Nonalcoholic fatty liver disease (NAFLD) from pathogenesis to treatment concepts in humans

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) comprises hepatic alterations with increased lipid accumulation (steatosis) without or with inflammation (nonalcoholic steatohepatitis, NASH) and/or fibrosis in the absence of other causes of liver disease. NAFLD is developing as a burgeoning health challenge, mainly due to the worldwide obesity and diabetes epidemics.

Scope of review: This review summarizes the knowledge on the pathogenesis underlying NAFLD by focusing on studies in humans and on hypercaloric nutrition, including effects of saturated fat and fructose, as well as adipose tissue dysfunction, leading to hepatic lipotoxicity, abnormal mitochondrial function, and oxidative stress, and highlights intestinal dysbiosis. These mechanisms are discussed in the context of current treatments targeting metabolic pathways and the results of related clinical trials.

Major conclusions: Recent studies have provided evidence that certain conditions, for example, the severe insulin-resistant diabetes (SIRD) subgroup (cluster) and the presence of an increasing number of gene variants, seem to predispose for excessive risk of NAFLD and its accelerated progression. Recent clinical trials have been frequently unsuccessful in halting or preventing NAFLD progression, perhaps partly due to including unselected cohorts in later stages of NAFLD. On the basis of this literature review, this study proposed screening in individuals with the highest genetic or acquired risk of disease progression, for example, the SIRD subgroup, and developing treatment concepts targeting the earliest pathophysiological alterations, namely, adipocyte dysfunction and insulin resistance.

Keywords Fatty liver; Lipotoxicity; Inflammation; Fibrosis; Insulin resistance; Clinical trials

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, with a prevalence approaching 25% in the general population [1] and mainly affecting individuals with obesity and type 2 diabetes mellitus (T2DM) [2]. The spectrum of NAFLD encompasses different abnormalities, ranging from a simple increase in intrahepatic lipid content (steatosis, nonalcoholic fatty liver, NAFL) to nonalcoholic steatohepatitis (NASH) with various degrees of necrotic inflammation, fibrosis, and ultimately cirrhosis [3]. NAFLD is not only associated with an increased risk of hepatocellular carcinoma but also cardiovascular diseases and complications related to T2DM, such as nephropathy and neuropathy [4–6]. Thus, delineating the mechanisms underlying the pathogenesis of NAFLD is crucial to managing NAFLD and its comorbidities.

Concepts in the literature have suggested that NAFLD results from a double-hit lesion [7]. The first hit was considered the intrahepatic accumulation of fatty acids (FA), which increased the vulnerability of the hepatic cells to a variety of secondary insults, leading to inflammation and ultimately fibrosis [8]. Several observations have challenged this view, for example, by showing that inflammation may precede hepatic triacylglycerol (TAG) accumulation and that steatosis may protect from liver damage, suggesting multiple hits acting simultaneously rather than sequentially to drive NAFLD progression [9]. This review addresses mechanisms underlying the development and progression of NAFLD from studies in humans. Furthermore, no pharmacological treatment has been approved for NAFLD [10], but recent evidence from well-designed randomized controlled trials (RCTs) points to the efficacy of certain pharmacological agents already in use to treat T2DM [11–13]. Thus, this review also summarizes current concepts to treat NAFLD in humans.

2. POPULATIONS AT RISK

Despite the general association among obesity, dysglycemia, and NAFLD, the presence of metabolic comorbidities coexisting with NAFLD varies substantially across populations. In a population-based cohort study of more than 4 million individuals, with a median follow-up period of 4.7 years, overweight and obese individuals without further metabolic abnormalities including diabetes, hypertension, and dyslipidemia, presented with a 3.3- and an almost 7-fold higher risk of incident NAFLD, respectively, than their normal-weight counterparts [14]. These findings underline the key role of body weight, before the development of further metabolic abnormalities. By contrast, obesity...
cannot be regarded as the sole criterion for the screening of NAFLD, because the prevalence of nonobese NAFLD varies in the general population from 25% to 50% [15] and the presence of ≥1 metabolic abnormalities even in the absence of obesity doubles the risk for NAFLD [14]. However, even among individuals with known diabetes, the prevalence of NAFLD may differ markedly.

A recent analysis of the German Diabetes Study (GDS) confirmed the presence of 5 diabetes subgroups (clusters) [18] by comprehensive phenotyping of 1105 humans with newly diagnosed diabetes. These subgroups comprised in addition to severe autoimmune diabetes, age-related diabetes, mild obesity-related diabetes, severe insulin-deficient diabetes, and severe insulin-resistant diabetes (SIRD), classically termed T2DM [4]. Members of the SIRD are not only characterized by high body mass index (BMI) and the highest degree of whole-body and adipose tissue insulin resistance but also the highest hepatocellular TAG content (assessed using magnetic resonance spectroscopy, MRS) at times of diagnosis. Notably, the SIRD group also shows evidence of mild fibrosis at the 5-year follow-up [4]. This subgroup further exhibits a more frequent prevalence of the rs738409(G) polymorphism in the patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene, which was associated with augmented adipose tissue lipolysis and insulin resistance [17]. This points to a complex interaction between genetic and lifestyle factors in the development of NAFLD.

The importance of discriminating between populations at risk is further underlined by differences in the risk of long-term complications, specifically when comparing NAFLD cohorts presenting with or without obesity and metabolic syndrome. For instance, humans with NAFLD and metabolic syndrome have a greater risk for incident T2DM and cardiovascular events as well as a higher left ventricular mass index during a follow-up of 16 years, than humans with hepatic steatosis only do [18]. In line with these findings, a recent meta-analysis reported significantly lower rates of hypertension, lower uric acid, and fasting plasma glucose and a higher level of high-density lipoprotein among humans who are lean/nonobese with NAFLD than for those who are obese with NAFLD [19]. When body composition is categorized according to BMI and waist circumference, a higher risk for cardiovascular mortality is observed in humans with NAFLD who have a normal BMI but are characterized as obese by waist circumference than in those who are overweight (by BMI) with NAFLD with normal waist circumference [20]. Nevertheless, a high risk of new-onset cardiovascular disease of 18.7 per 1000 persons-years was reported in individuals with lean or nonobese NAFLD, underlining the severity of the condition even in the absence of obesity [15].

3. GENE VARIANTS

Human genetic analyses have provided evidence for some degree of heritability of NAFLD, which could at least partly explain the broad variability in phenotypes and risk of disease progression [21]. The most commonly described genetic variants associated with NAFLD have been identified in the PNPLA3, transmembrane 6 superfamily member 2 (TM6SF2), and glucokinase regulatory protein (GCKR) genes (Table 1) [22—26]. Different PNPLA3 gene alleles have been shown to either confer susceptibility (rs738409(G), encoding I148M, Hispanics), or protection from NAFLD (rs6006460[T], encoding S453I, African-American populations) [27]. Importantly, the presence of the mutant I148M seems to increase NAFLD risk, specifically in the context of body weight gain [28]. Many candidate gene studies of ethnic groups have reported on the association with histological severity, including hepatic fibrosis [23,29—35]. A cross-sectional study of 42 pairs of
Table 1 — Studies targeting specific genetic polymorphisms and large epidemiological genome-wide association studies, which evaluated the association of gene variants with histological severity of human NAFLD.

| Gene       | Function                                         | Polymorphism | MAF  | Protein variant | Hepatic effect of variant | Steatosis | Inflammation | Fibrosis | Ref.       |
|------------|--------------------------------------------------|--------------|------|-----------------|---------------------------|-----------|--------------|----------|------------|
| PNPLA3     | Hepatic TAG composition                          | rs738409     | 0.230| 148M            | ↑ PPARA in TG              | ↑          | ↑            | ↑        | [24,27,30–35,37] |
|            |                                                  | rs2294918 G > A | 0.370| E434K           | PNPLA3 downregulation      | ↑          | ↑ (when combined with 148M) | ↑        | [20]       |
|            |                                                  | rs1260326 C > T | 0.293| P446L           | ↑ G K activity             | ↔/↑       | ↔/↑         | ↑        | [44]       |
| TM6SF2     | Extracellular TAG transport                      | rs5842396 C > T | 0.067| E167K           | ↑ TAG                      | ↑          | ↑ (when combined with 148M) | ↑        | [25,35,38]  |
| GCKR       | Hepatocyte specific glucokinase (GK)             | rs1260326 C > T | 0.293| P446L           | ↑ G K activity             | ↔/↑       | ↔/↑         | ↑        | [44]       |
| IRS1       | Insulin signaling                                | rs1801278     | 0.053| Gly972Arg       | ↑ Akt Thr308 (27%)/Ser473P (21%) | NR        | ↔            | ↑        | [49]       |
| ENPP1      | Insulin receptor activity                        | rs1044498     | 0.342| Lys121Gln       | ↑ Akt Thr308 (31%)/Ser473P (46%) | NR        | ↔            | ↑        | [49]       |
| MBOAT7     | Lipid acyl-chain remodeling                     | rs641738 C > T | 0.40 |                  | ↑ PIPn acyl-chain remodeling | ↑          | ↑            | ↑        | [47,48,55]  |
| HSD17B13   | Enzymatic activity against bioactive lipid species | rs72613567:TA | 0.180| Hepatic         | ↓ HSD17B13 enzymatic activity | ↑          | ↓            | ↓        | [56,57]     |
|            |                                                  |              |      | HSD17B13        | ↓ PNPLA3 mRNA expression   |           |              |          |            |
| SOD2       | Protection from oxidative stress                 | rs6834314 G/G | 0.194|                  | ↑ HSD17B13 enzymatic activity | ↑          | ↔            | ↑        | [58]       |
| UCP2       | Regulation of mitochondrial lipid fluxes and ROS production | rs695386 G > A | 0.264| C47T            | ↑ MnSOD activity           | ↑          | ↔            | ↑        | [50]       |
| MERTK      | HSC activation                                   | rs4374383 G > A | 0.41 |                  | ↑ HSC migration and procollagen expression | ↑          | ↓            | ↓        | [51,53]     |
| LYPAL1     | TG catabolism                                    | rs12137855 C > T | 0.164|                  | ↑                         | ↑          | ↔            | ↑        | [59]       |
| FND5C      | HSC activation                                   | rs3480 A > G | 0.41 |                  | ↓ irisin expression in HSC | ↔          | ↔            | ↓        | [60]       |
|            |                                                  | rs3480 G      | 0.41 |                  | ↑ FND5C mRNA stability     | ↑          | ↔            | ↔        | [61]       |

Studies that provided no evidence of association (either positive or negative) between genetic determinants and liver disease (either steatosis, inflammation or fibrosis); studies addressing fibrogenesis, interferon and tumor necrosis factor production, nuclear receptors and hepatic iron deposition; and studies examining humans with NAFLD-related hepatocellular carcinoma and genetic determinants with a minor allele frequency <0.001 were excluded from this analysis.

AA: arachidonic acid, APOC3: apolipoprotein C3, DM: diabetes mellitus, DNL: de novo lipogenesis, dys.: dysfunctional, ENPP1: ectoenzyme nucleotide pyrophosphate phosphodiesterase, FND5C: fibronectin type III domain-containing protein 5, GCKR: glucokinase regulatory protein, DNL: de novo lipogenesis, HSC: hepatic stellate cells, HSD17B13: hydroxysteroid 17β-dehydrogenase 13, Ins-S: insulin sensitivity, intracell: intracellular, IRS1: insulin receptor substrate 1, LDs: lipid droplets, LYPAL1: lysophospholipase-like 1, MBOAT7: membrane-bound O-acyltransferase domain-containing 7 gene, MERTK: myeloid-epithelial-reproductive tyrosine kinase, metab: metabolism, MnSOD: manganese-dependent superoxide dismutase, mRNA: messenger ribonucleic acid, NR: not reported, PIPn: phosphatidylinositol, PNPLA3: patatin-like phospholipase domain-containing protein 3, P: phosphorylation, Ref.: references, Ser: serine, SOD2: superoxide dismutase 2, TAG: triacylglycerol, Thr: threonine, TM6SF2: transmembrane 6 superfamily member 2, Tg, triglycerides, UCP: uncoupling protein 2.
monzygotic twins and 18 pairs of dizygotic twins was conducted on the heritability of hepatic steatosis and hepatic fibrosis; the assessment used imaging techniques in humans with and without the PNPLA3 risk genotype and revealed no association, which might be because of low statistical power [21]. In another study, humans who were homozygotes for the 148M allele had higher steatosis scores (33.3% ± 4.0%) than those who were heterozygotes for the 148M allele (26.3% ± 3.5%) and 148I allele (14.9% ± 3.9%) [36]. Human studies have suggested that the PNPLA3-148M allele remodels liver TAG by transferring polyunsaturated FA (PUFA) from diacylglycerol (DAG) species to feed phosphatidylcholine synthesis [37] (Figure 1). By contrast, the I148 wild type protein hydrolyses triglycerides from lipid droplets [27], and I148 variant was reported to downregulate PNPLA3 expression, reducing the effect of I148M and the protective I148 variant on lipid droplet formation [28]. The I148 variant was associated with a lower hepatic steatosis score [29]. Therefore, the I148 variant was associated with a lower hepatic steatosis score [29]. Therefore, the I148 variant was associated with a lower hepatic steatosis score [29]. Therefore, the I148 variant was associated with a lower hepatic steatosis score [29]. Therefore, the I148 variant was associated with a lower hepatic steatosis score [29].
triglycerides, independent of age, sex, and BMI, as aforementioned [17]. Although the PNPLA3 polymorphism did not directly associate with insulin sensitivity, the G allele carriers also featured greater adipose tissue insulin resistance, which could contribute to NAFLD via excessive lipolysis and subsequently augmented FA flux to the liver. Among other genes, the most widely studied TM6SF2 variant, rs58542926 [C > T] encoding E167K, is associated with histological severity of steatosis and all the components of the NAFLD activity score [38–40], but in one study [35], not with fibrosis. This variant leads to an inability to mobilize neutral lipids for the very low density (VLDL) lipoprotein assembly in hepatocytes, resulting in increased TAG levels and lipid droplet formation [38,39] (Figure 1). Notably, recent data point to a greater proportion of lean humans with histologically proven NAFLD carrying the TM6SF2 58542926 T allele than overweight humans with NAFLD, despite similar PNPLA3 rs738409 GG genotype distribution. This difference persisted upon adjustment for multiple confounding factors, supporting the relevance of the genetic background even in the absence of adiposity [41]. The GCKR gene plays a central role in hepatic lipid metabolism through modulation of activity and intracellular localization of glucokinase [42]. The rs1260326 C > T polymorphism (P446L variant) enhances glucokinase activity in the liver in response to fructose-6-phosphate [43] and was shown to correlate with the severity of liver fibrosis but not with other NAFLD histological features [44]. In another study, the GCKR rs1260326 and rs780094 allele T were associated with susceptibility to NAFLD, NASH, and NASH with relevant fibrosis [45], and the GCKR rs1260326 variant explained 9.2% of the percentage of hepatic TAG content variance in Caucasians [46].

The genetics underlying the pathogenesis of NAFLD, ranging from inflammation to altered mitochondrial function, in oxidative stress and impaired insulin sensitivity, is less well defined [47–54] (Table 1). Genetic variants influencing insulin receptor activity point to an independent association with fibrosis but not with NASH with the ecto-enzyme nucleotide pyrophosphate phosphodiesterase (ENPP1) 121Gln and insulin receptor substrate-1 (IRS-1) 972Arg polymorphisms [49]. The membrane-bound 0-acyltransferase domain-containing 7 gene (MBOAT7) rs641738 T allele was shown to increase the risk of steatosis, necroinflammation, and fibrosis stage (F) 2–4 [47,48]. MBOAT7, also known as lysophosphatidylinositol acyltransferase 1, is an enzyme involved in the phospholipid acyl-chain remodeling, which transfers PUFAs, such as arachidonoyl-CoA, to lysophosphatidylinositol and other lysophospholipids. An independent association of the MBOAT7 genotype across the entire spectrum of NAFLD-related liver damage was reported after adjustment for BMI [47], and further evidence underlined the association of this gene variant with fibrosis independent of the presence of histological inflammation [55]. Each copy of the T allele was reported to increase the odds of hepatic steatosis by 20% after correction for demographic and anthropometric, clinical, and genetic factors [47]. It was suggested that lower hepatic protein expression of MBOAT7 mediates the association with NAFLD severity via changes in the hepatic phosphatidylino- linositols acyl-chain remodeling [47,48]. Furthermore, the splice variant rs72613567:TA encoding the hepatic lipid droplet protein hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13), which has enzymatic activity against several bioactive lipid species implicated in lipid-mediated inflammation, reduced the risk of fibrosis [56] and NASH but not steatosis [57]. Each copy of the minor G allele of the rs6834314 splice variant of the HSD17B13 gene further increased the risk of liver TAG content >33% by 32% [58]. Carriage of the superoxide dismutase 2 (SOD2) C47T polymorphism, the gene encoding manganese-dependent superoxide dismutase, which protects cells from oxidative stress, was also associated with steatosis and more advanced fibrosis in NASH [59], and variants on the lysophospholipase-like 1 (LYPLAL1) were reported to increase hepatic steatosis, potentially because of a reduced triglyceride breakdown [59]. Uncoupling protein 2 (UCP2) rs695366 G genotype correlates with reduced steatosis and NASH risk, particularly in humans with fasting normoglycemia [54], whereas myeloid-epithelial-reproductive tyrosine kinase (MERTK) rs4374383 polymorphism decreased the risk of steatosis, NASH, and fibrosis [51,53]. The rs3480 A > G polymorphism of fibronectin type III domain-containing protein 5 (FNDC5) gene, which encodes irisin, was further associated with protection from clinically significant fibrosis in patients with NAFLD, in a study reporting an increased expression of irisin in activated human hepatic stellate cells (HSC), where irisin promoted the expression of proinflammatory and fibrogenic markers [60]; however, a more recent study reported an increased risk of steatosis with the same allele and a lack of association with other histological features [61]. The reasons for this discrepancy remain elusive. Other genes with links to autophagy are also being investigated [62,63]. Unfortunately, some of these studies have examined the association only with certain NAFLD stages [49] or were limited by the lack of validation in ethnic populations [44,47].

4. Nutrition

Excessive caloric intake, a nutritional pattern rich in saturated fat, carbohydrates, and sugar-sweetened beverages have all been implicated in the development of NAFLD [1,64–68] (Figure 1). (Table 2) Hypercaloric and high-fat diets seem to increase intrahepatic fat content [69,70]. Although a single monounsaturated fat load does not affect hepatic steatosis among glucose-tolerant volunteers [71], a single oral lipid load rich in saturated fat augments hepatic glucose-neogenesis, induces insulin resistance and tends to augment hepatic lipid accumulation in healthy lean men [69], effects potentially ascribed to the lipid-mediated inhibition of insulin signaling [72]. Furthermore, robust effects of fructose consumption on hepatic steatosis were reported in healthy male humans [73,74] (Table 2). In a nonrandomized crossover study, a 7-day hypercaloric fructose diet increased intrahepatic TAG content and augmented hepatic steatosis more than an isocaloric diet did, with more potent stimulation of hepatic de novo lipogenesis (DNL) in 16 healthy males with a family history of T2DM than in 8 controls [75]. This finding indicates that fructose induces its detrimental effects, especially under hypercaloric conditions. In support, a systematic review and meta-analysis of studies in healthy humans revealed a fructose-induced increase in hepatic TAG content only in the context of excessive caloric intake and concluded that isocaloric exchange of fructose for other carbohydrates would not induce NAFLD at least in healthy participants [76]. Intriguingly, the available evidence points to similar detrimental effects of glucose and fructose in hypercaloric diets on liver fat content and liver enzymes in healthy men [77]. In long-term studies (Table 2), a high-fat diet [78,79], particularly rich in saturated fat [84,85,80], a high caloric intake [86], fructose [87], and beverage [88] consumption, all increase hepatic steatosis (Table 2), and further cohort studies point to the involvement of red and processed meat [81]. Evidence for a potential role of insufficient dietary fiber intake was also presented [82]. Notably, few dietary interventions have evaluated the effects on liver fibrosis in detail.

5. Gut microbiota

The body of evidence supporting an association between gut microbiome and NAFLD (Figure 1) is increasing, as illustrated by a
Review

Table 2 — Effects of dietary interventions in randomized controlled and randomized parallel group clinical trials demonstrating increases in intrahepatic fat content by magnetic resonance methods.

| Diet                          | Design, Ref. Cohort | Intervention Duration | Δ body mass vs BL vs CTRL | Liver TAG | Metabolic effects |
|-------------------------------|---------------------|-----------------------|---------------------------|-----------|------------------|
| Monounsaturated fat           | R/C ([7])           |                       |                           |           |                  |
| 14 ([14], 0%)                 |                     |                       |                           |           |                  |
| 22 ± 1                       | 14 (14), 0%         |                       |                           |           |                  |
| Polyunsaturated fat           | R/P, [64]           |                       |                           |           |                  |
| 37 (26), 0%                  |                     |                       |                           |           |                  |
| 18–27                        |                     |                       |                           |           |                  |
| 22.6 ± 0.5                   | 30.3 ± 3.7          | 3 w                   |                           |           |                  |
| Saturated fat                 | R/P, [6]            |                       |                           |           |                  |
| 61 (21), NR                   |                     |                       |                           |           |                  |
| 22.4 ± 1                      | 31.3 ± 3.9          | 4 w                   |                           |           |                  |
| High fat/high protein diets   | R/C, [11]           |                       |                           |           |                  |
| 10 (10), NR                   |                     |                       |                           |           |                  |
| 22.6 ± 0.6                    | 27                  | 4 w                   |                           |           |                  |
| High fat diet                 | R/P, [7]            |                       |                           |           |                  |
| 38 (17), NR                   |                     |                       |                           |           |                  |
| 3 ± 0.6                      | 4.6 ± 0.6           | 4 d                   |                           |           |                  |
| Energy-dense diet             | R/P, [6]            |                       |                           |           |                  |
| 36 (20), 0%                  |                     |                       |                           |           |                  |
| 19.5–24.5                    | 21 ± 2              | 6 w                   |                           |           |                  |
| Fructose                      | R/P, [67]           |                       |                           |           |                  |
| 32 (32), NR                   |                     |                       |                           |           |                  |
| 2 w                           | 2 ± 1               | 2 w                   |                           |           |                  |
| R/C, [7]                      | Std ± Glu (25% TE)  | 2 w                   |                           |           |                  |
| 10 (10), NR                   | Std ± Glu (25% TE)  | 19–25                 |                           |           |                  |
| Beverages                     | R/P, [6]            |                       |                           |           |                  |
| 47 (17), 0%                  |                     |                       |                           |           |                  |
| 24 w                          | 1 L/d of cola, semi-skinned milk, diet cola, or water | 24 w |                      |           |                  |

Single group assignment studies and non-randomized parallel group studies were excluded. Subject data presented as mean (±SEM) unless otherwise stated. AAs: amino acids, ALA: alpha-linolenic acid, BW: body weight, BG: between groups, BL: baseline, BMI: body mass index, CARB: simple sugars, CTRL: control, d: day, DHA: docosahexaenoic acid, dxa: dual-energy X-ray absorptiometry, EA: esacosapentaenoic acid, EGP: endogenous glucose production, FITP: fasting endogenous glucose production, FFM: free mass, FG: fasting glucose, Fru: fructose, g: grams, Glu: glucose, HDAC: glycated hemoglobin, HEC: hyperinsulinemic euglycemic clamp, HF: high fat, HFP: high fat protein, HOMA-IR: homeostasis model assessment of insulin resistance, 1H-MRS: proton magnetic resonance spectroscopy, HS: high sugar, Hypercal: hypercalcic, IMCLC: intramyocellular lipid content, Ins: insulin, IS: insulin sensitivity, isoacil: isoacilic, kg: kilograms, LF: low fat, min: minutes, MRI/m1H-MRS: magnetic resonance imaging, MD: Mediterranean diet, N: number, NA: not assessed, NAFLD: non-alcoholic fatty liver disease, NASH: non-alcoholic steatohepatitis, NEFA: non esterified fatty acids, NR: not reported, PLC: placebo, PUFA: polyunsaturated fatty acids, Ref: references, R/C: randomized controlled, R/P: randomized parallel group, SAT: subcutaneous adipose tissue, SG: single group assignment study, SFA: saturated fatty acids, StdD: standard diet, TAG: triglycerol, 120M: type 2 diabetes mellitus, TE: total energy, TG: triglycerides, VAT: visceral adipose tissue, UFA: unsaturated fatty acids, VCL: vehicle, vs: versus, w: weeks, WB: whole body.

a Estimated from table/graph.
b Median.
c Pooled data.
d mean ± SD.
e Significant effect of intervention (p < 0.05) according to the manuscript.
f Significant difference between groups (p < 0.05) according to the manuscript.
surprisingly robust diagnostic accuracy (area under the curve 0.936) of gut microbiota-derived signature for predicting the presence of advanced fibrosis F3 or F4 in 86 well-characterized individuals with biopsy-proven NAFLD [83]. Moreover, individuals who are obese and have NASH seem to exhibit a different microbiota signature, especially pertaining to the taxa of Proteobacteria, Enterobacteriaceae, and Escherichia, than obese and lean individuals without NASH [84]. The NASH microbiome can be characterized by an increased abundance of alcohol-producing bacteria along with higher blood ethanol levels in individuals with NASH [84]. This endogenously produced alcohol is immediately transported through the portal blood to the liver and could thereby contribute to the generation of ROS and hepatic inflammation [85]. Enhanced presence of alcohol-producing bacteria was also found in advanced NAFLD, suggesting a role of these bacteria in disease progression [83]. The gut is also a major site of gut bacterial fermentation of dietary carbohydrates, leading to the production of short chain FA. Although these FA are generally considered to exert beneficial effects on glucose and lipid metabolism, they may stimulate nutrient absorption, favoring obesity and steatosis [86]. Altered gut microbiota composition has been further associated with increased intestinal permeability and disruption of intercellular tight junctions in human NAFLD [87], permitting translocation of bacterial lipopolysaccharide (LPS) into the circulation (Figure 1). Animal studies have demonstrated the operation of this mechanism facilitating hepatic FA accumulation and NASH progression [88].

In human studies, intrahepatic localization of LPS was reported to correlate with liver inflammation, through the interaction with the toll-like receptor-4 (TLR4)-mediated pathway [88]. Liver sinusoidal endothelial cells (LSEC) and tissue resident macrophages would be expected to eliminate blood-borne pathogens including gut-derived bacteria and other components entering the liver [89]. Thus, the mechanisms of impaired defense in NAFLD are unknown. A recent study identified differences in liver tissue 16S ribosomal RNA gene bacterial metatranscriptomic signatures between humans with NAFLD who are severe and moderately obese [90]. Reduction in liver bacterial DNA from the Lachnospiraceae family was associated with histological disease severity [90]. This study did not assess the origin of the detected microbiome. Nevertheless, the biological relevance of liver bacterial DNA fragments, the potential contribution of particular taxa, and finally, the causality of microbiota alterations for NAFLD progression in humans require dedicated studies. Furthermore, among other intestinal actions, primary bile acids are metabolized in the small intestine into secondary bile acids. These are further reabsorbed through the portal circulation back to the liver, where they act as signaling molecules by interacting with receptors, for example, the farnesoid X receptor (FXR) and members of the G-protein-coupled receptor superfamily, which self-modulate their synthesis, glucose and lipid metabolism, and energy expenditure (Figure 1). For instance, interaction with FXR represses de novo FA synthesis in the liver and stimulates FA oxidation [96]. Finally, glucagon-like peptide-1 is an incretin secreted postprandially by L-cells in the small intestine, stimulating insulin secretion and inhibiting glucagon secretion, with positive effects on body weight loss and cardiovascular outcomes [13].

6. ADIPOSE TISSUE DYSFUNCTION

Adipose tissue is the principal sink of TAG and the main source of circulating FA during fasting because of lipolysis [72]. A systematic analysis of 798 humans undergoing bariatric surgery demonstrated that the percentage of body fat decreases while the ratio of trunk-to-limb fat increases with the progression from healthy liver to NASH (activity score >2) [91]. This points to limited subcutaneous adipose tissue expandability as an important mediator of the excess lipid fluxes to ectopic tissues, including the liver. Furthermore, subcutaneous adipose tissue hypertrophic adipocytes, which are more exposed to lipolysis and the production of proinflammatory mediators, correlated with NAFLD severity at least among female participants. Insulin-resistant states are specifically associated with blunted insulin-mediated restriction of adipose tissue lipolysis, which increases the flux of FFA and glycerol to the liver [13], where they serve as substrates for re-esterification, generation of lipid mediators (e.g., DAG) and DNL (Figure 1), or allosterically stimulate gluconeogenesis [72]. A recent study employing [1,1,2,3,3-2H5]glycerol in obese volunteers showed that insulin-mediated suppression of the rate of glycerol appearance and circulating FFA strongly correlates with cytosolic DAG concentration [92]. Obesity-related insulin resistance is further accompanied by macrophage infiltration and local increase in inflammatory cytokines in the adipose tissue [93]. However, adipose tissue insulin resistance can exist without concomitant adipose tissue inflammation, because oral lipid ingestion acutely induced adipose tissue insulin resistance without a concomitant increase in the expression and secretion of inflammatory cytokines including tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) from subcutaneous adipose tissue of healthy humans [69]. Nevertheless, long-standing insulin resistance and NAFLD generally associate with circulating proinflammatory cytokines, contributing to the adipose tissue—liver cross talk. The adipokines adiponectin and leptin contribute to this cross talk and glucose production, lipogenesis, and FA oxidation (Figure 1). Although higher levels of serum leptin and decreases in the soluble leptin receptor have been reported in human biopsy-proven NAFLD [94], no association has been observed between the leptin and fibrosis stage, after a correction for the important confounder, age, gender, BMI, diabetes, and insulin resistance [95]. By contrast, adiponectin was found to be reduced during NAFLD progression [96], probably reflecting the increasing insulin resistance. In addition to endocrine acting factors, adipocytes release small extracellular vesicles, exosomes, containing among others small noncoding RNA possibly serving as novel signals of interorgan communication. Severely obese and lean humans displayed more than 55 exosomal microRNA derived from visceral adipose tissue, differentially expressed in 2 cohorts. These microRNA (miR –23b, miR-140-3p, miR-148b, and miR-182) may regulate liver fibrosis through the upregulation of transforming growth factor-β (TGF-β), which activates the ECM secreting fibroblasts [97].

7. HEPATIC LIPID ACCUMULATION, INSULIN RESISTANCE, AND MITOCHONDRIAL ADAPTATION

Net hepatic TAG accumulation is the result of hepatic lipid supply and demand. Circulating FFA and glycerol serve as lipid sources for hepatic re-esterification and DNL, and hepatic TAG can be disposed of through oxidation or export as VLDL particles [98]. The FA transporters (FATP2 and FATP5) and the cluster of differentiation 36 (CD36) [99] facilitate hepatocyte FFA uptake (Figure 1). Studies have reported conflicting findings on the expression patterns of FATP2 and CD36 in humans with steatosis and NASH [99–101]. Recently, evidence was provided for an inverse association between hepatic FATP5 messenger RNA expression and histologic changes in NAFLD, including ballooning and fibrosis, suggesting reduced FATP5-mediated TAG accumulation during NASH progression [101].

DNL, namely, the conversion of acetyl-CoA over malonyl-CoA via acetyl coenzyme A carboxylase (ACC) and finally to palmitate (Figure 1), was
independently associated with hepatic TAG in individuals with NAFLD [102]. Comprehensive isotopic tracer studies have highlighted that DNL accounts for up to 26% of intrahepatic palmitate production in humans with NAFLD [102—104] and up to 10% in humans without steatosis [102,105—107]. Prolonged ingestion of 2H2O over 3—5 weeks, with correction for adipose tissue FA synthesis, revealed an even higher contribution of 38% of hepatic DNL to hepatic TAG among obese glucose-intolerant individuals with NAFLD, compared with the 19% and 11% in glucose-tolerant obese and lean individuals without NAFLD, compared with the prior appreciations [108]. Moreover, hepatic DNL negatively correlated with hepatic and skeletal muscle insulin sensitivity. DNL is regulated by the transcription factors sterol regulatory element-binding protein 1c (SREBP1c), which is activated by insulin and liver X receptor α (LXrα), and carbohydrate regulatory element-binding protein (ChREBP), mainly activated by carbohydrates [72] (Figure 1). Enhanced expression of SREBP1c has been demonstrated in human NAFLD [109]. From mouse studies, SREBP1c-stimulated DNL has been related to selective insulin resistance, by which insulin would continue to maintain elevated DNL despite its inability to suppress hepatic gluconeogenesis [72,110]. Subsequent mouse studies, however, provided evidence that chronic excessive lipid supply rather than insulin increases hepatic DNL [72]. ChREBP stimulates the expression of glycolytic genes, augmenting substrate availability for DNL, and induces the expression of steroyl-CoA desaturase 1 (SCD1), the enzyme responsible for the conversion of saturated to monounsaturated FA, increasing TAG accumulation [111]. Elevated expression levels of ChREBP were found in human NASH [111], which decline with severe insulin resistance. Individuals with NAFLD may exhibit downregulated ChREBP more than healthy humans do [112]. Lipid metabolites also relate to hepatic insulin resistance in the context of NAFLD. Liver of obese humans presents with increased hepatic DAG and activation of the novel protein kinase C (PKC) ε isoform, which also negatively relate to their insulin sensitivity [113—115]. In liver, PKCε was shown to induce serine phosphorylation, inhibiting insulin receptor kinase activity [116,117]. Ongoing research has addressed the role of specific DAG isoforms and their localization in subcellular compartments [92,113]. Interestingly, other lipid metabolites, including ceramides or acylcarnitines, or markers of endoplasmic reticulum (ER) stress or inflammation, were not increased or did not relate to hepatic insulin sensitivity [113,114,118]. However, specific ceramides and other sphingolipids were elevated only in obese individuals with NASH, not those with or without steatosis [118,119]. Interestingly, some sphingolipids correlated with markers of hepatic oxidative stress and inflammatory pathways, suggesting an important role of these lipid metabolites for NAFLD progression [118]. Studies have also highlighted the ability of insulin to induce connective tissue growth factor expression of the HSC in liver biopsies of patients with NASH (Figure 1), inferring that insulin resistance–associated hyperinsulinemia may directly accelerate fibrogenesis during NAFLD development [120].

Any increase in lipid overload is expected to stimulate its disposal. Mitochondrial oxidative capacity, as assessed with high-resolution respirometry, is up to 5-fold higher in human liver biopsies of obese insulin-resistant participants without or with histologically proven NAFLD than for lean humans [121]. Compared with the steatosis group, the NASH group had higher mitochondrial mass but a 31%—40% lower maximal respiration associated with mitochondrial uncoupling, leaking activity, augmented hepatic oxidative stress, and oxidative DNA damage [121]. Accordingly, recovery from hepatic adenosine triphosphate (ATP) depletion was shown to be severely impaired among humans who were both obese and had NASH [122,123]. Notably, individuals with T2DM feature reductions in fasting and postprandial absolute concentrations of γ-ATP and inorganic phosphate in hepatic ATP turnover were compared with age-and body mass index-matched and lean young humans [124—126]. This suggests that abnormal energy metabolism could contribute to the accelerated progression of NAFLD known for T2DM.

The aforementioned evidence of oxidative DNA damage in NASH highlights the importance of effective anti-oxidant mechanisms [121]. Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the scavenging capacity of the antioxidative mechanisms [127] (Figure 1). Production of ROS, including major free radicals such as the superoxide anion radicals (O2·) and the major nonradical species hydrogen peroxide (H2O2), occurs continuously through energy metabolism in the intracellular environment of liver cells [128]. ROS that are maintained in normal values through clearance pathways (e.g., catalase, glutathione peroxidase, and peroxiredoxin), act as signaling molecules and are involved in many vital mechanisms for cellular survival [129]. Increased ROS levels have been demonstrated by using cell lines to modulate lipid metabolism, through downregulation of peroxisome proliferator-activated receptor (PPAR)(α) activity, a key transcriptional factor of FA oxidation, leading to subsequent disturbed lipid homeostasis of human liver cells exposed to H2O2 [130]. In NASH, augmented hepatic oxidative stress (H2O2, lipid peroxides) and oxidative DNA damage was paralleled by reduced antioxidant defense capacity and increased inflammatory response [121]. Finally, export via water-soluble VLDL particles represents another way to remove hepatic TAG [131,132] (Figure 1). Although VLDL secretion is increased in human NAFLD [132—134], it plateaus when hepatic TAG accumulation exceeds 10% [132]. Formation of VLDL particles occurs in the ER through lipidation of the apolipoprotein B100 (apoB100), catalyzed by microsomal triglyceride transfer protein (MTTP) [135]. There is evidence that apoB100 synthesis rates are lower in humans with NASH than in obese and lean humans without NASH [136]. Additionally, hepatic MTTP levels are lower in humans with NAFLD with hepatic TAG content exceeding 30% than in healthy humans [137]. Humans with NASH have lower hepatic messenger RNA levels of apoB100 and MTTP and serum VLDL-triglycerides than those without NASH do, suggesting a potential contribution of the compromised VLDL export to NAFLD progression [138]. Nevertheless, another study reported similar expression levels of MTTP and apoB100 between humans without/with steatosis or NASH [139]. Thus, NAFLD heterogeneity might also result from the variability of the individual plateau of ER stress-related exposure to FA, but this needs to be further investigated.

8. INTRAHEPATIC MECHANISMS DRIVING INFLAMMATION AND FIBROSIS

The mechanistic concepts of intrahepatic inflammation and fibrosis have been recently reviewed [140,141]. Gut-derived LPS, translocated gut bacteria, certain metabolites (e.g., saturated FA, cholesterol), and injured hepatocytes activate the resident hepatic macrophage cells [142], the Kupffer cells, which release proinflammatory cytokines including interleukin-1β (IL-1β), IL-6 and TNF-α via TL4 activation [143] (Figure 1). Experimental mouse models have revealed that the release of chemokines from the injured liver cells promotes the infiltration of circulating C-C chemokine receptors (CCR) 2 monocytes, which differentiate into macrophages. These cells further contribute to NASH progression by maintaining an inflammatory environment and activating HSC, releasing CCR5 and thereby regulating their activation and proliferation [144]. HSC are the main source of extracellular matrix
(ECM) proteins and considered the main mediators of fibrosis development in human NAFLD [145]. Lipid accumulation in human-injured hepatocytes may trigger a profibrogenic response of HSC [146,147], activated among other mediators by the transforming growth factor-β (TGF-β) to express ECM gene expression [148]. Fibrosis progresses in parallel to altered angiogenesis, eventually driving the architectural changes characteristic of the development of cirrhosis [149], with recent evidence showing that mechanical stretching of human hepatic endothelial cells suffices to trigger angiocrine signals that induce the release of hepatocyte growth-promoting signals [150]. Further evidence from human studies points to enhanced ER stress (increased expression of the ER stress markers Chop and Grp78) and inflammasome activation in obese patients with NASH than in those with/without NAFL, with a positive correlation between ER stress and level of inflammasome transcripts [151].

LSECs, the most abundant nonparenchymal liver cells, are involved in metabolite transfer via fenestrations, which can be lost during the transition from healthy to fibrotic conditions as a result of increasing liver stiffness due to a phenomenon called capillarization, as reported for human LSECs [152].

9. CLINICAL DIAGNOSIS OF NAFLD

Understanding the results of clinical studies and the relevance of reported effects of treatments requires a short reflection on the different approaches to diagnose NAFLD in humans. Currently, liver biopsy remains the gold standard technique for the comprehensive diagnosis of NAFLD, because it allows for identifying inflammation and classifying fibrosis stages F0—4 [13]. Nevertheless, the invasive nature and—at least—risk of complications limits its widespread use. Liver biopsies have other methodological shortcomings, for example, access to a small volume of the liver and different classifications [13]. As a result, most—earlier—clinical studies applied ultrasonography, liver enzymes, or various indices for the diagnosis of NAFLD. With regard to steatosis, such approaches are inferior to 1H MRS and magnetic resonance imaging—estimated proton density fat fraction (MRI-PDFF), which allow for exact noninvasive quantification of hepatic TAG [153–155]. The NAFLD liver fat score and fatty liver index demonstrated modest diagnostic accuracy against 1H MRS [154] and offer limited potential to detect changes in hepatic TAG, especially when examining low-carbohydrate diets [156]. Additionally, indices to assess liver fibrosis, such as fibrosis-4 index, NAFLD fibrosis score, or aspartate aminotransferase to platelet ratio index, offer only moderate diagnostic efficacy, particularly in individuals with T2DM [157]. Imaging-based methods such as transient (ultrasound) or magnetic resonance elastography are promising novel tools [10] that require further testing in large prospective studies.

10. TREATMENT CONCEPTS

10.1. Strategies to induce body weight loss

10.1.1. Lifestyle modification

Hypocaloric nutrition in individuals who are overweight or obese and exercise training are considered essential to the nonpharmacological management of NAFLD [10] (Figure 1), with some evidence showing that even fibrosis and NASH resolution may be expected through lifestyle interventions [158]. There is evidence that a 10% weight loss can induce complete resolution of NAFLD in humans [159]. However, in the context of obesity and human NAFLD, the goal of 10% weight loss might be difficult to accomplish and even more difficult to sustain, with the majority of the studies reporting weight regain during the 2-year follow-up [159]. That said, there seem to be long-lasting beneficial effects of body weight loss on liver fat, despite body weight regain [159]. Another early study showed that a 12-week lifestyle intervention with an average of 8 kg of weight loss reversed hepatic steatosis [160]. Nevertheless, the study revealed no effects on whole-body insulin sensitivity or circulating markers of inflammation and adiponectin [160]. Subsequent studies have examined the role of different macronutrients, reporting beneficial effects of low carbohydrates [161], n-6 PUFA enriched fat [64,65], high protein [162,163], low fat [164], and Mediterranean diets [165,166], not n-3 PUFA enriched diets [167,168]. However, a recent meta-analysis showed little evidence that low-carbohydrate diets are more beneficial for NAFLD than differently composed diets with similar caloric intake [169]. Moderate-carbohydrate diets yielded changes in liver enzymes similar to those of low/moderate-fat diets [170]. In line with that finding, one RCT showed that the decrease in energy consumption rather than dietary composition, including modification of red meat, fiber, and caffeine, is the driver of the reduction of intrahepatic TAG accumulation, at least in individuals with T2DM and NAFLD [171]. By contrast, a moderate macronutrient shift, induced by substituting carbohydrates with protein and fat for 6 weeks reduced intrahepatic TAG content in weight-stable humans with T2DM [172]. Further lifestyle interventions have demonstrated decreases in liver TAG content independently of weight loss with supplementation of n-6 PUFA among abdominally obese participants [65] and high-protein diets in volunteers without [70] or with T2DM [163]. This may be of particular importance for lean humans with NAFLD. By contrast, some macronutrients may have deleterious effects, as reported with an excess of n-6 PUFA with regards to cardiovascular disease, cancer, and inflammatory and autoimmune diseases [173]. Additionally, increased consumption of animal protein has been linked to increased all-cause mortality [174]. Regular physical activity can also exert specific effects on NAFLD by various mechanisms, as reviewed recently [175]. For example, one 8-week resistance training study led to a robust decrease of 13% of hepatic TAG content in humans with NAFLD who maintained a stable body weight [176]. A meta-analysis including RCTs with 1073 patients with NAFLD revealed that exercise training improves transaminases and liver fat content, also irrespective of weight change [170]. There was no difference between aerobic and resistance exercise types, whereas moderate-to-high volume seems more beneficial than other exercise volumes. Nevertheless, interventions combining exercise and diet are more efficient to decrease transaminases (ALT) and to improve the NAFLD activity score (SMD = −0.61, 95% CI: −1.09 to −0.13) [170]. However, in the context of obesity, excessive lipid peroxidation, a known source of ROS, has been reported for acute intense aerobic and resistance exercise sessions [177]. Even more, higher oxidative lipid damage as observed upon chronic combined (aerobic and resistance) exercise training in obese humans [177] suggests that not all individuals may equally benefit from certain modes of exercise.

10.1.2. Bariatric (metabolic) surgery

Because of its superior efficacy to cause sustained weight loss, bariatric surgery is another nonpharmacological therapeutic option in obese humans with NAFLD [13] (Figure 1). A recent systematic review and meta-analysis of 32 cohort studies comprising >3000 biopsy specimens reported a biopsy-proven resolution of steatosis in 66%
inflammation in 50%, ballooning in 76%, and fibrosis in 40% of all patients, yielding a significant decrease in mean NAFLD activity score [178]. Notably, 12% of the participants developed new or worsening features of NAFLD [178], but the lack of RCTs represents an important limitation of this meta-analysis. It remains unclear whether any worsening of NAFLD could be linked to the transient increase in lipolysis and circulating FFA, which also prevents early improvement in insulin resistance after bariatric surgery [179] but may be required for the subsequent beneficial epigenetic remodeling demonstrated in human skeletal muscle [179,180]. In addition, higher BMI, lower serum albumin, and an open surgical approach was independently associated with increased risk of complications, which include infections (mainly at the wound site), intra-abdominal fluid collections requiring drainage, and small bowel obstruction or stenosis of the surgical anastomosis [181].

10.2. Anti-hyperglycemic agents

Studies investigating the effects of anti-hyperglycemic drug classes in clinical trials to evaluate their efficacy for patients with NAFLD and with or without T2DM are discussed in this section, with a special focus on studies applying state-of-the-art methods, such as H MRS and MRI-PDFF, for the quantification of TAG content.

10.2.1. Peroxisome proliferator-activated receptor agonists

PPAR agonists target nuclear transcription factors that primarily stimulate adipocyte differentiation and adipose tissue remodeling, modulating lipid fluxes to improve insulin signaling and glucose homeostasis [3,10]; although PPAR expression has further been reported in inactivated HSC, which declines upon HSC activation [182]. The best evidence for an improvement of histologically proven NAFLD has been reported for the PPARγ agonist, pioglitazone, which demonstrated reductions in hepatic steatosis and lobular inflammation, without significant effects on fibrosis scores after treatment for 96 weeks [183]. The results were replicated in smaller RCTs with a shorter duration [184—186] (Table 3). Importantly, some safety concerns such as body weight gain, decompensation of preexisting heart failure, and atypical bone fractures in women limit the broad use of pioglitazone [182]. Lobeglitazone, another PPARγ agonist, recently licensed in South Korea for the treatment of T2DM, also showed promising reduction of hepatic steatosis, assessed by controlled attenuation parameter with transient liver elastography [187], with an overall satisfactory safety profile, but still with an average body weight gain of 1.4 kg. Several other PPAR agonists with different pharmacodynamic profiles are being tested in clinical trials [188]. Elafibraran, a dual PPARα/δ agonist, initially one of the most promising anti-NASH drugs, recently failed to fulfill the predefined primary endpoint of NASH resolution without worsening of fibrosis in a population of 1070 patients with NASH, according to the interim analysis of the phase III trial [189]. Results from saroglitazar, a dual PPARα/γ agonist, and lanifibranor, a pan-PPAR agonist, in phase II trials have not been reported [13]. Intriguingly, lanifibranor inhibited the activation and proliferation of human HSC [182]. The development of seladopar, a PPARδ agonist, was suspended because of unexpected histological findings in a phase II trial of a NASH cohort [182].

10.2.2. Sodium glucose cotransporter (SGLT)-2 inhibitors (SGLT2i)

SGLT2i were initially developed because of their glucose-lowering effects, but empagliflozin, canagliflozin, and dapagliflozin later also showed reductions of cardiovascular endpoints in T2DM in large RCTs [190]. Recently, dapagliflozin treatment was associated with a significant risk reduction of worsening heart failure or cardiovascular mortality among heart failure patients, irrespective of the presence of T2DM [191]. The metabolic effects of SGLT2i resulting from renal glucosuria and energy loss with concomitant body weight loss include a shift of substrate utilization with increased ketogenesis and hepatic FFA use but might also attenuate oxidative stress and inflammatory pathways [192]. SGLT2i have an overall satisfactory safety profile, but some concerns are the risk of genitourinary tract infections and candida vulvovaginitis, bone fractures (particularly with canagliflozin), and normoglycemic ketoadiposi, mainly in the context of low body weight, severely impaired insulin secretion, and low carbohydrate intake [190]. A recent study showed that empagliflozin treatment also decreases circulating uric acid and significantly increases adiponectin secretion in individuals with T2DM, suggesting improvement of adipose tissue function [11]. Several RCTs have reported a decrease in hepatic fat content with empagliflozin [11], dapagliflozin [193,194] and the dual SGLT1/SGLT2i, licagliflozin [188], and some studies with dapagliflozin [195,196] and canagliflozin [197] have revealed no effect (Table 3). This could be explained by different efficacy to induce weight loss, but some studies have demonstrated a significant hepatic TAG reduction independently of any weight change [11]. Although no RCTs testing SGLT2i on histological features of NAFLD has been conducted, 2 small scale single-center studies reported some improvements in NAFLD activity from serial liver biopsies [198]. Preliminary data from a recent RCT also suggested that irapagliflozin may benefit ballooning and fibrosis in a small Japanese cohort [199]. Among humans without diabetes, a recent clinical study reported improvement in hepatic fibrosis and steatosis (assessed using transient elastography) with 24 weeks of empagliflozin treatment, compared with placebo with no major adverse events [200].

10.3.3. Incenmimetics and co-agonists

GLP-1 agonists mimic the action of the endogenously produced GLP-1 [13,188] and associate with—mostly transient—gastrointestinal discomfort and a questionable risk of acute pancreatitis [201]. Liraglutide was associated with significant relative hepatic TAG reduction of 31%, as assessed by MRI-PDFF after 6 months of treatment in 68 humans with uncontrolled T2DM [202] and with higher rates of histological resolution of NASH without worsening of fibrosis than with placebo in 26 humans with and without T2DM after 48 weeks [12], with transient, mild, or moderate side effects including diarrhea, constipation, and loss of appetite. In a subgroup analysis, liraglutide was reported to reduce adipose tissue, as well as hepatic insulin resistance and DNL [203]. Notably, another pre-specified secondary analysis showed that liraglutide did not reduce hepatic TAG accumulation in individuals with T2DM, when compared with placebo treatment added to standard care [204] (Table 3). Exenatide treatment showed a reduction of hepatic TAG content among obese humans with T2DM in comparison to reference treatment [205] or no significant histologic features in biopsy-proven NAFLD [201], with 2 out of the 8 participants in this study reporting abdominal discomfort. Studies with other GLP-1 agonists such as semaglutide and dulaglutide are ongoing [13].

In a 26-week phase IIb study of cutasitide, a dual glucagon receptor/GLP-1 agonist showed superior results pertaining to body weight reduction and transaminases improvement than with liraglutide among overweight humans with T2DM [188]. Similarly, the glucose-dependent insulinotropic polypeptide/GLP-1 combined agonist, tirzepatide, improved NASH-related biomarkers and increased circulating adiponectin at 26 weeks of treatment in humans with T2DM [206].
Randomized double-blinded placebo-controlled trials on drug treatment in adult patients with NAFLD with liver-related outcomes, as assessed by either liver histology or magnetic resonance methods, with > 20 participants in the intervention arm.

| Class | Control/ Treatment | Method | Intervention | Primary outcome | Steatohepatitis | Inflammation | Fibrosis | Metabolite effects |
|-------|---------------------|--------|--------------|-----------------|-----------------|-------------|----------|-------------------|
| PPARγ agonist | NAS1 [104] | PIO 45 mg/d (28) | MRS, PL (12) | Histology | NASH | ↓ | ↓ | ↓ | ↓ | ↓ |
| 6 m | NAS1 [105] | PIO 30 mg/d (68) | MRS, PL (25) | Histology | NASH histology | ↓ | ↓ | ↓ | ↓ | ↓ |
| 96 w | NAS1 [106] | PIO 45 mg/d (58) | MRS, PL (31) | Histology | NAS ↓ ≥ 2 points without F worsening | ↓ | ↓ | ↓ | ↓ | ↓ |
| 18 m (open 18 m) | NAS1 [107] | PIO 30 mg/d (31 of 37) | MRS, PL (30 of 37) | Histology | Hepatobiliary injury (8) | ↑ | ↓ | ↓ | ↓ | ↓ |
| 12 m | PPARα/δ agonist | NAS1 [108] | EILA 80 mg/d (58) | MRS/PL (43) | Histology | NASH resolution without F worsening | ↓ | ↓ | ↓ | ↓ | ↓ |
| 52 w | NAS2 [110] | DPIA 10 mg/d (38 of 91) | MRS, PL (24 of 91) | Histology | BW | ↑ | ↑ | ↑ | ↑ | ↑ |
| 24 w | NAS2 [111] | DPIA 10 mg/d (21) | MRS, PL (21) | Histology | Metabolic function | ↑ | ↑ | ↑ | ↑ | ↑ |
| 12 w | NAS3 [112] | CASA 300 mg/d (26) | MRS, PL (30) | Histology | MRS | ↑ | ↑ | ↑ | ↑ | ↑ |
| 7 days | GLP-1 RA | NAS1 [113] | LRA 1.8 mg/d (23 of 24) | MRS, PL (22 of 26) | Histology | NASH resolution without F worsening | ↑ | ↑ | ↑ | ↑ | ↑ |
| 48 w | NAS1 [114] | DPIA 10 mg/d (24) | MRS, PL (24) | Histology | MRS | ↑ | ↑ | ↑ | ↑ | ↑ |
| 26 w | NAS2 [115] | VLDL 100 mg/d (22) | MRS, PL (22) | Histology | MRS | ↑ | ↑ | ↑ | ↑ | ↑ |
| 6 m | Metformin | NAS1 [116] | MET 2.5-5.0 g/d (20 of 24) | MRS, PL (24) | Histology | S | ↑ | ↑ | ↑ | ↑ | ↑ |
| 24 w | FXR agonist | NAS2 [117] | OBCA 25 mg/d (141) | MRS, PL (142) | Histology | NAS ↓ ≥ 2 points without F worsening | ↑ | ↑ | ↑ | ↑ | ↑ |
| 72 w | NAS2 [118] | OBCA 10 mg/d (132) | MRS, PL (132) | Histology | OBGA 25 mg/d (303) | F improv. ≥ 1 stage without NASH worsening | ↑ | ↑ | ↑ | ↑ | ↑ |
| 18 m (opening) | NAS2 [119] | VLDL 100 mg/d (22) | MRS, PL (22) | Histology | NAS resolution without F worsening | ↑ | ↑ | ↑ | ↑ | ↑ |
| 12 w | CORS2/5 antagonist | NAS2 [120] | Arm A: DIO 150 mg/d (140) | Arm B | Histology | NASH ↓ ≥ 2 points with B/I ↓ > 1 point without F worsening | ↑ | ↑ | ↑ | ↑ | ↑ |
| 2 years (arm A) | SC17 inhibitor | NAS1 [121] | Aram 400 mg/d (101) | MRS/PL (121) | Histology | Aram 400 mg/d (98) | LFC | ↓ | ↑ | ↑ | ↑ |
| 1 year (arm B) | 1 year (arm B) | Aram 400 mg/d (101) | Arm B | Histology | Arm C (PLC 72) | LFC | ↓ | ↑ | ↑ | ↑ |
| 52 w | ACC γ inhibitor | NAS1 [122] | GS-0976 5 mg/d (51) | MRS/PL (51) | Histology | Safety | ↓ | ↑ | ↑ | ↑ | ↑ |
| 12 w | MRE2 | GS-0976 5 mg/d (51) | MRE2 | Histology | GS-0976 20 mg/d (49) | Safety | ↓ | ↑ | ↑ | ↑ | ↑ |
| 36 w | THR-β agonist | NAS2 [123] | Res 80 mg/d (76 of 84) | MRS/PL (84) | Histology | Res 80 mg/d (76 of 84) | PFDR | ↓ | ↑ | ↑ | ↑ |
| 12 m | MPC inhibitor | NAS2 [124] | MEDC-0620K 62.5 mg/d (99) | MRS/PL (99) | Histology | MEDC-0620K 62.5 mg/d (99) | MEDC-0620K 62.5 mg/d (99) | MEDC-0620K 62.5 mg/d (99) | ↑ | ↑ | ↑ |
| 250 mg/d (101) | MRS/PL (101) | Histology | MEDC-0620K 250 mg/d (101) | MRS/PL (101) | Histology | MEDC-0620K 250 mg/d (101) | MEDC-0620K 250 mg/d (101) | ↑ | ↑ | ↑ |
| 200 mg/d (26) | MRS/PL (26) | Histology | MEDC-0620K 200 mg/d (26) | MRS/PL (26) | Histology | MEDC-0620K 200 mg/d (26) | MEDC-0620K 200 mg/d (26) | ↑ | ↑ | ↑ |
| 16 w | FGF21 agonist | NAS2 [125] | Peg 35 mg/24 (24) | MRS/PL (24) | Histology | Peg 35 mg/24 (24) | Safety | ↓ | ↑ | ↑ | ↑ |
| 12 m | 16 w | Peg 10 mg/d (20) | Safety | ↓ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |

ACC: acetyl coenzyme A carboxylase, Aram: aramchol, B: balloning, BW: body weight, CANA: canagliflozin, COMB: combination, CCR: chemokine receptors, d: daily, Ccv: cenicriviroc, DAPA: dapagliflozin, DM: diabetes mellitus, DPI-4: dipethylpeptide-4 inhibitors, ELA: elafiranor, EMPA: empagliflozin, FGF: fibrilastin growth factor, FXR: farnesoid X receptor X, GLP-1RA: glucagon-like peptide 1 receptor agonist, HbA1c: glycated haemoglobin, eHDL: high density lipoprotein, HOMA-IR: homeostatic model of assessment of insulin resistance, improv.: improvement, INS-S: insulin sensitivity, KKH: ketohexokinase, LDL: low density lipoprotein, LFC: liver fat content, LIRA: iraglutide, M, M/I, Rd, Glu clearance: whole body (muscle) insulin sensitivity, m: months, MET: metformin, mg: milligrams, MRE: magnetic resonance elastography, MRS: magnetic resonance imaging, MS: metabolic syndrome, OBCA: obeticholic acid, OGTT: oral glucose tolerance test, Om3FA: omega 3 fatty acids, Peg: pegbelfermin, PIO: pioglitazone, PLC: placebo, PFDR: protein density fat fraction, PPAR: peroxisome proliferator activated receptor, Res: resmetirom, Ref: references, SCD: stearoyl CoA desaturase, SGLT2I: sodium glucose co-transporter 2 inhibitor, SITA: sitagliptin, TG: triglycerides, THR: thyroid hormone receptor, VLDL: very low density lipoprotein, W: weeks.

a Primary outcome measure achieved.
b Primary outcome measure not achieved.
10.2.4. Dipeptidyl peptidase-4 (DPP-4) inhibitors (DPP-4i)
DPP4i inhibit the degradation of GLP-1, prolonging the action of the endogenously produced GLP-1 [207], and have a low risk of adverse effects such as upper respiratory tract infections and maybe acute pancreatitis [208]. Sitagliptin failed to ameliorate hepatic steatosis and fibrosis in several human RCTs [209–213] (Table 3). However, when combined with metformin, 26-week sitagliptin treatment was equally efficient as iraglutide and superior to insulin glargine to reduce intrahepatic lipids in individuals with T2DM and NAFLD [214]. Vildagliptin treatment for 6 months decreased hepatic steatosis without serious adverse events in a double-blind RCT involving 44 patients with well-controlled T2DM [215], but evidence from other DPP-4i remains scarce (Table 3).

10.2.5. Metformin
Metformin is the drug of choice for the first-line management of T2DM, with gastrointestinal intolerance being the most frequently reported side effect [216]. Despite its beneficial effects on body weight reduction, metformin was not associated with improvement of liver histology in a 6-month RCT examining participants with NAFLD [217] (Table 3). A systematic review and meta-analysis of 9 RCTs confirmed the lack of improvement of NAFLD-related histological features [218]. Importantly, retrospective studies have suggested a reduction in the rates of hepatocellular carcinoma in metformin-treated humans [10], but no RCTs demonstrating the effect of metformin on hepatocellular carcinoma have been published.

10.2.6. α-glycosidase inhibitors
Inhibition of intestinal α-glycosidases flattens the postprandial rise of blood glucose by delaying dietary carbohydrate absorption, which also accounts for dose-related gastrointestinal adverse effects [219]. Acarbose was reported to improve liver fat content, and histology, when combined with ezetimibe [220,221]. A 12-week treatment with acarbose decreased elevated hepatic fat content, measured with 1H MRS in humans with metabolic syndrome [222]. Likewise, a 12-month treatment with miglitol revealed significant improvement in steatosis, lobular inflammation, portal inflammation scores, and NAFLD activity score in 17 humans with T2DM and histologically confirmed NASH [223].

10.3. Anti-inflammatory drugs
10.3.1. FXR agonists
FXR agonists improve cholestatic liver disease and are one of the most promising drug classes to treat NAFLD, with animal studies pointing to FXR-induced inhibition of DNL [224]. Treatment with obeticholic acid, a potent semisynthetic and selective FXR agonist, for 18 months resulted in fibrosis resolution without worsening of NAS in humans with F 2–3 [225] and in resolution of NAS without worsening of fibrosis in humans with F1. In another 76-week multicenter, RCT, obeticholic acid also improved NAS histology but caused major dose-dependent adverse events including pruritus in 23% of the participants and dyslipidemia [226] (Table 3). Thus, and because only 1 of 4 humans with NASH responds to treatment with FXR agonists [186], combination treatments with other drugs are being explored. Ongoing studies are also investigating other FXR agonists, such as cilofexor, EDP-305, and EYP 001, in human NASH [186].

10.3.2. CCR 2/5 antagonists
The anti-inflammatory and anti-fibrotic properties of these agents were the basis for the investigation of cenicriviroc, an oral, dual CCR2/CCRS5 receptor inhibitor in the phase IIb CENTAUR trial. Cenicriviroc showed no effect on the primary outcome measure, namely, a ≥2-point improvement in NAS with no worsening of fibrosis at 1 year. Nevertheless, the primary endpoint was achieved in 24% of patients who switched to cenicriviroc and in 17% of those who remained on placebo. Importantly, 60% of patients who achieved fibrosis improvement at year 1 maintained it during the next year, 30% in the placebo arm [227,228] (Table 3). The frequency of adverse events was comparable to placebo [227], except for a higher frequency of nasopharyngitis [228]. The efficacy and safety of cenicriviroc are being tested in patients with NASH in a phase III clinical trial [186] and as a combination treatment with the FXR agonist tropsifexor in patients with NASH and F 2–3 fibrosis.

10.4. Metabolic enzyme inhibitors
10.4.1. SCD1 inhibitors
Aramchol is a liver-directed SCD1 modulator that in a 52-week placebo RCT promoted NASH resolution and fibrosis stage reduction in a dose–response manner with a favorable biochemical and safety profile [229] (Table 3). Notably, pruritus was reported in 6.9% and 11.2% with aramchol 400 mg and 600 mg daily, respectively, and in 6.3% with placebo, without changes in body weight or circulating lipids. These findings led to further testing in a phase III clinical trial.

10.4.2. ACC 1/2 inhibitors
ACC is a rate-controlling enzyme of DNL such that inhibition of ACC decreases the synthesis of PUFA and increases beta-oxidation in mitochondria because the product of ACC2, malonyl-CoA, inhibits carnitine palmitoyltransferase 1, facilitating mitochondrial FA uptake. Firsocostat (GS-0976), a dual ACC 1/2 inhibitor, led to an almost 30% relative reduction of hepatic TAG content in 126 patients with NASH after 12 weeks but also to an increase in plasma triglycerides, particularly in individuals with preexisting hypertriglyceridemia [230] (Table 3). The most common adverse events included nausea, abdominal pain, diarrhea, and headache [230]. The second-generation liver-targeted ACC inhibitor, PF-05221304, is also associated with increased serum triglyceride levels in healthy humans but only at doses inhibiting fructose-stimulated hepatic DNL by >90% [231]. This finding has stimulated the further investigation of optimal dosing and combination treatments.

10.4.3. Ketohexokinase inhibitors
Ketohexokinase, the principal enzyme responsible for fructose metabolism, catalyzes the conversion of fructose to fructose-1-phosphate, which channels dietary sugar into DNL. Ketohexokinase inhibitor, was evaluated in a phase Ila RCT for its safety, tolerability, and pharmacodynamics in once-daily administration for 6 weeks in 47 humans with NAFLD. The trial reported greater mean reduction of hepatic TAG content, by MRI-PDFF, than and a profile for PF-06835919 similar to placebo [232].

10.5. Modulation of energy metabolism
10.5.1. Thyroid hormone receptor (THR)-β agonists
Thyroid THR-β is involved in hepatic lipid metabolism. Thyroid hormones exert their catabolic actions on hepatic lipids mediated through mobilization and subsequent beta-oxidation of hepatic FFA stored as TAG [233]. The phase II study with the THR-β agonist, VK2809, reported >50% relative reduction in hepatic TAG content in individuals with NAFLD, with a transient increase in transaminases [234]. The THR-β agonist resmetirom (MGL-3196) was similarly effective to reduce hepatic TAG content in biopsy-proven NASH along with a NASH
resolution of 27%, 6% in the placebo group, over 12 and 36 weeks [235] (Table 3). The most commonly reported adverse effects were diarrhea and nausea, with no changes in routine laboratory parameters from baseline and between treatment arms [235]. Both agents are being investigated in further clinical studies [188], which also address the safety profile of these agents, given the report of impaired skeletal muscle insulin sensitivity in animal studies [236].

10.5.2. Mitochondrial pyruvate carrier inhibitors
Recent evidence points to the mitochondrial pyruvate carrier as an important mediator of nutritional overload signals. MSDC-0602K, a mitochondrial pyruvate carrier inhibitor, is a second-generation insulin sensitizer designed to prevent side effects of PPARγ agonists. A 52-week phase IIb RCT revealed no significant superiority in the primary histological liver outcome in humans with biopsy-proven NASH with F1-3 and no difference for the incidence of hypoglycemia and PPARγ agonist-associated side effects [237]. Because of an improved NAFLD activity score and favorable effects on metabolic parameters [237] (Table 3), MSDC-0602K is undergoing a phase III RCT in individuals with T2DM and NASH [188].

10.5.3. Fibroblast growth factor (FGF) analogs
FGF 21 stimulates mitochondrial beta-oxidation in hepatocytes and modulates lipids and glucose metabolism. FGF21 and its related subfamily member, FGF19, are potent modulators of bile acid metabolism and cholesterol metabolism. Pegbelfermin (BMS-986036), a pegylated subcutaneously administered recombinant FGF21 analog, decreased hepatic fat fraction in a 16-week phase II study of 75 overweight/obese participants, with generally mild adverse events, who most frequently reported diarrhea and nausea [238] (Table 3). The efficacy and safety of pegbelfermin are currently being tested in advanced NAFLD, NASH with bridging fibrosis, and NASH with compensated cirrhosis [188]. NGM-282, an engineered FGF-19 analog, significantly reduced the NAFLD activity score and fibrosis score above one stage, without worsening of steatohepatitis in 43 humans with histologically proven NASH upon 12 weeks’ treatment [239]. Diarrhea occurred as a relevant side effect, which might have contributed to the beneficial effect on the liver via body weight loss.

11. CONCLUSIONS
During recent years, the awareness of NAFLD has markedly increased, leading to discoveries of pathogenetic mechanisms and innovative therapeutic opportunities. On the other hand, current treatments do not advance much beyond the recommendation of sustained body weight loss. The lack of efficacy or adverse effects of treatments are probably contributing to the proportional global increase in metabolic diseases and NAFLD, making them a progressive pandemic, which poses an important health and economic burden and significantly affects health-related quality of life [240]. A proposal is to screen individuals with the highest genetic or acquired risk of disease progression, for example, the SIRD subgroup, initiate early prevention and treatment, and develop therapeutic concepts, targeting the earliest pathophysiological alterations, namely, adipocyte dysfunction and insulin resistance.

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Idea and concept, MR, KP; Literature search, KP, MR; Manuscript writing, KP, MR; Manuscript editing, KP, MR.

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CONFLICT OF INTEREST
KP declares no conflicts of interest, MR is on the scientific advisory boards of Bristol-Myers Squibb, Eli Lilly, Gilead Sciences, NovoNordisk, Servier Laboratories, Target Pharmasolutions, and Terra Firma and receives investigator-initiated support from Boehringer Ingelheim, Nutricia/Danone, and Sanofi–Aventis.

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