessment of statin safety by hepatologists. Am J Cardiol 2006;97:77C–81C.
14. Dam-Larsen S., Becker U., Franzmann M. B., Larsen K., Christoffersen P., and Bendtsen F., "Final results of a long-term,clinical follow-up in fatty liver patients," *Scandinavian Journal of Gastroenterology*, vol. 44, no. 10, pp. 1236–1243, 2009.
15. Day CP. Natural history of NAFLD: remarkably benign in the absence of cirrhosis. Gastroenterology 2005; 129:375–8.
16. Day CP, James OF. Steatohepatitis: a tale of two 'hits'?Gastroenterology 1998; 114:842–5.
17. Day CP. From fat to inflammation. Gastroenterology 2006;130:207–10.
18. De Alwis NM, Day CP. Non-alcoholic fatty liver disease: the mist gradually clears. J Hepatol 2008; 48 (Suppl. 1):S104–12.
19. Feldstein AE, Werneburg NW, Canbay A, Guicciardi ME,Bronk SF, Rydzewski R, et al.Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. Hepatology 2004; 40:185–94.
20. Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes.Hepatology 2006;44:865–73.
21. Fracanzani, A.L.; Valenti, L.; Bugianesi, E.; Andreoletti, M.; Colli, A.; Vanni, E.; Bertelli, C.; Fatta, E.; Bignamini, D.; Marchesini, G.; *et al.* Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: A role for insulin resistance and diabetes. *Hepatology* 2008, *48*, 792–798.
22. Gastaldelli, A.; Cusi, K.; Pettiti, M.; Hardies, J.; Miyazaki, Y.; Berria, R.; Buzzigoli, E.; Sironi, A.M.; Cersosimo, E.; Ferrannini, E.; *et al.* Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* 2007, *133*, 496–506.
23. Jou J, Choi SS, Diehl AM. Mechanisms of disease progression in nonalcoholic fatty liver disease. Semin Liver Dis 2008; 28:370–9.
24. Kotronen, A.; Juurinen, L.; Hakkarainen, A.; Westerbacka, J.; Corner, A.; Bergholm, R.; Yki-Jarvinen, H. Liver fat is increased in type 2 diabetic patients and underestimated by serum alanine aminotransferase compared with equally obese nondiabetic subjects. *Diabetes Care* 2008, *31*, 165–169.
25. Kotronen A, Yki-Jarvinen H. Fatty liver. A novel component of the metabolic syndrome. Arterioscler Thromb Vasc Biol 2008;28:27–38.
26. Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. J Hepatol 2006; 45:600–6.
27. Neuschwander-Tetri BA, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 2003; 37:1202–19.
28. Olufadi R, Byrne CD. Clinical and laboratory diagnosis of the metabolic syndrome. J Clin Pathol 2008; 61:697–706.
29. Reynolds K, He J. Epidemiology of the metabolic syndrome. Am J Med Sci 2005;330:273–9.
30. Rocha R., Cotrim H. P., Carvalho F. M., Siqueira A. C., Braga H., and Freitas L. A., "Body mass index and waist circumference in non-alcoholic fatty liver disease," *Journal of Human Nutrition and Dietetics*, vol. 18, no. 5, pp. 365–370, 2005.
31. Roskams T, Yang SQ, Koteish A, Durnez A, DeVos R,Huang X, et al. Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. Am J Pathol 2003; 163:1301–11.
32. Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: Selected practical issues in their evaluation and management. Hepatology 2009; 49:306–17.
33. Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK,et al. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. Hepatology 2007; 45:1366–74.