

## LETTER TO THE EDITOR

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# Ideal Diagnostic Tool and Proper Statistical Analysis Improve the Credibility of the Study

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### Dear Editor,

We read with great interest the recently published article by Haidari et al. in which the authors aimed to compare the dietary intakes of essential fatty acids (EFAs) and serum levels of inflammatory factors in asthmatic and healthy adults, and to examine the potential relationship between inflammatory markers and dietary fatty acids (FAs). They concluded that higher intake of omega-3 and lower levels of inflammatory factors in the healthy control group compared to asthmatic group may explain the protective role of EFAs in asthma.<sup>1</sup> However; we think that there are some points that should be emphasized about this study.

First, in the original study, it was indicated that dietary intakes were assessed by semiquantitative food frequency questionnaire (FFQ) which had previously been validated and adapted by Mirmiran et al. to assess intakes of the main nutrients and food groups.<sup>2</sup> According to literature, this FFQ is based on 24 h dietary recalls. However, this 24-hour questionnaire is not enough to have an opinion about the process of inflammation in the pathogenesis of asthma. Although, the importance of FAs in cellular homeostasis demands an efficient uptake system for these FAs, their metabolism in cells and tissues still play the most

important role in the pathogenesis of diseases like asthma. Therefore, quantitating the level of FAs in plasma and tissues, particularly of EFAs, besides assessing dietary intake, can provide more accurate results about the inflammation process for asthma.<sup>3</sup> As it is known, asthma is a disease with bronchial inflammation. Accordingly, detailed analysis of FAs can provide valuable information in the management of dietary intake or supplementation of FAs, which are believed to be deficient and are considered responsible for the pathogenesis of asthma.

Second, typically, plasma/serum samples are more commonly analyzed for the assessment of an individual's FA status because the FA composition of plasma reflects recent dietary fat intake.<sup>4</sup> However, plasma FA levels are subject to multiple different dietary influences. The plasma FA profile may be determined not only by the time elapsed between the ingestion of fat-containing foods, but also by the type of dietary lipids ingested.<sup>5</sup> Consequently, because erythrocytes have a rather long lifespan (~ 120days), the FA profile of erythrocytes is considered a better indicator of long-term FA intake compared to the intermediate lifespan (max ~3 weeks) of platelet or plasma lipids.<sup>4-6</sup> In this respect, the erythrocytes are more stable, and it would be more valuable/proper to measure their FA concentration to assess the functional activity of FAs. Therefore, measurement of erythrocyte membrane fatty acid levels is extremely valuable in such studies.<sup>3, 7</sup>

Third, although authors have stated that they have done normality analysis (Kolmogorov-Smirnov test)

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for the distribution of the groups, we have doubts about omega-6, omega-3 and omega-6/omega-3 ratio in asthmatic group. Since mean of total omega-3 ( $1.28 \pm 0.86$  gr) is more than 1.00, the average value of omega-6/omega-3 ( $29.20 \pm 20.53$ ) is expected to be smaller than the mean of Omega-6 ( $24.41 \pm 5.34$  gr). We think that this is all because of using incorrect descriptive parameters. Instead of using the mean  $\pm$  standard deviation (SD) for all parameters, authors should have used mean  $\pm$  SD for normally distributed variables and median (25th-75th interquartile range) for the non-Gaussian distributed variables. Moreover, an error in normality analysis will lead to the use of the improper tests in group comparisons, which will cause incorrect *p* values. Additionally, as seen in table 2 of the original study, there was no significant difference between the two groups regarding the mean omega-6 levels. The main reason for the statistically significant difference between the omega-6/omega-3 values is arising from the significant difference between omega-3 values of the two groups.

In conclusion, quantitative analysis of erythrocyte FAs should have been preferred to evaluate FA profile and proper use of statistical analysis for correct and reliable results should have been used.

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