GI Labs

Final Report Protocol GIL-1651 Revised September 16, 2016

Revolution and Natural Life Specialty Pvt Ltd

Glycemic Index determination of:

SugarLIF - A Herbal Treated Sugar

PREPARED FOR:

September 16, 2016

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Summary

The glycemic index value of SugarLIF was determined in 10 healthy subjects using the standard ISO method (ISO 26642:2010). SugarLif is an herbal treated Sugar, and is a brand owned by Natural Life Specialty Pvt Ltd. The GI values are expressed on the glucose scale where the GI of glucose=100 and white bread=71. The GI value (Mean±SEM) of the food tested was:

Test Meal	Glycemic Index*	GI Category**
Dextrose control (50g)	100	High
SugarLIF	50±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.

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Background

The glycemic index (GI) was proposed in the 1980s as a means to classify carbohydrate foods according to their effect on postprandial blood 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 blood 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 glycemic index 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 Glycemic Index Laboratories 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 glycaemic index (GI) and recommendation for food classification".

Study Objective: The objective of this study was to determine the glycemic index of an herbal treated sugar: SugarLIF a brand owned by Natural Life Specialty Pvt Ltd

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Methods

Subjects

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

Exclusion and withdrawal criteria: Subject less than 18 years old or older than 75 years; 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 blood 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 GI labs 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 Glycemic Index Laboratories (20 Victoria Street, 3rd floor) in the morning after a 10-14 hour overnight fast. 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 snack and then allowed to leave.

Informed Consent

The GI Laboratories protocol has been approved by the Western Institutional Review Board® 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.

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Test Meals

The test meals consisted of a portion of the test food or dextrose containing 50g available carbohydrate (defined as total carbohydrate minus dietary fiber/non-digestible carbohydrates). The portion size of the test foods was calculated based on the results of nutrition analysis provided by the client (Table 1). Each subject was given a choice of a beverage (water, coffee or tea with 30ml of 2% milk and a non-caloric sweetener if desired) to consume with the test meal; the beverage chosen was kept the same for all test meals. The control dextrose meal was tested 2 times by all subjects. Test meals were given in random order.

Food preparation:

Dextrose control: 54.6g of monohydrous dextrose (ADM Clintose® A Dextrose, Decatur, IL, USA) was dissolved in 250g of water to yield a 50g available carbohydrate solution.

SugarLIF: 50g of the herbal treated sugar containing 50g available carbohydrate was weighed out and mixed with 250mL of water and served 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	Dex50	54.6	0.0	0.0	50.0	0	50.0
SugarLIF	SugLIF	50.0	0.0	0.0	50.0	0.0	50.0

Palatability

After consuming the test meal, subjects rated the palatability of the test meal using a visual analogue scale consisting of a 100mm line anchored at the left end by "very unpalatable" and at the right end by "very palatable". Subjects made a vertical mark along the line to indicate their perceived palatability. The distance from the left end of the line to the mark made by the subject is the palatability rating; the higher the value, the higher the perceived palatability.

Blood Samples

Blood samples (2-3 drops each) were collected into 5 mL tubes containing a small amount of anticoagulant (sodium fluoride/potassium oxalate). The samples were mixed by rotating the

tube vigorously and then refrigerated during the testing session. After completion of the test session, samples were stored at -20°C prior to glucose analysis. Blood glucose analysis, using a YSI (Yellow Spring Instruments, OH) analyzer, took place within five days of collection.

Data Analysis

Data were entered into a spreadsheet by 2 different individuals and the values compared to assure accurate transcription. 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 SD of the analytical variation as follows:

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

The glycemic index was calculated by expressing each subject's glucose IAUC for the test food as a percentage of the same subject's mean response after reference meal and, if required, adjusting the GI to the glucose scale where glucose = 100 and white bread =71. 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. After demonstrating significant heterogeneity, 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 least significant difference (LSD) differ significantly.

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Results

Ten (10) subjects (7 males and 3 females), aged 36±13 years with a body mass index of 25.1±2.6 kg/m² participated in 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)	
337	F	Caucasian	48	155.5	60.6	69.1	152.0	28.6
467	M	Caucasian	48	167.0	65.1	80.5	177.1	28.9
558	M	Caucasian/Aboriginal	58	177.0	69.0	79.8	175.6	25.5
609	M	Arab/West Asian	46	165.4	64.5	63.3	139.3	23.1
633	M	Black	30	183.0	71.4	86.7	190.7	25.9
636	F	Chinese	34	156.8	61.2	57.9	127.4	23.5
637	M	Korean	23	177.6	69.3	75.4	165.9	23.9
640	M	Chinese	24	166.2	64.8	73.4	161.5	26.6
645	F	Chinese	29	164.7	64.2	67.8	149.2	25.0
649	M	Chinese/Caucasian	23	182.0	71.0	67.0	147.4	20.2
MEAN			**36.3**	**169.5**	**66.1**	**72.1**	**158.6**	**25.1**
SD			**12.7**	**9.9**	**3.8**	**8.7**	**19.2**	**2.6**

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

Analytical variation: Duplicate analysis was performed on 28 samples taken at 0min. The mean ± SD of blood glucose in these samples was 4.36 ± 0.046 mmol/L for a CV of 1.1%, which is <3.6% and, thus, satisfactory (ISO 26642:2010). The mean±SD for the 30 -5 and 0min samples was 4.39 ± 0.101 mmol/L for a CV of 2.3%, which is greater than analytical variation because it reflects both analytical variation and minute-to-minute variation in blood 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 17.7%. The tests appeared to be technically satisfactory, as judged by the average within-subject variation of glycemic responses for the repeated dextrose tests. Values less than 30% are considered to be satisfactory (ISO 26642:2010).

Adverse Events and Protocol Deviations

All test meals were well tolerated and no adverse events were reported

Palatability

Palatability scores are given in Table 3. There were no significant differences in palatability between the SugarLIF and the control dextrose drink.

Blood Glucose Response

The blood glucose responses to the test meals are shown in figure 1. Mean fasting blood glucose was identical before each test meal within each series. The tabulated blood glucose responses can be viewed on the analysis pages (GICalc Report - GIL-1651 SugarLIF.pdf).

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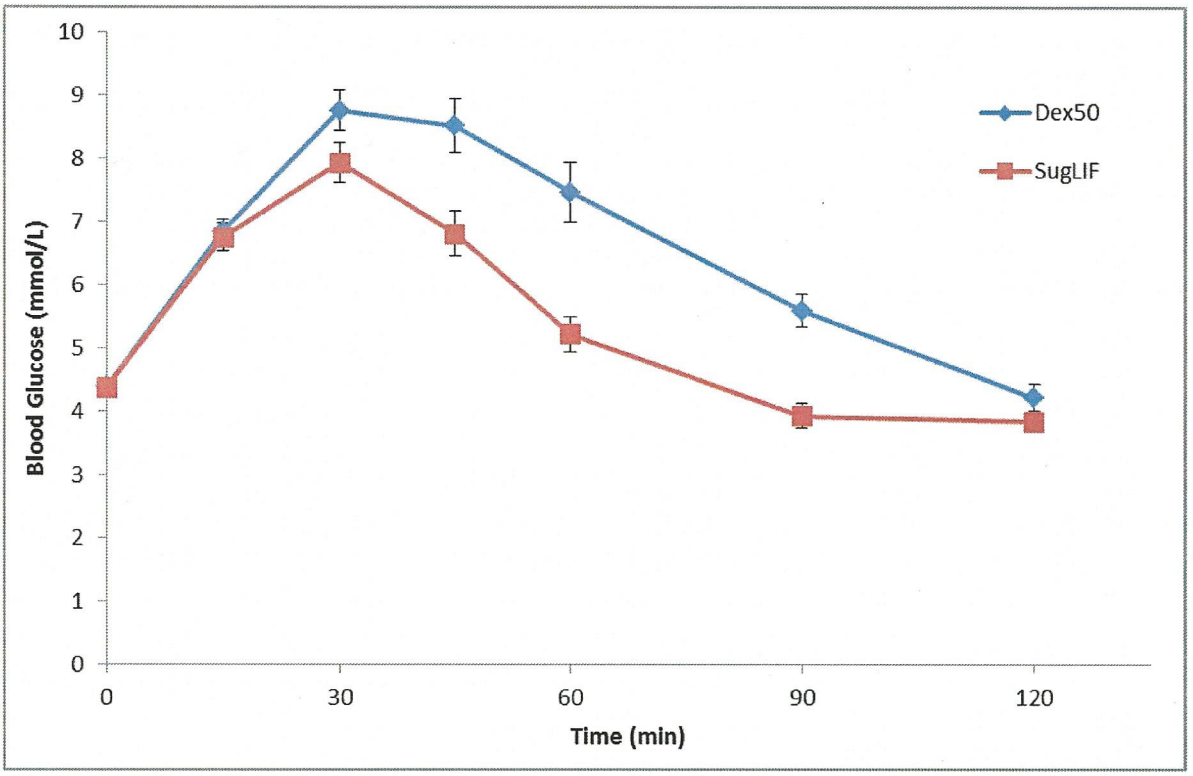

Figure 1: Postprandial glucose responses to SugarLIF (SugLIF) and the 50g Dextrose control (Dex50) (mean of 2 meals, 10 subjects each). All meals contained 50g of available carbohydrate. Data are expressed as Mean±SEM.

Glycemic Index

The data and calculations for the glycemic index are given in the pdf sheets entitled: GICalc Report - GIL-1651 SugarLIF.pdf. There was one outlier: subject 633 in the SugarLIF series whose results were excluded from the final GI calculation.

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

Test Meal	Abbr	Palatability (mm)	IAUC (mmol×min/L)	GI	GI Category
Dextrose	Dex25	47±6	275.6±34.7[a]	100[a]	High
SugarLIF	SugLIF	53±10	141.7±13.5[b]	50±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).

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Appendix 1

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
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 (ie. humans)

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

Postprandial
following a meal

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