Unraveling the digestion of milk protein$^{1,2}$

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Milk has often been touted as the perfect food—designed by Mother Nature not just to nourish the newborn but, as we now know, to “educate” them through various mechanisms for promoting infant health (1). For example, human milk contains an array of complex oligosaccharides known to promote beneficial intestinal microbiota colonization and bioactive peptides that exhibit a range of biological activities from neuroactive to antimicrobial. These peptides are generally encrypted in larger proteins that are released only following hydrolysis during intestinal digestion (2). Milk contains 2 major protein groups, caseins and whey proteins, the latter of which is traditionally a valuable by-product of cheesemaking. Both types are highly valuable protein sources for human nutrition and are also major potential sources of bioactive peptides that can elicit a variety of physiologic effects in humans. However, most studies to date (including those from our own group) have concentrated on the in vitro bioactivity of isolated peptides and then speculated on a potential role in human health, nutrition, and disease. In contrast, there are only a few examples in which digestion of milk protein has been studied in detail in vivo in humans (3, 4). In this sense, what happens during digestion within the milieu of the human intestine has been more of a “black box” given the sheer number and bioactivity of peptides that could be generated from complex protein sources such as milk.

In this issue of the Journal, Boutrou et al (5), a research team from the Institut National de la Recherche Agronomique in France, have attempted to decipher the complexities of the breakdown to peptides as a consequence of the ingestion of milk protein and in so doing unravel what is happening in this black box. To accomplish this, the milk protein was labeled with stable isotope and fed to humans, who were subsequently sampled from the jejunum through a nasogastric tube. The peptides were subsequently identified by nano liquid chromatography–electrospray ionization tandem mass spectrometry and quantified. Through use of this approach, the derivative peptides from digestion could be mapped onto their source milk proteins so that a picture of in vivo digestion could be established. The main findings of the study were as follows:

1) Whey proteins were completely released from the stomach within 3 h, whereas it took at least twice that time to allow gastric emptying of casein to occur.

2) The size of peptides generated from both types of protein also varied considerably. In general, digestion of casein gave rise to medium-sized peptides in the range of 750–1050 Da, whereas the whey-derived peptides were larger (1050–1800 Da) and fewer.

3) Digestion gave rise to a whole host of previously identified bioactive peptides including casomorphins and angiotensin-converting enzyme (ACE)–inhibitory and antithrombotic peptides. Moreover, some of these peptides were found to be present at concentrations likely to exhibit some biological activity.

There are some unanswered questions in the study, however—the main one being why there are many regions of the proteins from which few or no peptides of significant size were detected. The authors suggest that these were possibly hotspots for digestion and were almost completely digested or, alternatively, that any peptides generated were simply too large to be analyzed—2 very different explanations with completely differing consequences from a nutritional perspective. In addition, although some bioactive peptides might reach a concentration at which they are biologically active in the lumen, they still have to be absorbed into the bloodstream to exert a biological effect in vivo. An example given by the authors is the presence of ACE-inhibitory peptides at 1.5 times their half maximal inhibitory concentration (IC$_{50}$) in the lumen, but it is estimated that these peptides would have an absorption rate of 0.1–1%. However, it is probably even a lot more complicated than that, considering that ACE inhibition itself would be due not to a single peptide but rather to a complex mixture of peptides, each containing some biological activity. Undoubtedly, the same complication would arise for other types of bioactive peptide combinations in which there may be a number of molecules contributing to the biological effect, not to mention the possible presence of peptides that might counteract this effect. In the case of antimicrobial antagonism, for example, milk protein is known to contain a number of antimicrobial peptides, which when released through digestion could act synergistically to give a more (or less) potent effect. Nonetheless, even given these considerations, we feel that the evidence provided in this article indicating that certain known bioactive peptides are reaching concentrations in the intestine, where they potentially could be having some physiologic effect,
is a significant finding. However, as always, one would need to be cautious in terms of what conclusions can be drawn from such observations, especially with respect to the physiologic consequences mediated by individual components of what are very complex peptide mixtures. Such considerations become even more complicated when one takes into account the whole diet and not just an isolated aspect, such as milk protein. In the words of Alexander Pope (1688–1744), “a little knowledge [can be] a dangerous thing,” and one needs to consider the impact of the whole food and not just the released peptides in terms of human nutrition and biological effects.

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REFERENCES