**Name of journal:** ***World Journal of Gastroenterology***

**Manuscript NO: 47672**

**Manuscript Type: MINIREVIEWS**

**Biomarkers and subtypes of deranged lipid metabolism in non-alcoholic fatty liver disease**

Mato JM *et al*. Biomarkers and subtypes of NAFLD

José M Mato, Cristina Alonso, Mazen Noureddin, Shelly C Lu

**José M Mato,** CIC bioGUNE, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), Technology Park of Bizkaia, Derio 48160, Bizkaia, Spain

**Cristina Alonso,** OWL Metabolomics, Technology Park of Bizkaia, Derio 48160, Bizkaia, Spain

**Mazen Noureddin, Shelly C Lu,** Division of Digestive and Liver Diseases, Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA 90048, United States

**ORCID number:** José M Mato (0000-0003-1264-3153); Cristina Alonso (0000-0002-2019-678X); Mazen Noureddin (0000-0003-2127-2040); Shelly C Lu (0000-0003-2128-5407).

**Author contributions:** All authors contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

**Conflict-of-interest statement:** The authors have declared no conflicts of interest.

**Open-Access:** This is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Invited manuscript

**Corresponding author: José M Mato, PhD, Director, Professor,** CIC bioGUNE, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), Technology Park of Bizkaia, Derio 48160, Bizkaia, Spain. director@cicbiogune.es

**Telephone:** +34-946-572517

**Fax:** +34-944-061-301

**Received:** March 22, 2019

**Peer-review started:** March 22, 2019

**First decision:** April 11, 2019

**Revised:** May 6, 2019

**Accepted:** May 18, 2019

**Article in press:**

**Published online:**

**Abstract**

Nonalcoholic fatty liver disease (NAFLD) is a heterogeneous and complex disease that is imprecisely diagnosed by liver biopsy. NAFLD covers a spectrum that ranges from simple steatosis, nonalcoholic steatohepatitis (NASH) with varying degrees of fibrosis, to cirrhosis, which is a major risk factor for hepatocellular carcinoma. Lifestyle and eating habit changes during the last century have made NAFLD the most common liver disease linked to obesity, type 2 diabetes mellitus and dyslipidemia, with a global prevalence of 25%. NAFLD arises when the uptake of fatty acids (FA) and triglycerides (TG) from circulation and de novo lipogenesis saturate the rate of FA β-oxidation and very-low density lipoprotein (VLDL)-TG export. Deranged lipid metabolism is also associated with NAFLD progression from steatosis to NASH, and therefore, alterations in liver and serum lipidomic signatures are good indicators of the disease’s development and progression. This review focuses on the importance of the classification of NAFLD patients into different subtypes, corresponding to the main alteration(s) in the major pathways that regulate FA homeostasis leading, in each case, to the initiation and progression of NASH. This concept also supports the targeted intervention as a key approach to maximize therapeutic efficacy and opens the door to the development of precise NASH treatments.

**Key words:** S-adenosylmethionine; Methionine adenosyltransferase; Lipid metabolism; Multiomics; Lipidomics; Nonalcoholic steatohepatitis; One-carbon metabolism; Very low-density lipoproteins; Steatosis; Precision medicine

**© The Author(s) 2019.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Nonalcoholic fatty liver disease (NAFLD) is a heterogeneous and complex disease that is imprecisely diagnosed by liver biopsy. The advent of metabolomics has shown that NAFLD progression from simple steatosis to nonalcoholic steatohepatitis (NASH) associates with profound alterations in liver and serum lipidomic signatures that are good indicators of the disease’s development and progression. Lipidomics has also permitted the classification of NAFLD patients into different subtypes corresponding to the main alteration(s) leading, in each case, to the initiation and progression of NASH based on the identification of specific lipid signatures, opening the door to the development of precise NASH treatments.

Mato JM, Alonso C, Noureddin M, Lu SC. Biomarkers and subtypes of deranged lipid metabolism in non-alcoholic fatty liver disease. *World J Gastroenterol* 2019; In press

**INTRODUCTION**

Fat storing is common to many different species. The desert locust stores lipids in the “fat body”, a dynamic tissue that plays an essential role in energy storage and utilization in insects[1], to migrate from south-western Morocco to the Iberian Peninsula covering a distance of 600 miles without settling down. Some fish also store fat for their survival. Without eating and powered only by stored fat, salmons swim 2000 miles up the fresh waters of the Yukon River from the Bering Sea to reach their spawning grounds. Long distance migrating birds, such as the bar-tailed godwit, the ruby-throated hummingbirds and the bar-headed geese, accumulate large amounts of fat prior to departing. Likewise, the gray whale increases its fat stores prior to swimming more than 10000 miles between feeding grounds in the Artic to the nursery lagoons of Mexico´s Baja Peninsula; and hibernating mammals such as the grizzly bears, after a period of incomparable hyperphagia, do not eat for 5 to 7 mo subsisting solely on stored fat.

The energy source for these prodigious feats are fatty acids (FA) stored as triglycerides (TG) into lipid droplets (LD) primarily in the adipose tissue and liver. The mobilization of FA from adipose tissue TG stores requires the activity of TG lipases that generate FA, which are then released into the blood and taken up by hepatocytes, where are reincorporated into TG (Figure 1). Some of these re-esterified TG combine with apolipoprotein-B (APOB) to form very low-density lipoproteins (VLDL), and are exported into circulation. This process is regulated by microsomal TG transfer protein (MTTP) and accompanied by encapsulating the neutral lipid core with a phospholipid (PL) monolayer enriched in phosphatidylcholine (PC) molecules containing polyunsaturated FA (PUFA), such as arachidonic acid (AA; 20:4n-6) and docosahexaenoic acid (DHA; 22:6n-3)[2,3]. APOB, cholesterol and other apolipoproteins (like APOC) are also found decorating the surface of the VLDL-TG particle[2,3]. The largest amount of TG used for the synthesis of VLDL (VLDL-TG) is synthesized from FA entering the liver from the adipose tissue, even under conditions where the synthesis of FA from glucose and fructose by *de novo* lipogenesis (DNL) is high (see below). Humans preferentially oxidize carbohydrate over fat, a process that helps to maintain blood glucose homeostasis. Most of the TG in circulation during the post-absorptive phase are associated with VLDL-TG[2]. This mechanism uncouples hepatic TG synthesis (energy storing) from TG secretion and maintains a low blood content of FA, which are cytotoxic.

**NONALCOHOLIC FATTY LIVER DISEASE**

TG are energy dense and chemical stable compounds. By weight, FA provide more than twice as much energy (9 kcal/g) as carbohydrates and proteins (4 kcal/g), and match the caloric density of diesel (8 kcal/g). From this perspective, fatty liver may be considered a physiological adaptation and an evolutionary advantage to anticipate periods of prolonged food (energy) shortage. However, lifestyle and eating habit changes during the last century have made fatty liver the most common liver disease linked to obesity, type 2 diabetes mellitus (T2D) and dyslipidemia, with a prevalence of 25%[4-7]. Nonalcoholic fatty liver disease (NAFLD) covers a spectrum that ranges from simple steatosis (NAFL), nonalcoholic steatohepatitis (NASH) with varying degrees of fibrosis, to cirrhosis, which is a major risk factor for hepatocellular carcinoma (HCC). NASH is distinguished from steatosis by the presence of inflammation and hepatocyte injury. Approximately 25% of individuals with NAFL progress to NASH. Of those that develop NASH, 25% progress to cirrhosis, of whom at least 1%-2% per year develop HCC[4-6]. NASH is now the leading cause of liver transplantation in women[8] and projected to be the leading indication in the United States by 2020[4-6]. Degree of liver fibrosis is the major factor linked to all-cause mortality[9]. However, NAFLD does not always follow an orderly progression. For instance, it is possible for NAFLD patients to develop fibrosis without going through the NASH stage, or to develop liver cancer despite absence of fibrosis or histologic NASH[4-6,10]. Studies have reported 10-70% of HCC cases in NAFLD occurred without cirrhosis[11]. The annual direct medical cost is > $100 billion in the United States alone for NAFLD[4-6]. Despite the huge investment by the pharmaceutical industry there are still no approved therapies targeting NASH[12]. Lifestyle changes are the only therapeutic strategy that can halt the progression of NAFLD[4-6]. Clearly, both a better understanding of the factors that promote progression from simple steatosis to NASH, fibrosis and liver cancer is sorely needed to improve our therapeutic strategy.

**LIVER LIPID METABOLISM IN NAFLD**

Consisting with its energy storage function, the relationship between the intrahepatic TG (IHTG) content and VLDL-TG secretion rate is curvilinear. In subjects with normal IHTG (up to 5% of liver weight), VLDL-TG export increases linearly with IHTG content; but in individuals with steatosis, VLDL-TG secretion reaches a plateau independently of the amount of IHTG[13,14]. Genetic defects (*APOB, APOC3, MTTP, TM6SF2*) that impair hepatic VLDL-TG secretion cause hepatic steatosis that may progress to NASH with fibrosis, even without obesity or T2D[15-19]; and impaired APOB synthesis has been observed in NASH patients as compared to obese controls[20]. These results indicate that a reduction in the capacity to export VLDL-TG increases the risk to develop NASH. Consistently, patients treated with antisense APOB or MTTP inhibitors, which lower VLDL assembly and secretion, are associated with hepatic steatosis, inflammation and fibrosis, which limit their utility[21,22]. The discovery that the effect of defective VLDL-TG secretion extends well beyond the management of liver energy storage to promote the development of NASH and fibrogenesis emphasizes the importance of identifying therapeutic targets for NASH reversal in the setting of impaired VLDL-TG secretion. It is important to note, however, that the increase in susceptibility to develop NASH in obese subjects that are carriers of the TM6SF2 E167K variant, which impairs VLDL-TG export, is accompanied by protection from cardiovascular disease due to the reduced serum levels of atherogenic lipoproteins[23]. This is important when designing treatments that aim to increase VLDL-TG export in NASH.

Hepatic steatosis arises when the uptake of FA and TG from circulation and DNL saturate the rate of FA β-oxidation (in the mitochondria and peroxisomes) and VLDL-TG export (Figure 1). NAFLD subjects often show an increase in DNL[13,24], and it has been proposed by many that DNL is a major pathway in the pathogenesis of NAFLD[25]. On this premise the pharmacological inhibition of DNL that include (1) downregulating SREBP-1c, the major transcriptional regulator of the enzymes involved in DNL, (2) decreasing the activity of the DNL rate-limiting enzyme, specifically acetyl-CoA carboxylase (ACC), and (3) inhibiting stearoyl-CoA dehydrogenase 1 (SCD1), the first irreversible step committing FA to TG synthesis, are being studied in phase 2 and 3 clinical trials of NASH[26]. However, a potential limitation of this approach is that a decrease in DNL may induce an increase in FA uptake to the liver from circulation, the major source of hepatic lipids, or a decrease in FA oxidation as compensatory mechanisms[27,28]. From an evolutionary stand point, it seems unlikely that an increase in DNL would be a major pathway in the development of NAFLD. FA from the adipose tissue and from the diet contribute about 59% of TG in the livers of patients with NAFLD, while DNL contributes 26% of intrahepatic FA, and dietary TG transported by chylomicrons 15% of liver fat[29]. Accordingly, the inhibition of liver FA uptake has been shown to improve NASH in experimental models[30]; albeit at the risk of increasing FA in circulation, peripheral FA stores, and weight gain, which may limit its potential therapeutic application. The importance of increased DNL in NASH development should, however, not be minimized since increased DNL may just as well overwhelm a deficient VLDL-TG exporting system which, presumably, is already saturated caused by increased hepatocellular lipid uptake. The increase in DNL in NAFLD may be an adaptive mechanism for the generation of metabolic signals that direct lipids toward beneficial pathways to improve energy balance even in the setting of excess FA accumulation, a concept known as lipoexpediency (the antonym to lipotoxicity[31,32]). For instance, it has been shown that FA synthase, the DNL enzyme that catalyzes the conversion of acetyl-CoA to the 16-carbon FA palmitate, is involved in the activation of PPARα (an activator of FA oxidation that is expressed at high concentrations in the liver) via the synthesis of its ligand, palmitoyl-stearoyl-phosphatidylcholine (PC-16:0/18:1)[33]. NAFLD subjects also show an increase in the rate of hepatic FA oxidation[34,35] because of mitochondrial uncoupling between FA oxidation and ATP synthesis[36]. Increased FA oxidation in NAFLD may be, however, detrimental to the liver due to the excessive generation of reactive oxygen species. Together, these results suggest that different individuals (NAFLD subtypes) could have different alterations in the major pathway(s) that regulate FA homeostasis leading to NAFLD[37]. Evidence from clinical trials indicating that only a small percent (20%-50%) of NASH patients benefit from the different treatments supports this concept[26]. Thus, the identification of noninvasive metabolic biomarkers that would allow the classification of patients into different subtypes that correspond to the main alteration(s) leading to the initiation and progression of NASH would be of great help for the development of precise treatments.

**S-ADENOSLYMETHIONINE AS A LINK BETWEEN LIPID METABOLISM AND HEPATOCELLULAR ONE CARBON METABOLISM**

Assessing the hepatic lipid metabolism, it is important to note that LD are not only critically important for energy metabolism in terms of TG storage, but are also a major supply of (1) PL precursors, such as diacylglycerols (DG) and other lipids of the monoalk(en)yl diacylglycerol family, that give rise to diacyl-PL and plasmalogens, respectively; (2) cholesterol, which is stored as cholesteryl-esters (CE); and (3) FA, not only saturated FA, such as palmitate (16:0) and stearate (18.0), which are cytotoxic, but also PUFA, such as AA, that gives rise to the eicosanoid family of inflammatory mediators (prostaglandins, thromboxanes, and leukotrienes), and DHA, which is anti-inflammatory[38]. The main lipid classes found in the core of liver LD are TG, DG, and CE, which are enveloped by a PL monolayer (mainly made of PC) decorated with proteins that are important in lipid remodeling, signaling and energy storing[39,40]. PC found in LD are synthesized both by the Kennedy route, whose last step is the reaction of CDP-choline with DG to form PC and cytidine monophosphate; and the PE *N*-methyltransferase (PEMT) pathway, which converts PE rich in PUFA (mainly AA and DHA) into PC through three successive *N*-methylations of the PE amino group, with S-adenosylmethionine (SAMe) as the methyl donor[41] (Figure 1). SAMe is a versatile molecule which is the source of essentially all methyl transfer reactions in cells[42]. Liver plays a central role in SAMe metabolism, as this is where up to half of the daily intake of methionine is catabolized via its conversion to SAMe[43]. This reaction is catalyzed by methionine adenosyltransferase (MAT). Two genes encode for MAT, *MAT1A* is expressed in normal differentiated liver and *MAT2A* is expressed in all extrahepatic tissues as well as in fetal liver[43]. In liver, SAMe homeostasis is controlled by MAT-mediated synthesis and utilization, largely accomplished by glycine *N*-methyltransferase (GNMT)[43] (Figure 2). Accordingly, GNMT deletion in mice induces a massive increase in intrahepatic SAMe content[44] that accelerates the flux of methyl groups through multiple pathways, including PEMT and DNA-methylation, leading to aberrant liver lipid signatures, development of NASH, fibrosis and HCC[45].

SAMe metabolism is coupled to the folate cycle and together they form the so called one carbon metabolism (1CM) (Figure 3). 1CM circulates 1-carbon units from different nutritional and amino acids inputs (choline, betaine, folate, glucose, methionine, serine, glycine and threonine), via SAMe and 5-methyltetrahydrofolate (MTHF), into a large variety of outputs, such as PL-, protein- and DNA-methylation, and glutathione (GSH), polyamines, reduced nicotinamide adenine dinucleotide phosphate (NADPH), and nucleotide synthesis, that regulate key biological processes ranging from VLDL-TG export, gene expression and redox homeostasis, to DNA synthesis and cell growth. *Mat1a* knockout (KO) mice have chronically low hepatic SAMe level (75% lower)[46], show reduced content of PC-PUFA (mainly AA and DHA)[37] and, as expected, impaired synthesis and release of VLDL-TG, which leads to the accumulation of TG, DG and FA, accumulation of oxidized FA, oxidative stress, and abnormal hepatic lipid signatures, which trigger the spontaneous development of steatosis and its progression to NASH, fibrosis and HCC[37,43,46]. In *Mat1a* KO mice, low SAMe also associates with increased serum levels of amino acids methionine, serine and glycine; increased hepatic MTHF, decreased GSH content, and altered protein and DNA methylation[37]. *MAT1A* is often downregulated in NAFLD patients with more advanced fibrosis[47]. Consistently, several studies showed human NASH have reduced transmethylation[48], hepatic PC/PE ratio[49], and abnormal VLDL-TG assembly and export[50]. These results suggest that SAMe deficiency may be a critical driver of NASH in a subgroup of NAFLD patients. Importantly, SAMe treatment of the *Mat1a* KO mice after onset of NASH for two months corrected many of the abnormalities, nearly normalized the liver histology, and reduced blood ALT, AST and TG levels without altering cholesterol content[37]. SAMe treatment of rats fed a methionine and choline deficient (MCD) diet, which reduces hepatic SAMe content and induces steatohepatitis, also improved liver histology[51]. Taken together, these results support the concept that 1) a reduction in SAMe is a common driver of NAFLD initiation and progression to NASH in humans, and 2) that NAFLD patients with M-subtype serum metabolomic profile (see below) will likely benefit from SAMe treatment, but this has not yet been examined.

**CIRCULATING BIOMARKERS OF NAFLD**

The advent of lipidomics has taught us that each lipid class (*e.g.*, TG, PC) is made of a multitude of different lipid molecular species varying in the length and number of double bonds of their FA chains[52,53]; and that the lipid homeostatic status is implemented by a large family of FA desaturases and elongases in conjunction with lipases, acyl-transferases, PL and sphingolipid synthesizing enzymes, and phospholipases[54,55]. Changes in lipid signatures (lipid molecular species compositions) can have profound effects on cell function, regulating processes such as oxidative phosphorylation[56]. A sequence variant in PNPLA3 that is strongly associated with NAFLD has been related to TG remodeling and VLDL-TG secretion in hepatocytes[57,58], suggesting that abnormal lipid remodeling may be key to the development and progression of NAFLD. Accordingly, mice modify the liver lipid profile in response to a variety of conditions that induce steatosis and its progression to NASH, such as ablation of methionine adenosyltransferase 1A (*Mat1a*)[46], fasting or feeding a high fat diet[41], or feeding an MCD diet[59]. It has also been observed that the serum lipidomic profile reflects the liver lipidome[37], a finding which supports the search of noninvasive NAFLD biomarkers in blood. At present, liver biopsy is the “gold standard” to diagnose NASH, an invasive, imprecise and expensive procedure with possible complications. As a result, numerous studies have been published aiming to the identification of panels of circulating biomarkers (using genomics, transcriptomics, proteomics and metabolomics) for steatosis, NASH and fibrosis diagnosis, as well as for risk prediction of NAFLD progression and response to therapy[60,61]. Some studies have shown that lipidomic patterns can differentiate between normal liver and NAFLD[62,63]. Interestingly, recent studies also focus on the discrimination between simple steatosis and NASH[64] or the detection of advance fibrosis[65]. However, a burning challenge in NAFLD research is the identification of which patients with NAFLD will develop NASH and, for those with NASH, how fast the disease will progress. At present, it is premature to conclude which of these blood biomarkers, alone or in combination, would be best to precisely and rapidly diagnose the severity of NASH and monitor the liver’s response to treatment[60,61].

**IDENTIFICATION OF NAFLD SUBTYPES**

Despite the current essential role of biopsy for NAFLD diagnosis, its use as a tool for determining the different metabolic pathways that lead to the initiation and progression of NAFLD is certainly limited. Recently, lipidomics has permitted the classification of NAFLD patients into different subtypes corresponding to the main alteration(s) leading, in each case, to the initiation and progression of NASH based on the identification of specific lipid signatures. We identified a unique serum metabolomic profile that distinguished between *Mat1a* KO and wild type (WT) mice and observed, using a large cohort of 535 serum samples from biopsied NAFLD patients, that nearly half of them showed this *Mat1a* KO-type (M-subtype) metabolomic signature[37] (Table 1). Although classification based on this approach is not indicative of disease progression (M-subtype is equally distributed among patients with steatosis and NASH), a small group of serum metabolites that could differentiate simple steatosis from NASH in the *Mat1a* KO and in NAFLD patients was also identified. This work defined, for the first time, the metabolic landscape affected by a chronically reduced hepatic SAMe level and demonstrated key abnormalities that were corrected by SAMe treatment, which led to resolution of NASH.

The MCD diet model is a widely-used murine model of NASH but animals lose weight rapidly, have low serum TG levels, and do not become insulin resistant[66]. The addition of 0.1% methionine (normal diet contains 0.3% methionine) minimizes weight loss and yet mice fed the 0.1MCD diet have low liver SAMe content and developed steatosis, inflammation and fibrosis[59]. The mechanism for steatosis included impaired VLDL-TG secretion and reduced GSH, due to the decrease in SAMe content, the concomitant reduction in the synthesis of PC-PUFA through the PEMT pathway, and increased uptake of FA via CD36. Despite the existence of important differences between both models [(1) the protein content of SCD1 is increased in *Mat1a*KO and decreased in 0.1MCD; and 2) mitochondrial FA β-oxidation is decreased in *Mat1a*KO and increased in 0.1MCD], the reduction in hepatic SAMe content is the common driver of NAFLD initiation and progression to NASH in both of them and, accordingly, NAFLD patients classified as M-subtype were found to have a metabolic profile similar to the 0.1MCD model[59] (Table 1). Treatment of the 0.1MCD mice for two weeks, after the onset of NAFLD, with the SCD1 inhibitor arachidyl amido cholanoic acid (Aramchol, a Phase 2b test drug candidate in a clinical trial for NASH)[67], improved the liver histology[59] (Table 1). Aramchol has been shown to improve the three key pathologies associated to NASH: (1) steatosis, by reducing TG synthesis and increasing VLDL-TG export and FA β-oxidation; (2) inflammation, by decreasing lipotoxicity; and (3) fibrosis, by downregulation of collagen production by stellate cells[59]. We speculate *Mat1a* KO mice, and therefore NAFLD patients with M-subtype serum metabolomic profile, will likely benefit from Aramchol treatment.

Interestingly, nearly all NAFLD patients classified as having a non-M-subtype, according to both the *Mat1a* KO and 0.1MCD metabolomics models of NASH, were found to have a lipidomic signature similar to that found in low-density lipoprotein receptor (*Ldlr*) KO mice fed a high fat diet (HFD)[68] (Table 1). This mouse model (*Ldlr* KO/HFD) shows high serum levels of cholesterol and TG, normal liver SAMe, and develop NASH and fibrosis. Treatment of the *Ldlr* KO/HFD mice for ten weeks, after the onset of NAFLD, with the Farnesoid X Receptor agonist Obeticholic acid (OCA, a Phase 3 test drug candidate in a clinical trial for NASH)[69], nearly normalized the liver histology, reduced blood ALT, AST and TG levels and tended to lower cholesterol content[68]. It would be interesting to determine if in the Aramchol and OCA clinical trials for NASH, patients that responded to treatment were enriched in M- and non-M-subtype, respectively.

However, this approach also results in a certain number of unclassified patients (named as indeterminate)[37,59], which can be inherently linked to the unsupervised classification methodology and validation procedure. Potential integation of other omics data as well as clinical parameters may improve this novel subtyping approach of NAFLD patients, allowing further interpretation of the complex biochemical processes and the heterogeneity of the disease.

**CONCLUSION**

To understand the pathogenesis of NASH, a useful conceptual framework is that the liver’s capacity to accumulate and export TG supports two crucial physiological functions (1) storing highly energetic, but also highly cytotoxic, FA stably as TG, and (2) placing into circulation the right amount of VLDL-TG to meet the energy needs of extrahepatic tissues. Both functions collide when the IHTG content exceeds 5% and hepatocytes must safely handle and accumulate excess FA into TG without increasing the rate of VLDL-TG export[13,14]. The maximum capacity to safely handle FA by the liver in the presence of increasing levels of IHTG may vary between individuals depending on the variable contributions from different molecular pathway(s) that result in TG accumulation; and NASH may develop when this maximum capacity is exceeded. The observation that it is possible for NAFLD patients to develop NASH at various grades of steatosis, supports this notion. However, clinical trials currently designed for the treatment of NASH are based on the mechanism of action of a drug that is administered to patients without confirming if that specific molecular pathway is altered; which is against the view that NASH pathogenesis has diverse drivers. Understandably, no more than 40% of patients in these trials have shown a positive response to treatment[26]. Alternatively, a comprehensive landscape of the main NASH drivers may be obtained, for example, by integrating multiomics data of well-defined mouse models of NASH, for which the efficacy of different drugs have been validated, with the multiomics data of a large cohort of well-characterized NASH patients following a similar procedure to that previously described[37]. Such a strategy would associate patients’ multiomics signatures to specific therapies that could be validated reanalyzing the data of clinical trials where the efficacy of these drugs has been tested. In addition, this approach may allow advances in our understanding of the complex biochemical processes and pathophysiological responses in NAFLD[70,71]. Moreover, it will be also important to integrate gene products, mRNA, proteins and metabolites, with environmental factors, such as diet and life style[72,73]. Finally, this strategy may be extended to the identification of optimal therapeutic drug combinations.

**REFERENCES**

1 **Arrese EL**, Soulages JL. Insect fat body: energy, metabolism, and regulation. *Annu Rev Entomol* 2010; **55**: 207-225 [PMID: 19725772 DOI: 10.1146/annurev-ento-112408-085356]

2 **Gibbons GF**, Wiggins D, Brown AM, Hebbachi AM. Synthesis and function of hepatic very-low-density lipoprotein. *Biochem Soc Trans* 2004; **32**: 59-64 [PMID: 14748713 DOI: 10:1042/]

3 **Sundaram M**, Yao Z. Recent progress in understanding protein and lipid factors affecting hepatic VLDL assembly and secretion. *Nutr Metab* (Lond) 2010; **7**: 35 [PMID: 20423497 DOI: 10.1186/1743-7075-7-35]

4 **Suzuki A**, Diehl AM. Nonalcoholic Steatohepatitis. *Annu Rev Med* 2017; **68**: 85-98 [PMID: 27732787 DOI: 10.1146/annurev-med-051215-031109]

5 **Setiawan VW**, Stram DO, Porcel J, Lu SC, Le Marchand L, Noureddin M. Prevalence of chronic liver disease and cirrhosis by underlying cause in understudied ethnic groups: The multiethnic cohort. *Hepatology* 2016; **64**: 1969-1977 [PMID: 27301913 DOI: 10.1002/hep.28677]

6 **Younossi ZM**. Non-alcoholic fatty liver disease - A global public health perspective. *J Hepatol* 2019; **70**: 531-544 [PMID: 30414863 DOI: 10.1016/j.jhep.2018.10.033]

7 **Trovato FM**, Martines GF, Brischetto D, Catalano D, Musumeci G, Trovato GM. Fatty liver disease and lifestyle in youngsters: diet, food intake frequency, exercise, sleep shortage and fashion. *Liver Int* 2016; **36**: 427-433 [PMID: 26346413 DOI: 10.1111/liv.12957]

8 **Noureddin M**, Vipani A, Bresee C, Todo T, Kim IK, Alkhouri N, Setiawan VW, Tran T, Ayoub WS, Lu SC, Klein AS, Sundaram V, Nissen NN. NASH Leading Cause of Liver Transplant in Women: Updated Analysis of Indications For Liver Transplant and Ethnic and Gender Variances. *Am J Gastroenterol* 2018; **113**: 1649-1659 [PMID: 29880964 DOI: 10.1038/s41395-018-0088-6]

9 **Dulai PS**, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, Sebastiani G, Ekstedt M, Hagstrom H, Nasr P, Stal P, Wong VW, Kechagias S, Hultcrantz R, Loomba R. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. *Hepatology* 2017; **65**: 1557-1565 [PMID: 28130788 DOI: 10.1002/hep.29085]

10 **Cholankeril G**, Patel R, Khurana S, Satapathy SK. Hepatocellular carcinoma in non-alcoholic steatohepatitis: Current knowledge and implications for management. *World J Hepatol* 2017; **9**: 533-543 [PMID: 28469809 DOI: 10.4254/wjh.v9.i11.533]

11 **Michelotti GA**, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 656-665 [PMID: 24080776 DOI: 10.1038/nrgastro.2013.183]

12 **Cassidy S**, Syed BA. Nonalcoholic steatohepatitis (NASH) drugs market. *Nat Rev Drug Discov* 2016; **15**: 745-746 [PMID: 27807356 DOI: 10.1038/nrd.2016.188]

13 **Fabbrini E**, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology* 2008; **134**: 424-431 [PMID: 18242210 DOI: 10.1053/j.gastro.2007.11.038]

14 **Mittendorfer B**, Yoshino M, Patterson BW, Klein S. VLDL Triglyceride Kinetics in Lean, Overweight, and Obese Men and Women. *J Clin Endocrinol Metab* 2016; **101**: 4151-4160 [PMID: 27588438 DOI: 10.1210/jc.2016-1500]

15 **Bonnefont-Rousselot D**, Condat B, Sassolas A, Chebel S, Bittar R, Federspiel MC, Cazals-Hatem D, Bruckert E. Cryptogenic cirrhosis in a patient with familial hypocholesterolemia due to a new truncated form of apolipoprotein B. *Eur J Gastroenterol Hepatol* 2009; **21**: 104-108 [PMID: 19060634 DOI: 10.1097/MEG.0b013e3282ffd9f8]

16 **Qin W**, Sundaram M, Wang Y, Zhou H, Zhong S, Chang CC, Manhas S, Yao EF, Parks RJ, McFie PJ, Stone SJ, Jiang ZG, Wang C, Figeys D, Jia W, Yao Z. Missense mutation in APOC3 within the C-terminal lipid binding domain of human ApoC-III results in impaired assembly and secretion of triacylglycerol-rich very low density lipoproteins: evidence that ApoC-III plays a major role in the formation of lipid precursors within the microsomal lumen. *J Biol Chem* 2011; **286**: 27769-27780 [PMID: 21676879 DOI: 10.1074/jbc.M110.203679]

17 **Di Filippo M**, Moulin P, Roy P, Samson-Bouma ME, Collardeau-Frachon S, Chebel-Dumont S, Peretti N, Dumortier J, Zoulim F, Fontanges T, Parini R, Rigoldi M, Furlan F, Mancini G, Bonnefont-Rousselot D, Bruckert E, Schmitz J, Scoazec JY, Charrière S, Villar-Fimbel S, Gottrand F, Dubern B, Doummar D, Joly F, Liard-Meillon ME, Lachaux A, Sassolas A. Homozygous MTTP and APOB mutations may lead to hepatic steatosis and fibrosis despite metabolic differences in congenital hypocholesterolemia. *J Hepatol* 2014; **61**: 891-902 [PMID: 24842304 DOI: 10.1016/j.jhep.2014.05.023]

18 **Kozlitina J**, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjærg-Hansen A, Vogt TF, Hobbs HH, Cohen JC. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014; **46**: 352-356 [PMID: 24531328 DOI: 10.1038/ng.2901]

19 **Dongiovanni P**, Romeo S, Valenti L. Genetic Factors in the Pathogenesis of Nonalcoholic Fatty Liver and Steatohepatitis. *Biomed Res Int* 2015; **2015**: 460190 [PMID: 26273621 DOI: 10.1155/2015/460190]

20 **Jiang ZG**, Robson SC, Yao Z. Lipoprotein metabolism in nonalcoholic fatty liver disease. *J Biomed Res* 2013; **27**: 1-13 [PMID: 23554788 DOI: 10.7555/JBR.27.20120077]

21 **Cuchel M**, Bloedon LT, Szapary PO, Kolansky DM, Wolfe ML, Sarkis A, Millar JS, Ikewaki K, Siegelman ES, Gregg RE, Rader DJ. Inhibition of microsomal triglyceride transfer protein in familial hypercholesterolemia. *N Engl J Med* 2007; **356**: 148-156 [PMID: 17215532 DOI: 10.1056/NEJMoa061189]

22 **Cuchel M**, Bruckert E, Ginsberg HN, Raal FJ, Santos RD, Hegele RA, Kuivenhoven JA, Nordestgaard BG, Descamps OS, Steinhagen-Thiessen E, Tybjærg-Hansen A, Watts GF, Averna M, Boileau C, Borén J, Catapano AL, Defesche JC, Hovingh GK, Humphries SE, Kovanen PT, Masana L, Pajukanta P, Parhofer KG, Ray KK, Stalenhoef AF, Stroes E, Taskinen MR, Wiegman A, Wiklund O, Chapman MJ; European Atherosclerosis Society Consensus Panel on Familial Hypercholesterolaemia. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *Eur Heart J* 2014; **35**: 2146-2157 [PMID: 25053660 DOI: 10.1093/eurheartj/ehu274]

23 **Dongiovanni P**, Petta S, Maglio C, Fracanzani AL, Pipitone R, Mozzi E, Motta BM, Kaminska D, Rametta R, Grimaudo S, Pelusi S, Montalcini T, Alisi A, Maggioni M, Kärjä V, Borén J, Käkelä P, Di Marco V, Xing C, Nobili V, Dallapiccola B, Craxi A, Pihlajamäki J, Fargion S, Sjöström L, Carlsson LM, Romeo S, Valenti L. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* 2015; **61**: 506-514 [PMID: 25251399 DOI: 10.1002/hep.27490]

24 **Diraison F**, Moulin P, Beylot M. Contribution of hepatic de novo lipogenesis and reesterification of plasma non esterified fatty acids to plasma triglyceride synthesis during non-alcoholic fatty liver disease. *Diabetes Metab* 2003; **29**: 478-485 [PMID: 14631324 DOI: 10.1016/S1262-3636(07)70061-7]

25 **Lambert JE**, Ramos-Roman MA, Browning JD, Parks EJ. Increased de novo lipogenesis is a distinct characteristic of individuals with nonalcoholic fatty liver disease. *Gastroenterology* 2014; **146**: 726-735 [PMID: 24316260 DOI: 10.1053/j.gastro.2013.11.049]

26 **Friedman SL**, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 2018; **24**: 908-922 [PMID: 29967350 DOI: 10.1038/s41591-018-0104-9]

27 **Solinas G**, Borén J, Dulloo AG. De novo lipogenesis in metabolic homeostasis: More friend than foe? *Mol Metab* 2015; **4**: 367-377 [PMID: 25973385 DOI: 10.1016/j.molmet.2015.03.004]

28 **Sanders FWB**, Acharjee A, Walker C, Marney L, Roberts LD, Imamura F, Jenkins B, Case J, Ray S, Virtue S, Vidal-Puig A, Kuh D, Hardy R, Allison M, Forouhi N, Murray AJ, Wareham N, Vacca M, Koulman A, Griffin JL. Hepatic steatosis risk is partly driven by increased de novo lipogenesis following carbohydrate consumption. *Genome Biol* 2018; **19**: 79 [PMID: 29925420 DOI: 10.1186/s13059-018-1439-8]

29 **Donnelly KL**, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005; **115**: 1343-1351 [PMID: 15864352 DOI: 10.1172/JCI200523621]

30 **Stremmel W**, Staffer S, Wannhoff A, Pathil A, Chamulitrat W. Plasma membrane phospholipase A2 controls hepatocellular fatty acid uptake and is responsive to pharmacological modulation: implications for nonalcoholic steatohepatitis. *FASEB J* 2014; **28**: 3159-3170 [PMID: 24719358 DOI: 10.1096/fj.14-249763]

31 **Virtue S**, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the Metabolic Syndrome--an allostatic perspective. *Biochim Biophys Acta* 2010; **1801**: 338-349 [PMID: 20056169 DOI: 10.1016/j.bbalip.2009.12.006]

32 **Lodhi IJ**, Wei X, Semenkovich CF. Lipoexpediency: de novo lipogenesis as a metabolic signal transmitter. *Trends Endocrinol Metab* 2011; **22**: 1-8 [PMID: 20889351 DOI: 10.1016/j.tem.2010.09.002]

33 **Chakravarthy MV**, Lodhi IJ, Yin L, Malapaka RR, Xu HE, Turk J, Semenkovich CF. Identification of a physiologically relevant endogenous ligand for PPARalpha in liver. *Cell* 2009; **138**: 476-488 [PMID: 19646743 DOI: 10.1016/j.cell.2009.05.036]

34 **Bugianesi E**, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S, Ponti V, Pagano G, Ferrannini E, Rizzetto M. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. *Diabetologia* 2005; **48**: 634-642 [PMID: 15747110 DOI: 10.1007/s00125-005-1682-x]

35 **Marra F**, Gastaldelli A, Svegliati Baroni G, Tell G, Tiribelli C. Molecular basis and mechanisms of progression of non-alcoholic steatohepatitis. *Trends Mol Med* 2008; **14**: 72-81 [PMID: 18218340 DOI: 10.1016/j.molmed.2007.12.003]

36 **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 [PMID: 11266382 DOI: 10.1053/gast.2001.23256]

37 **Alonso C**, Fernández-Ramos D, Varela-Rey M, Martínez-Arranz I, Navasa N, Van Liempd SM, Lavín Trueba JL, Mayo R, Ilisso CP, de Juan VG, Iruarrizaga-Lejarreta M, delaCruz-Villar L, Mincholé I, Robinson A, Crespo J, Martín-Duce A, Romero-Gómez M, Sann H, Platon J, Van Eyk J, Aspichueta P, Noureddin M, Falcón-Pérez JM, Anguita J, Aransay AM, Martínez-Chantar ML, Lu SC, Mato JM. Metabolomic Identification of Subtypes of Nonalcoholic Steatohepatitis. *Gastroenterology* 2017; **152**: 1449-1461.e7 [PMID: 28132890 DOI: 10.1053/j.gastro.2017.01.015]

38 **Calder PC**. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol* 2013; **75**: 645-662 [PMID: 22765297 DOI: 10.1111/j.1365-2125.2012.04374.x]

39 **Su W**, Wang Y, Jia X, Wu W, Li L, Tian X, Li S, Wang C, Xu H, Cao J, Han Q, Xu S, Chen Y, Zhong Y, Zhang X, Liu P, Gustafsson JÅ, Guan Y. Comparative proteomic study reveals 17β-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. *Proc Natl Acad Sci USA* 2014; **111**: 11437-11442 [PMID: 25028495 DOI: 10.1073/pnas.1410741111]

40 **Welte MA**, Gould AP. Lipid droplet functions beyond energy storage. *Biochim Biophys Acta Mol Cell Biol Lipids* 2017; **1862**: 1260-1272 [PMID: 28735096 DOI: 10.1016/j.bbalip.2017.07.006]

41 **Chitraju C**, Trötzmüller M, Hartler J, Wolinski H, Thallinger GG, Lass A, Zechner R, Zimmermann R, Köfeler HC, Spener F. Lipidomic analysis of lipid droplets from murine hepatocytes reveals distinct signatures for nutritional stress. *J Lipid Res* 2012; **53**: 2141-2152 [PMID: 22872753 DOI: 10.1194/jlr.M028902]

42 **Walsh CT**, Tu BP, Tang Y. Eight Kinetically Stable but Thermodynamically Activated Molecules that Power Cell Metabolism. *Chem Rev* 2018; **118**: 1460-1494 [PMID: 29272116 DOI: 10.1021/acs.chemrev.7b00510]

43 **Lu SC**, Mato JM. S-adenosylmethionine in liver health, injury, and cancer. *Physiol Rev* 2012; **92**: 1515-1542 [PMID: 23073625 DOI: 10.1152/physrev.00047.2011]

44 **Luka Z**, Capdevila A, Mato JM, Wagner C. A glycine N-methyltransferase knockout mouse model for humans with deficiency of this enzyme. *Transgenic Res* 2006; **15**: 393-397 [PMID: 16779654 DOI: 10.1007/s11248-006-0008-1]

45 **Martínez-Chantar ML**, Vázquez-Chantada M, Ariz U, Martínez N, Varela M, Luka Z, Capdevila A, Rodríguez J, Aransay AM, Matthiesen R, Yang H, Calvisi DF, Esteller M, Fraga M, Lu SC, Wagner C, Mato JM. Loss of the glycine N-methyltransferase gene leads to steatosis and hepatocellular carcinoma in mice. *Hepatology* 2008; **47**: 1191-1199 [PMID: 18318442 DOI: 10.1002/hep.22159]

46 **Lu SC**, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, Avila MA, Kanel G, Mato JM. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. *Proc Natl Acad Sci USA* 2001; **98**: 5560-5565 [PMID: 11320206 DOI: 10.1073/pnas.091016398]

47 **Moylan CA**, Pang H, Dellinger A, Suzuki A, Garrett ME, Guy CD, Murphy SK, Ashley-Koch AE, Choi SS, Michelotti GA, Hampton DD, Chen Y, Tillmann HL, Hauser MA, Abdelmalek MF, Diehl AM. Hepatic gene expression profiles differentiate presymptomatic patients with mild versus severe nonalcoholic fatty liver disease. *Hepatology* 2014; **59**: 471-482 [PMID: 23913408 DOI: 10.1002/hep.26661]

48 **Kalhan SC**, Edmison J, Marczewski S, Dasarathy S, Gruca LL, Bennett C, Duenas C, Lopez R. Methionine and protein metabolism in non-alcoholic steatohepatitis: evidence for lower rate of transmethylation of methionine. *Clin Sci* (Lond) 2011; **121**: 179-189 [PMID: 21446920 DOI: 10.1042/CS20110060]

49 **Li Z**, Agellon LB, Allen TM, Umeda M, Jewell L, Mason A, Vance DE. The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. *Cell Metab* 2006; **3**: 321-331 [PMID: 16679290 DOI: 10.1016/j.cmet.2006.03.007]

50 **Fujita K**, Nozaki Y, Wada K, Yoneda M, Fujimoto Y, Fujitake M, Endo H, Takahashi H, Inamori M, Kobayashi N, Kirikoshi H, Kubota K, Saito S, Nakajima A. Dysfunctional very-low-density lipoprotein synthesis and release is a key factor in nonalcoholic steatohepatitis pathogenesis. *Hepatology* 2009; **50**: 772-780 [PMID: 19650159 DOI: 10.1002/hep.23094]

51 **Oz HS**, Im HJ, Chen TS, de Villiers WJ, McClain CJ. Glutathione-enhancing agents protect against steatohepatitis in a dietary model. *J Biochem Mol Toxicol* 2006; **20**: 39-47 [PMID: 16498637 DOI: 10.1002/jbt.20109]

52 **Psychogios N**, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, Young N, Xia J, Knox C, Dong E, Huang P, Hollander Z, Pedersen TL, Smith SR, Bamforth F, Greiner R, McManus B, Newman JW, Goodfriend T, Wishart DS. The human serum metabolome. *PLoS One* 2011; **6**: e16957 [PMID: 21359215 DOI: 10.1371/journal.pone.0016957]

53 **Quehenberger O**, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, Bandyopadhyay S, Jones KN, Kelly S, Shaner RL, Sullards CM, Wang E, Murphy RC, Barkley RM, Leiker TJ, Raetz CR, Guan Z, Laird GM, Six DA, Russell DW, McDonald JG, Subramaniam S, Fahy E, Dennis EA. Lipidomics reveals a remarkable diversity of lipids in human plasma. *J Lipid Res* 2010; **51**: 3299-3305 [PMID: 20671299 DOI: 10.1194/jlr.M009449]

54 **Guillou H**, Zadravec D, Martin PG, Jacobsson A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice. *Prog Lipid Res* 2010; **49**: 186-199 [PMID: 20018209 DOI: 10.1016/j.plipres.2009.12.002]

55 **Lee JM**, Lee H, Kang S, Park WJ. Fatty Acid Desaturases, Polyunsaturated Fatty Acid Regulation, and Biotechnological Advances. *Nutrients* 2016; **8**: [PMID: 26742061 DOI: 10.3390/nu8010023]

56 **Budin I**, de Rond T, Chen Y, Chan LJG, Petzold CJ, Keasling JD. Viscous control of cellular respiration by membrane lipid composition. *Science* 2018; **362**: 1186-1189 [PMID: 30361388 DOI: 10.1126/science.aat7925]

57 **Ruhanen H**, Perttilä J, Hölttä-Vuori M, Zhou Y, Yki-Järvinen H, Ikonen E, Käkelä R, Olkkonen VM. PNPLA3 mediates hepatocyte triacylglycerol remodeling. *J Lipid Res* 2014; **55**: 739-746 [PMID: 24511104 DOI: 10.1194/jlr.M046607]

58 **BasuRay S**, Smagris E, Cohen JC, Hobbs HH. The PNPLA3 variant associated with fatty liver disease (I148M) accumulates on lipid droplets by evading ubiquitylation. *Hepatology* 2017; **66**: 1111-1124 [PMID: 28520213 DOI: 10.1002/hep.29273]

59 **Iruarrizaga-Lejarreta M**, Varela-Rey M, Fernández-Ramos D, Martínez-Arranz I, Delgado TC, Simon J, Juan VG, delaCruz-Villar L, Azkargorta M, Lavin JL, Mayo R, Van Liempd SM, Aurrekoetxea I, Buqué X, Cave DD, Peña A, Rodríguez-Cuesta J, Aransay AM, Elortza F, Falcón-Pérez JM, Aspichueta P, Hayardeny L, Noureddin M, Sanyal AJ, Alonso C, Anguita J, Martínez-Chantar ML, Lu SC, Mato JM. Role of Aramchol in steatohepatitis and fibrosis in mice. *Hepatol Commun* 2017; **1**: 911-927 [PMID: 29159325 DOI: 10.1002/hep4.1107]

60 **Iruarrizaga-Lejarreta M**, Bril F, Nouredin M, Ortiz P, Lu SC, Mato JM, Alonso C. Emerging circulating biomarkers for the diagnosis and assessment of treatment responses in patients with hepatic fat accumulation, NASH and liver fibrosis. In: Krentz AJ, Weyer C, Hompesch M. Translational Research Methods in Diabetes, Obesity, and Nonalcoholic Fatty Liver Disease. Switzerland: Springer Nature, 2019: 423-448

61 **Pirola CJ**, Sookoian S. Multiomics biomarkers for the prediction of nonalcoholic fatty liver disease severity. *World J Gastroenterol* 2018; **24**: 1601-1615 [PMID: 29686467 DOI: 10.3748/wjg.v24.i15.1601]

62 **Barr J**, Caballería J, Martínez-Arranz I, Domínguez-Díez A, Alonso C, Muntané J, Pérez-Cormenzana M, García-Monzón C, Mayo R, Martín-Duce A, Romero-Gómez M, Lo Iacono O, Tordjman J, Andrade RJ, Pérez-Carreras M, Le Marchand-Brustel Y, Tran A, Fernández-Escalante C, Arévalo E, García-Unzueta M, Clement K, Crespo J, Gual P, Gómez-Fleitas M, Martínez-Chantar ML, Castro A, Lu SC, Vázquez-Chantada M, Mato JM. Obesity-dependent metabolic signatures associated with nonalcoholic fatty liver disease progression. *J Proteome Res* 2012; **11**: 2521-2532 [PMID: 22364559 DOI: 10.1021/pr201223p]

63 **Gitto S**, Schepis F, Andreone P, Villa E. Study of the Serum Metabolomic Profile in Nonalcoholic Fatty Liver Disease: Research and Clinical Perspectives. *Metabolites* 2018; **8**: [PMID: 29495258 DOI: 10.3390/metabo8010017]

64 **Mayo R**, Crespo J, Martínez-Arranz I, Banales JM, Arias M, Mincholé I, Aller de la Fuente R, Jimenez-Agüero R, Alonso C, de Luis DA, Vitek L, Stritesky J, Caballería J, Romero-Gómez M, Martín-Duce A, Mugüerza Huguet JM, Busteros-Moraza JI, Idowu MO, Castro A, Martínez-Chantar ML, Ortiz P, Bruha R, Lu SC, Bedossa P, Noureddin M, Sanyal AJ, Mato JM. Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: Results from discovery and validation cohorts. *Hepatol Commun* 2018; **2**: 807-820 [PMID: 30027139 DOI: 10.1002/hep4.1188]

65 **Caussy C**, Ajmera VH, Puri P, Hsu CL, Bassirian S, Mgdsyan M, Singh S, Faulkner C, Valasek MA, Rizo E, Richards L, Brenner DA, Sirlin CB, Sanyal AJ, Loomba R. Serum metabolites detect the presence of advanced fibrosis in derivation and validation cohorts of patients with non-alcoholic fatty liver disease. *Gut* 2018; : [PMID: 30567742 DOI: 10.1136/gutjnl-2018-317584]

66 **Anstee QM**, Goldin RD. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int J Exp Pathol* 2006; **87**: 1-16 [PMID: 16436109 DOI: 10.1111/j.0959-9673.2006.00465.x]

67 **Leikin-Frenkel A**, Gonen A, Shaish A, Goldiner I, Leikin-Gobbi D, Konikoff FM, Harats D, Gilat T. Fatty acid bile acid conjugate inhibits hepatic stearoyl coenzyme A desaturase and is non-atherogenic. *Arch Med Res* 2010; **41**: 397-404 [PMID: 21044742 DOI: 10.1016/j.arcmed.2010.09.001]

68 **Morrison MC**, Verschuren L, Salic K, Verheij J, Menke A, Wielinga PY, Iruarrizaga-Lejarreta M, Gole L, Yu WM, Turner S, Caspers MPM, Martínez-Arranz I, Pieterman E, Stoop R, van Koppen A, van den Hoek AM, Mato JM, Hanemaaijer R, Alonso C, Kleemann R. Obeticholic Acid Modulates Serum Metabolites and Gene Signatures Characteristic of Human NASH and Attenuates Inflammation and Fibrosis Progression in Ldlr-/-.Leiden Mice. *Hepatol Commun* 2018; **2**: 1513-1532 [PMID: 30556039 DOI: 10.1002/hep4.1270]

69 **Neuschwander-Tetri BA**, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV, McCullough A, Terrault N, Clark JM, Tonascia J, Brunt EM, Kleiner DE, Doo E; NASH Clinical Research Network. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015; **385**: 956-965 [PMID: 25468160 DOI: 10.1016/S0140-6736(14)61933-4]

70 **Mardinoglu A**, Uhlén M. Liver: Phenotypic and genetic variance: a systems approach to the liver. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 439-440 [PMID: 27329803 DOI: 10.1038/nrgastro.2016.93]

71 **Mardinoglu A**, Boren J, Smith U, Uhlen M, Nielsen J. Systems biology in hepatology: approaches and applications. *Nat Rev Gastroenterol Hepatol* 2018; **15**: 365-377 [PMID: 29686404 DOI: 10.1038/s41575-018-0007-8]

72 **Maldonado EM**, Fisher CP, Mazzatti DJ, Barber AL, Tindall MJ, Plant NJ, Kierzek AM, Moore JB. Multi-scale, whole-system models of liver metabolic adaptation to fat and sugar in non-alcoholic fatty liver disease. *NPJ Syst Biol Appl* 2018; **4**: 33 [PMID: 30131870 DOI: 10.1038/s41540-018-0070-3]

73 **Mardinoglu A**, Wu H, Bjornson E, Zhang C, Hakkarainen A, Räsänen SM, Lee S, Mancina RM, Bergentall M, Pietiläinen KH, Söderlund S, Matikainen N, Ståhlman M, Bergh PO, Adiels M, Piening BD, Granér M, Lundbom N, Williams KJ, Romeo S, Nielsen J, Snyder M, Uhlén M, Bergström G, Perkins R, Marschall HU, Bäckhed F, Taskinen MR, Borén J. An Integrated Understanding of the Rapid Metabolic Benefits of a Carbohydrate-Restricted Diet on Hepatic Steatosis in Humans. *Cell Metab* 2018; **27**: 559-571.e5 [PMID: 29456073 DOI: 10.1016/j.cmet.2018.01.005]

**P-Reviewer:** Baffy G, Cardoso CRL Musumeci G

**S-Editor:** Ma RY **L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** Spain

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C, C

Grade D (Fair): 0

Grade E (Poor): 0

**Table 1 Nonalcoholic fatty liver disease subtype classification**

|  |  |  |  |
| --- | --- | --- | --- |
| **NAFLD subtype** | **Characteristics** | **Mouse model-based classification** | **Treatments tested** |
| M-Subtype | Increased fatty acid uptake.Low liver glutathione and SAMe content.Reduced synthesis of PC-PUFA.Abnormal VLDL-TG assembly and export. | *Mat1a* KO[37]0.1MCD[59] | SAMeAramchol |
|  |  |
| Non-M-Subtype | Increased DNL.Normal hepatic SAMe levels.Normal VLDL-TG secretion.High serum levels of cholesterol and TG. | *Ldlr* KO/HFD[68] | Obeticholic acid |

NAFLD: Nonalcoholic fatty liver disease; SAMe: S-adenosylmethionine; PC-PUFA: Phosphatidylcholines containing polyunsaturated fatty acids; VLDL-TG: Very low-density lipoprotein-triglycerides; DNL: *de novo* lipogenesis; KO: Knockout; MCD: Methionine and choline deficient.

****

**Figure 1 Lipid metabolism.** The mobilization of fatty acids (FA) from their triglyceride (TG) storage in the adipose tissue is promoted by TG lipases. The resultant FA are then released into the blood and taken up by hepatocytes. Other sources of hepatic FA are the dietary lipids in chylomicrons and *de novo* lipogenesis induced by carbohydrates. These FA are metabolized by mitochondrial or peroxisomal β-oxidation, accumulated in the cytoplasm inducing lipotoxicity, or subsequently elongated, desaturated and re-esterified for synthesis of complex lipids such us phospholipids (PL), diglycerides or TG. Some of the re-esterified TG are packed into very low-density lipoproteins combined with apolipoprotein-B and exported into circulation. This process is regulated by microsomal triglyceride transfer protein and accompanied by encapsulating the neutral lipid core with a PL monolayer enriched in phosphatidylcholine molecules containing polyunsaturated FA. Enzyme reactions regulated by S-adenosylmethionine (SAMe) and pathways in which SAMe deficiency may lead to the accumulation of TG and progression to nonalcoholic steatohepatitis are indicated in blue. APOB: Apolipoprotein-B; DG: Diglycerides; ER: Endoplasmic reticulum; FA: Fatty acids; MTTP: Microsomal triglycerides transfer protein; PC-PUFA: Phosphatidylcholines containing polyunsaturated fatty acids; PL: Phospholipids; SAMe: S-adenosylmethionine; TG: Triglycerides; VLDL: Very low-density lipoproteins.



**Figure 2 Regulation of hepatic S-adenosylmethionine homeostasis.** Hepatic S-adenosylmethionine (SAMe) content is regulated by the concerted activity of methionine adenosyltransferase (MAT) and glycine *N*-methyltransferase (GNMT). Methionine is mainly metabolized by the liver where is converted to SAMe by the enzyme MAT using ATP as co-substrate. SAMe, the main cellular methyl donor, is converted to S-adenosylhomocysteine (SAH) by a legion of methyltransferases (MTs) that catalyze the methylation of multiple substrates (DNA, proteins, phospholipids, small molecules, toxic and waist products). Excess SAMe is catabolized by GNMT, the most abundant hepatic MT, to prevent undesirable methylations. The GNMT-sarcosine dehydrogenase (SDH) pathway recycles the excess of methyl groups via generation of methylene-tetrahydrofolate (CH2-THF) and the methylation of homocysteine to regenerate methionine (not shown) to maintain SAMe homeostasis. SAH is converted to homocysteine, a metabolic crossroad that can be used for the regeneration of methionine (not shown) or the synthesis of glutathione depending on whether the concentration of SAMe is low or high, respectively. SAMe is an allosteric activator of GNMT and an inhibitor of the re-synthesis of methionine *via* the CH2-THF pathway (broken lines). CH2-THF: 5,10-methylene-tetrahydrofolate; Gly: Glycine; GNMT: Glycine *N*-methyltransferase; MAT: Methionine adenosyltransferase; Me-Gly: Methylglycine (sarcosine); Me-R: Methylated product; MTs: Methyltransferases; MTHF: 5-methyltetrahydrofolate; R: Methylation substrate; SAH: S-adenosylhomocysteine; SAMe: S-adenosylmethionine; SDH: Sarcosine dehydrogenase; THF: Tetrahydrofolate.



**Figure 3 Schematic representation of one carbon metabolism.** One carbon metabolism involves multiple physiological processes in which one carbon units circulate from different nutritional and amino acids inputs (choline, betaine, folic acid, glucose, methionine, serine, glycine and threonine), mediated by S-adenosylmethionine and 5-methyltetrahydrofolate, and are converted into a wide variety of outputs, such as the methylation of phospholipids, protein and DNA, and the synthesis of glutathione, polyamines, nucleotides, and reduced nicotinamide adenine dinucleotide phosphate. CH2-THF: Methylene tetrahydrofolate; Gly: Glycine; GSH: Glutathione; Hcy: Homocysteine; Met: Methionine; MTHF: 5-Methyltetrahydrofolate; NAPDH: Reduced nicotinamide adenine dinucleotide phosphate; SAH: S-denosylhomocysteine; SAMe: S-adenosylmethionine; Ser: Serine; THF: Tetrahydrofolate; Thr: Threonine.