

June, 30nd 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3463-edited.doc).

Title: The relationship between changes of liver fat content and transaminases in obese children after 12-month nutritional intervention.

Author: Elvira Verduci, Carlo Pozzato, Giuseppe Banderali, Giovanni Radaelli, Chiara Arrizza, Antonio Rovere, Enrica Riva, Marcello Giovannini

Name of Journal: *World Journal of Hepatology*

ESPS Manuscript NO: 3463

We would like to thank you for your evaluation of our manuscript and your helpful and insightful comments both on its content and structure.

We have made several changes to the manuscript in accordance with your recommendations;

1 Format has been updated

2

Revision has been made according to the suggestions of the **reviewer 1 (02444743)**:

Major comments

- (1) Abstract has been shortened in the conclusion section as suggested by the Editor Office.

Minor comments

- (1) The spelling of paediatric or pediatric in text has been uniformed
- (2) How to measure HDL-C levels (Page 5) has been added.
- (3) The units of blood parameters have been uniformed.
- (4) The abbr. of gamma glutamyltransferase has been uniformed
- (5) The full name of NASH has been added
- (6) In text, "...Children spent 2h 20min (1h40min)/die of physical activity..." has been reworded as "...Children spent 2h 20min (1h40min)/day of physical activity..." (Page 2).
- (7) In text, "...biochemical parameter was not was associated with change in liver echogenicity...." has been reworded as "...biochemical parameter was not associated with change in liver echogenicity...." (Page 11).
- (8) In text, "...longitudinal changee in liver fat..." has been reworded as "...longitudinal changes in liver fat...." (Page 12).

Revision has been made according to the suggestions of the **reviewer 2 (01809219)**:

1. Some details on the nutritional intervention has been added in the abstract.
2. About the diet the content of monosaturated and polyunsaturated (PUFA) fats have been added.

3. In the present study there was a reduction in triglycerides without changes in any cholesterol fraction despite a decrease in ApoB after dietary intervention. Please note that these results are in agreement with results of others' study and also to our previous studies evaluating the effect of dietary intervention. These results are explained considering that triglyceride levels depend mostly from dietary intakes than cholesterol levels that, instead, are determined from endogenous synthesis and therefore it is necessary more time of intervention to demonstrate an effect. The decrease in ApoB, a component of LDL-cholesterol, should be read as an earlier effect on cholesterol metabolism than reduction of cholesterol levels.
4. No adipokine measurement has been performed in this study for financial constraints.
5. Typo errors scattered through the manuscript have been corrected.

Revision has been made according to the suggestions of the **reviewer 3 (02461842)**

1. Please consider that the aim of the present study was to assess whether any association may exist of change in liver fat content, based on MRI or ultrasonography, with change in liver biochemical parameters in obese children who underwent a one-year nutritional intervention. In particular in the present study the association of liver biochemical parameters with fat fraction was stronger than with liver echogenicity both at baseline and the end of intervention. Change in any liver biochemical parameter was not associated with change in liver echogenicity. On the contrary, change in AST and ALT was associated with change in liver fat fraction. Indeed as MRI may agree better than US with the diagnosis of steatosis based on biopsy, MRI may be a reasonable way to follow liver fat content in NAFLD.
2. Among the limitations of the study, the fact the Frequency Food Questionnaire is not the gold standard to assess food intake, although it is largely used, and that the gold standard is the three day food record has been added.
3. Why they used a cut off for FF% of 9%, while the most widely used is 5%?

Very thanks for having pointed out this doubt. Indeed, we choose *a priori* a cut off for FF% of 9% when planning the study design in 2009, according to Fishbein et al. (e.g., Fishbein MH, Gardner KG, Potter CJ, Schmalbrock P, Smith MA. Introduction of fast MR imaging in the assessment of hepatic steatosis. *Magn Reson Imag* 1997; 15: 287-93. Fishbein MH, Miner M, Mogren C, Chalekson J. The spectrum of fatty liver in obese children and the relationship of serum aminotransferases to severity of steatosis. *J Ped Gastroenterol Nutr* 2003; 36: 54-61. Fishbein MH, Mogren C, Gleason T, Stevens R. Relationship of hepatic steatosis to adipose tissue distribution in pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr*. 2006; 42: 83-88.) Moreover other studies then conducted in the pediatric age used this cut off (e.g., Pacifico L, Celestre M, Anania C, Paolantonio P, Chiesa C, Laghi A. MRI and ultrasound for hepatic fat quantification: relationships to clinical and metabolic characteristics of pediatric nonalcoholic fatty liver disease *Acta Paediatr*. 2007;96:542-547).

Really, already in 2006 a cut off of 5.5% has been used in obese adolescents (e.g., Burgert TS, Taksali SE, Dziura J, Goodman TR, Yeckel CW, Papademetris X, Constable RT, Weiss R, Tamborlane WV, Savoye M, Seyal AA, Caprio S. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 2006;91:4287-4294) estimated on a large cohort of 392 subjects, and further supported by results regarding a direct measure of hepatic triglyceride content using magnetic resonance spectroscopy (Szczeplaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 2005;288:E462-E468). Recently, Pacifico et al. (Pacifico L, Martino MD, Catalano C, Panebianco V, Bezzi M, Anania C, Chiesa C. T1-weighted dual-echo MRI for fat quantification in pediatric nonalcoholic fatty liver disease. *World J Gastroenterol* 2011;17:3012-3019) found in obese children the cut off for FF of 4.85%

having sensitivity of 95.8% and specificity of 100%, but the sample size analyzed (25 obese children with biopsy-proven NAFLD and 25 age and sex matched obese without NAFLD at ultrasonography) does not allow to ultimately infer in the general pediatric population about this cut off value.

Anyway, we recognize that nowadays the cut off for FF 5% may be increasingly used and reporting data also related to this cut off might therefore make simpler comparison among different studies. For this reason we have now re-analyzed the data also considering a cut off value of 5%. The main numerical results related to this cut off have been reported in the text and Table 1 and a comment has been included in the Discussion section.

1. In the text has been specified that APOB is APOB-100.

Revision has been made according to the suggestions of the **reviewer 4 (00071687)**

1. Please note that no Pediatric NAFLD Histological Score (PNHS) has been reported because in the present study the liver biopsy has not been performed due to ethical problems.
2. No correlation was found about the pediatric NAFLD fibrosis index (PNFI).
3. Please, consider that assessment by MRI agrees better than US with the diagnosis of steatosis based on biopsy. In the present study the association of liver biochemical parameters with FF was stronger than with liver echogenicity both at baseline and the end of intervention, in accordance with the literature. Change in any liver biochemical parameter was not was associated with change in liver echogenicity, supporting previous findings.. On the contrary, change in AST and ALT was associated with change in liver fat fraction. These results may be expected. Indeed as MRI may agree better than US with the diagnosis of steatosis based on biopsy, MRI may be a reasonable way to follow liver fat in NAFLD.

Revision has been made according to the suggestions of the **reviewer 5 (00742311)**

1. Several typing errors, such as glycemix... ..it should be note...sierologic., have been checked and correted.

3 References and typesetting were corrected

Thank you again for considering our manuscript for pubblication in the *World Journal of Hepatology*.

Sincerely yours,

Dr. Elvira Verduci PhD MD,
Pediatric Department San Paolo Hospital
Via Di Rudinì 8,
20142 Milano Italy.
elvira.verduci@unimi.it
Tel: +393498623769
Fax: +390289150125