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Report on the accuracy and precision of the CSIRO laboratory method for predicting Glycemic Index

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Contents

List of Figures

List of Tables

1. SUMMARY

The CSIRO in vitro assay for predicting glycemic index is accurate, robust and precise, with both high repeatability and reproducibility. A large variety of foods, including commercially processed products, have been tested and the results correlate very closely with values obtained in human studies using standardised in vivo testing protocols. Also, classification accuracy is consistent with that achieved by human studies. Statistical analyses demonstrate that the methodology yields GI values which are essentially no different from those obtained using the human (in vivo) testing procedure.

2. INTRODUCTION

The Glycemic Index (GI) is a physiological construct which ranks foods according to their ability to raise blood glucose concentration. As such, determination of the GI of a food item or mixed meal involves testing in humans. However, this approach is laborious, slow and expensive. It is especially unsuited to product development, quality assurance and research purposes which require reasonably precise, high throughput, rapid assays. Indeed, even under the most stringent testing conditions the in vivo GI method lacks the necessary discriminatory power to discern subtle differences in carbohydrate digestibility between test foods. Accordingly, this approach is not at all suitable for product development.

To overcome the practical limitations as well as ethical constraints of conventional GI testing, CSIRO Food Futures Flagship developed a laboratory-based method that allows for rapid prediction of the GI of foods. The in vitro assay in essence models the process of food digestion as typically occurs in the upper gastrointestinal tract of healthy people (Bird, Usher, Klingner, Topping and Morell, unpublished data). More recently, CSIRO Food Futures Flagship automated the method for rapid prediction of GI values for foods. The prototype instrument is based on a modular design that features a central rectangular, temperature-controlled chamber constructed of stainless steel and Perspex. The chamber houses a carousel that holds multiple disposable reaction vessels. The reaction system involves a series of incubations, at physiological pH and temperature that essentially mimics the buccal, gastric and pancreatic phases of food digestion. Protein, fat and starch are sequentially hydrolysed using a combination of hydrolytic enzymes. Representative samples of foods, in a form in which they are normally consumed, are prepared for testing by chopping them using an automated mechanical device. The predicted GI of the sample is calculated as the percentage of available carbohydrate converted to glucose and released during the time course of the incubation. For certain foods an algorithm is applied to improve the predictive power of the assay. Reference foods are included in each assay run for quality control.

The new method offers considerable potential for expediting development of healthier foods and food ingredients, including nutritionally enhanced cereal products and low digestibility starches.

3. VALIDATION PROCESS AND STUDIES

A preliminary assessment against in vivo GI values available in public databases for foods of the same description (Foster-Powell et al., 2002) indicates that the CSIRO method generated results which were similar to those obtained in vivo. An extensive, systematic validation strategy to objectively substantiate the predictive accuracy and reliability of the in vitro procedure was then implemented and a large number of foods comprising a broad range of GI values and food types have been tested to date. Briefly, in vivo and in vitro testing on identical samples was conducted on more than 30 different foods. The foods were selected on the basis that they encompassed as wide a range of GI values as possible and that all the major food type categories were included. Whereas the majority of foods tested were shelf-stable products manufactured by large commercial concerns, a number of staple starchy foods which had to be cooked first before testing were also included. The foods were representative of the type of starchy foods commonly eaten in many industrialised countries and that would meet the requirements for GI testing. Foods containing minimal carbohydrate content were not included in the studies. The GI of each of the reference foods was established using standardised testing procedures (Standards Australia, 2007; International Standards Organisation, 2010) and the studies were performed by trained staff at the CSIRO Clinical Research Unit (Adelaide, South Australia) and the International Diabetes Institute (Caulfield, Victoria). For in vitro GI testing, duplicate samples of reference foods essentially identical to those tested in vivo were used. Appropriate statistical methodology was applied to establish the precision and accuracy of the CSIRO instrument.

4. RESULTS

Validation studies have shown that the in vitro assay has high predictive power:

- Correlation analysis showed that there is a strong relationship between predicted and "true" GI values for the large dataset comprising a wide diversity of food types (r^2>0.8; n = 30; see Figure 1).
- For foods tested to date all GI values were within ± 17 GI units of the corresponding reference (in vivo) values (see Figure 2).
- More than 80% of foods tested were within ± 10 GI units and about 55% were within ± 5 GI units of the corresponding reference values.

Variation in repeated measurements on the same foods using the in vitro method is minor, especially in comparison to the in vivo technique:

- The assay is precise – it has both high repeatability and reproducibility (see Table 1 and 2, respectively).
- Repeatability (within assay precision; intra-assay variability) was excellent especially for homogeneous samples (CV ~2% for homogeneous foods, CV<5% for most heterogeneous foods; Table1).
- Assay reproducibility was also very good (see Table 2). The mean inter-assay CV was 4% and ranged from 2.2% to 10.0% (based on an average of three repeated assays for a total of 19 different test foods).
- Understandably, precision was lower for certain heterogeneous foods. This is largely a consequence of sampling error. Triplicate determinations can be used to improve assay precision if necessary.
- The in vitro assay is an order of magnitude more precise than the in vivo procedure for GI determination (which has a CV of approximately 30%).

The results correlate well with values obtained in humans for the full range of food products that were tested. Classification accuracy for the method was also high, as was precision. The performance parameters of the assay are acceptable given the multiplicity of factors that potentially contribute to analytical error, the small sample size in particular, and the variation and hence imprecision inherent in the reference values derived using the in vivo procedure.

Figure 1 In vitro GI vs in vivo GI

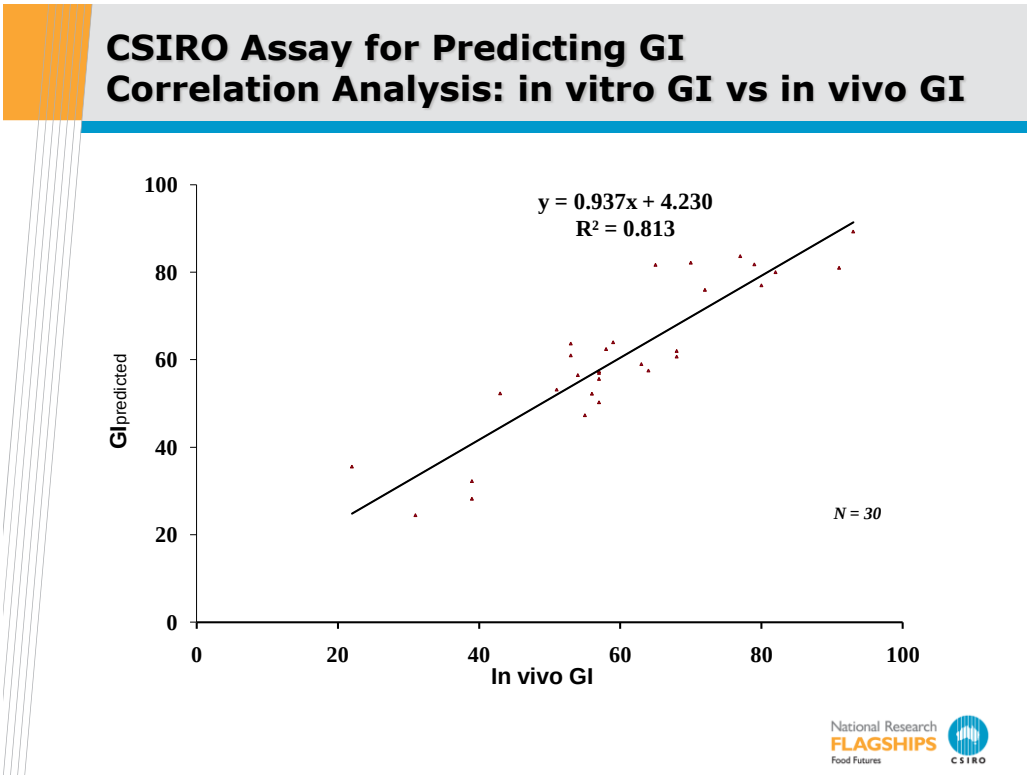

Figure 2 Bland-Altman plot for GI prediction

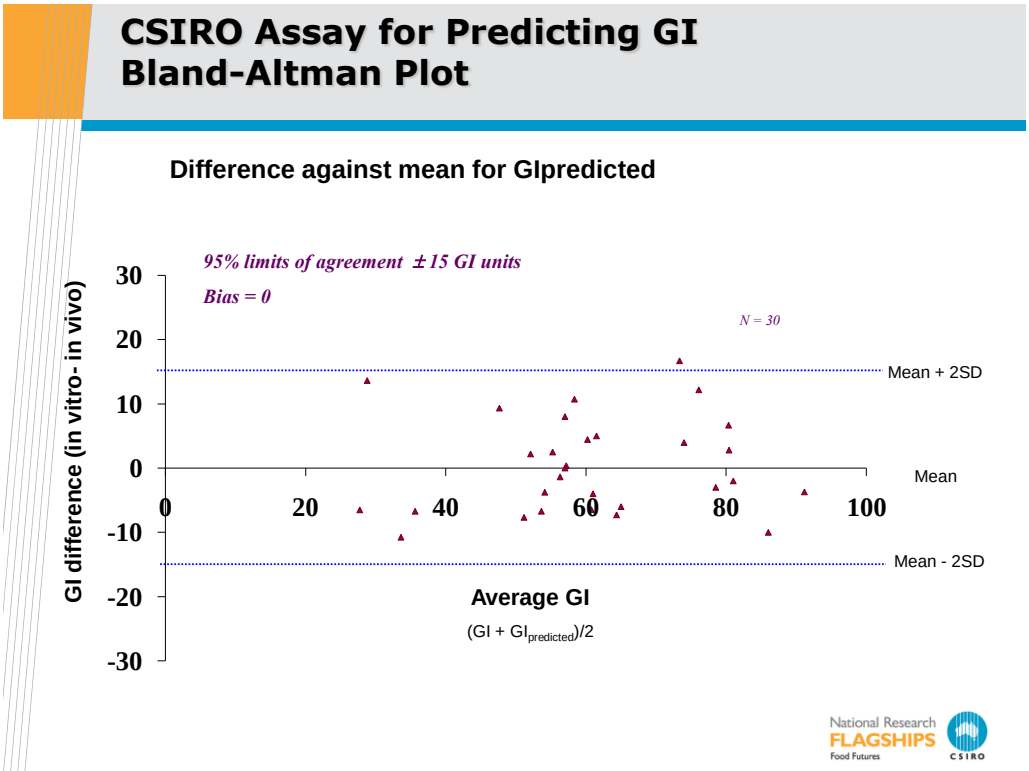

Table 1 Within-assay precision estimates for the in vitro GI assay[1]

Food Group	Product	Mean GI	Intra-assay CV[2]	Range
Breakfast Cereals	Flaked cereal	74	1.5	73-76
	Processed wheat bran	26	1.8	26-27
	Flaked cereal	59	3.2	57-62
	Muesli	53	3.3	50-55
Biscuit	Rice cake	85	1.6	82-86

[1]Average of 8 values for GI measured on the one occasion for 5 reference foods.

[2]Intra-assay coefficient of variation based on n = 8 replicates.

Table 2 Between-assay precision estimates for the in vitro GI assay instrument

Food Group	Product	GI (predicted)	SEM[1]	RSD (%)[2]
Breakfast Cereals	Flaked cereal	82	1	2.2
	Wheat bran	46	1	2.2
	Flaked, high sugar	51	1	3.8
Bread	Wholegrain rye	79	2	4.4
	Wholemeal wheat	80	1	2.4
Biscuit	Rice cake	87	1	2.5
	Shortbread biscuit	62	1	2.2
	Oatmeal biscuit	49	2	6.0
Snack bar	Fruit bar	46	2	8.8
	Muesli bar	90	4	6.1
Snack foods	Savoury product	72	1	4.0
Beans & legumes	Canned beans	59	2	7.5
Pasta	Thin spaghetti	61	1	4.1
Rice	White rice variety	80	2	3.9
	White rice variety	59	0	0.4
Dried Fruit	Sultanas	44	1	2.9

[1]SEM, standard error of the mean.

[2]Residual standard deviation (inter-assay coefficient of variation) is based on between 2 to 3 determinations for each food; individual values used in the calculations are the mean of duplicate determinations.

5. CONCLUSION

The CSIRO in vitro method enables the rapid, reliable and accurate prediction of GI of foods and provides a cheaper and much more precise option to testing in humans. Statistical analyses of the results produced by this method demonstrate close agreement with the corresponding results obtained using the very best testing methods in humans.

This low-cost predictive method offers significant potential for reformulating existing food products and accelerating development of new foods and food ingredients likely to improve the metabolic health and wellbeing of consumers.

REFERENCES

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