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Nonalcoholic fatty liver disease: Updates on associations with the metabolic syndrome and lipid profile and effects of treatment with PPAR- γ 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 γ 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Our manuscript is an original and previously unpublished work and no other submission or publication of the same material has been or will be made before completion of the review process by “Metabolism” and, in the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,

Stergios A. Polyzos, MD, MSc, PhD
Endocrinologist

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

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

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Abstract

Not required

Keywords: hyperlipidemia; metabolic syndrome; nonalcoholic fatty liver disease; nonalcoholic steatohepatitis; pioglitazone; PPAR- γ .

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

1. Introduction

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

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

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

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

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

2. NAFLD and metabolic syndrome

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

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

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

3. NAFLD and lipid profiles

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

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

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

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

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

4. NAFLD and pioglitazone

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

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

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

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

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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256 **References**

- 257 [1] Bugianesi E, Rosso C, Cortez-Pinto H. How to diagnose NAFLD in 2016. *J Hepatol*
- 258 2016;10.1016/j.jhep.2016.05.038.
- 259 [2] Bugianesi E. EASL-EASD-EASO Clinical Practice Guidelines for the management of
- 260 non-alcoholic fatty liver disease: disease mongering or call to action? *Diabetologia*
- 261 2016;59:1145-7.
- 262 [3] Fazel Y, Koenig AB, Sayiner M, Goodman ZD, Younossi ZM. Epidemiology and natural
- 263 history of nonalcoholic fatty liver disease. *Metabolism* 2016;65:1017-25.
- 264 [4] Zoller H, Tilg H. Nonalcoholic fatty liver disease and hepatocellular carcinoma.
- 265 *Metabolism* 2016;65:1151-60.
- 266 [5] Khan RS, Newsome PN. Non-alcoholic fatty liver disease and liver transplantation.
- 267 *Metabolism* 2016;65:1208-23.
- 268 [6] Petta S, Valenti L, Bugianesi E, Targher G, Bellentani S, Bonino F. A "systems medicine"
- 269 approach to the study of non-alcoholic fatty liver disease. *Dig Liver Dis* 2016;48:333-42.
- 270 [7] Vanni E, Marengo A, Mezzabotta L, Bugianesi E. Systemic Complications of
- 271 Nonalcoholic Fatty Liver Disease: When the Liver Is Not an Innocent Bystander. *Semin Liver*
- 272 *Dis* 2015;35:236-49.
- 273 [8] Marengo A, Jouness RI, Bugianesi E. Progression and Natural History of Nonalcoholic
- 274 Fatty Liver Disease in Adults. *Clin Liver Dis* 2016;20:313-24.
- 275 [9] Polyzos SA, Kountouras J, Zavos C. The multi-hit process and the antagonistic roles of
- 276 tumor necrosis factor-alpha and adiponectin in nonalcoholic fatty liver disease. *Hippokratia*
- 277 2009;13:127.
- 278 [10] Polyzos SA, Mantzoros CS. An update on the validity of irisin assays and the link
- 279 between irisin and hepatic metabolism. *Metabolism* 2015;64:937-42.
- 280 [11] Dongiovanni P, Valenti L. Genetics of nonalcoholic fatty liver disease. *Metabolism*
- 281 2015;65:1026-37.
- 282 [12] Mahady SE, George J. Exercise and diet in the management of nonalcoholic fatty liver
- 283 disease. *Metabolism* 2016;65:1172-82.
- 284 [13] Polyzos SA, Kountouras J, Zavos C. Nonalcoholic fatty liver disease: the pathogenetic
- 285 roles of insulin resistance and adipocytokines. *Curr Mol Med* 2009;72:299-314.
- 286 [14] Spahis S, Delvin E, Borys JM, Levy E. Oxidative Stress as a Critical Factor in
- 287 Nonalcoholic Fatty Liver Disease Pathogenesis. *Antioxid Redox Signal*
- 288 2016;10.1089/ars.2016.6776.
- 289 [15] Polyzos SA, Kountouras J, Mantzoros CS. Adipokines in nonalcoholic fatty liver disease.
- 290 *Metabolism* 2016;65:1062-79.
- 291 [16] Petrasek J, Csak T, Ganz M, Szabo G. Differences in innate immune signaling between
- 292 alcoholic and non-alcoholic steatohepatitis. *J Gastroenterol Hepatol* 2013;28 Suppl 1:93-8.
- 293 [17] Goel A, Gupta M, Aggarwal R. Gut microbiota and liver disease. *J Gastroenterol Hepatol*
- 294 2014;29:1139-48.
- 295 [18] Polyzos SA, Kountouras J, Deretzi G, Zavos C, Mantzoros CS. The emerging role of
- 296 endocrine disruptors in pathogenesis of insulin resistance: a concept implicating nonalcoholic
- 297 fatty liver disease. *Curr Mol Med* 2012;12:68-82.
- 298 [19] Karajamaki AJ, Bloigu R, Kauma H, Kesaniemi YA, Koivurova OP, Perkiomaki J, et al.
- 299 Non-alcoholic fatty liver disease with and without metabolic syndrome: Different long-term
- 300 outcomes. *Metabolism* 2016;10.1016/j.metabol.2016.06.009.
- 301 [20] Du T, Sun X, Yuan G, Zhou X, Lu H, Lin X, et al. Lipid phenotypes in patients with
- 302 nonalcoholic fatty liver disease. *Metabolism* 2016;65:1391-8.
- 303 [21] Cusi K, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, et al. Long-Term
- 304 Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or
- 305 Type 2 Diabetes Mellitus: A Randomized, Controlled Trial. *Ann Intern Med*
- 306 2016;10.7326/M15-1774.
- 307 [22] Mantovani A, Zoppini G, Targher G, Golia G, Bonora E. Non-alcoholic fatty liver
- 308 disease is independently associated with left ventricular hypertrophy in hypertensive Type 2
- 309 diabetic individuals. *J Endocrinol Invest* 2012;35:215-8.

- [23] Lallukka S, Yki-Jarvinen H. Non-alcoholic fatty liver disease and risk of type 2 diabetes. *Best Pract Res Clin Endocrinol Metab* 2016;30:385-95.
- [24] National Cholesterol Education Program expert p. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143-421.
- [25] Katsiki N, Mikhailidis DP, Mantzoros CS. Non-alcoholic fatty liver disease and dyslipidemia: An update. *Metabolism* 2016;65:1109-23.
- [26] Toth PP. Triglyceride-rich lipoproteins as a causal factor for cardiovascular disease. *Vasc Health Risk Manag* 2016;12:171-83.
- [27] Wadhera RK, Steen DL, Khan I, Giugliano RP, Foody JM. A review of low-density lipoprotein cholesterol, treatment strategies, and its impact on cardiovascular disease morbidity and mortality. *J Clin Lipidol* 2016;10:472-89.
- [28] Polyzos SA, Kountouras J, Zavos C, Deretzi G. Nonalcoholic fatty liver disease: Multimodal treatment options for a pathogenetically multiple-hit disease. *J Clin Gastroenterol* 2012;46:272-84.
- [29] Zhang QQ, Lu LG. Nonalcoholic Fatty Liver Disease: Dyslipidemia, Risk for Cardiovascular Complications, and Treatment Strategy. *J Clin Transl Hepatol* 2015;3:78-84.
- [30] Tziomalos K, Athyros VG, Paschos P, Karagiannis A. Nonalcoholic fatty liver disease and statins. *Metabolism* 2015;64:1215-23.
- [31] Polyzos SA, Mantzoros CS. Adiponectin as a target for the treatment of nonalcoholic steatohepatitis with thiazolidinediones: A systematic review. *Metabolism* 2016;65:1297-306.
- [32] Lutchman G, Modi A, Kleiner DE, Promrat K, Heller T, Ghany M, et al. The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. *Hepatology* 2007;46:424-9.
- [33] Ratziu V, Charlotte F, Bernhardt C, Giral P, Halbron M, Lenaour G, et al. Long-term efficacy of rosiglitazone in nonalcoholic steatohepatitis: results of the fatty liver improvement by rosiglitazone therapy (FLIRT 2) extension trial. *Hepatology* 2010;51:445-53.
- [34] EASL–EASD–EASO. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *Diabetologia* 2016;59:1121-40.
- [35] Lewis JD, Ferrara A, Peng T, Hedderston M, Bilker WB, Quesenberry CP, Jr., et al. Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. *Diabetes Care* 2011;34:916-22.
- [36] Higgins LS, Mantzoros CS. The Development of INT131 as a Selective PPARgamma Modulator: Approach to a Safer Insulin Sensitizer. *PPAR Res* 2008;2008:936906.
- [37] Dunn FL, Higgins LS, Fredrickson J, DePaoli AM. Selective modulation of PPARgamma activity can lower plasma glucose without typical thiazolidinedione side-effects in patients with Type 2 diabetes. *J Diabetes Complications* 2011;25:151-8.
- [38] DePaoli AM, Higgins LS, Henry RR, Mantzoros C, Dunn FL. Can a selective PPARgamma modulator improve glycemic control in patients with type 2 diabetes with fewer side effects compared with pioglitazone? *Diabetes Care* 2014;37:1918-23.
- [39] Xue L, Zhu XH, Yang XF, Bao XC, Gao XQ, Qiu YH, et al. Effect of pioglitazone combined with simvastatin on the CD40-CD40 ligand system in rabbits with atherosclerosis. *Eur Rev Med Pharmacol Sci* 2015;19:322-7.
- [40] Grosso AF, de Oliveira SF, Higuchi Mde L, Favarato D, Dallan LA, da Luz PL. Synergistic anti-inflammatory effect: simvastatin and pioglitazone reduce inflammatory markers of plasma and epicardial adipose tissue of coronary patients with metabolic syndrome. *Diabetol Metab Syndr* 2014;6:47.
- [41] Abraldes JG, Villanueva C, Aracil C, Turnes J, Hernandez-Guerra M, Genesca J, et al. Addition of Simvastatin to Standard Therapy for the Prevention of Variceal Rebleeding Does Not Reduce Rebleeding but Increases Survival in Patients With Cirrhosis. *Gastroenterology* 2016;150:1160-70 e3.
- [42] Alkhatatbeh MJ, Lincz LF, Thorne RF. Low simvastatin concentrations reduce oleic acid-induced steatosis in HepG2 cells: An in vitro model of non-alcoholic fatty liver disease. *Exp Ther Med* 2016;11:1487-92.

365 [43] Wang W, Zhao C, Zhou J, Zhen Z, Wang Y, Shen C. Simvastatin ameliorates liver
366 fibrosis via mediating nitric oxide synthase in rats with non-alcoholic steatohepatitis-related
367 liver fibrosis. PLoS One 2013;8:e76538.

368 [44] Valenti L, Bugianesi E, Pajvani U, Targher G. Nonalcoholic fatty liver disease: cause or
369 consequence of type 2 diabetes? Liver Int 2016;10.1111/liv.13185.

370 [45] Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver
371 disease and diabetes. Metabolism 2016;65:1096-108.

372 [46] Athyros VG, Mikhailidis DP, Didangelos TP, Giouleme OI, Liberopoulos EN,
373 Karagiannis A, et al. Effect of multifactorial treatment on non-alcoholic fatty liver disease in
374 metabolic syndrome: a randomised study. Curr Med Res Opin 2006;22:873-83.

375 [47] Polyzos SA, Mantzoros CS. Nonalcoholic fatty future disease. Metabolism
376 2016;65:1007-16.