Title: OBESITY, FATTY LIVER DISEASE AND INTESTINAL MICROBIOTA

Corrections on the manuscript were done:

1. Telephone and fax numbers were corrected.
2. **Author contribution was added:** Nur Arslan designed and wrote the manuscript.
3. **Abstract was rewritten and it contains 209 words:** "Nonalcoholic fatty liver disease (NAFLD) is a chronic liver disorder that is increasing in prevalence with the worldwide epidemic of obesity. NAFLD is the hepatic manifestation of the metabolic syndrome. The term NAFLD describes a spectrum of liver pathology ranges from simple steatosis to steatosis with inflammation (nonacoholic steatohepatitis; NASH) and even cirrhosis. Metabolic syndrome and NAFLD also predict hepatocellular carcinoma. Many genetic and environmental factors have been suggested to contribute to the development of obesity and NAFLD, but the exact mechanisms are not known. Intestinal ecosystem contains trillions of microorganisms including bacteria, Archaea, yeasts and viruses. Several studies support the relationship between the intestinal microbial changes and obesity and also its complications, including insulin resistance and NAFLD. Given that the gut and liver are connected by the portal venous system, it makes the liver more vulnerable to translocation of bacteria, bacterial products, endotoxins or secreted cytokines. Altered intestinal microbiota (dysbiosis) may stimulate hepatic fat deposition through several mechanisms: regulation of gut permeability, increasing low-grade inflammation, modulation of dietary choline metabolism, regulation of bile acid metabolism and producing endogenous ethanol. Regulation of intestinal microbial ecosystem by diet modifications or by using probiotics and prebiotics as a treatment for obesity and its complications might be the issue of further investigations."
4. **Coretip was added to the manuscript**: "There is increasing evidence for the relation between dietary habits, gut microbiota and obesity. Nonalcoholic fatty liver disease is a common complication of obesity. This manuscript

summarizes the relationship betwen intestinal microbial dysregulation and fatty liver disease related with obesity, and their proposed mechanisms."

5. **Author's name and the manuscript name were added:** Arslan N. Obesity, fatty liver disease and intestinal microbiota.
6. **Each key word was separated by a semicolon**.
7. **Comment: A ppt version of the figures should be added to the text.**

Answer: All figures were added as an attached ppt file to the text for editing easily.

To Reviewer 1:

1. **Comment**: I suggest to delete the word "severe" (NAFLD is a common disease)

   Answer: The term "severe" was changed with the word "chronic"
2. **Comment**: After every citation please add point: fort example Spencer et al [103].

   Answer: A point was added to the end of all sentences.

To Reviewer 2:

1. **Comment (Abstract)**: Nonalcoholic fatty liver disease (NAFLD) is not always a severe liver.

   Answer: The term "severe" was changed the word "chronic"
2. **Comment (Abstract)**: It would more appropriate to explain also the evolution for cirrhosis and hepatocellular carcinoma.

   Answer: Two sentences were added to the abstract: "The term NAFLD describes a spectrum of liver pathology ranges from simple steatosis to steatosis with inflammation (nonacoholic steatohepatitis; NASH) and even cirrhosis. Metabolic syndrome and NAFLD also predict hepatocellular carcinoma."
3. **Comment (Introduction)**: Recent evidence suggests that enteric microbiota may play a significant role in the development of obesity and its complications. (reference here)

Answer: A reference was added: **Ridaura VK**, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* 2013; **341**: 1241214 [PMID: 24009397 doi: 10.1126/science.1241214]

4. **Comment**: Fatty liver disease is dramatically increasing in childhood and adolescent obesity, and it has become the most common form of chronic liver disease in these age groups (ref).

   Answer: Two reference were added to the text:

   Berardis S, Sokal E. Pediatric non-alcoholic fatty liver disease: an increasing public health issue. *Eur J Pediatr* 2014; **173**: 131-139 [PMID: 24068459 doi: 10.1007/s00431-013-2157-6]

   Widhalm K, Ghods E. Nonalcoholic fatty liver disease: a challenge for pediatricians. *Int J Obes (Lond)* 2010; **34**: 1451-1467 [PMID: 20838401 doi: 10.1038/ijo.2010.185]

5. **Comment**: It would be appropriate to tell some figures about the world dimension of childhood obesity.

   Answer: A sentence and references were added:

   Global prevalance of childhood overweight/obesity varies from 5.7% to 40% in different populations

   Gulati S, Misra A, Colles SL, Kondal D, Gupta N, Goel K, Bansal S, Mishra M, Madkaikar V, Bhardwaj S. Dietary intakes and familial correlates of overweight/obesity: a four-cities study in India. *Ann Nutr Metab* 2013; **62**: 279-290 [PMID: 23689065 doi: 10.1159/000346554]

   Jafar TH, Qadri Z, Islam M, Hatcher J, Bhutta ZA, Chaturvedi N. Rise in childhood obesity with persistently high rates of undernutrition among urban school-aged Indo-Asian children. *Arch Dis Child* 2008; **93**: 373-378 [PMID: 17942586 doi: 10.1136/adc.2007.125641]

Moraes SA, Beltrán Rosas J, Mondini L, Freitas IC. Prevalence of overweight and obesity, and associated factors in school children from urban area in Chilpancingo, Guerrero, Mexico, 2004. *Cad Saude Publica* 2006; **22**: 1289-1301 [PMID: 16751968]

Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA* 2014; **311**: 806-814 [PMID: 24570244 doi: 10.1001/jama.2014.732]

6. **Comment:** Based on these results, the Authors concluded that dietary inulin-type fructans could play a role in the management of obesity and diabetes through their capacity to promote secretion...This type of text is not very appropriate i.e. the Authors...

   Answer : Two corrections were made: **1)** Based on these results, it can be concluded that dietary inulin-type fructans could play a role in the management of obesity and diabetes through their capacity to promote secretion.. **2)** "Since both groups of animals had been fed with the same diet, the Authors suggested that obesity might affect the diversity of gut microbiota" was changed "Since both groups of animals had been fed with the same diet, it was suggested that obesity might affect the diversity of gut microbiota"

7. **Comment**: There must be some discussion about oncogenicity of obesity, risk of cirrhosis, hepatocellular carcinoma.

   Answer: These sentences were added to the text and references were added to the references:

   Nonalcoholic steatohepatitis progress to advanced fibrosis and cirrhosis in 37% patients

   Several case reports, human and animal studies demonstrated that obesity is an important risk factor for carcinogenesis in many malignant neoplasms and also in hepatocellular carcinoma. Moreover, childhood obesity was shown to be related with increased risk of primary liver cancer in later adulthood

   Bugianesi E. Non-alcoholic steatohepatitis and cancer. *Clin Liver Dis* 2007; **11**: 191-207 [PMID: 17544979]

Kikuchi L, Oliveira CP, Carrilho FJ. Nonalcoholic fatty liver disease and hepatocellular carcinoma. *Biomed Res Int* 2014; **2014**: 106247 [PMID: 24738043]

Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625-1638 [PMID: 12711737 doi: 10.1056/NEJMoa021423]

Nair S, Mason A, Eason J, Loss G, Perrillo RP. Is obesity an independent risk factor for hepatocellular carcinoma in cirrhosis? *Hepatology* 2002; **36**: 150-155 [PMID: 12085359 doi: 10.1053/jhep.2002.33713]

Berentzen TL, Gamborg M, Holst C, Sørensen TI, Baker JL. Body mass index in childhood and adult risk of primary liver cancer. *J Hepatol* 2014; 60: 325-630 [PMID: 24076363 doi: 10.1016/j.jhep.2013.09.015]

Argo CK, Northup PG, Al-Osaimi AM, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. *J Hepatol* 2009; **51**: 371-379 [PMID: 19501928 doi: 10.1016/j.jhep.2009.03.019]

8. **Comment**: In the introduction to justify the importance the theme, the authors should emphasize the reduction in the life expectancy in the near future.

   Answer: A sentence and a reference was added to the manuscript:

   Obesity and related complications including fatty liver disease, cardiovascular disorders and hepatocellular carcinoma were found associated with the reduction in the life expentancy compared to general population

   Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005; **129**: 113-121 [PMID: 16012941]

9. **Comment:** It is necessary to put some more figures, color infography and tables to help explain this difficult and complex subject. Like for examples hormones, factors, cascades, etc.

   Answer: One table (table 1) and one figure (figure 3) were added to the text.

Table 1. Some key host proteins and factors those their expressions were changed by intestinal microbial changes and those play role in the development of obesity

Host protein/factor	Function
• **Fiaf** (fasting-induced adipocyte factor)	A protein that inhibits lipoprotein lipase activity.
• **ChREBP** (carbohydrate response element-binding protein)	A transcription factor that recognizes monosaccharides in the portal vein and plays a key role in the hepatic carbohydrate metabolism.
• **SREBP-1** (liver sterol response element-binding protein type-1)	A transcription factor family that controls the lipid synthesis in the liver and other tissues.
• **G-protein coupled receptors** (GPR43 and GPR41)	Proteins expressed in enteroendocrine L-cells those recognize luminal short-chain fatty acids (SCFAs) and mediate SCFA-induced GLP-1 release. They also present in adipocytes and promote adipogenesis by increasing lipid accumulation and inhibiting lipolysis and stimulate leptin production in response to SCFAs.
• **Toll like receptors**	Transmembrane molecules those recognize bacterial breakdown products.
• **GLP 1** (Glucagon-like peptide 1)	A protein produced by intestinal epithelial endocrine L-cells that stimulates insulin secretion, inhibits gastrointestinal motility, regulates appetite and food intake.
• **Peptide YY**	A peptide hormone produced by intestinal epithelial endocrine L-cells that inhibits intestinal motility.
• **Farnesoid X receptor**	A receptor expressed in liver and intestine that regulates bile acid synthesis, transport and detoxification.

Figure 3. Probable mechanisms of action of the antiobesity effects of modulated intestinal microbiota. CLA: conjugated linoleic acid, Fiaf: fasting-induced adipocyte factor