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Fermentable Carbohydrate Intake and Differences in Health Parameters in US College Students

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FERMENTABLE CARBOHYDRATE INTAKE AND DIFFERENCES IN HEALTH PARAMETERS IN US COLLEGE STUDENTS

BY

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ABSTRACT

Background

Indigestible, fermentable carbohydrates are bioactive dietary carbohydrates not digested by human enzymes but are fermented into short chain fatty acids and gases by colonic bacteria. These carbohydrates display prebiotic effects and may influence body mass index (BMI), glucose regulation, blood pressure, and blood cholesterol. However, intakes of fermentable carbohydrates have not been explored in the general US population and potential metabolic effects have not been well elucidated. The purpose of this cross-sectional study was to examine consumption of total fermentable carbohydrates and subclasses, oligosaccharides and polyols, in US college students (n=359, body fat=25.4±9.3%, 83% female) and potential health differences between high and low consumers.

Methods

Intake of total fermentable carbohydrates, oligosaccharides, polyols and their subclasses were quantified by the Comprehensive Nutrition Assessment Questionnaire. Subjects were classified into lower median (LM) and upper median (UM) groups using median split for total grams consumed and grams consumed per 1000kcal diet (g/1000kcal). Blood glucose and lipids were measured by Cholestech LDX®, body fat percent by BODPOD®, and waist circumference and blood pressure by standardized instruments and protocols. Median differences in dependent variables were analyzed by analysis of variance (ANOVA) and covariance (ANCOVA).

Results

Average fermentable carbohydrate intake was 8.0±4.9 grams with approximately even amounts coming from oligosaccharides and polyols. The LM for total grams of fermentable carbohydrate had higher BMI (24.4±4.6 vs. 23.3±3.7kg/m^2 respectively; p=.022), body fat percent (26.6±9.8 vs. 24.1±8.5%; p=.016), and blood glucose (84±8 vs. 82±7mg/dL; p=.024) than the respective UM. Using ANCOVA, the LM had higher systolic blood pressure than the UM (117±14 vs. 114±14mmHg; p=.028). Findings were similar with fermentable carbohydrate expressed as g/1000kcal. Using ANCOVA, the LM for OS as g/1000kcal had higher BMI (p=.021) and systolic blood pressure (p=.036) than the UM. The LM for polyols as g/1000kcal had higher diastolic blood pressure than the UM (p=.045).

Conclusion

Fermentable carbohydrate intake was low in this population. However, within this range, results suggest higher intake may impact BMI, blood glucose, and blood pressure in healthy US college students. Long term and mechanistic studies are needed to assess potential relationships, including in at-risk populations.

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PREFACE

This thesis was written to comply with the University of Rhode Island Graduate Manuscript Thesis Format. This thesis contains one manuscript entitled “Fermentable carbohydrate intake and differences in health parameters in us college students”. This manuscript has been written in a form suitable for publication in *The Nutrition Journal.*

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MANUSCRIPT INTRODUCTION PAGE

MANUSCRIPT- 1

To be submitted to The Nutrition Journal

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Conclusion

Fermentable carbohydrate intake was low in this population. However, within this range, results suggest higher intake may impact BMI, blood glucose, and blood pressure in healthy US college students. Long term and mechanistic studies are needed to assess potential relationships, including in at-risk populations.

INTRODUCTION

Obesity has increased in the United States (US) in the past 20 years, with approximately 75% of the population age 25-54 years classified as overweight or obese [1, 2]. Abdominal obesity is a strong component of metabolic syndrome, a group of underlying risk factors which predispose an individual to cardiovascular disease (CVD) development [3]. Elevated blood pressure, poor glucose tolerance, and atherogenic dsylipidemia are also components of metabolic syndrome related to CVD risk [3] as well as risk for hypertension and diabetes [1].

A previous observational study of college students found that 46.9% of participating males and 27.2% of females were overweight or obese [4]. Additionally, the study found that college adults were at risk for development of metabolic syndrome. Obesity prevention measures such as healthy diet and regular physical activity have been recommended by the Institute of Medicine in order to combat risk of obesity [2, 5]. However, analysis of self reported measures by U.S. college students indicated that 52% consumed two or more servings of high saturated fat containing foods per day and 36% participated in cardiovascular exercise two or fewer times per week [6]. These students exhibited several risk factors for cardiovascular disease indicating the need for effective counseling and intervention [6].

The human gut may provide a secondary mechanism for influencing obesity as well as blood pressure, glucose tolerance, and dyslipidemia [7-12]. Colonic microflora break down substrates otherwise indigestible by the human gastrointestinal system, creating new metabolically active products [9-11, 13, 14]. Existing concentrations of

gut bacterial colonies may be modified by ingestion of indigestible, fermentable carbohydrates [15-17].

Fermentable carbohydrates are carbohydrates consumed in the human diet, not digested by the human digestive tract, and ultimately fermented by colonic bacteria [18]. This can include resistant starches, non-starch polysaccharides, hemicelluloses, pectins, gums, some mono- and disaccharides, polyols, and oligosaccharides (OS) [18-20]. Of these, non-starch polysaccharides, OS, and some mono- and disaccharides are selectively fermented by beneficial colonic bacteria to promote growth and colonization of more beneficial bacteria [18].

Past research has shown that increased consumption of fermentable carbohydrates enhances concentrations of the beneficial gut microflora, bifidobacteria [21-23]. In mouse models, changes in dietary carbohydrate intake, specifically increasing fermentable carbohydrate intake, are associated with increased concentrations of bifidobacteria, a beneficial gut bacteria, and improvement in body composition [9, 13]. Additionally, fermentation of carbohydrates in the colon yields short chain fatty acid (SCFA) byproducts, which increase circulation of satiety hormones [7]. Changes in gut hormone circulation and perceived satiety could result in changes in weight and energy balance [7-9]. Satiety, postprandial blood glucose, and insulin have improved following short-term consumption of fermentable carbohydrates such as oat-bran, barley kernel, or other OS-rich foods [8, 9]. Overall, human studies involving supplementation of fermentable carbohydrate have shown increases in perceived satiety, decreased energy intake, improved glucose regulation and improved lipid profiles [8, 10, 11].

Current studies focus on the implications of poorly digested, fermentable carbohydrates in relation to decreasing adverse gastrointestinal symptoms in subjects with irritable bowel syndrome or intestinal bowel disease [14, 19, 24, 25]. In contrast, others promote the prebiotic effects exhibited by particular fermentable carbohydrates including OS and polyols [20, 26]. However, these are mostly short-term controlled laboratory studies. No estimation of fermentable carbohydrate consumption has been established in a free-living US population. Furthermore, intakes of subclasses of such carbohydrates, including the short-chain polyols and the longer-chain OS, have not been explored, especially with regard to their potential impacts on health parameters. Given that fermentable carbohydrate consumption may play a role in obesity prevention and glucose regulation, further research is needed to fully elucidate the potential health effects and average consumption of these carbohydrates [8, 9, 12, 27].

The purpose of this study was to, for the first time to our knowledge, estimate intake of fermentable carbohydrates in healthy US college students consuming typical western diets in order to observe differences in health parameters between groups of high and low consumers. It was hypothesized that individuals with higher intake of quantified fermentable carbohydrates (estimated OS and polyols consumed) would have lower BMI compared to those consuming less. Secondary hypotheses were that individuals consuming more grams of polyols or more grams of OS would have lower BMI compared to subjects with low polyol or OS consumption. Exploratory variables in this study were blood pressure, blood glucose, and low density lipoprotein cholesterol (LDL-C).

METHODS

Research Design:

This study was a continuation of the Nutrition Assessment Study, an ongoing investigation of health risk factors in college students, which was approved by the University of Rhode Island (URI) Institutional Review Board (IRB) (IRB HU1112-069). This ancillary study of the larger cross-sectional study examined dietary intake of indigestible, fermentable carbohydrates and their potential relationships to anthropometric, biochemical, and other health related variables. Demographic and anthropometric data, collected as a part of the Nutrition Assessment Study, were used in statistical analysis along with dietary intake measured via the Comprehensive Nutrition Assessment Questionnaire (CNAQ) [28]- an online food frequency questionnaire (FFQ).

Subjects and Recruitment:

University of Rhode Island students from an introductory and an advanced nutrition course completed anthropometric, dietary, biochemical, and health assessments as required course activities, and had the choice of allowing these data to be used for research. Course teaching assistants (TAs) informed the students of their eligibility to participate, described the study, performed informed consent processes and collected signed informed consent forms from those who accepted the invitation to participate. Consenting students in fall 2013, spring 2014, fall 2014, and spring 2015 were given the opportunity to complete the CNAQ [28] as a dietary assessment activity. Data were analyzed from consenting students who completed the CNAQ and were apparently free from GI disorders (determined by survey response). Other

exclusion criteria included pregnancy and noncompliance with fasting or other data collection protocols. All data analyses were conducted on the deidentified data.

Data Collection Procedure:

Data Collection Staff-

Assessment was conducted by trained teaching assistants and supervised by course faculty. Students underwent anthropometric and biochemical assessment at the beginning of the semester and completed the CNAQ during regular lab periods. The CNAQ was analyzed by the FFQ developers [28] and students were provided with dietary analysis results to use for their dietary project.

Measures-

The CNAQ is a 297-item online questionnaire validated in 2010 for use in adults, which evaluates intake of 52 nutritional indices [28]. It was designed to analyze dietary macronutrients, selected micronutrients, indigestible fermentable carbohydrates, starch, glycemic index, and glycemic load [28]. Responses to the CNAQ were processed using a food composition database [28]. Fermentable carbohydrates estimated by the CNAQ include OS, OS subclasses fructooligosaccharide (FOS) and galactooligosaccharide (GOS), polyols, and polyol subclasses sorbitol and mannitol.

Students completed the CNAQ within 20-40 minutes, but the survey could be saved, stopped, and continued over multiple sessions if necessary. Students were prompted to evaluate their average intake of foods over a one year duration (responses include, but were not limited to “daily”, “weekly”, “monthly”, or “never or rarely”). The CNAQ survey page briefly instructed students in documenting conditional items

such as those consumed only when in season. Prompts encouraged students to identify quantities of foods consumed. It was not possible to submit the FFQ with unanswered items. A translation sheet was provided for students to explain differences in Australian and American food terminology. Graduate students were present during lab time to answer questions.

Anthropometric and biochemical measurements were obtained according to standardized protocols. Height was measured using a calibrated stadiometer and weight using a calibrated scale. Body mass index was calculated from height and weight. Body composition measures were obtained after an overnight fast using air displacement plethysmography via BODPOD™ (LMI, CA) [29] to obtain body fat percentage. Before use, the BODPOD™ instrument was calibrated according to manufacturer instruction, subjects were weighed, and height was measured. Blood pressure was measured using an electronic sphygmomanometer- the first reading was done after the participant had been sitting for at least five minutes, the second and any follow-up readings were done at least one minute apart. Biochemical assessment consisted of total lipid profile, including LDL-C and blood glucose (mg/dL), measured via finger stick using the Alere Cholestech LDX System (Serial No. SNAA122881, Alere Inc., Waltham MA). After an overnight fast, blood samples of 40uL were collected from students using capillary tubes for analysis.

In addition to the above assessments, consenting students completed a brief standardized demographic survey at the time of anthropometric assessments. Demographic information including age, gender, ethnicity, college major, and school

year were collected via the survey. These data were used for descriptive purposes and analysis of covariance when necessary.

Statistical Analysis:

Sample Size Calculation-

Sample size was calculated using G*Power (version 3.1.9.2). Sample size was estimated using difference in LDL-C (mmol/L) between a group consuming cereal containing oat-beta glucan (LDL-C = 3.59 ± .06mmol/L) and a group consuming cereal containing wheat bran (LDL-C = 3.84 ± .05mmol/L) after four weeks of daily consumption [30]. Subjects were 154 males and females age 35-70 with a fasting LDL-C between 3.0 and 5.0mmol/L. An effect size of 4.527 was calculated. A sample size of six was estimated in order to find significance for a power of .8 when alpha equals .05.

Descriptives-

Data analysis was conducted using IBM SPSS Statistics, version 22. Categorical variables age, gender, ethnicity, major were presented as frequencies and percentages. Continuous variables including average fermentable carbohydrate intake, biochemical, and anthropometric data were presented as means and standard deviations. Normality was also assessed for continuous variables (BMI, body fat percent, LDL-C, blood glucose, and blood pressure) using skewness and kurtosis. Variables that were not normally distributed were log10 transformed and reassessed for normality as previously described.

Median Split-

Participants who reported consuming less than 500 or more than 5,000kcal per day were excluded from analyses [31]. Consumption of OS and polyols quantified by the CNAQ were added together in order to quantify fermentable carbohydrate intake. Intake of quantified fermentable carbohydrates, oligosaccharides, and polyols were quantified per gram (g) and as grams per 1000kcal (g/1000kcal) to control for the possibility of increased intake from total consumption. High and low intake of quantified fermentable carbohydrates and their subclasses (oligosaccharides and polyols) were then determined by median split. Gram intakes of fermentable carbohydrate consumed per 1000kcal diet were calculated and high and low intakes were determined by median split.

Analysis of Hypotheses-

Analysis of variance (ANOVA) was used to observe differences in BMI between high and low intake groups. Covariates were then determined using correlations and Mann-Whitney U tests. For correlations, in the case of normal distribution, Pearson chi-squared test was applied. If distribution was not normal, Spearman's rho was used. Analysis of covariance (ANCOVA) was then used to observe differences in BMI between high and low intake groups. Exploratory dependent variables (blood pressure, blood glucose, and LDL-C) were assessed similarly.

RESULTS

Participant Demographics

Demographic, anthropometric, biochemical, and nutrient intake data were collected from 431 participants. Of these, 36 did not complete the CNAQ. In some cases, students enrolled in both the introductory and senior course and data were collected twice. For these cases, the most complete data set was used for each participant. Participants who reported consuming less than 500 or more than 5,000kcal per day were excluded from analyses (n = 26) [31]. One participant was excluded from the study due to pregnancy. Two participants were excluded from blood pressure analyses and two from blood panel analyses due to non-compliance with data collection protocols.

Data from 359 participants were used in analysis. Ninety-three percent of the population was between 18 and 24 years old (Table 2). The participant population was 83% female, 84% Caucasian/white, 40% freshman, and 28% were seniors or higher education. Average participant BMI was 23.9 ± 4.3 kg/m^2 and average body fat percentage was 25.4 ± 9.3%.

Average Intakes

Mean quantified fermentable carbohydrate intake was 8.0±4.9g and 3.4±1.4 g/1000kcal (Table 3). Mean oligosaccharide intake was 4.0±2.5g and 1.7±0.7 g/1000kcal. Mean polyol intake was 4.0±3.1g and 1.7±0.7 g/1000kcal. Average intakes for subclasses of oligosaccharides and polyols are listed in Table 3.

Determination of Covariates

Mann-Whitney U tests between median groups initially identified student gender, enrollment in either the introductory or senior course, and whether or not students majored in nutrition as potential covariates ($p<.05$). Using correlations, fiber was identified as a covariate ($p<.000$) for total fermentable carbohydrates, oligossacharides, and polyols consumed in total grams or g/1000kcal. When using ANCOVA to examine differences in mean intake, enrollment in the introductory or senior course was not significantly different between groups. Independent variables discussed below were run in ANCOVA with gender, major, and fiber as covariates.

Differences between High and Low Fermentable Carbohydrate Intakes

Gram intake-

The mean lower median (LM) intake of quantified fermentable carbohydrates was 4.9±1.6g and the mean upper median (UM) intake was 11.8±4.9g (Table 4). Using analysis of variance, the LM had higher BMI (24.4±4.6 vs. 23.3±3.7 kg/m^2 respectively; p=.022), body fat percent (26.6±9.8 vs. 24.1±8.5%; p=.016), and blood glucose (84±8 vs. 82±7 mg/dL; p=.024) than the respective UM groups. After using ANCOVA to adjust for gender, major, and fiber intake, systolic blood pressure was higher in the LM than the UM (117±14 vs. 114±14 mmHg respectively; p=.028).

Gram/1000kcal intake-

Mean LM intake of quantified fermentable carbohydrates was 2.4±0.6 g/1000kcal and mean UM intake was 4.4±1.2 g/1000kcal (Table 4). Using analysis of variance, the LM had higher BMI (24.5±4.6 vs. 23.3±3.8 kg/m^2; p=.009), systolic blood pressure (118±15 vs. 113±13 mmHg; p=.001), and diastolic blood pressure

(75±9 vs. 72±9 mmHg; p=.004) than the respective UM groups. After using ANCOVA to adjust for gender, major, and fiber intake, differences between LM and UM groups remained significant only for systolic blood pressure (p=.027) and diastolic blood pressure (p=.020).

Differences between High and Low Oligosaccharide Intakes

Gram intake-

Mean LM intake of oligosaccharides was 2.3±0.7g and UM intake was 5.8±2.5g (Table 5). No differences between groups were statistically significant in ANOVA or ANCOVA.

Gram/1000kcal intake-

Mean LM intake of oligosaccharides was 1.2±0.3 g/1000kcal and UM intake was 2.2±0.6 g/1000kcal (Table 5). Using analysis of variance, the LM had higher BMI (24.7±4.6 vs. 23.1±3.8 kg/m^2; p=.001), systolic blood pressure (118±15 vs. 113±14 mmHg; p=.002), and diastolic blood pressure (75±9 vs. 73±9 mmHg; p=.038) than the respective UM groups. After using ANCOVA to adjust for gender, major, and fiber intake, differences between LM and UM remained significant for BMI (p=.021) and systolic blood pressure (p=.036).

Differences between High and Low Polyol Intakes

Gram intake-

Mean LM intake of polyols was 2.0±0.8g and UM intake was 6.0±3.2g (Table 6). Using analysis of variance, blood glucose was higher in the LM than the UM (84±7 and 82±7 mg/dL respectively; p=.018). After using ANCOVA to adjust for gender,

major, and fiber intake, systolic blood pressure was higher in the LM than the UM (117±14 and 115±14 mmHg respectively; p=.019).

Gram/1000kcal intake-

Mean LM intake of polyols was 1.0±0.3 g/1000kcal and UM intake was 2.5±1.0 g/1000kcal (Table 6). Using analysis of variance, the LM had higher blood glucose (84±7 vs. 82±8 mmHg; p=.020), systolic blood pressure (117±14 vs. 114±15 mmHg ; p=.045), and diastolic blood pressure (75±9 vs. 72±9 mmHg; p=.018) than the respective UM groups. After using ANCOVA to adjust for gender, major, and fiber intake, diastolic blood pressure remained significantly different between LM and UM groups (p=.045).

DISCUSSION

This study is the first to estimate habitual intake of fermentable carbohydrates and fermentable carbohydrate subclasses in a large sample of 359 US college students. To our knowledge, this is the first study to observe differences in body mass and blood pressure between groups with high and low fermentable carbohydrate intake. This is also the first study to use the CNAQ for applied research purposes.

Participants consuming more quantified fermentable carbohydrates as measured by the CNAQ displayed a lower average BMI using ANOVA, but this did not remain significant when applied in ANCOVA. Participants consuming more oligosaccharides g/1000kcal displayed a lower average BMI using both ANOVA and after correcting for confounding variables with ANCOVA. Potential mechanisms leading to differences in BMI could be related to an increase in circulation of satiety

hormones after consumption of fermentable carbohydrate such as OS [8, 27]. The SCFA produced during fermentation may increase circulation of satiety hormones [7]. Intake of single meals that included fermentable carbohydrate have shown decreased plasma ghrelin and decreased energy intake at subsequent meals compared to meals consumed with little fermentable carbohydrate [8]. Additionally, consumption of 50 grams indigestible carbohydrate at an evening meal resulted in increased perceived satiety ratings in healthy subjects [27]. Similar increases in satiety and decreases in energy intake were seen in healthy adults age 21-39 of normal BMI after 2 weeks of supplementation with 16g indigestible carbohydrate as oligofructose [32]. The current study did not measure satiety or plasma levels of satiety hormones, but this should be pursued in future work to determine if observed differences in BMI might be explained through such satiety-related mechanisms.

Systolic and diastolic blood pressures were lower with higher intakes of quantified fermentable carbohydrates as well as their subclasses, oligosaccharides and polyols. Few clinical trials have investigated the effects of fermentable carbohydrates on blood pressure. Potential mechanisms responsible for the differences observed pertain to regulation of angiotensin-converting enzyme (ACE) activity, glucose tolerance, and systemic inflammation [33, 34]. These mechanisms have been investigated in studies using probiotic supplementation and foods fermented with probiotics [33, 35, 36], but need to be explored with prebiotics such as fermentable carbohydrate.

While, prebiotic substances such as OS can be used to improve intestinal microbiota concentrations by acting as a substrate for existing bacteria [23], probiotics

are live cultures that can achieve the same goal by directly providing beneficial bacteria through the diet [33]. Probiotic supplementation in a controlled, randomized double-blind study has been associated with a 6.7mmHg decrease in systolic blood pressure after 21 weeks in hypertensive individuals in a study by Seppo et al. [36]. This association was also seen in a four week randomized, placebo-controlled double-blind study by Aihara et al. [35]. These observations could be partially due an ACE-inhibitory activity of fermentation byproducts [37], however more investigation is still necessary. Fermented dairy products have exhibited increased ACE-inhibitory activity [38, 39]. Increased ACE activity can increase blood pressure by stimulating the production of a vasoconstrictor and degradation of a vasodilator [40, 41], therefore, decreased ACE activity could inhibit increases in blood pressure.

Soluble fibers can exhibit a prebiotic effect similar to fermentable carbohydrates such as OS and polyols [20]. The observed differences in blood pressure between groups in this study are consistent with past research associating total dietary or soluble fiber with decreases in blood pressure [42-44]. A meta-analysis of randomized controlled trials found a negative association between total dietary fiber (soluble and insoluble) intake and blood pressure [44]. Both systolic and diastolic blood pressures were lowered more in hypertensive populations than in normotensive. Sex and BMI did not affect these differences. A previous study in normotensive subjects found small decreases in blood pressure after 12 weeks of psyllium fiber (a source of viscous, soluble fiber fermented similarly in the colon) supplementation, however this was not significantly different from the control group [43]. In the current study, differences in blood pressure of the observed population groups existed even

though the majority of participants were not hypertensive. The potential mechanism by which insoluble fiber or fermentable carbohydrate intake impacts blood pressure is not fully understood and is still being investigated [43]. Arterial stiffness has been associated with increases in systolic and diastolic blood pressure [45] and is a major risk factor for cardiovascular disease [45, 46]. Dietary fiber can impact insulin regulation and vascular function which may both alter blood pressure [42].

In this study, there was a 2mg/dL difference in fasting blood glucose between groups of high and low intake for total fermentable carbohydrates and for polyols. However, these differences were not significantly different when applied in ANCOVA. Increased fiber intake is associated with increased insulin regulation and can slow absorption of complex carbohydrates, which may impact blood glucose regulation [42]. Previous studies have observed lower postprandial blood glucose peaks and total glucose circulation following meals containing fermentable carbohydrate [8, 27].

In this study, no observed differences in LDL-C were observed. It has been established that water soluble fibers bind to and interfere with resorption of bile acids [47], however the mechanistic role of SCFA byproducts on cholesterol lowering effects of fermentable carbohydrate are still being explored [7, 48-51]. Both SCFA and fermentable sugar beet fiber containing diets have shown lowered plasma cholesterol compared to a fiber-free control diet in rats [49]. However, one study in 12 men (average age 23 years) did not show significant change in blood lipid profiles after 3 weeks of 15g inulin, FOS or GOS supplementation [52]. This could potentially be due to the study population being apparently healthy and non-diabetic, or because

baseline lipid values were low [52]. The population examined in the present study was also apparently healthy.

Study limitations included a mainly female, Caucasian population, so future work should include more males and a more racial-ethnic diversity. As mentioned previously, it included only oligosaccharides and polyols as fermentable carbohydrates. Although additional work is needed on other types of fermentable carbohydrates, such as resistant starches and soluble fibers, focusing on oligosaccharides and polyols can also be seen as a study strength since, to date, so little human work has been published on these. Additionally, the observed intakes of fermentable carbohydrates were low and differences between high and low median intake groups were relatively small, so increased intakes may make more of a difference. To our knowledge this research provides the first quantification of oligosaccharide and polyol intake in a free-living US population and has suggested some potential health benefits despite low consumption. This is the first time this has been documented outside controlled laboratory intervention studies and has tested the largest number of subjects to date for these hypotheses. This study was also the first to observe higher BMI and blood pressure in a group of low fermentable carbohydrate consumers compared to high consumers.

Few instruments are available to quantify intakes of fermentable carbohydrates and their subclasses in large populations. No US-validated FFQ or nutrient database provides all of these carbohydrate subclasses, so the Australian-validated CNAQ was used in this study [28]. Intakes of fermentable carbohydrates were found to be low, as indicated by the CNAQ. However, the amounts consumed in this study were high

enough to elicit differences in some health indices, even within this apparently healthy population.

The results of this study found significantly lower BMI and systolic blood pressure in participants consuming more grams or grams per 1000 kcal of fermentable carbohydrates and oligosaccharides. Subjects with higher polyol intakes also showed potentially lower systolic blood pressure and fasting blood glucose. Despite some differences of only a few points, it is important to note that a small change in health parameters can translate to decreased health risk. For example, difference of 2mmHg, as observed in this study, can significantly decrease mortality from stroke and CVD [53]. Observations in this study were made using healthy, young adult participants, so may not represent changes seen in at-risk or older populations

As this study was cross-sectional no causality can be inferred. Further large scale, longitudinal studies and intervention studies are necessary to understand the potential health impacts of fermentable carbohydrates and their subclasses. Additionally, further investigation into the mechanisms of action leading to observed differences is necessary to elucidate the function and potential uses of fermentable carbohydrates. Longitudinal and mechanistic information could be used when further researching fermentable carbohydrate consumption and its action in at risk populations.

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Table 1. Participants

Total Participant n	**431**
Excluded Participants:	**n**
No FFQ	36
Repeat participants*	7
Pregnant	1
Error in FFQ results	2
<500 kcal/day reported	2
>5000kcal/ day reported	24
Total Excluded	**72**
Final n	**359**

* For repeat participants, the most complete data set was used for analysis

Table 2. Participant Demographics (n =359)

		n	**%**
Age	18-24 years	334	93%
	>24 years	25	7%
Gender	Male	62	17%
	Female	297	83%
Ethnicity	Caucasian	302	84%
	African American	4	1%
	Hispanic/Latino	17	5%
	Asian	14	4%
	Other	22	6%
School Year	Freshman	142	40%
	Sophomore	63	17%
	Junior	34	9%
	Senior or higher edu.	99	28%
	Non-response	21	6%
Major	Nutrition only*	196	55%
		Mean	**Std dev**
BMI	(kg/m^2)	23.9	4.3
Body Fat	(%)	25.4	9.3

BMI=body mass index

*Participants who declared themselves as nutrition majors with no other additional major.

Table 3. Average Fermentable Carbohydrate Intake (n = 359)

	Min	Max	Mean ± std dev
Total Fermentable CHO (g)	1.1	39.2	8.0 ± 4.9
Total Fermentable CHO (g/1000kcal)	0.7	10.7	3.4 ± 1.4
OS (g)	0.7	20.1	4.0 ± 2.5
OS (g/1000kcal)	0.3	5.2	1.7 ± 0.7
FOS (g)	0.4	9.4	2.6 ± 1.6
FOS (g/1000kcal)	0.2	4.4	1.1 ± 0.4
GOS (g)	0.1	11.5	1.4 ± 1.3
GOS (g/1000kcal)	0.1	2.6	0.6 ± 0.4
Polyols (g)	0.4	26.4	4.0 ± 3.1
Polyols (g/1000kcal)	0.2	7.1	1.7 ± 1.1
Sorbitol (g)	0.2	22.9	3.0 ± 2.6
Sorbitol (g/1000kcal)	0.1	6.3	1.3 ± 0.9
Mannitol (g)	0.0	6.9	1.0 ± 0.9
Mannitol (g/1000kcal)	0.0	3.8	0.4 ± 0.4

CHO=carbohydrate; OS=oligosaccharide; FOS=fructooligosaccharide; GOS=galactooligosaccharide

Table 4. Differences in High vs Low Fermentable Carbohydrate Intake

	n	**mean ± std dev**		**significance (p)**	
	LM / UM	LM	UM	ANOVA	ANCOVA
IFCHO (g)	**197/162**	**4.9 ± 1.6**	**11.8 ± 4.9**		
BMI (kg/m2)	166/130	24.4 ± 4.6	23.3 ± 3.7	.022*	.109
Body Fat (%)	177/147	26.6 ± 9.8	24.1 ± 8.5	.016*	.256
LDL-C (mg/dL)	166/139	84 ± 24	86 ± 32	.799	.204
GLC (mg/dL)	178/150	84 ± 8	82 ± 7	.024*	.476
SBP (mmHg)	179/151	117 ± 14	114 ± 14	.177	.028*
DBP (mmHg)	179/151	74 ± 9	73 ± 8	.466	.445
IFCHO (g/1000kcal)	**179/180**	**2.4 ± 0.6**	**4.4 ± 1.2**		
BMI (kg/m2)	147/149	24.5 ± 4.6	23.3 ± 3.8	.009*	.110
Body Fat (%)	160/164	25.5 ± 9.7	25.4 ± 9.0	.881	.342
LDL-C (mg/dL)	150/155	84 ± 25	86 ± 31	.557	.254
GLC (mg/dL)	161/167	84 ± 7	82 ± 7	.127	.798
SBP (mmHg)	159/171	118 ± 15	113 ± 13	.001**	.027*
DBP (mmHg)	159/171	75 ± 9	72 ± 9	.004**	.020*

IFCHO=indigestible, fermentable carbohydrate; BMI=body mass index; LDL-C= low density lipoprotein cholesterol; GLC=blood glucose; SBP=systolic blood pressure; DBP=diastolic blood pressure; LM=lower median; UM=upper median

* significance $p<.05$

** significance $p<.01$

Table 5. Differences in High vs Low Oligosaccharide Intake

	n	**mean ± std dev**		**significance (p)**	
	LM / UM	LM	UM	ANOVA	ANCOVA
OS (g)	**180/179**	**2.3 ± 0.7**	**5.8 ± 2.5**		
BMI (kg/m2)	153/143	24.2 ± 4.2	23.6 ± 4.3	.228	.734
Body Fat (%)	162/162	26.4 ± 9.3	24.5 ± 9.3	.077	.660
LDL-C (mg/dL)	150/155	87 ± 26	83 ± 31	.135	.274
GLC (mg/dL)	163/165	83 ± 8	83 ± 7	.457	.355
SBP (mmHg)	164/166	116 ± 14	115 ± 15	.856	.655
DBP (mmHg)	164/166	73 ± 9	74 ± 9	.379	.206
OS (g/1000kcal)	**179/180**	**1.2 ± 0.3**	**2.2 ± 0.6**		
BMI (kg/m2)	148/148	24.7 ± 4.6	23.1 ± 3.8	.001**	.021*
Body Fat (%)	161/163	26.2 ± 9.9	24.7 ± 8.7	.155	.146
LDL-C (mg/dL)	151/154	86 ± 27	84 ± 30	.512	.789
GLC (mg/dL)	162/166	84 ± 7	82 ± 7	.160	.840
SBP (mmHg)	161/169	118 ± 15	113 ± 14	.002**	.036*
DBP (mmHg)	161/169	75 ± 9	73 ± 9	.038*	.097

OS=oligosaccharide; BMI=body mass index; LDL-C= low density lipoprotein cholesterol; GLC=blood glucose; SBP=systolic blood pressure; DBP=diastolic blood pressure; LM=lower median; UM=upper median

* significance p<.05

** significance p<.01

Table 6. Differences in High vs. Low Polyol Intake

	n	mean ± std dev		significance (p)	
	LM / UM	LM	UM	ANOVA	ANCOVA
Polyol (g)	**181/178**	**2.0 ± 0.8**	**6.0 ± 3.2**		
BMI (kg/m2)	150/146	24.2 ± 4.6	23.6 ± 3.9	.193	.565
Body Fat (%)	162/162	26.4 ± 10.1	24.5 ± 8.4	.063	.888
LDL-C (mg/dL)	155/150	84 ± 25	86 ± 32	.685	.275
GLC (mg/dL)	163/165	84 ± 7	82 ± 7	.008**	.108
SBP (mmHg)	164/166	117 ± 14	115 ± 14	.213	.016*
DBP (mmHg)	164/166	74 ± 9	73 ± 9	.579	.611
Polyol (g/1000kcal)	**180/179**	**1.0 ± 0.3**	**2.5 ± 1.0**		
BMI (kg/m2)	150/146	24.2 ± 4.5	23.6 ± 4.0	.218	.746
Body Fat (%)	164/160	25.4 ± 10.0	25.5 ± 8.6	.895	.211
LDL-C (mg/dL)	156/149	83 ± 24	87 ± 32	.201	.098
GLC (mg/dL)	165/163	84 ± 7	82 ± 8	.020*	.132
SBP (mmHg)	164/166	117 ± 14	114 ± 15	.045*	.080
DBP (mmHg)	164/166	75 ± 9	72 ± 9	.018*	.045*

BMI=body mass index; LDL-C= low density lipoprotein cholesterol; GLC=blood glucose; SBP=systolic blood pressure; DBP=diastolic blood pressure; LM=lower median; UM=upper median

* significance p<.05

** significance p<.01

APPENDIX 1: REVIEW OF LITERATURE

Indigestible Fermentable Carbohydrates

Fermentable Carbohydrates Defined-

Indigestible, fermentable carbohydrates are carbohydrates consumed in the human diet, not digested by the human digestive tract, and ultimately fermented by colonic bacteria [18]. This can include indigestible oligosaccharides (OS), polyols, resistant starches, non-starch polysaccharides, hemicelluloses, pectins, gums, and plant cell wall polysaccharides [18]. Of the total fermentable carbohydrate consumed daily, approximately 8-40 grams are consumed as resistant starch, 8-18 grams as non-starch polysaccharides, 2-10 grams as unabsorbed sugars, and 2-8 grams as OS [21, 54].

Fermentation of particular types of these carbohydrates selectively fermented to promote the growth of beneficial bifidobacteria and lactobacilli in the colon [15, 17, 55, 56]. Resistant starch as well as non-starch polysaccharides are varieties of fermentable carbohydrates that are not selectively fermented and can therefore promote growth of beneficial as well as pathogenic bacteria [11, 13]. While resistant starch makes up the majority of colonically fermented carbohydrate it is not yet quantifiable in the free-living human diet because resistant starch availability and digestibility can be altered by numerous environmental factors such as age of food items, cooking method, cooling, and reheating [57]. Conversely, while OS and polyols are typically consumed in smaller amounts, they are quantifiable and are selectively fermented [21, 23, 28, 58, 59].

Oligosaccharides-

Oligosaccharides are carbohydrate chains consisting of 2-10 monosaccharide units linked by β-glycosidic bonds, which cannot be broken down by human gastrointestinal enzymes [60]. As these bonds are not digested or absorbed in the small intestine, OS pass to the colon.

Two subtypes of OS are galactooligosaccharides (GOS) and fructooligosaccharides (FOS). Galactooligosaccharides are monosaccharide chains found in soy, beans, peas, and lentils [61], but they can also be commercially produced from lactose [62]. These carbohydrates are more readily used by bifidobacteria than any other OS [56]. Fructooligosaccharides are monosaccharide chains present in grains and pastas, fruits, scallions, onions, artichoke, and garlic [63, 64].

Oligosaccharides escape hydrolysis by mammalian enzymes due to the β (2-1) links that connect the monosaccharide units [55]. Bifidobacteria produce the intracellular enzymes, B-fructofuranosidase, B-galactosidase, and other enzymes that hydrolyze β (2-1) links and α (1-2) links found in OS [23, 65]. Because bifidobacteria produce enzymes capable of OS breakdown, growth of bifidobacterial colonies can be selectively stimulated [55].

Polyols-

Along with fibers and resistant starches, polyols or "sugar alcohols" are the most prevalent low digestible carbohydrates found in the US food supply [66]. They are found naturally in some fruits, mushrooms, and cauliflower, but can also be synthesized for addition to food products [66, 67]. Polyols are the reduced forms of different mono- and disaccharides [66]. Two polyols, sorbitol and mannitol, are

hydrogenated forms of their monosaccharide counterparts, glucose and mannose respectively [66]. While some polyols can be absorbed in the small intestine, hydrogenated monosaccharides and dissacharides are more resistant to enzymatic activity in the human intestine, resulting in passage to the large intestine [66]. Different polyols range in sweetness from 40-100% that of sucrose [67], but are lower in caloric density because of their low digestibility making them viable sugar substitutes [66, 68, 69]. Large amounts of polyols in the colon may result in diarrhea due to their rapid fermentation increasing luminal osmolarity [66].

FODMAP Diets-

Current research focuses on the implications of fermentable carbohydrates in relation to adverse symptoms of irritable bowel syndrome or intestinal bowel disease [14, 19, 24, 25]. This is achieved by reducing particular types of fermentable oligo- di- monosaccharides, and polyols in the diet, often referred to as the low FODMAP diet. Reduced FODMAP intake decreases the amount of indigestible, fermentable carbohydrate that passes undigested through the small intestine and into the colon as substrate for bacterial fermentation. This decreases the osmotic effects experienced in the colon and can decrease adverse symptoms experienced by individuals with gastrointestinal disorders.

Indigestible Fermentable Carbohydrates, the Microbiome, and Health

Prebiotics-

Some fibers and certain types of fermentable carbohydrates exhibit prebiotic properties [20, 26]. Prebiotics are a specific type of colonic carbohydrate. They are food substances consumed or added to the diet that selectively promote the growth of

certain types of pre-existing bacterial colonies [18]. Like general colonic carbohydrates, they escape human digestion and travel to the colon, but not all bacteria present in the colon are equally able to ferment and utilize these carbohydrates. Prebiotics promote growth of beneficial bacteria only [18]. Compounds including FOS, soybean OS, raffinose, and stachyose have been established as prebiotics [18, 70].

The Microbiome-

Bacteroides and firmicutes are the predominant types of bacteria found in the microbiome [26, 71]. Lactobacilli, an order of firmicutes, as well as bifidobacterium, a bacteria found in lesser concentrations, are known to be the most beneficial types of gut microbes [72]. Bifidobacteria is thought to be one of the most beneficial types of bacteria due to its ability to inhibit growth of pathogenic bacteria, antibacterial properties, and immunomodulatory potential [18, 22, 73].

Ratios of microbiome bacterial concentrations vary on an individual basis due to a variety of modifiable and unmodifiable factors. In mice, genetics plays a prominent role in determining gut bacteria ratios [74]. Bifidobacteria concentrations are lower in overweight subjects compared to lean subjects [9, 11]. As previously discussed, alterations in diet can also alter concentrations of gut bacteria [11, 13, 71].

Human studies on healthy subjects and those at metabolic risk have shown consumption of fermentable carbohydrate can change bifidobacteria concentrations and improve metabolic risk factors such as glucose regulation and lipid profiles [8, 10, 11]. In general, existing concentrations of gut bacterial colonies remain stable over time, but can be modified by ingestion of indigestible, fermentable carbohydrates [15-

17]. In mouse models, changes in dietary carbohydrate and fat are associated with alteration of gut bacteria ratios and changes in body composition indicating that gut microflora modulation may play a role in management of weight and energy balance [13].

Due to high variation in bacteria concentrations between individuals, variable responses to prebiotic supplementation have been documented [17]. Greater promotion of beneficial bacterial growth and improved intestinal function are seen in those with low initial bifidobacteria [17]. Humans who consumed 6.6g FOS and 3.4g partially hydrolyzed guar gum from biscuits exhibited significantly higher concentrations of beneficial colonic bacteria after 21 days [17]. Additionally, subjects who have initially low concentrations of bifidobacteria are likely to benefit more from fermentable carbohydrate consumption. Tuohy et al. [17] found that individuals who had a lower initial concentration of bifidobacteria had greater increases in concentration after 21 days of fermentable carbohydrate supplementation.

Health Applications-

Including the beneficial bacteria discussed, the more than 500 types of microflora in the human gut provide a secondary mechanism for energy balance, glucose tolerance, satiety hormone regulation, and altering disease risk by breaking down substrates otherwise indigestible by the human gastrointestinal system [7, 9-11, 13, 14]. Fermentation of indigestible carbohydrates by these bacteria produces hydrogen gas, carbon dioxide, methane, and short chain fatty acids [7]. Short chain fatty acid products of bacterial fermentation may improve blood lipid profile and serve to decrease intestinal pH, which improves absorption of minerals [75]. Due to the

large potential impact of colonic bacterial colonies, understanding environmental effects on the microbiome and their resultant effects on the human body is a necessity.

Products and Effects of Dietary Fermentable Carbohydrate Metabolism

It is known that particular fermentable carbohydrate, including polyols, fructans, and oligosaccharides, have health benefits and prebiotic uses [19, 20, 71]. Increased consumption of fermentable carbohydrate has shown lower low density lipoprotein cholesterol (LDL-C) [12, 76] and also improve fasting and postprandial glucose regulation [8, 11, 13]. Studies have demonstrated lower caloric intake and decreased body weight post-intervention with increased consumption of fermentable carbohydrates [8, 76]. However, studies on these carbohydrates have been relatively small ($n<100$) and most have not assessed fermentable carbohydrate intake in populations without complicating factors (ex. hypercholesterolemia, irritable bowel syndrome, diabetes).

Byproducts of Bacterial Fermentation-

Consumption and colonic fermentation of indigestible carbohydrates produces short chain fatty acids (SCFA), hydrogen gas, and carbon dioxide [7]. These SCFA products may play physiological roles in colon health, satiety, and cholesterol metabolism [7, 48, 77]. The human host is able to absorb and utilize SCFA in body tissues [78]. Additionally, increased concentration of SCFA can decrease colonic pH and create an acidic environment that discourages growth of pathogenic microbes and promotes growth of beneficial bacteria [77]. Different SCFA products may influence lipid and cholesterol metabolism by binding of bile salts and other unidentified

impacts on the cholesterol biosynthesis pathway actuated or inhibited by SCFA [7, 48-51].

Because of the extensive diversity of its microbiota, the human colon has a large capacity to produce various metabolites, both beneficial and harmful [7]. Although the complete pathogenesis is unclear, colonic microbiota dysfunction has been associated with diabetes, colon cancer, Inflammatory Bowel Disease (IBD), Irritable Bowel Syndrome (IBS), and changes in immune response [79]. Specific products of microbial fermentation include the SCFAs: acetate, propionate, and butyrate [80]. Acetate is typically produced in the largest quantity [80].

The SCFAs produced in the colon are either used by the colonocytes or absorbed and utilized by the host [81, 82]. Ninety percent of all SCFAs, including the majority of butyrate, produced are absorbed by the colonic microbiota [81]. Propionate is taken up by the human liver and acetate passes into host circulation freely [82].

Changes in Energy Balance-

Short chain fatty acid products are thought to play a role in regulation of satiety hormone release through the activation of free fatty acid receptors [7]. Free fatty acid receptors 2 and 3 (FFA2 and FFA3, respectively) found in human colonic cells, respond to the presence of particular SCFAs [83]. Acetate stimulates the FFA2 receptor [84] and butyrate activates FFA3 [85]. Propionate acts as an agonist for both receptors. In mice, guinea pigs, and humans, enteroendocrine L cells that produce the satiety hormones peptide- YY (PYY) and Glucagon-Like Peptide-1 (GLP-1) are associated with greater expression of FFA2 and FFA3 [86-89]. Intravenous and rectal supplementation of SCFA increased release of GLP-1 and PYY in animal models [90].

The alteration in GLP-1 and PYY resultant of increased SCFA concentrations with carbohydrate fermentation decreases insulin secretion and increases satiety [91]. Long term intake of fermentable carbohydrates increases colonic SCFA concentration. Increased SCFA in the colon is associated with higher L cell proliferation rates, possibly due to the increased expression of promoters for cell production [92, 93]. Understanding of the entire physiological role played by SCFAs and their interactions with enteroendocrine cells with respect to satiety hormones requires further research [7].

An experimental randomized cross-over study of 19 human subjects (age 20-35) compared voluntary food intake and serum levels of glucose, insulin, GLP-1, and ghrelin with different types and amounts of indigestible carbohydrate intake [8]. Peak blood glucose concentration levels after the subsequent breakfast meal were lower when subjects consumed the barley kernel wheat bread that contained more fermentable carbohydrate. Additionally, calorie intake at the following lunch was decreased by 15% in the barley kernel wheat bread compared to the whole wheat bread. Analysis showed a 16% decrease in plasma ghrelin when subjects consumed the barley kernel bread. Fasting blood glucose and insulin levels were not significantly different. The decrease in plasma ghrelin as a result of increased fermentable carbohydrate consumption could play a part in the reduced energy intake at subsequent meals during this study and may, after long term habitual consumption of fermentable carbohydrate, lead to improved energy balance.

In a study by Nilsson et al., [27] the effects of 50g indigestible carbohydrate (not resistant starch) at evening meals were examined in 15 healthy human subjects

age 22-32 years. Subjects consumed a standardized diet for two weeks prior to start of the study and then consumed an evening meal containing either barley kernel (containing 50g fermentable carbohydrate per meal) or white wheat bread. Bread made from barley kernel resulted in a greater decrease in post-breakfast glucose response ($p<0.05$), and lower glucose peaks ($p<0.05$) compared to white wheat bread. Bread made with barley kernels and higher amount of beta-glucans, a type of fermentable carbohydrate, resulted in a greater satiety ratings ($p<0.05$) and reduced inflammatory markers. Improved satiety as a result of increased fermentable carbohydrate consumption as seen in this study could lead to reduced energy intake and therefore improve energy balance over time.

Changes in Blood Cholesterol-

While insoluble fibers have been shown to reduce blood cholesterol by inhibition of bile acid and cholesterol absorption as well as cholesterol synthesis [94, 95], fermentable carbohydrates have been shown to more effectively decrease blood cholesterol levels [96]. It has been established that water soluble fibers bind to and interfere with resorption of bile acids [47], however the mechanistic role of SCFA byproducts on cholesterol lowering effects of fermentable carbohydrate are still being explored [7, 48-51].

Rat cecal contents cultured with sugar beet fiber (SBF) in aerobic conditions to produce fermentation products, primarily SCFA, were fed to rats (80g fermentation product/ 100kg diet) [49]. SCFA and SBF containing diets both lowered plasma cholesterol significantly compared to a fiber-free control diet [49]. Additionally, rats fed a SCFA diet containing acetate had lower plasma cholesterol than the control diet,

whereas rats fed a SCFA diet without acetate did not differ in plasma cholesterol compared to the control. This study identifies acetate as the potential SCFA responsible for the cholesterol lowering effect of fermentable carbohydrates [49].

Another study group fed male rats one of five diets: control, pectin containing, guar gum, gum arabic, or B-cyclodextrin [51]. Of the four diets containing fermentable carbohydrate, the guar gum and B-cyclodextrin diet fed rats exhibited lower triglyceride, LDL-C, and high density lipoprotein 1 cholesterol as well as decreased HMG-CoA reductase activity. Rats fed either guar gum or B-cyclodextrin also had significantly higher cecal propionate concentrations. In opposition to the previous study by Hara et al., conclusions from this study implicate propionate as a primary SCFA involved in reduction of blood cholesterol [51].

A follow-up study using SCFA and SBF diets in rats also resulted in lower plasma total cholesterol in both diets compared to a fiber free control [48]. Greater in vitro cholesterol synthesis was observed in the SBF group compared to control while the SCFA diet resulted in lower synthesis rates. This is possibly due to the SBF diet stimulating increased bile acid excretion and SCFA inhibiting a downstream increase in hepatic cholesterol synthesis that accompanies increased bile acid excretion via regulation of HMG-CoA reductase regulation [48].

Previous studies corroborate the cholesterol lowering effect of fermentable carbohydrates. When fed a combination of guar gum, apple pectin, wheat bran, soybean fiber, and raw potato starch for 3 weeks, rats exhibited decreased blood cholesterol levels and triglycerides [50]. When fed a diet containing lard, oil, or dietary cholesterol in addition to fermentable carbohydrate, elevation of blood

cholesterol and triglycerides was not observed. All rats fed fermentable carbohydrate diets displayed increased cecal SCFA. Despite lower observed levels of HDL-C, plasma clearance of injected LDL-C was increased in the fermentable carbohydrate diet rats compared to a fiber free diet control group [50]. Additionally, liver triglycerides and cholesterol levels were lower and HMG-CoA reductase activity was higher in the fermentable carbohydrate fed rats [50].

Other recent studies have shown changes in blood lipids with differing levels of OS. A randomized trial consisting of 75 hypercholesterolemic human subjects receiving a pill containing 6 grams oat beta-glucan (an indigestible, fermentable carbohydrate) exhibited lower total cholesterol and significantly decreased LDL-C ($p=0.026$) after 6 weeks of supplementation.

One study in 12 men (average age 23 years) did not show significant change in blood lipid profiles after 3 weeks of 15g inulin, FOS or GOS supplementation [52]. This could potentially be due to the study population being apparently health and non-diabetic or because baseline lipid values being healthy or low (although baseline values were not reported).

Changes in Blood Pressure-

Hypertension has been associated with increased CVD morbidity and mortality risks [97]. Reductions of 2-5mmHg of blood pressure have been shown to reduce stroke and CVD mortality risks [53]. The World Health Organization has recommended increasing dietary fiber in hypertensive individuals as a means to reduce risk of CVD [98]. Observational studies have shown an inverse relationship between fiber intake and hypertension [99, 100].

A meta-analysis of randomized controlled trials examined the effect of total fiber intake on blood pressure [44]. Older populations exhibited greater reductions in systolic blood pressure with fiber supplementation. Both systolic and diastolic blood pressures were lowered more in hypertensive populations than in normotensive. Sex and BMI did not affect these differences. Overall, researchers concluded that increasing fiber intake in populations consuming less than the recommendations may play a role in prevention of hypertension [44].

While high fiber intakes have been associated with lower blood pressure, other factors including fruit and vegetable intake, sodium, potassium, magnesium, and calcium can also affect blood pressure [44, 101]. These factors could, therefore, confound potential associations of fiber with lower blood pressure [44].

Fiber intake and cardiovascular events were monitored in more than 69,000 male and female participants followed for over 10 years as a part of the Swedish Mammography Cohort and Cohort of Swedish Men [102]. Nutrient intake was measured using a food frequency questionnaire. After dividing participants into quintiles based on total dietary fiber intake, lower numbers of stroke and hemorrhage occurred in the highest quintile of fiber intake compared to those in the lowest quintile. Gender did not affect these results. Adjustments for intake of vitamin C, folate, B-carotene, magnesium, and potassium did not affect these results. Researchers noted that the observations of this study do differ with past research in which the inverse association of fiber intake and stroke were not persistent after adjustment for potassium and magnesium intakes [102, 103].

Insulin resistance and fasting glucose have been positively associated with risk of hypertension as part of the Insulin Resistance Atherosclerosis Study [104]. The prevalence of hypertension was also higher in those with type 2 diabetes [104]. For both diabetic and healthy subjects, the blood pressure lowering effects of water soluble fibers have been attributed to reductions in insulin resistance [105, 106]. Dietary fiber can impact insulin and vascular endothelial function which, in turn, alters blood pressure [42]. Arterial stiffness has been associated with increases in systolic and diastolic blood pressure [45, 107] and is a major risk factor for cardiovascular disease [46].

Changes in Glucose and Insulin-

Soluble fibers have a bulking effect that increases the viscosity of food passing through the gastrointestinal tract [108, 109]. This process slows gastric emptying, digestion, and absorption [108, 109]. The SCFA produced from colonic fermentation of fermentable carbohydrates can alter insulin sensitivity [110]. Recent research has examined the effects of these carbohydrates on postprandial blood glucose as well as long-term effects.

One experimental study of 88 human subjects (age 30-65) showed an alteration in dietary carbohydrate or fat changed the composition of gut microbiota [11]. Subjects were eligible if they were at risk for development of metabolic syndrome (two or more features of metabolic syndrome). After four weeks of high saturated fat/ high glycemic index (GI) diet, subjects were randomly assigned to diets including: 1) the control reference diet of high saturated fat/ high GI, 2) high monounsaturated fatty acid (MUFA)/high GI, 3) high MUFA/low GI, 4) high carbohydrate/high GI, or 5)

high carbohydrate/low GI. Diets 2, 3, 4, and 5 resulted in decreases in LDL-C compared to the initial four week diet ($p<0.05$). Increased consumption of non-starch polysaccharides was observed in the two low GI diets, ($p<0.01$). Fasting plasma glucose decreased in high carbohydrate diets ($p<0.05$ for both diets). Plasma insulin concentrations decreased in subjects consuming a high carbohydrate/ high GI diet ($p<0.05$). Increased carbohydrate in the diet resulted in a shift of intestinal microbiota to include a greater number of bifidobacteria. Overall, researchers determined that both type and quantity of dietary carbohydrate resulted in a shift in bacterial composition and activities in individuals at risk for metabolic syndrome [11].

Tools of Measuring Fermentable Carbohydrate

The Comprehensive Nutrition Assessment Questionnaire (CNAQ) is a 297-item online questionnaire validated in 2010 for use in adults. The CNAQ evaluates intake of 52 nutritional indices designed to analyze dietary macronutrients, selected micronutrients, fermentable carbohydrate, starch, glycemic index, and glycemic load [28]. Completers of the CNAQ are prompted to evaluate their average intake of foods over a one-year duration. Potential responses include, but are not limited to "daily," "weekly," "monthly," or "never or rarely". Subjects are prompted to contemplate and identify quantities consumed of each food item on an annual basis with respect to abstract concepts such as dietary rotation of foods and seasonal items. The CNAQ survey page briefly instructs completers in documentation of quantities and conditional items such as those consumed only when in season. This FFQ may not be submitted for analysis with unanswered items.

Responses to the CNAQ are processed by developers of the FFQ at Monash University in Australia using a food composition database [28]. The CNAQ survey generates feedback including the estimated daily intake of total and individual mono- and disaccharides (g), oligosaccharides (g), fructans (g), galactooligosaccharides (g), raffinose (g), stachyose (g), sorbitol (g), mannitol (g), glycemic load, glycemic index, total energy (kJ), macronutrients (g), vitamins and minerals (mg), dietary fiber (g), and cholesterol (mg). Compared to three day diet records, the CNAQ validation study indicated that nutrients were overestimated by an average of 140% (with a range of 95-249%).

The CNAQ's ability to measure OS and OS subtypes in addition to monosaccharides, disaccharides, and polyols allows researchers to estimate fermentable carbohydrate intakes in these categories. Other dietary recall and analysis programs may contain more food items compared to the 297 items addressed in the CNAQ but do not quantify all types of fermentable carbohydrates. For example, the Nutrition Data System for Research contains over 23,000 foods and quantifies monosaccharides, disaccharides, polyols, and fibers, some of which can be fermented, but does not quantify oligosaccharides or their subtypes [111]. Comparatively, the CNAQ is a tool to estimate present intake of fermentable carbohydrate and particular subtypes.

The CNAQ was validated in Australia for quantification of FODMAP carbohydrates [28]. It has not been used for applied research purposes in the US to date. Additionally, the researchers responsible for validation of the CNAQ stated that

the 297 items required to accurately assess FODMAP intake were excessive compared to other FFQs which could lead to loss of concentration and accuracy [28].

Implications of Fermentable Carbohydrates for Health

As discussed previously, indigestible fermentable carbohydrates and the byproducts of fermentation have multiple potential health benefits related to obesity, glucose regulation, blood pressure, and blood cholesterol. Obesity, poor glucose regulation, hypertension, and dyslipidemia are risk factors related to metabolic syndrome, which may be improved by increased consumption of fermentable carbohydrate. According to the American Heart Association [112], criteria for clinical diagnosis of metabolic syndrome includes any 3 of five factors:

1. Obesity: waist circumference >102cm in males or >88cm in females
2. Blood triglycerides > 150mg/dL
3. High density lipoprotein (HDL) cholesterol < 40mg/dL in males or < 50mg/dL in females
4. Blood pressure > 130/85mmHg
5. Fasting glucose >100mg/dL

Presence of metabolic syndrome can increase risk for the development of CVD. Decreasing blood LDL-C is also a major focus of therapy in patients with CVD and those at cardiovascular risk [68]. Cardiovascular disease is currently the leading cause of death in the US with 1 in 3 Americans dying of heart disease or stroke [113]. From 2007 to 2010 approximately 7.3% of male adolescents (age 12-19 years) and 7.6% of female adolescents had high blood LDL-C (>130mg/dL) [114]. Some

research subjects have decreased blood LDL-C after the inclusion of fermentable carbohydrate, such as dietary oat-bran, or other fibers [11, 68, 76].

There is evidence to suggest that fermentable carbohydrates have beneficial health effects including reduction of factors associated with hyperlipidemia as well as body weight and insulin resistance [8, 12, 27]. Current studies focus on the implications of poorly digested, fermentable carbohydrates relating to decreasing adverse symptoms in subjects with irritable bowel syndrome or intestinal bowel disease [14, 19, 24, 25] while others promote the prebiotic effects of these indigestible molecules [20, 26]. However, these are mostly short-term studies. There is no research regarding average intake of total indigestible, fermentable carbohydrates in the US young adult population. Furthermore, intakes of subclasses of such carbohydrates, including the short-chain polyols and the longer-chain oligosaccharides, have not been explored, especially with regard to their differential impacts on health parameters.

Need for Examining Fermentable Carbohydrate Intake

Indigestible, fermentable carbohydrates display prebiotic effects and may influence obesity, glucose regulation, blood pressure, and blood cholesterol. Resistant starch is the most quantitatively important fermentable carbohydrate, however this carbohydrate type does not selectively promote the growth of beneficial bacteria [20]. Furthermore, it cannot yet be quantified in free-living individuals due to the various internal and external factors that affect structure and digestibility [57]. Particular fermentable carbohydrates including oligosaccharides and some di- and monosaccharides, such as polyols, are consumed in smaller amounts but may elicit some health benefits. A review by Gibson [20] predicts that 4-8g of these

carbohydrates may be needed to see significant impact, however habitual intake in the US population has not been quantified. As habitual intakes of fermentable carbohydrates and their subclasses have not been explored in the general US population, observation of habitual intake is necessary to further understand their potential metabolic effects.

APPENDIX 2: CONSENT FORM

THE
UNIVERSITY
OF RHODE ISLAND

COLLEGE OF
THE ENVIRONMENT
AND LIFE SCIENCES

THINK BIG WE DO

NUTRITION AND FOOD SCIENCES
112 Ranger Hall, Kingston, RI 02881 USA p: 401.874.2253 f: 401.874.5974 cels.uri.edu/nfs

Title of Project: Nutrition Assessment Secondary Data Analysis

INFORMED CONSENT TO PARTICIPATE IN RESEARCH

You are invited to take part in a research project described below. Students enrolled in NFS 210 and NFS 443 currently have anthropometric and biochemical assessments and complete dietary assessment as part of their coursework. These assessments are used for classroom assignments. We are asking you to give us permission to use these data for research. In addition, we are asking you to complete a few additional demographic and dietary questions. The purposes of the research is to validate assessment methodologies and to investigate the relationship between anthropometric, biochemical, and dietary variables that are related to chronic disease risk. If you have questions you may contact the Geoffrey Greene, the person mainly responsible for this study at 874-4028 or email him at gwg@uri.edu.

Description of the Project:
The purpose of the study is to use nutrition assessment data for research to help us understand the relationship between diet and disease risk in college students.

My Participation
You must sign this informed consent form for the data collected as part of this class to be used for research, and must complete the additional brief questionnaire.

What will be done:
If you take part in this study, your information entered into a password protected computer. Your data will be identified by code number only. Once all data have been entered and verified, the link between code number and identifying data will be destroyed. All data analysis will be conducted by code number only. Assessments that we will be using are listed below (these are collected as part of your class and the additional brief demographic questionnaire):

Demographics	✓
Dietary Assessment	✓
Height, Weight	✓
Waist and Hip Circumference	✓
Air Displacement Plethysmography (BodPod)	✓
Sonographic Measurement of the Heel (bone density)	✓
Standard Blood Tests (TG, HDL, LDL, Total Cholesterol, Glucose)	✓

Risks or discomfort:
The risks are minimal. The only risks would be loss of confidentiality and that will be minimized as described below.

Benefits of this study:
You will not receive any direct benefit. Allowing us to use your data and filling out the brief questionnaire will help us with research to better understand the relationship between diet and chronic disease in college students.

Confidentiality:
Your part in this study is confidential. None of the information will identify you name. We will keep all consent forms in a locked cabinet in Room 307 Ranger for five years. All information collected for the class will be identified by code numbers and will not include any link to your name. This information will be confidential.

Decision to quit at any time:
Your have been given the opportunity to decide whether or not to participate in this study. Your decision to participate will not affect your grade in the class or your relationship with your class instructor. Your instructor will not know who is participating in this study. You have the right to stop participating at any time, but once data have been entered and verified and the link between participant and code has been destroyed, we will not be able to remove your data.

Rights and Complaints:
If you are not satisfied with the way this study is performed, you may discuss your complaints with Geoffrey Greene (401-874-4028) anonymously, if you choose. In addition, if you have questions about your rights as a research participant, you may contact the Office of Research Integrity, 70 Lower College Road, Suite 2, University of Rhode Island, Kingston, RI, telephone: (401) 874-4328.

You have read this Consent Form. Your questions have been answered. Your signature on this form means that you understand the information and you agree to participate in the study. Please note that you must be at least 18 years of age in order to participate.

Print Your Name: _____

_____ _____ _____
Signature of Participant Date Signature of Researcher

Please sign both consent forms, keeping one for yourself

APPENDIX 3: NUTRITION ASSESSMENT STUDY SURVEY

Name:_____ Date:_____
Please Print

Nutrition Assessment Study Survey

1. What is your age (in years)?
<18, 18, 19, 20, 21, 22, 23, 24, 24+

2. What is your gender?
Male, female, choose not to answer

3. Which one of the following best applies to you?
-White
-Black or African American
-Hispanic/Latino
-Asian
-Native Hawaiian or other Pacific Islander
-American Indian or Alaskan Native
-Mixed
-Other (please specify):
-Choose not to answer

4. What is your year in school?
Freshman, Sophomore, Junior, Senior, Graduate

5. What is your current major?
- Agricultural Sciences
- Biological Sciences
- Business/Communication
-Education
-Exercise Science/Kinesiology
-Fine Arts/Humanities
-Health/Nursing
-Nutrition
- Social Sciences
- Undeclared
- Graduate Student
- Other (please specify):

6. Place of residence during the academic year?
- On campus
- Off campus

7. Green Eating is: Eating locally grown foods, limited amounts of processed/fast foods, eating meatless meals at least one day per week, choosing organic foods as much as possible, and only taking what you plan on eating.

Are you a green eater?

- No, and I do not intend to start within the next 6 months
- No, but I am thinking about becoming a green eater within the next 6 months
- No, but I am planning on becoming a green eater within the next 30 days
- Yes, I am a green eater and have been for less than 6 months
- Yes, I am a green eater and have been doing so for 6 months or more
- I choose not to answer

8. Which of the following best describes the MAJORITY of your meals during the academic year?
- I eat meals prepared at home.
- I purchase frozen or ready-to-eat meals
- I eat at dining halls/restaurants
- I get fast food/take-out

9. Do you have a campus meal plan? - Yes
-No

10. What is your usual rate of eating?

Very slow	Slow	Medium	Fast	Very fast
1	2	3	4	5

11. Do you experience abdominal discomfort such as cramping, bloating, or excess gas? (this refers to gastrointestinal discomfort, NOT menstrual discomfort)

- Never or very seldom
- Seldom, less than once per month
- Occasionally, a few times per month
- Fairly often, once or twice per week
- Very often, several times per week or daily

12. If you experience abdominal discomfort , how severe is it?

- I do not experience abdominal discomfort
- Very mild –not very noticeable
- Moderate – noticeable but not too bad
- Somewhat uncomfortable – it's kind of bad, but manageable
- Very uncomfortable – I cannot carry out my normal activities

13. Please select the answer that BEST describes your usual behavior.

	Barely ever to never	Rarely (25%)	Sometimes (50%)	Often (75%)	Almost always
- Locally grown foods are grown within 100 miles of your location. Based on this, how often do you eat locally grown foods?	o	o	o	o	o
- When in season, how often do you shop at farmer's markets?	o	o	o	o	o
- How often do you choose foods that are labeled certified organic?	o	o	o	o	o
- How often do you select meats, poultry, and dairy products that are raised without antibiotics or hormones?	o	o	o	o	o
- How often do you select food or beverages that are labeled fair trade certified?	o	o	o	o	o
- How often do you buy meat or poultry products labeled "free range" or "cage free"?	o	o	o	o	o

APPENDIX 4: CNAQ TRANSLATION SHEET

CNAQ Food Frequency Questionnaire Translation Sheet

Department of Nutrition & Food Sciences, University of Rhode Island, USA

Translation of Australian food terms to American food terms:

1. 'whole meal' = 'whole grain'
2. 250 milliliters (ml) = ~1 cup
3. Fibre = Fiber
4. Spirit = Alcoholic beverage (e.g. whiskey, vodka, rum)
5. Cordial/Squash Drink = Non Alcoholic mixer (usually fruit based, containing sugar and water).
6. Muesli Bar = Granola bar made primarily of rolled oats, fruits, nuts and seeds.
7. Takeaway meal = Fast Food
8. *Chocolate Biscuits = Chocolate cookies and other chocolate confectionaries (e.g. kitkats)
9. *Fruit Biscuits = Cookies made with fruit
10. Beetroot = Beet
11. Silver beet = Chard
12. Capsicum = Red Bell Pepper
13. Rocket = Arugula
14. Swede = Turnip or Rutabaga
15. Broad Bean = Faba or Fava bean
16. *Butter bean = Lima Bean
17. Minced meat = ground meat
18. Yoghurt = Yogurt

Places to include American foods not listed:

1. Greek yogurt - include under 'yogurt'
2. Edamame - include under 'soy products'
3. Almond milk include under 'almonds'
4. Oatmeal - include under cooked cereal, eg porridge

Other foods you do not recognize at all:

Answer 'never' because if you do not know what it is, it is likely that you have not eaten it.

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