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Letter to the Editor

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Can Pre-analytical Mistake Bearing Irisin Concentrations Be an Indicator of Coronary Artery Disease?

Suna Aydin (iD), **MD, PhD**

Cardiovascular Surgery Department, Elazig Education and Research Hospital, Heath Science University, Elazig, Turkey

> ► See the article "Serum Irisin Level Can Predict the Severity of Coronary Artery Disease in Patients with Stable Angina" in volume 47 on page 44.

Correspondence to
Suna Aydin, MD, PhD
Cardiovascular Surgery Department, Elazig Education and Research Hospital, Heath Science University; Rızaiye Mh., İnönü Caddesi (Hastane Cd.) No. 74, Elazığ 23100, Turkey.
E-mail: cerrah52@hotmail.com

ORCID iDs
Suna Aydin (iD)
https://orcid.org/0000-0002-5857-0888

Conflict of Interest
The author has no financial conflicts of interest.

Dear Editor,

It was with great interest that I read the article, entitled "Serum Irisin Level Can Predict the Severity of Coronary Artery Disease in Patients with Stable Angina," published in the *Korean Circulation Journal*,[1] a leading journal in cardiology and cardiac surgery. I would like to congratulate Efe et al.[1] on their contributions to our knowledge of the circulatory physiology and biochemistry of the heart, as well as the mechanisms underlying stable angina. However, considering a number of pre-analytical errors and the incorrect citation of some sources, which I specify below, I think it would be useful to revisit and reinterpret this article.

Irisin is a peptide molecule containing 112 amino acids.[2] Peptide/protein molecules are destroyed very rapidly by proteases. The human body produces more than 700 proteases and their concentration varies from one disease to another. In order to correctly measure the concentrations of peptide and protein molecules, biological samples taken from patients must be put into tubes containing a protease inhibitor like aprotinin. Otherwise, because the degree of destruction by proteases remains unclear, accurate measurement of peptide/protein concentrations is not possible.[3] In the study, researchers put 10-mL blood samples into tubes containing ethylenediaminetetraacetic acid (EDTA) and stored the tubes at −80°C until analysis; they did not add any protease inhibitor to the tubes.[1] EDTA in this context is a calcium-binding anticoagulant molecule. When samples taken from the patients are placed into tubes containing anticoagulants, the resulting biological sample will be plasma, not serum. Consequently, Efe et al.[1] studied the irisin molecule not in serum (as indicated in their title), but in plasma. When the reported irisin concentrations were evaluated, the standard deviation value was very high. For instance, the standard deviation of irisin value (299.54±123.20 ng/mL) in the control group is almost half of the main value(299.54 ng/mL). This can be explained by 1) insufficient sampling protocols or 2) by varying destruction of irisin by proteases, the content of which may vary in different samples.[3] Additionally, although there is no worldwide consensus on normal irisin concentrations, tandem mass spectrometry (LC-MS/MS) measurements found normal irisin concentrations range between 3.6 and 4.3 ng/mL[4] and 3rd-generation enzyme-linked immunosorbent assay (ELISA) irisin kits indicate normal irisin concentrations in the 6.8 to 12.0 ng/mL range. The mean irisin value in the control subjects of the researchers was 299.54±123.20 ng/mL.[1] This value was 50

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to 60 times higher than normal. This was likely due to different ELISA kits being used for the measurement of irisin concentration (AdipoGen ELISA kit; per the manufacturer, normal irisin measurement ranges of 0.2–2.0 µg/mL can be calculated 200–2,000 ng/mL). Cross-reactions with non-specific serum proteins can also influence the accuracy of ELISA kits.[5] However, the researchers did not address the reasons for these high values. Efe et al.[1] noted that the irisin decrease they found in stable coronary artery disease in their study resulted from the need for adenosine triphosphate (ATP) synthesis, as irisin overproduction would cause more heat and block ATP synthesis. Thus, they stated that they agreed with Bostrom et al.[2] and Park et al.,[6] who claimed that decreases in irisin served as a compensatory mechanism for increased ATP demand. However, the mechanism they explain was suggested by Aydin and colleagues,[7] not by Bostrom et al.[2] or Park et al.[6] Likewise, that the heart muscle produces more irisin than the skeletal muscle was reported first by Aydin et al.,[7] not by Bostrom et al.[2]

In consideration of the pre-analytical mistakes indicated above, irisin concentrations well outside of previously reported ranges, and failure to correctly cite sources, we think it will be useful to revisit and reinterpret the article.

REFERENCES

1. Efe TH, Açar B, Ertem AG, et al. Serum irisin level can predict the severity of coronary artery disease in patients with stable angina. *Korean Circ J* 2017;47:44-9.
   PUBMED | **CROSSREF**

2. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012;481:463-8.
   PUBMED | **CROSSREF**

3. Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. *Peptides* 2015;72:4-15.
   PUBMED | **CROSSREF**

4. Jedrychowski MP, Wrann CD, Paulo JA, et al. Detection and quantitation of circulating human irisin by tandem mass spectrometry. *Cell Metab* 2015;22:734-40.
   PUBMED | **CROSSREF**

5. Albrecht E, Norheim F, Thiede B, et al. Irisin - a myth rather than an exercise-inducible myokine. *Sci Rep* 2015;5:8889.
   PUBMED | **CROSSREF**

6. Park SE, Park CY, Sweeney G. Biomarkers of insulin sensitivity and insulin resistance: past, present and future. *Crit Rev Clin Lab Sci* 2015;52:180-90.
   PUBMED | **CROSSREF**

7. Aydin S, Kuloglu T, Aydin S, et al. Cardiac, skeletal muscle and serum irisin responses to with or without water exercise in young and old male rats: cardiac muscle produces more irisin than skeletal muscle. *Peptides* 2014;52:68-73.
   PUBMED | **CROSSREF**