RESEARCH ARTICLE

REVISED Glucose induced ApoB100/ApoAI ratio changes in cultured HepG2 cells *in vitro* [version 2; peer review: 2 not approved]

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Abstract

Background: Numerous *in vivo* human cohort studies have suggested that the apolipoprotein B100/apolipoprotein AI (ApoB100/ApoAI) ratio might be a risk factor in coronary heart disease. The aim of this study was to measure ApoB100/ApoAI ratio changes in cell secretions by incubating HepG2 cells with various amounts of glucose *in vitro*.

Methods : HepG2 cells were cultured in low-, normal- or high-glucose Dulbecco's Modified Eagle Medium (DMEM) (1, 4.5 and 10g/L, respectively). Levels of ApoAI and ApoB100 were measured with commercial sandwich enzyme-linked immunosorbent assay kits (cat#: H0123 and H0124) from ShangHai MEIXUAN Biological Science and Technology Ltd (Shanghai, China). Experiments were repeated six times for each assay.

Results: The results showed that ApoB100/ApoAI ratio have positive correlations with the glucose concentration increase.

Conclusions: A higher concentration of glucose induced an undesirable ApoB100/ApoAI ratio change, which suggests a new regulatory pathway in lipoprotein catabolism and provides a cell model for further mechanism study. This finding may lead to novel therapeutic ways for diagnosis and treatment for coronary artery disease.

Keywords

ApoB100/ApoAI ratio, ApoB100, ApoAI, Hepatic lipase, Glucose

Open Peer Review**Approval Status**

	1	2
version 2 (revision) 10 Jan 2022	 view	 view
version 1 23 Aug 2021	 view	 view

1. **Anne Tailleur** , University of Lille, Lille, France
2. **Li Lin**, Hubei University of Chinese Medicine, Wuhan, China

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Hu M: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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First published: 23 Aug 2021, 10:842 <https://doi.org/10.12688/f1000research.55215.1>

REVISED Amendments from Version 1

The paper was revised in response to the reviewers' comment. Since it is too early to publish the hepatic lipase (HL) data in this research, all the content related to HL was removed.

Any further responses from the reviewers can be found at the end of the article

Introduction

Atherosclerotic coronary artery occlusion is the most frequent cause of coronary heart disease (CHD); numerous epidemiologic studies and randomized clinical trials have established that lipoprotein metabolism is a major contributor to CHD.¹ Conventionally, it was thought that increases in plasma low-density lipoprotein cholesterol (LDL-C) and decreases in high-density lipoprotein cholesterol (HDL-C) were the major factors causing CHD.² However, numerous *in vivo* human cohort studies have suggested that another factor in CHD risk might be the apolipoprotein B100/apolipoprotein AI (ApoB100/ApoAI) ratio.^{3–7}

ApoB100 is a large surface protein present on low-density lipoprotein (LDL) and serves as a ligand for the LDL receptor, which facilitates its clearance from the plasma. Apolipoprotein AI (ApoAI) is the major protein constituent on high-density lipoprotein (HDL) and plays a central role in reverse cholesterol transport by stabilizing the HDL particle, interacting with the ATP-binding cassette transporter I, activating lecithin cholesterol acyl transferase and acting as a ligand for the hepatic scavenger receptor.²

In order to prove those *in vivo* human cohort findings that ApoB100/ApoAI ratio is a CHD risk factor and understand the molecular mechanism of how ApoB100/ApoAI ratios are modulated in atherosclerosis, an *in vitro* cell assay model was built in the current study. Extracellular expression of ApoB100 and ApoAI were measured in cultured HepG2 cells in response to different concentrations of glucose. This research may provide the targets of many novel therapeutics and is an area with great potential for the prevention and treatment of CHD.

It has been shown that a high-carbohydrate (high-CHO) diet can reduce the risk of CHD. Further analysis found that this high-CHO diet affects the ApoB100/ApoAI ratio instead of the HDL-C related ratios.^{6–8} In the current study, various amounts of glucose were used to stimulate the secretion of ApoB100 and ApoAI in cultured HepG2 cells. It was hypothesized that the different concentration of glucose could modulate ApoB100/ApoAI ratio *in vitro*.

Methods

Cell culture and treatment

HepG2 cells (American Type Culture Collection, ATCC, Rockville, MD, USA) were routinely cultured in DMEM + 10% FBS (contains 4.5 g/L glucose, normal glucose group) at 37°C in a 5% CO₂ incubator up to 70% confluency. For ApoB100 and ApoAI expression assays, cell culture media that contained different concentrations of glucose (1 and 10g/L) were then added to the cultured cells in order to make the low glucose group and high glucose group besides the original normal glucose group. All the cell groups then continued to be cultured for eight hours before the assays.

ApoB100 and ApoAI expression assays

The culture media of HepG2 cells were collected. ApoB100 and ApoAI expression were measured with commercial sandwich enzyme-linked immunosorbent assay (ELISA) kits (cat. no. H0124 and H0123) from ShangHai MEIXUAN Biological Science and Technology Ltd (Shanghai, China). The microplate provided in this kit had been pre-coated with a monoclonal antibody specific for ApoB100 or ApoAI. Standards or samples were then pipetted into the microplate wells, and ApoB100 or ApoAI present in the samples or standards binds to antibodies adsorbed to the microplate wells. To quantitatively determine the amount of ApoB100 or ApoAI present in the samples, the horseradish peroxidase (HRP)-conjugated polyclonal antibody specific for ApoB100 or ApoAI was added to each well. The microplate was incubated for one hour, and then the wells were thoroughly washed to remove any unbound components. The substrate solutions A and B were respectively added to each well. After the enzyme (HRP) and substrate reacting over a short period, this reaction was stopped by addition of a sulphuric acid solution and the color change is measured at a wavelength of 450 nm. The proportion to amount of ApoB100 or ApoAI bound in the initial step develops in the color change. Color intensities were measured using a MK3 microplate reader (Thermo Fisher, MA, USA).

Statistical analysis

Experimental results were expressed as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using one-way ANOVA to determine significant differences among groups. IBM SPSS Statistics version 22 was used for data analysis.

Table 1. Hepatic lipase activity, ApoB100, ApoAI and ApoB100/ApoAI ratio in HepG2 cell cultured in low, normal and high glucose concentration.

	Low glucose concentration (1 g/L)	Normal glucose concentration (4.5 g/L)	High glucose concentration (10 g/L)	F	P
ApoB100 (g/ml)	131.31 ± 10.39	146.77 ± 3.82**	167.02 ± 10.84***#	24.052	<0.001
ApoAI (μg/ml)	12.06 ± 0.47	10.31 ± 0.19**	8.18 ± 0.31***#	190.710	<0.001
ApoB100/ApoAI (×10 ⁶)	10.92 ± 1.15	14.24 ± 0.23**	20.49 ± 1.95***#	82.211	<0.001

**p < 0.05 for glucose concentration (4.5 g/L) vs. (1 g/L) or (10 g/L) vs. (1 g/L) (one-way ANOVA).

##p < 0.05 for glucose concentration (1 g/L) vs. (4.5 g/L) or (10 g/L) vs. (4.5 g/L) (one-way ANOVA).

Results

Glucose modulates secretion of ApoB100 and ApoAI in HepG2 cells

To explore the impacts of glucose on ApoB100 and ApoAI secretion over different concentrations, HepG2 cells were exposed to low (1 g/L), medium (4.5 g/L) and high (10 g/L) concentrations of glucose. As shown in Table 1, ELISA revealed that with the glucose concentration increase, ApoB100 concentrations were significantly increased (1 g/L < 4.5 g/L < 10 g/L), while ApoAI concentrations were significantly decreased (1 g/L > 4.5 g/L > 10 g/L). ApoB100/ApoAI ratios were significantly increased (1 g/L < 4.5 g/L < 10 g/L). ANOVA showed that the differences are significant ($p < 0.001$).¹⁸

Discussion

While studying the glucose-induced ApoB100 and ApoAI expression in cultured HepG2 cell *in vitro*, in the culture media that contained a higher concentration of glucose, ApoB100/ApoAI ratios were found to be significantly increased. In the culture media that contained a lower concentration of glucose, however, ApoB100/ApoAI ratios were found to be significantly decreased. Although previous *in vivo* human cohort studies indicated that the ApoB100/ApoAI ratios might associated with the CHD risk from statistical analysis,^{6–8} in the current study, the ApoB100/ApoAI ratios were quantitatively measured *in vitro* at the first time, which solidly proved that ApoB100/ApoAI ratio is a CHD risk factor.

However, a high-CHO diet was found to have generally favorable effects on the ApoB100/ApoAI ratio, reducing CHD risk, in a previous human cohort study *in vivo*,⁶ while the higher concentration of glucose induced an undesirable ApoB100/ApoAI ratio change, increasing CHD risk, in the present *in vitro* study. We can reach a reasonable explanation to this seemingly contradictory data if we look at what is happening inside the body after the meal; the rich viscous fiber contained in the high-CHO diet we administered in our cohort study has been shown to significantly reduce postprandial glucose excursions,^{9–11} which means the cells inside the body were actually exposed to a lower level of glucose after the high-CHO diet. Thus, the results we obtained from the lower concentration of glucose in the HepG2 cell culture experiment *in vitro* should correspond to the results we obtained from the high-CHO diet experimental group *in vivo*.

Lipoproteins are spherical particles that carry lipids in the body. These particles contain both lipids and proteins. LDL and HDL are the two main types of lipoproteins. LDL delivers fat molecules to cells. A high LDL level means too much LDL cholesterol in the blood. This extra LDL, along with other substances, forms plaque which can build up in the arteries to form atherosclerosis, causing heart disease. HDL-C carries cholesterol from other parts of the body back to the liver. The liver then processes the cholesterol for excretion to reduce the risk of heart disease. It is well known that both LDL and HDL consist of heterogeneous particles of different size and density.¹² ApoB100 is the primary protein in LDL and ApoAI is the primary protein in HDL. Normally, it is proteins instead of lipids which perform essential functions within organisms, including catalyzing metabolic reactions, providing structure to cells and organisms, and transporting molecules from one location to another. It is likely that lipoprotein metabolism is regulated by directly modulating their protein components like ApoB100 and ApoAI rather than their lipid components like triglycerides, phospholipids, and cholesterol molecules. Although previous *in vivo* studies have already indicated this,^{6–8} further *in vitro* studies are needed to prove it on a molecular level. The current study is the first to explore this issue in an *in vitro* cell culture system, and the result positively supports this hypothesis.

For many years, the ApoB100/ApoAI ratio rather than ApoB100 or ApoAI alone has been extensively reported as a risk factor of CHD.^{13–15} However, no one has understood the mechanism or even made any hypothesis about it. Atherosclerosis, the process involved in LDL oxidation within the walls of arteries, might be due to the more powerful function of ApoB100 to deliver lipids to the cells than ApoAI to transport lipids out of the cells and take them back to the liver. The dynamic equilibrium of these two proteins, which can be described in their ratios, is essential to our health.

This finding confirmed *in vitro* helps to explain why the existing clinical treatments based on the regulation of lipoproteins themselves cannot achieve the desired results in many clinical cases. For example, although statins have documented efficacy in reducing clinical events and angiographic disease progression in patients with coronary atherosclerosis, the results of subsequent large prospective clinical trials using different types of statins clearly demonstrate that statins do not have a short-to-medium term effect on prevention of restenosis after successful conventional percutaneous transluminal coronary angioplasty.¹⁶ Current available data also indicate that increased HDL-C levels do not always correlate with enhanced HDL functions because HDL is highly heterogeneous and there are different HDL subpopulations exist.¹⁷

Conclusions

In conclusion, *in vitro* HepG2 cell culture revealed ApoB100/ApoAI ratios change in response to the fluctuations of glucose concentration. Higher amounts of glucose can induce significantly increased ApoB100/ApoAI ratio, while lower amounts of glucose can induce significantly decreased, ApoB100/ApoAI ratio. The result suggests a new and central cell signal transduction pathway in lipoprotein metabolism and might provide molecular targets for clinical diagnoses and treatments of CHD.

Data availability

Underlying data

Harvard Dataverse: Replication Data for: Glucose induced ApoB100/ApoAI ratio changes in cultured HepG2 cells *in vitro*, <https://doi.org/10.7910/DVN/AHEAES>.¹⁸

This project contains the following underlying data:

- dataset–Glucose Induced ApoAI-ApoB100.tab

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

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Open Peer Review

Current Peer Review Status:

Version 2

Reviewer Report 10 February 2022

<https://doi.org/10.5256/f1000research.119823.r119129>

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Li Lin

Laboratory of Medical Molecular and Cellular Biology, College of Basic Medical sciences, Hubei University of Chinese Medicine, Wuhan, China

The author does not directly answer some of my relevant questions. Comments 1 and 2, in which the research content of Version 2 is not in-depth but more simple and the statistical method is simpler rather than detailed. So, I don't think the manuscript version can be accepted.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 18 January 2022

<https://doi.org/10.5256/f1000research.119823.r119128>

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Anne Tailleux

U1011, Pasteur Institute of Lille, Inserm, University of Lille, Lille, France

- Very little has been changed in the revised version, apart from the removal of the HL part.
- The answers to comments 1, 4, 6, 9, 10, 12, 13, and 14 are not at all satisfactory, and/or answers beside the matter.

- In particular, the rationale for studying different glucose concentrations and the link to T2D is never mentioned.
- As already stated, the article is not currently acceptable for indexing.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cardiometabolic diseases, bile acids and their receptors, NAFLD

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Version 1

Reviewer Report 26 November 2021

<https://doi.org/10.5256/f1000research.58773.r98246>

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Li Lin

Laboratory of Medical Molecular and Cellular Biology, College of Basic Medical sciences, Hubei University of Chinese Medicine, Wuhan, China

In this study, Minshan Hu reported that glucose may increase or decrease ApoB100/ApoAI ratio through up-and down-regulation of hepatic lipase activity, which suggests a new regulatory pathway in lipoprotein catabolism.

The observations in this work are interesting with some merits. However, there are some major issues that remain to be addressed.

1. The overall design of the experiment is too simple, it is best to complement the relevant mechanism of the experiment.
2. The statistics and statistical identification (Table 1) in the MS are not clearly described. In addition, the statistical drawing of Fig.1 is not standardized.
3. The MS was not very carefully prepared in organization and writing, for example, the discussion should focus on the experimental results and analyze the reasons and possible mechanisms.
4. An English language edit is required.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Prevention, treatment and pathophysiological study of senile and chronic diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 26 Dec 2021

Minshan Hu, Sichuan University, Chengdu, China

Dear Dr. Lin,

I appreciate the time and effort that you have dedicated to providing your valuable feedback on my manuscript. I am grateful to you for your insightful comments on my paper. I have been able to incorporate changes to reflect most of the suggestions provided by you. I have highlighted the changes within the manuscript.

Here is a point-by-point response to your comments and concerns.

Comment 1: The overall design of the experiment is too simple, it is best to complement the relevant mechanism of the experiment.

Response: Thank you for pointing this out. The current experimental data are far less enough to prove the association, so we only reach the conclusion of a higher concentration of glucose-induced an undesirable ApoB100/ApoAI ratio change in the revised version and rule out all the data related to HL.

Comment 2: The statistics and statistical identification (Table 1) in the MS are not clearly described. In addition, the statistical drawing of Fig.1 is not standardized.

Response: Thank you for pointing this out. Figure 1 has been deleted, and only Table 1 is kept in the revised version.

Comment 3: The MS was not very carefully prepared in organization and writing, for example, the discussion should focus on the experimental results and analyze the reasons and possible mechanisms.

Response: Thank you for pointing this out. The manuscript can be better prepared when more experimental results will be obtained in the future.

Comment 4: An English language edit is required.

Response: Thank you for this suggestion. English language has been edited.

Competing Interests: No Competing Interest exists.

Reviewer Report 02 November 2021

<https://doi.org/10.5256/f1000research.58773.r96276>

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Anne Tailleux

U1011, Pasteur Institute of Lille, Inserm, University of Lille, Lille, France

Comments to the author:

The objective of the study was to analyse the impact of glucose on hepatic lipase activity, ApoB100 and ApoA1 secretion in hepatocytes. The author performed a single *in vitro* study, using a well-documented human hepatocyte cell line, namely HepG2, incubated with different levels of glucose. HL activity, ApoB100 and ApoA1 were determined in the supernatant of the cell culture.

- The argument of why to watch the impact of glucose on HL/Apos parameters is not clearly stated in the introduction.
- A number of details are missing for the understanding of the methodological part.
- "Experiments were repeated six times for each assay" is stated in the Abstract. Is it the cell experiment or the dosages of HL, ApoB100 and ApoA1 that have been performed 6 times? This point should be clarified in the methods section.

- What is the duration of incubation for the cell experiment? Are the results time-dependant? A kinetic study should be informative.
- Methods section: HL activity is expressed as 1 μ mol FA with 1 ml reaction volume. In Table 1, HL is expressed as U/mg. The author should clarify.
- Statistical analysis: the author used a one-way ANOVA test to see differences between groups by global analysis. What is the post-hoc test used?
- Table 1 and Figure 1 represent exactly the same information and are redundant. The author should choose. If the figure is chosen, the unit of abscise should be added and the legend of the figure completed.
- The author has shown an association between HL activity and ApoB100/ApoA1, and they conclude that HL is a factor modifying Apos. What are the arguments to say that it's not the opposite?
- HepG2 is a cancer cell line. Cautions should be taken before extending the results obtained with a cancer line to hepatocytes. This point should be introduced in a "limitation of the study" paragraph in the discussion.
- ApoB100 is synthetised exclusively by the liver, in contrast to ApoA1 is synthetised by the liver and the intestine. However, the author makes a parallel between the results obtained with a hepatocyte cell line and what appends *in vivo* in the circulation of patients. This point should be modified.
- In the discussion, the author states that "HL regulates lipoprotein metabolisms by directly modulating... rather than lipid content like triglyceride". An HL is a lipase, it is very surprising! The author should modulate.
- Even if the author does not look for the molecular mechanisms underlying the association between HL and Apos, in the presence of glucose, they could at least speculate and state their hypothesis. Does the HL act via the FFA released that could modify gene expression of the Apos? Do Apos physically interact with HL? How could glucose modify the process? Others?
- What is the phenotype of mice knock-out for HL (whole body or hepato-specific)? Do they have an altered concentration of circulating ApoB and ApoA1?
- The discussion is a kind of juxtaposition of paragraphs without a narrative line. It is largely redundant and could be better structured.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

No

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cardiometabolic diseases, bile acids and their receptors, NAFLD

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 26 Dec 2021

Minshan Hu, Sichuan University, Chengdu, China

Dear Dr. Tailleux,

I appreciate the time and effort that you have dedicated to providing your valuable feedback on my manuscript. I am grateful to you for your insightful comments on my paper. I have been able to incorporate changes to reflect most of the suggestions provided by you. I have highlighted the changes within the manuscript.

Here is a point-by-point response to your comments and concerns.

Comment 1: The argument of why to watch the impact of glucose on HL/Apos parameters is not clearly stated in the introduction.

Response: I agree with this. Although the impact of HL on ApoB100/ApoAI ratio changes has been shown in a previous human cohort study *in vivo*, we have not yet obtained enough solid evidence that HL contributes to ApoB100/ApoAI ratio changes *in vitro*. I have incorporated your suggestion throughout the manuscript by eliminating HL data, and limiting the current report to only observe the ApoB100/ApoAI ratio changes in response to glucose concentration changes.

Comment 2: A number of details are missing for the understanding of the methodological part.

Response: You have raised an important point here. The changes have been made, please refer to the response to comment 5.

Comment 3: "Experiments were repeated six times for each assay" is stated in the Abstract. Is it the cell experiment or the dosages of HL, ApoB100 and ApoA1 that have been performed 6 times? This point should be clarified in the methods section.

Response: For those parameter measurements (HL, ApoB100 or ApoAI), six individual wells of cells were used for each concentration of glucose (please refer to Data availability in manuscript).

Comment 4: What is the duration of incubation for the cell experiment? Are the results time-dependant? A kinetic study should be informative.

Response: The experimental design of the duration of incubation for the cell experiment was according to PMID: 18758746. After cells reach 70% confluency, all the cell groups were then continued to be cultured for eight hours before the assays. Thank you for pointing out that it is better to measure those parameters in a time-dependent manner. I agree with this comment. Therefore, I will conduct those experiments in the ongoing research work.

Comment 5: Methods section: HL activity is expressed as 1 μmol FA with 1 ml reaction volume. In Table 1, HL is expressed as U/mg. The author should clarify.

Response: Thank you for pointing this out. I agree with this comment. Enzyme activity is the amount of substrate converted by the enzyme in moles per unit time. It measures the amount of active enzyme present in a mixture at a given time. On the other hand, specific activity is the activity of the enzyme per mg of total protein. Protein content was quantified by Coomassie Brilliant Blue assay, using bovine serum albumin (BioRad) as standard. Although specific HL activity was calculated, total protein measurement was missing from the Methods section.

Comment 6: Statistical analysis: the author used a one-way ANOVA test to see differences between groups by global analysis. What is the post-hoc test used?

Response: The post-hoc test is used to dive in and look for differences between groups testing each possible pair of groups.

Comment 7: Table 1 and Figure 1 represent exactly the same information and are redundant. The author should choose. If the figure is chosen, the unit of abscise should be added and the legend of the figure completed.

Response: Thank you for pointing this out. I agree with this comment. Figure 1 has been deleted, and only Table 1 is kept and revised.

Comment 8: The author has shown an association between HL activity and ApoB100/ApoA1, and they conclude that HL is a factor modifying Apos. What are the arguments to say that it's not the opposite?

Response: Thank you for pointing this out. The current experimental data are far less enough to prove the association, so we only reach the conclusion of a higher concentration of glucose-induced an undesirable ApoB100/ApoAI ratio change in the revised version and rule out all the data related to HL.

Comment 9: HepG2 is a cancer cell line. Cautions should be taken before extending the results obtained with a cancer line to hepatocytes. This point should be introduced in a "limitation of the study" paragraph in the discussion.

Response: Thank you for pointing this out. Cancer cell lines and primary cells do behave differently in some aspects, particularly in cell proliferation. HepG2 is used in this research by referring to numerous other similar published work related to hepatic cell signal transduction pathways (PMID: 30466006, PMID: 29886733, PMID: 31853220, etc.). But certainly, I am going to check this result in hepatocytes in future research.

Comment 10: ApoB100 is synthesized exclusively by the liver, in contrast to ApoA1 is synthesized by the liver and the intestine. However, the author makes a parallel between the results obtained with a hepatocyte cell line and what happens in vivo in the circulation of patients. This point should be modified.

Response: Thank you for this suggestion. However, the hypothesis of this study is that a major contributor to coronary heart disease is the dynamic equilibrium of apolipoproteins which deliver lipids to the cells and transport lipids out of the cells and take them back to the liver. Considering there are many other apolipoproteins involved in this "to the cells" and "back to the liver" process, the main event that happens in the liver is what I am exploring. And thank you for pointing this out, regarding the dynamic equilibrium, other apolipoproteins secreted from the entire body should be studied in the future.

Comment 11: In the discussion, the author states that "HL regulates lipoprotein metabolisms by directly modulating... rather than lipid content like triglyceride". An HL is a lipase, it is very surprising! The author should modulate.

Response: Thank you for pointing this out. The current experimental data are far less enough to prove this. So I have ruled out all the research work related to HL in the revised version.

Comment 12: Even if the author does not look for the molecular mechanisms underlying the association between HL and Apos, in the presence of glucose, they could at least speculate and state their hypothesis. Does the HL act via the FFA released that could modify gene expression of the Apos? Do Apos physically interact with HL? How could glucose modify the process? Others?

Response: Thank you for pointing this out. The current experimental data are far less enough to prove these and I am going to explore these in the future.

Comment 13: What is the phenotype of mice knock-out for HL (whole body or hepato-

specific)? Do they have an altered concentration of circulating ApoB and ApoA1?

Response: The *in vivo* human cohort studies were conducted instead of mice.

Comment 14: The discussion is a kind of juxtaposition of paragraphs without a narrative line. It is largely redundant and could be better structured.

Response: Thank you for this suggestion. I have restructured as suggested.

Competing Interests: No Competing Interests exist.

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