Reply to Yamauchi *et al.*: Analyses of lysine aldehyde cross-linking in collagen reveal that the mature cross-link histidinohydroxylysinonorleucine is an artifact

DOI 10.1074/jbc.RL119.010692

David R. Eyre[1], **MaryAnn Weis, and Jyoti Rai**

From the Department of Orthopaedics and Sports Medicine, University of Washington, Seattle, Washington 98195

Edited by Gerald W. Hart

Yamauchi *et al.* (1) question the data in our recent paper (2) as support for our conclusions. We disagree and maintain that histidinohydroxylysinonorleucine (HHL)[2] (3, 4) does not exist as a natural product in collagen.

In Point 1, "these cross-linked peptides" presumably refers to peptides that Mechanic *et al.* (3) thought were linked by HHL based on Edman N-terminal sequencing and amino acid analysis. In our opinion, this did not rule out a mixture of physically associated peptides or a peptide remnant of the C-telopeptide aldol adduct that we conclude creates HHL on acid hydrolysis (see Fig. 7) (2). Quantifying HHL in acid hydrolysates of equal amounts of starting tissue collagen, a proteolytic digest of it, and subsequent fractions is highly instructive. For example, from bovine cornea and dermis, the yields of HHL in a bacterial collagenase digest are 6 and 17% based on the LC/MS assay method used in Fig. 8 (2).[3] This is consistent with the low yield of the C-telopeptide aldol adduct (see Figs. 5*A* and 7) (2) and its HHL artifactual product on LC/MS (see Fig. 8*F*) (2). Thus, with progressive chromatography under denaturing and acidic conditions, the labile adduct that creates HHL on acid hydrolysis continues to dissociate.

With regard to Points 2 and 3, we explain above and in Eyre *et al.* (2) why peptides prepared from collagen yield so little HHL. Such concerns do not apply to HHMD because tissue borohydride reduction quantitatively could convert the N-telopeptide dimer pool (see Fig. 5) (2) to HHMD-linked N-telopeptide to α1(I)K930 structures (see Fig. 6) (2). The latter do match exactly the peptide structures shown including the mass of HHMD, as does the peptide in Fig. 7 with its cross-link (Fig. 10) (2).

Finally, the peptides in Fig. 6 are dominant, not minor partial cleavage products, from the N-telopeptide to the helix site under the conditions we use (10% acetonitrile in the digest) to limit the range of products.

The authors declare that they have no conflicts of interest with the contents of this article.

[1] To whom correspondence should be addressed. E-mail: deyre@uw.edu.

[2] The abbreviations used are: HHL, histidinohydroxylysinonorleucine; HHMD, histidinohydroxymerodemosine.

[3] D. R. Eyre, M. Weis, and J. Rai, unpublished observations.

References

1. Yamauchi, M., Taga, Y., and Terajima, M. (2019) Letter to the Editor: Analyses of lysine aldehyde cross-linking in collagen reveal that the mature cross-link histidinohydroxylysinonorleucine is an artifact. *J. Biol. Chem.* **294,** 14163–14163 CrossRef
2. Eyre, D. R., Weis, M., and Rai, J. (2019) Analyses of lysine aldehyde cross-linking in collagen reveal that the mature cross-link histidinohydroxylysinonorleucine is an artifact. *J. Biol. Chem.* **294,** 6578–6590 CrossRef Medline
3. Mechanic, G. L., Katz, E. P., Henmi, M., Noyes, C., and Yamauchi, M. (1987) Locus of a histidine-based, stable trifunctional, helix to helix collagen cross-link: stereospecific collagen structure of type I skin fibrils. *Biochemistry* **26,** 3500–3509 CrossRef Medline
4. Yamauchi, M., London, R. E., Guenat, C., Hashimoto, F., and Mechanic, G. L. (1987) Structure and formation of a stable histidine-based trifunctional cross-link in skin collagen. *J. Biol. Chem.* **262,** 11428–11434 Medline