REPLY TO SOULILLOU ET AL :

Difficulties in extrapolating from animal models exemplify unusual human atherosclerosis susceptibility and mechanisms via *CMAH* loss

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Soulillou et al. 1) address our report 2) regarding intrinsic and extrinsic atherosclerotic cardiovascular disease ASCVD) in mice with human-like loss of *Cmah* cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase)—eliminating *N*-glycolylneuraminic acid Neu5Gc) production. While our findings may help explain the unusual human propensity for ASCVD, extrapolation from animal models to humans has limitations. However, the intrinsic hyperimmune reactivity and diabetic tendency of these mice refs. 3 and 4 and citations therein) are consistent with the human condition. The extrinsic mechanism involving metabolic incorporation and endogenous cell surface presentation of diet-derived Neu5Gc in humans is an example of a "xenoautoantigen"–"xenoautoantibody" reaction—which, contrary to Soulillou et al., is not induced by dietary Neu5Gc 4), but likely a response to exposure to Neu5Gc incorporated into human-specific commensals like nontypeable *Haemophilus influenzae* 4). All our data are internally consistent, including antibody activation of human endothelium displaying Neu5Gc 5), xenosialitis-mediated acceleration of atherosclerosis 2), and cancer promotion 4). Other concerns of Soulillou et al. are effectively addressed by emphasizing that all studies were internally controlled by feeding and/or immunization of congenic mice with the nonimmunogenic *N*-acetylneuraminic acid Neu5Ac) precursor, differing by a single oxygen atom. We acknowledge an error in our methods description—we used a single immunization with Freund s complete adjuvant and two boosts with incomplete adjuvant to mimic in a short time span a human disease developing over many decades. The low-density lipoprotein receptor LDLR)-deficient background is a standard mouse model for atherosclerosis research developed by Nobel Laureates Joseph L. Goldstein and Michael S. Brown 6). Notably, *Ldlr* deficiency alone did not predispose mice to type 2 diabetes-associated insulin resistance even on high-fat diet nor result in differences in body or organ weights between our *Ldlr* $^{-/-}$ and *Cmah* $^{-/-}$ *Ldlr* $^{-/-}$ mice on a C57BL/6N background not the diabetogenic C57BL/6J background).

We acknowledge we did not cite a study focused on type 1 diabetes treatment, reporting absence of glucose and insulin abnormalities in a small number of adolescent male pigs deficient for *CMAH* and 1-3-galactosyltransferase, fed an unspecified regular energy balanced chow, when compared to nonlittermate wild-type pigs, a species evolutionarily more distant to humans than mice 7). We also did not cite reports of extremely high anti-Neu5Gc titers elicited by xenotransplants, bioprostheses, or rabbit polyclonal IgG 8–10), as red meat consumption data were lacking and follow-up periods short, making it difficult to interpret relevance for ASCVD. Another not mutually exclusive explanation comes from our finding of "inverse hormesis" in xenosialitis-cancer models, wherein lower levels of anti-Neu5Gc antibodies stimulated, but very high levels suppressed tumor progression 11).

Ongoing correlational studies of diverse polyclonal anti-Neu5Gc antibodies, red meat consumption, and ASCVD in well-defined long-term follow-up populations are needed to validate the translational implications of our findings, and address whether our model accurately mimics the human-specific red meat-associated cancer and ASCVD risk via "xenosialitis." Until conclusive human data are available, differing opinions are expected, but this does not constitute a "controversy." Meanwhile, the great difficulties in extrapolating from animal models of ASCVD reemphasize the unusual susceptibility of humans and support proposed mechanisms involving *CMAH* loss.

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