

ANSWERING REVIEWERS



January 3, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5768-review.doc).

Title: The effect of resistance training on non-alcoholic fatty-liver disease a randomized-clinical trial

Author: Shira Zelber-Sagi, Assaf Buch, Hanny Yeshua, Nahum Vaisman, Muriel Webb, Gil Harari, Ofer Kis, Naomi Fliss Isakov, Elena Izkhakov, Zamir Halpern, Erwin Santo, Ran Oren, Oren Shibolet

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 as detailed below.
3. References and typesetting were corrected.

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

Sincerely yours,

Dr Oren Shibolet MD

Reviewer 1

Comment: This randomized trial is of potential interest given the paucity of data on effective NAFLD treatment. Review of previous contributions on this topic s appropriately referenced and the submission is professionally written.

Response: Thank you.

Comment: In particular, the discussion needs major editorial changes. The Authors state that “HRI has been validated versus liver biopsy and provides a highly sensitive, objective and quantitative tool for liver fat evaluation with a high correlation ($r=0.82$, $p<0.001$) and a kappa of 0.75 as compared with histological steatosis”. However, several lifestyle changes and drug treatments are associated with intrahepatic fat reduction in NAFLD and nevertheless this is not the chief aim of therapy. In discussing the limitations of their study, the Authors should highlight that, at variance with other ultrasonographic indices (Liver Int. 2012;32:1242-52.), HRI values do not predict NASH, which is the NAFLD variant which needs to be treated more urgently. The Author state “we were unable to perform repeated liver biopsies”. However, they should probably state better that they failed to characterize their patients: did they have simple steatosis or NASH ? On the grounds that not even base-line biopsy was performed. This is of major importance given the major differences in the natural history of such conditions (Curr Pharm Des. 2013;19:5177-92; Aliment Pharmacol Ther. 2011;34:274-85. ; Hepatology. 2011;53:1792-4.; Hepatology. 2009;49:306-17.)

Response: This comment was added with the relevant references to the discussion in the limitations of this study on page 16 and the US-FLI method was indicated.

Comment: The Authors need to acknowledge that their submission is limited in not providing any mechanistic explanation as to why RT is associated with reduced serum cholesterol and ferritin values.

Response: The mechanisms are indeed unknown; however there are several suggested hypotheses that were added to the paper on pages 14-15.

Comment: The statement (discussion, last paragraph) “demonstrated a significant reduction in steatosis” needs to be softened by specifying “as assessed through HRI values”.

Response: The sentence was modified as suggested (page 16).

Comment: The Authors speculate that “The modest effect on liver steatosis could have been more impressive with longer or more intense training” such a statement is not based on findings and should better be omitted.

Response: The sentence was omitted.

Comment: Non-diabetic patients were recruited in this trial. The reasons for such a choice need to be discussed given that diabetes worsens NAFLD course (Hepatol Res. 2013;43:51-64). making this category of individuals at special need for treatment.

Response: Adults with diabetes were excluded to avoid a confounding effect, since it is unclear whether they would have the same response to physical training and since changes in antidiabetic medications during the trial might occur. This explanation was added to the methods section on pages 6-7.

Comment MINOR: Authors might be willing to highlight that, although there seems to be universal consensus on lifestyle changes in NAFLD (J Hepatol. 2013;59:859-71.), nevertheless, how this should be obtained in clinical practice remains to be shown. Studies have reported that NAFLD patients prefer dieting and tend to avoid increases in physical activity (Clin Res Hepatol Gastroenterol. 2013;37:353-8.). Are the Authors willing to comment on this ?

Response: Indeed it seems that physical activity is more threatening to patients than diet when they are asked about it. This was shown also in another publication demonstrating that self-efficacy (Frith J, et al. J of Hepatology 2010) and readiness to lifestyle changes is low in NAFLD patients even as compared to readiness to diet (Centis E, et al. Journal of Hepatology 2013). We hope that resistance training would be less threatening to the NAFLD patients (as mentioned on page 6 first paragraph). Either way, even if they prefer to diet and avoid PA this should not be a hindrance to recommending it. Patients with NAFLD need PA for many hepatic and cardiovascular reasons.

Comment: Radiological examination for determination of NAFLD and quantification of steatosis – As far as I may understand, no X-rays are involved in performing liver ultrasonography. Accordingly, this technique cannot be defined a “Radiological” examination but it is better called “Ultrasound scanning”; “Ultrasonography” or “US Liver Imaging”.

Response: Thank you, the term was corrected on page 8.

Reviewer 2

Comment: it would have been important to quantify the amount of liver fat by liver biopsy or by transient elastography with CAP measurement or by

MRI spectroscopy and triglyceride level rather than by liver ultrasound. the accuracy of liver ultrasound in the grading of fat is not optimal. this should be added as a limitation of the study.

Response: This comment was added to the study limitations (page 16). As mentioned in the paper (page 16, see also comments to reviewer 1), the HRI was validated for *grading* the amount of liver fat with comparison to both liver biopsy and ¹H-MRS showing an excellent correlation. Furthermore, HRI highly correlates with biochemical surrogate markers of liver steatosis: the fatty liver index (FLI) and the SteatoTest. The controlled attenuation parameter (CAP) is indeed a promising screening technique, but requires by itself further validation (Nat Rev Gastroenterol Hepatol. 2013 Nov;10(11):666-75).

Comment: explain the mechanism of reducing serum ferritin levels in the resistance training program.

Response: There are several suggested hypotheses that were added to the paper on pages 14-15.

Reviewer 3

Comment: The aim of the study was to evaluate the effect of RT on NAFLD patients. The manuscript shows important finding regarding improvement of hepatic fat content.

Response: Thank you.

Comment: In the discussion authors state the following: "Our study showed significant improvement in the liver enzymes (ALT and aspartate aminotransferase-AST) within group but with no difference between arms.

Interestingly, there seems to be a limited correlation between exercise and liver enzyme reduction. Aerobic PA led to a significant reduction in liver enzymes in some trials [42] while no reduction was seen in other trials despite reduced steatosis [8, 34, 35]. ". The authors give no further clarification on this point. In spite of decreased levels within the RT arm, the lack of differences between arms of the study could be attributed to that liver enzyme levels are released from extrahepatic tissue in response to resistance training regardless of fatty liver masking the liver enzyme decrease compared to the stretching arm. One limitation in the study is that there is no reference to a control group without liver steatosis whom have undergone similar resistance training to address this issue which may explain the lack of enzyme level differences, as liver enzymes may have been released from extrahepatic (muscle) tissue in response to resistance training. This does not however undermine the major findings of the study but should be highlighted in the discussion considering liver enzymes which may provide a better explanation regarding their liver enzyme findings. The following reference is of significant importance regarding justification of liver enzyme findings. Pettersson J, Hindorf U, Persson P, Bengtsson T, Malmqvist U, Werkström V and Ekelund M. Muscular exercise can cause highly pathological liver function tests in healthy men. *Br J Clin Pharmacol.* 2008 February; 65(2): 253–259.

Response: Thank you for bringing up this important explanation. This was added to the discussion section (page 14) with the reference suggested.

Comment: Minor comments: Abstract: The abstract is lacking a brief background. The results section of the abstract is too detailed considering patients dropping out, quitting and exclusion of patients. This is not necessary in the abstract.

Response: According to the WJG instructions for authors, there is no background in the abstract. "Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words.....)".

The description of the patient's flow in the abstract was shortened.

Comment: The 3rd line in the results withdraw should be withdrew.

Response: Thank you, it was fixed.

Reviewer 4

Comment: Abstract; contents in abstract are confusing. Overall, there is pointless conclusion without strong support evidence and it suffers from poor design and analysis.

Response: The abstract and the paper are written according to the Consolidated Standards of Reporting Trials (CONSORT) Statement^[1]. However, we made several changes to make it clearer. The study was designed as a RCT, one of only a few in this subject. The analysis was performed by an expert in statistics which is in charge of many RCTs in national and international level.

Comment: Title is vague and should be revised.

Response: The title describes the type of treatment provided to the disease of interest (NAFLD) and state on the study design, as accepted^[1]. If the reviewer and editors prefer, the title can be changed to: Resistance training improves fat content and body composition in patients with NAFLD a randomized controlled trial.

Comment: Introduction; many portions should be condensed and should be moved into Discussion.

Response: The introduction was revised according to the comments of the reviewers.

Comment: Methods; is this study really a RCT study?

Response: Absolutely, as stated. The randomization method is described on page 6 " We conducted a RCT (sealed envelopes randomization stratified by gender) ". Furthermore, the study was pre-registered in the NIH registration website as an RCT (TRIAL no. NCT01264198)

Comment: What is sample size justification for this RCT study?

Response: The sample size calculation is explained on page 7: " A sample size of 32 patients in each group was calculated to be needed for a 90% power to detect a difference of 0.25 with a standard deviation of 0.30, based on previously published data on HRI change following weight change [2], with a 0.050 two-sided significance level. Additional 20% patients were recruited taking into consideration attrition or protocol violation".

Comment: Why did the authors choose the control group? This is very confusing. Is this also considered in sample size estimation?

Response: The controls were not chosen, since this is a randomized trial, the subjects were randomized to either resistance training or controls. The sample size was calculated taking under consideration a controlled trial design.

Reviewer 5

Comment: The study of Zelber-Sagi et al entitled "The effect of resistance training (RT) on non-alcoholic fatty liver disease randomized clinical trial" is an independent study focusing on the physical exercise pattern and the improvement of NAFLD patients in the vital parameters. The study demonstrates an important aspect of Resistance training in addition to the therapeutic approach benefiting patients in dealing with the NAFLD. The

manuscript is written clearly and highlights all the parameters necessary to document the study in a scientific manner.

Response: Thank you.

Comment: The only drawback the reviewer could point out is that it would be better if a correlation is drawn between the two exercise parameters, i.e., the Strength training or the resistance training versus the vital outcomes, and serum parameters. A separate graph of correlation coefficients with each of these parameters/indices will be helpful in understanding the advantage(s) of one treatment arm over the other.

Response: The correlation can be performed; for example the correlation between treatment arm and reduction in the Hepato-Renal index is 0.321, $P=0.01$, and for liver enzymes it is not significant as there was no significant difference between treatment arm in reduction of liver enzymes (only significant for within group comparisons), as described on table 2. A more suitable and accepted way to analyze the data in a clinical trial is to do analysis of variance using repeated measurements model for testing the group X time interactions, as we did. The individual changes in HRI in every arm, presented one next to the other, is described in Figure 2, and a correlation (scatter plot) graph would add little information.

Comment: More importantly, in the future, to conduct a possible follow-up study with the same patients criteria, this sort of charting will also help to execute a population-based study on the lifestyle, dietary habits in NAFLD patients (with or without diabetics or alcoholics) in response to multiple physical exercise benefits. The study is very important for the journal's readership and can be accepted with the additional analytical parameter of Correlation Coefficients as mentioned above.

Response: Thank you.

1 Bian ZX, Shang HC. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Annals of internal medicine* 2011; **154**(4): 290-291; author reply 291-292 [PMID: 21320945 DOI: 10.7326/0003-4819-154-4-201102150-00016]

2 Zelber-Sagi S, Lotan R, Shlomai A, Webb M, Harrari G, Buch A, Nitzan Kaluski D, Halpern Z, Oren R. Predictors for incidence and remission of NAFLD in the general population during a seven-year prospective follow-up. *Journal of hepatology* 2012; **56**(5): 1145-1151 [PMID: 22245895 DOI: 10.1016/j.jhep.2011.12.011]