Angiotensin Converting Enzyme-1 inhibitory activity of milk proteins evaluated after *in vitro* digestion and peptidomic analysis

Carlotta Giromini1, Raffaella Rebucci1, Gabriella Tedeschi2, Tamil Selvi Sundaram1, Federica Cheli1, Antonella Baldi1.

1Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, University of Milan, Italy;
2Dipartimento di Medicina Veterinaria, University of Milan, Italy

Corresponding author: carlotta.giromini@unimi.it

Milk proteins are relevant sources of bioactive peptides. However, many hurdles still exist regarding the widespread utilization of milk protein-derived bioactive peptides as they may be degraded during gastrointestinal digestion. A crucial issue in this field is the demonstration of a cause-effect relationship, from the ingested intact form to the bioactive form.
The aim of this study was to study *in vitro* digestion, digestibility (IVD, using two different hydrolysis methods) and Angiotensin Converting Enzyme-1 inhibitory activity (ACE-1i) of milk and plant proteins (used as control). Based on ACE-1i effect, a peptidomic and proteomic profile analysis was performed on permeate and retentate samples.
In particular, milk and plant protein samples were *in vitro* digested and the total digest was filtered using a 3KDa membrane. A permeate fraction (<3KDa) and retentate fraction (>3KDa) were obtained. ACE-1i activity was measured as the ability of protein fractions (pre-digested, permeate and retentate) to decrease the hydrolysis of furanacroloyl-Phe-Glu-Glu (FAPGG) synthetic substrate for ACE enzyme. Furthermore, permeate were characterized by LC-nano ESI MS/MS using a shotgun-peptidomic approach, whereas retentate was further trypsin-digested prior the analysis with mass spectrometry using a shotgun-proteomic approach.
We found a positive correlation among the IVD methods tested ($P<0.05$; r=0,85). Milk proteins exhibiting higher values of IVD (>82.5%) with both methods used, compared with plant proteins. Milk proteins after *in vitro* digestion exhibited a significant increase in ACE-1i ($P<0.05$) (> 23.91 ± 0.64%) compared with plant protein tested (10.40 ± 1.07 %). Based on proteomic and peptidomic analysis performed, specific peptides associated with anti-hypertensive and ACE-1i effect have been identified in permeate and retentate of milk proteins. Our results demonstrated that milk and plant proteins are highly digestible and, in particular milk proteins may represent valuable sources of ACE-1i and anti-hypertensive peptides which may confers the ability to decrease blood pressure *in vivo*.