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Prof Ya-Juan Ma
Science Editor
World Journal of Gastroenterology

Dear Prof Ma,

Re: Manuscript *"***18884: Oily fish, coffee and walnuts: Dietary treatment for Nonalcoholic fatty liver disease"**

We would like to thank the reviewers and editor for thoughtfully reviewing our manuscript. We have found the reviewers' suggestions helpful and have incorporated them into the manuscript, which we feel is now substantially improved with the additions. In line with the editorials comments, we have addressed all noted concerns.

Please find accompanying, a point by point response to the reviewers comments as well as the revised manuscript with changes underlined.

All requested files have been uploaded apart from the language certificate (all authors are native English speakers) and CrossCheck document. I am not able to access the CrossCheck service as my institution has no login, but would be more than happy to submit the document to this check if you are able to provide me with login details.

Thankyou for your further review. Yours Sincerely

Dr David van der Poorten on behalf of the authors

EDITORS COMMENTS:

1. **Title:** as suggested this has been amended to be 10-12 characters in length: "**Oily fish, coffee and walnuts: Dietary treatment for Nonalcoholic fatty liver disease"**

2. **Conflict of interest statement:** has been added. All authors have no conflict to report.

3. Audio core tip file attached.

4. Tables have been reformatted as requested to comply with the journal style.

REVIEWER 1

Overall, I loved reading this paper. Nonetheless, I feel that the paper deserves some comments for further improvement.

1. Section oily fish and fish oil The authors should comment the recommended intake of omega-3 FAs for children.

The following has been added to address this.

For young children, the situation is somewhat altered with regulatory agencies recommending less than 60g of fish per week, due to the potential risk of environmental contaminants, but also the avoidance of fish oil supplements without a doctor's prescription[25]. Nonetheless the World Health Organization recommends consumption of at least 400mg per 10kg bodyweight Omega-3 each day, while the International Society for the Study of Fatty Acids and Lipids suggests 350-750 mg per 10 kg of body weight[25].

The authors should comment the impact of omega-3 FA supplements to treat NAFLD in children. In that vein, they may wish to look up the recent review by Pacifico L et al. (Mini-Reviews in Medicinal Chemistry 2014;vol.14,791-804).

Thank you. This has been added as below.

A limited number of studies have examined Omega-3 PUFA supplementation in patients with pediatric NAFLD with results largely mirroring those seen in adult populations[20]. Nobili *et al* prospectively followed 60 children with biopsy confirmed NAFLD and showed that both short (6 month)[21] and longer term (24 month)[22] DHA supplementation, reduced liver steatosis as measured by ultrasound in addition to improving ALT and triglycerides, irrespective of DHA dose (250mg vs 500mg/day). In a follow up study the same authors showed that supplementation of 250mg DHA for 18 months improved hepatic steatosis and ballooning as measured on paired biopsies, but did not affect fibrosis[23]. Importantly however, a marked anti-inflammatory effect was noted in these biopsies, characterized by reductions in hepatic progenitor cell activation, reduced numbers of inflammatory macrophages and G-protein receptor changes associated with inhibition of TNF and toll like receptor pathways[20]. In pediatric patients with obesity or metabolic syndrome the addition of Omega-3 has also variably been associated with reductions in hypertension, improvement in lipid profile and reductions in insulin resistance[20] suggesting it is likely to be of benefit in NAFLD.

Reference #14: as suggested, the optimal dose of omega-3 PUFA is currently not known. Most trials have used ultrasound or liver enzymes as a semi-quantitative or non-specific measure of NAFLD severity. This should be emphasized.

Thank you. It has been noted in the text that studies used ultrasound as a measure of liver fat and the following change has been made to the conclusion of this section.

While there appears to be ample evidence that regular consumption of oily fish has metabolic benefit, the effect of additional oily fish or fish oil supplementation in NAFLD is uncertain and the current optimal dose is not known. Despite studies showing consistent improvement in liver fat content as semi-quantitatively measured by ultrasound or other imaging, recent RCTs have not shown a significant benefit in the harder endpoints of liver histology or fibrosis.

Reference #16:almost 25% of subjects did not complete the trial!

This has been noted in the paper.

The largest study involving 243 biopsy-proven NASH subjects given placebo, low-dose E-EPA (1.8g/day) or high-dose E-EPA (2.7g/day) for 12 months did not show any significant effects on liver steatosis, inflammation or fibrosis across treatment groups[16]. There was also no significant improvement in metabolic parameters including HbA1c, total cholesterol and BMI. The exception was serum triglycerides, which was lower in the high-dose E-EPA group compared to placebo. It is worthwhile noting, however, that almost 25% of subjects did not complete the trial.

Section Coffee

Reference #25.This study shows an inverse associations between coffee drinking and most major causes of death, with the exception of cancer. Please include limitations of this study. Coffee consumption was assessed by self-report at a single time point and may not reflect long-term patterns of consumption. The distinction between persons who drank caffeinated coffee and those who drank decaffeinated coffee was subject to misclassification. The authors of reference #25 lacked data on how coffee was prepared (espresso, boiled, or filtered), and the constituents of coffee may differ according to the method of preparation.

Thank you. This important information has been added to the manuscript.

Coffee drinking was first reported to have a protective effect against the development of cirrhosis close to two decades ago[30], with a recent study pointing towards an inverse relationship with total and non-cancer related mortality[31]. This latter data however was limited by self reporting of coffee consumption at a single time point, uncertainty over caffeine content and a lack of information on the method of coffee

preparation[31].

In several case-control studies, coffee consumption has shown an inverse association with the incidence or diagnosis of liver cirrhosis, with significant trends in risk with dose and duration (Klatsky and Armstrong, 1992; Klatsky et al, 1993; Corrao et al, 1994, 2001; Gallus et al, 2002). Since liver cirrhosis is strongly related to the incidence of hepatocellular carcinoma (Adami et al,1992; La Vecchia et al, 1998, Kuper et al, 2000), the apparent protective effect of coffee consumption on hepatocellular carcinogenesis may be due to its inverse relation with liver cirrhosis.

Effect of coffee on the development of liver cancer. A large Finnish study, including over 60,000 individuals across a 19 year follow-up period, was able to show a dose-dependent decrease of the rate of HCC-development in the consumption of up to 6 cups of coffee per day (Hepatology 2008;48:129-136). Please mention it. A meta-analysis of the impact of coffee consumption on the risk of HCC-development was able to confirm this association (BMC Gastroenterol 2013;13:34).

Thank you. This has been added and addressed below.

It also protects against the development of hepatocellular carcinoma (HCC) irrespective of the aetiology of liver disease,[37] with a dose dependant decrease in the incidence of HCC seen with consumption of up to 6 cups of coffee per day[38, 39]. This relationship may well be driven by the inverse relationship between coffee and liver cirrhosis[30, 33, 40], given the strong relationship between cirrhosis and the incidence of HCC[41, 42].

Reference #27 is relevant. It shows a robust inverse relation of coffee drinking to risk of alcoholic cirrhosis, independent of several potential confounders. In contrast, there was no statistically significant relation of coffee drinking to risk of nonalcoholic cirrhosis. Cross-sectional data from reference #27 suggest that coffee is more specifically protective against severe chronic liver disease (ie, cirrhosis) when alcohol is the noxious agent.

Thank you. This has been noted that the association was for alcohol alone and the paragraph rearranged as follows:

Initially the association between coffee drinking and lower risk of liver disease was found in the context of alcoholic liver disease alone[30, 32],

Reference #29. The main strength of this analysis is that the study was based on prospective collection of exposure history (coffee or tea consumption) and clinical outcome (hospitalization or death due to chronic liver disease) in a large

population-based sample (NHANES-I). Methodologically, however, this study was limited for a number of reasons. Firstly, a relatively large proportion of subjects were excluded from the analysis because of a lack of data on coffee or tea consumption; secondly, ascertainment of liver disease was based on hospital discharge records and death certificates, as opposed to verifiable clinical records; and thirdly, there was a lack of detail regarding the amount and type of beverage consumed. Weighing up these strengths and limitations, however, the reader becomes intrigued that coffee or tea consumption might, indeed, be good for the liver. Caution must be exercised, however, before physicians begin to advise patients with liver disease to consume more tea or coffee. Although these observational data show a consistent association between coffee or tea consumption and chronic liver disease, it is premature to conclude a causal relationship between the two (i.e that these beverages reduce the risk of liver disease). Firstly, no known ingredients of coffee or tea have been linked with a protective effect in the pathogenesis of CLD. Caffeine might not be responsible, as caffeine-containing beverages other than coffee did not show any benefit in a study by Corrao et al. (reference #28). Despite the recent interest in the antioxidant and other potentially beneficial properties of catechins in tea, a protective effect against chronic liver disease remains to be determined. (Cooper R et al. Medicinal benefits of green tea: part I. Review of noncancer health benefits. J Altern Complement Med 2005; 11: 521– 5283). Secondly, it is possible that the association revealed in this study might have been confounded with other dietary or behavioral factors that are indeed responsible for the reduced risk of CLD. For example, coffee or tea consumption was inversely associated with BMI. It could be that the consumption of these beverages is associated with healthier dietary practices, which might reduce the risk of the metabolic syndrome—itself associated with nonalcoholic fatty liver disease. Similarly, consumption of coffee or tea might be inversely correlated with heavy alcohol consumption. The lack of a consistent pattern between coffee or tea consumption and alcohol intake in the Ruhl and Everhart study might be attributable to the fact that all levels of alcohol consumption >2 drinks per day were lumped together; patients with alcoholic liver disease who consumed >2 alcoholic drinks per day could not be separately analyzed. Please consider and discuss these limitations when reviewing the effects of coffee or tea on NAFLD.

Thank you. Some of the limitations of this study have been noted to put it in context and the caveats surrounding coffee and NAFLD have been alluded to in the conclusions of this section. Benefits and risks of tea have been added in the appropriate section of the manuscript.

A subsequent large epidemiological study based on NHANES-I clinical outcome data associated prospectively collected coffee consumption with a decreased risk of liver enzyme derangement and reduced mortality and hospitalisations in all cirrhotics[34]. The limitations of this study were noteworthy, with a significant proportion of subjects excluded from the analysis due to a lack of data on coffee or tea consumption, ascertainment of liver disease based on hospital discharge records and death certificates as opposed to verifiable clinical records, and a lack of detail regarding the amount and type of beverage consumed[34]. Furthermore, the inverse association between coffee consumption and BMI meant healthier dietary practices and reduced

incidence of metabolic syndrome (and hence NAFLD) could not be excluded.

Based on recent literature, a growing and pervasive argument is mounting that coffee may protect against the development of NAFLD and reduce NASH severity. It would appear that the effects of coffee on the aetiology of liver disease are multifactorial, and whilst more detailed mechanistic studies are required to elucidate this further, the addition of filtered unsweetened coffee may be a reasonable adjunct to diet and exercise in patients on the fatty liver spectrum[27-29].

Despite the recent interest in antioxidant and other properties of catechins that may have potential benefit, a definite protective effect against chronic liver disease remains to be determined in human subjects[90].

Effect of coffee on the progression of NAFLD. A meta-analytic review of the evidence for preventing development and progression of NAFLD by coffee consumption was able to substantiate the protective effect of coffee on NAFLD in the experimental as well as the clinical setting (Aliment Pharmacol Ther 2013;38:1038-1044). Please comment this review.

This has been added.

Furthermore, a recent meta-analysis, including three animal studies and eleven epidemiological and clinical studies, supported the concept that coffee intake protects against the development of metabolic syndrome and NAFLD in experimental models and clinical settings[27]

On the basis of available data, the effects of coffee on the etiology of liver disease are indeed multi- factorial, necessitating detailed mechanistic studies to understand its exact impact. Please take it into account.

Noted in the conclusions.

Based on recent literature, a growing and pervasive argument is mounting that coffee may protect against the development of NAFLD and reduce NASH severity. It would appear that the effects of coffee on the aetiology of liver disease are multifactorial, and whilst more detailed mechanistic studies are required to elucidate this further, the addition of filtered unsweetened coffee may be a reasonable adjunct to diet and exercise in patients on the fatty liver spectrum[27-29].

The study by Birerdinc A et al (reference #37) : caffeine consumption was found to be an independent predictors of NAFLD, even after adjustment for race, gender and metabolic syndrome components.

This has been Included

Epidemiological data suggests that coffee may have a protective effect against the development of NAFLD. Data published in 2012, based on four continuous cycles of the US National Health and Nutrition Examination Survey (NHANES 2001-2008) showed caffeine consumption and plain water intake to be independently associated with a lower risk of NAFLD, even when patient demographics (e.g. race and gender), clinical parameters such as metabolic syndrome components and other dietary constituents were considered[47].

As observed by Molloy et al. (reference #40), Bambha et al. also observed a beneficial effect of regular coffee consumption on liver fibrosis in NAFLD patients, however, only in patients with a low level of insulin resistance (Hepatology 2012;56:242A).

Thank you. I believe this was from a 2014 paper as below. We have inserted this important bit of information.

A further study of 782 adults with NAFLD was also able to demonstrate a reduced risk for advanced NASH in patients who regularly consumed coffee, but interestingly this effect was only noted in patients with low but not high levels of insulin resistance (HOMA-IR < 4.3).[51]

51 Bambha K, Wilson LA, Unalp A, Loomba R, Neuschwander-Tetri BA, Brunt EM, Bass NM. Nonalcoholic Steatohepatitis Clinical Research Network Research Coffee consumption in NAFLD patients with lower insulin resistance is associated with a lower risk of severe fibrosis. *Liver Int.* 2014; **34**(8)1250-8

NUTS Reference #67 shows that a low intake of nuts and seeds (OR, 3.66) was associated with a significantly higher risk for developing NAFLD.

This has been included.

An additional epidemiological study from Korea has shown that a low intake of nuts and seeds (OR: 3.66) was associated with a significantly higher risk for developing NAFLD in male subjects[77],

Reference #68: Vitamin E was superior to placebo for the treatment of nonalcoholic steatohepatitis in adults without diabetes.

This has been noted.

There are, however, numerous trials testing the therapeutic value of vitamin E, including the landmark PIVENS trial where vitamin E was seen to significantly improve LFTs, increase adiponectin and reduce hepatic steatosis and lobular inflammation, demonstrating superiority to placebo for the treatment of NASH in adults without diabetes[78].

Reference #65 shows that significant inverse associations were also observed between nut consumption and deaths due to cancer, heart disease, and respiratory disease. This study supports the health benefits of nut consumption for many chronic diseases. As the study lacked data on how nuts were prepared (e.g., salted, spiced, roasted, or raw), the authors of reference #65 were unable to examine the influence of preparation method on mortality. As outlined in reference #65, nutrients in nuts, such as unsaturated fatty acids, high-quality protein, fiber, vitamins (e.g., folate, niacin, and vitamin E), minerals (e.g., potassium, calcium, and magnesium), and phytochemicals (e.g., carotenoids, flavonoids, and phytosterols), may confer cardioprotective, anticarcinogenic, antiinflammatory, and antioxidant properties. Indeed, clinical trials have shown that nut consumption has beneficial effects on some intermediate markers of chronic diseases, such as high cholesterol levels, oxidation, endothelial dysfunction, hyperglycemia, and insulin resistance.

Thank you. Some additional information has been noted about this study as follows:

In the largest epidemiological study to date that followed over 118,000 patients for up to 30 years, overall nut consumption correlated with reduced all-cause mortality for both men and women and with reduced deaths due to cancer, heart disease and respiratory disease, effects most pronounced in those who consumed higher quantities of nuts[75]. No conclusions could be drawn as to the influence of preparation methods (roasted, salted, spiced or raw) on mortality, as this data was not collected.[75]

Reference #70 evaluated the effect of an almond-enriched (high monounsaturated fat, MUFA) or complex carbohydrate-enriched (high carbohydrate) formula-based low-calorie diet (LCD) on anthropometric, body composition and metabolic parameters in a weight reduction program. Ketone levels increased only in the almond-LCD group (+260 vs 0%, P<0.02). Glucose, insulin, diastolic blood pressure, total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) and LDL-C to HDL-C ratio decreased significantly to a similar extent in "both" dietary interventions. Homeostasis model analysis of insulin resistance (HOMAIR) decreased in "both" study groups over time.

Thank you. We have now modified the paragraph to reflect this and have removed reference to improved insulin sensitivity from nuts in the conclusion.

In one small RCT of 50 patients, incorporation of mixed raw nuts (walnuts, almonds and hazelnuts) into a healthy diet significantly reduced fasting insulin levels from baseline, decreased insulin resistance and improved serum LDL compared to controls[80]. These results were not replicated in another study of 60 patients with the metabolic syndrome however, where the addition of a handful of almonds to a low calorie diet resulted in reductions in insulin resistance and lipids that were no different to control, albeit with more substantial improvements in BMI and waist circumference seen[81]. No benefit was observed in T2DM patients fed almonds daily as part of a low-fat or high-fat diet (HFD), perhaps due to the lack of statistical power in the latter study (only 20 patients)[65].

RED WINE Reference #93: this study was not placebo- controlled.

This has been added.

Napoli *et al*[104] demonstrated improved insulin-mediated glucose disposal with the addition of 360mL of red wine per day (compared to abstinence) in 17 diabetic adults treated for 2 weeks, however, this study was not placebo controlled.

Reference #94: The main findings of this study are that red wine rich in polyphenols with or without alcohol (red wine and dealcoholized red wine interventions) but not gin, an alcoholic beverage devoid of polyphenols, improved glucose metabolism, as measured by HOMA-IR.

Done

In a subsequent randomised crossover trial, 66 men at high risk of cardiovascular disease were supplemented with 30g/day of red wine, 30g/day of gin or an equivalent amount of dealcoholised red wine. The authors found that supplementation with red wine or dealcoholised red wine (both rich in polyphenols), but not gin, which does not contain polyphenols, led to significant reductions in plasma insulin and insulin resistance (measured by HOMA-IR) while glucose levels remained constant.

OLIVE OIL Reference #118 found a dose-response effect, whereby the highest quartile of olive oil intake showed the greatest reduction in risk (reducing mortality risk by 44%). Please stress that the authors of reference #118 were able to analyze the effect of virgin and ordinary olive oil separately, although they did not observe any difference in their association with overall mortality.

Thank you. This has been added.

Buckland *et al*[129] showed that this occurs in a dose dependent fashion with those in the highest quartile of olive oil consumption having the greatest reduction in overall mortality (26%) and CVD mortality (44%) irrespective of the type of olive oil (virgin or ordinary) used[129].

Olive oil and decreased risk of certain cancers, in particular breast cancer (Curr Pharm Des 2011;17:805–12).

This has been added.

Regular consumption of olive oil can decrease the risk of certain cancers, particularly breast cancer[130],

Mediterranean diet and primary prevention of CVD: please mention and discuss the article by Ramón Estruch et al (NEJM April 4, 2013)

A recent study furthered these observations, showing that a Mediterranean diet supplemented with virgin olive oil or nuts substantially reduced the risk of serious cardiac events or death by up to 30% in the primary prevention setting of patients at high risk for cardiovascular disease[62].