STANDARDISATION OF *IN VITRO* CARBOHYDRATE DIGESTION METHODS FOR PREDICTING THE RELATIVE GLYCEMIC RESPONSE TO FOODS

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ABSTRACT

Global incidence of type II diabetes is driving the need for communication, via food-labelling, of the likely glycemic effects of foods. *In vivo* methods for measuring the glycemic response are costly, time-consuming and hence unsuitable for routine food analysis. *In vitro* carbohydrate digestion methods offer an alternative to *in vivo* testing. Foods are incubated sequentially with pepsin and pancreatin under simulated *in vivo* conditions and the pattern of sugar release used as a predictor of the food's likely glycemic effect. *In vitro* methods are well-suited to routine food analysis since they are inexpensive, high-throughput and yield highly precise results. Application of *in vitro* technology is hindered by the lack of standardised methodology. Countless *in vitro* methods are described in the literature. All differ in their approach to replicating *in vivo* conditions. It is not known what effect such differences in methodology might exert on relative estimates of glycemic response.

A systematic investigation was undertaken to characterise the relative effect of method variables on subsequent *in vitro* digestion results, using five standard test foods. Variables investigated include mode of comminution, pepsin inclusion versus omission, amylolytic enzyme concentration, incubatum pH and stirring method. A rudimentary framework for a standardised *in vitro* method is proposed. Comminution and stirring were the method factors most influential to *in vitro* starch digestion kinetics. Thus, the standardised method features differing approaches to comminution and incubatum stirring depending on the structural properties of the food to be analysed.

In vitro methods, in their current format, do not account for the effect of gastric emptying rate on the glycemic response. The glycemic response and gastric emptying rate of ^{13}C-labelled flatbreads containing either 5, 15 or 30 % fat, known to slow gastric emptying, was measured in ten healthy subjects via a GI test and breath testing. The objective was to obtain *in vivo* data for gastric emptying that might be applied as a correction to parallel *in vitro* digests of the flatbreads improving their predictive power. Gastric emptying rate reduced significantly with increased flatbread fat content. There was no difference in the glycemic response to each flatbread. Due to the lack of glycemic effect *in vivo*, no adjustments to *in vitro* curves could be made.

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PUBLICATIONS, PRESENTATIONS ARISING FROM THIS THESIS

Glycemic Carbohydrates: Standardisation of In Vitro Methods
J W Woolnough, J A Monro, A R Bird, C S Brennan
Asia Pacific Journal of Clinical Nutrition
Volume 16 Supplement 3, Page S47, 2007

Simulating Human Carbohydrate Digestion In Vitro: a Review of Methods and the Need for Standardisation
J W Woolnough, J A Monro, C S Brennan, A R Bird
International Journal of Food Science and Technology
Volume 43 Issue 12, Pages 2245 – 2256, 2008

Impact of Guar and Wheat Bran on the Physical and Nutritional Quality of Extruded Breakfast Cereals
M A Brennan, I Merts, J A Monro, **J W Woolnough**, C S Brennan
Starch/Stärke
Volume 60 Issue 5, Pages 248 – 256, 2008

The Effect of a Brief Salivary α-Amylase Exposure During Chewing on Subsequent In Vitro Starch Digestion Curve Profiles
J W Woolnough, A R Bird, J A Monro, C S Brennan
International Journal of Molecular Sciences
Volume 11 Issue 8, Pages 2780 – 2790, 2010

Predicting Glycemic Response In Vitro – Oral Presentation
NZIFST Annual Conference
Wellington, NZ
June 2007

Glycemic Carbohydrates: Standardisation of In Vitro Methods – Oral Presentation
Joint NZ & Australian Nutrition Societies Conference and Annual Scientific Meeting
Auckland, NZ
December 2007

Physiological Determinants of the Glycemic Response to Foods – Oral Presentation
CSIRO Food Futures Flagship Annual Scientific Meeting
Canberra, ACT
March 2010

A Comparison of the Effect of Comminution Techniques and Stirring Methods on Subsequent In vitro Starch Digestion Kinetics
J W Woolnough, C S Brennan, J A Monro, A R Bird
2011 – yet to be submitted

The Effect of Fat on Gastric Emptying Rate and the Glycemic Response to Flatbreads: Reflecting this Effect In Vitro
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%dose/h	Per cent recovery of the original ^{13}C dose administered per hour
AACC	American Association of Cereal Chemists
abs	Absorbance
AC	Available carbohydrate
A_{GR}	Amplitude (mmol/L) of the glycemic response
AMG	Amyloglucosidase
ANOVA(s)	Analysis of variance
AUC	Area under the curve
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CPDR	Cumulative percentage of the original dose (^{13}C) recovered
DNS	3,5-dinitrosalicyclic acid
DF	Dietary fibre
DP	Degree of polymerisation
DS	Digestible starch
EE	End-over-end inverter
EE, fast	End-over-end inverter, fast treatment (2 rotations every 30 seconds)
EE, slow	End-over-end inverter, slow treatment (1 rotation every 30 seconds)
ESA	Enzyme solution A
FAO	Food and Agriculture Organisation
g	Gram(s)
GGEs	Glycemic glucose equivalents
GI	Glycemic index
GIP	Glucose-dependent insulinotropic polypeptide
GL	Glycemic load
GLP-1	Glucagon-like peptide 1
GR	Glycemic response
h	Hour(s)
HI	Hydrolysis index
HPLC	High-performance liquid chromatography
IAUC	Incremental area under the curve
imm	immediate

inv	Invertase
min	Minute(s)
mmol/L	Millimoles per litre
MS	Magnetic stirrer
MS, fast	Magnetic stirring, fast treatment (260 rpm)
MS, slow	Magnetic stirring, slow treatment (130 rpm)
NSP(s)	Non-starch polysaccharides
PG	pregelatinised (starch)
RDS	Rapidly digestible starch
ReS	Reducing sugar(s)
rpm	Rotations per minute
RS	Resistant starch
s	Seconds
sal	Salivary
SD	Starch digestion
SDS	Slowly digestible starch
SE	Standard error of the mean
SEM	Scanning electron microscopy
SWB	Shaking water bath
SWB, fast	Shaking water bath, fast treatment (140 strokes/min)
SWB, slow	Shaking water bath, slow treatment (70 strokes/min)
T_0	Time zero
T_{20}	20 min of pancreatic digestion
T_{120}	120 min of pancreatic digestion
$T_{1/2}GE$	Gastric half-emptying time (min)
T_{GRP}	Time (min) to glycemic response peak
T_{lag}	Gastric emptying lag time (min)
T_{max}	Peak gastric excretion time (min)
TS	Total starch
U	Units (of enzyme)
WHO	World Health Organisation