PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cross-sectional association between prolactin levels and nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: a retrospective analysis of patients from a single hospital in China
AUTHORS	Yuanyuan, Zhang; Liu, Huaizhen

VERSION 1 – REVIEW

REVIEWER	Jean-Paul Kovalik Duke-NUS Medical School, CVMD
REVIEW RETURNED	11-Apr-2022

GENERAL COMMENTS	This manuscript examines the relationship between serum prolactin levels and the presence of non-alcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes. This study is retrospective and uses data gathered from patients at a single center. Inclusion and exclusion criteria are appropriate and sufficient numbers of subjects are used in the study. The prevalence of NAFLD is ~60% in both men and women. As expected, subjects with NAFLD has more components of metabolic disease compared to subjects with no NAFLD. Prolactin levels were lower in patients with NAFLD. Prolactin levels significantly correlated with multiple markers of metabolic disease. Regressions analysis showed that prolactin levels were negatively associated with NAFLD after correction for the usual markers of metabolic disease. The discussion reviews prior work on prolactin and human metabolic disease and highlights some potential mechanisms for this relationship based on pre-clinical studies. Finally, limitations of a retrospective analysis in trying to determine causality are mentioned. I have no major comments for this manuscript. I found it easy to read and understand the findings from this article. As a native English speaker I noticed some places in the manuscript where grammar and diction seemed a bit off.

REVIEWER	Shaminie Athinarayanan Virta Health, Research
REVIEW RETURNED	24-Apr-2022

GENERAL COMMENTS	This manuscript needs extensive grammar, sentence structure and English language editing. It was difficult to read and follow the paper since it was poorly written and requires extensive grammatical improvement Abstract Line 11-12 Objective: remove the word "probe" and change it to "assess"

	Line 20-22: negatively correlated- negative coefficient and what does the author mean by "inversely related"- positively correlated Introduction: Need grammatical and sentence structure correction Line 32 -35: The sentence is too long and confusing, simplify the sentence Line 39-40: " T2D can boost"; I would rephrase the whole sentence: "T2D is an important risk factor associated in the progression of NAFLD to NASH, fibrosis" Line 41-45: Very hard to read, sentence structure and grammar Line 46: hormone is a protein, remove "protein" Line 5: What does the author mean by studies at home and abroad? Line 57-63: Rephrase, clearly list the hypothesis and the goal of research. This sentence is not clear. Is there any other studies associating PRL to NAFLD, NASH or Met S-please list them to justify your hypothesis and research goal Materials and methods: Needs extensive grammar and sentence structure correction Line 78: what is the cut-off for "too much drink", alcohol? Line 83: :were included in this study" Line 84: What the author mean by "General clinical message and laboratory test targets" Line 100-101: PRL were measured on different days using fasting samples. How many fasting samples collected for each patient? Which of the two values selected? And how was the two values determined and selected? This requires some analytical explanation on how they determine which samples to use to calculate the average PRL Line 105-111: Lot of grammar and sentence structure issue, spelling as well Line 112-128: Sentence structure and grammar Statistical analysis: grammar and sentence structure Line 139: Counting data or is it categorical variable? Results: Need extensive grammar and sentence structure correction - hard to read In Table 1: please indicate which of the variables normally versus not normally distributed Discussion Please correct sentence structure and grammar The discussion is all over the place, please focus on the main findings and discuss as appropriately Line 224-225: rephrase Line 234-235: rephrase The manuscript would benefit getting help with English language editing.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Comments to the Author:

This manuscript examines the relationship between serum prolactin levels and the presence of non-alcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes. This study is retrospective and uses data gathered from patients at a single center. Inclusion and exclusion criteria are appropriate and sufficient numbers of subjects are used in the study. The prevalence of NAFLD is ~60% in both men and women. As expected, subjects with NAFLD has more components of metabolic disease compared to subjects with no NAFLD. Prolactin levels were lower in patients with NAFLD. Prolactin levels significantly correlated with multiple markers of metabolic disease. Regressions analysis showed that prolactin levels were negatively associated with NAFLD after correction for the usual markers of metabolic disease. The discussion reviews prior work on prolactin and human metabolic disease and highlights some potential mechanisms for this relationship based on pre-clinical studies. Finally, limitations of a retrospective analysis in trying to determine causality are mentioned.

I have no major comments for this manuscript.

I found it easy to read and understand the findings from this article. As a native English speaker I noticed some places in the manuscript where grammar and diction seemed a bit off.

Answer: Thank you very much for your comments on our manuscript. We have asked a colleague whose native language is English to help improve the English quality of the manuscript. See the main text for details.

Reviewer: 2

Comments to the Author:

This manuscript needs extensive grammar, sentence structure and English language editing. It was difficult to read and follow the paper since it was poorly written and requires extensive grammatical improvement

Answer: I'm sorry, because of our poor English writing ability, it is difficult for you to understand and read the manuscript. We have asked a colleague whose native language is English to help improve the English quality of the manuscript. See the main text for details.

1. Abstract

1.1 Line 11-12 Objective: remove the word "probe" and change it to "assess"

Answer: In line 12 of the text, we have removed the word "probe" and changed it to "assess".

1.2 Line 20-22: negatively correlated- negative coefficient and what does the author mean by "inversely related"- positively correlated

Answer: We used Spearman correlation analysis to discuss the relationship between PRL levels and metabolic syndrome related parameters, and listed the correlation coefficient r, r> 0 was positive correlation, r< 0 was negative correlation. In line nos. 25 to 32 of the text, we modified it as follows: In male subjects, the levels of PRL were negatively correlated with hip circumference, homeostasis model assessment for insulin resistance (C-peptide) and triglyceeide (TG), and inversely correlated with high density lipoprotein (HDL) (r=-0.141, p=0.032, r=-0.141, p=0.032, r=-0.252, p=0.000, r=0.147, p=0.025, respectively). In female subjects, PRL levels were negatively related with body mass index, diastolic blood pressure, waist circumference, hip circumference and TG (r=-0.192, p=0.011, r=-0.220, p=0.003, r=-0.152, p=0.044, r=-0.157, p=0.037, r=-0.258, p=0.001, respectively).

2. Introduction: Need grammatical and sentence structure correction

2.1 Line 32 -35: The sentence is too long and confusing, simplify the sentence

Answer: In line nos. 55 to 58 of the text, we have simplified and modified the sentences as follows: The liver is an important organ of glycolipid metabolism in the body. When triglyceride deposition in hepatocytes increases and exceeds 5% and other factors causing liver steatosis (such as alcohol consumption and viral hepatitis) are excluded, NAFLD can be diagnosed.

2.2 Line 39-40: " T2D can boost"; I would rephrase the whole sentence: "T2D is an important risk factor associated in the progression of NAFLD to NASH, fibrosis"

Answer: In line nos. 62 to 63 of the text, we have changed " T2D can boost" to "T2DM is an important factor associated with the progression of NAFLD to NASH and fibrosis".

2.3 Line 41-45: Very hard to read, sentence structure and grammar

Answer: In line nos. 64 to 68 of the text, we have changed this paragraph to "NAFLD is closely related to central obesity, hypertension, hyperlipidaemia, T2DM and metabolic syndrome (MetS)[5]. Among

MetS-related diseases, only NAFLD is considered a strong predictor of MetS, and the incidence of MetS in fatty liver patients is more than 4 times that in nonfatty liver patients[6]. Therefore, NAFLD is considered the expression of MetS in the liver".

2.4 Line 46: hormone is a protein, remove "protein"

Answer:In line 69 of the text, We have remove "protein".

Line 5: What does the author mean by studies at home and abroad?

Answer: In line nos. 75 to 77 of the text, we have revised it as follows:Studies in China and abroad have found that the decrease in serum PRL at the physiological level is closely related to the occurrence of T2DM.

Line 57-63: Rephrase, clearly list the hypothesis and the goal of research. This sentence is not clear. Is there any other studies associating PRL to NAFLD, NASH or Met S-please list them to justify your hypothesis and research goal

Answer: In line nos. 79 to 86 of the text, we have revised it as follows: The researchers further pointed out that the decrease in physiological levels of PRL was related to an increased risk of T2DM[9]. Manshaei et al[12] also found that the serum PRL concentration of patients with T2DM was lower than that of healthy people. Because of the high incidence of NAFLD in patients with T2DM, T2DM is also an important factor in MetS. The relationship among PRL, NAFLD and MetS at the physiological level has not been explored. The goal of this research was to explore the relationship among PRL, NAFLD and MetS in patients with T2DM.

3. Materials and methods: Needs extensive grammar and sentence structure correction

3.1 Line 78: what is the cut-off for "too much drink", alcohol?

Answer: In line 96 of the text, too much drink means that intake of alcohol exceeding 140 g/week for men and 70 g/week for women

3.2 Line 83: were included in this study"

Answer: In line 101 of the text, we have changed the “were taken into the research” to “were included in this study”.

3.3 Line 84: What the author mean by "General clinical message and laboratory test targets"

Answer: In line 105 of the text, we have changed the "General clinical message and laboratory test targets" to "Data collection",which included basic information of patients and biological sample data.

3.4 Line 100-101: PRL were measured on different days using fasting samples. How many fasting samples collected for each patient? Which of the two values selected? And how was the two values determined and selected? This requires some analytical explanation on how they determine which samples to use to calculate the average PRL.

Answer: In line nos. 120 to 125 of the text, we have revised this paragraph as follows:

PRL levels are affected by many conditions including the use of various drugs, stress, and exercise, so we took blood samples at 9: 00 am after the patients were admitted to the hospital on the first day and the next morning. We took 2 ml blood samples each time. The patients fasted and rested in a sitting position for 30 minutes, and then the average value of two blood pressure readings was taken

3.5 Line 105-111: Lot of grammar and sentence structure issue, spelling as well

Answer: In line nos. 133 to 139 of the text, we have revised this paragraph as follows: NAFLD was diagnosed by ultrasound[13] by a senior technician. The ultrasonic diagnosis of fatty liver is as follows: near-field of liver permeate punctiform hyperecho, composition of the intrahepatic duct not clearly demonstrated by ultrasonography, and weak echo in the distal echo. Diagnosis of NAFLD is based on the following requirements: no history of alcohol consumption, no other types of liver diseases, and unexplained increase in serum alanine aminotransferase (ALT), aspartic acid aminotransferase (AST) or glutamyltransferase (GGT) over 6 months[14].

3.6 Line 112-128: Sentence structure and grammar

Answer: In line nos. 140 to 159 of the text, We have revised this paragraph as follows: The diagnosis of MetS conformed to the standard put forward in the ninth edition of internal medicine in China [15], and the diagnostic standard included the following three or more items: ① central obesity and/or abdominal obesity: waist circumference is greater than 90 cm for men and 85 cm for women; ② hyperglycaemia: FBG > 6.1 mmol/L or 2-hour blood glucose >7.8 mmol/L and/or confirmation of diabetes diagnosis and treatment with hypoglycaemic therapy; ③Hypertension: blood pressure

exceeding 130/85 mmHg and/or diagnosis of hypertension and treatment with antihypertensive therapy; ④ fasting triglyceride (TG) level exceeding 1.7 mmol/l; and ⑤ fasting high-density lipoprotein (HDL) Level below 1.04 mmol/l. Body mass index (BMI) was computed by dividing the body weight (kg) by the square of the height (m^2). homeostasis model assessment of insulin resistance (C-peptide) (HOMA-IR (CP)) was determined by FCP as a substitute for fasting insulin as follows: HOMA-IR (CP) =1.5+FBG (mmol/L)xFCP (pmol/L)/2800. HOMA-β (CP-DM) =0.27x FCP (pmol/L) (FBG (mmol/L) -3.5)[16].

In conformity with ultrasonic diagnosis, patients with T2DM who met the inclusion criteria were segmented into the without NAFLD group (77 men, 66 women) and the with NAFLD group (153 men, 110 women).

Patient and public involvement

Patients and the public were not involved in the design, conduction, reporting, or dissemination plans of our research.

4. Statistical analysis: grammar and sentence structure

4.1 Line 139: Counting data or is it categorical variable?

Answer: In line 167, it is categorical variable.

5. Results: Need extensive grammar and sentence structure correction - hard to read

In Table 1: please indicate which of the variables normally versus not normally distributed

Answer: In line nos. 190 to 192 of the text, we have indicated in the note in Table 1 which variables are normally distributed variables: BMI, diastolic blood pressure, waist circumference, hip circumference, TC, LDL; nonnormally distributed variables: Age, diabetes course, systolic blood pressure, ALT, AST, GGT, FBG, TG, HDL, HOMA-IR (CP), HOMA-β (CP-DM), HbA1C, and PRL.

6. Discussion

Please correct sentence structure and grammar

The discussion is all over the place, please focus on the main findings and discuss as appropriately

6.1 Line 224-225: rephrase

Answer: In line nos. 257 to 258 of the text, we have revised this paragraph as follows:

PRL is a hormone closely related to metabolism[20]. Recent findings have shown that there is a close association between PRL and T2DM.

6.2 Line 234-235: rephrase

Answer:In line nos. 268 to 269 of the text, we have revised this paragraph as follows: Jha et al[23] also found that serum PRL had a significant correlation with liver disease and predicted mortality.

VERSION 2 – REVIEW

REVIEWER	Shaminie Athinarayanan Virta Health, Research
REVIEW RETURNED	10-Jun-2022

GENERAL COMMENTS	Abstract: Participants (153 men, 110 women)= total is 263, why did the author list n=406 patients with T2D/ Spelling triglyceeide to triglyceride Maybe list the r and p-value after the variable, easier to follow Grammar correction in the conclusion section of the abstract Introduction- nothing to change Results Line 91 - "due to the use of medications that affect PRL levels" Paragraph lines 140 to 150- change the structure Discussion- nothing to add

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Comments to the Author:

Abstract:

1. Participants (153 men, 110 women)= total is 263, why did the author list n=406 patients with T2DM

Answer: I'm very sorry, there is a mistake here. In line nos. 16 to 17 of the text, we have made the following changes: **Participants**: A total of 406 patients with T2DM (230 men and 176 women) were selected.

2. Spelling triglyceeide to triglyceride

Answer: In line 27 of the text, we have modified triglyceeide to triglyceride.

3. Maybe list the r and p-value after the variable, easier to follow

Answer: In line nos. 25 to 32 of the text, we have listed the r and p-value after the variable as follows: In male subjects, the levels of PRL were negatively correlated with hip circumference (r=-0.141, p=0.032), homeostasis model assessment for insulin resistance (C-peptide) (r=-0.141, p=0.032) and triglyceride (TG) (r=-0.252, p=0.000) values and inversely correlated with high-density lipoprotein (HDL) (r=0.147, p=0.025) levels. In female subjects, PRL levels were negatively related to body mass index (r=-0.192, p=0.011), diastolic blood pressure (r=-0.220, p=0.003), waist circumference (r=-0.152, p=0.044), hip circumference (r=-0.157, p=0.037) and TG (r=-0.258, p=0.001) values.

4. Grammar correction in the conclusion section of the abstract

Answer: In line nos. 37 to 39 of the text, we have corrected the grammar in the conclusion section of the abstract as follows: Our results supported that low levels of PRL in the physiological range were markers of NAFLD in T2DM patients and suggested that PRL within the biologically high range may play a protective role in the pathogenesis of NAFLD.

Results

1. Line 91 - "due to the use of medications that affect PRL levels"

Answer: In line nos. 91 to 92 of the text, we have corrected the sentence "due to the use of that affect PRL" to the sentence "due to the use of medications that affect PRL levels".

2. Paragraph lines 140 to 150- change the structure

Answer: Answer: In line nos. 141 to 155 of the text, we have changed the structure as follows: The diagnosis of MetS conformed to the standard put forward in the ninth edition of internal medicine in China [15], and the diagnostic standard included three or more of the following items: ① central obesity and/or abdominal obesity: a waist circumference greater than 90 cm for men and 85 cm for women; ② hyperglycaemia: an FBG level > 6.1 mmol/L or a 2-hour blood glucose level >7.8 mmol/L and/or the confirmation of a diabetes diagnosis and treatment with hypoglycaemic therapy; ③ hypertension: a blood pressure exceeding 130/85 mmHg and/or a diagnosis of hypertension and treatment with antihypertensive therapy; ④ a fasting triglyceride (TG) level exceeding 1.7 mmol/l; and ⑤ a fasting high-density lipoprotein (HDL) level below 1.04 mmol/l. Body mass index (BMI) was computed by dividing the body weight (kg) by the square of the height (m2). The homeostasis model assessment of insulin resistance (C-peptide) (HOMA-IR (CP)) value was determined by the FCP level as a substitute for the fasting insulin level as follows: HOMA-IR (CP) =1.5+FBG (mmol/L)xFCP (pmol/L)/2800. HOMA-β (CP-DM) =0.27x FCP (pmol/L) (FBG (mmol/L) -3.5)[16].