LETTER

Reply to Diet-responsive MicroRNAs Are Likely Exogenous

This is a response to a letter by Zempleni et al. (1).

We here address concerns raised in a letter to the *Journal of Biological Chemistry* by Zempleni *et al.* (1). First, exosomal encapsulation of milk miRNAs confers limited protection against degradation by the digestive system (Fig. 5 of Ref. 2). Exosomal transport into intestinal cells has only been demonstrated *in vitro* (3) and is not supported by our *in vivo* data. Second, detection of dietary miRNAs has been linked to sample contamination and oversensitivity of sequencing methods (4). Furthermore, analysis of the cited paper reveals that total plant miRNAs represent only 0.07 and 0.007% on average of small RNA sequences in human and porcine milk, respectively, suggesting they are artifactual or present in trace amounts. Third, miRNAs are only subject to degradation at high sponge site expression (5) and upon extensive sequence complementarity between miRNA and target, rarely reached in mammalian cells (6). It is unlikely that in murine tissues, sufficient complementarity is achieved to degrade miR-375 post-uptake and prevent its subsequent detection. Fourth, we studied milk miRNA uptake at early (D3) and late (D14) lactation. While we cannot exclude that uptake occurs on other days, we believe this is unlikely. Fifth, much of this research involves *in vitro* or non-physiological *in vivo* analysis (7). In our study, we never detect any significant evidence of uptake (Fig. 3 of Ref. 2). Although we cannot exclude that a low miR-375 copy number remains undetectable, analysis of the highly endocytotic liver reveals no difference in target gene expression (Fig. 4 of Ref. 2), demonstrating that low copy number is unlikely to lead to canonical miRNA gene regulation.

Alexandra Title, Remy Denzler, and Markus Stoffel[1]
Institute of Molecular Health Science, Swiss Federal Institute of Technology in Zurich (ETH Zurich), Otto-Stern Weg 7, 8093 Zurich, Switzerland

1. Zempleni, J., Baier, S., and Hirschi, K. (2015) Diet-responsive microRNAs are likely exogenous. *J. Biol. Chem.* **290,** 25197
2. Title, A. C., Denzler, R., and Stoffel, M. (2015) Uptake and function studies of maternal milk-derived microRNAs. *J. Biol. Chem.* **290,** 23680–23691
3. Wolf, T., Baier, S. R., and Zempleni, J. (2015) The intestinal transport of bovine milk exosomes is mediated by endocytosis in human colon carcinoma Caco-2 cells and rat small intestinal IEC-6 cells. *J Nutr.* 10.3945/jn.115.218586
4. Witwer, K. W., and Hirschi, K. D. (2014) Transfer and functional consequences of dietary microRNAs in vertebrates: concepts in search of corroboration. Negative results challenge the hypothesis that dietary xenomiRs cross the gut and regulate genes in ingesting vertebrates, but important questions persist. *Bioessays* **36,** 394–406
5. Xie, J., Ameres, S. L., Friedline, R., Hung, J. H., Zhang, Y., Xie, Q., Zhong, L., Su, Q., He, R., Li, M., Li, H., Mu, X., Zhang, H., Broderick, J. A., Kim, J. K., Weng, Z., Flotte, T. R., Zamore, P. D., and Gao, G. (2012) Long-term, efficient inhibition of microRNA function in mice using rAAV vectors. *Nature Methods* **9,** 403–409
6. Ameres, S. L., Horwich, M. D., Hung, J. H., Xu, J., Ghildiyal, M., Weng, Z., and Zamore, P. D. (2010) Target RNA-directed trimming and tailing of small silencing RNAs. *Science* **328,** 1534–1539
7. Bryniarski, K., Ptak, W., Martin, E., Nazimek, K., Szczepanik, M., Sanak, M., and Askenase, P. W. (2015) Free extracellular miRNA functionally targets cells by transfecting exosomes from their companion cells. *PLoS ONE* **10,** e0122991

DOI 10.1074/jbc.L115.688358

[1]E-mail: stoffel@biol.ethz.ch