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**Nonalcoholic fatty liver disease: Updates on associations with the metabolic syndrome and lipid profile and effects of treatment with PPAR- $\gamma$  agonists**

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Dear Editor,

We should be glad if you consider our manuscript entitled “Nonalcoholic fatty liver disease: Updates on associations with the metabolic syndrome and lipid profile and effects of treatment with PPAR $\gamma$  agonists” for publication in “Metabolism” as an "Editorial".

Our manuscript is an original and previously unpublished work and no other submission or publication of the same material has been or will be made before completion of the review process by “Metabolism” and, in the event the submitted is accepted by “Metabolism”, before its publication.

We also declare that all authors have participated in the study to a significant extent and are in agreement with the content of the manuscript. As requested in the instructions to authors, any conflict of interest is clearly stated in the title page.

Sincerely yours,

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Endocrinologist

1 **Nonalcoholic fatty liver disease: Updates on associations with the**  
2 **metabolic syndrome and lipid profile and effects of treatment with PPAR $\gamma$**   
3 **agonists**

4

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14 **Running title:** NAFLD, Mets and pioglitazone

15

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21 **Abstract**

22 Not required

23

24 **Keywords:** hyperlipidemia; metabolic syndrome; nonalcoholic fatty liver disease;  
25 nonalcoholic steatohepatitis; pioglitazone; PPAR- $\gamma$ .

26

27 **List of abbreviations:** CVD, cardiovascular disease; HOMA-IR, homeostasis model of  
28 assessment insulin resistance; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio;  
29 IR, insulin resistance; LDL-C, low-density lipoprotein cholesterol; LVH, left ventricular  
30 hypertrophy; LVMI, left ventricular mass index; MetS, metabolic syndrome; NAFLD,  
31 nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic  
32 steatohepatitis; OR, odds ratio; PNPLA, patatin-like phospholipase domain-containing  
33 protein; PPAR, peroxisome proliferator activated receptor; RCT, randomized controlled trial;  
34 SS, simple steatosis; T2DM, type 2 diabetes mellitus.

35 **1. Introduction**

36 Nonalcoholic fatty liver disease (NAFLD) is a disease of increasing prevalence and  
37 has been recognized as a global public health problem, affecting approximately one third of  
38 the general adult population and one tenth of children [1,2]. The prevalence of NAFLD  
39 increases with obesity, type 2 diabetes mellitus (T2DM) and/or metabolic syndrome (MetS)  
40 [1,2]. NAFLD is now among the leading causes of cirrhosis [3], hepatocellular carcinoma [4]  
41 and liver transplantation [5]. Besides hepatic morbidity, NAFLD has been associated to extra-  
42 hepatic morbidity too [6], including metabolic complications, chronic kidney, malignancies  
43 and cardiovascular disease (CVD), which all contribute to the higher mortality observed  
44 among NAFLD patients [7]. Specifically, evidence from recent studies strongly emphasizes  
45 the importance of assessing the global CVD risk in patients with NAFLD and that NAFLD  
46 might be both a marker and an early mediator of CVD.

47 NAFLD encompasses a spectrum of phenotypes, ranging from nonalcoholic simple  
48 steatosis (SS), histologically defined as intrahepatic lipid accumulation with or without mild  
49 inflammation, to nonalcoholic steatohepatitis (NASH), characterized by the addition of  
50 hepatic necroinflammatory features and/or fibrosis, up to NASH-related cirrhosis and its  
51 complications, including hepatocellular carcinoma [8].

52 The pathogenesis of NAFLD is multifactorial, since various factors (“hits”)  
53 contribute to its development and progression [9]. Genetic predisposition (e.g.,  
54 polymorphisms of patatin-like phospholipase domain-containing protein (PNPLA3 gene)  
55 [10,11], lifestyle factors (e.g., lack of exercise, high fructose and saturated fat intake etc.  
56 [12]), insulin resistance (IR) [13], redox imbalance [14] and certain adipokines [15] are  
57 regarded as established “hits”, whereas other factors, including impaired innate and adaptive  
58 immunity [16], dysbiosis of the gut microbiota [17] and endocrine disruptors [18], have been  
59 linked with NAFLD, although further validation is needed.

60 “*Metabolism, Clinical and Experimental*” has recently published two studies  
61 advancing our knowledge in NAFLD, which are presented and commented hereby: one of  
62 them focuses on NAFLD association with MetS [19] and the other on NAFLD association

63 with different types of dyslipidemia [20]. A third study reporting on the long-term efficacy  
64 and safety of pioglitazone in NASH patients with T2DM [21], recently published in “*Annals*  
65 *of Internal Medicine*”, is also discussed herein.

66

## 67 **2. NAFLD and metabolic syndrome**

68 Karajamaki et al. [19] analyzed a subset of data from a cohort study of a middle-aged  
69 Finnish population (Oulu Project Elucidating Risk of Atherosclerosis [OPERA] study),  
70 aiming to evaluate the dynamic interaction between NAFLD and MetS on left ventricular  
71 mass index (LVMI), a surrogate of left ventricular hypertrophy (LVH) and a predictor of  
72 cardiac morbidity and mortality in hypertensive patients, major cardiovascular events  
73 (coronary heart disease, stroke or death), as well as new incidence of T2DM. More  
74 specifically, Karajamaki et al. [19] divided the population at baseline (1991-1993; n=958) into  
75 four groups: a) coexistence of NAFLD and MetS (19%); b) NAFLD without MetS (7%); c)  
76 MetS without NAFLD (17%); and d) neither NAFLD nor MetS (57%). After a mean follow-  
77 up of 16.3 years, major cardiovascular events occurred in 30% (hazard ratio [HR]: 2.8; 95%  
78 CI: 1.9-3.9), 20% (HR: 1.7; 95% CI: 1.0-3.1), 22% (HR: 2.1; 95% CI: 1.4-3.1) and 12%  
79 (reference group), respectively. Interestingly, in a multivariate Cox regression model, MetS  
80 with and without NAFLD could predict the risk for cardiovascular events, whereas NAFLD  
81 without MetS could not. Change in LVMI was statistically more significant in groups with  
82 both NAFLD and MetS, and MetS without NAFLD.

83 Regarding new cases of T2DM, the rates were 47% (NAFLD and MetS), 24%  
84 (NAFLD without MetS), 40% (MetS without NAFLD) and 19% (neither NAFLD nor MetS),  
85 being statistically higher in groups with both NAFLD and MetS, and MetS without NAFLD.  
86 Interestingly, in the subset of individuals without MetS at baseline, the incidence of MetS  
87 during the follow-up was higher in those with (71%) than without (48%) NAFLD. Another  
88 important observation of this study is that the unfavorable genotype of *PNPLA3* gene  
89 polymorphism, which is strongly associated with the susceptibility and severity of NAFLD  
90 [10,11], was most prevalent in individuals with NAFLD without MetS.

91 To the best of our knowledge, this is the first cohort study evaluating the combined  
92 effect of NAFLD and MetS on cardiovascular events and T2DM incidence. Although limited  
93 by the fact that OPERA was not specifically designed for this aim and by the small number of  
94 individuals in the NAFLD without MetS group (7%), this study indicates that NAFLD affects  
95 cardiovascular morbidity and T2DM incidence mainly when it is combined with MetS,  
96 thereby implying that IR may be the pathogenetic common denominator resulting in higher  
97 cardiovascular morbidity and not NAFLD itself. However, other investigators, also mentioned  
98 by the authors [19], reported that hypertensive patients with T2DM and with NAFLD exhibit  
99 a remarkably higher frequency of LVH than hypertensive diabetic patients without NAFLD,  
100 and that NAFLD is related with LVH independently of conventional cardiovascular risk  
101 factors and other potential co-founders [22]. Therefore, due to the limited number of  
102 individuals in the NAFLD without MetS group [19], further large-scale relative studies are  
103 warranted to elucidate the potential impact of NAFLD on cardiovascular morbidity and  
104 T2DM incidence when or not combined with MetS. Furthermore, this study strengthens  
105 existing evidence that *PNPLA3* gene polymorphism predisposes to NAFLD, but not MetS or  
106 T2DM. In this regard, *PNPLA3* gene polymorphism promotes advanced liver damage in  
107 NAFLD [10,11], increasing hepatic morbidity, but it is not associated with IR or T2DM [23],  
108 thereby not increasing NAFLD-related cardiovascular morbidity. This study also reinforces  
109 the concept that NAFLD itself is able to favor the onset of MetS. Therefore, it could be  
110 speculated that, when NAFLD is not efficiently managed, it may foster the development of  
111 MetS and both of them jointly increase the risk of cardiovascular morbidity. However, this  
112 hypothesis should be confirmed by specifically designed future cohort studies.

113

### 114 **3. NAFLD and lipid profiles**

115 Du et al. [20] performed a cross-sectional study in a large sample (n=9560) of adult  
116 Chinese seen for routine health check-up. Individuals with T2DM or other liver disease and  
117 those on lipid-lowering medications were excluded. Based on liver ultrasound examination,  
118 approximately 39% of them were diagnosed with NAFLD. Lipid abnormalities were defined



119 according to National Cholesterol Education Program/Adult Treatment Panel (ATP)-III  
120 guidelines [24] and were subdivided into five mutually exclusive categories: a) isolated  
121 hypercholesterolemia (high low-density lipoprotein cholesterol [LDL-C], normal  
122 triglycerides; 2.9%); b) isolated hypertriglyceridemia (high triglycerides, normal LDL-C and  
123 high-density lipoprotein cholesterol [HDL-C]; 13.7%) c) dyslipidemia of MetS (normal LDL-  
124 C, low HDL-C, high triglycerides; 9.5%); d) combined hyperlipidemia (high LDL-C and high  
125 triglycerides; 2.0%); e) isolated low-HDL-C (low HDL-C, normal LDL-C and triglycerides;  
126 10.9%). Individuals with normolipemia (normal LDL-C, HDL-C and triglycerides; 61.0%)  
127 served as a reference group.

128         Within NAFLD patients, 3.2% had isolated hypercholesterolemia, 23.3% isolated  
129 hypertriglyceridemia, 17.7% MetS dyslipidemia, 3.8% combined hyperlipidemia, 10.2%  
130 isolated low HDL-C, whereas 41.8% had normolipemia, providing evidence for higher rates  
131 of lipid abnormalities in NAFLD. Inversely, all lipid abnormalities showed higher rates of  
132 NAFLD compared to individuals with normolipemia (reference group). More specifically,  
133 combined hyperlipidemia provided the higher rates (unadjusted odds ratio [OR]: 9.0; 95% CI  
134 6.4-12.7), followed by MetS dyslipidemia (unadjusted OR: 7.30; 95% CI: 6.2-8.5), isolated  
135 hypertriglyceridemia (unadjusted OR: 5.3; 95% CI: 4.7-6.1), isolated hypercholesterolemia  
136 (unadjusted OR: 2.1; 95% CI: 1.7-2.7) and isolated low HDL-C (unadjusted OR: 1.6; 95% CI:  
137 1.4-1.8). The association between lipid profiles and NAFLD remained robust after adjustment  
138 for potential co-founders for combined hyperlipidemia, MetS dyslipidemia and isolated  
139 hypertriglyceridemia, but not for isolated hypercholesterolemia and isolated low HDL-C [20].

140         To the best of our knowledge, this is the largest study evaluating the association  
141 between NAFLD and different lipid profiles. Although it is limited by its observational  
142 nature, thereby failing to prove causality, and by the fact that lipoprotein (a) (Lp(a)), an  
143 independent predictor of cardiovascular risk [25], was not evaluated, this study strengthens  
144 our knowledge on the close relationship between NAFLD and lipid profile and its potential  
145 impact on CVD. Combined hyperlipidemia also appears to be a risk factor for CDV; high  
146 triglyceride levels are associated with increased CVD risk [26] and high LDL-C has now

147 largely replaced total cholesterol as a risk marker for CVD from a biologic, epidemiologic,  
148 and genetic standpoint [27]. Noteworthy, NAFLD remained independently associated with all  
149 lipid abnormalities characterized by high triglyceride levels, which is commonly observed in  
150 the setting of IR. This study warrants further research. A deeper insight into lipid profiles in  
151 patients with NASH, especially those with liver fibrosis who have the poorer prognosis [28],  
152 would be of importance, and might have therapeutic perspectives. Implementation of an  
153 aggressive therapeutic strategy for dyslipidemia with hypolipidemic agents, also mentioned  
154 by the authors [20], might mitigate the risk for CVD among NAFLD patients [29]. However,  
155 to-date, contrasting data are available on hypolipidemic treatment in NASH [25,30] but in the  
156 end nor omega-3, fibrates or statins clearly proved to be effective in improving the features of  
157 liver damage other than steatosis in NASH. On the other hand, another point needing  
158 clarification is the selection of medications to treat different lipid profiles specifically in  
159 NAFLD subjects. Statins proved to be safe in NAFLD, thereby toning down previous fear for  
160 statin use in patients with abnormal liver function tests, while it remains unknown how to  
161 treat NAFLD patients with isolated hypertriglyceridemia, MetS dyslipidemia and isolated  
162 high HDL-C. Until further studies elucidate this issue in specifically NAFLD populations, it  
163 is suggested that we follow the recommendations published for general population.

164

#### 165 **4. NAFLD and pioglitazone**

166 Cusi et al. [21] performed a single-center, randomized placebo controlled trial (RCT;  
167 18 months) followed by a 18-month open-label extension (totally 36 months) evaluating the  
168 long-term safety and efficacy of pioglitazone (45 mg/d; added to a hypocaloric diet), a  
169 peroxisome proliferator activated receptor (PPAR)- $\gamma$  ligand, in patients with diabetes or  
170 prediabetes and biopsy-proven NASH (n=101). Previous studies had already shown a  
171 favorable effect of pioglitazone on hepatic steatosis and lobular inflammation, whereas its  
172 effect on hepatic fibrosis remained unclear, as we recently summarized [31]. At month 18  
173 (end of RCT), more patients in the pioglitazone than in the placebo group (58% vs. 17%,  
174 respectively) achieved the primary outcome, being the reduction of at least 2 points in the

175 NAFLD activity score (NAS) in 2 histologic categories, without worsening of fibrosis [21].  
176 Furthermore, resolution of NASH occurred in 51% of pioglitazone-treated patients vs. 19% of  
177 those receiving placebo. Regarding specific histological lesions, patients on pioglitazone  
178 improved hepatic steatosis, inflammation, ballooning and, notably, fibrosis more than those in  
179 placebo. Interestingly, progression of fibrosis occurred in less patients on pioglitazone (12%)  
180 than placebo (28%). As expected, pioglitazone improved hepatic, muscle and adipose tissue  
181 IR, liver function tests and circulating adiponectin. All 18-month metabolic and histological  
182 improvements persisted over 36 months of therapy (open-label extension). Although weight  
183 gain was greater with pioglitazone (mean 2.5 kg over placebo), the overall rate of adverse  
184 events did not differ between groups and no case of bladder cancer or osteoporotic fracture  
185 was observed in pioglitazone group [21].

186 This study confirms that long-term pioglitazone treatment in patients with NASH and  
187 T2DM or prediabetes is a safe and effective choice and, contrary to previous trials where  
188 discontinuation resulted in histological “rebound” [32], it shows for the first time that  
189 metabolic and histological improvements, including fibrosis, are maintained during long-term  
190 treatment with pioglitazone. Similarly to a previous open-label extension of a rosiglitazone  
191 trial in NASH [33], the Cusi et al. study did not show further histological improvement during  
192 the extension, a finding that should be cautiously interpreted, because of the open-label nature  
193 and relatively high drop-out rates at the end of the extension that possibly resulted in a  
194 relatively underpowered substudy.

195 The pharmacological treatment of NASH remains an unmet medical need [34], but  
196 the study by Cusi et al. [21] adds value by proposing the use of pioglitazone in subgroups of  
197 NASH patients with T2DM or prediabetes. However, candidates for pioglitazone treatment  
198 should be carefully selected because of its potentially adverse effect on CVD, osteoporosis  
199 and bladder cancer [31,35]. Notably, selective PPAR- $\gamma$  modulators have been developed,  
200 including INT131 (formerly AMG131) [36]. INT131 is designed to exhibit strong efficacy,  
201 but less side effects compared to PPAR- $\gamma$  full agonists, such as pioglitazone [36]. INT131 was  
202 well tolerated and improved glycated hemoglobin in T2DM patients vs. placebo in phase II

203 trials [37,38]. Less adverse effects, including edema, fluid retention and weight gain were  
204 observed compared with rosiglitazone [37] or pioglitazone [38]. Based on these observations,  
205 INT131 is one of the most promising candidates for clinical trials in NASH patients, along  
206 with other new compounds that are rapidly changing the landscape of the pharmacological  
207 treatment of NASH. Noteworthy, pioglitazone, simvastatin or a combination treatment may  
208 have synergistic effects by inhibiting different functions, such as inflammatory response and  
209 lipid regulation, by inhibiting the CD40-CD40L signaling pathway to suppress the formation  
210 of atherosclerosis, and reducing epicardial adipose tissue and plasma inflammatory markers in  
211 CVD and MetS patients [39,40]. Specifically, simvastatin, apart from exerting pleiotropic  
212 effects on the cardiovascular system, may improve the prognosis of NASH-related fibrosis by  
213 increasing the expression of endothelial nitric oxide synthase, decreasing the expression of  
214 inducible nitric oxide synthase, and inhibiting the activation of human hepatic stellate cells  
215 involved in liver fibrogenesis and carcinogenesis; a low simvastatin dose might have a role in  
216 preventing NAFLD and addition of simvastatin is associated with a survival benefit for  
217 patients with chronic liver disease [41-43].

218

## 219 **5. Closing remarks**

220         NAFLD is a complex disease with a growing prevalence and thus clinical importance  
221 affecting both hepatic and extra-hepatic morbidity and mortality. Despite the increasing  
222 prevalence of NAFLD, there is currently no definitive therapeutic modality, besides weight  
223 loss and exercise [34]. Both, weight loss and exercise, are difficult to achieve and sustain,  
224 which makes the need for pharmacological treatment of paramount importance [28]. In our  
225 opinion, a more holistic approach might probably lead to more efficient management.  
226 NAFLD is not a separate entity: it usually coexists with other components of MetS, including  
227 obesity, T2DM and various lipid abnormalities [20], but the cross-talk is probably bi-  
228 directional, i.e., NAFLD affects and is affected by other metabolic co-morbidities [44]. For  
229 example, T2DM patients have higher prevalence of NAFLD [45], but also hepatic lipid  
230 accumulation in NAFLD impairs hepatic glucose and lipid metabolism, thereby increasing the

231 risk of T2DM and CVD [7]. Because of the multifactorial nature of the disease, a combined  
232 treatment, simultaneously targeting more than one pathogenetic “hit”, might represent a more  
233 realistic management, as we previously suggested [28]. It would be advisable to effectively  
234 manage all related comorbidities, i.e., T2DM, lipid abnormalities, arterial hypertension, with  
235 a diabetes-like approach [28,46], though the impact on liver damage of such approach is  
236 currently unknown and more studies are needed, which however are implicated by the need  
237 for repeat biopsies and high drop-out rates [47].

238 Remarkably, due to its multifactorial nature, the same medications may not be  
239 suitable for all NAFLD patients. Du et al. showed the diversity of lipid abnormalities in  
240 NAFLD [20], possibly implying that the same hypolipidemic medications are not similarly  
241 effective in all NAFLD patients. Further, each NAFLD patient has a different genetic  
242 background and different related co-morbidities and, last but not least, each patient has a  
243 different time course of liver disease, often unpredictable. Therefore, beyond the search for  
244 the single, “magic bullet” medication, suitable for all NAFLD patients, research should be  
245 oriented to a more holistic approach and a more personalized management.

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