Author’s response to reviews

Title: Cholecystectomy versus central obesity or insulin resistance in relation to the risk of nonalcoholic fatty liver disease: the third US National Health and Nutrition Examination Survey

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Author’s response to reviews:

Replies to the comments of Reviewer 1 (Hidetaka Hamasaki)

1. Why did the authors use data between the years 1988 and 1994? The reviewer is afraid that the imaging quality of abdominal ultrasonography to detect NAFLD was poor.

Reply: Thank you for your critical comments. Since abdominal ultrasonography was only available in the third U.S. National Health and Nutrition Examination Survey (NHANES III) among all waves of NHANES, we have to use data between the years 1988 and 1994. Although the ultrasonography was conducted in the years of 1988 to 1994, for the diagnosis of NAFLD, the percent agreement for intra-and inter-rater reliability was 91% (with a kappa statistic (95% confidence interval (CI)) of 0.77 (0.73–0.82)) and 89% (kappa, 0.70 (0.64–0.76)), respectively [Third National Health and Nutrition Examination Survey: hepatic steatosis assessment procedure manual. Atlanta: National Center for Health Statistics; 2010.]. Hence, the imaging quality of ultrasonography was not a problem. In addition, there are many other papers using the abdominal ultrasonography data between the years 1988 and 1994 published in recent years [Neurology. 2016 Mar 22;86(12):1136-42; Am J Gastroenterol. 2013 Jun;108(6):952-8].

2. The authors stated "Changes in BAs (bile acids) and the microbiome, and the metabolic products of microbial digestion of nutrients…is known to contribute to the involvement of metabolic abnormalities" on page 12, line 257-259. This is an intriguing statement but unsupported by evidence. The reviewer would suggest discussing the mechanism of interaction between cholecystectomy, gut microbiota, and insulin resistance in more detail.
Reply: We appreciate your constructive suggestions. Alterations in gut microbiota have been linked to host insulin resistance, and diabetes [Nature 2013;500:541–6; Nature 2016;535:376–81]. Emerging evidence reported that altered composition of gut microbiota was noted in patients who underwent cholecystectomy; the community diversity of intestinal microbiota in cholecystectomized patients was decreased compared with healthy population. The abundance of some species changed after cholecystectomy: bacteroidetes increased, prevotella decreased, etc. [Environ Microbiol Rep, 2015. 7(6): p. 874-80. Front Microbiol, 2018. 9: p. 1402.]. A human study found that low richness of gut microbiome plays a role in insulin resistance, and the main species involved in insulin resistance are Prevotella copri and Bacterides vulgates [Nature, 2016. 535(7612): p. 376-81.]. Taken together, alterations in gut microbiota may contribute to the involvement of insulin resistance and metabolic abnormalities. We addressed the relevant information in the Discussion section in the revised manuscript by adding the following sentences (page 13-14, line 282-289). “Alterations in gut microbiota have been linked to host insulin resistance, and diabetes [Nature 2013;500:541–6; Nature 2016;535:376–81]. Emerging evidence reported that altered composition of gut microbiota was noted in patients who underwent cholecystectomy; the community diversity of intestinal microbiota in cholecystectomized patients was decreased compared with healthy population [Environ Microbiol Rep, 2015. 7(6): p. 874-80. J Clin Med, 2019. 8(1). Front Microbiol, 2018. 9: p. 1402]. A human study found that low richness of gut microbiome plays a role in insulin resistance [Nature, 2016. 535(7612): p. 376-81]. Taken together, alterations in gut microbiota after cholecystectomy may contribute to the involvement of insulin resistance and metabolic abnormalities.”

3. In the Background section, page 3, line 53, "...in limited" should be changed to "...is limited".

Reply: Thank you for carefully and patiently reviewing our manuscript. The relevant word had been corrected in the revised manuscript (page, line 55).

Replies to the comments of Reviewer 2 (Marika Menduni, M.D.)

1. I suggest to specify a few steps:

[1] - the data in the diabetic population: the diabetes could increase the incidence of NALFD not only because of the insulin-resistance or the obesity but also because of the numbers of years of the disease, the glycemic control and the drugs used;
Reply: Thank you for your constructive suggestion. We entirely agree with you that diabetes can increase the NALFD risk through insulin-resistance, obesity, the diabetes duration, and the glycemic control. Accordingly, we analyzed cholecystectomy-associated NALFD risk according to diabetes status. The corresponding results were described in the Results section of our revised manuscript (page 10, line 206-210, and Figure 3).

2. the numbers of years elapsed from the cholecystectomy to the ultrasonography and if there is a time in which the incidence of NAFLD changes drastically or it increases slower: if it's possible that the cholecystectomy exposes the patient to a change in lipid metabolism and bile acids secretion, it's probable these modifications could affect the structure of the liver in a few years.

Reply: Thank you for your insightful suggestion. One study using the same data have reported that the odds of NAFLD did not differ based on time since cholecystectomy [Am J Gastroenterol. 2013 Jun;108(6):952-8]. There are two longitudinal studies assessed the risk of liver steatosis after cholecystectomy [World J Surg, 2016. 40(6): p. 1412-21; Lipids Health Dis, 2017. 16(1): p. 129]. One study reported that hepatic steatosis developed 3 months after cholecystectomy [World J Surg, 2016. 40(6): p. 1412-21.]. Another study noted that hepatic fat content was significantly increased 24 months after cholecystectomy [Lipids Health Dis, 2017. 16(1): p. 129.]. Hence, changes in lipid metabolism and bile acids secretion after cholecystectomy can affect the structure of the liver. However, the exact time it takes to develop NAFLD remains to be determined. We elaborated the relevant information in the Discussion section of the revised manuscript (page 11-12, line 230-257).

Replies to the comments of Reviewer 3 (Roger Gutiérrez-Juárez, M.D., Ph.D.)

Main issues:

1. To assess the extent by which central obesity contributes to NAFLD the authors use only waist circumference (WC) determinations.

Is that enough to state that the "magnitude of the association of NAFLD with cholecystectomy was similar to that with central obesity" (p. 13, lines 274-275)?

What else would need to be done to further strengthen this conclusion?

Reply: Thank you for your insightful suggestion. We entirely agree with you that WC cannot sufficiently discriminate between visceral and subcutaneous fat. However, the magnetic resonance imaging (MRI) and computed tomography (CT), which constitute the gold standard...
for quantitative evaluation of visceral adiposity, were not conducted in NHANES III. Hence, we
can not assess the association of MRI- or CT-measured visceral adiposity with NAFLD risk.

To further strengthen the conclusion, we did additional statistical analysis. We analyzed the
relationship between WC/HC (hip circumference), another surrogate marker of central adiposity,
and NAFLD risk. We found that cholecystectomy had an effect on the risk of NAFLD similar to
central obesity assessed by WC/hip circumference (HC). For example, participants with only
WC/HC defined centrally obese participants were at 1.94-fold risk for NAFLD, and participants
with only cholecystectomy were at 2.31-fold risk of NAFLD (Supplementary Figure 1).

The relevant results were described in the Results section of the revised manuscript (page 9, line
191-198).

2. On page 10, lines 208-210, the authors state that "... the effects of gallstones on NAFLD were
mediated primarily by the presence of central obesity, IR or MetS."

Can the authors elaborate on how exactly they propose that gallstone effects would be mediated
by central obesity, IR or MetS?

Reply: Thank you for carefully and patiently reviewing our manuscript. In the current study, we
found that gallstones associated with an increased NAFLD risk only in the presence of central
obesity, IR or MetS. However, the association did not exist in those without central obesity, IR
or MetS. Previous reports also reported that gallstones were unrelated to NAFLD after correction
for central obesity or metabolic risk factors []. Hence, we propose that the effect of gallstones on
NAFLD may be mediated by central obesity, IR or MetS. Actually, the relevant information was
addressed in the Discussion section of the manuscript (page 11, line 220-226).

3. Besides being more aware of the effect of cholecystectomy on the development of NAFLD,
is there something that the authors would suggest regarding the treatment of gallstones,
especially in people with other risk factors?

Reply: Thank you for your valuable comments. According to the guideline, people with
asymptomatic gallbladder stones found in a normal gallbladder and normal biliary tree do not
need treatment unless they develop symptoms. Symptomatic gallbladder stones and common bile
duct stone whether symptomatic or asymptomatic should be offered cholecystectomy [Bmj,
2014. 349: p. g6241.]. Hence, for patients with gallstones together with other risk factors such as
obesity, hyperlipidemia, insulin resistance, and T2DM, which are predisposed to confer an
increased NAFLD risk, comprehensive treatment including lifestyle interventions, BP, and
glucose control should be take into consideration to keep body weight, BP and glucose in
optimal levels and thus to avoid the occurrence of a symptomatic gallbladder. The relevant
information was addressed in the Discussion section of the revised manuscript (page 14, line 292-299).

4. Do the authors think that a refinement in the detection of NAFLD (i.e., people with less than 30% fat infiltration of the liver) has the potential to influence their conclusions?

Reply: Thank you for your critical comment. The magnetic resonance imaging (MRI) and computed tomography (CT) are more sensitive than ultrasonography in detecting NAFLD. If MRI or CT was conducted in the survey, more NAFLD would be detected and the association between cholecystectomy and NAFLD may be more robust. However, MRI and CT were not conducted in the exam, we have no way to prove this.

Minor issues:

1. There is a number of English language issues throughout the manuscript that need to be corrected by someone familiar with the language.

Reply: Thank you for carefully and patiently reviewing our manuscript. We completely realized that there were many grammar, syntax, punctuation errors, and misleading statements in our manuscript. We have already edited the English in the revised manuscript.