Dear Editor,

An initial positive finding of mammalian uptake of dietary foreign microRNAs (miRNAs), or xenomiRs, has been followed by a raft of negative studies [reviewed in (1)]. However, several questions remain, including whether gut health affects uptake, and whether specific situations, such as breastfeeding of neonates, might facilitate miRNA transfer. In an interesting study, Baier et al. (2) orally administered bovine milk to healthy human volunteers and observed transient increases in 2 miRNAs (miRs)-29b and -200c, in participant blood. Since 1) these miRNAs are 100% identical from cow to human, 2) an apparent dose response was observed, and 3) a third miRNA, miR-1, did not change, the authors concluded that the increases were “unambiguous evidence” of absorption into human circulation of bovine miRNAs.

As a first reaction to this conclusion, one might note that identity of most cow and human miRNAs greatly complicates assignment of origin, especially as the assayed miRNAs are either abundant (miR-29b, miR-200c) or virtually absent (miR-1) in both milk and blood. In the context of a genetic miRNA knockout, ambiguity might be avoided. In just such a study, Snow et al. (3) fed a miR-21–replete diet to miR-21 knockout mice and observed no transfer. However, these considerations aside, a simple alternative explanation for the finding also presents itself: response of specific endogenous miRNAs to milk intake, mediated by the products of lactose breakdown or other diet-derived factors. Glucose upregulates miR-29 family members in a variety of cells and tissues (4–7), an increase of particular interest in nutrition studies because miR-29 contributes to regulation of insulin signaling. Glucose-responsive cells also have a higher level of a miR-200 family member than non-responsive cells (8), suggesting that miR-200c may be glucose-sensitive like miR-29b. Nor is glucose the only dietary factor at play. Fatty acids were recently reported to increase miR-29 (9), for example, and amino acids, too, can stimulate changes in miRNA profile.

What of miR-1, the negative control miRNA that was found to be unchanged in response to milk intake? Baier et al. suggested that the low concentration of miR-1 in milk was insufficient to produce a noticeable change in the circulating molecule. Yet miR-1, unlike miR-29, is restricted largely to muscle cells. These cells, under normal circumstances, are unlikely to contribute as significantly to the circulating extracellular miRNome as blood cells, which along with endothelia contribute the overwhelming bulk of blood exRNAs. miR-29 family members are found in many cells, including blood cells (10). Thus, even if miR-1 were confirmed to be as glucose-sensitive as miR-29, its levels in circulation would not necessarily respond robustly to dietary intake.

To an extent that remains unclear, some of the data not shown by Baier et al., including a small mouse study finding moderate depletion of miR-29b after long-term feeding with an “exosome-depleted” diet, might answer these questions. Future work in this area, in vitro and in vivo, will need to include controls to help detect the potential effects of dietary vesicular structures that are not mediated by RNA.

As we approach a decade after the first findings on circulating RNAs, many unknowns remain: normative profiles, diurnal variation, response to various dietary factors, and much more. The work of Baier et al. certainly furthers our understanding of dietary xenomiRs. Baier et al. joined many others (1) who did not observe uptake of plant xenomiRs by healthy individuals, in their case with a broccoli feeding study. Similarly, I would submit that the simplest explanation for post-prandial increases in specific animal miRNAs—in circulation and in defined cell populations—is response to nutrients, not direct RNA transfer.

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References
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Reply to Witwer1,2

Dear Editor,

We appreciate Witwer’s (1) interest in our work on the bioavailability of milk microRNAs (miRNAs) in humans and mice (2). Witwer raises concerns that the postprandial increases in plasma miRNA (miR)-29b and miR-200c concentrations after a milk meal might be due to milk-dependent endogenous miRNA synthesis, as opposed to absorption from milk (1). Although it is certainly a possibility that dietary compounds alter the expression of genes coding for miRNAs (3), we maintain our original position that mammals absorb bovine miRNAs from milk. This position is based on the following lines of evidence. First, milk miRNAs are encapsulated in exosomes (4–6). There is consensus that human cells transport human exosomes by using carrier-mediated processes and phagocytosis (7–10). Recently, we observed for the first time that human intestinal cells also transport milk exosomes through a process that follows saturation kinetics, is inhibited at low temperatures (4°C), and is inhibited when surface proteins are removed from exosomes or intestinal cells by proteinase K treatment (T. Wolf, S. R. Baier, J. Zempleni, unpublished data, 2014). Considering that these studies were conducted by using fluorophore-labeled exosomes (7), endogenous vesicles and miRNAs were eliminated as possible confounders. Witwer was not aware of these transport studies at the time of writing his letter.

Second, we fed mice milk miRNA-depleted diets or milk miRNA–sufficient control diets (2). The content of compounds other than miRNAs was identical in both diets [Supplemental Table 2 in (2)]. The plasma miR-29b concentrations were 61% lower in the deficient group than in sufficient controls. Because the content of nutrients other than miRNAs was identical in both diets, the 61% decrease in plasma miR-29b can only be explained by an insufficient supply of exogenous, dietary miRNAs.

Third, Witwer points out that Snow et al. (11) did not observe a transfer of dietary miR-21 in miR-21 knockout mice. We estimated the dietary supply of miR-21 in the studies by Snow et al. and arrived at the conclusion that, even for the lowest dose of milk used in our studies (a mere 0.25 L) (2), the dietary intake (normalized for body weight) of miR-29b exceeded that of miR-21 in studies by Snow et al. by >100 times. If we decreased the amount of milk in our human feeding studies by a factor of 100 to 2.5 mL, we also would not anticipate observing an increase in miR-29b plasma concentrations [see Fig. 1 and Table 1 in (2)]. However, a dose that low lacks nutritional relevance. As a side note, an absence of a postprandial increase in any dietary compound or drug in the peripheral circulation must not be confused with zero bioavailability, because these compounds might have been degraded or stored in the intestinal mucosa or liver in a process referred to as first-pass elimination (12).

Witwer raises some points that we fully endorse. Like him, we believe that the bioavailability of plant miRNAs is negligibly small in humans. We also agree that many unknowns remain to be addressed in the field of dietary miRNAs and are currently working toward creating protocols for distinguishing endogenous and exogenous (milk-borne) miRNAs in human body fluids and tissue samples.

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References


1 Supported by funds provided through the Hatch Act. Additional support was provided by the National Institute of Food and Agriculture (multistate grant W0002) and the NIH (RO1 DK063945 and RO1 DK077816).
2 Author disclosures: S. R. Baier, C. Nguyen, F. Xie, J. R. Wood, and J. Zempleni, no conflicts of interest.
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