The Effects of *Salacia oblonga* Extract on Postprandial Glycemia Following a Solid, High Starch Meal

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements
for Graduation with Distinction in Nutrition in the College
of Human Ecology at The Ohio State University

By
Matthew Washam

The Ohio State University
May 2006

Project Advisor: Dr. Steve Hertzler, Department of Human Nutrition

ABSTRACT

Attenuation of postprandial glycemia is hypothesized to reduce the risk of progression from impaired glucose tolerance to diabetes. It is also thought to reduce the number of complications associated with diabetes. *S. oblonga* extract has been shown to reduce postprandial glycemia when it is fed in addition to a liquid nutritional supplement containing mainly maltodextrin. No studies have been done, however, using *S. oblonga* extract on a solid meal. The purpose of this study was to measure the effects of *S. oblonga* extract on the postprandial glycemic and lactate responses along with the perceived gastrointestinal, satiety, and flatulence symptoms severity following a solid, high starch meal. Fourteen subjects (8 males, 6 females) ate two test meals after an overnight fast following a standardized dinner. The meals consisted of 112 g durum spaghetti noodles, one cup (8 oz.) of Meijer meatless spaghetti sauce, and one cup (8 oz.) of unsweetened, caffeine-free tea. In the treatment meal, the tea also contained 480 mg of *S. oblonga* extract. The subjects had their glycemic responses to the meals measured through two hours postprandial. The subjects then ate a standardized lunch and reported perceived satiety, gastrointestinal distress, and frequency of flatulence while fasting for an additional five hours. The serum glucose positive incremental area under the curve (AUC) response was reduced by 25% ($P = 0.022$) and the serum lactate AUC response was reduced by 29% ($P = 0.033$) in the treatment meal compared with the control meal. The serum glucose baseline-adjusted peak was reduced by 27% ($P < 0.001$) and the serum glucose excursion was reduced by 23% ($P = 0.002$) in the treatment meal compared with the control meal. Also, the total number of gas passages for hours 0-8 increased from 7.29 ± 1.76 in the control to 26.4 ± 4.59 in the treatment ($P = 0.001$) indicating the alpha-glucosidase inhibitory effects of *S. oblonga*. In summary, *S. oblonga* extract effectively lowered the postprandial glucose response to a high starch meal, consistent with previous studies on this herb. The extract may have value in the management of blood glucose for persons with diabetes. The development of mild to moderate flatulence is a limitation for the use of this herbal extract. More studies are needed to develop ways to improve gastrointestinal tolerance.

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INTRODUCTION

Overview of Diabetes Mellitus

Approximately 18.2 million people (6.3% of the population) in the United States have Diabetes Mellitus and its prevalence is on the rise (1, 2). Diabetes is defined as having a fasting blood glucose (FBG) of > 126 mg/dl and/or a glucose response of ≥ 200 mg/dl to a two hour, 75 g oral glucose tolerance test (OGTT, 3). Such glucose intolerance is due to defects in insulin production, insulin action, or both (1). Type 1 Diabetes Mellitus (T1DM) results from an autoimmune response to the body's own pancreatic beta cells, where insulin production occurs, and is thus treated with administration of insulin (4). T1DM occurs in approximately 10% of all diabetes cases. The other 90% of cases are of Type 2 Diabetes Mellitus (T2DM). T2DM results from a combination of insulin resistance and an inability of pancreatic beta cells to secrete enough insulin in response to a rise in plasma glucose concentrations (4). T2DM is treated using weight reduction, exercise, and dietary modification programs along with oral medications. The estimated cost of caring for diabetes was approximately $132 billion in the US in 2002, including $17.5 billion in outpatient medications (5).

Chronic hyperglycemia induced by diabetes is associated with higher risks of strokes, coronary heart disease, peripheral vascular disease, dyslipidemia, hypertension, and obesity. This chronic hyperglycemia is also associated with damage to and failure of the eyes, kidneys, nerves, heart, and blood vessels (3). These abnormalities occur due to the accumulation of glucose metabolites and the glycosylation of certain proteins, including hemoglobin (4). Elevated glycosylated hemoglobin (HbA1c) levels > 7% are associated with a glucose response of ≥ 228 mg/dl during an OGTT (6).

Treatment of Diabetes Mellitus

Treatment for T2DM aims to reduce the postprandial blood glucose response and HbA1c levels in the blood. Low Glycemic Index (GI) diets have been shown to improve blood-glucose control in patients with T1DM and T2DM (7). Other methods include:

- increasing insulin output from the pancreatic beta cells using sulfonylurea drugs, such as glimepiride, or herbs, such as fenugreek (8, 9)
- decreasing hepatic glucose output using biguanide drugs, such as metformin (8)
- increasing insulin sensitivity using thiazolidinedione drugs, such as pioglitazone, or supplements, such as chromium (8, 10)
- decreasing formation of glycylated proteins using acetylating agents and antioxidants, such as the herb ginseng (11)
- decreasing stomach emptying rates using viscous and soluble fiber, such as guar gum (12)
- decreasing the amount of carbohydrate absorbed from the small intestine using alpha-glucosidase inhibitor drugs, such as acarbose, and herbs, such as *Salacia oblonga* (8, 13)
- increasing hepatic glucose uptake using fructose (14)

Alpha-Glucosidase Inhibitors

Chiasson et al. measured the effects of chronic use of the alpha-glucosidase inhibitor acarbose over a 3 year period; Refer to Figure 1 (15). Subjects with impaired glucose tolerance were allocated into two groups: 714 were placed in the acarbose group and 715 were placed in the placebo group. The acarbose and placebo were taken 3 times a day for 3.3 years by the respective groups, immediately before beginning consumption of a meal. Chiasson et al. found a reduced risk of progression from impaired glucose tolerance to diabetes of 25% in the acarbose group compared with the placebo group. This reduction in risk was hypothesized to occur because of the decreased postprandial glucose response due to the blocked alpha-glucosidase enzyme by acarbose. Chiasson et al. also hypothesized that the increased reversion to normal glucose tolerance in the acarbose group was due to the decreased postprandial glucose response as well.

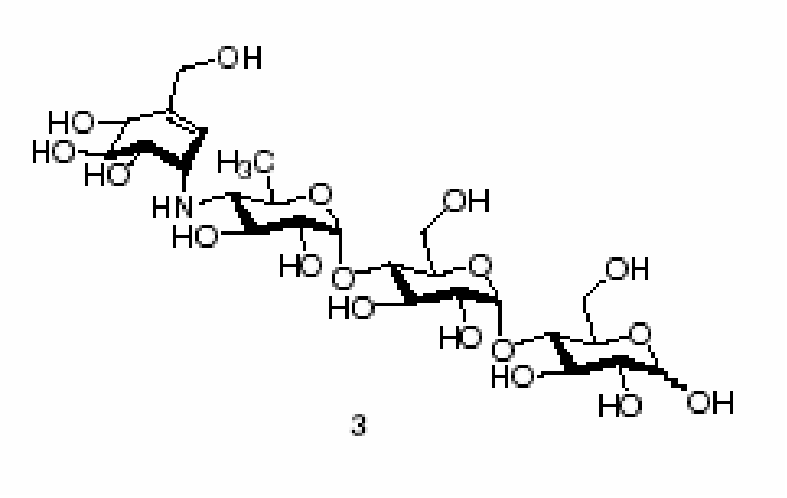


Figure 1: Structure of Acarbose. Acarbose mimics the oligosaccharide substrate and binds to alpha-glucosidase with a greater binding affinity than oligosaccharides (16).

Hanefeld et al. conducted a meta-analysis of seven randomized, placebo-controlled acarbose studies with a minimum duration of 52 weeks (17). All patients in these studies had diabetes, with the vast majority being type 2 diabetics. Under acarbose treatment, a significant prolongation of time in which patients remained free of any newly diagnosed cardiovascular events occurred compared with patients under the placebo treatment. Additionally, a 64% relative risk reduction for myocardial infarctions was observed for the acarbose treatment group. Long term glycemic control was observed in the acarbose treatment group compared with the placebo group, with a significant reduction in HbA1c levels, fasting and postprandial blood glucose levels in acarbose patients. Patients in the acarbose group also demonstrated lowered plasma triglycerides levels, body weight, BMI, systolic blood pressure, reduced insulin levels, and increased insulin sensitivity secondary to reduced postprandial glycemia (17).

LITERATURE REVIEW

Extracts from the roots and stems of *Salacia oblonga*, a woody climbing plant that grows in parts of India and Sri Lanka, have been shown to have alpha-glucosidase inhibitory activity *in vitro* and may be useful in the prevention and/or treatment of diabetes (18-22). Two thiosugars isolated from *S. oblonga* extract, salacinol and kotalanol, have inhibitory effects, *in vitro*, against maltase, isomaltase, and sucrase, with the inhibitory effect against sucrase being more potent than the prescription alpha-glucosidase inhibitors acarbose and voglibiose that are used in the treatment of diabetes (23). Through the reduction of the enzymatic breakdown of di-, tri-, and oligosaccharides by alpha-glucosidase, carbohydrate absorption is decreased, attenuating

the postprandial glycemic response. The undigested di-, tri-, and oligosaccharides pass through the small intestine into the colon where they are digested by the colonic microflora producing gaseous byproducts, demonstrated by the increased breath hydrogen responses in Heacock et al (24). Lowering of postprandial glycemia by *S. oblonga* extract has been observed in rats fed either maltose or sucrose, but not glucose, which is consistent with its alpha-glucosidase inhibitory effect in the small intestine (22).

Salacinol contains a zwitterion consisting of a sulfonium ion with an internal sulfate counterion; Refer to Figure 2 (19). It is hypothesized that the permanent positive charge on the sulfur atom in the 1,4-anhydro-4-thio-D-arabinitol moiety binds to alpha-glucosidase through mimicry of the shape and charge of the oxacarbenium-ion intermediate in the hydrolysis reaction mediated by alpha-glucosidase; Refer to Figure 3 (16). Kotalanol contains the same 1,4-anhydro-4-thio-D-arabinitol moiety and is believed to work via the same mechanism as salacinol (Figure 1).

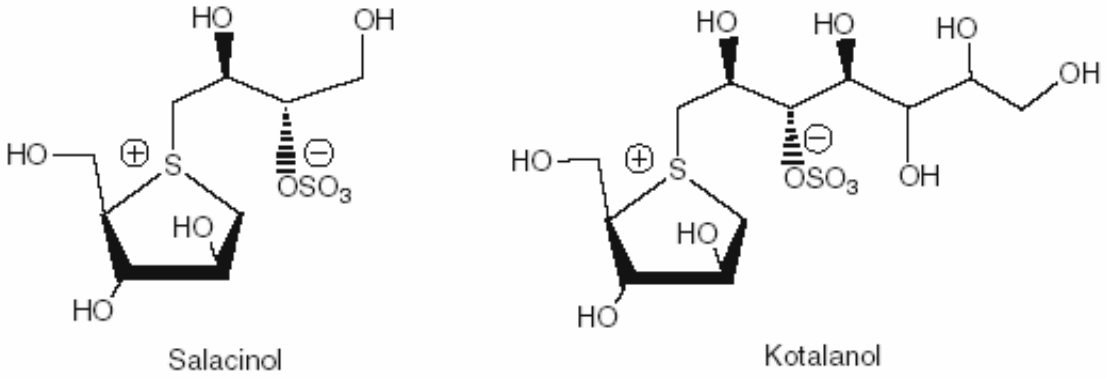


Figure 2: Structure of Salacinol and Kotalanol. The permanent positive charge residing on the sulfur atom in the 1,4-anhydro-4-thio-D-arabinitol moiety may be resposible for the alpha-glucosidase inhibition in vitro (25).

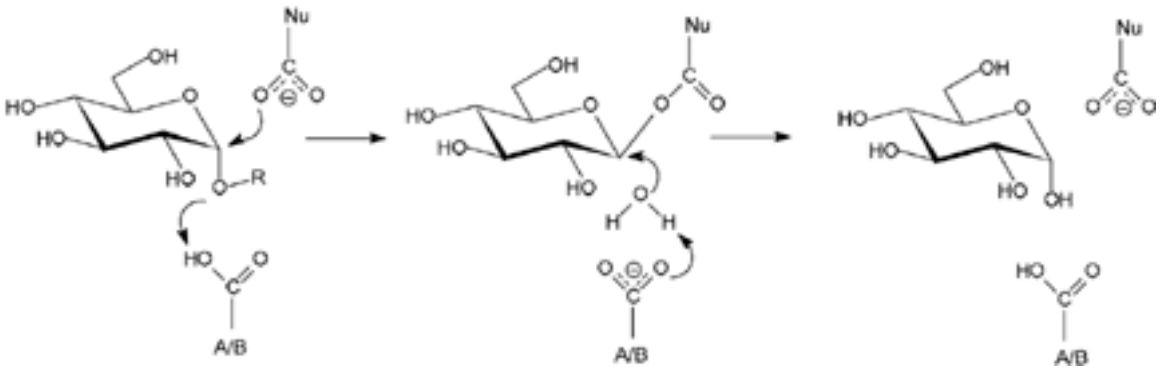


Figure 3: Mechanism of alpha-glucosidase hydrolysis of oligosaccharides into free monosaccharides. *S. oblonga* extract mimics the oxacarbenium-ion intermediate formed after step one of the reaction (26).

Heacock et al. and Collene et al. have demonstrated that *S. oblonga* extract reduces postprandial glycemia and insulinemia when it is fed in addition to a liquid nutritional supplement containing mainly maltodextrin (61% of available carbohydrate) as the carbohydrate source (13, 27). Heacock et al. measured the effects of varying doses of *S. oblonga* extract on postprandial glycemia in 39 healthy subjects (13). Subjects were fed a test beverage containing 49.5g maltodextrin with and without 1000 mg of *S. oblonga* extract. Serum glucose AUC was reduced by 23% and serum insulin AUC was reduced by 29% to the test beverage containing 1000 mg of extract compared with the test beverage alone. An increased breath hydrogen response was also measured with the 1000 mg treatment, indicating that carbohydrate malabsorption was at least partially responsible for the decreased glycemic response. These results are consistent with previous studies measuring postprandial glycemia using acarbose (13).

Collene et al. measured *S. oblonga* extract and free amino acids on postprandial glycemia in 43 healthy subjects (27). This study found a 24% decrease in postprandial AUC glycemic response to a test beverage containing 82 g carbohydrate, 20 g protein, and 14 g fat with 1000 mg *S. oblonga* extract compared with the test beverage alone.

Serum insulin response to the test beverage was also lower with the addition of *S. oblonga*. Extract fed with the beverage in addition to 3.5 g of phenylalanine and leucine led to a 27% decrease in postprandial AUC glycemia compared with the test beverage alone (27). Serum insulin response to the extract plus free amino acid meal was insignificantly lower compared with the serum insulin response to the control (27).

Other studies have determined that chronic use (daily use for 2-3 months) of *Salacia* herbs might also improve long-term glucose control (28-30). Jayawardena et al. tested *S. reticulata* use for 3 months on 51 subjects with type 2 diabetes mellitus. *S. reticulata* also contains the alpha-glucosidase inhibitors salacinol and kotalanol (30). Subjects in this study consumed Kothala Himbutu tea daily for six months: three months of which contained *S. reticulata* extract and three months of which did not (30). A decrease in HbA1c was observed in patients after the *S. reticulata* treatment than after the placebo treatment (6.29 +/- 1.02 in treatment vs. 6.65 +/- 1.04 in placebo). Jayawardena et al. also reported no significant abnormalities in liver or renal function after treatment with *S. reticulata*, with the main adverse effect reported being loose stools (30).

The safety profile of *Salacia* herbs has been studied in laboratory animals. Wolf and Weisbrode measured the effects of *S. oblonga* extract in a two week trial using Sprague-Dawley rats at a dose approximately 10 times higher than used in human trials (31). The rats fed the extract showed significantly reduced weight gain and relative liver and spleen weights; however no significant histopathological changes in hepatic or renal functions occurred. Shimoda et al. also showed no adverse effects on food intake, body weight, blood chemistries, organ weights, or histopathological findings on rats fed *S. reticulata* at doses up to 1000 mg/kg for 13 weeks of continuous intake (32). Results

observed by Ratnasooriya et al., however, show that *S. reticulata* fed to Wistar rats during early to mid-pregnancy was associated with increased post-implantations losses, reduced birth weight of the pups, reduced fetal survival ratio, and reduced viability ratio at a dose 170 times greater than doses fed previously in humans (33).

OBJECTIVE OF STUDY

While the effects on postprandial glycemia have been observed with carbohydrate test beverages, the effects of *S. oblonga* extract have not yet been evaluated in the context of a solid meal (13, 27). The method for delivering the carbohydrate load in postprandial glycemia studies is important for several reasons. The anti-hyperglycemic effects of acarbose are more apparent when the drug is given with a solid meal versus a liquid meal (34). This increased effectiveness of acarbose occurred in both healthy subjects and subjects with diabetes, although the effect was somewhat more pronounced in healthy subjects. The likely explanation for these findings is that liquid meals empty from the stomach too rapidly, allowing the carbohydrate from the meal to reach the intestine before the medication can become active. Additionally, 2-hour glucose tolerance tests performed with a standardized solid meal may result in less variability in postprandial glucose responses compared with a glucose solution due to the increased gastric-emptying time as well (21). The objective of this study is to evaluate the effects of *S. oblonga* extract on the postprandial glycemic response to a high starch, solid meal along with its effects on the postprandial lactate response and on perceived gastrointestinal tolerance, satiety and flatulence.

HYPOTHESES

It is anticipated that there will be a reduction in the postprandial glycemic AUC response to the high starch, solid meal using *S. oblonga* extract compared with the control meal. A similar reduction in the postprandial lactate AUC response is also anticipated using the *S. oblonga* extract. Additionally, it is anticipated that subjects will experience increased flatulence to the *S. oblonga* extract meal compared with the control meal, consistent to the increased breath hydrogen AUC response to *S. oblonga* extract in previous studies. Fermentation by colonic bacteria is the only significant source of hydrogen in the breath (13, 27). Lastly, it is anticipated that an increased feeling of bloating will occur with the *S. oblonga* extract while there will be no difference in nausea, headache, or abdominal cramping between the two treatments. Similar symptom ratings occurred in the study by Heacock et al.

METHODS

Subjects

Subjects were healthy adults and were enrolled in the study based on the following criteria:

1) Subject was male or a non-pregnant female between the ages of 18-45 of any race. Screening for pregnancy via a commercially available urine test (Fact Plus Select, Abbott Laboratories, Chicago, IL) was conducted on all females who met other eligibility criteria. Pregnant women were excluded due to effects of pregnancy on glucose

metabolism and the unknown effects that *S. oblonga* extract might have on the developing fetus.

2) Female subjects on oral contraceptives were taking these agents for at least 3 months prior to the study and were consistently using the medication throughout the study.
3) Subject was willing to complete all necessary study questionnaires and agreed to keep notes and records if required.
4) Subject was interested in participating in the study after: a) being fully informed about the experimental treatments and the trial procedures, b) reviewing the study methodology, and c) signing a subject consent form.
5) Subject had a body mass index (BMI) between 20 and 28 OR subject had a BMI > 28 and ≤ 30 and had a waist circumference ≤ 35 inches if female or ≤ 40 inches if male. BMI = weight (kg)/ height² (m^2) (35)
6) Subject did not have diabetes mellitus or glucose intolerance. Fasting (at least 10 hrs.) plasma glucose level was < 100 mg/dl (36). A capillary finger-stick blood sample (1 drop) was obtained from the subject at screening for measurement of plasma glucose using a portable blood glucose monitor (Accucheck Advantage, Roche, Inc., Indianapolis, IN).
7) Subject stated that he/she is free from active metabolic or gastrointestinal diseases which may interfere with nutrient absorption,

distribution, metabolism, or excretion and that the subject has no known food allergies.

8) Subject had not had a recent infection requiring medication or hospitalization; surgery; corticosteroid treatment in the last 3 months; or taken antibiotics in the last three weeks.
9) Subject was willing to consume a high carbohydrate diet (at least 150 g daily) for the three days prior to the test (37).
10) Subject was willing to fast at least 10 hours prior to the test. During fasting, the subject consumed only water.
11) Subject abstained from vigorous exercise 24 hours prior to testing, performing only usual activities, and minimized activity during the test.
12) Subject was not taking daily medications (e.g., acetaminophen, salicylates, diuretics, etc.) that would interfere with nutrient absorption, metabolism, excretion, or gastric motility.
13) Subject was not taking any dietary supplements that would affect serum glucose levels or glucose metabolism.
14) Subject did not have a history of drug or alcohol abuse in the previous six months.
15) Subject did not have an active weight loss of > 5 kg, intended or unintended, in the previous three months.

Sample Size

The sample for glycemic index studies typically consists of approximately 10 healthy adults (38). A power analysis was conducted using data from a previous study (Power Analysis Sample Size module, Number Cruncher Statistical System 2001, NCSS Computing, Kaysville, UT). Using a difference of 0.9 mmol/L in baseline-adjusted peak serum glucose, a standard deviation of 1.1 mmol/L, and an alpha level of 0.05, a total of 14 subjects were required for 80% power in a crossover type of design (each subject serving as his/her own control). Thus, our sample size estimate was 14 subjects (8 males, 6 females). Subject characteristics, displayed as mean ± SEM (range) were as follows: age was 23 ± 1 year (20-32 years); body weight was 72 ± 3 kg (53-94 kg); and body mass index (BMI) was 24 ± 1 kg/m² (20-27 kg/m²). Of the 14 subjects, 2 were Asian and 12 were white. Subjects gave informed consent and the study was approved by the Institutional Review Board Human Subjects Committee at The Ohio State University (see appendix).

Treatments

There were a total of 2 test meals in this protocol (treatment and control) and they were administered in random order. Each test meal was preceded by a standardized dinner the night before, which was followed by an overnight (at least 10 h) fast. The standardized dinner consisted of one can of Ensure plus a variable number of Zone Perfect bars such that 1/3 of the subject's estimated energy expenditure was met. Energy expenditure was based on the Harris Benedict equation multiplied by 1.3 to account for very light physical activity (39). This meal was consumed between 1600 and 1900 h on

the night before the test meal and no additional foods were consumed. The subject began the overnight fast following consumption of the standardized dinner.

The morning following the fasting period, at approximately 0700 h, subjects reported to the laboratory (Room 219, Campbell Hall). Their weight was recorded and they rested for 30 minutes. After the 30 minute rest period, the blood pressure and temperature was recorded for each subject and his/her baseline blood samples (1-2 mL) was collected via capillary finger-stick (baseline sample). Immediately following this sampling, subjects proceeded to the kitchen area (Room 215 Campbell Hall) and were fed the test meal. The timing for subsequent blood samples began with the first bite of the test meal. The test meals consisted of 8 fl. oz. (240 mL) of a beverage plus spaghetti. The beverages were unsweetened, instant decaffeinated tea (the control) or unsweetened, instant decaffeinated tea plus 480 mg *S. oblonga* extract (the treatment). The spaghetti (constant for each meal) consisted of 1 cup (2 servings) Meijer all natural meatless spaghetti sauce and 4 ounces (dry weight, 2 servings) Meijer 100% durum semolina spaghetti noodles. This meal provided approximately 106 g carbohydrate, 4 g fat, and 18 g protein.

Subjects resumed fasting after eating the test meal (preferably within 10 min) and then blood samples were collected from the subjects via the capillary finger-prick technique at 15, 30, 45, 60, 90, and 120 min postprandial (40) (Figure 4). The subjects completed ratings of perceived hunger and symptom record forms at each of the blood sampling interval time points. Subjects completed the rectal gas passage form at hourly intervals starting at 60 min postprandial. The subjects then waited in the laboratory for an additional hour after the last blood sample was collected. At the completion of that

hour, the subject completed the hunger, gas passage, and symptom record forms again and were fed a lunch that was to be consumed on site. The contents of the lunch were the same as for the standardized dinner the night before the test. Upon finishing the lunch, the subjects were free to leave the laboratory and completed further hunger and symptom record forms at hourly intervals for the next 5 hours. During that 5-hour period, the subjects were not allowed to consume additional foods except for water. After completing the last symptom and hunger rating questionnaires, the subjects were allowed to resume their normal diet and activity patterns. The two meal test visits to the laboratory were separated by a minimum of 4 days.

Figure 4: Data collection time points; all times refer to the beginning of the test meal

Data	**Time (min)**												
	0	15	30	45	60	90	120	180*	240	300	360	420	480
Blood Sample	*X*	*X*	*X*	*X*	*X*	*X*	*X*						
Satiety Rating Scale	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Rectal Gas Passage					*X*		*X*	*X*	*X*	*X*	*X*	*X*	*X*
Gastrointestinal Tolerance	*X*				*X*		*X*	*X*	*X*	*X*	*X*	*X*	*X*

*Time of Standardized Lunch

ANALYSES

Serum analysis

The whole blood samples (approximately 1-2 mL) were collected in serum separator tubes (BD Vacutainer, Becton Dickinson Scientific, Franklin Lakes, NJ) and allowed to clot for 5-10 min after collection. Samples were then centrifuged at 1168 X *g* to obtain serum and the serum was frozen at -20° C until analysis. Serum glucose and

lactate concentrations were determined in duplicate using the YSI 2700 Select Plus Biochemistry Analyzer. This instrument employs the glucose oxidase method and is often used in clinical studies as a "gold standard" for comparison with other methods (41).

The positive incremental areas under curve (AUC) for the serum glucose and serum lactate responses (0-120 min) were calculated using the method of Wolever et al (40). Briefly, the trapezoidal rule was used to calculate all the area between the subject's postprandial curve and that subject's baseline serum glucose or serum lactate value (any area below the baseline was discarded). In addition, the baseline-adjusted peaks for glucose and lactate (highest value minus the baseline value at time 0) and the excursions (highest value minus lowest value) were calculated.

Symptom ratings

At the intervals listed in Figure 4, subjects self-rated their perceived levels of severity for symptoms of nausea, headache, abdominal cramping, bloating/excessive fullness, and flatulence on a ranked scale with the following definitions: 0 indicates no symptoms, 1 indicates slight symptoms, 2 indicates mild symptoms, 3 indicates moderate symptoms, 4 indicates moderately severe symptoms, and 5 indicates severe symptoms (refer to appendix, 42). Data was presented as the sum of the ratings for hours 1 to 8. Thus, the maximum possible symptom score was 40 for each symptom (a rating of 5 every hour for 8 hours). For determination of flatus frequency, subjects counted the number of rectal gas passages at hourly intervals over the 8-hour period (refer to appendix). A hand-tally counting device (VWR Scientific, West Chester, PA) and a form

(see appendix) for recording the results was provided to the subjects to assist in obtaining these counts. These data were also presented as the sum of hours 1 to 8.

Hunger ratings

A 7-point, equilateral scale (Figure 5) was used to assess hunger of the subjects at each of the time points listed in Figure 4 (refer to appendix). The horizontal distance from the left side of the figure was measured and the data from these measurements was used to generate an average sum. This use of this scale to calculate a "satiety index" of foods has been validated against measured food intake at 120 min postprandial in a previous study (43).

Figure 5: Equilateral satiety rating scale (43)

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

Statistical Analyses

Descriptive statistics were calculated and normality tests were performed for all variables using SYSTAT. ANOVA for a randomized block design (subjects as the random factor and test food as the fixed factor) was used to test for overall significant differences among plasma glucose and plasma lactate treatment means for AUC, peak, and individual time points (44). Data were reported as mean ± standard error of the mean, and significance was determined at $P < 0.05$. For gastrointestinal tolerance factors and

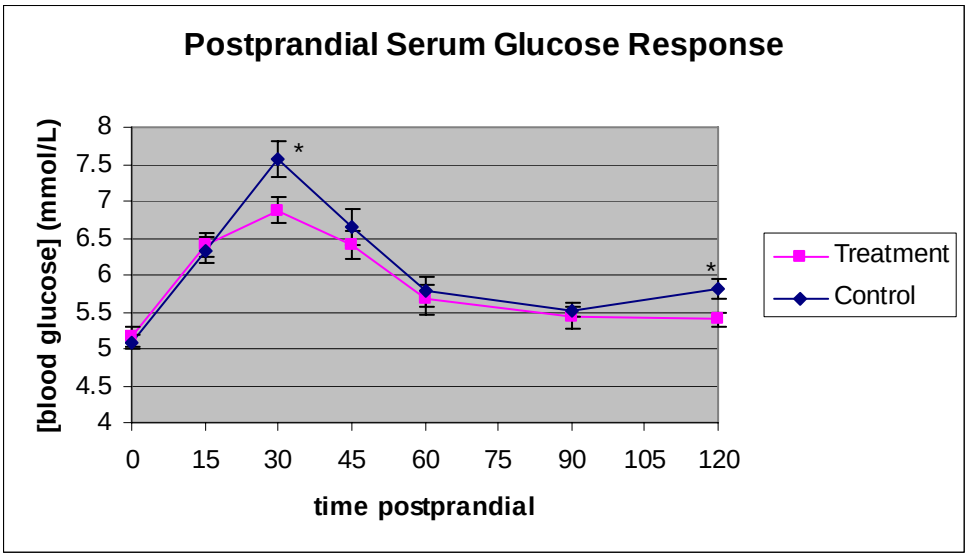
satiety ratings, randomized block ANOVA was used to compare the total rating for each factor between the different treatments. For the flatulence symptom ratings and number of gas passages, randomized block ANOVA was used to compare the total rating at each time point between the different treatments (44).

RESULTS

Serum Glucose Response

Results of the serum glucose response through 120 minutes postprandial to the control and treatment meals are shown in Figure 6. There were no significant differences between the fasting serum glucose concentration between the control and treatment meals ($P = 0.451$). Both meals produced a peak in serum glucose concentration at 30 minutes postprandial, with the control peak (7.57 ± 0.24 mmol/dl) being significantly higher than the treatment peak (6.88 ± 0.18 mmol/dl, $P = 0.005$). An additional reactive hyperglycemic peak occurred at 120 minutes postprandial (5.82 ± 0.13 mmol/dl) in the control while no peak occurred in the treatment (5.40 ± 0.10 mmol/dl, $P = 0.001$).

Figure 6: Postprandial Serum Glucose Response



*Denotes significant difference between treatment and control meals ($P < 0.05$)

Time Postprandial	Control mean [serum glucose] mmol/dl[1]	Treatment mean [serum glucose] mmol/dl[1]	*P* Level (Significance at $P < 0.05$)
0	5.09 ± 0.10	5.16 ± 0.14	0.451 NS
15	6.33 ± 0.17	6.41 ± 0.16	0.550 NS
30	7.57 ± 0.24	6.88 ± 0.18	**0.005 S**
45	6.65 ± 0.25	6.40 ± 0.19	0.324 NS
60	5.77 ± 0.20	5.67 ± 0.20	0.683 NS
90	5.52 ± 0.10	5.43 ± 0.15	0.523 NS
120	5.82 ± 0.13	5.40 ± 0.10	**0.001 S**

[1]Mean ± standard error of the mean (SEM), number of subjects = 14
S = Significant ($P < 0.05$), NS = Nonsignificant

Results of the mean serum glucose baseline-adjusted peak (BAP) and excursion of the control and treatment meals are shown in Figure 7. The mean BAP between the control meal (2.52 ± 0.24 mmol/dl) and the treatment meal (1.84 ± 0.16 mmol/dl) were significantly different ($P < 0.001$). Additionally, the mean excursion of the control (2.54 ± 0.24 mmol/dl) and the treatment (1.95 ± 0.14 mmol/dl) were significantly different ($P = 0.002$).

Figure 7: Serum Glucose Baseline Adjusted Peak and Excursion (Maximum-Minimum) Data

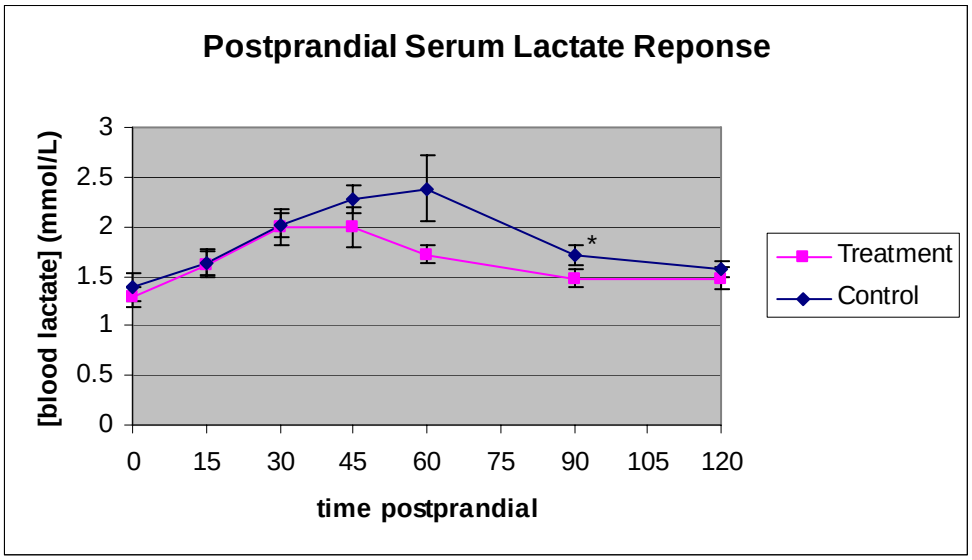
	Control [serum glucose] mmol/dl[1]	**Treatment [serum glucose] mmol/dl[1]**	***P* Level** (Significance at $P < 0.05$)
Baseline	5.09 ± 0.10	5.16 ± 0.14	0.451 NS
Maximum	7.62 ± 0.24	7.00 ± 0.17	**0.004 S**
Minimum	5.08 ± 0.10	5.05 ± 0.11	0.653 NS
BAP	2.52 ± 0.24	1.84 ± 0.16	**< 0.001 S**
Excursion	2.54 ± 0.24	1.95 ± 0.14	**0.002 S**

[1]Mean ± standard error of the mean (SEM), number of subjects = 14
S = Significant ($P < 0.05$), NS = Nonsignificant

Serum Lactate Response

Results of the serum lactate response through 120 minutes postprandial to the control and treatment meals are shown in Figure 8. There were no significant differences between the fasting serum lactate concentration between the control and treatment meals ($P = 0.298$). Both meals produced a peak in serum lactate concentration between 30 and 60 minutes postprandial, with the control peak (2.39 ± 0.33 mmol/dl) occurring at 60 minutes postprandial and the treatment peak (2.00 ± 0.18 mmol/dl) occurring at 30 minutes postprandial. The peaks in serum lactate concentration were not significantly different in the control versus the treatment meal ($P = 0.134$). At 90 minutes postprandial, the mean control serum lactate concentration (1.71 ± 0.09 mmol/dl) significantly differed from the mean treatment serum lactate concentration (1.48 ± 0.09 mmol/dl, $P = 0.011$).

Figure 8: Postprandial Serum Lactate Response



*Denotes significant difference between treatment and control meals ($P < 0.05$)

Time Postprandial	Control mean [serum lactate] mmol/dl[1]	Treatment mean [serum lactate] mmol/dl[1]	*P* Level (Significance at $P < 0.05$)
0	1.39 ± 0.14	1.29 ± 0.10	0.298 NS
15	1.64 ± 0.13	1.61 ± 0.13	0.890 NS
30	2.02 ± 0.12	2.00 ± 0.18	0.888 NS
45	2.28 ± 0.14	1.99 ± 0.20	0.084 NS
60	2.39 ± 0.33	1.72 ± 0.09	0.075 NS
90	1.71 ± 0.09	1.48 ± 0.09	**0.011 S**
120	1.57 ± 0.08	1.48 ± 0.11	0.300 NS

[1]Mean ± standard error of the mean (SEM), number of subjects = 14
S = Significant ($P < 0.05$), NS = Nonsignificant

Results of the mean serum lactate baseline-adjusted peak (BAP) and excursion of the control and treatment meals are shown in Figure 9. The mean BAP between the control meal (1.26 ± 0.24 mmol/dl) and the treatment meal (1.01 ± 0.16 mmol/dl) were not significantly different ($P = 0.249$). Additionally, the mean excursion of the control (1.40 ± 0.29 mmol/dl) and the treatment (1.10 ± 0.19 mmol/dl) were not significantly different ($P = 0.201$).

Figure 9: Serum Lactate Baseline Adjusted Peak and Excursion (Maximum-Minimum) Data

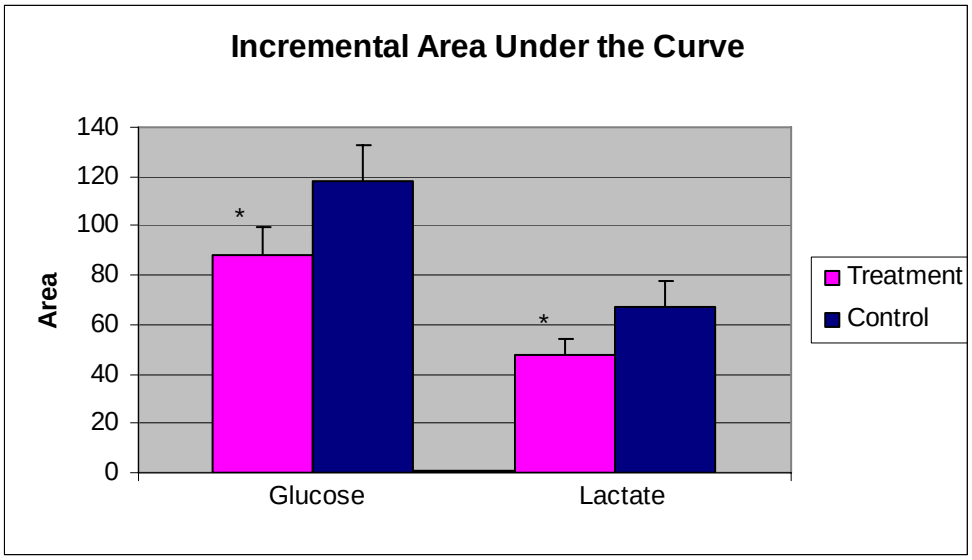
	Control [serum lactate] mmol/dl[1]	**Treatment [serum lactate] mmol/dl[1]**	***P* Level** (Significance at $P < 0.05$)
Baseline	1.39 ± 0.14	1.29 ± 0.10	0.298 NS
Maximum	2.65 ± 0.32	2.29 ± 0.22	0.134 NS
Minimum	1.24 ± 0.08	1.19 ± 0.06	0.478 NS
BAP	1.26 ± 0.24	1.01 ± 0.16	0.249 NS
Excursion	1.40 ± 0.29	1.10 ± 0.19	0.201 NS

[1]Mean ± standard error of the mean (SEM), number of subjects = 14
S = Significant ($P < 0.05$), NS = Nonsignificant

Positive Incremental AUC

Results of the positive incremental AUC serum glucose and lactate responses for both the control and treatment meals are shown in Figure 10. The mean serum glucose AUC showed a significant 25% reduction ($P = 0.022$) in postprandial glycemia between the treatment meal (AUC = 88.3 ± 11.0) and the control meal (AUC = 118.4 ± 14.0). The mean serum lactate AUC also displayed a significant 29% reduction ($P = 0.033$) in postprandial lactate response between the treatment meal (AUC = 47.6 ± 6.9) and the control meal (AUC = 67.4 ± 10.2).

Figure 10: Mean Serum Glucose and Lactate AUC

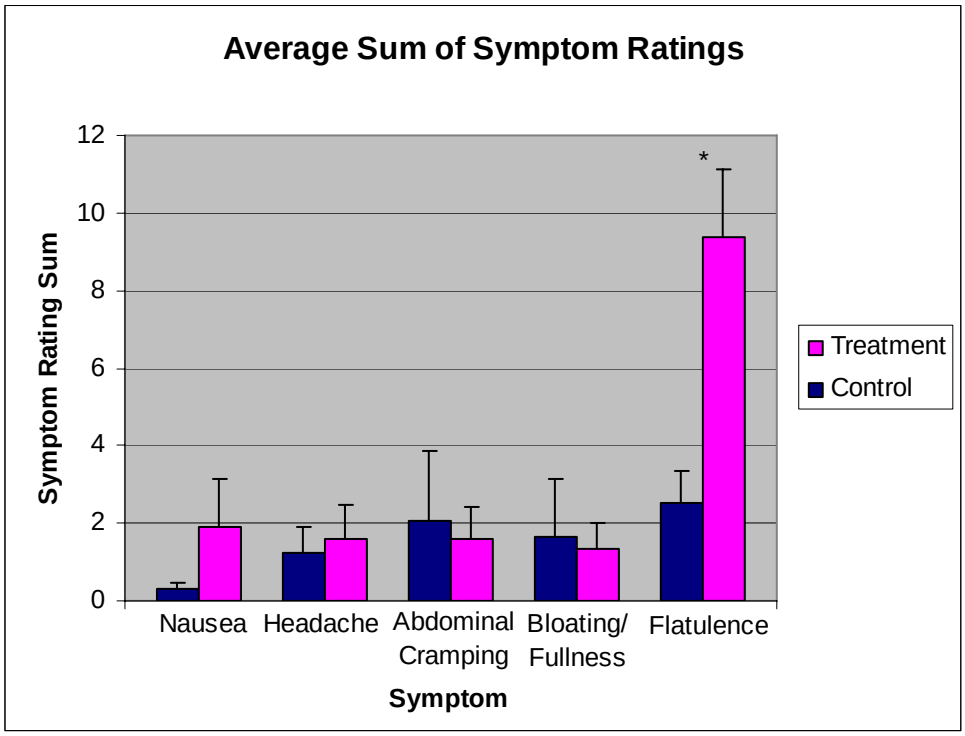


*Denotes significant difference between treatment and control meals ($P < 0.05$)

Symptom Responses

Figure 11 displays the mean sum of the symptom responses for the control and treatment meals from hours 0 through 8. The maximum response for nausea, headache, abdominal cramping, bloating/excessive fullness, and flatulence was 40 (a recording of 5 through the full 8 hours). The maximum response for satiety was 91 (a baseline recording of 7 followed by a recording of 7 through the full 8 hours at the specified time points). The symptoms of nausea ($P = 0.175$), headache ($P = 0.646$), abdominal cramping ($P = 0.729$), bloating/excessive fullness ($P = 0.811$), and satiety ($P = 0.953$) did not differ significantly between the control meal and the treatment meal. The symptom of flatulence ($P = 0.002$), however, differed significantly with the mean sum of the control responses being 2.50 ± 0.86 and the mean sum of the treatment responses being 9.36 ± 2.69.

Figure 11: Mean Symptom Response Sum (Hours 0-8)



*Denotes significant difference between treatment and control meals ($P < 0.05$)

Symptom	Mean Control Response[1]	Mean Treatment Response[1]	*P* Level (Significance at $P < 0.05$)
Nausea[2]	0.29 ± 0.19	1.93 ± 1.19	0.175NS
Headache[2]	1.21 ± 0.67	1.57 ± 0.88	0.646 NS
Abdominal Cramping[2]	2.07 ± 1.77	1.57 ± 0.86	0.729 NS
Bloating/ Excessive Fullness[2]	1.64 ± 1.49	1.36 ± 0.68	0.811 NS
Flatulence[2]	2.50 ± 0.86	9.36 ± 1.77	**0.002 S**
Satiety[3]	59.2 ± 2.93	59.4 ± 2.69	0.953 NS

[1]Mean ± standard error of the mean (SEM), number of subjects = 14
S = Significant ($P < 0.05$), NS = Nonsignificant

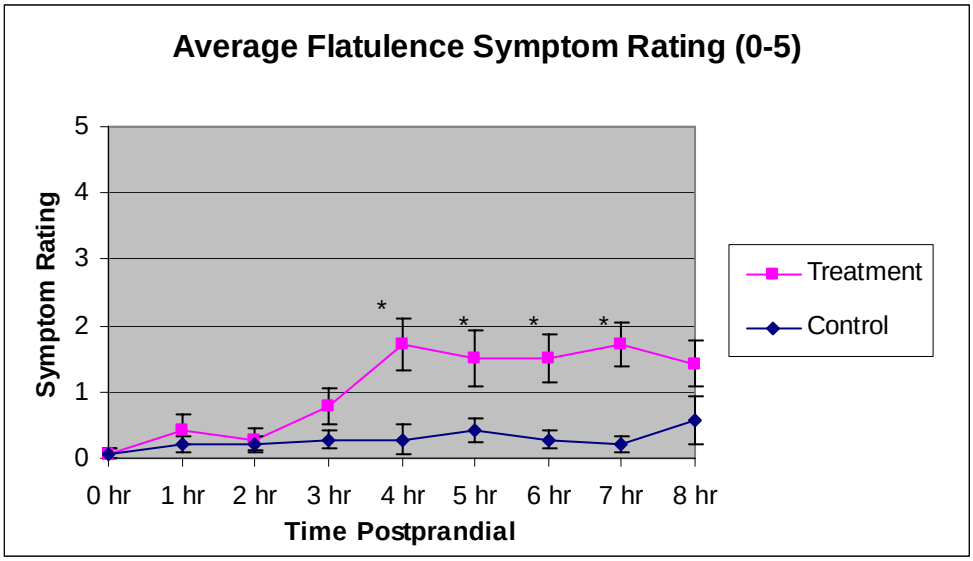
[2]The maximum possible sum for perceived symptom severity was 40 (a rating of 5 for each hour)

[3]The maximum possible sum for perceived satiety was 91 (a rating of 7 for each time point)

Flatulence Data

Figures 12 and 13 display the mean flatulence symptom ratings along with the total number of rectal gas passages recorded at each hourly interval from hours 0 to 8 for the control and treatment meals. The maximum mean flatulence symptom rating is 5. The mean flatulence symptom ratings for the control and treatment meals differed significantly at hours 4 ($P = 0.001$), 5 ($P = 0.022$), 6 ($P = 0.006$), and 7 ($P = 0.001$). The number of rectal gas passages differed significantly between the control and treatment meals at the time intervals of 3-4 hours ($P = 0.001$), 4-5 hours ($P = 0.015$), 5-6 hours ($P = 0.004$), and 6-7 hours ($P = 0.001$). The total rectal gas passage mean also differed significantly between the two meals ($P = 0.001$). Figure 14 displays the direct positive relationship ($R^2 = 0.8658$) between the number of gas passages recorded with the perceived severity of flatulence symptoms.

Figure 12: Mean Flatulence Symptom Rating at Baseline and at Each Hourly Interval Postprandial

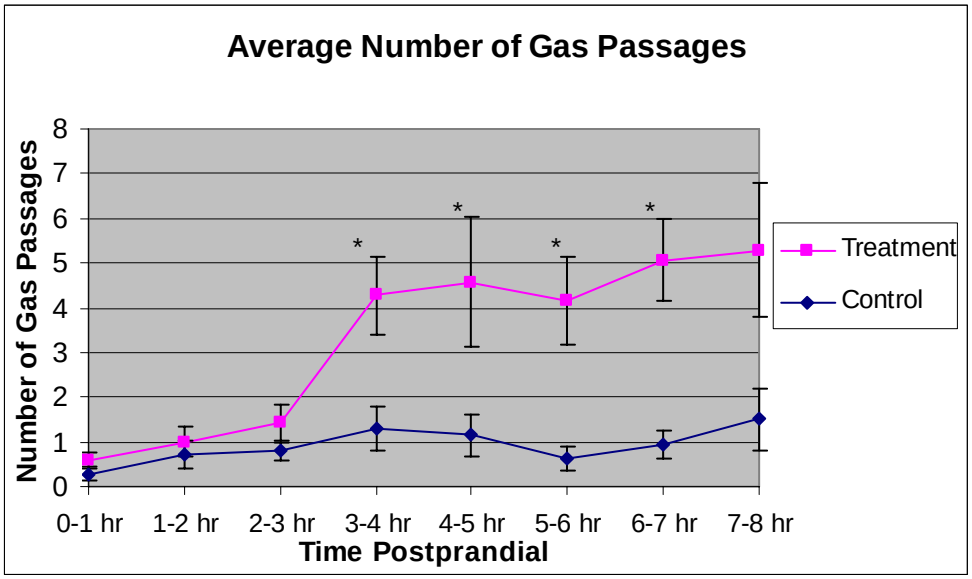


*Denotes significant difference between treatment and control meals ($P < 0.05$)

Time (Hour Postprandial)	**Control**[12]	**Treatment**[1]	***P* Level** (Significance at $P<0.05$)
0	0.07 ± 0.07	0.07 ± 0.07	1.00 NS
1	0.21 ± 0.11	0.43 ± 0.23	0.426 NS
2	0.21 ± 0.11	0.29 ± 0.16	0.671 NS
3	0.29 ± 0.13	0.79 ± 0.28	0.110 NS
4	0.29 ± 0.22	1.71 ± 0.40	**0.001 S**
5	0.43 ± 0.17	1.50 ± 0.43	**0.022 S**
6	0.29 ± 0.13	1.50 ± 0.36	**0.006 S**
7	0.21 ± 0.11	1.71 ± 0.34	**0.001 S**
8	0.57 ± 0.36	1.43 ± 0.36	0.152 NS

[1]Mean ± standard error of the mean (SEM), number of subjects = 14
S = Significant ($P < 0.05$), NS = Nonsignificant
[2]The maximum possible score for the perceived flatulence symptom rating was 5

Figure 13: Number of Gas Passages

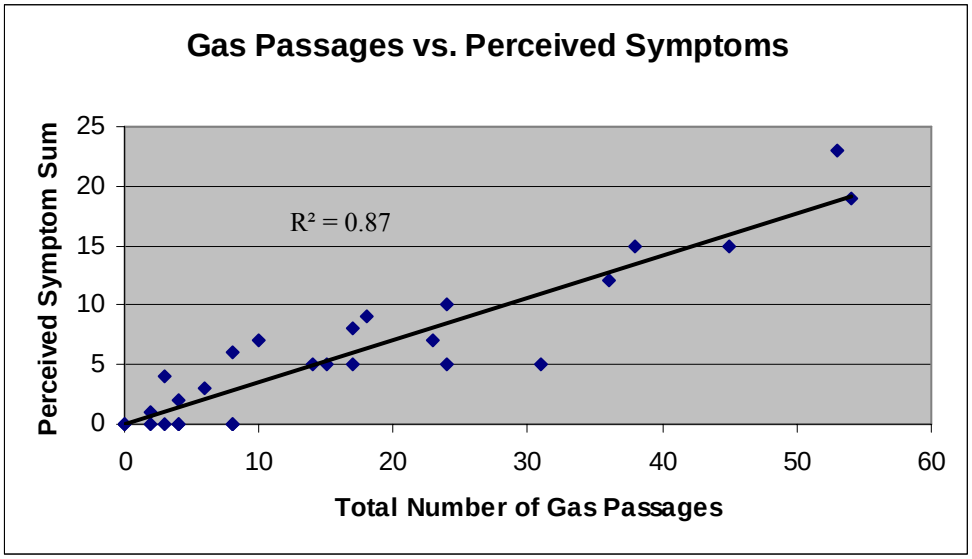


*Denotes significant difference between treatment and control meals ($P < 0.05$)

Time (Hour Postprandial)	**Control**[1]	**Treatment**[1]	***P* Level** (Significance at $P < .05$)
0-1	0.29 ± 0.16	0.57 ± 0.17	0.104 NS
1-2	0.71 ± 0.32	1.00 ± 0.33	0.525 NS
2-3	0.79 ± 0.21	1.43 ± 0.39	0.156 NS
3-4	1.29 ± 0.50	4.29 ± 0.87	**0.001 S**
4-5	1.14 ± 0.46	4.57 ± 1.45	**0.015 S**
5-6	0.64 ± 0.27	4.14 ± 0.98	**0.004 S**
6-7	0.93 ± 0.31	5.07 ± 0.92	**0.001 S**
7-8	1.50 ± 0.69	5.29 ± 1.49	0.050 NS
Total	7.29 ± 1.76	26.4 ± 4.59	**0.001 S**

[1]Mean ± standard error of the mean (SEM), number of subjects = 14
S = Significant ($P < 0.05$), NS = Nonsignificant

Figure 14: Relationship Between Number of Gas Passages and Perceived Symptoms



DISCUSSION

As the prevalence of T2DM and the associated costs in dealing with the induced complications of T2DM rises, it is becoming increasingly important to find alternative ways to prevent progression of impaired glucose tolerance into T2DM, delay the progression of those with T2DM, and also treat complications brought about by T2DM. The alpha-glucosidase inhibitor acarbose has been shown in a chronic study to reduce the

risk of progression from impaired glucose tolerance to diabetes by 25% (15). Acarbose has also been shown to reduce the risk of myocardial infarctions and other cardiovascular events in a meta-analysis (17). Patients using acarbose had reduced HbA1c levels, reduced fasting and postprandial blood glucose levels, reduced plasma triglyceride levels, reduced body weight and BMI, reduced systolic blood pressure, and reduced insulin levels (17).

In this study, the effects of the alpha-glucosidase inhibitors in *S. oblonga* extract, salacinol and kotalanol, on a solid, high starch meal were measured. Previous studies have demonstrated the effectiveness of *S. oblonga* extract on lowering postprandial glycemia to a liquid nutritional supplement containing 61% maltodextrin (13, 27). This study supports those findings, with an observed 25% reduction in positive incremental AUC ($P = 0.022$) being approximately equal to the 23% reduction of Heacock et al. and the 24% reduction of Collene et al. This result, however, does not support the increased anti-hyperglycemic effects to a solid meal versus a liquid meal observed with acarbose (34). A possible reason for this similar effectiveness to both liquid and solid meals is that the *S. oblonga* extract does not need the delayed gastric emptying offered by a solid meal for full alpha-glucosidase activity.

In addition to a reduced positive incremental AUC for serum glucose to the treatment meal, the peak serum glucose concentration was reduced by 8% ($P = 0.006$) and the 120 min serum glucose concentration was reduced by 7% ($P = 0.001$) in the treatment meal. These results also support the glucose-attenuating effects of *S. oblonga* extract found in previous studies. The decreased peak glucose concentration indicates that the alpha-glucosidase activity of salacinol and kotalanol inhibited full digestion and

absorption of the starch meal. The decreased peak also indicates that *S. oblonga* extract may reduce many of the complications of T2DM that occurred when the peak glucose concentrations decreased in studies with acarbose. The decrease in glucose concentration occurring at 120 min postprandial indicates that the *S. oblonga* extract prevented the mild reactive hypoglycemic event which occurred in the control meal. This effect is most likely related to the decrease in peak blood glucose in the treatment meal.

The positive incremental AUC serum lactate response also decreased by 29% (P = 0.033) in the treatment meal compared with the control meal. To the author's knowledge, no previous studies have been conducted relating the postprandial glycemic and lactate responses to each other. The decreased in serum lactate AUC is hypothesized to have occurred because of the decreased postprandial glucose load arriving to the liver, indicated by the reduction in serum glucose AUC. With a decreased glucose load, less glucose was available for use in metabolism creating a reduction in circulating lactate, a metabolite of glucose. The shape of the serum lactate concentration curve (Figure 8) supports this hypothesis. The peak in serum lactate concentration occurred between 30 and 60 minutes postprandial, compared with the peak serum glucose concentration occurring at 30 minutes postprandial. The delay in the lactate peak indicates the time required for the circulating glucose to be metabolized by cells, releasing lactate into circulation.

Decreases in the serum lactate concentrations of 13% (P = 0.084) and 28% (P = 0.075) were observed at 45 and 60 minutes postprandial, although these decreases were not significant. A significant 13% decrease (P = 0.011) was observed at 90 minutes postprandial, however. These reductions over a broad range of time indicate that *S.*

oblonga extract may have longer-term control of serum lactate levels via a reduced postprandial glycemic response. These findings may have significance in exercise science for athletes wishing to attenuate their blood glucose levels and minimize circulating lactate levels as well, although further studies need to be conducted to determine *S. oblonga* extract's effect on athletic performance.

The baseline adjusted peak and the excursion data showed significant differences for serum glucose concentrations but insignificant differences for serum lactate concentrations. Serum glucose BAP decreased 27% ($P < 0.001$) and the excursion decreased 23% for the treatment meals compared with the control meals. Despite similar AUC reductions for both glucose and lactate, the BAP and excursion data indicate that the postprandial glycemic responses provide a better indication of the possible anti-diabetic effects of *S. oblonga*. This supports the literature documenting the correlation between lowering postprandial glycemia and reducing complications associated with T2DM.

No significant differences were found with nausea ($P = 0.175$), headache ($P = 0.646$), or abdominal cramping ($P = 0.729$) symptoms between the treatment and the control meals. These findings support those of Heacock et al. However, Heacock et al. also found an increased perceived symptom of bloating/excessive fullness in the *S. oblonga* treatment. In this study, no significant difference in bloating/excessive fullness symptoms were observed ($P = 0.811$). This discrepancy may have occurred due to the use of a solid meal in this study rather than a liquid meal used by Heacock et al. The delayed gastric emptying from the solid meal may have led to a slower rate of entry of undigested carbohydrate into the large intestine as well, rather than the appearance of a

large bolus of undigested carbohydrate from a liquid meal. A slower, yet more constant rate of fermentation of the carbohydrate by colonic microflora may have led to a decreased perception of bloating and excessive fullness. Additionally, there were no significant differences in satiety ($P = 0.953$) between the treatment and control meals.

Consistent with the findings of Heacock et al. and Collene et al., the perceived flatulence symptoms in this study were significantly greater ($P = 0.002$) in the treatment meals compared with the control meals. Specifically, at hours 4 ($P = 0.001$), 5 ($P = 0.022$), 6 ($P = 0.006$), and 7 ($P = 0.001$) postprandial the perceived flatulence symptoms were significantly greater in the treatment. Additionally, the total number of rectal gas passages was significantly greater ($P = 0.001$) in the treatment compared with the control. During the time periods of 3-4 hours ($P = 0.001$), 4-5 hours ($P = 0.015$), 5-6 hours ($P = 0.004$), and 6-7 hours ($P = 0.001$) postprandial, there was a significant increase in the recorded number of gas passages to the treatment meal compared with the control meal. These data support the alpha-glucosidase effects of salacinol and kotalanol documented in the literature. The increased amount of flatulence occurring approximately 4 hours postprandial indicate that undigested carbohydrate from the solid meal was being fermented by the colonic microflora to produce gaseous byproducts. The 8 hour postprandial time point data shows an increase in perceived flatulence ($P = 0.152$) and the number of gas passages ($P = 0.050$), however this increase is not significant.

Plotting the total number of gas passages versus the perceived flatulence symptom ratings (Figure 14) showed a strong, positive linear correlation between the two ($R^2 = 0.8658$). This result verifies the use of subjective flatulence symptom perception rating forms to approximate the objective data of number of rectal gas passages. Future studies

should focus on the perceived flatulence symptom data, not only because it approximates the number of gas passages, but also because perceived symptoms by the subject is more important in the likelihood of use of *S. oblonga* extract as part of a treatment plan for diabetes.

Conclusion

A limitation of this study is the use of a small sample size (N = 14). Several of the subjects' postprandial glucose concentrations were unresponsive to the *S. oblonga* extract. There may be an unresponsive subgroup in the population, however the only way to determine this is to include a greater sample size. Time and funding limitations prevented measuring a possible carryover effect of the *S. oblonga* extract on postprandial glycemia to the standardized lunch. A residual amount of the extract may lower the response to a subsequent meal, compounding the anti-hyperglycemic effects. Another additional limitation caused by time and funding was the use of the number of gas passages and perceived flatulence symptoms to measure the degree of colonic fermentation. Previous studies by Heacock et al. and Collene et al. have measured breath hydrogen excretions as a more objective quantitative measurement for colonic microflora fermentation.

Future studies using *S. oblonga* extract can measure its effects on other sources of carbohydrates for the solid meal. Other studies can measure the effects of chronic use of the extract with type 2 diabetic and impaired glucose tolerance populations on diabetic complications and prevention of progression into T2DM. Chronic use studies of the extract can measure its effects on HbA1c concentrations in those subjects with T2DM as well. Similar studies have already been done using the prescription drug acarbose. Other

studies can measure a dosage effect of *S. oblonga* extract on postprandial glycemia to a solid meal. Heacock et al. measured the dosage effect of the extract using a liquid nutritional supplement; however no studies have been done using a solid meal. Additional studies measuring *S. oblonga* extract on exercise in athletes can also be conducted. There may be possible athletic implications to the lowered lactate response to the treatment meal.

In conclusion, *S. oblonga* extract lowered the postprandial glucose response by 25% ($P = 0.022$) and the postprandial lactate response by 29% ($P = 0.033$). The extract did not cause any significant differences in perceived nausea, headache, abdominal cramping, bloating/excessive fullness, or satiety. However, *S. oblonga* extract did cause an increase in perceived flatulence ($P = 0.002$) and in total number of rectal gas passages ($P = 0.001$), indicating the alpha-glucosidase activity of the extract. Despite the small sample size of the study (N = 14), these results can be used to support the use of *S. oblonga* extract in treating those with T2DM or with impaired glucose tolerance. Future studies can determine how to most effectively use *S. oblonga* extract as an alpha-glucosidase inhibitor.

ACKNOWLEDGEMENTS

I would like to thank Dr. Steve Hertzler for assisting me through all of the steps in conducting this research. Dr. Hertzler has not only taught me about the research process and about nutrition, but has helped guide me through my undergraduate career. I would also like to thank Dr. Josh Bomser for serving on my evaluation committee. Lastly, I would like to thank my family for their support and encouragement.

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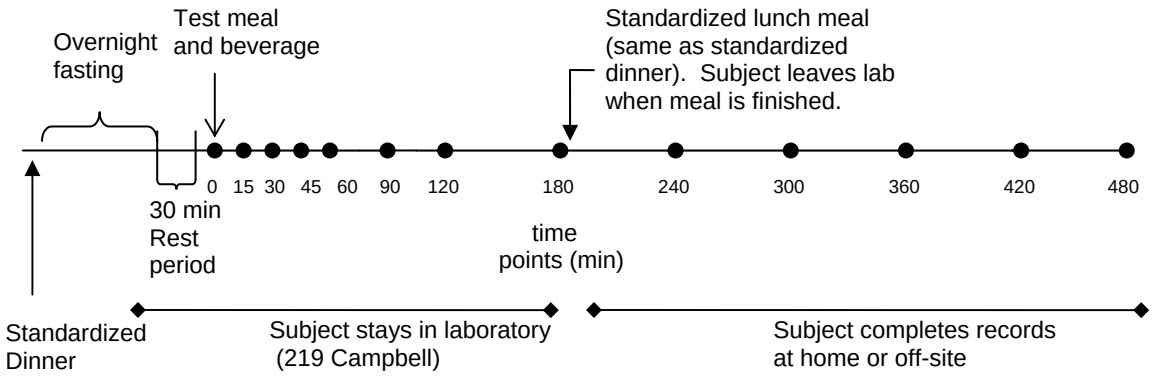
APPENDICES

Test Visit Sequence of Events

Diagram of the sequence of events for each meal test visit



Blood samples
Baseline (just prior to time 0), 15, 30, 45, 60, 90, 120 min

Symptom ratings
0, 60, 120, 180, 240, 300, 360, 420, 480 min

Hunger ratings
0, 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480 min

Collection of data on frequency
Of rectal gas passages (8 intervals)
0-60 min
60-120 min
120-180 min
180-240 min
240-300 min
300-360 min
360-420 min
420-480 min

***NOTE: ALL TIMINGS PERTAIN TO THE START OF THE TEST MEAL (SPAGHETTI AND BEVERAGE)**

Symptom Ratings Scale

Symptom ratings

Date :
Subject # :
Subject initials:
Treatment visit # :
Timepoint :

For each timepoint listed below, please describe how you feel for each of the symptoms listed. Use the following scale to rate your experience of the symptoms listed in the table:

0 - No Symptoms
1 - Slight Symptoms
2 - Mild Symptoms
3 - Moderate Symptoms
4 - Moderately Severe Symptoms
5 - Severe Symptoms

Timepoint	Nausea	Headache	Abdominal Cramping	Bloating/Excessive Fullness	Flatulence (gas)
0 hr					
1 hr					
2 hr					
3 hr					
4 hr					
5 hr					
6 hr					
7 hr					
8 hr					

Rectal Gas Passages Form

Rectal Gas Passages Data Collection Form

Date :
Subject # :
Subject initials:
Treatment visit # :
Timepoint :

For each interval listed below, record the number of rectal gas passages listed on the counter device that was provided. If a passage occurs exactly on the hour, include it in the next interval (or don't count it if it occurs exactly on the 8 hour time point).

Timepoint	Number of Rectal Gas Passages
From 0-1 hr after the test meal	
From 1-2 hrs after the test meal	
From 2-3 hrs after the test meal	
From 3-4 hours after the test meal	
From 4-5 hours after the test meal	
From 5-6 hours after the test meal	
From 6-7 hours after the test meal	
From 7-8 hours after the test meal	

Satiety Form

Hunger Rating Scale

Date :
Subject # :
Subject initials:
Treatment visit # :

Please indicate by a **slash** or **single stroke** through the line, the level of hunger/satiety experienced at each of the timepoints mentioned below:

Timepoint 0 minutes

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

Timepoint 15 minutes

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

Timepoint 30 minutes

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

Timepoint 45 minutes

1	2	3	4	5	6	7
extremely hungry		semi hungry	no particular feeling	semi satisfied		extremely full

Timepoint 60 minutes (1 hour)

1	2	3	4	5	6	7
extremely hungry		semi hungry	no particular feeling	semi satisfied		extremely full

Timepoint 90 minutes (1.5 hours)

1	2	3	4	5	6	7
extremely hungry		semi hungry	no particular feeling	semi satisfied		extremely full

Timepoint 120 minutes (2 hours)

1	2	3	4	5	6	7
extremely hungry		semi hungry	no particular feeling	semi satisfied		extremely full

Timepoint 180 minutes (3 hours)

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

Timepoint 240 minutes (4 hours)

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

Timepoint 300 minutes (5 hours)

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

Timepoint 360 minutes (6 hours)

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

Timepoint 420 minutes (7 hours)

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

Timepoint 480 minutes (8 hours)

1	2	3	4	5	6	7
extremely hungry	hungry	semi hungry	no particular feeling	semi satisfied	satisfied	extremely full

IRB Approval Form

BIOMEDICAL SCIENCES INSTITUTIONAL REVIEW BOARD
RESEARCH INVOLVING HUMAN SUBJECTS
THE OHIO STATE UNIVERSITY

__X__ **Original Review**
____ **Continuing Review**
____ **Amendment**

ACTION OF THE REVIEW BOARD

Research Protocol:

2006H0022 THE EFFECTS OF SALACIA OBLONGA EXTRACT ON POSTPRANDIAL GLYCEMIA FOLLOWING A SOLID, HIGH STARCH MEAL, Steven R. Hertzler, Human Nutrition

presented for review by the Biomedical Sciences Institutional Review Board to ensure the proper protection of rights and welfare of the individuals involved with consideration of the methods used to obtain informed consent and the justification of risks in terms of potential benefits to be gained.

The protocol was **APPROVED** by The Biomedical Institutional Review Board.

NOTE: Inclusion of vulnerable subjects (OSU students and employees) is approved as described in *45 CFR 46.111(b)*. Additional safeguards have been included in the study to protect the rights and welfare of these subjects.

Approval for proposed research includes all materials submitted by the investigator unless otherwise noted.

It is the responsibility of the principal investigator to retain a copy of each signed consent form for at least three (3) years beyond the termination of the subject's participation in the proposed activity. Should the principal investigator leave the University, signed consent forms are to be transferred to the Biomedical Sciences Institutional Review Board for the required retention period. This application has been approved for a period of not more than one year. You are reminded that you must promptly report any problems to the Review Board, and that no procedural changes may be made without prior review and approval. You are also reminded that the identity of the research participants must be kept confidential.

Date: February 27, 2006 Signed: Karla Zadi
Chairperson

hs-025h Biomedical approval letter (08.04)