NASH in lean individuals

TITLE

NASH in Lean Individuals

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Major Concepts

- The presence of non-alcoholic fatty liver disease (NAFLD) in subjects with a BMI within the ethnic-specific cut-off of 25 kg/m^2 BMI in Caucasian and 23 kg/ m^2 in Asian has been defined as 'Lean' NAFLD.
- 'Lean' NAFLD has been initially described in the Asian population; it can be diagnosed in approximately 5%–26% of the general population in Asia and 7-20% in the Western world.
- Pathophysiological mechanisms are not totally understood and may include a dysfunctional adipose tissue, altered body composition, genetic mutations, epigenetic changes occurring early in life and a different pattern of gut microbiota.
- Although this phenotype has generally a more favorable metabolic profile when compared to obese NAFLD, 'Lean' NAFLD patients can develop the full spectrum of liver damage that characterize non-lean NAFLD.
- Data on long-term prognosis of lean patients are insufficient and controversial but suggest that 'Lean' NAFLD is not a "benign" disease
- General recommendations include an adoption of a healthy lifestyle, but guidelines do not provide much information as to whether and to what extent prevention and treatment should be adapted in lean patients, given the harder correction of underlying risk factors.

Abstract

Non alcoholic Fatty Liver Disease (NAFLD) is generally associated with obesity and the related co-morbidities but it can also develop in subjects with a BMI within the ethnic-specific cut-off of 25 kg/ m^2 BMI in Caucasian and 23 kg/ m^2 in Asian subjects, the so-called 'lean' NAFLD. This sub-phenotype of NAFLD patients has been described across populations of different ethnicity, particularly in Asia, but it can be diagnosed in 10%-20% of non-obese Americans and Caucasians. Pathophysiological mechanisms underpinning the "lean" phenotype are not completely understood, but they may include a more dysfunctional fat (visceral obesity, differences in adipocyte differentiation and altered lipid turnover), altered body composition (decreased muscle mass), a genetic background, not limited to PNPLA3 C>G polymorphisms, epigenetic changes occurring early in life and a different pattern of gut microbiota. Lean subjects with NAFLD have milder features of the metabolic syndrome when compared to obese patients. Nonetheless they have a higher prevalence of metabolic alterations (e.g. dyslipidemia, arterial hypertension, insulin resistance and diabetes) compared to healthy controls. Data on histological severity are controversial, but they can develop the full spectrum of liver disease associated with Non Alcoholic Steatohepatitis (NASH). Since 'Lean' NAFLD usually present with less obesity-related comorbidities, it is commonly believed that this group would follow a relatively benign clinical course but recent data challenge this concept. Here we describe the current knowledge about NAFLD in lean individuals and highlight the unanswered questions and gaps in the field.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the most frequent cause of liver disease in Western countries, with an almost exponential increase in South America, Asia and in the Middle East. From its early description, we know that NAFLD is intimately connected with obesity, Type 2 diabetes (T2DM) and the Metabolic Syndrome (MetS), therefore its spread in parallel with the worldwide pandemic of obesity is not surprising. Nevertheless, NAFLD can develop in the absence of obesity, the so-called 'lean' NAFLD (i.e. BMI within the ethnic-specific cut-off of 25 kg/m^2 in Caucasian and 23 kg/m^2 in Asian subjects). By this definition, NAFLD in subjects with normal BMI has been frequently described in the Asian population, but it can be diagnosed in 10%-20% of non-obese Americans and Caucasians[1,2]. Despite the apparent 'healthier' phenotype, these patients may display the whole spectrum of the histopathological features of Non-alcoholic steatohepatitis (NASH), i.e. steatosis, lobular inflammation, hepatocyte ballooning and/or fibrosis[3]. Overall, many clinicians have a perception of 'lean' NAFLD being more "benign" in nature, but recent data challenge this view and suggest that it should not be overlooked.

It is conceivable that 'lean' NAFLD comprises an heterogeneous spectrum of different causes (Table 1), ranging from environmental cases (such as high fructose and high fat intake) , body fat distribution (visceral obesity as opposed to general obesity), body composition (acquired or congenital lipodistrophy, sarcopenia) and genetic risk factors, including rare congenital defects of metabolism such as Lysosomal Acid Lipase Deficiency (LAL-D) and Familial Hypobetalipoprotein B (FHLB). While the description of secondary causes of 'lean' NAFLD is beyond the scope of this review, we will describe the current knowledge about NAFLD in lean individuals and highlight the unanswered questions and gaps in the field.

Epidemiology

Epidemiological studies about prevalence, incidence and natural history of NAFLD and NASH in lean subjects suffer from several limitations: on one hand, the 'classic' bias of the criteria adopted to define NAFLD in the general population (liver function tests vs imaging vs algorythms) and the lack of non-invasive markers for NASH; on the other the heterogeneous definition of 'lean' across studies. In fact, while most of these studies compared patients using 25 as BMI cut-off, in Asian cohorts the term 'lean' should be used according to the ethnic-specific BMI cut-off of 23, while patients with BMI < 25 should be commonly indicated as "non-obese"[4]. In this regard, interpreting BMI values with the simplistic association between low BMI and low body fat is misleading because BMI is an imperfect index of adiposity, particularly in truncal fat accumulation, and does not take into account body composition. Adding to the confusion is that the definition of MetS, commonly used to detect metabolic abnormalities, varies across studies and even more at the individual level, so that the association between the degree of obesity and development of insulin resistance may not be so clear-cut.

Population studies had being describing NAFLD in non-obese subjects since 2006 (Table 2). In a study including 2520 residents of the Shengang Township in Taiwan, NAFLD (by abdominal ultrasound) was found in 61 over 1,444 non-obese participants (4.2%), in the absence of other etiologies of chronic liver disease[5]. In a prospective epidemiological study carried in a very poor, rural area of West Bengal, India, NAFLD was identified in 8.6% of the overall population, but 75% of NAFLD subjects belonged to the non-obese group[6]. The non-obese and lean individuals with NAFLD had more subcutaneous fat, higher fasting blood glucose, and higher levels of triglycerides. However this population also included 47% with malnutrition, which can be associated with NAFLD by a different mechanism (choline deficiency).

After these two pioneer epidemiological surveys, most of the studies investigating the non-obese pattern of NAFLD had been carried out in the Asian continent. In China, the Zhejiang Zhenhai Study evaluated the prevalence and risk factors of NAFLD in 6,905 non-obese individuals (BMI <

25). NAFLD was diagnosed by ultrasound in 7.27% of the study participants[7]. Similarly, in a cohort of 2,000 Chinese subjects who received annual physical examinations, NAFLD was found in nearly 18% of the non-obese subjects (BMI < 24)[8]. A large Korean population study recruiting 29,994 individuals who presented for a routine health evaluation, reported a prevalence of NAFLD of 12.6% in the non-obese participants (n=3,014)[9]. These findings were confirmed in another Korean general medical check-up program where 22.4%, of non-obese subjects (333/1,487) had NAFLD[10]. In Japan, the overall prevalence of NAFLD in 3,271 subjects who received health checkups from 2011 to 2012 was 68.5% in obese subjects and 15.2% in non-obese subjects. Metabolic factors such as waist circumference and triglycerides were predictors of non-obese NAFLD. Interestingly, weight gain since early adulthood (around the age of 20) was significantly associated with NAFLD in non-obese subjects of both genders[11]. In a community-based Hong-Kong cohort NAFLD was detected by Proton-Magnetic Resonance Spectroscopy (1H-MRS) in 19.3% of the lean cases (BMI < 23 kg/m^2), compared with 61% in those with a higher BMI[12].

Compared to the Asian surveys described above, studies in the Caucasian population are less numerous and, in general, involved a smaller number of subjects. In Italy, the Dionysus Study showed that the prevalence of 'lean' NAFLD (BMI < 25 kg/ m^2) assessed by ultrasonography was 16%, compared with 75.8% in obese[13]. In Iceland, the AGES-Reykjavik Study Investigators highlighted an association between the central axes of obesity with the presence of MetS in lean patients[14]. In this study, CT scan showed the presence of hepatic steatosis even in patients with a median BMI of 22.7. In the Dallas Heart Study, the prevalence of steatosis (defined as a hepatic triglyceride content > 5.5% by 1H-MRS) in subjects with a BMI < 30 kg/ m^2 was 17%, compared with 34% in the overall study subjects[15]. The prevalence of NAFLD was significantly lower in non-obese African Americans (11%) but comparable in non-obese Caucasians and non-obese Hispanics (20% vs 26%; P = .12)[15]. However, the largest epidemiological study analyzing prevalence and features of lean Caucasian NAFLD patients has been carried in the United States, using data of the Third National Health and Nutrition Examination Survey III (NHANES III)[16]. Among 11,613

subjects, the prevalence of NAFLD at ultrasonography was as high as 27.7% in overweight/obese subjects, significantly higher than the 7.4% observed in subjects with normal BMI (<25 kg/ m^2). The presence of a lean phenotype with NAFLD is observed across all age groups, including adolescents. Cross sectional data from 1482 lean subjects (body mass index <85th percentile) aged between 12 and 18 years, enrolled in the NHANES during the 2005 to 2014 cycles, reported a 8% prevalence of suspected NAFLD (by ALT above the low gender-specific cut-off) among lean adolescents. Low HDL, hypertriglyceridemia and presence of insulin resistance were more common among 'Lean' NAFLD compared with their non-NAFLD counterparts[17].

Overall, epidemiologic data suggest that the prevalence of 'lean' NAFLD is approximately 5%–26% in the Asian population and 7-20% in the Western one. This suggests that factors independent from body weight may be important in a subset of NAFLD subjects.

Pathophysiology of Lean NAFLD

Pathologic pathways underlying the development of NAFLD in lean subjects are not entirely understood. However, studies on adipose tissue functions, genetic analyses, studies in vitro and in vivo on animal models and finally gut microbiome research can provide some hints to mechanisms (Figure 1).

Insulin resistance and fat distribution

Despite a reduced likelihood of being associated with the components of MetS, subjects with 'lean' NAFLD are nonetheless insulin resistant when compared to healthy controls. In a cohort of subjects with biopsy-proven NAFLD, free of diabetes, obesity and MetS, the metabolic pattern of insulin resistance in the main target tissues (muscle, liver and adipose tissue) was similar to that observed in obesity, with adipose tissue insulin resistance playing an important role despite a low BMI and

normal subcutaneous fat[18]. This early finding was further supported by more recent studies, showing higher circulating concentration of free fatty acids (FFA) in 'lean' NAFLD patients compared to healthy controls and a higher portal FFA flow, which may induce intra-hepatic fat accumulation[19,20].

It is likely that a vast part of 'lean' NAFLD belong to the phenotype of "metabolically obese normal weight" (MONW) subjects, described in at least 5% of the occidental population, who display altered insulin sensitivity and increased cardiovascular risk[21]. When comparing metabolically healthy and unhealthy normal weight subjects, the latter population has increased liver fat content, visceral fat mass and carotid Intima Media Thickness (cIMT), but lower subcutaneous fat mass, insulin sensitivity and insulin secretion[22]. These key characteristic of MONW are consistent across studies in humans; increased liver fat is probably due to a decreased capacity for storing fat in subcutaneous adipose tissue, coupled with reduced mitochondrial function and increased de novo lipogenesis in the liver[22]. Further, MONW subjects also have a pro-inflammatory circulating milieu characterized by decreased adiponectin concentration and a unique T-cell signature[21]. Ethnicity has a significant impact on the variability of MONW across studies. In the Multi-Ethnic Study of Atherosclerosis, the prevalence of metabolically unhealthy with normal weight ranged from 21 % in whites, 32 % in Chinese Americans, 31 % in African Americans, 38.5% in Hispanics, and 43.6% in South Asians[23]. In the same study, the prevalence of non-obese NAFLD (BMI < 30 kg/ m^2) assessed by CT scan was 11%, including 9% among Caucasians, 6% among African Americans, and 18% among Hispanic Americans[23].

At the extreme end of the spectrum of MONW lays the lipodystrophy phenotype. These subjects typically display absence of fat in the classic subcutaneous depots but large ectopic accumulation of lipids in the skeletal muscle and in the liver associated with severe insulin resistance[24]. While the genetic forms are relatively rare, acquired lipodystrophy can be found in HIV patients under HAART therapy and can be also found in some lean people not having been diagnosed with lipodystrophy.

The importance of body composition in the onset and progression of NAFLD is also supported by the finding that sarcopenia, defined as a progressive and generalized loss of skeletal muscle mass, strength and function, is a novel risk factor for the development of NAFLD[25]. Recently we found elevated plasma aminoacids concentrations in NAFLD subjects either with and without obesity, likely to be related to peripheral resistance and resulting in increased muscle proteolysis during the fasting state, which lends support to the pathogenesis of sarcopenia in NAFLD[26].

Genetic and environmental factors

The data discussed above suggest that 'lean' NAFLD have an increased susceptibility to develop NAFLD that can be partially attributed to genetic factors or epigenetic changes induced early in life. The search for genetic causes that contribute to the incidence of NAFLD in lean patients is still in its infancy. As reported by genome-wide association analyses, firstly performed within the Dallas Heart Study and widely confirmed in literature, a single variant in the patatin-like phospholipase domain-containing protein 3 (PNPLA3), the rs738409[G], encoding I148M, is associated with an increase in both liver fat and hepatic inflammation[27]. Comparing obese and non-obese subjects with NAFLD, the prevalence of the PNPLA3 [G] allele was significantly higher among non-obese individuals (78.4% vs 59.8%) and was independently associated with NAFLD even in the multivariate analysis[12]. A study recruiting lean Japanese NAFLD patients (BMI < 23) confirmed the previous findings. Although there were no GG homozygous carriers in their population, in the lean group the G allele was a predictor of NAFLD in all multivariate analysis steps, while it was not in the obese population[28]. Another Japanese study recruiting 540 biopsy-proven NAFLD patients (134 non-obese) found a higher rs738409 GG homozygous genotype rate in non-obese NAFLD patients compared to obese individuals with fatty liver. Again, the GG-single-nucleotide polymorphism was an independent predictor of NAFLD, together with diabetes, in the non-obese cohort only[29]. In a retrospective study on patients with 'Lean' NAFLD, the only variable associated independently with NASH and a fibrosis score of 2 or higher was rs738409 C>G in PNPLA3[30]. However, in a

prospective general population study in Hong Kong including 565 cases (BMI < 23 in 72%) without evidence of NAFLD (by 1H-NMR) at baseline, the presence of the common variant in the PNPLA3 gene did not provide any relevant clue for incident NAFLD[31].

Other variants in different loci may be involved in individual cases. Cholesteryl ester transfer protein (CETP) is involved in triglyceride exchange between lipoproteins[32]. Two single-nucleotide polymorphisms (rs12447924 and rs12597002) were associated with fatty liver disease in adolescent lean Caucasian females (BMI < 25). The highest risk of NAFLD was found in the group with the lowest adiposity assessed by skinfold thickness, where the prevalence of NAFLD in lean homozygotes, heterozygotes and wild type was over 30%, 10-15% and 3.5% respectively[33]. A single-nucleotide polymorphism in transmembrane 6 superfamily member 2 (TM6SF2) was associated with NAFLD and fibrosis independent of age, diabetes, obesity and the PNPLA3 genotype[34]. In a retrospective cohort, a significantly greater proportion of patients with 'Lean' NAFLD carried rs58542926 C>T in TM6SF2 (4%) than obese or overweight individuals[30]. The interferon (IFN) lambda 4 rs368234815 TT>δG variant, influencing innate immunity regulation, has been linked to liver damage in patients with NAFLD. The impact of rs368234815 seems generally more marked in non-obese individuals, where an association with severe fibrosis, necroinflammation, and NASH has been observed[35].

Finally, a recent study on animal models hypothesized that a deficiency of the phosphatidylethanolamine N-methyltransferase (PEMT) could play a key role in the development of NASH in lean individuals[36]. PEMT is an enzyme involved in the synthesis of phosphatidylcholines in liver cells. PEMT -/- mice on high fat-high sucrose diet did not develop obesity or insulin resistance compared to the PEMT +/+ and presented normal cholesterol and triglyceride levels. Nonetheless, PEMT -/- developed NASH and after 90 weeks all PEMT -/- mice developed liver tumors. When PEMT mRNA expression in human liver biopsies were quantified, a lower expression of PEMT was found in patients with NASH. A correlation with lower BMI has been also reported, suggesting that PEMT deficiency could be an etiologic agent of lean NASH[36].

Insulin resistance in adipose tissue develops early during fetal growth restriction and is maintained during the neonatal period and adulthood[37]. Besides genetic factors, intrauterine growth might play a role in favoring NAFLD, particularly in children. An Italian group first described the association of intrauterine growth retardation with pediatric NAFLD and more severe disease activity at histology, independent of and in addition to insulin resistance. At an average age of 11 years, most study's subjects (80%) were insulin resistant, despite normal BMI and a very low prevalence of metabolic abnormalities. Notably, the family history of type 2 diabetes was less common, suggesting that genetic factors have lower relevance in the onset of NAFLD in this cohort[38].

The setting of a smaller adipocyte number during early life let lean population mostly change their adipocyte volume in adulthood, developing MetS features at a lower fat mass[39], partially explaining why these subjects easily develop NAFLD for small increases in body weight, still in the non-obese range[31].

Finally, among the most common environmental causes, a high fructose intake is an additional risk factors for NAFLD and NASH, particularly in children and adolescents. In a study on young non-obese subjects without obvious metabolic risk factors, the only independent predictor for the presence of NAFLD was a higher soft drink and juices consumption, up to 4-fold compared to healthy controls[40]. Thus, preventing fructose intake may represent a readily modifiable environmental factor, particularly in younger ‘lean’ NAFLD patients.

Gut Microbiome and Metabolomics

Fatty liver has been associated with a lower rate of *Bacteroidetes* and a higher rate of *Prevotella* and *Porphyromonas*, as well as a higher number of ethanol-producing bacteria[41]. Duarte et al. described a significant difference in the abundance of *Faecalibacterium*, *Ruminococcus*, *Lactobacillus* and *Bifidobacterium* in patients with NASH when compared to controls[44]. The subgroup of lean patients with NASH had had less abundance of *Ruminococcus* and a deficiency of Lactobacilli when compared to overweight and obese patients with NASH[42].

Lyso-phosphatidylcholines (lyso-PCs) are phospholipids with anti-inflammatory and insulin-sensitizer effects and lower levels of lyso-PCs are observed in obesity[43-45]. Metabolomic analyses demonstrated lower levels of lyso-PCs in both lean and obese NAFLD patients when compared to healthy controls[46]. On the other hand, when compared their obese counterpart, lean patients with NAFLD showed a higher level of lysine concentration[46]. Being related to visceral fat accumulation[47], lysine may represent a sign of the dysfunctional metabolic environment underpinning 'Lean' NAFLD individuals.

Clinical and Pathological Features

Compared to their healthy counterpart, 'lean' NAFLD have a reduced likelihood of being associated with the components of MetS; nonetheless subjects have an increased prevalence of metabolic derangements, above all diabetes and higher plasma triglycerides, although both abnormalities are usually less severe than in obese NAFLD patients[16,48]. In the NHANES III population, when compared with overweight/obese NAFLD subjects, 'lean' NAFLD was independently associated with younger age, female sex, insulin resistance and hypercholesterolemia. Among individuals who fulfilled the clinical definition of NASH as it was adapted for that cohort (i.e. NAFLD patients with elevated aminotransferases in the presence of either diabetes or insulin resistance), the prevalence of lean subjects was as low as 1.38%[16]. Another study in Caucasians confirmed that patients with 'lean' NAFLD have a better metabolic profile than overweight and obese, i.e. higher levels of high-density lipoprotein cholesterol (HDL-C), lower triglyceride and fasting glucose levels, in addition to a lower concentration of the pro-inflammatory cytokines IL6 and TNF-α. In addition, leptin levels were similar to healthy controls and significantly lower than in obese NAFLD patients. Conversely, in 'lean' NAFLD adiponectin levels have been found significantly lower than healthy controls, but similar to the obese NAFLD[46]. In Asian cohorts, features of MetS have been associated with the development of NAFLD across the entire BMI spectrum, but lean NAFLD patients presented the

strongest correlations[7,8]. Further, 'Lean' NAFLD seems to have a more active visceral adipose tissue, in terms of visceral adiposity index (an indicator of visceral fat function associated with cardiometabolic risk), when compared to overweight or obese population[8,49]. Noteworthy, in some lean healthy subjects relatively small changes in their metabolic profile and body weight can be associated with incident 'lean' NAFLD. A prospective cohort study from Hong Kong included a subgroup of 406 lean (BMI < 23.0 kg/m^2) subjects, of whom 7.9% developed incident steatosis (by 1H-MRS) after an interval of 3–5 years (median 47 months)[31]. At multivariable analysis, increased waist circumference and serum triglyceride levels during follow-up were associated with incident fatty liver, although some of these patients did not develop a full MetS.

Even though a better metabolic profile is supposed to be associated with a less severe histological damage, there is no agreement on this issue and some studies suggest the contrary in 'lean' NAFLD. A seminal Italian study[50] reported the presence of NASH in 50% of lean NAFLD patients (BMI <25 kg/ m^2). The prevalence of NASH was quite similar across BMI classes (normal weight, 65%; overweight, 73%; and obese, 84%; P = 0.184). Another study including 430 biopsy proven NAFLD, showed that 55% of patients without visceral obesity according to waist circumference had NASH and fibrosis ≥ F2, despite milder metabolic alterations[51]. On the contrary, in another retrospective series including 669 biopsy-proven Caucasian NAFLD patients, when compared to overweight and obese patients, NAFLD subjects with a BMI < 25 had a lower rate of MetS and diabetes, lower cardiovascular damage, expressed as cIMT and prevalence of carotid plaques as well as lower prevalence of histologically-diagnosed NASH and fibrosis F2 or higher[30]. Of interest, in a Turkish cohort of 483 biopsy-proven NAFLD patients, with a prevalence of 'Lean' NAFLD (BMI < 25) of 7.6%, high hemoglobin levels was the only independent predictor of NASH and advanced fibrosis in lean individuals and not in the obese/overweight group[52].

In a study from China, similar proportions of obese and non-obese patients had NASH (51.9% versus 43.5%, P = 0.217), although the latter ones had a lower degree of steatosis and hepatocyte ballooning, and the proportion of patients with advanced fibrosis was not different in the two

groups[3]. Triglyceride levels independently predicted disease severity in non-obese NAFLD group and were associated with both higher grade of steatosis and hepatocyte ballooning[3]. Similarly, Kumar et al. found no difference in NASH prevalence between lean (BMI < 23) and non-lean subjects among 110 biopsy-proven NAFLD patients of Indian ethnicity[53]. In a Japanese cohort of biopsy-proven NAFLD patients, lobular inflammation, hepatocyte ballooning and NAFLD activity score were strictly associated with the GG-PNPLA3 single-nucleotide polymorphism, which was more prevalent in the non-obese cohort and was not associated with histological severity in NAFLD obese patients[29].

Thus, lean NAFLD patients may present milder histological features or may show the same characteristics when compared to obese patients with NAFLD, but overall they can display the full spectrum of liver damage. This suggests that the risk of cirrhosis for lean NAFLD patients may not be that different after all, and that, once NASH is established, obesity may not be the main driver of fibrosis progression. The next important question is whether 'lean' NAFLD subjects have an adverse outcome related to liver-related morbidity and mortality.

Outcome and Prognosis of Lean NAFLD patients

Since 'Lean' NAFLD usually present with less obesity-related comorbidities, it is commonly believed that this group would follow a relatively benign clinical course. Within the cohort of the NHANES III[54], mortality of metabolically-normal NAFLD patients was similar to the cohort without liver disease. However, the longitudinal risk of mortality in 'Lean' NAFLD has been scarcely explored. In the Hong Kong cohort of Leung et al., where 307 patients with biopsy-proven NAFLD (23.5% non-obese, BMI < 25) had been followed up for a median period of 49 months, clinical events similarly occurred in obese (11.9%) and of non-obese patients (8.3%). Cardiovascular morbidity accounted for about two thirds of all major events in both groups. All deaths (n=6) occurred in the obese group, but definite conclusions are difficult to make as follow-up

was relatively short[3]. An international study with a longer follow-up period, published so far only in abstract form, challenged the concept that the prognosis of patients with NAFLD who have normal BMI is better than in those who are overweight or obese[55]. Over a total 1090 prospectively recruited patients, 125 (11.5%) were classified as lean (BMI < 25 for non-Asians and < 23 for Asians). 'Lean' NAFLD were more commonly men of non-Caucasian origin and, as expected, showed less features of the MetS. Histologically, they had less severe fibrosis but a higher grade of lobular inflammation. Interestingly, median survival free of liver transplantation was significantly shorter in lean than in non-lean patients (18.1 vs. 26.6 years, respectively, p<0.001). The higher risk of death/liver transplantation in 'Lean' NAFLD was independent of the classic risk factors that may influence the development of this outcome.

In another retrospective study including 646 patients with biopsy-proven NAFLD, at enrolment NAFLD 19% of patients were lean, 52% overweight and 29% obese. Patients with 'Lean' NAFLD were older, had lower transaminases, lower stages of fibrosis, and lower prevalence of NASH compared to patients with a higher BMI. During a mean follow-up of 19.9 years (range 0.4-40 years) patients with 'Lean' NAFLD had no increased risk for overall mortality, but they were more likely to develop severe liver disease than patients with NAFLD who were overweight (hazard ratio 2.69; P = 0.007), independent of available confounders[56]. Three prognostic indicators for mortality in 'Lean' NAFLD were identified: older age, fibrosis stage, and hypertension. Noteworthy, of the 19 patients with 'Lean' NAFLD who developed severe liver disease, 58% (n = 11) had fibrosis stage 0-2 at baseline. A limitation of the longitudinal retrospective studies is the limited ability to determine whether or not subjects developed additional risk factors over time that are known to predispose to advanced liver disease, such as diabetes or changes in alcohol intake and body weight. Certainly future longitudinal prospective studies are needed to define the prognosis in 'Lean' NAFLD and to elucidate potential pathophysiologic mechanisms underlying progression; nevertheless data available suggest that carefully selected patients with 'Lean' NAFLD likely require long-term follow-up and reassessment of progression of liver disease over time.

Management

When it comes to the prevention and treatment of 'Lean' NAFLD, current European guideline states that follow up is mandatory in obesity, but also suggest follow up in lean persons with NAFLD because of possible disease progression, even though they have less severe metabolic disturbance (B2 strength of evidence)[57] . Careful identification and correction of environmental causes, such as significant fructose consumption, may be effective particularly in young patients. General recommendations include an adoption of a healthy lifestyle and the initiation of pharmacological treatments for elevated blood pressure, dyslipidemia, and hyperglycemia, if necessary[57]. However, these guidelines do not provide much information as to whether and to what extent prevention and treatment should be adapted in lean patients, given the harder correction of underlying risk factors. Weight loss remains the background therapy in all cases with overweight/obesity, but in 'Lean' NAFLD the efficacy of calorie restriction has not been tested. Nevertheless, diet should be prescribed when a weight gain even within the non-obese BMI range was preceding the development of 'Lean' NAFLD. Importantly, habitual physical activity should certainly be indicated because it can specifically decrease visceral fat[58]. Treatment with the TZD pioglitazone also reduced the diabetes risk and improved insulin secretion in non-obese subjects with impaired glucose tolerance[59], but no analysis had been performed in normal weight individuals. Incretin-based treatments may be more effective in overweight and obese subjects than in normal weight individuals because its efficacy is associated with weight loss[60]. In the Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes (LEADER) study, which included 9,340 patients, treatment with liraglutide was associated with lower incidence of the primary composite outcome in obese patients with type 2 diabetes, but not in non-obese[61].

Conclusions

Lean patients with non-alcoholic fatty liver disease are a well-defined entity and are described by numerous studies both in the Eastern continent and in the Western world. Considering that lean NAFLD patients can develop the full spectrum of liver damage that characterize non-lean NAFLD, it is important to understand what phenotypes characterize this population. Compared to healthy individuals, lean subjects with NAFLD present metabolic risk factors (dyslipidemia, arterial hypertension, diabetes, insulin resistance) to a significantly greater extent, probably due to a more dysfunctional adipose tissue, not limited to its visceral component. Although literature data indicate that these patients have more favorable metabolic characteristics when compared to obese NAFLD patients, data on long-term survival and mortality are insufficient and controversial. Furthermore, genetic analyses suggest that metabolic risk appears to be determined by different pathways in normal weight and obese subjects and indicates that the genetic background could be the key to better characterize this type of patients. These findings may have several implications for clinical interventions and for drug development. Applying well-defined phenotyping strategies in clinical trials to separate the outcome in lean and obese NAFLD subjects will help to more precisely understand the pathophysiology of liver disease. Without a doubt, the challenges that the ‘Lean’ NAFLD raises are an excellent incentive for the development of future research.

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Tables

Table 1 Possible causes of NAFLD in lean subjects

High fructose intake
Metabolically Obese Normal Weight Subjects
Congenital and acquired (highly active antiretroviral therapy for HIV) lipodistrophy
Malnutrition (Kwashiorkor)
Genetic PNPLA 3 GG variant Congenital defects of metabolism – Familial Hypo-BetaLipoproteinaemia (FHBL) – Lysosomal Acid Lipase Deficiency (LAL-D)
Endocrine disorders (polycystic ovary syndrome, hypothyroidism or growth hormone deficiency)
Drug-related (amiodarone, methotrexate, tamoxifen)
Jejunoileal bypass, starvation, Total Parenteral Nutrition

Table 2 Main epidemiological, cross-sectional and longitudinal studies describing the characteristics of "lean" patients in NAFLD

Epidemiological Studies		
	Main findings*	**Ethnicity**
Chen et al.[5], 2006	2520 Taiwanese subjects included. NAFLD was found in 4.2% of non-obese participants.	Asian
Das K et al.[6], 2010	Of the 1911 individuals included, NAFLD was found in 8.6% of the population, of whom 75% were non-obese.	Indian
Kwon et al.[9], 2012	Large Korean population study (n=29944). NAFLD reported a prevalence of 12.6% in the non-obese participants.	Asian
Kim et al.[14], 2011	Icelandic study including 2945 patients. Hepatic steatosis was shown even in patients with a median BMI of 22.7	Caucasian
Browning et al.[15], 2004	In the Dallas Heart Study cohort, the prevalence of steatosis in subjects with BMI < 30 was 17%	Caucasian
Younossi et al.[16], 2012	Among the 11613 subjects of the NHANES III cohort, the prevalence of NAFLD in lean individuals was 7.4%	Caucasian
Cross-sectional Studies		
C.Selvakumar[17], 2018	Among 1482 US lean adolescence, 8% had NAFLD, and had a higher prevalence of low HDL, hypertriglyceridemia and insulin resistance.	Caucasian
Younossi et al.[16], 2012	'Lean' NAFLD was associated with younger age, female sex, insulin resistance and hypercholesterolemia	Caucasian
Feldman et al.[46], 2017	Although lean NAFLD patients have a better metabolic profile, they have lower adiponectin levels when compared to healthy controls.	Caucasian
Feng et al.[8], 2014	'Lean' NAFLD have a more active and pro-inflammatory visceral adipose tissue	Asian
Wong et al.[31], 2015	Waist circumference and triglyceride levels predicted the incidence of steatosis in lean individuals	Asian
Fracanzani et al.[30], 2017	Lean patients had a lower rate of MetS, diabetes, lower cardiovascular damage and lower hepatic histological damage	Caucasian
Akyuz et al.[52], 2015	Turkish cohort with lean prevalence of 7.6%. High hemoglobin levels were the only independent predictor of NASH and fibrosis in lean subjects.	Caucasian
Longitudinal Studies		
Leung et al.[3], 2017	Median follow up of 49-months. Hypertriglyceridemia and higher creatinine were associated with advanced liver disease in lean. Death and HCC were recorded only in the obese group.	Asian
Cruz et al.[55], 2014	Despite a better metabolic profile, less insulin resistance and fibrosis, lean subjects have a higher overall mortality than patients with NAFLD who are overweight or obese.	Mixed
Hagstrom et al.[56], 2018	After a median follow-up of 19.9 years, although patients with lean NAFLD showed lower fibrosis, they were at higher risk for development of severe liver disease compared to patients with NAFLD and a higher BMI, independent of available confounders.	Caucasian

*****in all studies, alternative etiologies of liver disease has been excluded.
BMI: Body Mass Index; NHANES: Third National Health and Nutrition Examination Surve; US: United States; HDL: high-density lipoprotein; HCC: hepatocellular carcinoma.

Figure 1 Pathophysiological Determinants of Non Alcoholic Fatty Liver Disease in Lean subjects

AT: Adipose Tissue; FFA: Free Fatty Acids; PNPLA3: patatin-like phospholipase domain-containing protein 3; TM6SF2: Transmembrane 6 Superfamily Member 2; CETP: Cholesteryl Ester Transfer Protein; IFN: Interferon; PEMT: phosphatidylethanolamine N-methyltransferase.

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