Final Report

PVMI

Protocol INQ-2424

DECEMBER 17, 2024
Revised January 2, 2025

INQUIS GLYCEMIC INDEX

GLYCEMIC INDEX DETERMINATION OF:

Huckleberry Gold Potatoes

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Summary

The glycemic index (GI) value of one test food was determined using the standard ISO method (ISO 26642:2010). Ten subjects consumed the test meal and the control meal (25g of Dextrose) twice. The GI values are expressed on the glucose scale where the GI of glucose=100 and white bread=71. The GI values (Mean±SEM) of the foods tested were:

Test Meal	Glycemic Index	GI Category
Dextrose Control	100	High
Huckleberry Gold Potatoes	52±3	Low

* The listed GI value(s) is/are only valid as long as product ingredients, formulation, processing, and/or any other material production factors remain unchanged.

** Using the classification of ISO 26642:2010, products with a glycemic index (GI) less than or equal to 55 are classified as being low GI, those with a GI of 56 to 69 are classified as medium, while those with a GI equal to or greater than 70 are high GI.

Background

The glycemic index (GI) was proposed in the 1980s as a means to classify carbohydrate foods according to their effect on postprandial plasma glucose responses (Jenkins et al, 1981). Low GI foods release their carbohydrate slowly and elicit a lower glycemic response while high GI foods are rapidly digested with a corresponding higher glycemic response. The rate of glucose absorption and extent and duration of elevated plasma glucose levels induce many hormonal and metabolic changes that may affect health or disease parameters. Low GI diets may help in weight maintenance (Larsen et al, 2010) and weight loss (Ebbeling et al, 2003) in addition to being protective against chronic disease such as diabetes (Salmeron et al, 1997a,b), heart disease (van Dam et al, 2000; Lui et al, 2002) and certain cancers (Augustin et al, 2001; Francheschi et al, 2001). Interest in identifying low GI foods and the food factors responsible for the low GI of foods has therefore increased. Several food factors have been identified that influence *in vivo* absorption and therefore potentially the GI of a food or meal. Some of these factors include: the gross matrix structure, cell wall and starch structure (i.e. ripening), amylose to amylopectin ratio, and viscous fibre (Brand et al, 1985).

The methodology for determining the GI is now well established (Wolever et al, 1991; Brouns et al, 2005) and has been shown to be reproducible by laboratories across the world (Wolever et al, 2003, 2008). The protocol used by INQUIS Clinical Research (INQUIS) to determine the GI of foods adheres to or exceeds the methods specified by the International Organization for Standardization in: ISO 26642:2010 “Food products - Determination of the GI and recommendation for food classification”.

Study Objective: The objective of this study was to determine the GI of Huckleberry Gold Potatoeses (Pot).

Methods

Subjects

Inclusion criteria: Subjects were males or non-pregnant females aged 18-75 years and in good health.

Exclusion and withdrawal criteria: Subject with a known history of AIDS, hepatitis, diabetes or a heart condition. Subject taking medication or with any condition which might, in the opinion of Dr. Wolever either make participation dangerous to the subject or to others or affect the results.

Number of Subjects: A total of ten (10) subjects were studied. Using the t-distribution and assuming an average coefficient of variation (CV) of within individual variation of incremental area under the plasma glucose curve (IAUC) values of 25%, n=10 subjects has 80% power to detect a 33% difference in IAUC with 2 tailed $p<0.05$.

Protocol

Glycemic Index Testing: The protocol used at INQUIS Clinical Research (INQUIS) for determining the GI of foods follows the methods described in ISO 26642:2010 - "Food products - Determination of the glycaemic index and recommendation for food classification".

The study used an open-label, randomized crossover design. Each subject underwent treatments on separate days, with each subject performing up to 3 tests per week separated by at least one day. On each test day, subjects came to INQUIS (20 Victoria Street, 3rd floor) in the morning after a 10-14 hour overnight fast. Upon arrival, subjects were asked to wash their hands and were then directed to their assigned study visit seat. After being weighed and having two fasting blood samples obtained by finger-prick five minutes apart, the subject then consumed a test meal within 15 minutes. Further blood samples were obtained at 15, 30, 45, 60, 90 and 120 minutes after the start of the test meal. Subjects remained seated quietly during the 2 hours of the test. After the completion of the test they were offered a packed snack to take with them.

Informed Consent

The INQUIS protocol has been approved by the WIRB-Copernicus Group ® (WCG) which meets all the requirements of the US Food and Drug Administration (FDA), the Department of Health and Human Services (DHHS), the Canadian Health Protection Branch (HPB), Canadian Institutes of Health Research (CIHR) and the European Community Guidelines. All subjects provided written informed consent prior to starting the study.

Test Meals

The test meals consisted of a portion of the test food or dextrose containing 25g available carbohydrate (defined as total carbohydrate minus dietary fiber/non-digestible carbohydrates). The portion size of the test food was calculated based on the results of nutrition analysis and ingredients list provided by the

client (Table 1 and Appendix 1). Each subject was given a choice of a beverage (water, coffee or tea with 30ml of 2% milk and non-caloric sweetener if desired) to consume with the test food; the beverage chosen was kept the same for all test meals. The control meal was tested two times by all subjects.

Food preparation:
Dextrose: 27.3g of monohydrous dextrose (ADM Clintose® A Dextrose, Decatur, IL, USA) was dissolved in 250g of water to yield a 25g available carbohydrate solution.

Huckleberry Gold Potatoes: 171.2g of pot was weighed and cut into 1-inch cubes. The Huckleberry Gold Potatoes were then added to a pot of boiling water and cooked for 12 minutes. Once cooked, the water was drained completely from the potatoes. As Huckleberry Gold Potatoes are usually used for making potato salad, the potatoes were left to cool before being stored in the refrigerator overnight. They were then served cold to the subjects.

Table 1. Nutrient content of test meals

Test Meal	Abbr	Weight (g)	Protein (g)	Fat (g)	T CHO (g)	Fibre (g)	AvCHO (g)
Dextrose Control	Dex25	27.3	0	0	25.0	0	25.0
Huckleberry Gold Potatoes	Pot	171.2	4.6	0.3	28.6	3.6	25.0

Blood Samples

Blood samples (300 µL each) were collected into tubes containing heparin to prevent clotting and fluoride to inhibit glycolysis. Upon collection, samples were immediately centrifuged at 2,000g for 5 min and refrigerated. Plasma glucose analysis, using a VITROS 350 analyzer (Ortho Clinical Diagnostics), took place within five days of collection.

Data Analysis

Data were imported by middleware software Instrument Manager (Data Innovations) from the VITROS 350 analyzer into a spreadsheet. IAUC values were calculated using the trapezoid rule, ignoring area beneath the baseline. For the purpose of the IAUC calculation, fasting glucose was taken to be the mean of the first measurement of the glucose concentration at times -5 and 0min. Glucose was measured in the 0min fasting sample 2 times and the data used to determine the standard deviation (SD) of the analytical variation as follows:

$$SD = \sqrt{(\sum d^2/n)}$$

Where d is the difference between the duplicate measures and n is the number of samples analyzed.

The GI was calculated by expressing each subject's glucose IAUC for the test food as a percentage of the same subject's mean IAUC after glucose. Data for GI, IAUC and serum glucose at each time point were analyzed by repeated-measures analysis of variance (ANOVA) examining for the main effect of treatment. After demonstrating significant heterogeneity among the means for the different treatments, the differences between individual means were assessed using Tukey's test to control for multiple comparisons, with the criterion for significance being 2-tailed $p<0.05$. Means which differ by more than the least significant difference (LSD) differ significantly. A second statistical analysis was done on the GI values after excluding those values >2SD above the mean in which case excluded values were replaced by the mean of the remaining values and the error degrees of freedom in the ANOVA was reduced by the number of outliers excluded.

Results

Ten (10) subjects (4 males and 6 females), aged 36.4±16.2 years with a body mass index of 25.9±5.9 kg/m² completed the study. The individual details are shown in Table 2.

Table 2. Subject details

ID	Sex	Ethnicity	Age (yrs)	Height		Weight		BMI (kg/m²)
				(cm)	(in)	(kg)	(lb)	
143	F	White - North American	63	160.6	62.6	98.4	216.5	38.2
299	F	White - European	65	162.5	63.4	90.5	199.1	34.3
611	M	Asian - East	28	167.6	65.4	58.5	128.7	20.8
692	F	Latin - American	34	167.1	65.2	62.3	137.1	22.3
695	M	Asian - East	48	175.3	68.4	84.9	186.8	27.6
738	F	Asian - East	23	160.1	62.4	58.9	129.6	23.0
742	M	Asian - East	25	169.0	65.9	74.6	164.1	26.1
743	F	Asian - South East	24	154.3	60.2	54.0	118.8	22.7
747	F	White - European	27	152.1	59.3	52.0	114.4	22.5
748	M	White - European	27	186.8	72.9	75.6	166.3	21.7
MEAN			**36.4**	**165.5**	**64.6**	**71.0**	**156.1**	**25.9**
SD			**16.2**	**10.2**	**4.0**	**16.3**	**35.8**	**5.9**

Analytical Variation for Plasma Glucose and Within Subject Variation of Reference Food

Analytical variation: Duplicate analysis was performed on 30 samples taken at 0min. The mean ± SD of plasma glucose in these samples was 5.29 ± 0.02 mmol/L for a CV of 0.4%, which is <3.6% and, thus, satisfactory (ISO 26642:2010). The mean±SD for the 30 samples taken at -5 and 0min was 5.30 ± 0.09 mmol/L for a CV of 1.8%, which is greater than analytical variation because it reflects both analytical variation and minute-to-minute variation in plasma glucose.

Within subject variation of reference food: There was no significant effect of order on the IAUC values after the repeated dextrose controls. The mean within-CV of the IAUC values after the 2 repeated dextrose tests was 24.6%. The tests appeared to be technically satisfactory, as judged by the average within-subject variation of glycemic responses for the repeated control meals. Values less than 30% are considered to be satisfactory (ISO 26642:2010).

Adverse Events and Protocol Deviations

There was one adverse event: ID 738 experienced cold sores which resolved after the study; this was deemed unrelated to the study product.

There were no protocol deviations.

Plasma Glucose Response

The plasma glucose response to the test meal is shown in Figure 1. Mean fasting plasma glucose was similar before the test meal and controls. The tabulated plasma glucose responses can be viewed on the analysis pages (GICalc Report - INQ-2424 PVMI Data.pdf).

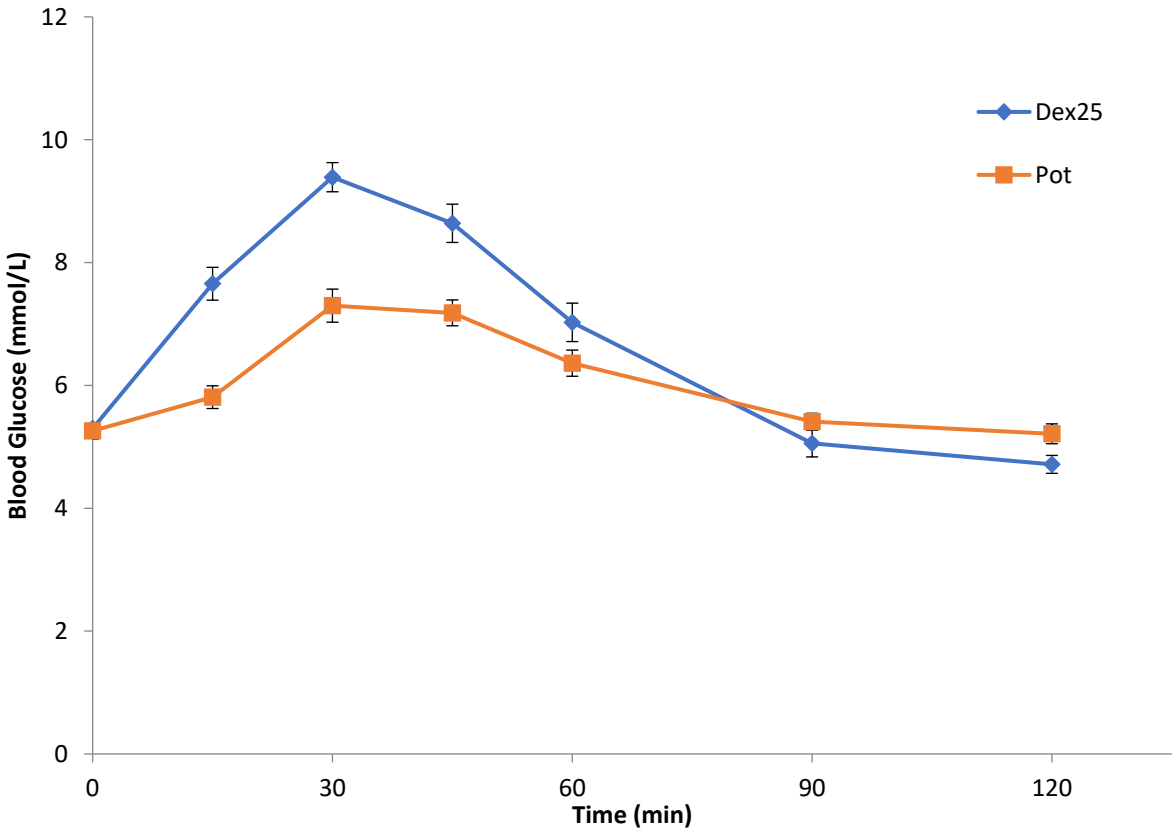

Figure 1: Postprandial glucose responses to: Huckleberry Gold Potatoes (Pot) and the Dextrose control (Dex25) (mean of 2 meals), 10 subjects each. All meals contained 25g of available carbohydrate. Data are expressed as Mean±SEM.

Glycemic Index

The data and calculations for the GI are given in the pdf sheets entitled: GICalc Report – INQ-2424 PVMI Data.pdf. There were no statistical outliers and all data were used to calculate the final GI.

Table 3. Incremental Area Under the Curve, Glycemic Index and Glycemic Index Category

Test Meal	Abbr	IAUC (mmol×min/L)	GI	GI Category
Dextrose	Dex25	190.9±18.6[a]	100[a]	High
Huckleberry Gold Potatoes	Pot	98.6±12.0[b]	52±3[b]	Low

Results are given as Mean±SEM.

* GI values are given after outliers were excluded and by category (Category: high (GI ≥70), medium (56≥GI≤-69), or low,(GI≤55) (ISO 26642:2010)

ab Numbers within the same column with a different letter in the superscript are statistically significantly different (P<0.05).

Areas for Future Scientific Study

This study demonstrated that the PVMI Huckleberry Gold Potato falls into the low GI category. This is unusual as most potatoes tend to fall into the medium or high GI category. As Huckleberry Gold Potatoes are often used to make potato salad, it was cooked and refrigerated overnight before serving it cold to the subjects. Refrigeration may increase starch gelatinization which, in turn, may decrease carbohydrate availability in the small intestine. Whether this is the reason that this potato fell into the low GI category is not known, further studies therefore would be of interest:

GI Testing: There are very few potatoes which fall into the low GI category. It would therefore be very interesting to re-test the Huckleberry Gold Potatoes prepared more conventionally, ie boiled and served hot. If the low GI value persists, this would single this potato out as rather unique.

Metabolic Health Outcomes: Low glycemic index diets have been associated with metabolic health benefits such as improving cholesterol, blood pressure and glucose management. A low GI potato is unusual, and it would be of interest to explore whether extended intake (e.g., 3 weeks) would lead to improvements in metabolic outcomes in healthy individuals or populations at increased risk of developing diabetes.

Microbiome: Gut health is a growing field of nutrition which offers numerous clinical applications. High fibre foods such as potatoes may positively impact the microbiota. A clinical trial exploring the benefits of regular Huckleberry Gold Potatoes consumption on the gut microbiome may be of interest.

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Appendix 1

Ingredients & Macronutrients of test foods

NUTRITIONAL ANALYSIS

Serving Size: 100.0 g

Analytes	Units	Results Per 100g	Amount Per Serving	Test Date	Test Location
Calories USA	Cal	79	79	11/26/2024	
Calories Canada	Cal	75	75	11/26/2024	
Fat	g	0.18	0.18	11/20/2024	
Carbohydrates	g	16.7	16.7	11/26/2024	
Protein (F=6.25)	g	2.7	2.7	11/19/2024	
Ash	g	0.75	0.75	11/19/2024	
Moisture	g	79.69	79.69	11/20/2024	

Appendix 2

Abbreviations

BMI	body mass index
CHO	carbohydrate
CV	coefficient of variation
GI	glycemic index
GL	glycemic load
IAUC	incremental area under the curve
min	minutes
SEM	standard error of the mean
SD	standard deviation
WB	white bread
ANOVA	analysis of variance

Definitions

Glycemic Index

a ranking of carbohydrate-containing foods according to the extent to which they raise blood glucose levels after being eaten

Glycemic Load

a ranking of carbohydrate-containing foods according to the amount of available carbohydrate in the serving and its glycemic index

In vivo

occurring in living organisms (i.e. humans)

Macronutrient

one of the nutritional components of the diet: fat, protein, or carbohydrate

Postprandial

following a meal

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- Measures for low-carbohydrate products

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- Microbiome
- Laxation
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- Appetite, satiety, satiation
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- Physiological markers
- Long term weight loss
- Changes in body composition

CHRONIC DISEASE RISK FACTORS

- Cardiovascular disease risk factors
- Cholesterol-lowering studies
- Diabetes risk factors

QUALITY OF LIFE

- Mood, wellbeing, sleep
- Energy & fatigue
- Cognition, memory, mental acuity

SCIENTIFIC EXPERTISE

- Scientific consulting
- Systematic review meta-analysis (SRMA)
- Manuscript preparation
- Protocol design
- Statistical analysis

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