Characterizing the *in vitro* digestion and biological activity of bovine casein variants

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Bovine caseins are a well-known source of bioactive peptides that exert a number of potentially health-enhancing effects, such as immune modulation, antimicrobial, antithrombotic, antihypertensive, opioid, and mineral binding. Bioactive peptides are encrypted within the primary structure of proteins, and may be released through various types of enzymatic action, i.e. the targeted action of microbial or plant derived enzymes; the action of microbial enzymes during fermentation; or the action of digestive enzymes, either *in vitro* or in the gastrointestinal tract. To investigate differences in their digestion and bioactive potential four variants of β-casein (A^1 ,A^2, B, and I), and three variants of κ-casein (A, B, and E) were purified from the milk of cows that were homozygous for these variants. The caseins were subjected to *in vitro* gastrointestinal digestion, and the resulting hydrolysates were characterized using various techniques. Obvious differences in digestion patterns were found, and as a consequence of amino acid substitutions, unique peptides were generated from some variants. Three such peptides from β-casein variants were synthesized (VYPFPGPIHN, VYPFPGPIPN, and TER). All hydrolysates and peptides were assessed for antioxidative capacity, and angiotensin-1 converting enzyme (ACE) inhibition. Extent of digestion had a significant effect on both antioxidative capacity and ACE inhibition, with some variants reaching higher levels than others. The three synthesized peptides also showed differences in both activities. TER (β-casein variant B, f[120-122]) showed a moderate ACE inhibitory capacity with

an IC_{50} of ≈90 μM, slightly lower than that of VYPFPGPIHN (β-casein variants A^1 and B, f[59-68]), which was ≈123 μM, whereas that of VYPFPGPIPN (β-casein variants A^2 and I, f[59-68]) was significantly higher at ≈650 μM. TER had no antioxidative capacity, however VYPFPGPIHN and VYPFPGPIPN did possess antioxidative capacities differing slightly. These investigations show that genetic variation at the casein locus has potential implications for the digestion and bioactive potential of bovine caseins. In addition, the novel ACE inbitory peptide TER was derived from the in vitro digestion of β-casein variant B.