Author's response to reviews

Title: Low fat intake is associated with pathological manifestations and poor recovery in patients with hepatocellular carcinoma

Authors:

Kazuki Yamada (kaduki-y@med.niigata-u.ac.jp)
Takeshi Takeshi Suda (suda@med.niigata-u.ac.jp)
Yuko S Komoro (cherry@adm.niigata-u.ac.jp)
Tomoyuki Kubota (t-kubota@med.niigata-u.ac.jp)
Toshiko Murayama (murato4@adm.niigata-u.ac.jp)
Hideaki Nakayama (hidenaka@med.niigata-u.ac.jp)
Yutaka Aoyagi (aoy@med.niigata-u.ac.jp)

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Author's response to reviews:

Dear Dr. Hiromichi Kumagai,

Thank you for reviewing our manuscript, MS: 2126761805878423, entitled "Low fat intake is associated with pathological manifestations and poor recovery in patients with hepatocellular carcinoma". We would like to express our profound thanks and appreciation to you and each Reviewer for your excellent comments and suggestions. In accord with the Reviewers' comments and suggestions, the manuscript has been revised. A point-by-point response to the comments and suggestions has been prepared and follows this cover letter. The corrections in the revised version were indicated in red in the text and figures. In order to increase the number of cases, we included one more author. We are very grateful if the revised version is judged to be suitable for publication in your prestigious journal, Nutrition Journal, now.

Sincerely,

Takeshi Suda
Associate Professor
Niigata University Graduate School of Medical & Dental Sciences
Division of Gastroenterology & Hepatology
suda@med.niigata-u.ac.jp

Reply to the Reviewers and Editor:
Reviewer #1:
R1-Q1: Only 23 HCC patients were studied. Since HCC is a clinically heterogeneous disease entity, the relatively small sample size greatly limits the power of statistical analysis.
R1-A1: Because many reviewers raised the limitation of this study due to the small number of subjects, we would like to reply all together. Please see our reply at the bottom of this section.

R1-Q2: Case no 1 and 2 (Table 1) did not receive any treatment. On what basis did the authors measure the nutritional changes?

R1-A2: In this study, all consecutive cases, which were planned to receive active treatments for HCC in admission, were enrolled before admission. Several cases, however, were judged to be out of indication for active treatments after admission due to various reasons such as poor performance status and/or residual liver function. Cases 1 and 2 are the cases that fell into this category. While these cases are not suitable for the evaluation of a recovery speed, still they are valid for the evaluation of nutritional state in association with clinicopathological features such as npRQ or MHE. In order to make our enrollment criteria clear, “consecutive” was inserted in the revised version (P5, L17).

Reviewer #2:

R2-Q1: Based on the number of patients, results about patients hospitalized for HCC treatments frequently suffered from protein-energy malnutrition is relatively convincing. However, results about minimal hepatic encephalopathy is associated with energy malnutrition is shaky due to a very small number of patients with MHE. Thus, the main conclusion that poor energy intake from fat is associated with MHE is problematic and needs to be further confirmed by including more cases.

R2-A1: Because many reviewers raised the limitation of this study due to the small number of subjects, we would like to reply all together. Please see our reply at the bottom of this section.

R2-Q2: The conclusion that deterioration of nmRQ over hospitalization is associated with poor recovery from invasive therapies for HCC is also questionable. To establish this conclusion, subjects with deterioration of nmRQ need to be compared with those without deterioration of nmRQ. The contributions from other factors such as age, sex, and severity of HCC to the poor recovery after hospitalization should be thoroughly investigated.

R2-A2: Thank you for the reviewer’s productive comments. As the cases were increased to 20 for the analysis in association with the recovery, npRQ was deteriorated or improved in 5 and 15 cases, respectively, after admission. In the result, Unpaired-t test revealed a significant longer hospital stay in the cases with deteriorated npRQ after admission (175 ± 76 days vs 40 ± 59 days in cases with improved npRQ, p=0.0006). In order to clarify the impact of other clinicopathological factors on the recovery speed, a multiple linear regression analysis was performed by employing the recovery speed as a dependent variable. Independent variables consists of age, gender, background liver diseases, TNM stage, Child-Pugh score, body mass index, BCAA supplementation, and npRQ difference between day 1 and day 4. In
the results, the npRQ difference was selected as an only significant explanatory
(p=0.001). These results were incorporated in the revised manuscript.

Reviewer #3:

R3-Q1: The authors state that “energy from fat intake was positively correlated
with npRQ” in their “Results” section but this is counter to known data that the
contribution of different sources of energy (fat, carbohydrate and protein) is
determined by npRQ. So it is surprising that the authors found a positive and
direct correlation between npRQ and fat energy because as the fat energy
increases, one would expect the npRQ will decrease.

R3-A1: As the reviewer indicated, at first we also expected that fat intake would
lead to npRQ reduction by inducing fat metabolism. The observation was,
however, opposite, and we learned that NEFA can enhance gluconeogenesis
roughly through two actions of energy source and insulin modulator as discussed
in the last paragraph of the discussion section in detail. Noteworthy, almost all
cases in our cohort depended on fat within general nutritional allowance, less
than 30% of total energy intake, and many were restricted in sugar and protein
usage. We believe that under these circumstances appropriate amount of fat is
beneficial for patients by inducing gluconeogenesis.

R3-Q2: The authors in their “Methods” section page 8 that the REE or BEE was
multiplied by stress and activity coefficients (1.1 and 1.3) but they do not provide
any data on how these were decided. Was this based on an activity score or
stress level? How was stress determined?

R3-A2: We defined stress and activity factors based on the general
recommendation when BEE is calculated based on Harris-Benedict formula. For
activity factor, the recommendation is 1.0, 1.2, and 1.4 - 1.8 for bed rest, walking,
and activity at various level, respectively. In our hospital, all patients are
encouraged to walk inside the hospital after admission to keep them physically
active and sometime need to take medical examination that requires physical
activity at some extent such as respiratory function test and neuropsychiatric test.
Therefore, we selected 1.3 for their activity factor. In terms of stress factor, 1.2 is
generally selected for patient with cancer. All cases in our cohort, however,
admited for invasive treatments, which mean their physical and psychological
states are relatively well tolerated. In comparison with 1.0 for surgical intervention
of inguinal herniation and appendectomy, we feel 1.2 is too much for our cohort.
Thus, we chose 1.1 as their stress factor. The selection reason was described in
the revised version (P8, L6).

R3-Q3: They use a single criterion for recovery based on the INR. However, after
interventions for HCC, there is likely to be some hepatocellular decompensation
and this may affect the INR rather than the nutritional status. This needs to be
discussed and the data interpreted with this limitation explicitly stated. Did the
authors consider any other recovery pattern outcome measure e.g.
Hospitalization, decompensation etc since it is recognized that complications of
cirrhosis are more frequent and severe in malnourished than well nourished
subjects?
R3-A3: First of all, we measured patient’s recovery based on hospital stay, not on PT-INR. We employed PT-INR to standardize the invasiveness of each treatment. Patient’s recovery from invasive treatments is generally evaluated on the basis of functional liver reserve, which includes protein synthesis and detoxification capabilities. PT-INR is one of the most common indicators for protein synthesis, while NH3 and bilirubin are usually measured to assess residual detoxification function. The decompensated state of the liver is expression of functional insufficiency of protein synthesis or detoxification or both. Furthermore, nutritional/energy state is the reflection of residual liver function as long as sufficient nutrients are provided via any route of enteral or parenteral. Thus, nutritional state and decompensation are two sides of the same coin in the patients with chronic liver diseases.

We completely agree with that the evaluation using a single criterion may draw incorrect conclusion. Actually, we tried to standardize therapeutic invasiveness using serum concentrations of NH3 or total bilirubin. These indicators were, however, not good enough for our purpose. The serum concentration of NH3 is affected by NH3 source especially from gut, and the concentration easily elevated under constipation or other extrahepatic conditions without any treatment. The serum concentration of total bilirubin is a good indicator for liver detoxification function, but it has a nature to alter at the very end stage of liver diseases. Rather, it sensitively increases in many cases according to constitutional jaundice with indirect bilirubin dominancy under various stresses. In this regard, so far we could not find a better indicator for therapeutic invasiveness than PT-INR. The limitation of our study using a single criterion is added in the revised version (P17, L11).

R3-Q4: The authors have used BIA as a single measurement tool for quantifying body composition but its validity in cirrhosis especially those with fluid overload may have limitations. Even though a number of authors have suggested that BIA is reliable in cirrhosis, were any other measures like skin fold thickness, arm area, CT measures of muscle and fat, or DEXA used to determine the functional impact of energy malnutrition at least at baseline?

R3-A4: We completely agree with reviewer’s comment that BIA is unreliable when patients are edematous especially when they are suffering from ascites. Because most cases enrolled in this study had relatively good condition without edema or ascites in facing active treatment for HCC, we believed that BIA is enough reliable for these cases. Now, we are planning a prospective study to see if nutritional intervention primarily supporting fat intake is beneficial for the cases with HCC after treatment. According to the reviewer’s comment, we would like to involve several quantification methods for body composition in addition to BIA.

R3-Q5: The authors interpret their npRQ to show that patients had protein and energy malnutrition. Using only a small number of control subjects to define cut offs, define energy needs based on prediction equations is fraught with limitations and these need to be acknowledged.

R3-A5: We used nitrogen balance instead of npRQ to evaluate protein malnutrition, and the cutoff is clear in this case, positive or negative. In terms of
the cutoff value for energy malnutrition in npRQ, we referenced Tajika’s report, in which 109 cirrhotic cases and 22 healthy control subjects were enrolled to see the effect of npRQ on survival over 8 years. They reported that cases with npRQ less than 0.85 showed poor survival. The report was included in the reference in the revised version (P8, L10). According to this additional reference, the reference numbers of following reference were changed in the revised version. Because many reviewers raised the limitation of this study due to the small number of subjects, we would like to reply all together. Please see our reply at the bottom of this section.

R3-Q6: The tables with the clinical details are very unwieldy, it could be summarized and summary data provided so that the 2 tables can perhaps be summarized into one.
R3-A6: Tables 1 and 2 were combined to a new Table 1, and only summarized data were presented. According to an additional multivariate analysis, a new Table 2 was inserted in the revised version.

R3-Q7: The authors also have 2 distinct measures that include response to therapy and minimal HE. The relation between these 2 with HCC is also not clear. The rationale for this needs to be clarified. The authors state that minimal HE alters food intake but is there evidence that it specifically alters fat intake or metabolism and if so what is the mechanism for this?
R3-A7: We explored two distinctive measures in this study; a recovery speed from invasive treatment for HCC and minimal hepatic encephalopathy (MHE). The recovery is a primary concern, when we applied invasive treatments, and it is not difficult to assume that nutritional/energy state has significant impact on the recovery, as nutritional intervention on cirrhotic patients significantly improved their survival. In general, hepatic encephalopathy, at least overt one, is observed in the cases with poor functional hepatic reserve, which frequently show protein/energy malnutrition. On the other hand, it is under debate if MHE is manifested due to the same mechanism with overt one. Thus, it is important to know if there is any association between nutritional/energy state and MHE. In terms of the mechanism that poor energy state is likely to associate with MHE, we discussed about ketone body in the discussion section, because NEFA links to the generation of ketone body, which in turn becomes a major energy source of brain.

R3-Q8: The selection bias in this is also not taken into consideration. How were these patients chosen? Were they consecutive patients or was there a reason for selecting these subjects?
R3-A8: All cases were consecutive patients, who admitted to our hospital for the interventional treatment against HCC. In order to make this point clear, “consecutive” was inserted in the revised version (P5, L17).

Reviewer #4:

R4-Q1: The major problem with this study is that it is entirely a small clinical trial. There is no way to know whether factors other than the low fat intake actually
played a role in the results, because all patients could administered branched-chain amino acids. Authors should be discussed about the relationship between BCAA and low fat intake. Is only BCAA enough to control HCC patients with chronic liver diseases? Or are you thinking that low fat diet should be necessary?

R4-A1: Because many reviewers raised the limitation of this study due to the small number of subjects, we would like to reply all together. Please see our reply at the bottom of this section.

In this study, on the other hand, no nutritional intervention including low fat diet or BCAA supplementation was conducted.

R4-Q2: Also why and what kind of low fat diet supplementation did you use in this study?

R4-A2: Again, low fat diet was not provided to any case in this study.

R4-Q3: Authors should be described about the mechanisms of improving patients status. Recently several reports were accepted about L-carnitine supplementation to diet as a new tool in treatment of chronic liver diseases. It might be co-related this study. So authors could be discussed about this new comments.

R4-A3: Because many observational cohort studies and prospective nutritional intervention have already indicated that poor energy state/intake diminishes survival of cirrhotic patients, it is not difficult to assume that a treatment under a favorable nutritional state leads to a successful recovery. The possible mechanism that explains a connection between fat intake and npRQ increment is discussed in the last paragraph of the discussion section. In terms of L-carnitine, it would be over statement for us to discuss in this report, because we did not measure or administer L-carnitine at all in our cohort.

R4-Q4: Table 1 was very busy. Also Table 2 was busy.

R4-A4: Tables 1 and 2 were combined to a new Table 1, and only summarized data were presented. According to an additional multivariate analysis, a new Table 2 was inserted in the revised version.

Reviewer #5:

R5-Q1: As it is well recognized that elderly people tend to take less energy from fat, it is necessary to compare the fat intake of patients with liver cirrhosis (subjects) and the control group without liver disease.

R5-A1: We appreciate the suggestive comment. In terms of energy intake in general population, we employed 2010 national surveillance data from Japan. Japanese general population with age of 70 or older took 22.9 ± 10.5% of energy from fat (n=562), and it was not significantly different from 24.0 ± 4.6% in our MHE-negative patients with the same age profile (p=0.69). The results were inserted in the revised version (P11, L18).

R5-Q2: The number of patients is limited and it would be better to increase the participant number.
R5-A2: Because many reviewers raised the limitation of this study due to the small number of subjects, we would like to reply all together. Please see our reply at the bottom of this section.

R5-Q3: Although the authors mentioned that the patients with minimal hepatic encephalopathy tended to take less energy from fat, the number of subjects is too small (4 patients). Thus it is difficult to conclude that this tendency is due to minimal hepatic encephalopathy and not aging. The author should increase the number of subjects.

R5-A3: Because many reviewers raised the limitation of this study due to the small number of subjects, we would like to reply all together. Please see our reply at the bottom of this section.

R5-Q4: The volume of discussion is quite long, it would be better to be more succinct.

R5-A4: According to the reviewer’s comment, the third paragraph of the discussion section was substantially shorten in the revised version.

R1-Q1: Only 23 HCC patients were studied. Since HCC is a clinically heterogeneous disease entity, the relatively small sample size greatly limits the power of statistical analysis.

R2-Q1: Based on the number of patients, results about patients hospitalized for HCC treatments frequently suffered from protein-energy malnutrition is relatively convincing. However, results about minimal hepatic encephalopathy is associated with energy malnutrition is shaky due to a very small number of patients with MHE. Thus, the main conclusion that poor energy intake from fat is associated with MHE is problematic and needs to be further confirmed by including more cases.

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R*-A1: After submitting the first version, we were trying to increase the number of cases, and in the revised version, 35 HCC cases were analyzed for energy intake, npRQ, nitrogen balance, hepatic encephalopathy, and so on, leading to 5 cases with MHE positive cases. In terms of the recovery from invasive treatments, 20 cases were enrolled in the revised version instead of 14 cases in the original one. According to the increase of the cases, several statistical results were changed from tendency to significant and vice versa. In general, however, the conclusions were basically the same between the first and revised versions. All figures have been replaced with new data especially for the figure 1B, which was exchanged with the figure that is completely different type from the original version. Although we are still continuing the study to confirm the results, the
number of cases that can be analyzed at this moment is all included in the revised version. We believe that the fact that similar results were obtained after including the additional cases further enhances the rationale of our implication.