

Original Research

Non-alcoholic fatty liver disease and its complications

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ABSTRACT:

Non-alcoholic fatty liver disease (NAFLD) comprise the steatosis with hepatitis, fibrosis, cirrhosis, and in some cases liver carcinoma. It is a growing epidemic worldwide in part due to obesity and insulin resistance leading to liver accumulation of triglycerides and fatty acids. Various risk factors for the development of NAFLD have been exposed with most having some form of metabolic derangement or insulin resistance. NAFLD patients are at increased risk of liver related as well as cardiovascular mortality, and is rapidly becoming indication for liver transplantation. Several medications are being used in treatment of NAFLD, but none seems to promising results in this growing problem. In this review current knowledge of NAFLD, risk factors, lipid metabolism, associations with gut microbiota, diagnosis, pathogenesis, and its treatment in order to understand this disease highlighted.

Key words:- NAFLD, metabolism, factors, treatment

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is present if at least 5% of liver weight is fat without excess alcohol consumption or secondary causes of fat accumulation in the background (European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), & European Association for the Study of Obesity (EASO), 2016). NAFL has been thought to be a benign disease, but an accumulating body of evidence has shown that NAFL may progress to Non alcoholic steatohepatitis (NASH) (Buzzetti et al., 2016; Calzadilla Bertot & Adams, 2016;)

Approximately 25% of adults around the world have NAFLD (Rinella & Charlton, 2016; Younossi, Koenig et al., 2016) and the prevalence is still increasing (Seyda Seydel et al., 2016; Younossi, Koenig et al., 2016).

NAFLD parallels with its associates, obesity, metabolic syndrome (MetS) and type 2 diabetes

(T2D) (Anstee, Targher, & Day, 2013; Seyda Seydel et al., 2016; Younossi, Koenig et al., 2016) and it entails a considerable risk for metabolic disorders (MetS, T2D, dyslipidemia) and cardiovascular diseases (CVDs) (Anstee et al., 2013; Byrne & Targher, 2015; European Association for the Study of the Liver (EASL) et al., 2016), cirrhosis and hepatocellular carcinoma (HCC) in the latter end (European Association for the Study of the Liver (EASL) et al., 2016).

NON INVASIVE DIAGNOSIS

Due to low cost and high availability without radiation exposure, ultrasound is commonly used as a first-line imaging method in the clinical practice. The increased liver-kidney contrast showing an echogenic (bright) liver is a widely accepted criterion to set the diagnosis of hepatosteatosis (Ballestri, Romagnoli, Nascimbeni, Francica, & Lonardo, 2015; Saverymattu, Joseph, & Maxwell, 1986;). The

nature of ultrasound in the assessment of hepatosteatosis is more or less qualitative, but the use of hepatorenal sonographic index may achieve more quantitative assessment (Webb et al., 2009). Magnetic resonance imaging (MRI) and 1 H-MRS are the best non-invasive tools to diagnose hepatosteatosis. For the diagnosis of fibrosis and its severity, a liver biopsy is the golden standard. However, its usefulness is limited due to costs and risk of complications. Thus, it is unrealistic to perform liver biopsy for all subjects with NAFLD, the global prevalence of which is about 25% (Younossi, Koenig et al., 2016). Ultrasound-based shear wave elastography can be used to assess fibrosis in subjects with NAFLD.

LIPID METABOLISM IN NAFLD

When the hepatic FFA availability (influx and de novo synthesis with esterification to triglycerides) is greater than FFA disposal (oxidation and secretion), hepatosteatosis develops (Kawano & Cohen, 2013; Valenti, Bugianesi, Pajvani, & Targher, 2016). In the Western world, the most common background for this is higher energy intake as compared to energy expenditure: lipids (mainly free fatty acids from adipose tissue) begin to accumulate in tissues and organs not designed to store fat, such as the liver or the omentum. This phenomenon is called ectopic fat accumulation (Byrne & Targher, 2015; Shulman, 2014; Valenti et al., 2016) and hepatosteatosis is thus an example of it. Straightforwardly, there are two types of NAFLD: obese/metabolic NAFLD, resulting from energy surplus, and genetic NAFLD due to genetic alterations in lipid metabolism in the liver – although combinations of these are also commonly met (Petaja & Yki-Jarvinen, 2015;).

LIPID METABOLISM IN OBESE/METABOLIC NAFLD

Hepatosteatosis is formed when VLDL secretion and Acyl-CoA oxidation are overwhelmed by enhanced FFA influx and de novo lipogenesis. The lipid accumulation in the liver is mainly formed of triglycerides (Alkhoury et al., 2009), which are not lipotoxic nor induce insulin resistance to the same extent as many other lipids do (Takamura, Misu, Ota, & Kaneko, 2012). Indeed, converting FFAs to triglycerides may be seen as the liver protecting itself from the toxic effects of the excess FFAs, cholesterols, diacylglycerols, phospholipids and its components (ceramides, sphingolipids, lysophosphatidyl choline) in the surrounding milieu leading to oxidative stress, insulin resistance and more severe NAFLD (Kikuchi & Takamura, 2016; Musso, Gambino, & Cassader, 2013;).

GLUCOSE METABOLISM IN NAFLD

Insulin suppresses hepatic gluconeogenesis during fasting (Yki-Jarvinen, 2014). NAFLD is associated

with insulin resistance (Shulman, 2014). Thus, individuals with NAFLD have higher rates of gluconeogenesis (Sunny et al., 2011), which leads to mild hyperglycemia . Over time, pancreatic β cells may be unable to secrete sufficient amounts of insulin, which provokes T2D. The pathogenesis of insulin resistance in NAFLD is manifold (Begrache, Igoudjil, Pessayre, & Fromenty, 2006). If mitochondria cannot handle the increased lipid influx, mitochondrial dysfunction, activation of pro-inflammatory c-Jun NH₂- terminal kinase-1, burst of oxidative stress and endoplasmic reticulum stress develop (Begrache et al., 2006; Buzzetti et al., 2016; Cusi, 2009). Especially, saturated fatty acids, ceramides, sphingolipids and free cholesterol are toxic lipids that provoke liver cell injury and insulin resistance through these mechanisms (Mota, Banini, Cazanaue, & Sanyal, 2016).

GUT MICROBIOTA

Majority of the microbiota remains stable during adulthood, the composition is somewhat altered in response to alterations in the environment such as diet, antibiotics or other drugs and host susceptibility (Ussar et al., 2016). If the equilibrium of the eubiosis is destabilized, quali-quantitative alterations in the gut microbiota take place. This condition in which ‘the good bacteria’ are no longer able to control ‘the bad bacteria’ is called dysbiosis . Dysbiosis is related to various gastrointestinal diseases, such as inflammatory bowel disease, colon carcinoma, celiac disease and irritable bowel disease (Nagao- Kitamoto, Kitamoto, Kuffa, & Kamada, 2016) – and NAFLD, which has been demonstrated by correlative studies and by transplant of microbiota from obese mice or humans into germ-free mice (Machado & Cortez-Pinto, 2016). The dysbiotic gut induces hepatosteatosis by resulting in increased intestinal permeability and thus in increased energy availability and inflammatory cytokines and portal endotoxemia in the liver (Haque & Barritt, 2016). Additionally, leaky gut and small intestine bacterial overgrowth are associated with NAFLD (V. W. Wong et al., 2015) and endotoxemia (a burst of endotoxins or lipopolysaccharides, a major component of the cell wall of Gram- negative bacteria in the blood) is shown to correlate with the existence of NAFLD (Kitabatake et al., 2017;).

ALCOHOL CONSUMPTION

According to the definition of NAFLD, alcohol consumption more than 30g a day in men or 20g a day in women is an exclusion criterion for the NAFLD diagnosis (European Association for the Study of the Liver (EASL) et al., 2016). If these limits are exceeded, the condition is called alcoholic fatty liver disease. However, even moderate alcohol consumption below these limits may predispose to NAFLD depending on many factors, such as drinking patterns and individual or genetic susceptibility as, for

example, heavy episodic alcohol intake may be associated with fibrosis progression (Ajmera, Terrault, & Harrison, 2017;). Especially, those at metabolic risk tend to have NAFLD even with moderate alcohol consumption (European Association for the Study of the Liver (EASL) et al., 2016). Indeed, from the liver point of view, there are no precise safety limits for alcohol use (European Association for the Study of Liver, 2012). On the other hand, there are epidemiological data showing moderate alcohol consumption to be beneficial in the prevention of NAFLD development (European Association for the Study of the Liver (EASL) et al., 2016;) and in the protection of cardiovascular diseases in the general population when compared to total abstinence (Ajmera et al., 2017).

TOXIC COMPOUNDS, DRUGS AND NAFLD

Of note, there are also reports from rodents and humans that different pollutants are capable of worsening obesity, insulin resistance, NAFLD and even HCC (Arciello et al., 2013), but hepatosteatosis may be induced by xenobiotics, such as anabolic-androgenic steroids, even without the induction of insulin resistance (Schwingel et al., 2011). Methotrexate, amiodarone, valproate, glucocorticoids, synthetic estrogens, tetracycline and tamoxifen are other known steatogenic medication (Arkkila, 2009). Additionally, some chemicals, specifically synthetic ones, may mimic endogenous hormones, leading to disruption of the endocrine homeostasis and development of hepatosteatosis (Arciello et al., 2013). Some environmental heavy metals (e.g. mercury, lead, cadmium, arsenic) and polychlorinated compounds also seem have direct toxic effects on NAFLD and insulin resistance through oxidative stress (Arciello et al., 2013).

GENETIC FACTORS PREDISPOSING FOR NAFLD

There is a great variability in the prevalence of NAFLD around the world (Rinella & Charlton, 2016). Furthermore, in the USA the prevalence of hepatosteatosis varies significantly by ethnicity (Hispanics, whites, blacks, respectively) irrespective of body mass index, insulin resistance, alcohol consumption, or medication use (Speliotes, 2015). The majority of the variability between the ancestries is explained by genetic differences (Kahali et al., 2015). The heritable component of NAFLD through ancestries is estimated to be 22–38% (Kahali et al., 2015). In recent years, the study of the genetic factors predisposing for NAFLD has been intense. Straightforwardly, there are two main strategies to do the genetic studies: genome-wide association studies (GWAS, genome-wide study of genetic variants associated with the disease) and candidate gene studies (study of the selected candidate gene) (Macaluso, Maida, & Petta, 2015). To date,

GWAS has introduced the three best verified genetic variants affecting NAFLD, patatin-like phospholipase domain-containing 3 (PNPLA3) transmembrane 6 superfamily member 2 E167K variant (TM6SF2) (Macaluso et al., 2015) and membrane bound O-acyltransferase domain containing 7 (MBOAT7) (Macaluso et al., 2015; Mancina et al., 2016).

HEPATIC COMPLICATIONS OF NAFLD

The average risk of cirrhosis in subjects with simple steatosis is under 4% in 20 years, but in NASH subjects the risk reaches up to 25% in nine years (Calzadilla Bertot & Adams, 2016).). Indeed, given the high prevalence, NAFLD is the leading cause of cryptogenic cirrhosis and the second or third most common cause of liver transplantation (Calzadilla Bertot & Adams, 2016; European Association for the Study of the Liver (EASL) et al., 2016). NAFLD is predicted to be the most common cause of liver transplantation in the near future (Calzadilla Bertot & Adams, 2016). Looking back, there has been a 5-fold increase in the transplantations for NASH during this millennium (Agopian et al., 2012). Liver-related deaths (cirrhosis or HCC) accounted for up to 9–28% of all deaths in three long- term longitudinal studies of biopsied NAFLD patients (Angulo et al., 2015), although CVDs remain the most common cause of death in NAFLD subjects (Ekstedt et al., 2015; European Association for the Study of the Liver (EASL) et al., 2016).

NAFLD AND METABOLIC SYNDROME

Metabolic syndrome (MetS) is a leading risk factor of cardiovascular and T2D- related mortality and morbidity (Simons, Simons, Friedlander, & McCallum, 2011). In a recent meta-analysis of 53,000 subjects from 7 studies, Ballestri et al. reported that serum liver enzyme-based NAFLD was associated with 2-fold risk of incident MetS and over 3- fold risk if the diagnosis was ultrasonography-based (Ballestri et al., 2016). The European DIONYSOS study cohort with 3,000 participants showed the strong association between NAFLD and obesity, a central component of MetS and insulin resistance.

CARDIOVASCULAR COMPLICATIONS OF NAFLD

Over the past decade NAFLD has been shown to affect extra-hepatic organs. For example, in addition to the hepatic morbidity related to NAFLD, i.e., NASH, fibrosis, cirrhosis and HCC, NAFLD increases the risk of CVDs, T2D and chronic kidney disease. Although the relative risk of hepatic-related death increases the most in NAFLD subjects, the greatest absolute risk of death among them is attributable of CVDs (Lonardo, Sookoian, Pirola, & Targher, 2016). There is solid epidemiological evidence of a causative association between NAFLD and CVDs. In a Medline search for CVD events, i.e

coronary heart disease (CHD) and ischemic stroke, in prospective studies of subjects with imaging- or histology-based NAFLD and after exclusions of dual studies from the same database, various studies were found (Fracanzani et al., 2016).

NAFLD AND OTHER CO MORBIDITIES

There are some diseases shown to be associated with NAFLD, such as a wide range of extrahepatic cancers (Sanna, Rosso, Marietti, & Bugianesi, 2016), periodontitis (Han, Sun, & Yang, 2016), psoriasis (Mantovani, Gisondi, Lonardo, & Targher, 2016) and celiac disease (Reilly, Lebwohl, Hultcrantz, Green, & Ludvigsson, 2015). Also sleep apnea, osteoporosis and polycystic ovary syndrome have been reported to be associated with NAFLD (Byrne & Targher, 2015).

MANAGEMENT OF NAFLD

Lifestyle modification with weight reduction in overweight or obese people, physical activity and diet control is at the core of all management of NAFLD. At present, the pharmacotherapeutic agents available for NAFLD are scarce, but some potential new drugs are seen in the horizon. The agents are targeting insulin resistance, weight reduction and fibrotic or inflammatory processes. Bariatric surgery or liver transplantation may be used for selected patients. The recommendations of pharmacotherapy in NAFLD guidelines are unestablished. However, there are some interesting agents with potential benefit available and some promising agents are seen in the horizon.

POTENTIAL FUTURE DRUGS

Several new treatment options for NAFLD and NASH are currently being evaluated and developed, specifically for NASH, targeting insulin resistance, dyslipidemia, hepatic inflammation or fibrosis (Ratziu, 2016). Glucagon-like peptide-1 analogues, especially liraglutide, are promising in NASH treatment due to their potential to induce weight loss and insulin sensitivity, which may have a direct beneficial hepatic effect leading to decreasing hepatocyte triglyceride accumulation and fibrosis (Armstrong et al., 2016; Barb et al., 2016). Obeticholic acid is an agonist of farnesoid X receptor (Ijssennagger et al., 2016), which has several beneficial metabolic effects on the liver (Ratziu, Goodman, & Sanyal, 2015). Elafibranor, a dual agonist of peroxisome proliferator-activated receptor α and δ , is being studied as a NASH treatment (Barb et al., 2016). To conclude NAFLD is widely present in general population, and subjects with NAFLD have cardiovascular diseases. A small proportion of NAFLD will die from hepatic reasons, but due to its widespread nature, it will become main reason for liver transplantation in coming years.

REFERENCES

1. European Association for the Study of the Liver

- (EASL), European Association for the Study of Diabetes (EASD), & European Association for the Study of Obesity (EASO). (2016). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *Diabetologia*, 59(6), 1121–1140. doi:10.1007/s00125-016-3902-y[doi]
2. Buzzetti, E., Pinzani, M., & Tsochatzis, E. A. (2016). The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism: Clinical and Experimental*, 65(8), 1038–1048. doi:10.1016/j.metabol.2015.12.012 [doi]
3. Calzadilla Bertot, L., & Adams, L. A. (2016). The natural course of non-alcoholic fatty liver disease. *International Journal of Molecular Sciences*, 17(5), 10.3390/ijms17050774. doi:10.3390/ijms17050774[doi]
4. Adams, L. A., Waters, O. R., Knuiman, M. W., Elliott, R. R., & Olynyk, J. K. (2009). NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: An eleven-year follow-up study. *The American Journal of Gastroenterology*, 104(4), 861–867. doi:10.1038/ajg.2009.67 [doi]
5. Rinella, M., & Charlton, M. (2016). The globalization of non alcoholic fatty liver disease: Prevalence and impact on world health. *Hepatology (Baltimore, Md.)*, 64(1), 19–22. doi:10.1002/hep.28524[doi]
6. Charlton, M. R., Burns, J. M., Pedersen, R. A., Watt, K. D., Heimbach, J. K., & Dierkhising, R. A. (2011). Frequency and outcomes of liver transplantation for non alcoholic steatohepatitis in the united states. *Gastroenterology*, 141(4), 1249–1253. doi:10.1053/j.gastro.2011.06.061[doi]
7. Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., & Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology (Baltimore, Md.)*, 64(1), 73–84. doi:10.1002/hep.28431[doi]
8. Seyda Seydel, G., Kucukoglu, O., Altinbasv, A., Demir, O. O., Yilmaz, S., Akkiz, H., Canbay, A. (2016). Economic growth leads to increase of obesity and associated hepatocellular carcinoma in developing countries. *Annals of Hepatology*, 15(5), 662–672. doi:10.5604/16652681.1212316 [doi]
9. Anstee, Q. M., Targher, G., & Day, C. P. (2013). Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nature Reviews Gastroenterology & Hepatology*, 10(6), 330–344. doi:10.1038/nrgastro.2013.41 [doi]
10. Byrne, C. D., & Targher, G. (2015). NAFLD: A multi system disease. *Journal of Hepatology*, 62(1 Suppl), S47–64. doi:10.1016/j.jhep.2014.12.012 [doi]
11. Ballestri, S., Romagnoli, D., Nascimbeni, F., Francica, G., & Lonardo, A. (2015). Role of ultrasound in the diagnosis and treatment of nonalcoholic fatty liver disease and its complications. *Expert Review of Gastroenterology & Hepatology*, 9(5), 603–627. doi:10.1586/17474124.2015.1007955[doi]
12. Saverymuttu, S. H., Joseph, A. E., & Maxwell, J. D. (1986). Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *British Medical Journal (Clinical Research Ed.)*, 292(6512), 13–15.

13. Webb, M., Yeshua, H., Zelber-Sagi, S., Santo, E., Brazowski, E., Halpern, Z., & Oren, R. (2009). Diagnostic value of a computerized hepatorenal index for sonographic quantification of liver steatosis. *AJR. American Journal of Roentgenology*, 192(4), 909–914. doi:10.2214/AJR.07.4016 [doi]
14. Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., & Wymer, M. (2016). Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology (Baltimore, Md.)*, 64(1), 73–84. doi:10.1002/hep.28431 [doi]
15. Kawano, Y., Nishiumi, S., Saito, M., Yano, Y., Azuma, T., & Yoshida, M. (2015). Identification of lipid species linked to the progression of non-alcoholic fatty liver disease. *Current Drug Targets*, 16(12), 1293–1300. doi:CDT-EPUB-66400 [pii]
16. Valenti, L., Bugianesi, E., Pajvani, U., & Targher, G. (2016). Non alcoholic fatty liver disease: Cause or consequence of type 2 diabetes? *Liver International : Official Journal of the International Association for the Study of the Liver*, 36(11), 1563–1579. doi:10.1111/liv.13185 [doi]
17. Byrne, C. D., & Targher, G. (2015). NAFLD: A multi system disease. *Journal of Hepatology*, 62(1 Suppl), S47–64. doi:10.1016/j.jhep.2014.12.012 [doi]
18. Shulman, G. I. (2014). Ectopic fat in insulin resistance, dyslipidemia, and cardio metabolic disease. *The New England Journal of Medicine*, 371(12), 1131–1141. doi:10.1056/NEJMr1011035 [doi]
19. Petaja, E. M., & Yki-Jarvinen, H. (2016). Definitions of normal liver fat and the association of insulin sensitivity with acquired and genetic NAFLD—A systematic review. *International Journal of Molecular Sciences*, 17(5), 10.3390/ijms17050633. doi:10.3390/ijms17050633 [doi]
20. Yki-Jarvinen, H., & Luukkonen, P. K. (2015). Heterogeneity of non-alcoholic fatty liver disease. *Liver International : Official Journal of the International Association for the Study of the Liver*, 35(12), 2498–2500. doi:10.1111/liv.12970 [doi]
21. Alkhoury, N., Dixon, L. J., & Feldstein, A. E. (2009). Lipotoxicity in nonalcoholic fatty liver disease: Not all lipids are created equal. *Expert Review of Gastroenterology & Hepatology*, 3(4), 445–451. doi:10.1586/egh.09.32 [doi]
22. Takamura, T., Misu, H., Ota, T., & Kaneko, S. (2012). Fatty liver as a consequence and cause of insulin resistance: Lessons from type 2 diabetic liver. *Endocrine Journal*, 59(9), 745–763. doi:DN/JST.JSTAGE/endocrj/EJ12-0228 [pii]
23. Kikuchi, A., & Takamura, T. (2016). Where does liver fat go? A possible molecular link between fatty liver and diabetes. *Journal of Diabetes Investigation*, doi:10.1111/jdi.12573 [doi]
24. Musso, G., Gambino, R., & Cassader, M. (2013). Cholesterol metabolism and the pathogenesis of non-alcoholic steatohepatitis. *Progress in Lipid Research*, 52(1), 175–191. doi:10.1016/j.plipres.2012.11.002 [doi]
25. Yki-Jarvinen, H. (2014). Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. *The Lancet .Diabetes & Endocrinology*, 2(11), 901–910. doi:10.1016/S2213-8587(14)70032-4 [doi]
26. Shulman, G. I. (2014). Ectopic fat in insulin resistance, dyslipidemia, and cardio metabolic disease. *The New England Journal of Medicine*, 371(12), 1131–1141. doi:10.1056/NEJMr1011035 [doi]
27. Sunny, N. E., Parks, E. J., Browning, J. D., & Burgess, S. C. (2011). Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. *Cell Metabolism*, 14(6), 804–810. doi:10.1016/j.cmet.2011.11.004 [doi]
28. Begriche, K., Igoudjil, A., Pessayre, D., & Fromenty, B. (2006). Mitochondrial dysfunction in NASH: Causes, consequences and possible mean stop revert it. *Mitochondrion*, 6(1), 1–28. doi:S1567-7249(05)00136-4 [pii]
29. Buzzetti, E., Pinzani, M., & Tsochatzis, E. A. (2016). The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism: Clinical and Experimental*, 65(8), 1038–1048. doi:10.1016/j.metabol.2015.12.012 [doi]
30. Cusi, K. (2009). Role of insulin resistance and lipotoxicity in non-alcoholics steatohepatitis. *Clinics in Liver Disease*, 13(4), 545–563. doi:10.1016/j.cld.2009.07.009 [doi]
31. Mota, M., Banini, B. A., Cazanave, S. C., & Sanyal, A. J. (2016). Molecular mechanisms of lipotoxicity and glucotoxicity in nonalcoholic fatty liver disease. *Metabolism: Clinical and Experimental*, 65(8), 1049–1061. doi:10.1016/j.metabol.2016.02.014 [doi]
32. Ussar, S., Fujisaka, S., & Kahn, C. R. (2016). Interactions between host genetics and gut microbiome in diabetes and metabolic syndrome. *Molecular Metabolism*, 5(9), 795–803. doi:10.1016/j.molmet.2016.07.004 [doi]
33. Nagao-Kitamoto, H., Kitamoto, S., Kuffa, P., & Kamada, N. (2016). Pathogenic role of the gut microbiota in gastrointestinal diseases. *Intestinal Research*, 14(2), 127–138. doi:10.5217/ir.2016.14.2.127 [doi]
34. Machado, M. V., & Cortez-Pinto, H. (2016). Diet, microbiota, obesity, and NAFLD: A dangerous quartet. *International Journal of Molecular Sciences*, 17(4), 481. doi:10.3390/ijms17040481 [doi]
35. Haque, T. R., & Barritt, A. S., 4th. (2016). Intestinal microbiota in liver disease. *Best Practice & Research. Clinical Gastroenterology*, 30(1), 133–142. doi:10.1016/j.bpg.2016.02.004 [doi]
36. Wong, V. W., Wong, G. L., Chan, H. Y., Yeung, D. K., Chan, R. S., Chim, A. M., ... Chan, H. L. (2015). Bacterial endotoxin and non-alcoholic fatty liver disease in the general population: A prospective cohort study. *Alimentary Pharmacology & Therapeutics*, 42(6), 731–740. doi:10.1111/apt.13327 [doi]
37. Kitabatake, H., Tanaka, N., Fujimori, N., Komatsu, M., Okubo, A., Kakegawa, K., ... Tanaka, E. (2017). Association between endotoxemia and histological features of non alcoholic fatty liver disease. *World Journal of Gastroenterology*, 23(4), 712–722. doi:10.3748/wjg.v23.i4.712 [doi]
38. European Association for the Study of the Liver (EASL), European Association for the Study of

- Diabetes (EASD), & European Association for the Study of Obesity (EASO). (2016). EASL-EASD-EASO clinical practice guidelines for the management of non-alcoholic fatty liver disease. *Diabetologia*, 59(6), 1121–1140. doi:10.1007/s00125-016-3902-y[doi]
39. Ajmera, V. H., Terrault, N. A., & Harrison, S. A. (2017). Is moderate alcohol use in non alcoholic fatty liver disease good or bad? A critical review. *Hepatology*(Baltimore,Md.),doi:10.1002/hep.29055 [doi]
40. European Association for the Study of Liver. (2012). EASL clinical practical guidelines: Management of alcoholic liver disease. *Journal of Hepatology*,57(2),399–420.doi:10.1016/j.jhep.2012.04.004[doi]
41. Arciello, M., Gori, M., Maggio, R., Barbaro, B., Tarocchi, M., Galli, A., & Balsano, C.(2013). Environmental pollution: A tangible risk for NAFLD pathogenesis. *International Journal of Molecular Sciences*,14(11),22052–22066.doi:10.3390/ijms141122052[doi]
42. Schwingel, P. A., Cotrim, H. P., Salles, B. R., Almeida, C. E., dos Santos, C. R., Jr, Nache, B.,Zoppi, C. C. (2011). Anabolic-androgenic steroids: A possible new risk factor of toxicant-associated fatty liver disease. *Liver International : Official Journal of the International Association for the Study of the Liver*, 31(3), 348–353. doi:10.1111/j.1478–3231.2010.02346.x[doi]
43. Arkkila,P.(2009). Uhkaakor asvamak sakansanterveyttä? *Suomen Lääkärilehti*,(12),1111–1119.
44. Rinella, M., & Charlton, M. (2016). The globalization of non alcoholic fatty liver disease: Prevalence and impact on world health. *Hepatology* (Baltimore, Md.), 64(1), 19–22.doi:10.1002/hep.28524[doi]
45. Speliotes, E. K., Butler, J. L., Palmer, C. D., Voight, B. F., GIANT Consortium, MI Gen Consortium,...Hirschhorn,J.N.(2010). PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. *Hepatology*(Baltimore,Md.),52(3),904–912.doi:10.1002/hep.23768[doi]
46. Kahali, B., Halligan, B., & Speliotes, E. K. (2015). Insights from genome-wide association analyses of nonalcoholic fatty liver disease. *Seminars in Liver Disease*,35(4),375–391.doi:10.1055/s-0035-1567870[doi]
47. Macaluso, F. S., Maida, M., & Petta, S. (2015). Genetic background in nonalcoholic fatty liver disease: A comprehensive review. *World Journal of Gastroenterology*, 21(39),11088–11111.doi:10.3748/wjg.v21.i39.11088[doi]
48. Mancina,R.M., Dongiovanni,P.,Petta,S.,Pingitore,P.,Meroni,M.,Rametta,R,...Romeo, S. (2016). The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of european descent. *Gastroenterology*,150(5),1219–1230.e6.doi:S0016-5085(16)00131-1 [pii]
49. Calzadilla Bertot ,L.,& Adams,L.A.(2016).The natural course of non-alcoholic fatty liver disease. *International Journal of Molecular Sciences*, 17(5), 10.3390/ijms17050774.doi:10.3390/ijms17050774[doi]
50. Agopian,V. G.,Kaldas, F. M., Hong,J. C.,Whittaker, M., Holt, C.,Rana,A., ... Busuttil, R.W.(2012). Liver transplantation for nonalcoholic steatohepatitis:The new epidemic. *Annals of Surgery*, 256(4), 624–633. doi:10.1097/SLA.0b013e31826b4b7e [doi]
51. Angulo,P.,Kleiner,D.E.,Dam-Larsen,S.,Adams,L.A.,Bjornsson,E.S.,Charatcharoenwithaya, P., Bendtsen, F. (2015). Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. *Gastroenterology*, 149(2), 389–97.e10. doi:10.1053/j.gastro.2015.04.043 [doi]
52. Ekstedt,M., Hagstrom,H., Nasr,P., Fredrikson,M., Stal,P., Kechagias,S.,& Hultcrantz, R. (2015). Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* (Baltimore, Md.), 61(5), 1547–1554.doi:10.1002/hep.27368 [doi]
53. Simons,L.A., Simons,J., Friedlander, Y., & McCallum,J. (2011). Is prediction of cardiovascular disease and all-cause mortality genuinely driven by the metabolic syndrome, and independently from its component variables? the dubbo study. *Heart & Lung & Circulation*, 20(4),214–219.doi:10.1016/j.hlc.2010.12.005 [doi]
54. Ballestri,S., Zona,S., Targher,G.,Romagnoli,D., Baldelli,E.,Nascimbeni,F,...Lonardo, A.(2016). Non alcoholic fatty liver disease is associated with an almost two fold increased risk of incident type 2 diabetes and metabolic syndrome. evidence from asystematic review and meta-analysis. *Journal of Gastroenterology and Hepatology*,31(5),936–944.doi:10.1111/jgh.13264 [doi]
55. Lonardo, A., Sookoian, S., Pirola, C. J., & Targher, G. (2016). Non-alcoholic fatty liver disease and risk of cardiovascular disease. *Metabolism: Clinical and Experimental*,65(8),1136–1150.doi:10.1016/j.metabol.2015.09.017 [doi]
56. Fracanzani,A.L., Tiraboschi,S., Pisano,G., Consonni,D., Baragetti,A., Bertelli,C,... Fargion,S. (2016). Progression of carotid vascular damage and cardiovascular events in non-alcoholic fatty liver disease patients compared to the general population during 10 years of follow-up. *Atherosclerosis*,246,208–213.doi:10.1016/j.atherosclerosis.2016.01.016[doi]
57. Sanna,C.,Rosso,C.,Marietti,M.,&Bugianesi,E.(2016) . Non-alcoholic fatty liver disease and extra-hepatic cancers. *International Journal of Molecular Sciences*,17(5),10.3390/ijms17050717.doi:10.3390/ijms17050717 [doi]
58. Han,P., Sun,D., & Yang,J.(2016).Interaction between periodontitis and liverdiseases. *BiomedicalReports*,5(3),267–276.doi:10.3892/br.2016.718[doi]
59. Mantovani, A., Gisondi, P., Lonardo, A., &Targher, G. (2016). Relationship between non-alcoholic fatty liver disease and psoriasis: A novel hepato-dermal axis? *International Journal of Molecular Sciences*, 17(2), 217. doi:10.3390/ijms17020217 [doi]
60. Reilly,N.R., Lebowhl,B., Hultcrantz,R., Green,P.H., & Ludvigsson,J.F.(2015). Increased risk of non-alcoholic fatty liver disease after diagnosis of celiac disease. *Journal of Hepatology*,62(6),1405–

- 1411.doi:10.1016/j.jhep.2015.01.013[doi]
61. Byrne,C.D., & Targher,G.(2015). NAFLD:A multisystem disease .Journal of Hepatology,62(1Suppl), S47–64.doi:10.1016/j.jhep.2014.12.012 [doi]
62. Ratzu,V., Harrison,S.A., Francque,S., Bedossa,P., Lehert,P., Serfaty,L,...GOLDEN-505 Investigator Study Group.(2016) .Ela fibrinor ,anagonist of the peroxisome proliferator-activated receptor-alpha and -delta, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. Gastroenterology, 150(5), 1147–1159.e5. doi:10.1053/j.gastro.2016.01.038[doi]
63. Armstrong,M.J., Gaunt,P., Aithal,G.P., Barton,D., Hull,D.,Parker,R,...Newsome,P. N.(2016). Lira glutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): A multicentre,double-blind, randomised, placebo-controlled phase 2 study. Lancet (London,England), 387(10019), 679–690.doi:10.1016/S0140–6736(15)00803-X[doi]
64. Barb,D., Portillo-Sanchez,P., & Cusi,K.(2016). Pharmacological management of nonalcoholic fatty liver disease. Metabolism: Clinical and Experimental, 65(8), 1183–1195.doi:10.1016/j.metabol.2016.04.004 [doi]
65. Ijssennagger, N., Janssen, A. W., Milona, A., Ramos Pittol, J. M., Hollman, D. A., Mokry,M,...Kersten,S.(2016). Gene expression profiling in human precision cut liver slices in response to the FXR agonist obeticholic acid. Journal of Hepatology, 64(5), 1158–1166.doi:10.1016/j.jhep.2016.01.016 [doi]