



Response to: “Utility of endogenous 4 β -hydroxycholesterol as a biomarker to assess cytochrome P 450 3A (CYP3A) activity: not quite ready for prime time”

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We thank Tung and Ma for their interest in our recently published paper presenting correlations between 4 β -hydroxycholesterol (4 β OHC) concentrations and three other CYP3A metrics, including intestinal and hepatic CYP3A4 protein concentrations and microsomal activity, and in vivo midazolam pharmacokinetics in primarily a population with obesity, to assess 4 β OHC’s potential as an endogenous CYP3A4 biomarker [1]. In the letter to the editor, our understanding is that Tung and Ma argue that correlation coefficients are unsuitable for the validation of probe drugs or biomarkers such as 4 β OHC. They are also concerned that the results presented may lead to inappropriate use of 4 β OHC in other studies, particularly studies evaluating CYP3A-mediated drug-drug interactions.

First, we agree that correlation coefficients have certain limitations and that predictive performance via assessment of bias and precision should be included in the validation of phenotyping biomarkers and/or probe drugs. However, the nature of our study was exploratory, and as such, correlations analyses were performed, which are useful to assess associations between variables. One of the major strengths of our study was the ability to investigate the relationship between 4 β OHC concentrations and both CYP3A4 protein expression and microsomal activity in paired liver and jejunum samples. This provides novel information, which is lacking in the literature and is actually suggested to be one of several validation criteria for phenotyping metrics [2].

Indeed, we do not believe that the results presented in our study may lead to inappropriate use of 4 β OHC as a biomarker, but rather provide increased knowledge on when or how 4 β OHC may be utilized as a CYP3A4 biomarker. Tung and Ma claim that there have been concerns regarding the utility of 4 β OHC as a CYP3A4 biomarker, e.g., due to the uncertainty with regard to the intestinal contribution in 4 β OHC formation and systemic level [3]. We have previously reported that intake of grapefruit juice, an inhibitor of intestinal CYP3A4, had no impact on 4 β OHC to cholesterol ratio [4], hence showing that variability in intestinal CYP3A4 activity does not reflect 4 β OHC levels. An important point that Tung and Ma seem to have missed is that the results of our current study support that 4 β OHC reflects hepatic CYP3A4 activity, but not intestinal. Thus, we have clarified that 4 β OHC is only appropriate as hepatic CYP3A4 biomarker.

Tung and Ma comment on the lack of correlation between 4 β OHC and systemic midazolam clearance as an argument against the value of 4 β OHC as CYP3A4 biomarker. While midazolam is considered to be the gold standard CYP3A probe drug, it is reported to have a highly variable extraction ratio in the literature [5], and hence midazolam clearance may not reflect hepatic CYP3A activity in all patient populations, such as patients with obesity having an increased hepatic blood flow. Thus, we believe the discrepancy between 4 β OHC and systemic midazolam clearance in our study was attributed to the fact that midazolam behaved as a medium to high extraction ratio drug, and thus clearance is also dependent on hepatic blood flow. This hypothesis is supported by the fact that there was no correlation between systemic midazolam clearance and hepatic microsomal CYP3A4 activity. Accordingly, midazolam does not seem to be optimal as a CYP3A4 biomarker in patients with obesity. This is supported by previous studies showing that midazolam clearance appears to be influenced by hepatic blood flow in patients with obesity (6, 7). Thus, 4 β OHC

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may be superior to midazolam as a hepatic CYP3A4 metric in patients with obesity, but not necessarily in other patient groups.

To date, the perfect CYP3A4 biomarker does not exist and any information obtained by the use of such metrics should be interpreted with care. However, our study suggests that 4 β OHC may serve as a valuable measure to obtain information on hepatic CYP3A4 activity in clinical studies.

Author contribution K.E.K., K.H., E.M., and I.R. drafted, made revisions, and reviewed the manuscript.

Data availability Not applicable as no data were used.

Declarations

Conflict of interest The authors declare no competing interests.

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References

1. Eide Kvitne K, Hole K, Krogstad V et al (2022) Correlations between 4 β -hydroxycholesterol and hepatic and intestinal CYP3A4: protein expression, microsomal ex vivo activity, and in vivo activity in patients with a wide body weight range. *Eur J Clin Pharmacol* 78(8):1289–1299. <https://doi.org/10.1007/s00228-022-03336-9>
2. Fuhr U, Jetter A, Kirchheiner J (2007) Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the “cocktail” approach. *Clin Pharmacol Ther* 81(2):270–283. <https://doi.org/10.1038/sj.clpt.6100050>
3. Penzak SR, Rojas-Fernandez C (2019) 4 β -hydroxycholesterol as an endogenous biomarker for CYP3A activity: literature review and critical evaluation. *J Clin Pharmacol* 59(5):611–624. <https://doi.org/10.1002/jcph.1391>
4. Gjestad C, Hole K, Haslemo T, Diczfalusy U, Molden E (2019) Effect of grapefruit juice intake on serum level of the endogenous CYP3A4 metabolite 4 β -hydroxycholesterol-an interaction study in healthy volunteers. *Aaps j* 21(4):58. <https://doi.org/10.1208/s12248-019-0330-1>
5. Rogers JF, Rocci ML Jr, Haughey DB, Bertino JS Jr (2003) An evaluation of the suitability of intravenous midazolam as an in vivo marker for hepatic cytochrome P4503A activity. *Clin Pharmacol Ther* 73(3):153–158. <https://doi.org/10.1067/mcp.2003.23>
6. Brill MJ, Vålitalo PA, Darwich AS et al (2016) Semiphysiologically based pharmacokinetic model for midazolam and CYP3A mediated metabolite 1-OH-midazolam in morbidly obese and weight loss surgery patients. *CPT Pharmacometrics Syst Pharmacol* 5(1):20–30. <https://doi.org/10.1002/psp4.12048>
7. van Rongen A, Brill MJE, Vaughns JD et al (2018) Higher midazolam clearance in obese adolescents compared with morbidly obese adults. *Clin Pharmacokinet* 57(5):601–611. <https://doi.org/10.1007/s40262-017-0579-4>

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