Author’s response to reviews

Title: Elevated circulating irisin is associated with lower risk of insulin resistance: association and path analyses of obese Chinese adults

Authors:

Xiulin Shi (shixiulin2002@163.com)
Mingzhu Lin (linmz65@126.com)
Changqin Liu (liuchangqin@126.com)
Fangsen Xiao (xfs888@163.com)
Yongwen Liu (yongwen_liu@163.com)
Peiying Huang (d860hp@163.com)
Xin Zeng (cynthia.zeng@aliyun.com)
Bing Yan (yanbing.sy@163.com)
Suhuan Liu (liush_xm@126.com)
Xiaoying Li (xiaoying_li@hotmail.com)
Shuyu Yang (xmyangshuyu@126.com)
Xuejun Li (xmlixuejun@163.com)
Zhibin Li (zhibinli33@hotmail.com)

Version: 2 Date: 05 Jul 2016

Author’s response to reviews:

Point-by-point response to comments to the author

C=Comments by reviewer in bold
R=Response
Reviewer #1:

In the present study, Shi and colleagues found that insulin-resistant subjects from a large Chinese cohort of 1,115 individuals exhibited lower circulating irisin concentrations as well as higher adiposity than insulin-sensitive individuals. The authors conclude that elevated irisin levels improves indirectly insulin resistance through lowering fasting insulin. This is an interesting study, but some specific points must be amended.

R: We thank for the reviewer’s appreciation of our efforts.

Specific comments

C1: Metformin reportedly promotes FNDC5 gene expression and irisin secretion from skeletal muscle (Li DJ et al. Acta Physiologica 2015, PMID 25382002; Yang Z et al. Am J Transl Res 2015, PMID 26692929), which seems to be in accordance with the results exposed in the present study. The authors should evaluate the impact of metformin treatment on their cohort in order to avoid confounding factors.

R1: We appreciate the comment that metformin may promote FNDC5 gene expression and irisin secretion from skeletal muscle and thus may act as a confounding factor for that association between irisin and insulin resistance. In the present study, all the subjects were community-living healthy obese Chinese adults without any previously diagnosed chronic diseases, and they did not take any medicine, even though they might have some undiagnosed diseases, such as T2DM. Therefore, we did not have data on medicine utilization, e.g. metformin, and could not address the issue about the effect of metformin treatment as the potential confounding factor. But we do acknowledge that this could be a limitation. Like all the observational study, confounding bias cannot be avoided completely because some kind of confounding factors that we did not know could not be adjusted for.

C2: As the authors state in the Introduction, the adipose tissue encodes FNDC5 gene and secretes irisin. Interestingly, it has been recently described that leptin downregulates FND5 expression in the adipose tissue (Gutiérrez-Repiso C et al. Eur J Clin Invest 2014, PMID 25112714; Rodríguez A et al. Int J Obes 2015, PMID 25199621). The insulin-resistant individuals from the present study showed higher body fat percentage than insulin-sensitive ones, and excess adiposity is reportedly associated with increased circulating leptin concentrations. Thus, the inhibitory effect
of leptin on adipose FNDC5 expression may also explain the decreased circulating irisin found in insulin-resistant individuals showing higher adiposity. The authors should discuss these comments.

R2: We appreciate this comment. We agree with the reviewer that leptin may down-regulate FNDC5 expression in the adipose tissue and thus may confound or mediate the association between irisin and insulin resistance. Unfortunately, we did not measure other cytokines, such as leptin, adiponectin, IL-6 and TNF-α, which may be related to serum irisin. In the revised manuscript, we have discussed this issue and acknowledged it as another limitation of our study.

Page 18 line 16: Excess adiposity has been reported to be associated with increased circulating adipokine concentrations, such as leptin, and leptin has been recently found to down-regulate FNDC5 expression in the adipose tissue [33,34]. In the present study, subjects with insulin resistance showed higher body fat percentage than their controls, therefore, the inhibitory effect of leptin on adipose FNDC5 expression may also explain the decreased circulating irisin in subjects with insulin resistant. Therefore, the fourth limitation is that we did not measure other cytokines, such as leptin, adiponectin, IL-6 and TNF-α, which may be related to serum irisin level and may confound the association between irisin and insulin resistance.

C3: The detection of circulating irisin remains largely controversial, since the commercial antibodies and ELISA assays reveal prominent cross-reactivity with non-specific proteins in human and animal sera (Raschke S et al. PLoS One 2013, PMID 24040023; Albretch E et al. Sci Rep 2015; PMID 25749243). Since the authors do not present FNDC5 gene expression studies, this potential limitation must be also included in the Discussion.

R3: We agree with the reviewer that the detection of circulating irisin remains largely controversial and ELISA assays have not been widely accepted as accurate and reproducible. We found the intra and inter assay variations in our assays were both less than 10%. Thus, the methods we used here are reliable. In the revised manuscript, we have discussed this issue and acknowledged it as another limitation of our study.

Page 19 line 2: We must acknowledge that the detection of circulating irisin remains largely controversial, since the commercial antibodies and ELISA assays reveal prominent cross-reactivity with non-specific proteins in human and animal sera and ELISA assays have not been
widely accepted as accurate and reproducible [35]. Although the intra and inter assay variations in the present study were both less than 10%, detection of circulating irisin could be another limitation of the present study.

Minor comments

1. Abstract, line 25: please change "as insulin resistance" by "as insulin-resistant".

2. Page 9, line 20: an space must be included between the number and the units in "-20°C".

3. Page 12, lines 34-36: the units of the BMI and waist circumference are missing.

4. Table 1, line 25 and Table 2, lines 17, 19, 30, 33, 44 and 47: please change "Body fat rate (%)" by "Body fat (%)".

R1-4: Thanks a lot for the reviewer’s suggestion on minor revisions. In the revised manuscript, we have made these revisions accordingly.

Reviewer #2:

The authors present a follow up study that expands on their previous two reports examining irisin in a large cohort of community-living obese Chinese adults. This cross-sectional analysis supports the idea that increased circulating irisin is correlated with lower insulin concentrations and reduced insulin resistance. The authors further used a structural equation modeling approach to arrive at a proposed pathway diagram linking irisin to insulin resistance, but not directly to adiposity or plasma glucose per se.

R: We thank for the reviewer’s appreciation of our works.

C1: The analysis is reasonable, but a limitation is that assays of circulating irisin are not widely accepted as accurate or reproducible. The authors note in their previous report, published in
PLoS One in 2014, that the intra and inter assay variations in their assays were both less than 10%. Thus, it may be that the methods used here are reliable, and the data fit well with the proposed role of irisin. Nonetheless, it would be appropriate to comment on the assay in this manuscript, and to acknowledge that this could be a limitation of the present study.

R1: We appreciate the comment. As the comment C3 from the first reviewer, we agree that the detection of circulating irisin remains largely controversial and assays has not been widely accepted as accurate and reproducible. In the revised manuscript, we have discussed this issue and acknowledged it as another limitation of our study.

Page 19 line 2: We must acknowledge that the detection of circulating irisin remains largely controversial, since the commercial antibodies and ELISA assays reveal prominent cross-reactivity with non-specific proteins in human and animal sera and ELISA assays have not been widely accepted as accurate and reproducible [35]. Although the intra and inter assay variations in the present study were both less than 10%, detection of circulating irisin could be another limitation of the present study.