Response to Letter to the Editor from Hoekstra: 'Adrenal Abcg1 Controls Cholesterol Flux and Steroidogenesis'.

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Disclosures

The authors have nothing to disclose

We are writing in response to Dr. Menno Hoekstra's recent letter (1) concerning our work "Adrenal Abcg1 Controls Cholesterol Flux and Steroidogenesis" published in Endocrinology last February (2).

In 2019, Dr. Hoekstra's team published findings on a mouse model demonstrating mild glucocorticoid insufficiency and a reduction in adrenal cholesteryl esters following systemic deletion of the transporter Abcg1 (3). Our research, however, showed that adrenal-specific inactivation of *Abcg1* results in mild hypersecretion of corticosterone without altering adrenal fat composition (2).

We believe that these contrasting results provide an opportunity for further exploration and understanding. In our discussion, we proposed three potential reasons for these discrepancies:

1. **Systemic vs. Adrenal-Specific Deletion**: We suggested that global deletion of *Abcg1* might influence corticotropin-releasing hormone and/or adrenocorticotropin hormone (ACTH). Dr. Hoekstra highlighted that their protocol included ACTH stimulation 3 hours before sample collection, which addresses acute exposure. However, the possibility of prolonged low ACTH levels contributing to adrenal hypofunction remains plausible, as documented in both human and mouse models of secondary adrenal insufficiency following corticosteroid treatment (4). Further research could clarify the impact of systemic *Abcg1* deletion on ACTH levels and/or other factors related to the hypophyseal-pituitary-adrenal axis.
2. **Adrenal Cortex Function/Development**: We hypothesized that global *Abcg1* inactivation could impair adrenal cortex function or cause dysgenesis. Dr. Hoekstra's letter did not address this possibility, suggesting an area for additional investigation to determine the broader effects of *Abcg1* deletion on adrenal cortex development.
3. **Degree of *Abcg1* Recombination**: Dr. Hoekstra noted residual *Abcg1* transcripts in the zona Fasciculata in Fig. 1C of our paper and argued that the remaining transcripts may account for phenotype discrepancies (2). Of note, we observed a significant reduction in signal dots compared to controls, and whole-adrenal quantitative PCR showed a marked decrease in *Abcg1* transcripts. Together, this indicates substantial *Abcg1* reduction across the cortex following tissue-specific recombination. Nevertheless, even residual levels of *Abcg1* transcripts are unlikely to explain the phenotypic gain-of-function (i.e., higher corticosterone levels) observed in our model of adrenal-specific *Abcg1* deletion.

In addition, Dr. Hoekstra raised concerns about the suitability of the Aldosterone Synthase (AS, Cyp11b2)-regulated *Cre* system for recombination in the zona Fasciculata, where AS is not expressed. We refer to numerous lineage-tracing studies over the past decade, which confirm that

AS-expressing cells in the zona Glomerulosa transdifferentiate into zona Fasciculata cells (5,6). Consistent with these findings, the specific *Cre*-expressing mouse strain we used achieves nearly complete renewal of the steroidogenic cortex from AS-expressing cell descendants by 11-12 weeks of age (7). Thus, our experiments were appropriately conducted at this developmental stage. Moreover, we are pleased to share that additional lineage-tracing experiments using an mTmG allele (8) in *Abcg1* conditional knock-out and control mice at 12 weeks of age showed no impact of *Abcg1* deletion on transdifferentiation of zona Glomerulosa cells into zona Fasciculata cells (data not shown).

We thank Dr. Hoekstra for prompting this discussion and for the opportunity to clarify our findings. We hope these points contribute to a deeper understanding of Abcg1's role in adrenal physiology and encourage further collaborative research in this area.

References

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