Author’s response to reviews

Title: THE FALLACY OF ENZYMATIC HYDROLYSIS FOR THE DETERMINATION OF BIOACTIVE CURCUMIN IN PLASMA SAMPLES AS AN INDICATION OF BIOAVAILABILITY: A COMPARATIVE STUDY

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Author’s response to reviews:

We sincerely appreciate the helpful comments regarding our manuscript. We believe that we have appropriately addressed each of the comments. All changes and additions to the manuscript are noted in red type. A detailed response to each of the comments is provided below, with the response noted in red type for ease of discernment.

Editor Comment: Please include the correct ethics statement in the 'Ethics and consent to participate' Declaration.

Response---The correct ethics statement has been provided in the Ethics and Consent to Participate Declaration. Approval by an ethics committee and written consent by the participants is noted in the Methods section. The correct information is provided.
Reviewer #1

In the "Methods" section, "The samples were analysed for free curcumin, curcumin sulfate, curcumin glucuronide, tetrahydrocurcumin, demethoxycurcumin and bis-demethoxycurcumin" is written. However, no results were presented for curcumin sulfate, demethoxycurcumin and bis-demethoxycurcumin. If these analytes were not found (as they may exist below of the detection limit of the analytical method), please include some comment in the revised manuscript. Otherwise, please include the values obtained.

We have noted in the second paragraph of the Results that the amounts of curcumin sulfate, demethoxycurcumin and bis-demethoxycurcumin were near the limits of detection in non-hydrolyzed samples, while demethoxycurcumin and bis-demethoxycurcumin were not detected in hydrolyzed samples, and therefore no data are presented.

Finally, in the section "Ethics approval and consent to participate", a declaration should be included as this research involved human subjects.

Response---As noted above, the correct ethics statement has been provided in the Ethics and Consent to Participate Declaration. Approval by an ethics committee and written consent by the participants is noted in the Methods section.

Reviewer #2

* The number of subjects included in the study (8) is rather small; for example, according to CPMP/EWP/QWP/1401/98 guideline, „the minimum no. of subject should not be smaller than 12, unless justified“. Authors should provide justification for the selected number of subjects.

Response--- Indeed, the sample size was small but adequate for a proof-of-concept study, aiming to test the impact of the enzymatic hydrolysis step on plasma curcumin profile. Thus, the CPMP/EWP/QWP/1401/98 guideline noted by the reviewer is not applicable to the reported human study because it was not a bio-equivalence trial testing bioavailability and pharmacokinetics of 2 or more formulations of the same nutrient or drug. A sentence has been added in the second paragraph of the Methods denoting this consideration.

* A figure depicting the possible metabolic pathways of curcumin (as described in the introduction) in the human body could be a useful addition to the manuscript.

Response---As suggested, a figure (Figure 1) depicting the metabolic pathways for curcumin has been added.
* Results should be discussed in correlation with the BMI of the patients (average of 30.3 kg/m²), based on literature data. Is there any difference between the pharmacokinetic data between normoponderal and overweight patients?

Response--- As this human study was conducted to test the effect of the enzymatic hydrolysis step used to prepare plasma samples for curcumin analysis and metabolic profiling, the study was not designed to test the effect of BMI on curcumin pharmacokinetics. Thus, we believe that a discussion on the correlation between BMI and curcumin pharmacokinetics does not fall in the scope of the manuscript. A sentence has been added to the Methods section noting that no attempt was made to assess the effects of age, gender or BMI relative to the effects of enzymatic hydrolysis of the plasma samples.

Respectfully yours on behalf of all authors,

Sidney J. Stohs, Ph.D., FACN, CNS, ATS, FAPHA