

Preanalytical, analytical, and postanalytical errors in the measurement of irisin levels

To the Editor I have read with great interest the article by Kałużna et al¹ published in your journal. However, because the article contains some problems relating to preanalytical, analytical, or postanalytical measurements, the results need to be carefully reinterpreted. These problems have been discussed below.

The minimum measurement concentration of the assay used for measuring irisin levels (AdipoGen ELISA Irisin Kit, AdipoGen, Liestal, Switzerland) was specified as 1 ng/ml (1000 pg/ml) both by the authors and by the manufacturer.^{1,2} However, the authors of the study¹ reported the irisin levels to range from 6.54 to 9.45 pg/ml in the control group and from 3.48 to 6.38 pg/ml in the patient group. Given the fact that the lowest measurement level of the assay is 1000 pg/ml, how was it possible for the investigators to report the above ranges? The levels of irisin in their study were below the minimum measurement concentration of the assay, and such low irisin concentrations cannot be measured with this assay. Therefore, in my opinion, the results of the study must be carefully reinterpreted.

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- 2 AdipoGen Life Sciences. Irisin Competitive ELISA Kit. Liestal, Switzerland; 2017: 1-15.

Authors' reply We would like to sincerely thank Dr. Mehmet Kalayci for his careful attention while reading our article. We appreciate his constructive comments and feedback. We addressed his concerns below.

In our article,¹ there was an unfortunate typographical error for the irisin unit that occurred during manuscript rewriting. Irisin levels were actually measured in µg/ml and not in pg/ml as reported. Therefore, the levels were from 6.54 to 9.45 µg/ml in the control group and from 3.48 to 6.38 µg/ml in patients with end-stage renal disease (ESRD). Serum samples were obtained from participants, and irisin levels were measured in duplicate with the AdipoGen ELISA Irisin Kit (Penta Plex, version 2, Cat. No. AG-45A-0046YPP-KI01, AdipoGen, Liestal, Switzerland). We chose the AdipoGen assay because it has a wide detection range (0.001–5 µg/ml) and the highest sensitivity (0.001 µg/ml).¹

The range of the curve was between 0.001 and 5 µg/ml. However, the tests were diluted 4 times with due diligence by an experienced laboratory diagnostician, and the results read from the curve were multiplied by the dilution factor. The expected irisin levels in human plasma and serum range from 0.2 to 2 µg/ml according to the manufacturer.² The levels were significantly lower in patients with ESRD compared with controls (4.57 µg/ml vs 7.90 µg/ml; $P = 0.000001$). On the other hand, irisin concentrations in the control group were higher than expected and quite high in comparison with irisin concentrations previously measured by the AdipoGen ELISA Irisin Kit.³⁻⁵ Yet, the values were comparable to those obtained using the same kit in healthy patients.⁶ Similarly, irisin levels were lower in patients with ESRD treated with peritoneal dialysis or hemodialysis than in the control group as reported by Rodríguez-Carmona et al⁷ and in nondiabetic patients on peritoneal dialysis as recently reported by Tan et al.⁸

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