PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The association between genetically determined leptin and blood lipids considering alcohol consumption: a Mendelian randomization study
AUTHORS	Shen, Luqi; Cordero, José; Wang, Jia-Sheng; Shen, Ye; Li, Shengxu; Liang, Lirong; Zou, Zhiyong; Li, Changwei

VERSION 1 – REVIEW

REVIEWER	Harry Freitag Luglio Muhammad Department of Nutrition and Health, Faculty of Medicine, Universitas Gadjah Mada, Indonesia
REVIEW RETURNED	09-Feb-2019

GENERAL COMMENTS	In principle, this is an interesting study offers an interesting research questions which involved around the interaction between GRS and alcohol consumption on leptin and lipid profile. One of the advantage of this study was the big sample size. Leptin is multifaceted protein which is complicate to understand in a population setting, which make it interesting to study Introductions: However, I found the authors did not explicitly state the connection between variables that is being analyzed: Authors did not mention the influence of leptin on systemic inflammation. This is important because inflammation plays a crucial role in development of insulin resistance, hiperlipidemia and atherosclerosis. The main goal was to elucidate factors that might influence hyperlipidemia, to me it is unclear why author decided to incorporate data on alcohol consumption. It will be more beneficial if author also mention other dietary related factors that has a direct impact on lipid profile such as refined sugar, glycemic index, trans fatty acid, PUFA and saturated fat. Methods: Despite of include physical activity as confounding factor, authors did not consider energy intake as a potential confounding factor. Energy is important because it might that the effect of alcohol is rather because amount of energy consumed as alcohol rather than the alcohol itself. Alcohol consumption is different with alcohol drinking status. In order to have data on alcohol cunsumption, authors need data for dietary intake using 24 hours recall or semi quantitative food questionnaire. I am not sure if only by asking subjects drinking status (“current drinker” and “not a current drinker) Author can

	imply this as an alcohol consumption. In even in alcohol consumption, there is a range of consumption. Results: Changing dataset into log is not helpful for the readers. It will be more informative if author can also provide data on leptin and lipid profile. Discussion: It seems to me that authors would like to infer that alcohol consumption impair the beneficial effect of Leptin influencing GRS on lipid proifle. However, in the western societies, alcohol usually consumed as wine or beers. It has been establshed that wine contains resveratrol and phytochemical which is beneficial protection against dyslipidemia. Beers are also previously shown has phyochemical effect. Can you elaborate this in your discussion? Authors need to provide readers with the explanation why GRSs were positively correlated with leptin, leptin was positively correlated with hyperlipidemia but GRSs were negativey correlated with hyperlipidemia.

REVIEWER	Parvin Mirmiran Nutrition and Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
REVIEW RETURNED	13-Mar-2019

GENERAL COMMENTS	I read with interest the present manuscript aiming to evaluate the effect of genetically determined leptin on lipids profiles. The design of the study, and the analyses are in general adequate. However, there is one concern as follows: Considering to the introduction, it seems that the associations between serum leptin concentrations and blood lipids are not completely established in the previous study. Why did not you first evaluate the relationship between serum leptin concentrations and blood lipid profiles?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Harry Freitag Luglio Muhammad
Institution and Country: Department of Nutrition and Health, Faculty of Medicine, Universitas Gadjah Mada, Indonesia
Please state any competing interests or state ‘None declared’: None declared

In principle, this is an interesting study offers an interesting research questions which involved around the interaction between GRS and alcohol consumption on leptin and lipid profile. One of the advantage of this study was the big sample size. Leptin is multifaceted protein which is complicate to understand in a population setting, which make it interesting to study
Response: Thank you for the encouraging comments.

Introductions:

However, I found the authors did not explicitly state the connection between variables that is being analyzed:
Authors did not mention the influence of leptin on systemic inflammation. This is important because inflammation plays a crucial role in development of insulin resistance, hiperlipidemia and atherosclerosis.
Response: Thank you for the comments. We agree with the reviewer that leptin plays a pivotal role in systematic inflammation. We've now added the following paragraph in the manuscript summarizing the role of inflammation on the associations between leptin and insulin resistance and hyperlipidemia. Please refer to lines 5-10 on page 5.

"As an extremely active endocrine organ, the adipose tissue secretes leptin playing a key role in immunometabolism.10 Leptin can regulate both innate and adaptive immune responses.11 12 Meanwhile, leptin and insulin interact to establish a regulatory feedback loop, the adipoinsular axis.14 Leptin suppresses insulin synthesis and secretion from β-cells14 and improves insulin sensitivity15. In turn, insulin can stimulate leptin secretion from adipocytes16 17. Both the immune responses and insulin are involved in lipid metabolism.1318"

The main goal was to elucidate factors that might influence hyperlipidemia, to me it is unclear why author decided to incorporate data on alcohol consumption. It will be more beneficial if author also mention other dietary related factors that has a direct impact on lipid profile such as refined sugar, glycemic index, trans fatty acid, PUFA and saturated fat.
Response: Thank you for the comments and suggestions. The association between serum leptin and blood lipids can be influenced by many factors, including drinking and those factors listed by the reviewer. Our group dedicate to delineate the role of those factors in the genetically determined association between leptin and lipids. The current manuscript reports findings from one of a series analyses. We'll report findings for those factors in the future.

Methods:
Despite of include physical activity as confounding factor, authors did not consider energy intake as a potential confounding factor. Energy is important because it might that the effect of alcohol is rather because amount of energy consumed as alcohol rather than the alcohol itself.
Alcohol consumption is different with alcohol drinking status. In order to have data on alcohol cunsumption, authors need data for dietary intake using 24 hours recall or semi quantitative food questionnaire. I am not sure if only by asking subjects drinking status ("current drinker" and "not a current drinker) Author can imply this as an alcohol consumption. In even in alcohol consumption, there is a range of consumption.
Response: Thank you for the insightful comments. We did not control for total energy intake due to the fact that leptin can combine with receptors in the hypothalamus to reduce appetite and increase energy expenditure. Therefore, total energy intake is in the pathway from leptin to lipids and may not meet the criteria of being a confounder. More importantly, food frequency questionnaire survey was only conducted during examination cycle 2 in 2008-2011 for the third generation cohort, while leptin was measured at baseline in 2002-2005. Therefore, we could not adjust for it either. Drinking status was measured as a lifestyle behavior in the third generation cohort. We've now discussed it in the limitation section of the manuscript. Please refer to lines 21-23 on page 14 and lines 1-3 on page 15.

"Third, we did not control for total energy intake in our analyses because food frequency questionnaire survey was not conducted in the third generation cohort at baseline when leptin was measured. However, leptin combines with receptors in the hypothalamus to reduce appetite and increase energy expenditure. Therefore, total energy intake is in the pathway from leptin to lipids metabolism and may not meet the criteria of being a confounder."

Results:

Changing dataset into log is not helpful for the readers. It will be more informative if author can also provide data on leptin and lipid profile.
Response: Thank you for the comments and suggestions. We've now added levels of leptin and lipid profile in Table 1 of the manuscript. Since, we have to log transform leptin and triglycerides in the regression models so that the data distribution can meet the assumptions of models, we cannot provide beta coefficients in non-log transformed values.

Discussion:
It seems to me that authors would like to infer that alcohol consumption impair the beneficial effect of Leptin influencing GRS on lipid proifle. However, in the western societies, alcohol usually consumed as wine or beers. It has been establshed that wine contains resveratrol and phytochemical which is beneficial protection against dyslipidemia. Beers are also previously shown has phyochemical effect. Can you elaborate this in your discussion?
Response: Thank you for the insightful comments and suggestion. In the current study, we identified that alcohol consumption can impair the beneficial effect of leptin on lipid profile. We hypothesize that the underlying mechanism may be due to that alcohol consumption can influence leptin level and, therefore, impair the association between leptin GRS and lipid profile. Although resveratrol and phytochemical can benefit lipid profiles, they may not influence leptin, therefore, they cannot influence the association between leptin GRS and lipid profile. We've now added the following text in the discussion section of the manuscript. Please refer to lines 3-8 on page 15.

"Forth, the type of alcohol consumed was not measured and cannot be considered in the current analyses. It is possible that the alcohol consumed in the studied population is mainly wine and/or beers, which contain high levels of resveratrol and phytochemical. The two chemicals may benefit lipid metabolism.59 60 However, the two chemicals do not share similar genetic profile with leptin, and consequently, they should not be correlated with leptin GRS and cannot affect the associations between leptin GRS and blood lipids."

Authors need to provide readers with the explanation why GRSs were positively correlated with leptin, leptin was positively correlated with hyperlipidemia but GRSs were negativey correlated with hyperlipidemia.
Response: Thank you for the suggestion. Physiologically, leptin is involved in lipid and glucose metabolism. Individuals with congenital leptin deficiency exhibit early-onset, severe obesity, hyperlipidemia, and/or hyperinsulinemia1-7. Therefore, a negative correlation between leptin and lipids is expected. The observed positive correlation between leptin and lipids may be due to known and unknown confounding factors. This also reflects the advantage of using Mendelian randomization approach to pinpoint a causal relationship between an exposure and an outcome. We've now elaborated this in the discussion section. Please refer to lines 14-15 on page 13.

Responses to Reviewer #2's comments:
I read with interest the present manuscript aiming to evaluate the effect of genetically determined leptin on lipids profiles. The design of the study, and the analyses are in general adequate. However, there is one concern as follows:

Considering to the introduction, it seems that the associations between serum leptin concentrations and blood lipids are not completely established in the previous study. Why did not you first evaluate the relationship between serum leptin concentrations and blood lipid profiles?
Response: Thank you for the important comment. Serum leptin and lipids associations were not completely established in previous studies. This is also the reason of conducting a Mendelian randomization analyses to provide more robust evidence. Our study is the first Mendelian randomization study on this topic.

References:
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3. Paz-Filho G, Mastronardi CA, Licinio J. Leptin treatment: facts and expectations. Metabolism: clinical and experimental 2015;64(1):146-56. doi: 10.1016/j.metabol.2014.07.014
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6. Ebihara K, Kusakabe T, Hirata M, et al. Efficacy and safety of leptin-replacement therapy and possible mechanisms of leptin actions in patients with generalized lipodystrophy. The Journal of clinical endocrinology and metabolism 2007;92(2):532-41. doi: 10.1210/jc.2006-1546
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VERSION 2 – REVIEW

REVIEWER	Harry Freitag Luglio Muhammad Universitas Gadjah Mada, Indonesia
REVIEW RETURNED	21-Jun-2019

GENERAL COMMENTS	Authors need to explain why they analysed data on log. To some readers, this might lead to confusion. Especially considering practicality of the data that are being reported. When you look at table 1, leptin values in all quartiles were the same except for Q4. But what is that mean? It is unclear why adjustment for education is needed. Does it have biological meaning? The conclusion is too strong. I am not convinced by the conclusion that leptin reduced triglyceride because of the study design. This study is an observation study. Unless they be able to modulate leptin and measured TG, the conclusion will be around the association. It is important to keep in mind that the GRS was made based on leptin production, not leptin receptors/gene associated with leptin effect. Initially, authors stated that leptin has immunological properties. Which is true. But they did not explain it connection with leptin in respect of lipid regulation. Can Authors explain why they get high value of Beta for linear regression.

REVIEWER	Parvin Mirmiran Nutrition and Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
REVIEW RETURNED	16-Jun-2019

GENERAL COMMENTS	the association between nutrition and genetic is novel and this area can open a new and interesting insight, and this is the main reason that i was interested to your manuscript

VERSION 2 – AUTHOR RESPONSE

Responses to Reviewer #1's comments:

Reviewer: 1

Reviewer Name: Harry Freitag Luglio Muhammad

Institution and Country: Universitas Gadjah Mada, Indonesia

Please state any competing interests or state 'None declared': None declared

Authors need to explain why they analysed data on log. To some readers, this might lead to confusion. Especially considering practicality of the data that are being reported. When you look at table 1, leptin values in all quartiles were the same except for Q4. But what is that mean?

Response: Thank you for the comments. We've now added the following paragraph in the manuscript to explain why we analyze the leptin and triglycerides on log scale. Please refer to lines 9-10 and 15-16 on page 8.

"Leptin were logarithmically transformed in the current study so that the data distribution can meet the assumptions of linear regression models.

Triglycerides were logarithmically transformed (log-TG) in the current study so that the data distribution can meet the assumptions of linear regression models."

The log-leptin value in the quartiles were not exactly the same. We have changed the decimal of log-leptin from 1 to 2 in Table 1 to make it more clear. When the quartiles of the leptin GRS increase, the log-leptin level increases in parallel.

It is unclear why adjustment for education is needed. Does it have biological meaning?

Response: Thank you for the comments. Education level represents social economic status of the participants. It has been shown to be a strong predictor for a range of physiological measures such as blood lipids1 and leptin levels2. Therefore, adding education level in the model can help control for residual confounding.

The conclusion is too strong. I am not convinced by the conclusion that leptin reduced triglyceride because of the study design. This study is an observation study. Unless they be able to modulate leptin and measured TG, the conclusion will be around the association.

Response: Thank you for the suggestion. We've now revised the descriptions in the conclusion section according to this comment. Please refer to line 23 on page 3.

It is important to keep in mind that the GRS was made based on leptin production, not leptin receptors/gene associated with leptin effect.

Response: Thanks for your kind reminder. It would be more interesting to study genetically determined ratio of leptin to leptin receptor and blood lipids. However, we could not identify any genome-wide study on the ratio. Therefore, GRS for the ratio cannot be established. We've now added the following statement in the limitation section of the manuscript. Please refer to lines 14-18 on page 15.

"Genetically determined ratio of leptin to leptin receptor may be a better measure to study the role of leptin in lipid metabolism. However, we could not find a genome-wide study on the ratio of leptin to leptin receptor, therefore, a GRS on the ratio cannot be calculated. Future genome-wide studies on the ratio of leptin to leptin receptor are warranted."

Initially, authors stated that leptin has immunological properties. Which is true. But they did not explain it connection with leptin in respect of lipid regulation.

Response: Thank you for the comments. We've now added the following paragraph in the manuscript to further explain the interactive role of immune system and leptin in lipid metabolism. Please refer to lines 5-12 on page 5.

"As an active endocrine organ, the adipose tissue secretes leptin and plays a key role in immunometabolism.3 Leptin can regulate both innate and adaptive immune responses4 5 and subsequently regulate lipid profiles. Animal study demonstrated that hyperleptinemia decreases the expression of SREBP-1c, a master regulator of lipid metabolism, in liver and adenovirus-induced hyperleptinemia decreases triglyceride synthesis through SREBP-1c down-regulation.6 Meanwhile, SREBP-1c is involved in innate immune response in Macrophages.7 Therefore, it is rational to see immune connects with leptin in respect of lipid regulation.

Can Authors explain why they get high value of Beta for linear regression.

Response: Thanks for your question. The genetic risk score (GRS) ranged from 0 to 0.15, with a mean of 0.07 and a standard deviation of 0.03. The beta coefficient was change in lipids per 1 unit increase in the GRS.

Reviewer: 2

Reviewer Name: Parvin Mirmiran

Institution and Country: Nutrition and Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Please state any competing interests or state ‘None declared’: None declared

the association between nutrition and genetic is novel and this area can open a new and interesting insight, and this is the main reason that i was interested to your manuscript

Response: The authors thank the reviewer for the encouraging comments.

Reference

1. Lara M, Amigo H. Association between education and blood lipid levels as income increases over a decade: a cohort study. BMC Public Health 2018;18(1):286. doi: 10.1186/s12889-018-5185-3 [published Online First: 2018/02/27]

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VERSION 3 – REVIEW

REVIEWER	Harry Freitag Luglio Muhammad Universitas Gadjah Mada, Indonesia
REVIEW RETURNED	09-Aug-2019

GENERAL COMMENTS	The manuscript can go further as it is