

January 8, 2014

Dear Editor,



Please find enclosed the edited manuscript in Word format (file name: 6892-review.doc).

**Title:** Relationship between non-alcoholic fatty liver disease and obesity: biochemical, metabolic and clinical presentation.

**Authors:** Sandra Milić, Davorka Lulić, Davor Štimac

**Name of Journal:** *World Journal of Gastroenterology*

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**The manuscript has been improved according to the suggestions of reviewers:**

**1 Format has been updated.**

**2 Revision has been made according to the suggestions of the reviewer 1 and 2.**

- (1) **Comments from the reviewer 1:** The section on NAFLD is too long. Clearly the presentation of this liver condition spans asymptomatic patients through cryptogenic cirrhosis with or without hepatocellular carcinoma. This easy concept should be presented in a direct manner, avoiding long and unnecessary sentences (147 words in present version).

**Answer:** In accordance with this suggestion, we have corrected the section on NAFLD avoiding long sentences and presenting the concept in a direct manner:

*Non-alcoholic fatty liver disease (NAFLD) includes a spectrum of disorders that are characterized by steatosis of the liver with > 5% of hepatocytes infiltrated with fat in people with no history of alcohol abuse (< 30 g/day in men and < 20 g/day in women) and no competing etiologies for hepatic steatosis<sup>[1]</sup>. The presentation of the disease ranges from 'silent liver disease' or fatty steatosis to non-alcoholic steatohepatitis (NASH)<sup>[3]</sup>. 10-25% of patients with silent liver disease develop NASH and 5-8% of patients with NASH will develop liver cirrhosis within 5 years<sup>[1,4]</sup>. 12.8% patients with liver cirrhosis will develop hepatocellular carcinoma (HCC) within 3 years<sup>[5]</sup>. NASH is considered a major cause of cryptogenic cirrhosis with 70% of patients with cryptogenic cirrhosis having risk factors for NAFLD<sup>[6]</sup>.*

- (2) **Comments from the reviewer 1:** The authors allude to endocrine DYSfunction/derangement (rather than MALfunction); however ref. 11 is inappropriate to support such a statement and needs to be replaced (e.g. Loria P, Nat Rev Gastroenterol Hepatol. 2009;6:236-47.). Moreover, allusion is made to the role of age, gender and ethnicity which has best been reviewed in detail elsewhere (e.g. Vernon G, Aliment Pharmacol Ther. 2011;34:274-85.).

**Answer:** In accordance with this suggestion, we replaced the term “malfunction” with term “dysfunction” and replaced reference 11, as well as the references considering age, gender and ethnicity role in NAFLD etiology:

*Age, gender, ethnicity, endocrine dysfunctions (hypothyroidism, hypopituitarism, hypogonadism, and polycystic ovary syndrome) and sleep apnea also affect the prevalence of NAFLD<sup>[12,13]</sup>.*

- (3) **Comments from the reviewer 1:** As far as the concept of NAFLD as “the hepatic manifestation of MS” I think this to be outdated and NAFLD should better be envisaged as an essential component of MS (e.g. Vanni E, Dig Liver Dis 2010; 42: 320–330; Anstee QM, Nat Rev Gastroenterol Hepatol. 2013;10:330-44.)

**Answer:** In accordance with this suggestion, we replaced the term “the hepatic manifestation of MS” with “an essential component of metabolic syndrome”:

*NAFLD, an essential component of metabolic syndrome is specifically associated to insulin resistance (IR) and obesity<sup>[10,14]</sup>*

- (4) **Comments from the reviewer 1:** The natural history of hepatic and extrahepatic complications is poorly defined. In particular the risk of hepatic and extrahepatic cancers and cardiovascular risk are poorly outlined (Targher G, Curr Pharm Des. 2013;19:5177-92. Anstee QM, Nat Rev Gastroenterol Hepatol. 2013;10:330-44.)

**Answer:** In accordance with this suggestion, we define hepatic and extrahepatic complications of NAFLD, including the risk of extrahepatic cancers and cardiovascular risk:

*NAFLD is also related with an increased risk of developing cardiovascular disease, type 2 diabetes (T2D), chronic kidney disease, post-operative complications after major liver surgery and colorectal cancer<sup>[10,11]</sup>.*

- (5) **Comments from the reviewer 1:** The spectrum of cancer types associated with obesity needs to be mentioned here. Emphasize the risk of HCC in those NAFLD obese individuals (Canbay A. J Hepatol. 2012 ;56:952-64.)

**Answer:** In accordance with this suggestion, we mention the spectrum of malignancies associated with obesity and the risk of HCC in obese NAFLD individuals:

*Obesity is associated with a spectrum of cancer types (colon, breast, endometrium, kidney, esophagus, stomach, pancreas and gallbladder) and together with IR represents a risk factor for developing HCC<sup>[20]</sup>.*

- (6) **Comments from the reviewer 1:** Ref 25. Please make sure to read and discuss: Stefan N, Diabetes. 2011;60:2011-7. ; Stefan N, Arch Intern Med. 2008;168:1609-16.

**Answer:** In accordance with this suggestion, we discuss the above mentioned literature:

*However, metabolically beneficial phenotype has been identified by Stefan et al. as metabolically benign obesity where obese individuals who are insulin sensitive have less percentage of accumulated liver fat when compared to IR obese subjects<sup>[27]</sup>. This implies that the prevention and reduction of hepatic fat accumulation may lower IR even in subjects with increasing adiposity<sup>[27,28]</sup>.*

- (7) **Comments from the reviewer 1:** The comment that the fatty hepatocyte resembles adipocyte has been reported earlier and this needs to be acknowledged (Caldwell SH Expert Review of Endocrinology & Metabolism 2010; 5:403-23).

**Answer:** In accordance with this suggestion, we acknowledged Caldwell et al. considering reported

fact that fatty hepatocyte resembles adipocyte:

*Both liver and VAT share resemblance in which hepatocytes and adipocytes are in close propinquity to immune cells (NK and NKT cells), Kupffer cells, hepatic stellate cells, and endothelial cells or macrophages, with prompt access to blood vessels and similar biochemical signaling pathways<sup>[29,30]</sup>.*

- (8) **Comments from the reviewer 1:** Authors may be willing to acknowledge the interactions between lipidome and insulin signalling (Quehenberger O, N Engl J Med. 2011;365:1812-23.).

**Answer:** In accordance with this suggestion, we acknowledged Quehenberger et al. with reported fact considering the interactions between lipidome and insulin signalling:

*Secretion of IL-6 by adipose tissue dependant of cJun NH2-terminal kinase 1 (JNK1), regulatory protein activated by increased serum FFAs, may cause increased expression of liver SOCS-3<sup>[38,39]</sup>.*

- (9) **Comments from the reviewer 1:** Is there an increate risk of developing type 2 diabetes in those obese with NAFLD? This is of importance given that diabetes worsens NAFLD course and predisposes to HCC (Anania F, Hepatol Res. 2013 ;43:51-64).

**Answer:** In accordance with this suggestion, we discuss the increate risk of developing type 2 diabetes in obese people with NAFLD and the predisposition to HCC:

*In proposed individuals, hepatic fat accumulation is a major determinant in T2D development and once developed, T2D further contributes not only to hepatic steatosis level, but also to progressive liver damage including NASH, fibrosis, cirrhosis and HCC<sup>[74]</sup>.*

*Together with IR, hepatic steatosis, oxidative stress and impaired adipocytokine ratio, subacute inflammation provides a favorable environment for HCC development through cell growth kinetics and DNA damage promotion<sup>[74]</sup>.*

- (10) **Comments from the reviewer 1:** Again, discuss the role of metabolic derangements in the development of hepatic cancerogenesis (Canbay A. J Hepatol. 2012 ;56:952-64.).

**Answer:** In accordance with this suggestion, we discuss the role of metabolic derangements in the development of hepatic cancerogenesis:

*Induction of hepatocyte apoptosis, the invasion and activation of inflammatory cells, as well as fibrogenesis lead to cirrhosis, and possibly to NASH-related HCC<sup>[20]</sup>.*

- (11) **Comments from the reviewer 1:** Do not repeat that NAFLD is associated with MS: this has already been pointed out elsewhere in the manuscript.

**Answer:** In accordance with this suggestion, we avoided repeating the fact that NAFLD is associated with MS:

*Most NAFLD patients clinically present without symptoms, even though some may present with fatigue, dyspepsia, dull pain in the liver and hepatosplenomegaly and they are often overweight or obese<sup>[85]</sup>.*

- (12) **Comments from the reviewer 1:** Authors should be aware that not all studies agree that NAFLD is more prevalent in women and more severe in men (e.g. Loria J Hepatol. 2006;44:1196-207.).

**Answer:** In accordance with this suggestion, we discuss the prevalence of NAFLD among women and men:

*It was initially thought to be more common in women, however oposing results have been reported on this subject<sup>[86]</sup>.*

- (13) **Comments from the reviewer 1:** The contention that ultrasonography detects only steatosis > 33% conflicts with modern views (Dasarathy S, J Hepatol. 2009 ;51:1061-7.).

**Answer:** In accordance with this suggestion, we discuss the role of ultrasonography in detection of steatosis:

*The most available method, ultrasonography, has sensitivity of 100% and specificity of 90% when fat on liver biopsy  $\geq 20\%$ <sup>[97]</sup>.*

- (14) **Comments from the reviewer 1:** Shortly discuss whether bariatric surgery reverses NAFLD histology and reduces diabetes risk.

**Answer:** In accordance with this suggestion, we discuss whether bariatric surgery reverses NAFLD histology and reduces diabetes risk:

*Treatment for NAFLD and NASH includes weight reduction through lifestyle modifications, antiobesity medication and bariatric surgery<sup>[99]</sup>. Besides significant weight loss, bariatric surgery provides improvement of metabolic syndrome including T2D and pathological liver histological features such as grade of steatosis, hepatic inflammation, and fibrosis in most obese patients with NAFLD<sup>[99,100]</sup>.*

- (15) **Comments from the reviewer 1:** Please provide some clinical indications as to how these obese NAFLD patients should be followed in clinical practice, with specific reference to diabetes risk and surveillance for HCC. Are there any practical methods to identify those NAFLD obese who are at particularly increased HCC risk?

**Answer:** In accordance with this suggestion, we provide clinical indications how obese NAFLD patients should be followed in clinical practice, with reference to diabetes risk and surveillance for HCC:

*Obese patients with NAFLD should be followed in clinical practice, with specific reference to diabetes risk and surveillance for HCC. Weight loss, exercise, diet and lifestyle changes should be evaluated after 6 months, blood and platelet count, as well as liver biochemical tests and prothrombin time twice per year, screening for cardiovascular risk every 1-2 years and staging of liver damage to fibrosis by newer non-invasive method like transient elastography or, if it is not available, liver biopsy every 3-5 years. Surveillance for HCC includes ultrasound and alpha-fetoprotein every six months.*

- (16) **Comments from the reviewer 2:** Although the importance of visceral adipose tissue-lipolysis for hepatic FFA-delivery is generally recognized, the authors should also mention that it is not solely visceral fat that contributes to FFAs in the circulation, but that subcutaneous fat is a major source of systemic FFA-release in the fasting state (e.g. Koutsari and Jensen 2006, J Lipid Res). Thus, the statement that “FFAs are derived from [...] stored lipids that are being released from VAT into the portal system, dietary sources, and de novo lipogenesis...” (page 8, lines 6-8) should be changed accordingly.

**Answer:** In accordance with this suggestion we discuss the role of subcutaneous fat in systemic FFA release:

*FFAs are derived from three distinct sources: stored lipids that are being released from VAT into the portal system and subcutaneous fat that is a major source of systemic FFA release in the fasting state, dietary sources, and de novo lipogenesis (DNL, production of FAs from carbohydrates or amino acids)<sup>[34,57]</sup>.*

(17) **Comments from the reviewer 2:** Question: Regarding the role of leptin in NAFLD-development (Page 6), are there any studies specifically demonstrating hepatic leptin resistance in NAFLD-patients?

**Answer:** Huang et al. discuss leptin resistance in NAFLD patients. Accordingly, we write about it in the article:

*Obesity is considered a state of central and peripheral leptin resistance and obese individuals have higher circulating levels of leptin, as well as individuals with NAFLD and NASH<sup>[42-44]</sup>.*

(18) **Comments from the reviewer 2:** Please correct references on page 3, line 14.

**Answer:** In accordance with the suggestion from Reviewer 1 we have shortened the section on NAFLD, which was too long, and therefore, the references are now corrected.

(19) **Comments from the reviewer 2:** Page 8, line 9: Please correct to „lipolysis“.

**Answer:** In accordance with this suggestion, we corrected the word „lypolysis“ with „lipolysis“.

**3 References and typesetting were corrected.**

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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