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**Brush border digestion:  
Development of a physiologically relevant *in vitro*  
model.**

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## Abstract

The majority of current *in vitro* digestion methods either exclude the small intestinal brush border (BB) phase of digestion or do not incorporate the entire array of BB enzymes that are required to achieve terminal endogenous digestion *in vivo*. Accordingly, the digestate, and its derivatives, may not be representative of the digestive process *in vivo*. In order to improve the fidelity of the *in vitro* digestion process this thesis developed a physiologically relevant small intestinal BB phase using enzymes isolated from rat small intestinal mucosal tissue. The activities of BB enzymes were assessed and compared with known values, and under conditions physiologically representative of the small intestine. Although there were significant differences in BB enzyme activities depending on pH, enzyme solubilisation, and upon prolonged exposure to biliopancreatic secretions the BB preparation was deemed suitable for use in an *in vitro* digestion method.

A rationale for the composition of the BB digestive phase was developed based on published physiological data, and was validated using glycosylated polyphenolic compounds as substrates. Liquid chromatography mass spectrometry (LC-MS) was used to assess the derivatisation products of BB digestion. In the absence of biliopancreatic secretions the onion flesh polyphenolic compounds quercetin-4'-glucoside and isorhamnetin-4'-glucoside, but not quercetin-3-glucoside or quercetin-3,4'-diglucoside were hydrolysed. The positive control quercetin-3-glucoside was hydrolysed, and the negative control quercetin-3-rutinoside was not hydrolysed. The deglycosylation of quercetin-3-glucoside was monitored under conditions representative of the small intestine, *i.e.* incorporating bile and pancreatin, while at the appropriate pH. Quercetin-3-glucoside was significantly deglycosylated in BB treatments (no treatment or pancreatin alone) compared to BB treatments with bile (bile alone or pancreatin and bile).

The mammalian digestive system is equipped to hydrolyse macronutrients from their polymeric form through to monomers and oligomers suitable for absorption across the epithelial layer. As such the inactivation or degradation of some BB enzymes during the BB digestive phase by bile or pancreatin was not unexpected, and does not preclude its use as an *in vitro* tool in the future.



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## List of acronyms

<b>Acronym</b>	<b>Protein name</b>	<b>Description / Function</b>
<b>ACE</b>	Angiotensin converting enzyme	Brush border peptidase, peptide hormone
<b>ALP</b>	Alkaline phosphatase	Hydrolyzes phosphate residues, regulatory
<b>APA</b>	Aminopeptidase A	Brush border peptidase
<b>APN</b>	Aminopeptidase N	Brush border peptidase
<b>APP</b>	Aminopeptidase P	Brush border peptidase
<b>AS</b>	Alkaline sphingomyelinase	Sphingolipid hydrolysis
<b>BB</b>	Brush border	Intestinal microvillar membrane
<b>BBMV</b>	Brush border membrane vesicle	Vesicle of microvillar membrane shed from the tips of microvilli
<b>BSAL</b>	Bile salt activated lipase	Lipase/Sterol esterase
<b>CPA</b>	Carboxypeptidase A	C-terminal pancreatic peptidase
<b>CPB</b>	carboxypeptidase B	C-terminal pancreatic peptidase
<b>DP1</b>	Dipeptidase 1	Brush border dipeptidase
<b>DPPIV</b>	Dipeptidylpeptidase IV	N-terminal dipeptidase
<b>GGT</b>	$\gamma$ -glutamyl transpeptidase	Brush border peptidase
<b>GPI</b>	Glycophosphatidylinositol	Covalently attached glycolipid that anchors the protein to the membrane
<b>LPH</b>	Lactase-phlorizin hydrolase	Enzyme complex: $\beta$ -glucosidase/glycosylceramidase
<b>MEP</b>	Meprin A subunit $\beta$	Brush border endopeptidase
<b>MGAM</b>	Maltase–glucoamylase	Enzyme complex: $\alpha$ -1,4-glucosidase
<b>NEP</b>	Nepriylsin	Brush border endopeptidase
<b>NC</b>	Neutral ceramidase	Sphingolipid hydrolysis
<b>NTC</b>	Sodium taurocholate	Bile salt
<b>PTL</b>	Pancreatic triacylglycerol lipase	Lipase
<b>PLA2</b>	Phospholipase A2	Phospholipid hydrolysis
<b>PLB1</b>	Phospholipase B1	Phospholipid hydrolysis
<b>RER</b>	Rough endoplasmic reticulum	Cytosolic organelle
<b>SC</b>	Soluble cytosolic	Cytosolic enzyme/protein that is soluble
<b>SI</b>	Sucrase-isomaltase	Enzyme complex: $\alpha$ -1,4 and $\alpha$ -1-6-glucosidase



