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 derivitives, 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-34′-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

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