AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1659230> since 2018-01-26T13:49:29Z

Published version:

DOI:10.1038/nrgastro.2017.109

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Title: Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention**

2 **Authors:**

3 Zobair Younossi, Quentin M. Anstee, Milena Marietti, Timothy Hardy, Linda Henry, Mohammed Eslam,
4 Jacob George and Elisabetta Bugianesi.

5 **Institutions:**

6 Zobair Younossi & Linda Henry, Center for Liver Diseases, Department of Medicine, Inova Fairfax Hospital,
7 Falls Church, VA and Center for Outcomes Research in Liver Disease, Washington, DC; Quentin Anstee &
8 Timothy Hardy, Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University and Liver
9 Unit, Newcastle Upon Tyne Hospitals NHS Trust, Freeman Hospital, Newcastle upon Tyne, United Kingdom;
10 Mohammed Eslam & Jacob George, Storr Liver Unit, University of Sydney and Westmead Hospital,
11 Westmead, Australia; Milena Marietti & Elisabetta Bugianesi, Division of Gastroenterology, Department of
12 Medical Sciences, University of Torino, AOU Città della Salute e della Scienza, Torino, Italy.

13 **Author contributions**

14 The authors contributed equally to all aspects of this article.

15 **Competing interests statement**

16 The authors declare no competing interests for this manuscript.

17 **Correspondence to:**

18 Elisabetta Bugianesi, elisabetta.bugianesi@unito.it

19

20
21

22

23

24
25
26
27

28

Key Words:

Prevalence, incidence, ethnicity, genes, environment, steatohepatitis, cost-effectiveness.

Review criteria

PubMed was searched for articles published between 1990 and 2017 using the keywords “non-alcoholic fatty liver disease”, “steatohepatitis” or “fatty liver” combined with “prevalence”, “incidence”, “natural history”, “gene”, “lifestyle”, “lean”, or “children”. Articles published in languages other than English were excluded from the analysis.

29

30 **Key points:**

- 31 • Non Alcoholic Fatty Liver Disease (NAFLD) has gained clinical recognition as one of the most
32 important causes of liver disease worldwide both in adults and children as a consequence of the
33 pandemic spread of obesity, though some patients are lean.
- 34 • The global prevalence of NAFLD in the general population is currently estimated to be 24%, but the
35 highest rates are reported from South America and the Middle East, followed by Asia, United States
36 and Europe, whereas NAFLD is less common in Africa.
- 37 • What clearly sets NAFLD apart from other common liver diseases is the sheer volume of patients. In
38 this context, the major focus of clinical care is discerning NAFLD subjects at highest risk for
39 progressive liver disease
- 40 • Being overweight in childhood/late adolescence is associated with an increased risk of liver disease
41 due to NAFLD later in life. As a consequence, the threshold of liver-related morbidity and/or
42 mortality is reached at a younger age.
- 43 • NAFLD subjects have a high risk of liver-related morbidity/mortality along with metabolic
44 comorbidities and may place a growing strain on health systems from their need for management.
- 45 • NAFLD is a complex disease, affected by inter-related environmental factors and genetic
46 predisposition which varies in different regions of the world. Knowledge of the exact contribution
47 of each of the genetic or environmental components in promoting the burden of NAFLD should be
48 a priority in the research agenda.
- 49 • While waiting for effective therapies, NAFLD warrants the attention of primary care physicians,
50 specialists and health policy makers, starting from prevention of excessive weight gain during
51 childhood.

52

53

54 **Abstract**

55 NAFLD is one of the most important causes of liver disease worldwide and is likely to emerge as the leading
56 cause of end-stage liver disease (ESLD) in the coming decades, with the disease affecting both adults and
57 children. The epidemiology and demographic characteristics of NAFLD vary worldwide, usually parallel to
58 the prevalence of obesity, but a significant proportion of patients are lean. The large number of NAFLD
59 patients with potential for progressive liver disease creates challenges for screening, as the diagnosis of
60 NASH necessitates invasive liver biopsy. Furthermore, NAFLD subjects have a high frequency of metabolic
61 comorbidities and may place a growing strain on health systems from their need for management. While
62 waiting for effective therapies, this liver disease warrants the attention of primary care physicians,
63 specialists and health policy makers.

64

65

66 **Introduction**

67 During the last century, dramatic modifications in lifestyle have radically changed the health priorities in
68 most areas of the world. The new epidemic in chronic liver disease is related to the burden of Nonalcoholic
69 fatty liver disease (NAFLD), paralleling the worldwide increase of obesity. The global prevalence of NAFLD is
70 currently estimated to be 24%.¹ Community surveys utilizing ultrasonography or proton NMR spectroscopy
71 have assessed the prevalence of NAFLD across geographic locales (Figure 1), while studies based on
72 elevated liver enzymes systematically underestimated the true prevalence. NAFLD is highly prevalent in all
73 continents, but the highest rates are reported from South America (31%) and the Middle East (32%),
74 followed by Asia (27%), United States (24%) and Europe (23%), whereas NAFLD is less common in Africa
75 (14%).¹

76 NAFLD, particularly its histological phenotype nonalcoholic steatohepatitis (NASH), can potentially progress
77 to advanced liver disease, cirrhosis and hepatocellular carcinoma (HCC).² The prevalence of NAFLD is
78 constantly increasing (15% in 2005 to 25% in 2010) and similarly the rate of NASH is almost doubled in the
79 most recent studies compared to the oldest ones (59.1% versus 33%).¹ NASH is now considered the second
80 most common indication for liver transplant in the United States after chronic hepatitis C and is still
81 growing.² This review will provide evidence of the global burden of NAFLD and its clinical and economic
82 implications, which should be considered by health policies to secure a better future for coming
83 generations.

84

85

86 **Prevalence of NAFLD in the United States of America**

87 Over the recent decades, there has been extensive research to accurately determine the prevalence of
88 NAFLD in the United States.³⁻¹⁰ These data have recently been summarized in a meta-analysis reporting the
89 worldwide prevalence of NAFLD.¹ In most of these studies, the prevalence of NAFLD in the general
90 population was determined by imaging or other indirect methods. In this context, the prevalence of NAFLD
91 in the United States diagnosed by ultrasound was estimated to be 24.13% [95% CI: 19.73-29.15]. On the
92 other hand, the prevalence of NAFLD as determined by any other non-invasive methods (blood tests, ICD 9
93 or ICD 10 coding) was reported to be about 21.09% [95% 15.03-28.8], suggesting that diagnosis of NAFLD
94 that is solely based on the blood testing or ICD coding can lead to under-reporting of its true prevalence.¹

95 In the United States, the prevalence of NAFLD can vary by the ethnicity. In this context, the prevalence of
96 NAFLD is reported to be highest in the Hispanic Americans, followed by Americans of European descent
97 followed by African Americans.³⁻¹¹ Although still not fully resolved, a number of factors may explain these
98 reported ethnic disparities in the prevalence of NAFLD in the United States. These include genetic factors,
99 environmental factors, access to health care, and presence of chronic diseases such as metabolic
100 syndrome.^{1,6-8}

101 This issue is elucidated by the lower prevalence of NAFLD among African American than the Hispanic
102 Americans.^{5,8} This is especially surprising because of the higher prevalence of obesity and hypertension in
103 African American subjects.^{5,8}

104 In contrast, a study using the Third National Health and Nutrition Examination Survey (1988-1994) data
105 reported that metabolic syndrome was the primary driver of NAFLD among the non-Hispanic Blacks and the
106 Mexican Americans, but not for the White Americans. Despite some contradictory data regarding the
107 interaction of NAFLD and components of metabolic syndrome in African Americans, this study suggests that
108 the association of metabolic syndrome with NAFLD may be influenced by ethnicity.¹²

109 In another study using data from Non-alcoholic Steatohepatitis Clinical Research Network (NASH CRN),
110 investigators compared Latino subjects with NASH to non-Latino White subjects with NASH.¹³ The study
111 found that Latinos with NASH were younger, had less physical activity, but higher carbohydrate intake.
112 Furthermore, they reported that the effect of HOMA-IR on the risk of NASH was modified by ethnicity. In
113 this context, HOMA-IR was not a significant risk factor for NASH among Latinos (odds ratio [OR] = 0.93; 95%
114 confidence interval [CI]: 0.85-1.02), but was an important risk among non-Latino whites with NASH (OR,
115 1.06; 95% CI: 1.01-1.11). These data confirm that factors associated with NAFLD can be influenced by ethnic
116 background of the patient.¹³

117 It is also important to recognize that even within a certain ethnic group in the United States; there may be
118 differences in the prevalence of NAFLD. In fact, the prevalence of NAFLD among the Hispanic Americans can
119 vary according to the country of origin.^{8,14,15} In one such study, investigators compared the prevalence rates
120 of NAFLD subjects between Hispanics of Mexican origin and Hispanics of Dominican and Puerto Rican
121 origins (Caribbean area). Using data from the multi-ethnic study of atherosclerosis (MESA) cohort, the
122 overall Hispanic prevalence of NAFLD was 29%.¹⁴ On the other hand, Hispanics of Mexican origin had a
123 significantly higher prevalence of NAFLD at 33% while Hispanics of Dominican origin had a prevalence rate
124 of only 16% and Hispanics of Puerto Rican origin had a prevalence rate of 18%. After multivariate analysis,
125 Hispanics of Mexican origin continued to remain at higher risk of having NAFLD than the individuals of
126 Dominican and Puerto Rican origin.¹⁴

127 Although the ethnic and country of origin data regarding the prevalence of NAFLD is interesting, the exact
128 explanation for these ethnic differences remains unknown. Some of these differences can be explained by
129 the genetic factors that are described later in this review (nature) while others can be explained by
130 environmental factors (nurture), such as diet, exercise and alcohol consumption.

131 Finally, the prevalence of the progressive form of NAFLD or NASH in the general population remains
132 unknown. Nevertheless, there are indirect estimates for these rates by calculating prevalence of NASH in
133 NAFLD and prevalence of NAFLD in the general population. In this context, the prevalence of NASH among

134 subjects with NAFLD in the United States is reported to be 21% (95% CI: 19.85%- 22.95%).¹ Using this rate,
135 prevalence of NASH in the US population is estimated to be around 3-4%.¹ The corresponding prevalence
136 rates of the comorbid conditions associated with NASH have been reported to be obesity 82%, type 2
137 diabetes mellitus (T2DM) 48%, hyperlipidemia 82%, metabolic syndrome 76%, and hypertension 70%.¹

138

139 **Prevalence of NAFLD in South America**

140 The prevalence rate of NAFLD in South America seems to be higher than the rate reported for the United
141 States. Specifically, NAFLD prevalence (using ultrasound) for South America has been estimated to be
142 around 30.45% [95% CI: 22.74-39.4].¹ The majority of studies reporting the prevalence of NAFLD from
143 South America have been performed in Brazil.¹⁵⁻¹⁷ Nevertheless, in a study reported from Chile, the
144 prevalence of NAFLD (using ultrasound) was estimated to be 23%.¹⁸ Another study from Columbia also
145 using ultrasound, reported a prevalence rate of 26.6% in male subjects.¹⁹ Furthermore, the same
146 investigators have estimated that the prevalence of “probable NAFLD” based on the rates of obesity in
147 Peru, Argentina, Ecuador, Paraguay, and Uruguay could be as low as 13% (Peru) to as high as 24%
148 (Uruguay).¹⁹ Although there are estimates for the prevalence of NAFLD in South America, the data on the
149 prevalence of NASH is even more scarce. Nevertheless, in one study, 61% of the patients with NAFLD in
150 South America were found to have NASH which could make the prevalence of NASH from 6% to 18%.²⁰
151 These rates again can be influenced by genetic predisposition, as described later.

152 In summary, NAFLD prevalence rates do differ by ethnicity within the United States.^{1,18-21} The Hispanic
153 population has the highest prevalence while African Americans are reported to have the lowest prevalence,
154 despite having higher prevalence rates of hypertension and obesity, both NAFLD risk factors. There are also
155 ethnic differences noted within South America as well with Brazil reporting the highest prevalent rate and
156 Peru the lowest.

157

158 ***Incidence of NAFLD and Future Projections the United States and in South America***

159 Longitudinal studies of the general population are lacking in both the United States and South America. As a
160 result, there is no true population-based incidence rates reported NAFLD. However, Kanwal and colleagues
161 have suggested that the annual incidence of NAFLD within the USA should generally be stable at 2.2% to
162 3.2%.¹⁰ On the other hand, we know that NAFLD is highly associated with several metabolic conditions
163 [type 2 diabetes mellitus (T2DM), obesity, metabolic syndrome, hypertension, and hyperlipidemia].^{22,23}
164 Therefore, it is expected that the incidence of NAFLD should rise in parallel to the increasing incidence of
165 obesity and T2DM.²⁴ In addition to the data about the true incidence rates for NAFLD, it is also important to
166 determine the long -outcomes of patients with NAFLD. In this context, developing algorithms to define
167 which patients with NAFLD will develop the progressive form of NASH, cirrhosis, liver-related mortality or
168 cardiovascular mortality will be of great importance.

169

170
171

Epidemiology of NAFLD in Europe

172 In Europe a North-to-South gradient has been described for most chronic viral or non-viral hepatitis, but
173 this does not hold true for NAFLD. Rather, the globalization of NAFLD runs parallel to the prevalence rates
174 of obesity and varies accordingly, with degree of hepatic triglyceride accumulation being directly
175 proportional to the severity of each element of the metabolic syndrome.²⁵ Although prevalence varies
176 according to the modality used to detect NAFLD, approximately a quarter of the European population is
177 affected by this liver disease. A recent meta-analysis reported an average prevalence of 23.71% in Europe,
178 with variations ranging from 5% to 44% in different countries.¹

179 Data from the Study of Health in Pomerania (SHIP) cohort in North-eastern Germany, estimates the
180 prevalence of NAFLD to be around 30% when diagnosed by ultrasound.²⁶ A UK-based community study
181 determined that NAFLD was the most common aetiology for asymptomatic abnormal liver biochemistry,
182 accounting for 26.4% of cases (of whom 7.6% were predicted to have advanced liver disease).²⁷ Similarly, a
183 French series of liver biopsies in subjects with unexplained abnormal liver tests reported simple steatosis in
184 26.8% of cases, of whom 32.7% had NASH.²⁸ In the Dyonisos Study, the prevalence of NAFLD assessed by
185 ultrasound in Northern Italy was similar in subjects with and without suspected liver disease (25 vs. 20%, P
186 =0.203), defined as altered liver enzymes or positivity of HBsAg and/or anti-HCV. Notably, only 54% of
187 NAFLD cases occurred in patients with elevated ALT,²⁹ but the vast majority of them had many features of
188 the metabolic syndrome. Epidemiological data from Spain describe similar rates, with a NAFLD prevalence
189 of 25.8% in the adult population.³⁰ Only few studies are available from Eastern Europe. In Romania, NAFLD
190 assessed by ultrasound has been found in 20% of 3005 hospitalized patients without liver disease,³¹ while a
191 study on healthy Hungarian adult confirmed a 22.6% overall prevalence of sonographically detected fatty
192 liver.³²

193 As expected, the prevalence of NAFLD increases substantially in “at-risk” groups such as patients with type
194 2 diabetes (T2DM).² The two major available studies, conducted on a large cohort of Italian patients with
195 T2DM, reported NAFLD prevalence rates of 60–70%,^{33,34} while UK data suggests that ultrasound detected

196 NAFLD is present in 42.6% of patients with T2DM.³⁵ Similarly, prevalence increases with body mass index
197 (BMI) so that 91% of obese patients (BMI ≥ 30 kg/m²), 67% of overweight (BMI 25-30 kg/m²) and 25% in
198 normal weight individuals had ultrasound evidence of NAFLD in an unselected Italian population sample.³⁶
199 The prevalence of NAFLD among subjects matching at least one of the ATP III criteria for lipid alterations is
200 similarly high (78.8 %).³³

201 Data regarding the prevalence of advanced forms of NAFLD and NASH in the general population is more
202 limited. A community-based study from the Netherlands using a transient elastography reading of ≥ 8 kPa
203 for the diagnosis of liver fibrosis, estimated that clinically significant fibrosis was present in 5.6% (169/3041)
204 of total subjects and 8.4% (69/822) of those with NAFLD, and was positively associated with steatosis and
205 T2DM.³⁷ In this respect, the influence of T2DM on risk of progressive NAFLD is supported by a UK-based
206 paired-biopsy study that showed incident T2DM to be the strongest predictor of progressive disease.³⁸ A
207 Greek post-mortem study from 498 cases of ischaemic heart disease or traffic accident deaths revealed
208 simple steatosis in 31.3% and NASH in 39.8% of cases.³⁹ In a recent study performed on Spanish patients
209 with gallstone disease scheduled for cholecystectomy, 51.6% of them had histological evidence of NAFLD
210 and 19.8% of NASH.⁴⁰ Of note, in this cohort ultrasonography confirmed a fatty liver in only 67.6% of the
211 histologically diagnosed NAFLD subjects. Similar data are reported in healthy people evaluated in
212 Transplant Units as potential living liver donors. In a single retrospective study performed in a mixed
213 American and Italian cohort, the histological prevalence of steatosis was 48.5%, including a 15.5% of
214 steatohepatitis.⁴¹ However, both NAFLD and NASH were more frequently found in Americans compared
215 with Italians (54% vs 34% for NAFLD and 17.6% vs 16.2% for NASH, respectively). The rates of NASH are
216 clearly increased in patients referred to tertiary centers for NAFLD. In a recent meta-analysis the pooled
217 NASH prevalence in Europe among NAFLD patients with an indication for biopsy was 69.25% (95% CI:
218 55.93-79.98).¹

219 ***Incidence of NAFLD and future projections in Europe***

220 Only a small number of studies explored the incidence of NAFLD in the general population. Over a follow up
221 of 8.5 years, the incidence of ultrasound detected NAFLD was 18.5 per 1,000 person-years in a sample
222 representative of the general Italian population.³⁶ On the contrary, recent data are clarifying the natural
223 history of histologically diagnosed NAFLD. In a Swedish cohort of 229 biopsy-proven NAFLD patients with a
224 mean longitudinal follow-up of 26.4±5.6 years, compared to a matched reference population sample,
225 NAFLD patients had significantly increased all-cause mortality (hazard ratio [HR] 1.29, 95%CI 1.04-1.59),
226 exhibited an increased risk of cardiovascular disease (HR 1.55, 95%CI 1.11-2.15), hepatocellular carcinoma
227 (HR 6.55, 95%CI 2.14-20.03) and cirrhosis (HR 3.2, 95%CI 1.05-9.81).⁴² The presence of fibrosis was found to
228 be the strongest prognostic factor for liver-related events and mortality.⁴² Consistent with this, the burden
229 of NAFLD-related hepatocellular carcinoma is also increasing dramatically. A study from North East England
230 found that NAFLD associated HCC accounted for 35% of all cases (41/118) in 2010, representing a >10-fold
231 increase in 10 years.⁴³ Finally, results from the UK NHS Blood and Transplant Agency show that
232 decompensated NASH cirrhosis accounted for an increased proportion of patients undergoing liver
233 transplant (12% in 2013 compared with 4% in 1995).⁴⁴

234

235

236 **Epidemiology of NAFLD in the Asia-Pacific and Africa**

237 Within the Asia-Pacific Region, NAFLD prevalence varies widely as would be expected from a region of at
238 least 55 countries with marked disparities in rates of development within the economic, political and
239 educational spheres, and tied to this, variations in nutrition, lifestyle and sedentary behaviour. Further,
240 there is a bias towards reporting studies that emanate from more affluent economics in the region with
241 better health systems. Unlike from Europe and North America, data from the Asia Pacific and Africa is not
242 as comprehensive both between and within countries and there is a total absence of this information from
243 many countries.

244 Though there are no nationwide epidemiological surveys that include an assessment of liver fat, even
245 within a single country such as China, there are striking differences according to region and over time in the
246 prevalence of NAFLD. As an example, NAFLD prevalence in the populations of Chengdu (Southwest China),
247 Shanghai (East China), Guangdong (South China) and central China were 12.5%, 15%, 17% and 24.5%,
248 respectively.⁴⁵⁻⁴⁷ On the other hand, a more recent ultrasound based study of 7152 employees from
249 Shanghai suggested that NAFLD prevalence was as high as 38.17%.⁴⁸ In Hong Kong, a community-based
250 study employing state of the art proton-magnetic resonance spectroscopy to quantify liver fat estimated a
251 NAFLD prevalence of 28.8%; 19.3% in non-obese subjects and 60.5% among the obese.⁴⁹ Similarly in
252 Taiwan, the prevalence of NAFLD was reported to be 11.4% in the general community⁵⁰ but higher in
253 certain sub-populations including the elderly (50.1%)⁵¹ and in those with a typically inactive lifestyle (66.4%
254 in Taxi drivers).⁵²

255 In the Far East, the community prevalence of NAFLD was ~25% in Japan, increasing from 12.6% before 1990
256 to 30.3% in 1998.⁵³ More recent reports suggest that 23-26% of subjects undergoing routine health
257 screening have fatty liver by abdominal ultrasonography.⁵⁴ Using similar methodology, the reported
258 prevalence of NAFLD in Korea in 141,610 subjects was 27.3%.⁵⁵

259 South Asia and the Indian sub-continent are currently in the throes of rapid economic and social change,
260 with trends towards urbanization and an urban/rural economic divide. Not unexpectedly, in rural India, a

261 region characterized by traditional diets and lifestyles, the prevalence of NAFLD is remarkably low (~9%),
262 while it mimics western prevalence rates in urban populations, with rates varying between 16% and 32%.⁵⁶⁻
263 ⁵⁸ A similar dramatic variation in NAFLD prevalence (5-30%) was observed from smaller surveys in Sri Lanka
264 Malaysia, Singapore and Indonesia.⁵⁹⁻⁶²

265 Overall, while NAFLD prevalence rates are varied but increasing across Asia, given that this region is subject
266 to the same global forces of change towards energy dense food consumption and reduced physical activity,
267 NAFLD rates between the East and West are more similar than different, in the context of a similar
268 obesogenic environment.

269 The scanty available data on the prevalence of NAFLD in Africa suggests that Africans tend to have lower
270 prevalence, consistent with what has been reported in African Americans. In Nigeria, prevalence between
271 9.5%-16.7% in diabetics and 1.2%-4.5% in non-diabetics has been reported.^{63,64} Similarly, in South Africa,
272 the prevalence in obese and overweight was 45-50%.⁶⁵ A recent small population based study suggested a
273 prevalence of 20% in the Sudanese population.⁶⁶

274

275

276

277 **Lean NAFLD**

278 Initially described in Asian populations, NAFLD in the absence of obesity, i.e., the so-called ‘lean’ NAFLD,
279 can develop in around 10%-20% of non-obese Americans and Caucasians^{5,67} (Figure 2 and Supplementary
280 Tables A and B)^{45,49,50,56,67-78}. It deserves clinical attention as many physicians have a perception that lean
281 NAFLD is more “benign” in nature. Lean NAFLD encompasses a heterogeneous spectrum of disease arising
282 from different aetiologies, as listed in Table 1. Visceral obesity as opposed to general obesity, high fructose
283 and high fat intake and genetic risk factors, including congenital defects of metabolism, may be associated
284 with lean NAFLD. It is likely that a vast bulk of lean NAFLD cases belong to the phenotype of “metabolically
285 obese normal weight” subjects,⁷⁹ described in at least 5% of the occidental population. It consists of a
286 subgroup of non-obese, frequently sedentary subjects who display altered insulin sensitivity, increased
287 cardiovascular risk and increased liver lipid, the consequence of decreased capacity for storing fat and
288 reduced mitochondrial function in adipose tissue and increased de novo lipogenesis in liver.⁷⁹ When
289 compared to overweight-obese NAFLD patients, lean NAFLD subjects are younger and have a lower
290 prevalence of MetS (2%-48% versus 22-64% in overweight-obese).^{67,80} However, these patients are usually
291 insulin-resistant and have higher plasma triglycerides when compared to matched controls without
292 NAFLD.^{74,80} In a cohort of non-obese, non-diabetic subjects with biopsy-proven NAFLD, the metabolic
293 pattern was similar to that observed in obesity, with adipose tissue IR playing an important role.⁸¹

294 Since lean NAFLD is usually present with less obesity-related comorbidities, it is commonly believed that
295 this group would follow a relatively benign clinical course. Within the cohort of the National Health and
296 Nutrition Examination Survey III (NHANES III),⁷⁹ mortality of metabolically-normal NAFLD patients was
297 similar to the cohort without liver disease. Unfortunately, most reports are limited by the use of imaging
298 modalities rather than liver biopsy to confirm the diagnosis of fatty liver.^{5,74,79-84} In an Italian study⁸²
299 including 430 biopsy proven NAFLD, 55% of patients without visceral obesity had NASH and fibrosis $\geq$ F2
300 despite milder metabolic alterations. In a recent study⁸³ similar proportions of obese and non-obese

301 patients had NASH (51.9% versus 43.5%, $P = 0.217$), although the latter group had a lower degree of
302 steatosis and hepatocyte ballooning. Consistent with earlier reports ⁷⁹, the proportion of patients with
303 advanced fibrosis at baseline was not different between obese and non-obese subjects, suggesting that
304 once an individual declares him or herself as having NASH, obesity may not be the main driver of fibrosis
305 progression. Genetic factors might be involved in lean NAFLD, however the presence of NASH in these
306 subjects was not explained by mutations able to influence either IR (ENPP1 and IRS-1 polymorphisms) or
307 the severity of steatosis (PNPLA3 and TM6SF2 polymorphisms).⁸²

308 The longitudinal risk of mortality in lean NAFLD has been scarcely explored. In the above-mentioned study,
309 ⁸² after a median follow-up of 49 months, clinical events occurred in 11.9% of obese patients and 8.3% of
310 nonobese patients ($P = 0.190$). Cardiovascular events accounted for about two thirds of all major events in
311 both groups. All deaths ($n=6$) occurred in the obese group, but definitive conclusions are difficult to make
312 as follow-up was relatively short. An international cohort study including 483 cases with a mean follow-up
313 period of over 11 year ⁸⁵, published so far in abstract form, challenged the concept that the prognosis of
314 patients with NAFLD who have normal BMI is benign. Despite presenting with a healthier metabolic profile
315 and less advanced liver fibrosis, median survival free of liver transplantation was significantly shorter in
316 lean than in non-lean patients (18.1 vs. 26.6 years, respectively, $p<0.001$).

317 The final question is how to manage lean subjects diagnosed with NAFLD, given that it might be harder to
318 correct the underlying risk factors. Careful identification and correction of environmental causes, such as
319 significant fructose consumption, may be effective particularly in young patients. Weight loss remains the
320 background therapy in all cases with overweight/obesity, but in lean NAFLD patients habitual physical
321 activity should also be emphasized. A call for more studies to understand the natural history of the disease
322 but also for greater awareness among practitioners about the potential health risks associated with lean
323 NAFLD is urgently needed.

324

325

326

327 **Table 1**

Causes of NAFLD in lean subjects	
Environmental	
<ul style="list-style-type: none">• high-fructose diet, high fat diet• DAFLD (Dual alcoholic and nonalcoholic fatty liver disease)	
Metabolically Obese Normal Weight Subjects	
Congenital and acquired lipodistrophy (HIV-HAART)	
Genetic	
<ul style="list-style-type: none">• PNPLA3• Congenital defects of metabolism (FHLB, LAL-D)	
Endocrine disorders (PCOS, hypothyroidism, GH deficiency)	
Drug-related (amiodarone, methotrexate, tamoxifen)	
Jejunoileal bypass, starvation, TPN	

328 FHLB, Familial Hypobetalipoprotein B; GH, Growth Hormone; LAL-D, Lysosomal Acid Lipase Deficiency; PCOS,

329 Polycystic Ovary Syndrome; TPN, Total Parenteral Nutrition.

330

331 **The future impact of pediatric NAFLD**

332 Obesity in children has risen from 5.0% in 1960 to 16.9% in 2009-2010.⁸⁶ The obesity-related risk of future
333 liver disease is alarming, as a weight gain during school-years carries a higher risk of NAFLD than weight-
334 gain in late adulthood. In a large longitudinal Danish study,⁸⁷ a weight increase during childhood and early
335 adolescence was related to all histological features of adult NAFLD even after adjusting for initial as well as
336 attained BMI. Among children with similar attained BMIs at 13 years of age, the risk of cirrhosis in
337 adulthood was increased by 16% per each unit gain in BMI z-score at every age from 7 through 13 years.⁸⁷
338 Similarly, also a weight gain during late adolescence is able to induce an increased susceptibility of
339 developing NAFLD later in life. Another study tested the association of basal BMI on the development of
340 End-Stage-Liver-Disease (ESLD) or liver-related death in a general population cohort of 44,248 men aged
341 18-20 years that attended military service in Sweden between 1969 and 1970.⁸⁸ After a follow-up of almost
342 38 years, being overweight in late adolescence increased the risk of liver-related outcomes by 64%
343 compared with a low-normal range BMI, with a 5% increased risk for each unit of BMI above normal
344 range.⁸⁸ Obesity early in life also increases the adulthood risk of HCC. Another Danish study including
345 schoolchildren 7 through 13 years old ⁸⁹ showed that each unit increase in BMI z-score increased by 20–
346 30% the risk of liver cancer 30 years later. In other words, compared with an average height and weight 13-
347 year-old boy, a boy of similar height but who weighed 6 kg more would have a 30% increased risk of liver
348 cancer.⁸⁹ Besides the weight trajectory, other mechanisms appear to influence the spectrum of liver
349 damage in NAFLD later in life. In the Cardiovascular Risk in Young Finns Study ⁹⁰, after a follow-up of 31
350 years, adult NAFLD was predicted by modifiable as well as non-modifiable risk factors during childhood,
351 including BMI and insulin levels, male sex, genetic background (that is, *PNPLA3* and *TM6SF2* variants) and
352 low birth weight, an emerging risk factor for adulthood NAFLD probably related to intrauterine epigenetic
353 regulations. Overall, this means that NAFLD and its complications, including HCC, are more likely to be
354 anticipated at a fairly young age, foreseeing a possible reduction of life expectancy and an additional
355 societal burden.

356 **Risk factors: nature or nurture?**

Evidence from patients that have undergone serial liver biopsies over an interval of several years demonstrates that the progression of NAFLD from steatosis to NASH and fibrosis is not linear and is likely to be more dynamic than previously thought.^{38,91} Furthermore, evidence from familial aggregation and twin studies have shown a significant heritable component to NAFLD.^{92,93} Interestingly, the genetic susceptibility for the development of steatosis and fibrosis may be shared.⁹⁴ Different ethnic groups have disparate propensities to advanced disease, with Hispanics being more susceptible than whites, while the lowest susceptibility is observed in blacks.⁹⁵ An interesting systematic review suggested that the leading explanations for the lowest incidence and prevalence of both NAFLD and NASH in African-Americans in the United States is related to genetic differences in lipid metabolism, i.e. lower triglyceride levels and significantly higher serum HDL-c in this ethnic group compared to Hispanics and Caucasians with NAFLD.⁹⁶

In NAFLD, genome wide association studies (GWAS) have identified novel loci associated with disease severity phenotypes. A full discussion is beyond the scope of this article but the available literature has recently been reviewed elsewhere.⁹⁷ To date, non-synonymous SNPs in two genes in particular: *patatin-like phospholipase domain-containing 3 (PNPLA3)* and *transmembrane 6 superfamily member 2 (TM6SF2)* have most consistently been validated in separate large cohorts.^{98,99} Figure 1 shows the distribution of *PNPLA3* genotypes in NAFLD patients according to geographical areas. *PNPLA3* is presented as MAF frequency. Amongst the emerging newly discovered risk loci, variants near the *membrane bound O-acyltransferase domain-containing 7 gene (MBOAT7)* and *transmembrane channel-like 4 gene (TMC4)* have been shown to be associated with development and severity of NAFLD in patients of European descent.¹⁰⁰ Similarly, within the Latino population in South America, the TM6SF2 E167K and PNPLA3 I148M gene variants seem to be responsible for susceptibility to progressive NASH.¹⁰¹

Whilst there have been major advances uncovering the genetic basis for the heritability of NAFLD, heritable mechanisms other than those encoded within the nucleotide sequence of genes are emerging. Small non-coding RNAs such as microRNAs (miRNAs), recently been shown to explain discordant NAFLD in genetically identical twins.¹⁰² Epigenetic factors may also be a mechanism through which environmental exposures exert a heritable effect on disease risk. Remodelling of DNA methylation at key fibrosis modifier genes

underpinned murine ancestral protection to liver fibrosis.¹⁰³ Remarkably, similar remodelling occurred in NASH patients with mild fibrosis and there is intriguing data to suggest that epigenetic signatures present in the blood on circulating cell-free DNA may be a potential biomarker of this effect and thus disease severity.

103,104

Genetic predisposition must be placed in the context of environmental factors that also play an important role. The most relevant factors are dietary habits, activity and socio-economic factors.

Although a large amount of data suggests that dietary composition may predispose subjects to NAFLD, evidence at the population level is less well characterised. In this context, a recent study reported that subjects with NAFLD tended to reside in areas with many food source options including grocery stores, restaurants and fast food places. Furthermore, those with NAFLD were more likely to report having the unhealthiest eating habits (eating foods with high fat, high salt, high sugar/corn syrup, processed foods) and reported eating more frequently at restaurants.¹⁰⁵ Other studies focused on the nutritional assessments of subjects with NAFLD have further documented consumption of low-nutrient, high sodium and high fat foods, especially diets with high-fat food from meat and lower amounts of fresh fruits.^{106,107} In addition to the dietary habits, subjects with fatty liver were found to have very low physical activity levels and increased sitting times.¹⁰⁸⁻¹¹⁰

The prevalence of NAFLD is also related to socio-economic factors, but their exact role is debated. In a study exploring the role of environmental factors in different ethnic groups with NAFLD, acculturation, educational level, healthcare use, and income along with dietary and lifestyle factors and sleep, were not found to be independently associated with risk of developing NAFLD, suggesting that the environmental factors may play a role on a background of genetic predisposition.¹⁶

Alcohol consumption in the context of NAFLD should be carefully considered. Data from the SHIP study demonstrates that the presence of obesity and alcohol consumption are not mutually exclusive. In subjects with radiologically diagnosed hepatic steatosis, 27.3% of males and 9.7% of females fulfilled criteria for both obesity and high alcohol consumption (i.e. Dual Aetiology Fatty Liver Disease, 'DAFLD').¹¹¹ Prospective

408 data from the UK in 9559 men with up to 42-years of follow up unequivocally show that alcohol
409 consumption and the presence of obesity act synergistically to increase the risk of liver disease morbidity
410 and mortality.¹¹² Ultimately, to fully address the impact of even moderate alcohol consumption on NAFLD
411 will require prospective, longitudinal studies recording cumulative lifetime alcohol consumption.

412 Overall, these data confirm the concept that NAFLD is a complex disease and is affected by inter-related
413 environmental factors and genetic predisposition. The exact contribution of each of the genetic or
414 environmental components in the promoting the burden of NAFLD is not known and may vary in different
415 regions of the world. Therefore, future studies need to focus on this gap of knowledge in order to better
416 determine treatment and improve patient outcomes.

417

418 **Global perspectives, healthcare challenges and prevention strategies**

419 With an estimate of 64 million individuals affected in the United States and 52 million in European
420 countries,^{1,113} what clearly sets NAFLD apart from other common liver diseases is the sheer volume of
421 patients. In this context, the major focus of clinical care is discerning NAFLD subjects at highest risk for liver-
422 related complications. The recently released EASL-EASD-EASO guidelines recommend that individuals with
423 obesity or any component of the metabolic syndrome should have an ultrasound, steatosis biomarkers and
424 liver enzymes measured.¹¹⁴ Further, a burning issue is the development of HCC in non-cirrhotic NASH. As
425 the clinical consequences of NAFLD grow, the economic consequences will also increase. A recent model on
426 the population of US and of four European countries (Germany, France, Italy, and United Kingdom)
427 estimated the annual burden associated with all incident and prevalent NAFLD cases at \$103 billion in the
428 United States (\$1,613 per patient) and at €35 billion in the Europe-4 countries (from €354 to €1,163 per
429 patient).¹¹³ In a study of Medicare NAFLD patients, the mean yearly inflation-adjusted charges from the
430 outpatient setting increased from \$2,624 ± \$3,308 in 2005 to \$3,608 ± \$5,132 in 2010.¹¹⁵ If we assume the
431 annual rate of increase in the costs due to NAFLD to parallel the annual growth in the prevalence of obesity,
432 the expected 10-year burden of NAFLD could increase to an estimated \$1.005 trillion in the United States

433 and €334 billion in the Europe.¹¹³ In addition to the direct annual cost of NAFLD, there is a societal cost
434 related to the loss of QALYs and the burden of metabolic complications, including cardiovascular disease.

435 The main question is whether this enormous cost would be justified, provided it will be affordable. Cost-
436 utility analysis of NASH screening is hampered by the lack of evidence around the early stages of the
437 disease progression, uncertainties around the non-invasive markers of liver damage and the lack of
438 effectiveness data relating to the impact of treatment in patients with NASH. Steatosis testing has not been
439 recommended by the UK National Institute for Health and Care Excellence (NICE) NAFLD Guideline
440 Committee (GC), due to the uncertainty both in the cost effectiveness results for all tests and in the clinical
441 evidence base.¹¹⁶ On the other hand, both the EASL-EASO-EASD and the UK NAFLD CG recommend
442 biomarkers and transient elastography/ARFI to screen subjects with NAFLD for advanced fibrosis and
443 cirrhosis.^{114,116} In the end, screening for NASH will likely be cost-effective when medications with
444 reasonable efficacy and side effects will be available.

445

446 **Conclusions**

447 NAFLD is now the leading cause of chronic liver disease in the United States and Europe and increasing
448 worldwide, but there is a paucity of prospective population-based cohort studies from other geographical
449 areas, including South America, Asia, Australia and Africa, which are needed to better understand the
450 global burden of disease. Understanding the genetic and environmental risk factors of NAFLD and NASH
451 and their distribution across different countries is of paramount importance to develop strategies to
452 implement a multipronged public health policy and deal with this important chronic liver disease.

453

454

455 **Figure 1:** Worldwide estimated prevalence of NAFLD and the distribution of PNPLA3 genotypes. PNPLA3 is
456 presented as MAF frequency (red section of the pie chart).

457

458 **Figure 2:** A) The proportion of NAFLD in lean Subjects as compared to obese Subjects; B) The proportion of
459 NAFLD in lean Subjects. Data taken from references (45, 49, 50, 56 and 67-87).

461 **Supplementary Table A: Summary of studies showing the proportion of NAFLD in lean as compared to**
462 **obese patients.**

Country	Number	NAFLD prevalence			Method of diagnosis	Reference
		Overall	Non-obese	Obese		
India (lucknow)	280 subjects were screened and 150 were enrolled	53%	20%	80%	Ultrasonography	Bhat, et al. (68)
China (Heilongjiang)	2000 subjects were screened and 1779 were enrolled	44.9%	18.33	72.9%	Ultrasonography	Feng, et al. (69)
Japan (Kyoto)	5433 subjects were screened and 3271 were enrolled	24.6%	15.2%	68.5%	Ultrasonography	Nishioji, et al. (70)
South Korea (Seoul)	3123 subjects were screened and 2307 were enrolled	36%	22.4%	60.9%	Ultrasonography	Kim, et al. (71)
Hong Kong	3000 subjects were invited and 911 were enrolled	28.8%	19.3%	60.5%	¹ H-MRS	Wei, et al. (49)
Japan (Nagasaki)	3579 subjects were screened and 3432 were enrolled	21.8%	11%	60%	Ultrasonography	Omagari, et al. (72)
South Korea (Seoul)	59,771 subjects were screened and 29,994 were enrolled	20.1%	12.6%	50.1%	Ultrasonography	Kwon, et al. (73)
China (Shanghai)	4205 subjects were screened and 3175 were enrolled	20.82%	21%	39%	Ultrasonography	Fan, et al. (45)
Taiwan (Shengang)	12,474 residents were invited and 3245 were enrolled	11.5%	4.2%	30.8%	Ultrasonography	Chen, et al. (50)

464 **Supplementary Table B: Summary of studies showing the proportion of NAFLD in lean patients.**

Country	Number	NAFLD prevalence in Non-obese	Method of diagnosis	Reference
Belgium	1,777	2.8% ((38%) of cryptogenic liver disease)	Liver biopsy	Vos, et al. (74)
USA	11,613 were considered, 2492 fulfilled the definition of NAFLD.	7.39%	Ultrasonography	Younossi, et al. (67)
Greece	185	12%	Ultrasonography and/or liver histology	Margariti, et al. (75)
Korea (Changwon)	2,058 subjects were considered, 1,711 fulfilled the definition of NAFLD	12.4%	Ultrasonography	Cho et al., (76)
Australia	422	14%	Liver biopsy	Personal communication
Spain	262	21%	Blood test (OWLiver® test)	Ortiz, et al. (78)
Korea (Seoul)	768	23.4%	Ultrasonography	Kim, et al. (77)
Korea (Seoul)	3,123 subjects were screened and 2307 were enrolled	30.3% at follow-up assessment. The mean duration of follow-up was 28.7 ± 13.2 months	Ultrasonography	Kim, et al. (71)
India (Kolkata)	1,911	75%	Ultrasonography and CT	Das, et al. (56)

465

466
467
468
469
470
471
472
473

474

475

476

477

Acknowledgements

Work by the authors of this Review was funded by Horizon 2020 Framework Program of the European Union (under grant agreement no. 634413 for the project EPoS to E. Bugianesi and Q.M. Anstee) and by the Robert W. Storr Bequest to the Sydney Medical Foundation, University of Sydney; a National Health and Medical Research Council of Australia (NHMRC) Program Grant (1053206) and Project grants (APP1107178 and APP1108422)(to M. Eslam and J. George). T. Hardy is the recipient of a Clinical Research Training Fellowship from the Medical Research Council, UK.

479 **References**

- 480 1 Younossi, Z. M. *et al.* Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic
481 assessment of prevalence, incidence, and outcomes. *Hepatology* **64**, 73-84, doi:10.1002/hep.28431
482 (2016).
- 483 2 Anstee, Q. M., Targher, G. & Day, C. P. Progression of NAFLD to diabetes mellitus, cardiovascular
484 disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* **10**, 330-344, doi:10.1038/nrgastro.2013.41
485 (2013).
- 486 3 Lazo, M. *et al.* Prevalence of nonalcoholic fatty liver disease in the United States: the Third National
487 Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol* **178**, 38-45,
488 doi:10.1093/aje/kws448 (2013).
- 489 4 Pan, J. J. & Fallon, M. B. Gender and racial differences in nonalcoholic fatty liver disease. *World J*
490 *Hepatol* **6**, 274-283, doi:10.4254/wjh.v6.i5.274 (2014).
- 491 5 Browning, J. D. *et al.* Prevalence of hepatic steatosis in an urban population in the United States:
492 impact of ethnicity. *Hepatology* **40**, 1387-1395 (2004).
- 493 6 Schneider, A. L., Lazo, M., Selvin, E. & Clark, J. M. Racial differences in nonalcoholic fatty liver
494 disease in the U.S. population. *Obesity (Silver Spring)* **22**, 292-299, doi:10.1002/oby.20426 (2014).
- 495 7 Sherif, Z. A. *et al.* Global Epidemiology of Nonalcoholic Fatty Liver Disease and Perspectives on US
496 Minority Populations. *Dig Dis Sci* **61**, 1214-1225, doi:10.1007/s10620-016-4143-0 (2016).
- 497 8 Saab, S., Manne, V., Nieto, J., Schwimmer, J. B. & Chalasani, N. P. Nonalcoholic Fatty Liver Disease in
498 Latinos. *Clin Gastroenterol Hepatol* **14**, 5-12; quiz e19-10, doi:10.1016/j.cgh.2015.05.001 (2016).
- 499 9 Balakrishnan, M., Kanwal, F., El-Serag, H. B. & Thrift, A. P. Acculturation and Nonalcoholic Fatty Liver
500 Disease Risk Among Hispanics of Mexican Origin: Findings From the National Health and Nutrition
501 Examination Survey. *Clin Gastroenterol Hepatol* **15**, 310-312, doi:10.1016/j.cgh.2016.09.149 (2017).
- 502 10 Kanwal, F. *et al.* Trends in the Burden of Nonalcoholic Fatty Liver Disease in a United States Cohort
503 of Veterans. *Clin Gastroenterol Hepatol* **14**, 301-308 e301-302, doi:10.1016/j.cgh.2015.08.010
504 (2016).
- 505 11 Carroll, J. F. *et al.* Impact of race/ethnicity on the relationship between visceral fat and inflammatory
506 biomarkers. *Obesity (Silver Spring)* **17**, 1420-1427, doi:10.1038/oby.2008.657 (2009).
- 507 12 Smits, M. M., Ioannou, G. N., Boyko, E. J. & Utzschneider, K. M. Non-alcoholic fatty liver disease as
508 an independent manifestation of the metabolic syndrome: results of a US national survey in three
509 ethnic groups. *Journal of gastroenterology and hepatology* **28**, 664-670, doi:10.1111/jgh.12106
510 (2013).
- 511 13 Bambha, K. *et al.* Ethnicity and nonalcoholic fatty liver disease. *Hepatology* **55**, 769-780,
512 doi:10.1002/hep.24726 (2012).
- 513 14 Fleischman, M. W., Budoff, M., Zeb, I., Li, D. & Foster, T. NAFLD prevalence differs among hispanic
514 subgroups: the Multi-Ethnic Study of Atherosclerosis. *World J Gastroenterol* **20**, 4987-4993,
515 doi:10.3748/wjg.v20.i17.4987 (2014).
- 516 15 Karnikowski, M., Cordova, C., Oliveira, R. J., Karnikowski, M. G. & Nobrega Ode, T. Non-alcoholic
517 fatty liver disease and metabolic syndrome in Brazilian middle-aged and older adults. *Sao Paulo*
518 *Med J* **125**, 333-337 (2007).
- 519 16 Kallwitz, E. R. *et al.* Prevalence of suspected nonalcoholic fatty liver disease in Hispanic/Latino
520 individuals differs by heritage. *Clin Gastroenterol Hepatol* **13**, 569-576,
521 doi:10.1016/j.cgh.2014.08.037 (2015).
- 522 17 Oni, E. T. *et al.* Relation of physical activity to prevalence of nonalcoholic Fatty liver disease
523 independent of cardiometabolic risk. *Am J Cardiol* **115**, 34-39, doi:10.1016/j.amjcard.2014.09.044
524 (2015).
- 525 18 Riquelme, A. *et al.* Non-alcoholic fatty liver disease and its association with obesity, insulin
526 resistance and increased serum levels of C-reactive protein in Hispanics. *Liver international : official*

527 *journal of the International Association for the Study of the Liver* **29**, 82-88, doi:10.1111/j.1478-
528 3231.2008.01823.x (2009).

529 19 Lopez-Velazquez, J. A. *et al.* The prevalence of nonalcoholic fatty liver disease in the Americas. *Ann*
530 *Hepatol* **13**, 166-178 (2014).

531 20 Feijo, S. G. *et al.* The spectrum of non alcoholic fatty liver disease in morbidly obese patients:
532 prevalence and associate risk factors. *Acta Cir Bras* **28**, 788-793 (2013).

533 21 Hernaez, R. *et al.* Association between variants in or near PNPLA3, GCKR, and PPP1R3B with
534 ultrasound-defined steatosis based on data from the third National Health and Nutrition
535 Examination Survey. *Clin Gastroenterol Hepatol* **11**, 1183-1190 e1182,
536 doi:10.1016/j.cgh.2013.02.011 (2013).

537 22 Ballestri, S., Nascimbeni, F., Romagnoli, D. & Lonardo, A. The independent predictors of non-
538 alcoholic steatohepatitis and its individual histological features.: Insulin resistance, serum uric acid,
539 metabolic syndrome, alanine aminotransferase and serum total cholesterol are a clue to
540 pathogenesis and candidate targets for treatment. *Hepatology research : the official journal of the*
541 *Japan Society of Hepatology* **46**, 1074-1087, doi:10.1111/hepr.12656 (2016).

542 23 Stepanova, M. *et al.* Predictors of All-Cause Mortality and Liver-Related Mortality in Patients with
543 Non-Alcoholic Fatty Liver Disease (NAFLD). *Dig Dis Sci* **58**, 3017-3023, doi:10.1007/s10620-013-2743-
544 5 (2013).

545 24 Motamed, N. *et al.* Non-alcoholic fatty liver disease (NAFLD) and 10-year risk of cardiovascular
546 diseases. *Clin Res Hepatol Gastroenterol* **41**, 31-38, doi:10.1016/j.clinre.2016.07.005 (2017).

547 25 Kotronen, A., Westerbacka, J., Bergholm, R., Pietilainen, K. H. & Yki-Jarvinen, H. Liver fat in the
548 metabolic syndrome. *J Clin Endocrinol Metab* **92**, 3490-3497, doi:10.1210/jc.2007-0482 (2007).

549 26 Haring, R. *et al.* Ultrasonographic hepatic steatosis increases prediction of mortality risk from
550 elevated serum gamma-glutamyl transpeptidase levels. *Hepatology* **50**, 1403-1411,
551 doi:10.1002/hep.23135 (2009).

552 27 Armstrong, M. J. *et al.* Presence and severity of non-alcoholic fatty liver disease in a large
553 prospective primary care cohort. *J Hepatol* **56**, 234-240, doi:10.1016/j.jhep.2011.03.020 (2012).

554 28 de Ledinghen, V. *et al.* Diagnostic and predictive factors of significant liver fibrosis and minimal
555 lesions in patients with persistent unexplained elevated transaminases. A prospective multicenter
556 study. *Journal of hepatology* **45**, 592-599, doi:10.1016/j.jhep.2006.05.008 (2006).

557 29 Bedogni, G. *et al.* Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos
558 nutrition and liver study. *Hepatology* **42**, 44-52, doi:10.1002/hep.20734 (2005).

559 30 Caballeria, L. *et al.* Prevalence and factors associated with the presence of nonalcoholic fatty liver
560 disease in an adult population in Spain. *European journal of gastroenterology & hepatology* **22**, 24-
561 32, doi:10.1097/MEG.0b013e32832fcd0 (2010).

562 31 Radu, C. *et al.* Prevalence and associated risk factors of non-alcoholic fatty liver disease in
563 hospitalized patients. *J Gastrointest Liver Dis* **17**, 255-260 (2008).

564 32 Tarnoki, A. D. *et al.* Heritability of non-alcoholic fatty liver disease and association with abnormal
565 vascular parameters: a twin study. *Liver international : official journal of the International*
566 *Association for the Study of the Liver* **32**, 1287-1293, doi:10.1111/j.1478-3231.2012.02823.x (2012).

567 33 Soresi, M. *et al.* Nonalcoholic fatty liver and metabolic syndrome in Italy: results from a multicentric
568 study of the Italian Arteriosclerosis society. *Acta Diabetol* **50**, 241-249, doi:10.1007/s00592-012-
569 0406-1 (2013).

570 34 Targher, G. *et al.* Prevalence of nonalcoholic fatty liver disease and its association with
571 cardiovascular disease among type 2 diabetic patients. *Diabetes Care* **30**, 1212-1218,
572 doi:10.2337/dc06-2247 (2007).

573 35 Williamson, R. M. *et al.* Prevalence of and risk factors for hepatic steatosis and nonalcoholic Fatty
574 liver disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. *Diabetes Care* **34**,
575 1139-1144, doi:10.2337/dc10-2229 (2011).

576 36 Bedogni, G. *et al.* Incidence and natural course of fatty liver in the general population: the Dionysos
577 study. *Hepatology* **46**, 1387-1391, doi:10.1002/hep.21827 (2007).

578 37 Koehler, E. M. *et al.* Presence of diabetes mellitus and steatosis is associated with liver stiffness in a
579 general population: The Rotterdam study. *Hepatology* **63**, 138-147, doi:10.1002/hep.27981 (2016).

580 38 McPherson, S. *et al.* Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using
581 paired biopsies: Implications for prognosis and clinical management. *J Hepatol* **62**, 1148-1155,
582 doi:10.1016/j.jhep.2014.11.034 (2015).

583 39 Zois, C. D. *et al.* Steatosis and steatohepatitis in postmortem material from Northwestern Greece.
584 *World J Gastroenterol* **16**, 3944-3949 (2010).

585 40 Garcia-Monzon, C. *et al.* Prevalence and risk factors for biopsy-proven non-alcoholic fatty liver
586 disease and non-alcoholic steatohepatitis in a prospective cohort of adult patients with gallstones.
587 *Liver international : official journal of the International Association for the Study of the Liver* **35**,
588 1983-1991, doi:10.1111/liv.12813 (2015).

589 41 Minervini, M. I. *et al.* Liver biopsy findings from healthy potential living liver donors: reasons for
590 disqualification, silent diseases and correlation with liver injury tests. *J Hepatol* **50**, 501-510,
591 doi:S0168-8278(08)00796-4 [pii]10.1016/j.jhep.2008.10.030 (2009).

592 42 Ekstedt, M. *et al.* Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD
593 after up to 33 years of follow-up. *Hepatology* **61**, 1547-1554, doi:10.1002/hep.27368 (2015).

594 43 Dyson, J. *et al.* Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary
595 team. *J Hepatol* **60**, 110-117, doi:10.1016/j.jhep.2013.08.011 (2014).

596 44 Williams, R. *et al.* Addressing liver disease in the UK: a blueprint for attaining excellence in health
597 care and reducing premature mortality from lifestyle issues of excess consumption of alcohol,
598 obesity, and viral hepatitis. *Lancet* **384**, 1953-1997, doi:10.1016/S0140-6736(14)61838-9 (2014).

599 45 Fan, J. G. *et al.* Prevalence of and risk factors for fatty liver in a general population of Shanghai,
600 China. *J Hepatol* **43**, 508-514, doi:10.1016/j.jhep.2005.02.042 (2005).

601 46 Zhou, Y. J. *et al.* Prevalence of fatty liver disease and its risk factors in the population of South China.
602 *World J Gastroenterol* **13**, 6419-6424 (2007).

603 47 Li, H. *et al.* Prevalence and risk factors of fatty liver disease in Chengdu, Southwest China.
604 *Hepatobiliary Pancreat Dis Int* **8**, 377-382 (2009).

605 48 Hu, X. *et al.* Prevalence and factors associated with nonalcoholic fatty liver disease in Shanghai
606 work-units. *BMC Gastroenterol* **12**, 123, doi:10.1186/1471-230X-12-123 (2012).

607 49 Wei, J. L. *et al.* Prevalence and Severity of Nonalcoholic Fatty Liver Disease in Non-Obese Patients: A
608 Population Study Using Proton-Magnetic Resonance Spectroscopy. *Am J Gastroenterol* **110**, 1306-
609 1314; quiz 1315, doi:10.1038/ajg.2015.235 (2015).

610 50 Chen, C. H. *et al.* Prevalence and risk factors of nonalcoholic fatty liver disease in an adult
611 population of taiwan: metabolic significance of nonalcoholic fatty liver disease in nonobese adults. *J*
612 *Clin Gastroenterol* **40**, 745-752 (2006).

613 51 Hung SC, L. S., Chen MC, Li PC, Lin KC. Prevalence and related factors of non-alcoholic fatty liver
614 disease among the elderly in Taiwan. *Eur Geriatr Med* **4**, 78-81 (2013).

615 52 Tung, T. H. *et al.* Clinical correlation of nonalcoholic fatty liver disease in a Chinese taxi drivers
616 population in Taiwan: Experience at a teaching hospital. *BMC Res Notes* **4**, 315, doi:10.1186/1756-
617 0500-4-315 (2011).

618 53 Kojima, S., Watanabe, N., Numata, M., Ogawa, T. & Matsuzaki, S. Increase in the prevalence of fatty
619 liver in Japan over the past 12 years: analysis of clinical background. *J Gastroenterol* **38**, 954-961,
620 doi:10.1007/s00535-003-1178-8 (2003).

621 54 Hamaguchi, M. *et al.* The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Ann*
622 *Intern Med* **143**, 722-728 (2005).

623 55 Jeong, E. H. *et al.* Regional prevalence of non-alcoholic fatty liver disease in Seoul and Gyeonggi-do,
624 Korea. *Clin Mol Hepatol* **19**, 266-272, doi:10.3350/cmh.2013.19.3.266 (2013).

625 56 Das, K. *et al.* Nonobese Population in a Developing Country Has a High Prevalence of Nonalcoholic
626 Fatty Liver and Significant Liver Disease. *Hepatology* **51**, 1593-1602, doi:10.1002/hep.23567 (2010).

627 57 Singh, S. P. *et al.* Prevalence of nonalcoholic fatty liver disease in coastal eastern India: a preliminary
628 ultrasonographic survey. *Trop Gastroenterol* **25**, 76-79 (2004).

629 58 Amarapurkar, D. *et al.* Prevalence of non-alcoholic fatty liver disease: population based study. *Ann*
630 *Hepatol* **6**, 161-163 (2007).

631 59 Dassanayake, A. S. *et al.* Prevalence and risk factors for non-alcoholic fatty liver disease among
632 adults in an urban Sri Lankan population. *J Gastroenterol Hepatol* **24**, 1284-1288,
633 doi:10.1111/j.1440-1746.2009.05831.x (2009).

634 60 Mohan, V., Farooq, S., Deepa, M., Ravikumar, R. & Pitchumoni, C. S. Prevalence of non-alcoholic
635 fatty liver disease in urban south Indians in relation to different grades of glucose intolerance and
636 metabolic syndrome. *Diabetes Res Clin Pract* **84**, 84-91, doi:10.1016/j.diabres.2008.11.039 (2009).

637 61 Goh, S. C., Ho, E. L. & Goh, K. L. Prevalence and risk factors of non-alcoholic fatty liver disease in a
638 multiracial suburban Asian population in Malaysia. *Hepatol Int* **7**, 548-554, doi:10.1007/s12072-012-
639 9359-2 (2013).

640 62 Chow, W. C. *et al.* Significant non-alcoholic fatty liver disease is found in non-diabetic, pre-obese
641 Chinese in Singapore. *Singapore Med J* **48**, 752-757 (2007).

642 63 Onyekwere, C. A., Ogbera, A. O. & Balogun, B. O. Non-alcoholic fatty liver disease and the metabolic
643 syndrome in an urban hospital serving an African community. *Ann Hepatol* **10**, 119-124 (2011).

644 64 Olusanya, T. O., Lesi, O. A., Adeyomoye, A. A. & Fasanmade, O. A. Non alcoholic fatty liver disease in
645 a Nigerian population with type II diabetes mellitus. *Pan Afr Med J* **24**, 20,
646 doi:10.11604/pamj.2016.24.20.8181 (2016).

647 65 Kruger, F. C. *et al.* Non-alcoholic fatty liver disease (NAFLD) in the Western Cape: a descriptive
648 analysis. *S Afr Med J* **100**, 168-171 (2010).

649 66 Almobarak, A. O. *et al.* Non alcoholic fatty liver disease (NAFLD) in a Sudanese population: What is
650 the prevalence and risk factors? *Arab J Gastroenterol* **15**, 12-15, doi:10.1016/j.ajg.2014.01.008
651 (2014).

652 67 Younossi, Z. M. *et al.* Nonalcoholic fatty liver disease in lean individuals in the United States.
653 *Medicine (Baltimore)* **91**, 319-327, doi:10.1097/MD.0b013e3182779d49 (2012).

654 68 Bhat, G., Baba, C. S., Pandey, A., Kumari, N. & Choudhuri, G. Insulin resistance and metabolic
655 syndrome in nonobese Indian patients with non-alcoholic fatty liver disease. *Trop Gastroenterol* **34**,
656 18-24 (2013).

657 69 Feng, R. N. *et al.* Lean-non-alcoholic fatty liver disease increases risk for metabolic disorders in a
658 normal weight Chinese population. *World J Gastroenterol* **20**, 17932-17940,
659 doi:10.3748/wjg.v20.i47.17932 (2014).

660 70 Nishioji, K. *et al.* Prevalence of and risk factors for non-alcoholic fatty liver disease in a non-obese
661 Japanese population, 2011-2012. *J Gastroenterol* **50**, 95-108, doi:10.1007/s00535-014-0948-9
662 (2015).

663 71 Kim, N. H. *et al.* Clinical and metabolic factors associated with development and regression of
664 nonalcoholic fatty liver disease in nonobese subjects. *Liver Int* **34**, 604-611, doi:10.1111/liv.12454
665 (2014).

666 72 Omagari, K. *et al.* Fatty liver in non-alcoholic non-overweight Japanese adults: incidence and clinical
667 characteristics. *J Gastroenterol Hepatol* **17**, 1098-1105 (2002).

668 73 Kwon, Y. M. *et al.* Association of Nonalcoholic Fatty Liver Disease With Components of Metabolic
669 Syndrome According to Body Mass Index in Korean Adults. *American Journal of Gastroenterology*
670 **107**, 1852-1858, doi:10.1038/ajg.2012.314 (2012).

671 74 Vos, B. *et al.* Lean non-alcoholic fatty liver disease (Lean-NAFLD): a major cause of cryptogenic liver
672 disease. *Acta Gastroenterol Belg* **74**, 389-394 (2011).

673 75 Margariti, E., Deutsch, M., Manolakopoulos, S. & Papatheodoridis, G. V. Non-alcoholic fatty liver
674 disease may develop in individuals with normal body mass index. *Ann Gastroenterol* **25**, 45-51
675 (2012).

676 76 Cho, H. C. Prevalence and Factors Associated with Nonalcoholic Fatty Liver Disease in a Nonobese
677 Korean Population. *Gut Liver* **10**, 117-125, doi:10.5009/gnl14444 (2016).

678 77 Kim, H. J. *et al.* Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic
679 adults. *Arch Intern Med* **164**, 2169-2175, doi:10.1001/archinte.164.19.2169 (2004).

680 78 Ortiz P, M. R., Pérez-Cormenzana M, Martínez-Arranz I, Martínez-Chantar M L, Lu SC; Mato JM. in
681 AASLD meeting.

682 79 Conus, F., Rabasa-Lhoret, R. & Peronnet, F. Characteristics of metabolically obese normal-weight
683 (MONW) subjects. *Appl Physiol Nutr Metab* **32**, 4-12, doi:10.1139/H07-926 (2007).

684 80 Younossi, Z. M., Otgonsuren, M., Venkatesan, C. & Mishra, A. In patients with non-alcoholic fatty
685 liver disease, metabolically abnormal individuals are at a higher risk for mortality while
686 metabolically normal individuals are not. *Metabolism* **62**, 352-360,
687 doi:10.1016/j.metabol.2012.08.005 (2013).

688 81 Bugianesi, E. *et al.* Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease:
689 sites and mechanisms. *Diabetologia* **48**, 634-642, doi:10.1007/s00125-005-1682-x (2005).

690 82 Fracanzani, A. L. *et al.* Risk of nonalcoholic steatohepatitis and fibrosis in patients with nonalcoholic
691 fatty liver disease and low visceral adiposity. *Journal of hepatology* **54**, 1244-1249,
692 doi:10.1016/j.jhep.2010.09.037 (2011).

693 83 Leung, J. C. *et al.* Histological severity and clinical outcomes of nonalcoholic fatty liver disease in
694 nonobese patients. *Hepatology* **65**, 54-64, doi:10.1002/hep.28697 (2017).

695 84 Kumar, R. *et al.* Clinicopathological characteristics and metabolic profiles of non-alcoholic fatty liver
696 disease in Indian patients with normal body mass index: Do they differ from obese or overweight
697 non-alcoholic fatty liver disease? *Indian J Endocrinol Metab* **17**, 665-671, doi:10.4103/2230-
698 8210.113758 (2013).

699 85 Dela Cruz AC, B. E., George J, Day CP, Liaquat H, Charatcharoenwitthaya P, *et al.* . 379 characteristics
700 and long-term prognosis of lean patients with nonalcoholic fatty liver disease. *Gastroenterology*
701 **146**, doi:10.1016/S0016-5085(14)63307-2. (2014).

702 86 Ogden, C. L., Carroll, M. D., Kit, B. K. & Flegal, K. M. Prevalence of obesity and trends in body mass
703 index among US children and adolescents, 1999-2010. *JAMA : the journal of the American Medical*
704 *Association* **307**, 483-490, doi:10.1001/jama.2012.40 (2012).

705 87 Zimmermann, E. *et al.* Body mass index in school-aged children and the risk of routinely diagnosed
706 non-alcoholic fatty liver disease in adulthood: a prospective study based on the Copenhagen School
707 Health Records Register. *BMJ Open* **5**, e006998, doi:10.1136/bmjopen-2014-006998 (2015).

708 88 Hagstrom, H., Stal, P., Hultcrantz, R., Hemmingsson, T. & Andreasson, A. Overweight in late
709 adolescence predicts development of severe liver disease later in life: A 39years follow-up study.
710 *Journal of hepatology* **65**, 363-368, doi:10.1016/j.jhep.2016.03.019 (2016).

711 89 Berentzen, T. L., Gamborg, M., Holst, C., Sorensen, T. I. & Baker, J. L. Body mass index in childhood
712 and adult risk of primary liver cancer. *Journal of hepatology* **60**, 325-330,
713 doi:10.1016/j.jhep.2013.09.015 (2014).

714 90 Suomela, E. *et al.* Childhood predictors of adult fatty liver. The Cardiovascular Risk in Young Finns
715 Study. *Journal of hepatology* **65**, 784-790, doi:10.1016/j.jhep.2016.05.020 (2016).

716 91 Singh, S. *et al.* Fibrosis Progression in Nonalcoholic Fatty Liver vs Nonalcoholic Steatohepatitis: A
717 Systematic Review and Meta-analysis of Paired-Biopsy Studies. *Clin Gastroenterol Hepatol* **13**, 643-
718 654 e649, doi:10.1016/j.cgh.2014.04.014 (2015).

719 92 Loomba, R. *et al.* Heritability of Hepatic Fibrosis and Steatosis Based on a Prospective Twin Study.
720 *Gastroenterology*, doi:10.1053/j.gastro.2015.08.011 (2015).

721 93 Schwimmer, J. B. *et al.* Heritability of nonalcoholic fatty liver disease. *Gastroenterology* **136**, 1585-
722 1592 (2009).

723 94 Cui, J. *et al.* Shared genetic effects between hepatic steatosis and fibrosis: A prospective twin study.
724 *Hepatology* **64**, 1547-1558, doi:10.1002/hep.28674 (2016).

725 95 Anstee, Q. M. & Day, C. P. The genetics of NAFLD. *Nat Rev Gastroenterol Hepatol* **10**, 645-655,
726 doi:10.1038/nrgastro.2013.182 (2013).

727 96 Foster, T., Anania, F. A., Li, D., Katz, R. & Budoff, M. The prevalence and clinical correlates of
728 nonalcoholic fatty liver disease (NAFLD) in African Americans: the multiethnic study of
729 atherosclerosis (MESA). *Dig Dis Sci* **58**, 2392-2398, doi:10.1007/s10620-013-2652-7 (2013).

730 97 Anstee, Q. M. & Day, C. P. The Genetics of Nonalcoholic Fatty Liver Disease: Spotlight on PNPLA3
731 and TM6SF2. *Semin Liver Dis* **35**, 270-290, doi:10.1055/s-0035-1562947 (2015).

732 98 Liu, Y. L. *et al.* TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-
733 alcoholic fatty liver disease. *Nature communications* **5**, 4309, doi:10.1038/ncomms5309 (2014).
734 99 Valenti, L. *et al.* Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M
735 polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology*
736 **51**, 1209-1217, doi:10.1002/hep.23622 (2010).
737 100 Mancina, R. M. *et al.* The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver
738 Disease in Individuals of European Descent. *Gastroenterology* **150**, 1219-1230 e1216,
739 doi:10.1053/j.gastro.2016.01.032 (2016).
740 101 Chen, L. Z. *et al.* PNPLA3 I148M variant in nonalcoholic fatty liver disease: demographic and ethnic
741 characteristics and the role of the variant in nonalcoholic fatty liver fibrosis. *World J Gastroenterol*
742 **21**, 794-802, doi:10.3748/wjg.v21.i3.794 (2015).
743 102 Zarrinpar, A., Gupta, S., Maurya, M. R., Subramaniam, S. & Loomba, R. Serum microRNAs explain
744 discordance of non-alcoholic fatty liver disease in monozygotic and dizygotic twins: a prospective
745 study. *Gut* **65**, 1546-1554, doi:10.1136/gutjnl-2015-309456 (2016).
746 103 Zeybel, M. *et al.* Multigenerational epigenetic adaptation of the hepatic wound-healing response.
747 *Nature Medicine* **18**, 1369-1377, doi:10.1038/nm.2893 (2012).
748 104 Hardy, T. *et al.* Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in
749 non-alcoholic fatty liver disease. *Gut*, doi:10.1136/gutjnl-2016-311526 (2016).
750 105 Leslie, T. *et al.* Survey of health status, nutrition and geography of food selection of chronic liver
751 disease patients. *Ann Hepatol* **13**, 533-540 (2014).
752 106 Kim, C. H. *et al.* Nutritional assessments of patients with non-alcoholic fatty liver disease. *Obesity*
753 *surgery* **20**, 154-160, doi:10.1007/s11695-008-9549-0 (2010).
754 107 McCarthy, E. M. & Rinella, M. E. The role of diet and nutrient composition in nonalcoholic Fatty liver
755 disease. *J Acad Nutr Diet* **112**, 401-409, doi:10.1016/j.jada.2011.10.007 (2012).
756 108 Gerber, L. *et al.* Non-alcoholic fatty liver disease (NAFLD) is associated with low level of physical
757 activity: a population-based study. *Alimentary pharmacology & therapeutics* **36**, 772-781,
758 doi:10.1111/apt.12038 (2012).
759 109 Hallsworth, K. *et al.* Non-alcoholic fatty liver disease is associated with higher levels of objectively
760 measured sedentary behaviour and lower levels of physical activity than matched healthy controls.
761 *Frontline gastroenterology* **6**, 44-51, doi:10.1136/flgastro-2014-100432 (2015).
762 110 Keating, S. E., George, J. & Johnson, N. A. The benefits of exercise for patients with non-alcoholic
763 fatty liver disease. *Expert review of gastroenterology & hepatology* **9**, 1247-1250,
764 doi:10.1586/17474124.2015.1075392 (2015).
765 111 Volzke, H. Multicausality in fatty liver disease: is there a rationale to distinguish between alcoholic
766 and non-alcoholic origin? *World J Gastroenterol* **18**, 3492-3501, doi:10.3748/wjg.v18.i27.3492
767 (2012).
768 112 Hart, C. L., Morrison, D. S., Batty, G. D., Mitchell, R. J. & Davey Smith, G. Effect of body mass index
769 and alcohol consumption on liver disease: analysis of data from two prospective cohort studies.
770 *BMJ* **340**, c1240, doi:10.1136/bmj.c1240 (2010).
771 113 Younossi, Z. M. *et al.* The economic and clinical burden of nonalcoholic fatty liver disease in the
772 United States and Europe. *Hepatology* **64**, 1577-1586, doi:10.1002/hep.28785 (2016).
773 114 European Association for the Study of the Liver . Electronic address, e. e. e., European Association
774 for the Study of, D. & European Association for the Study of, O. EASL-EASD-EASO Clinical Practice
775 Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* **64**, 1388-1402,
776 doi:10.1016/j.jhep.2015.11.004 (2016).
777 115 Younossi, Z. M. *et al.* Trends in outpatient resource utilizations and outcomes for Medicare
778 beneficiaries with nonalcoholic fatty liver disease. *Journal of clinical gastroenterology* **49**, 222-227,
779 doi:10.1097/MCG.000000000000071 (2015).
780 116 National Institute for Health and Care Excellence. . *Non-alcoholic fatty liver disease (NAFLD):*
781 *assessment and management. NICE clinical guideline NG49. London. National Clinical Guideline*
782 *Centre* (2016).