Clinical Application of Basic Science

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Phenotyping the effect of diet on non-alcoholic fatty liver disease

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Summary

Non-alcoholic fatty liver disease (NAFLD) is associated with the growing incidence of metabolic syndrome. Diet is an important contributor to the pathogenesis of NAFLD. In this review, we focused on recent publications reporting on the effect of macro- and micronutrients on development and progression of NAFLD. In general, saturated fat and fructose seem to stimulate hepatic lipid accumulation and progression into NASH, whereas unsaturated fat, choline, antioxidants, and high-protein diets rich in isoflavones seem to have a more preventive effect. Knowledge of the underlying mechanisms by which diet affects NAFLD is expanding, not in the least due to innovative techniques, such as genomics tools that provide detailed comprehensive information on a large high-throughput scale. Although most nutrients seem to interfere with the balance between hepatic *de novo* lipogenesis (endogenous synthesis of fatty acids) and lipid oxidation (burning fat for energy), there are also indications that diet can trigger or prevent hepatic lipid accumulation by influencing the interaction between liver, gut, and adipose tissue. This review now gives a current detailed overview of diet-mediated mechanisms underlying NAFLD development and progression and summarizes recent results of genomics (transcriptomics, proteomics and metabolomics) studies that contribute to improved staging, monitoring and understanding of NAFLD pathophysiology.

Introduction

Metabolic syndrome is an emerging global epidemic, which comprises a cluster of metabolic disorders such as abdominal obesity and insulin resistance. One of the other metabolic diseases often associated with metabolic syndrome is non-alcoholic fatty liver disease (NAFLD). NAFLD ranges from steatosis to non-alcoholic steatohepatitis (NASH), with or without fibrosis and cirrhosis. The prevalence of NAFLD in Western society is 20–30% and the probable increasing incidence of NAFLD can be linked to lifestyle habits. An important determinant of lifestyle is diet. In this review, we summarize recent novel data on the effects of nutrients and certain food bioactive compounds on development and/or progression of NAFLD, and potentially also in prevention of the disease. Potential underlying mechanisms are discussed and summarized in Fig. 1. Moreover, this review discusses innovative genomics techniques that are useful for improvement of diagnosis and monitoring of NAFLD pathophysiology.

Keywords: NAFLD; NASH; Macronutrients; Micronutrients; Genomics techniques.
Received 26 April 2012; received in revised form 3 July 2012; accepted 3 July 2012
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Influence of nutrients on NAFLD

Fat

In NAFLD patients, a reduced intake of saturated fat that leads to weight loss is linked to improved NAFLD pathophysiology. Recently, it was also found that the ratio of polyunsaturated/saturated fatty acid intake in NASH patients was lower than in the healthy population [1]. In this association between saturated fat and NASH, the intestinal incretin glucose-dependent insulinotropic polypeptide (GIP) seems to play an important role. NASH patients exhibit a prolonged elevation of GIP after saturated fat ingestion and this increased GIP response to saturated fat intake is associated with the severity of liver disease (Fig. 1) [2]. Additionally, higher saturated fat intake seems to be correlated with an impaired glutathione metabolism towards an oxidant status in NASH patients [3]. Monounsaturated fatty acids (MUFA) induce a more favorable plasma lipid profile, with reduced oxidized LDL, LDL cholesterol, triglycerides, and a lower total cholesterol/HDL ratio [4]. Replacement of saturated fat in the diet by MUFA to improve lipid profiles and thereby the health status is still debated. In a recent review, it has been concluded that MUFA in the diet can indeed be useful for NAFLD patients, but further human studies are required to ascertain the beneficial effect of MUFA/olive oil on NAFLD [4].

Among polyunsaturated fatty acids (PUFA), in particular the n-3 fatty acids seem to reduce the accumulation of triglycerides and ameliorate hepatic steatosis [5]. Especially, docosahexaenoic acids (DHA), which are long chain n-3 fatty acids, were found to lower triglyceride levels in liver and improve the metabolic characteristics of obesity in general. Mechanistically, MUFA and PUFA might influence NAFLD by activation of peroxisome proliferator-activated receptors (PPARs) and inhibition of transcription factor sterol regulatory element binding protein-1 (SREBP-1) (Fig. 1). Activation of PPARs stimulates lipid oxidation and decreases inflammation and insulin resistance, leading to amelioration of hepatic steatosis [5]. Inhibition of SREBP-1 can decrease the expression of genes involved in hepatic *de novo* lipogenesis and

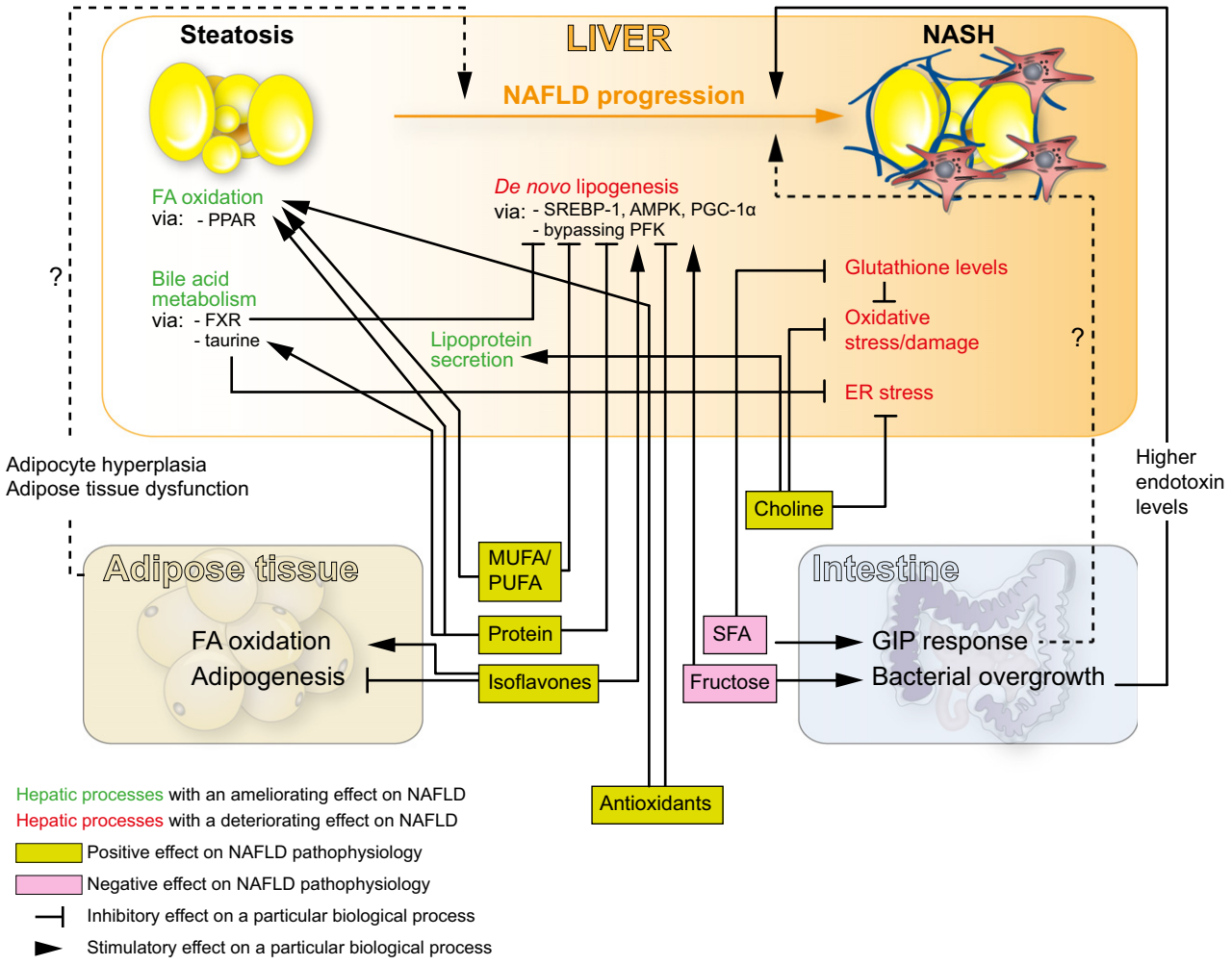

Fig. 1. Schematic overview of diet-induced mechanisms underlying the pathophysiology of NAFLD. Diet contributes to development and/or progression of NAFLD by affecting several biological processes in the liver, but also in other peripheral organs like adipose tissue and intestine. The dashed lines indicate a correlation with NAFLD progression, but the underlying mechanisms remain unclear.

thereby reduce liver fat [5]. Supplementation of MUFA and/or PUFA is currently investigated as a potential treatment against NAFLD.

Carbohydrates

A recent study has shown that sucrose-sweetened beverages, with a daily intake of 1 L, result in a higher relative amount of visceral adipose tissue (VAT), and liver fat accumulation than isocaloric semi-skim milk, aspartame-sweetened beverages, and water [6]. Body weight and total fat mass are not significantly different between the four groups, indicating that sucrose has a primary effect on expansion of VAT, including hepatic lipid accumulation. A study comparing fructose-sweetened and glucose-sweetened beverages revealed that mainly fructose-containing drinks increase visceral adiposity and lipids in overweight subjects [7]. The mechanism behind the fructose-induced expansion of ectopic fat is still under investigation, but it is hypothesized that fructose is a strong inducer of *de novo* lipogenesis (Fig. 1). This is supported by the fact that *de novo* lipogenesis only produces saturated fatty acids and that in humans a fatty liver predominantly exists of saturated fatty acids. Fructose consumption might induce hepatic lipid accumulation by activating lipogenic gene expression, but another explanation for its lipogenic nature might be the direct flow of fructose carbon into the glycolytic pathway, bypassing a key regulatory enzyme of glycolysis, phosphofructokinase [8]. For this reason, a higher proportion of the carbon from ingested fructose, as compared with glucose, is metabolized into triglycerides. Fructose consumption can also contribute to the inflammatory progression of NAFLD into NASH by inducing bacterial overgrowth in the small intestine with a concomitant increase in endotoxin levels in the portal vein (Fig. 1) [9]. This might trigger the NASH pathology.

Protein

Until now, only a few human studies addressed protein intake and intrahepatocellular lipids (IHCL) or NASH. Bortolotti *et al.* have recently observed in sedentary obese women, that consumed 60 g/day whey protein and were otherwise nourished on a spontaneous diet, a 20% reduction in IHCL [10]. This change was not related to changes in body weight or body composition.

Rodent data more clearly demonstrate the hepatic and metabolic adaptations to an increased dietary protein intake. In particular, dietary whey and soy protein intake have been repetitively shown to prevent or retard liver fat accumulation and NASH [10,11].

Several mechanisms can explain the beneficial effect of dietary protein on NAFLD (Fig. 1). As amino acid catabolism is an energy requiring process, a high protein intake might trigger an increased hepatic lipid oxidation through an increase in hepatic energy expenditure. Secondly, taurine, a derivative of the amino acid cysteine and a bile acid conjugate, is found to reduce hepatic lipid accumulation and inflammation in animals by reducing ER-stress associated with liver disease [12]. It is speculated that taurine supplementation might serve as a preventative treatment for NAFLD. Finally, amino acids or their metabolites can influence hepatic metabolism by regulating the expression of numerous metabolic genes. SREBP-1, bile acid-activated farnesoid X receptor (FXR), adenosine monophosphate-activated protein kinase and PPARγ co-activator are important regulators of these diet-induced changes in gene expression [13]. Interestingly, both the involvement of taurine and FXR suggest that the protein-mediated effect on NAFLD might be associated with bile acid metabolism.

Micronutrients and other food components

Choline is an essential nutrient, mainly metabolized and stored in the liver. Choline deficiency can affect NAFLD pathophysiology by inducing abnormal phospholipid synthesis, defects in lipoprotein secretion, oxidative damage due to mitochondrial dysfunction and ER-stress [14]. It has been recently discovered that the individual requirement of choline to prevent liver dysfunction is dependent on genetic variation and estrogen status [14]. Furthermore, gut microbiota may influence bioavailability of dietary choline to the host and thereby contribute to development of NAFLD [14].

An antioxidant that might be beneficial to NAFLD is resveratrol. In animal studies, it was shown that resveratrol could reduce hepatic oxidative stress and ameliorate NAFLD by inhibiting cytokines and reducing fatty acid content, probably by stimulating fatty acid oxidation [15]. Resveratrol has considerable potential to improve NAFLD in humans, but one has to keep in mind that substantial variation in metabolism between subjects affects the bioavailability of resveratrol after consumption [15]. Additional human studies should demonstrate the potential protective effect of resveratrol on NAFLD.

Isoflavones are also potentially involved in NAFLD because of its abundant presence in soy protein and the reduction in liver fat after soy intake [11]. The major soy isoflavones are genistin and daidzin and most studies have been performed with one of these components. Human intervention studies have not been reported yet, but in most animal studies isoflavones improved NAFLD [16]. The underlying mechanism has been suggested to occur partly via PPARs (fatty acid oxidation), carbohydrate responsive element binding protein (lipogenesis) and anti-adipogenic Wnt signaling [16].

Phenotyping of NAFLD

There is a growing interest in the large potential of comprehensive genomics tools for clinical purposes such as diagnosis, staging, and monitoring of NAFLD. Using transcriptomics, proteomics, and metabolomics, genome-wide changes can be assessed on the level of transcriptome, proteome and metabolome, respectively. Until now, most genomics studies are performed to optimize or validate the usage of these less invasive tools for appropriate application in monitoring NAFLD. However, genomics tools are also highly valuable for studying the role of diet in the NAFLD pathophysiology and unraveling the underlying (molecular) mechanisms.

Transcriptomics

Transcriptomics, which employs microarray analysis to study changes in messenger RNA expression, is a sensitive and well-validated technique. Transcriptomics enables detection of small diet-induced changes in gene expression and is an essential technique for human nutrigenomics studies [17]. Stefano *et al.* recently analysed human liver biopsies from different stages of NAFLD and found a progressively lower survivin expression in advanced stages of NAFLD [18]. Transcriptomics studies to mechanistically unravel the effect of diet on NAFLD are mainly conducted in mice, as human liver biopsies are scarce. Recently, Duval *et al.* demonstrated an association between adipose tissue dysfunction and NASH pathogenesis and identified several novel potential predictive plasma biomarkers for NASH [19].

Proteomics

In humans, proteomics studies are mainly conducted to identify novel biomarkers that facilitate accurate staging of NAFLD. Serum proteomics identified fibrinogen, RBP4, and lumican as biomarker candidates to distinguish between simple steatosis, NASH, and NASH with advanced fibrosis [20]. By studying the underlying mechanisms in animals, proteomics has recently revealed that when a pronounced diet-induced hepatic accumulation of triglycerides is visible, expression and cleavage of intermediate microfilaments are increased [21]. Changes in keratin 8 and 18 and vimentin seemed to be linked to hepatic steatosis leading to NASH. In line with these findings, Shen *et al.* found increased serum levels of keratin 18 fragments in NAFLD and NASH patients, probably due to enhanced proteolysis [22]. These fragments could serve as novel human serum biomarkers.

Metabolomics

Metabolomics comprises the high-throughput quantification and characterization of small metabolites in tissues and biofluids. Recently, plasma metabolite profiles of subjects with NAFLD have been compared to healthy controls. NAFLD patients showed lower plasma levels of glutathione and higher levels of bile acids, total carnitines, and γ-glutamyl peptides [23]. Additionally, γ-glutamyl peptides were found to have the potential to discriminate between the different progression stages of NAFLD, making it an interesting candidate biomarker. Comparison of metabolomics results of mouse NAFLD models and patients in different stages of NAFLD also showed a significant overlap in serum metabolites. In particular, the combination of glucose, lactate, glutamate/glutamine and taurine was found to be indicative of the risk of NAFLD progression [24].

One study combined transcriptomics, proteomics and metabolomics to identify changes during high-fat diet-induced hepatic

steatosis [25]. Major changes were found in hepatic one-carbon metabolism and pathways related to hepatic lipid accumulation. In accordance with previously mentioned studies [23,24], they also found a hepatic increase in taurine. These increased taurine levels, together with changes in one-carbon metabolism could reduce *de novo* glutathione synthesis, resulting in lower glutathione levels, which is indicative of an increased ROS burden in diet-induced hepatic steatosis. This study shows that the combination of genomics tools facilitates the understanding of NAFLD pathophysiology.

In conclusion, there is growing evidence that diet can affect the pathophysiology of NAFLD. Saturated fat and fructose are more likely to stimulate hepatic lipid accumulation and progression into NASH, whereas unsaturated fat, choline, antioxidants and high-protein diets rich in isoflavones seem to have a more preventive effect. However, whether specific dietary intervention strategies with or without other changes in lifestyle (e.g., more exercise) can also be used for treatment of NAFLD has still to be proven. More human studies need to be performed and additional tools for monitoring and comprehensive phenotyping NAFLD are essential, to extensively explore the potential role of healthy dietary patterns for treatment of patients. Non-invasive innovative high-throughput screening methods like genomics tools are valuable techniques to gain more insight into the underlying mechanisms of the pathophysiology of NAFLD and to identify novel biomarkers, which solely, or even more likely in combinations, can be used to distinguish the different stages of NAFLD.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Toshimitsu K, Matsuura B, Ohkubo I, Niiya T, Furukawa S, Hiasa Y, et al. Dietary habits and nutrient intake in non-alcoholic steatohepatitis. Nutrition 2007;23:46–52.
[2] Musso G, Gambino R, Pacini G, De Michieli F, Cassader M. Prolonged saturated fat-induced, glucose-dependent insulinotropic polypeptide elevation is associated with adipokine imbalance and liver injury in nonalcoholic steatohepatitis: dysregulated enteroadipocyte axis as a novel feature of fatty liver. Am J Clin Nutr 2009;89:558–567.
[3] Machado MV, Ravasco P, Jesus L, Marques-Vidal P, Oliveira CR, Proenca T, et al. Blood oxidative stress markers in non-alcoholic steatohepatitis and how it correlates with diet. Scand J Gastroenterol 2008;43:95–102.
[4] Assy N, Nassar F, Nasser G, Grosovski M. Olive oil consumption and non-alcoholic fatty liver disease. World J Gastroenterol 2009;15:1809–1815.
[5] Masterton GS, Plevris JN, Hayes PC. Review article: omega-3 fatty acids – a promising novel therapy for non-alcoholic fatty liver disease. Aliment Pharmacol Ther 2010;31:679–692.
[6] Maersk M, Belza A, Stodkilde-Jorgensen H, Ringgaard S, Chabanova E, Thomsen H, et al. Sucrose-sweetened beverages increase fat storage in the liver, muscle, and visceral fat depot: a 6-mo randomized intervention study. Am J Clin Nutr 2012;95:283–289.
[7] Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. J Clin Invest 2009;119:1322–1334.
[8] Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab 2005;2:5.
[9] Nomura K, Yamanouchi T. The role of fructose-enriched diets in mechanisms of nonalcoholic fatty liver disease. J Nutr Biochem 2012;23:203–208.
[10] Bortolotti M, Maiolo E, Corazza M, Van Dijke E, Schneiter P, Boss A, et al. Effects of a whey protein supplementation on intrahepatocellular lipids in obese female patients. Clin Nutr 2011;30:494–498.
[11] Yang HY, Tzeng YH, Chai CY, Hsieh AT, Chen JR, Chang LS, et al. Soy protein retards the progression of non-alcoholic steatohepatitis via improvement of insulin resistance and steatosis. Nutrition 2011;27:943–948.
[12] Gentile CL, Nivala AM, Gonzales JC, Pfaffenbach KT, Wang D, Wei Y, et al. Experimental evidence for therapeutic potential of taurine in the treatment of nonalcoholic fatty liver disease. Am J Physiol Regul Integr Comp Physiol 2011;301:R1710–R1722.
[13] Hashidume T, Sasaki T, Inoue J, Sato R. Consumption of soy protein isolate reduces hepatic SREBP-1c and lipogenic gene expression in wild-type mice, but not in FXR-deficient mice. Biosci Biotechnol Biochem 2011;75:1702–1707.
[14] Corbin KD, Zeisel SH. Choline metabolism provides novel insights into nonalcoholic fatty liver disease and its progression. Curr Opin Gastroenterol 2012;28:159–165.
[15] Smoliga JM, Baur JA, Hausenblas HA. Resveratrol and health – A comprehensive review of human clinical trials. Mol Nutr Food Res 2011;55:1129–1141.
[16] Kim MH, Kang KS. Isoflavones as a smart curer for non-alcoholic fatty liver disease and pathological adiposity via ChREBP and Wnt signaling. Prev Med 2012;54(Suppl.):S57–S63.
[17] Afman LA, Muller M. Human nutrigenomics of gene regulation by dietary fatty acids. Prog Lipid Res 2012;51:63–70.
[18] Stefano JT, de Oliveira CPMS, Corrêa-Giannella ML, Soares IC, Kubrusly MS, Bellodi-Privato M, et al. Decreased immunoexpression of survivin could be a potential marker in human non-alcoholic fatty liver disease progression? Liver Int 2011;31:377–385.
[19] Duval C, Thissen U, Keshtkar S, Accart B, Stienstra R, Boekschoten MV, et al. Adipose tissue dysfunction signals progression of hepatic steatosis towards nonalcoholic steatohepatitis in C57Bl/6 mice. Diabetes 2010;59:3181–3191.
[20] Bell LN, Theodorakis JL, Vuppalanchi R, Saxena R, Bemis KG, Wang M, et al. Serum proteomics and biomarker discovery across the spectrum of nonalcoholic fatty liver disease. Hepatology 2010;51:111–120.
[21] Park J-E, Kim HT, Lee S, Lee Y-S, Choi U-K, Kang JH, et al. Differential expression of intermediate filaments in the process of developing hepatic steatosis. Proteomics 2011;11:2777–2789.
[22] Shen J, Chan HL-Y, Wong GL-H, Choi PC-L, Chan AW-H, Chan H-Y, et al. Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers. J Hepatol 2012;56:1363–1370.
[23] Kalhan SC, Guo L, Edmison J, Dasarathy S, McCullough AJ, Hanson RW, et al. Plasma metabolomic profile in nonalcoholic fatty liver disease. Metabolism 2011;60:404–413.
[24] Li H, Wang L, Yan X, Liu Q, Yu C, Wei H, et al. A proton nuclear magnetic resonance metabonomics approach for biomarker discovery in nonalcoholic fatty liver disease. J Proteome Res 2011;10:2797–2806.
[25] Rubio-Aliaga I, Roos B, Sailer M, McLoughlin GA, Boekschoten MV, van Erk M, et al. Alterations in hepatic one-carbon metabolism and related pathways following a high-fat dietary intervention. Physiol Genomics 2011;43:408–416.