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LETTER TO THE EDITOR

Evaluating Melatonin's Effects on Hepatocyte Lipidome: A Critique of Analytical Methods

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To the Editor,

Migni et al. investigated the restorative effects of melatonin on the lipidome of human hepatocytes exposed to cadmium (Cd) and lipotoxicity induced by free fatty acids [1]. Their analysis utilized MetaboAnalyst 6.0, implementing both principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) to examine the data. Lipid species with a variable importance in projection (VIP) score exceeding one were selected to highlight melatonin's overall impact on the cellular lipid profile. Additionally, pairwise comparisons of lipid expression across various treatments were conducted using an FDR-corrected moderated *t*-test (adjusted $p < 0.01$, fold change cutoff = 1.5). The significantly modulated lipids were visualized through volcano plots, and the overall lipid composition was depicted in dot charts mapping the number of carbon atoms and double bonds across each lipid class [1].

This study underscores important theoretical concerns regarding the reliance on PCA and PLS-DA, both of which are predicated on linear assumptions that may result in misleading interpretations when applied to biological data characterized by nonlinear and nonparametric relationships. When linear methodologies are misapplied to nonlinear data or when parametric models are inappropriately used for nonparametric analysis, the resulting interpretations can become flawed and distorted [2–9]. It is crucial for researchers, including Migni et al., to have a solid understanding of the fundamental theoretical principles underlying the data analysis tools they employ. Violations of these analytical assumptions can compromise the reliability and validity of their findings.

PCA fundamentally assumes that relationships among variables are linear, that the data is centered (mean of zero) and ideally standardized (unit variance), and that the principal components effectively capture the data's variability. It also presumes independence among observations and that the scales of variables do not disproportionately influence the results, emphasizing the need for proper scaling when dealing with variables of different units. Furthermore, PCA presupposes that latent principal components account for the observed variance, typically exhibiting characteristics of normal distribution, although strict adherence to normality is not required. Misapplying PCA to nonlinear, nonparametric data can severely undermine the validity of the conclusions drawn due to breaches of these foundational assumptions [2–5].

In a similar vein, PLS-DA relies on several essential assumptions: linear relationships among variables, independence of observations, normality of residuals, homoscedasticity of variances, a sufficiently large sample size, additivity of predictor effects, appropriate data scaling, and the absence of measurement error in predictor variables. When PLS-DA is applied to nonlinear, nonparametric data, the outcomes may also be distorted [6–9]. Researchers must remain vigilant in recognizing these assumptions to ensure the robustness and accuracy of their analyses.

To address these limitations for true associations between variables, this paper recommends the use of nonlinear nonparametric robust methods such as total correlation (TC) [10, 11] and effective transfer entropy (ETE) [12, 13]. These advanced techniques are adept at capturing complex interactions among

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multiple variables, especially when those interactions exhibit nonmonotonic patterns that traditional methods may overlook. TC, for instance, allows for a more comprehensive understanding of dependencies between variables by assessing the total amount of information shared among them, rather than merely focusing on pairwise correlations. ETE further enhances this framework by quantifying the directed information flow between variables, thereby providing insights into causal relationships. By employing these methods, researchers can obtain a more nuanced and accurate analysis of biological systems, leading to a better understanding of the underlying mechanisms and interactions that govern cellular responses to various stimuli.

Author Contributions

Yoshiyasu Takefuji completed this study and wrote this article. According to ScholarGPS, Yoshiyasu Takefuji holds notable global rankings in several fields. He ranks 54th out of 395 884 scholars in neural networks (AI), 23rd out of 47 799 in parallel computing, and 14th out of 7222 in parallel algorithms. Furthermore, he ranks highest in AI tools and human-induced error analysis, underscoring his significant contributions to these domains.

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Ethics Statement

The author has nothing to report.

Consent

The author has nothing to report.

Conflicts of Interest

The author has no conflicts of interest.

Data Availability Statement

The author has nothing to report.

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