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Chocolate #9

FINAL REPORT

Protocol GIL-8036

Determination of glycemic index of:

Chocolate #9

28 July, 2008

DISCLAIMER

GI Labs has taken due care to ensure the accuracy of the results provided in this report. However, the results of glycemic response tests in human subjects are subject to biological variability and may vary depending on the methods used. Thus, these results may not be able to be reproduced exactly either by GI Labs or by others.

SUMMARY

The GI value of Chocolate #9 syrup was determined using standard methods in 10 normal subjects. The mean \pm SEM GI value obtained was 45.6 \pm 7.6.

METHODS

SUBJECTS

Inclusion criteria

Subjects are males or non-pregnant females aged 18-75 years and in good health.

Exclusion/withdrawal criteria

- age less than 18 years
- known history or AIDS, hepatitis, diabetes or a heart condition
- subjects using medications or with any condition which might, in the opinion of Dr. Wolever, either: 1) make participation dangerous to the subject or to others, or 2) affect the results
- subjects who cannot or will not comply with the experimental procedures or do not follow GI Laboratories safety guidelines.

Number of subjects

Ten (10) subjects were studied. Using the t-distribution and assuming an average CV of within-individual variation of incremental AUC values of 25%, n=10 subjects has 80% power to detect a 33% difference in incremental AUC with 2-tailed $p < 0.05$.

PROCEDURES

Subjects were studied between 7:00 and 9:30am after an overnight fast of 10-14h. On each test occasion the subject was weighed, and a fasting blood sample obtained by finger-prick. Then the subject started to consume a test meal. At the first bite a timer was started and additional blood samples taken at 15, 30, 45, 60, 90 and 120 min. Before and during the test, a blood glucose test record was filled out with the subject's initials, ID number, date, body weight, test meal, beverage, time of starting to eat, time it took to eat, time and composition of last meal, and any unusual activities. During the 2 hours of the test, subjects remain seated quietly.

After finishing each test meal, subjects rated its palatability using a visual analogue scale consisting of a 100mm line anchored at the left end with the words "Very Unpalatable" and at the right end with "Very Palatable". Subjects were asked to make a mark along the line to represent how palatable they considered the meal. The results are given in mm from the left end of the line: ie. a score of 0 means very unpalatable and a score of 100 means very palatable.

Blood Samples

Each blood sample consisted of 2-3 drops of blood obtained by finger-prick. Blood samples were taken into flat-bottomed 5ml plastic tubes with a push cap containing a small amount of sodium fluoride and potassium oxalate as an anticoagulant and preservative. Glucose analysis was done using a YSI model 2300 STAT analyzer (Yellow Springs, OH). Fasting blood glucose was measured in duplicate and the average used; if the duplicates differed by >0.2 mmol/L, fasting glucose was measured in triplicate and the average of the 2 closest values used. Glucose was measured in other samples in singlicate only.

Test Meals

Test meals consisted of a 50g anhydrous glucose dissolved in 250ml water or 110.8g Chocolate #9 syrup. Each subject tested glucose three times, and the 4 test meals were fed in randomized order. The portion size of Chocolate #9 was based on analytical data provided by the manufacturer to provide 50g available carbohydrate (Table 1).

Subjects were allowed to choose to have, with the test meals, a drink of one or 2 cups of water, coffee or tea with 30ml 2% milk per cup if desired. The drink chosen remained constant for all 3 test meals.

Table 1: Composition of Agave Syrup.

	per 15g	per 110.8g
Energy (Kcal)	35.9	265
Total Fat (g)	0.450	3.3
Total Carbohydrate (g)	7.440	55.0
Sugars (g)	6.450	47.6
Dietary Fiber (g)	0.675	5.0
Protein (g)	0.495	3.7
Available Carbohydrate (g)	6.765	50.0

Statistical Analysis

Data were entered into a spreadsheet by 2 different individuals and the values compared to assure accurate transcription. The reproducibility of duplicate measures of fasting glucose was expressed as the coefficient of variation, $CV=100 \times SD/mean$, with SD being calculated as:

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

where d is the difference between duplicates and n is the number of duplicate pairs included. Incremental areas under the glucose response curves (AUC), ignoring area below fasting, were calculated. The GI was calculated by expressing the AUC for each food as a percentage of the mean AUC elicited by glucose in the same subject. Blood glucose concentrations at each time and AUC and GI values were compared by two-way analysis of variance examining for the effects of subject and test food. 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 LSD (least significant difference) differ significantly.

Informed Consent

The GI Laboratories protocol has been approved by the Western Institutional Review Board® which meets all 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 for Health Research (CIHR) and the European Community Guidelines. Informed consent is obtained from all subjects for each series of tests they participate in.

RESULTS

Details of the subjects studied are shown in Table 2 below.

Table 2: Subject Characteristics.

ID	Age (yr)	Sex	Ethnicity	Height		Weight		BMI (kg/m ²)
				(cm)	(in)	(kg)	(lb)	
129	33	M	Caucasian	172	67.8	78.9	174	26.6
161	43	F	Latin American	157	61.9	63.0	139	25.5
313	35	M	Caucasian	174	68.6	70.0	154	23.0
351	22	M	African-American	180	71.0	96.9	213	29.8
352	43	F	South Asian	158	62.2	46.5	102	18.6
367	34	F	Latin American	157	61.7	69.0	152	28.1
382	20	F	Caucasian	168	66.1	63.0	139	22.3
390	29	M	Caucasian	184	72.4	90.0	198	26.6
392	30	F	Caucasian	168	66.2	66.2	146	23.4
393	38	M	Caucasian	171	67.3	77.1	170	26.4
Mean	32.7			169	66.5	72.1	159	25.0
SEM	2.5			3	1.2	4.6	10	1.0

Blood Glucose Analysis

There was not enough blood to allow duplicate analysis of fasting glucose in 2 blood samples. The mean±SD for the 38 fasting samples for which duplicates were measured was 4.190±0.057 (CV=1.4%).

Glycemic Response Elicited by Glucose

The AUC results for the repeated tests of glucose are shown on page 4 of the appendix (attached separate file). There was a significant effect of order, with the first test having a significantly higher AUC than the 2nd test, with the 3rd being intermediate and not significantly different from the other 2. A significant effect of order is unusual – these were trained subjects – and we can only suggest it was due to chance. The difference was quite small, but was able to be detected because the average within-individual variation, as assessed by coefficient of variation (CV), was also small, with a mean value of 15.8±3.5%, which is well below the average of 25% for normal subjects.

Palatability

There was no significant difference in palatability between glucose and Chocolate #9 (Table 3).

Table 3: Means±SEM Palatability, AUC and GI Values for the test foods.

Test Meal	Abbr	Palatability (mm)	AUC (mmol×min/L)	Glycemic Index (%)
Chocolate #9	Choc9	45±9	71±14*	46±8* (L)
Glucose	Glu	45±6	168±20	100 (H)

Values are means±SEM.

AUC = incremental area under the blood glucose response curve.

GI (glucose = 100). H = high GI (70-100), M=intermediate GI (55-69), L=low GI (<55).

* Significant difference between Agave and glucose ($p < 0.05$).

Blood Glucose Responses

Blood glucose was significantly lower after Chocolate #9 than glucose at 15, 30, 45 and 60min (attached file), and significantly higher at 120min. In addition in AUC after Chocolate #9 was significantly less than that after glucose (Table 3).

Glycemic Index Value

The GI value of Chocolate #9 syrup was 46±8. The SEM for the GI value is a little higher than usual, but there are no outliers (more than 2SD from the mean). If the mean is trimmed (ie. the highest and lowest individual values are removed), the mean is not affected but the SEM becomes smaller: 45.4±5.9 (n=8). This suggests the overall mean is accurate.

GIL-8036: Analysis of Variance

Palatability

	Choc9	Glu
129	79.0	67.0
161	6.0	60.7
313	67.0	67.7
351	32.0	18.7
352	56.0	53.0
367	73.0	57.0
382	14.0	19.3
390	35.0	51.3
392	9.0	29.0
393	83.0	22.7

Mean	45.4	44.6
SEM	9.4	6.3

ANOVA	Source	SS	df	MS	F	p
	Subj	7680.762	9	853.418	1.94	0.1682
	Food	2.888	1	2.888	0.01	0.937133
	Error	3951.282	9	439.0313		
	Total	11634.93			LSD=	21.2

Fasting Blood Glucose

	Choc9	Glu
129	4.57	4.66
161	4.02	4.05
313	4.01	4.13
351	4.52	4.52
352	4.20	3.81
367	4.69	4.33
382	5.29	3.93
390	4.26	4.08
392	3.79	3.90
393	4.05	4.37

Mean	4.34	4.18
SEM	0.14	0.09

ANOVA	Source	SS	df	MS	F	p
	Subj	1.430276	9	0.15892	1.40	0.311794
	Food	0.128801	1	0.128801	1.14	0.314368
	Error	1.020936	9	0.113437		
	Total	2.580014			LSD=	0.34

15 minutes

	Choc9	Glu
129	4.85	6.23
161	5.64	6.94
313	5.56	6.60
351	5.10	6.20
352	5.38	6.67
367	5.28	6.33
382	5.65	5.98
390	4.95	5.28
392	4.88	5.60
393	4.58	6.30

Mean	5.19	6.21
SEM	0.12	0.16

ANOVA	Source	SS	df	MS	F	p
	Subj	2.5438	9	0.282644	2.79	0.070892
	Food	5.26338	1	5.26338	52.04	0.00005
	Error	0.91022	9	0.101136		
	Total	8.7174			LSD=	0.32

30 minutes

	Choc9	Glu
129	4.90	7.00
161	6.12	8.30
313	6.43	8.12
351	5.69	8.05
352	6.65	8.01
367	5.19	4.77
382	5.80	7.46
390	5.34	7.71
392	4.96	6.35
393	5.45	6.69

Mean	5.65	7.25
SEM	0.19	0.34

ANOVA	Source	SS	df	MS	F	p
	Subj	10.86874	9	1.207638	3.58	0.035709
	Food	12.68824	1	12.68824	37.57	0.000173
	Error	3.039905	9	0.337767		
	Total	26.5969			LSD=	0.59

45 minutes

	Choc9	Glu
129	5.41	7.18
161	6.21	7.15
313	5.44	7.12
351	5.46	6.98
352	6.40	6.95
367	5.03	4.57
382	4.30	6.54
390	5.06	7.43
392	4.49	5.08
393	5.87	6.16

Mean	5.37	6.52
SEM	0.22	0.31

ANOVA	Source	SS	df	MS	F	p
	Subj	8.821005	9	0.980112	2.34	0.110285
	Food	6.601005	1	6.601005	15.78	0.003241
	Error	3.764045	9	0.418227		
	Total	19.18606			LSD=	0.65

60 minutes

	Choc9	Glu
129	5.34	7.35
161	5.32	6.29
313	4.07	5.05
351	5.36	5.26
352	5.75	5.93
367	4.29	4.87
382	4.36	5.65
390	3.81	6.50
392	4.02	4.15
393	5.06	5.45

Mean	4.74	5.65
SEM	0.22	0.29

ANOVA	Source	SS	df	MS	F	p
	Subj	8.35738	9	0.928598	2.36	0.108059
	Food	4.15872	1	4.15872	10.59	0.009942
	Error	3.53598	9	0.392887		
	Total	16.05208			LSD=	0.63

90 minutes

	Choc9	Glu
129	4.87	6.73
161	4.08	5.30
313	3.61	2.90
351	4.88	5.85
352	5.05	5.41
367	5.13	4.88
382	3.74	4.48
390	3.92	5.07
392	4.10	4.00
393	4.20	3.46

Mean	4.36	4.81
SEM	0.18	0.36

ANOVA	Source	SS	df	MS	F	p
	Subj	11.09382	9	1.232647	3.18	0.050163
	Food	1.0125	1	1.0125	2.61	0.140775
	Error	3.4939	9	0.388211		
	Total	15.60022			LSD=	0.63

120 minutes

	Choc9	Glu
129	4.75	4.81
161	4.17	3.24
313	4.10	3.83
351	4.20	4.18
352	4.89	4.64
367	4.98	3.73
382	3.56	2.80
390	4.15	3.09
392	3.59	3.40
393	4.68	3.89

Mean	4.31	3.76
SEM	0.16	0.21

ANOVA	Source	SS	df	MS	F	p
	Subj	5.17098	9	0.574553	5.31	0.010252
	Food	1.49058	1	1.49058	13.78	0.004829
	Error	0.97352	9	0.108169		
	Total	7.63508			LSD=	0.33

Incremental Area Under the Curve

	Choc9	Glu
129	50.8	221.6
161	122.5	234.7
313	81.8	154.8
351	67.5	167.7
352	158.2	261.8
367	34.9	64.7
382	10.5	172.8
390	36.4	200.6
392	57.6	92.5
393	92.9	107.4

Mean	71.3	167.9
SEM	14	20.4

ANOVA	Source	SS	df	MS	F	p
	Subj	39903.73	9	4433.748	2.64	0.082392
	Food	46609.51	1	46609.51	27.72	0.000517
	Error	15131.84	9	1681.316		
	Total	101645.1			LSD=	41.5

Glycaemic Index

	Choc9	Glu
129	22.9	100.0
161	52.2	100.0
313	52.8	100.0
351	40.3	100.0
352	60.4	100.0
367	53.9	100.0
382	6.1	100.0
390	18.1	100.0
392	62.3	100.0
393	86.5	100.0

Mean	45.6	100.0
SEM	7.6	0.0

ANOVA	Source	SS	df	MS	F	p
	Subj	2597.443	9	288.6047	1.00	0.5
	Food	14824.01	1	14824.01	51.36	0.000053
	Error	2597.4	9	288.6047		
	Total	20018.9			LSD=	17.2

Reproducibility of Repeat Tests of Reference Food

ID	Test #1	Test #2	Test #3	Mean	SD	CV
129	255.5	191.7	217.5	221.6	32.1	14.5
161	233.3	242.2	228.5	234.7	7.0	3.0
313	188.8	139.3	136.3	154.8	29.5	19.0
351	172.4	178.8	151.8	167.7	14.1	8.4
352	295.8	255.2	234.5	261.8	31.2	11.9
367	85.2	48.5	60.4	64.7	18.7	28.9
382	167.9	168.0	182.4	172.8	8.3	4.8
390	197.9	170.9	233.1	200.6	31.2	15.5
392	128.8	72.2	76.6	92.5	31.5	34.0
393	123.9	87.0	111.3	107.4	18.8	17.5

Mean	185.0	155.4	163.2	167.9	22.2	15.8
SEM	32.9	29.7	30.2	30.6	4.3	3.5

Dates of Repeated Tests of Reference Food

ID	Test #1	Test #2	Test #3
129	09/07/2008	11/07/2008	21/07/2008
161	17/06/2008	19/06/2008	02/07/2008
313	10/07/2008	14/07/2008	23/07/2008
351	17/07/2008	21/07/2008	24/07/2008
352	24/06/2008	02/07/2008	17/07/2008
367	26/06/2008	07/07/2008	14/07/2008
382	16/06/2008	23/06/2008	15/07/2008
390	02/07/2008	08/07/2008	16/07/2008
392	24/06/2008	03/07/2008	10/07/2008
393	11/07/2008	16/07/2008	22/07/2008

ANOVA*

Source	SS	df	MS	F	p
Subj	112238.9	9	12470.99	31.96	0.0000
Order	4694.255	2	2347.128	6.02	0.0100
Error	7023.605	18	390.2003		
Total	123956.8				

* Excluding subject(s) with missing reference test(s) (if any).

GIL-8036: Blood Glucose Responses and Glycaemic Index (Glucose = 100)

Chocolate #9

ID	Date	Pal	0min	15min	30min	45min	60min	90min	120min	AUC	GI
129	15/07/2008	79.0	4.57	4.85	4.90	5.41	5.34	4.87	4.75	50.8	22.9
161	22/07/2008	6.0	4.02	5.64	6.12	6.21	5.32	4.08	4.17	122.5	52.2
313	16/07/2008	67.0	4.01	5.56	6.43	5.44	4.07	3.61	4.10	81.8	52.8
351	18/07/2008	32.0	4.52	5.10	5.69	5.46	5.36	4.88	4.20	67.5	40.3
352	15/07/2008	56.0	4.20	5.38	6.65	6.40	5.75	5.05	4.89	158.2	60.4
367	09/07/2008	73.0	4.69	5.28	5.19	5.03	4.29	5.13	4.98	34.9	53.9
382	10/07/2008	14.0	5.29	5.65	5.80	4.30	4.36	3.74	3.56	10.5	6.1
390	14/07/2008	35.0	4.26	4.95	5.34	5.06	3.81	3.92	4.15	36.4	18.1
392	08/07/2008	9.0	3.79	4.88	4.96	4.49	4.02	4.10	3.59	57.6	62.3
393	17/07/2008	83.0	4.05	4.58	5.45	5.87	5.06	4.20	4.68	92.9	86.5

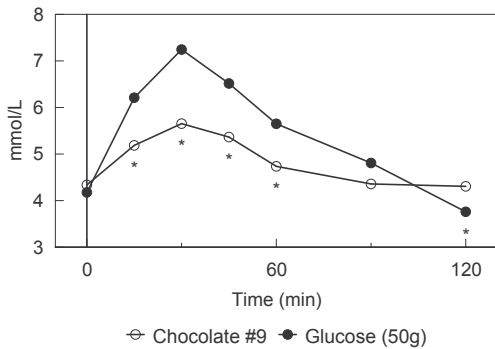
Mean	45.4	4.34	5.19	5.65	5.37	4.74	4.36	4.31	71.3	45.6
SEM	9.4	0.14	0.12	0.19	0.22	0.22	0.18	0.16	14.0	7.6
p=	ns	ns	0.000	0.000	0.003	0.010	ns	0.005	0.001	0.000

Glucose (50g)

ID	n	Pal	0min	15min	30min	45min	60min	90min	120min	AUC
129	3	67.0	4.66	6.23	7.00	7.18	7.35	6.73	4.81	221.6
161	3	60.7	4.05	6.94	8.30	7.15	6.29	5.30	3.24	234.7
313	3	67.7	4.13	6.60	8.12	7.12	5.05	2.90	3.83	154.8
351	3	18.7	4.52	6.20	8.05	6.98	5.26	5.85	4.18	167.7
352	3	53.0	3.81	6.67	8.01	6.95	5.93	5.41	4.64	261.8
367	3	57.0	4.33	6.33	4.77	4.57	4.87	4.88	3.73	64.7
382	3	19.3	3.93	5.98	7.46	6.54	5.65	4.48	2.80	172.8
390	3	51.3	4.08	5.28	7.71	7.43	6.50	5.07	3.09	200.6
392	3	29.0	3.90	5.60	6.35	5.08	4.15	4.00	3.40	92.5
393	3	22.7	4.37	6.30	6.69	6.16	5.45	3.46	3.89	107.4

Mean	44.6	4.18	6.21	7.25	6.52	5.65	4.81	3.76	167.9
SEM	6.3	0.09	0.16	0.34	0.31	0.29	0.36	0.21	20.4

Blood Glucose Concentrations



Blood Glucose Increments

