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Targeted plasma metabolome response to variations in dietary glycemic load in a randomized, controlled, crossover feeding trial in healthy adults

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Abstract

Low versus high glycemic load (GL) diet patterns are inversely associated with obesity and chronic diseases such as cancer and cardiovascular disease. These associations persist beyond the protection afforded by increased fiber alone, representing an important gap in our understanding of the metabolic effects of GL. We conducted a randomized, controlled, crossover feeding trial of two 28-day diet periods of high and low GL. Using LC-MS, targeted metabolomics analysis of 155 metabolites was performed on plasma samples from 19 healthy adults aged 18-45 years. Fourteen metabolites differed significantly between diets ($P<0.05$), with kynurenate remaining significant after Bonferroni correction ($P<4\times10^{-4}$). Metabolites with the largest difference in abundance were kynurenate and trimethylamine-*N*-oxide (TMAO), both significantly higher after consumption of the low GL diet. Partial least squares-discriminant analysis showed clear separation between the two diets; however no specific pathway was identified in pathway analyses. We found significant differences in 14 plasma metabolites suggesting a differing metabolic response to low and high GL diets. Kynurenate is associated with reduced inflammation, and may be one mechanism through which protective effects of a low GL diet are manifested and warrants further evaluation. This trial was registered at clinicaltrials.gov as NCT00622661.

Keywords

dietary intervention; fiber; glycemic load; kynurenine; metabolomics

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Introduction

Obesity and chronic diseases such as cancer and cardiovascular disease (CVD), collectively account for roughly half of the deaths in the United States [1-3]. Numerous epidemiologic studies find that low glycemic load (LGL) diets, characterized by consumption of foods that result in a relatively small postprandial increase in blood glucose[4], are associated with reduced risk for obesity[5], some types of cancer[5, 6], diabetes[5], CVD[5], and coronary heart disease (CHD)[5, 7]. In contrast, high glycemic load (HGL) diets are associated with an increased postprandial glucose response, post-prandial hyperglycemia, and oxidative stress, which are thought to contribute to the negative health outcomes linked to chronic consumption of HGL diets[6, 8].

Several explanations have been proposed to explain the protective effects of LGL diets. In addition to averting the hyperglycemic and hyperinsulinemic oxidative stress of HGL diets, higher fiber content is a suggested means by which LGL mitigates chronic disease risk. While LGL diets are inherently higher in dietary fiber, human studies have shown that increased fiber alone does not always confer the same degree of risk reduction for chronic disease as does lowering the GL of the diet[6]. Other factors, such as type of fiber (e.g., soluble vs insoluble), structural form of foods (e.g, whole grain vs milled), or the type of foods selected (high- vs low-phytochemical) may also contribute to response to a LGL diet. The protective effects of LGL beyond increased fiber represent an important gap in our understanding of the metabolic effects of differing GL.

Metabolomics is becoming a commonly used approach to identify metabolic differences resulting from various conditions, such as a diseased or non-diseased state, or exposures, e.g., before and after a drug or dietary treatment[9]. Metabolomics captures the current status of an organism's biochemistry and can illuminate consequences of environmental exposures (e.g., food), gene expression, and their combined implications[10]. In nutritional research, metabolomics has been used to identify biomarkers of dietary intake[11], validate food frequency questionnaires (FFQs)[12], and in dietary intervention studies to elucidate dietary effects on metabolic pathways[13]. A few studies have used metabolomics to identify biomarkers associated with glycemic index (GI) or exposure of dietary fiber intake[14, 15]. For example, after 77 overweight adults were randomized to high or low glycemic index diet for 6 months, authors found urinary formate discriminated between the two dietary patterns [14]. In another study, plasma concentrations of 2,6-dihyroxybenzoic acid and 2-aminophenol sulfate were increased after 5 weeks of consuming a high fiber diet compared to a low fiber diet[15]. However, no controlled feeding study has investigated metabolic changes in the plasma metabolome based on dietary GL in a crossover design.

In the present study we take an agnostic approach to evaluate the extent to which HGL and LGL diets modify the plasma metabolome using a targeted metabolomics approach on specimens collected from a controlled feeding trial. We hypothesize that LGL diets influence metabolism differently than HGL diets and that these effects will result in differences in metabolite abundances between the two diets. Results from this study may provide insight on the protective benefits of consuming low glycemic load diets.

Materials and Methods

The metabolomics analysis was a secondary analysis using samples derived from the "Carbohydrate and Related Biomarkers" study (CARB) conducted between June 2006 and July 2009 at the Fred Hutchinson Cancer Research Center (FHCRC) in Seattle, Washington. This trial was registered at clinicaltrials.gov as NCT00622661. CARB was a crossover dietary intervention with two 4-week feeding periods of HGL and LGL diets given in a computer-generated, randomly assigned order[16]. Metabolomics analysis was conducted on plasma collected from a subset of 20 participants at the end of each intervention period, from which samples from 19 pairs could be analyzed. The study protocol was approved by the FHCRC Institutional Review Board and all participants gave written informed consent.

Participants and Study Design

Details on recruitment and study design have been published elsewhere [16]. In brief, non-smoking, healthy individuals between the ages of 18-45 years were recruited from the Seattle area. Exclusion criteria included impaired fasting glucose (fasting blood glucose ≥ 5.6 mmol/L), any physician-diagnosed condition requiring a restricted diet, food allergies, regular use of hormones or anti-inflammatory medication, pregnancy or lactation, or heavy use of alcohol (>2 drinks per day). A total of 82 individuals completed all study activities. The study population was 50% male and was evenly distributed between normal weight (BMI >18.5 ≤ 25.0 kg/m^2) and overweight/obese (BMI ≥ 28.0 ≤ 40.0 kg/m^2)[17]. Diet order was randomly assigned, with half of the participants receiving the HGL diet first. Each intervention was four weeks long, with a four week washout period in between study periods[16]. A subset of 20 individuals was selected for this pilot metabolomics analysis. The group was selected to represent the larger study population: 50% male, evenly distributed between normal and overweight, with half having received the HGL diet first. Blood was drawn at the end of each intervention period for a total of 40 samples; however, one sample was compromised during sample preparation. Thus both time-points for that individual were excluded and final analysis was conducted on the remaining 19 pairs.

Study Diets

The study diets were based on a repeating seven-day menu, with identical distribution of macronutrients for both the HGL and LGL diets (see Table 1 for sample menu). The diets were designed to differ largely in GL, with the LGL diet being half the GL of the HGL intervention (125 and 250 GL/d respectively), and differed notably in fiber (55 and 28 g/d for the LGL and HGL, respectively). Total GL for each mixed meal was calculated by multiplying the grams of carbohydrate in each food by that food's GI value. The diets were isocaloric for each individual, although minor adjustments were made as necessary to keep participants' weight stable during the intervention (Table 2). Further details of the study diet have been published previously[16]. All food was provided by the FHCRC during the intervention, with weekday dinners consumed under supervision at the Center and the next day's breakfast and lunch taken home for consumption. On Fridays, participants received all weekend meals. Any unconsumed food was returned to the study center, weighed and recorded. During the washout period individuals returned to their habitual diet patterns.

Sample Collection And Analysis

At baseline, height and weight measurements were recorded for assignment to the overweight + obese (BMI > 28 kg/m^2) or normal weight (BMI < 25 kg/m^2) category. Those with BMI 25-27.9 kg/m^2 were excluded to ensure sufficient contrast between the normal weight and overweight/obese groups. Whole-body dual-energy X-ray absorptiometry (DEXA) scans (GE Lunar DPX-Pro) were also completed for each participant to accurately assess body fat percent. Weight measurements were taken regularly throughout the study period, with adjustments made to the study food provided as necessary to keep participants weight stable[16]. Blood was taken from each participant on Day 28 of both treatment periods after a 12-hour overnight fast, and processed and stored at -80°C using a standard protocol.

Metabolomics analysis

Metabolite profiling of plasma was completed at the University of Washington's Northwest Metabolomics Research Center. Targeted metabolomics analysis on the plasma of the subset of 19 participants was carried out using a liquid chromatography tandem mass spectrometry (LC-MS/MS) platform in both positive and negative ion modes against 155 standard metabolites from 25 metabolic pathways, (e.g., Glycolysis, TCA cycle, Amino Acid Metabolism, etc.) of potential significance to monitor diet effects (see Supplemental Table)[18-20].

All plasma samples were prepared at the same time. A standard protocol was used, where 25 μL plasma and 150 μL high performance liquid chromatography (HPLC) grade methanol were combined in an Eppendorf vial and vortexed for 2 min. After 20 min storage at -20 °C the samples were centrifuged at 18,000 g for 10 min. A fixed volume of 150 μL supernatant was collected and placed in a new Eppendorf vial. The protein pellets were mixed with another 300 μL HPLC grade methanol, then vortexed for 10 min and centrifuged for 10 min at 18,000 g. 250 μL was collected and combined with the previous 150 μL sample. Samples were then dried at 30 °C in a Speed Vac for 3 h.

The samples were analyzed in two sequential batches, each containing two quality control (QC) samples so that batch variation could be assessed. Prior to each LC run, samples were reconstituted with 100 μL 5 mM ammonium acetate in 95% water/5% acetonitrile + 0.5% acetic acid, and filtered through 0.45 μm PVDF filters (Phenomenex, Torrance, CA) prior to analysis on an AB Sciex QTrap 5500 LC-MS/MS system (AB Sciex, Toronto, ON, Canada)[21-23]. The LC system was composed of two Agilent 1260 binary pumps, an Agilent 1260 auto-sampler and Agilent 1290 column compartment containing a column-switching valve (Agilent Technologies, Santa Clara, CA). Each sample was injected twice, 10 μL for analysis using negative ionization mode and 2 μL for analysis using positive ionization mode. Both chromatographic separations were performed in reverse phase (RP) on Thermo Accucore PFP columns (150 × 2.1 mm, 2.6 μm particle size, Thermo Fisher Scientific Inc., Waltham, MA). The flow rate was 0.250 mL/min, auto-sampler temperature was kept at 4 °C, and the column compartment was set at 40 °C. The mobile phase was composed of Solvents A (5 mM ammonium acetate in H2O + 0.5% acetic acid + 0.5% acetonitrile) and B (acetonitrile + 0.5% acetic acid + 0.5% water). After chromatographic separation, MS ionization and data acquisition was performed using AB Sciex QTrap 5500 mass

spectrometer (AB Sciex, Toronto, ON, Canada) with electrospray ionization (ESI) source. The collision gas was 99.99% pure nitrogen. The data gathered through the multiple reaction monitoring were integrated using MultiQuant 2.1 software (AB Sciex, Toronto, ON, Canada).[20] Intra-assay CVs were 9.4% and 6.3 for negative and positive ion mode, respectively and 7.4% across all samples.

Statistical Analysis

Of the 155 metabolites measured, 125 had detectable signal in all study samples and were retained for analysis. Plasma data showed between-batch variability, and were therefore normalized using the mean signal among all study samples for a given batch as the constant by which to standardize the values for that metabolite within-batch. Paired t-tests were conducted on log-transformed metabolite values to identify statistically significant ($P<0.05$) within-person differences in plasma metabolite abundances, comparing Day 28 measurements from the LGL versus the HGL diet. Bonferroni and Benjamini-Hochberg (false discovery rate, FDR) methods were used to correct for multiple comparisons (Stata v13.1, College Station, TX)[24]. To compare the relative abundance of metabolites (within-person) between diet interventions, geometric mean ratios of detected metabolite concentrations after the LGL diet versus the HGL diet were calculated by exponentiating the mean within-person differences in log-transformed metabolite signals.

While the study protocol attempted to achieve weight maintenance, most participants experienced a minor change in weight (~1%). Up to 3% change was permitted, consistent with many dietary intervention studies as it represents minor fluctuations in fluid balance and normal day to day variation. To ensure that these minor fluctuations did not impact the findings, a linear mixed regression model was used to test the effects of weight change and diet on metabolite concentrations, modeled as a fixed effect, and individual baseline metabolite concentrations as a random effect (R statistical package v3.01, with package lme4 1.0). Models were also carried out to evaluate metabolites adjusted for fat distribution as determined by android to gynoid body fat % ratio, and body fat % using data derived from DEXA scans. Finally, to ensure that there were no carry over effects, diet sequence and period were evaluated. As these covariates did not have any effect on point estimates, data are presented without adjustment.

To consider differences occurring beyond the level of individual metabolites, pathway level analyses were conducted using the global test for changes among groups of metabolites described by Goeman, et al.[25] (*Globaltest* R statistical package v3.02). *A priori* pathways assessed were Krebs cycle, Gluconeogenesis and Glycolysis. Among inflammation-related pathways, the Tryptophan pathway was the only one with a sufficient number of metabolites to allow for meaningful analysis. We evaluated each pathway using 4 to 7 metabolites from the respective pathways, as identified in the Human Metabolome Database (HMDB)[26] and Kyoto Encyclopedia of Genes and Genomes (KEGG)[27] databases.

To determine whether the intervention diets led to holistic differences in metabolic responses, partial least squares discriminant analysis (PLS-DA) plots were generated via class separation in MetaboAnalyst 2.0 (http://www.metaboanalyst.ca/) with input of all metabolites, using the method described by Westerhuis, et. al., which considers the paired

data structure.[28] Leave one out cross-validation method was used to test the predictive performance of the model, and R^2 and Q^2 values were calculated. The variable importance in projection (VIP) score was used to estimate the influence of each metabolite to the first two components of the PLS model. Variables with VIP scores >1 are generally considered to have an important contribution to the model.[29]

Results

Characteristics for the 19 participants stratified by sex are given in Table 3. There are approximately an equal number of men and women evenly distributed between normal and overweight/obese.

Of the 125 plasma metabolites detected, a total of 14 metabolites differed significantly between the LGL and HGL interventions ($P<0.05$; Table 4). After Bonferonni correction for multiple comparisons ($P<0.05/125=4\times10^{-4}$), one metabolite (kynurenate) remained significant, while two additional metabolites, cystamine and methyl succinate, satisfied the less stringent threshold of FDR $q<0.20$. Geometric mean changes for metabolites in LGL compared to HGL ranged from 0.77-1.37 with a near equal number of analytes increasing as decreasing after the LGL diet relative to the HGL diet. Metabolites with the greatest fold change between diets were kynurenate and trimethylamine N-oxide (TMAO). Adjusting for weight change, body fat %, and fat distribution did not alter these findings (data not shown). Pathway analyses for Krebs cycle, Glycolysis and Gluconeogenesis, and Tryptophan metabolism did not yield significant findings (data not shown).

PLS-DA using all 125 detected metabolites showed good separation between the diets by the primary and secondary components (Figure 1). Together, these two components accounted for ∼23% of the variability (11.7% and 10.7% for the first and second components, respectively). The R^2 and Q^2 values were 0.72 and 0.45, and 0.91 and 0.75, for components 1 and 2 respectively. The metabolites with the greatest contribution in distinguishing the diets in the first component were kynurenate, cystathionine, glycocholate, glycochenodeoxycholate, adenylosuccinate, glyceraldehyde 3-phosphate, and biotin, all with VIP scores >2. The metabolites with the greatest contribution in the second component based on VIP scores of >2, were kynurenate, cystathionine, glycochenodeoxycholate, hippuric acid, glycerol-3-phosphate, and biotin.

Discussion

In this randomized crossover feeding trial, concentrations of 14 plasma metabolites differed significantly in abundance between the HGL and LGL intervention diets, with a near-equal number increasing as decreasing after the LGL. Differentially-abundant metabolites could be classified into several different metabolic pathways, but we were not able to unequivocally identify pathways that would be uniquely related to GL. Further analyses, focused on energy metabolism and the Tryptophan pathway using all retained metabolites, did not reveal statistically significant pathway perturbations between the diets. Differences in some metabolites between diets were small (5-37%), yet all but one were larger than the overall intra-assay variation (7%). Although modest, such differences compounded over

decades may, in part, account for observed differences in disease risk associated with differing habitual dietary patterns. Further, these differences may increase over time.

Despite the lack of significant pathway differences, the PLS-DA plot showed good separation between the diets with several metabolites, including kynurenate contributing considerably (VIP scores >2) to the model. Kynurenate was also identified in univariate analyses as significantly different between interventions, satisfying the stringent Bonferroni significance threshold ($P<0.05/125=4\times10^{-4}$). While plasma kynurenate was ~40% higher after the LGL diet compared with the HGL diet, its precursor, tryptophan, was not appreciably altered.

Tryptophan is converted to kynurenine, and then further degraded to quinolinate or kynurenate, which agonize and antagonize *N*-methyl-D-aspartate (NMDA) receptors, respectively[30]. While the effect of tryptophan metabolites is most pronounced in cerebrospinal fluid, NMDA receptors are also found in peripheral tissue, where they elicit the same excitatory and inhibitory responses[31]. Among other functions (e.g., cognition and memory), these receptors modulate inflammation pathways[31, 32]. Whereas quinolinate activates NMDA receptors, stimulating immune activation, kynurenate inhibits these effects. Modulatory effects on inflammation have been noted apart from the effects these metabolites have on receptor activity. For example, quinolinate is also produced by macrophages in the periphery in response to cytokine activation which, in turn upregulates other inflammatory mediators, such as MCP-1, a potent chemoattractant[33], as well as enzymes involved in the conversion of tryptophan to quinolinate[31]. Therefore, quinolinate not only stimulates pro-inflammatory pathways, but also upregulates the enzymes which lead to its further production, in a forward-feed cycle. Further, quinolinate, through interaction with hydroxyl kynurenine, an intermediate in the quinolinate pathway, is a pro-oxidant[34]. Consequently, a shift towards the kynurenate, or inhibitory side of the pathway, as observed after the LGL diet, suggests potential anti-inflammatory effects of the intervention[35, 36]. This finding is consistent with our previous report of reduced C-reactive protein concentrations after the LGL diet [16].

When considering directionality of all plasma metabolites in the Tryptophan pathway measured in our metabolite panel, regardless of significance (e.g., tryptophan, L-kynurinine, kynuranate, 3-hydroxykynurenine, xanthurinate, and quinolinate), a consistent shift away from quinolinate, and toward the kynurenate side of the pathway was observed; however, there was no significant difference in quinolinate concentrations between the diets. Although we did not determine absolute concentrations in plasma, studies suggest that the ratio of kynurenate to quinolinate is what determines its protective effects. Indeed, a higher ratio of quinolinate to kynurenate has been implicated in many neurodegenerative diseases thought to have an inflammatory basis, e.g., Alzheimer's disease, Parkinson's disease and Huntington's [34, 37]. Thus, increases in kynurenate relative to quinolinate, as observed after the LGL diet in the present study, is hypothesized to be beneficial[35, 36, 38], and may be one mechanism by which LGL diets contribute to reduced chronic disease risk.

TMAO was 37% higher at the end of the LGL compared to the HGL intervention. Generation of TMAO occurs via oxidation of gut bacterially-derived trimethylamine (TMA)

in the liver. Therate of TMA to TMAO conversion is determined by a combination of genetics and substrate availability[39]. Given the crossover nature of this study, differences are likely due to substrate availability, although diet itself may alter the gut microbial community, in turn affecting TMAO production[40]. Precursors for TMA include carnitine, choline and betaine[41, 42]. These substrates are absorbed by a combination of active and passive transport in the small intestine, only reaching the microbiota responsible for their conversion to TMA in the large intestine when doses are high enough to saturate these transport mechanisms[43-45]. The LGL diet had slightly less protein from animal sources, a rich source of carnitine, than the HGL diet (daily average of 47% of total animal protein compared to 59%) to accommodate other foods associated with lower GL (e.g., beans and whole grains). As these lower GL foods are higher in protein, meat content was reduced to keep macronutrient ratios constant between the diets. Carnitine was therefore lower in the LGL diet (Table 4). In contrast, the inclusion of more whole grains and leafy greens on the LGL diet resulted in ∼80% higher betaine, based on the nutrient data available, although it should be noted that betaine content of diet is not as well-documented as that of other micronutrients. Dietary intake of choline appeared to be similar between the diets. Thus, only one of the TMAO precursors —betaine, was higher in the LGL diet. It is possible that consuming a LGL diet, inherently high in fiber, results in changes to the gut microbiome community structure that enhance production of TMAO, however studies done on individuals who consume vegan or vegetarian diets, or other diets characteristically high in fiber, would suggest otherwise[46, 47].

TMAO is hypothesized to be associated with CVD risk due to its interference with cholesterol clearance, and the association between red meat intake and CVD is attributed, in part, to carnitine-derived TMAO [42, 48]. Therefore, the increase in TMAO after the LGL diet is contrary to what might be expected. Increased intakes of whole grains and leafy greens are widely accepted as healthy dietary behaviors[49], with betaine specifically identified as contributing beneficially as a methyl group donor to homocysteine[50]. A recent review evaluating dietary betaine and choline intakes not only found no association between increased intake and CVD risk, but reported reduced inflammation and other CVD risk factors[51]. Solanky et al. reported similar findings of increased TMAO after a dietary intervention with soy[52], and TMAO is found in high quantities in fish[53], both foods to which health benefits have been ascribed.

To our knowledge, this is the first study to use metabolomics to interrogate metabolic differences in specific pathways as a result of high and low GL dietary patterns in a crossover study design. While not specifically evaluated in other studies of GL, targeted metabolomics has been applied in the context of glycemic index, fiber and whole grain intake, mainly for dietary biomarker discovery[14, 15, 54]. An additional strength is the crossover design where individuals serve as their own control. The 4-week duration of the controlled diet intervention, where all foods were provided, was of sufficient length to allow us to capture changes in metabolism[16]. The participants largely maintained their weight on the standardized diets where macronutrient levels, with the exception of fiber, were held constant, differing only in GL. Adherence to the diets was excellent, with 97% of the participants consuming > 90% of the study provided food, with no difference in compliance between the two study diets. Targeted metabolomics, unlike global metabolomics, also

enabled us to be more certain about the identities and relative abundances of the metabolites, although our targeted profile was limited to 125 mostly aqueous metabolites.

This study was limited in that LC-MS was used, and some compounds, such as some volatile compounds, are not well detected with this method. Additional analysis with GC-MS and NMR, as well as lipidomics could provide more comprehensive insight into metabolic changes between the two GL diets. Fitness and activity level impact the metabolome[55] and while participants were asked to keep physical activity constant, detailed data on daily physical activity were not collected, and thus no adjustments were made. Although we were able to adjust for body fat and weight change, these variables account for only a small proportion of total metabolome variation. Finally, although fiber is a fundamental determinant of GL, it is difficult to ascertain whether the results of consuming a LGL diet pattern are due to differences in amount of fiber, type of fiber, structure of the foods, or other dietary constituents associated with low GI.

Conclusion

In summary, we found significant differences in plasma metabolites between a high and low GL diet which appear to suggest a metabolic response to differing GL; however, there were no statistically significant discernable shifts in the specific pathways examined that explain reported associations between LGL diet and reduced chronic disease risk. While the first two components in the PLS-DA analysis accounted for ∼22% of the variance, and only 14 metabolites were significantly altered between the diets, our ability to distinguish between the diets in this discriminate analysis suggests the GL of a diet affects plasma metabolites broadly. The metabolite with the largest difference in abundance was kynurenate, which increased after the LGL interventionm was identified as one of the small number of metabolites driving the separation between diets seen in the PLS-DA. Kynurenate, the only metabolite remaining statistically significant after Bonferonni correction, is associated with reduced inflammation and may be one mechanism through which protective effects of a LGL dietary pattern are manifested.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

GI	glycemic index
GL	glycemic load
LGL	low glycemic load

HGL	high glycemic load
TMAO	Trimethylamine-N-oxide
TMA	Trimethylamine
CVD	cardiovascular disease
CHD	coronary heart disease
FFQ	food frequency questionnaire
CARB	Carbohydrate and Related Biomarkers
FHCRC	Fred Hutchinson Cancer Research Center
DEXA	dual-energy X-ray absorptiometry
LC-MS	liquid chromatography mass spectrometry
GC-MS	gas chromatography mass spectrometry
NMR	nuclear magnetic resonance
HPLC	high performance liquid chromatography, QC, quality control
FDR	false discovery rate
PLS-DA	partial least squares discriminant analysis
VIP	variable importance in projection

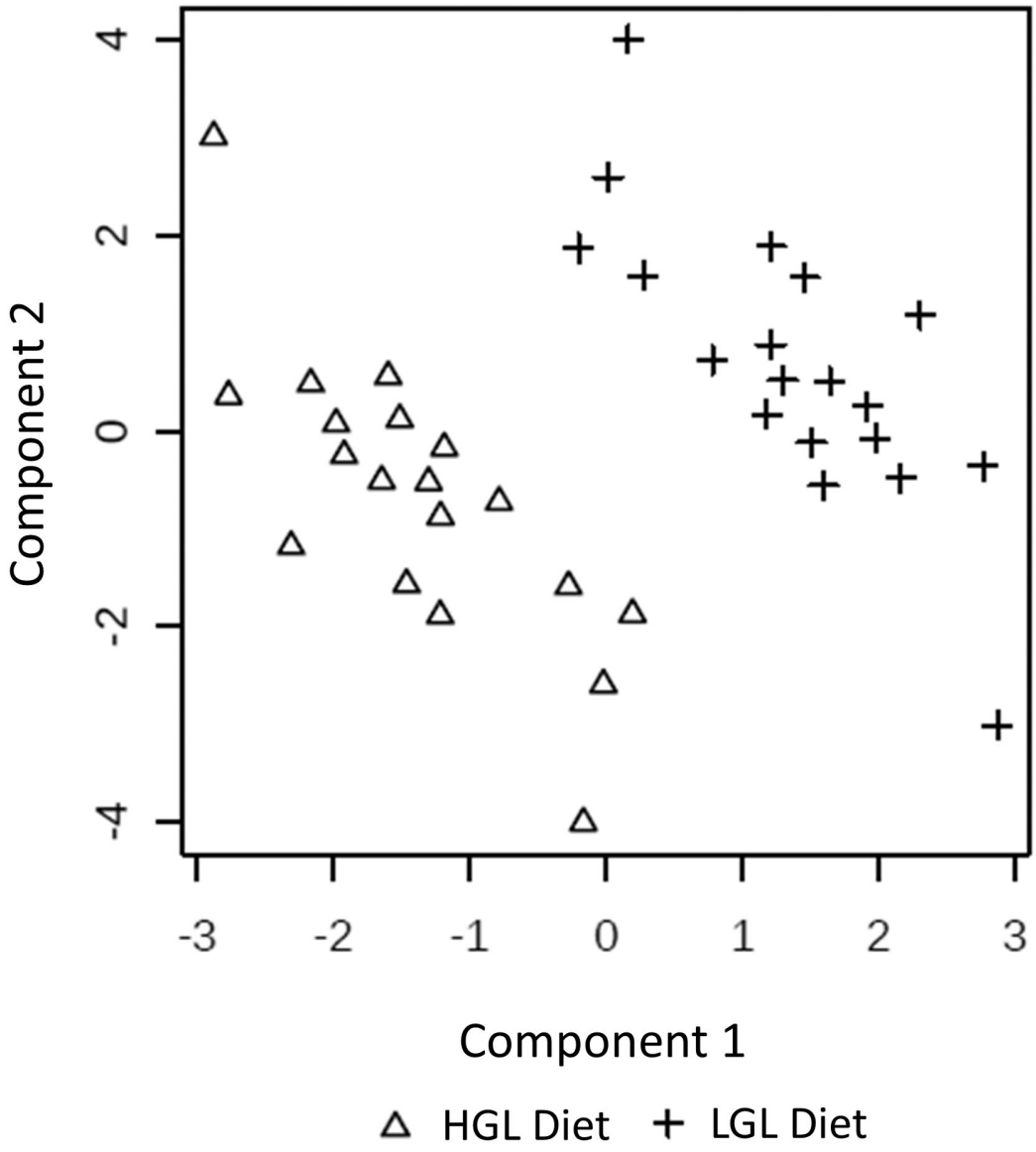

Figure 1.

PLS-DA score plot of plasma metabolites after 28-d consumption of high glycemic load (HGL, Δ) and low glycemic load (LGL, +) diets in a randomized crossover-feeding study. The within individual variation and class-separated score plot between the selected components showed clear separation. The first component accounted for 11.7% and the second for 10.7% of the variance. R^2 and Q^2 values were 0.0.72 and 0.45, and 0.91 and 0.75 for components 1 and 2, respectively.

Table 1

Sample daily meal plans for high glycemic load (HGL) and low glycemic load (LGL) intervention diets in the Carbohydrate and Related Biomarkers study.[1]

HGL Breakfast		**LGL Breakfast**	
Grape-nut cereal	Dates	All Bran	Strawberries/blueberries
2% milk	Dried cranberries	2% milk	Nut crunch
Sweetener		Sweetener	Tomato juice
HGL Lunch		**LGL Lunch**	
White bread	Cauliflower	Pumpernickel bread	Carrots
Roast beef	Potato salad	Roast beef	Tabouli
Mayo	Ranch dressing	Mayo	Hummus
Mustard		Mustard	
Tomatoes	*Dessert*	Tomato	*Dessert*
Lettuce	Fruit roll ups	Lettuce	M&Ms
Onions	Jellybeans	Onions	Pears
Pickles	Apricots	Pickles	
HGL Dinner		**LGL Dinner**	
Chicken breast	Sour cream	Chicken breast	Sour cream
Green pepper	White rice	Green pepper	Tortilla
Red pepper	Taco shell	Red pepper	
Onions	*Dessert*	Onions	*Dessert*
Mexican sauce	Rice pudding	Mexican sauce	Chocolate mousse
Salsa	Cranberry juice	Salsa	Apple juice
HGL Snack		**LGL Snack**	
Energy bar		Dried apple	Chocolate power bar

[1]Menuswere designed to contain similar foods with specific items substituted to alter glycemic load (GL) of overall meal so as to minimize change to the diet outside of GL

Table 2

Summary of average daily intake macronutrient and glycemic load (GL)in high glycemic load (HGL) and low glycemic load (LGL) intervention diets.[1]

Daily intake	HGL diet		LGL diet	
Energy, kcal	2610 (476)		2719 (563)	
Carbohydrate, g; %	364 (69)	67%	386 (84)	68%
Protein, g; %	95 (18)	17%	98 (22)	17%
Fat, g; %	86 (16)	16%	87 (20)	15%
GL	259 (50)		125 (27)	
Fiber, g	28 (5)		56 (12)	

[1] Mean (SD)

Table 3
Baseline characteristics of study participants (n=19)

Demographics	Men	Women
N	9	10
Age, y[1]	31.3 (9.2)	31.9 (9.5)
BMI[2] group (N)		
Normal (BMI < 25)	4	4
Overweight/obese (BMI > 28)	5	6
Mean body fat %[3]		
BMI < 25	20.57 (4.4)	28.74 (5.6)
BMI > 28	36.19 (6.0)	45.58 (4.6)
Mean waist/hip ratio[4]		
BMI < 25	0.86 (0.0)	1.08 (0.2)
BMI > 28	(0.11)	1.21 (0.1)
Fasting blood glucose, mmol/L[1]		
BMI < 25	4.9 (0.3)	4.9 (0.4)
BMI > 28	4.8 (0.8)	4.8 (0.7)

[1] Mean (SD)

[2] BMI, body mass index= weight in kg/m^2

[3] Body fat % (SD), measured by DEXA

[4] Waist to hip ratio (SD), calculated using android body fat % divided by gynoid body fat % from DEXA scan data

Table 4

Plasma metabolites significantly different at Day 28 between LGL/HGL dietary intervention (N=19)

Plasma Metabolite	Pathway	Function	Ion mode	Ratio[1]	*P*-value[2]	q-value[3]	
Kynurenate	Tryptophan metabolism	NMDA receptor antagonist; modulates inflammation	-	1.40	0.0002*	0.02	
Methyl succinate	Dicarboxylic acids and derivatives	Energy production	-	1.14	0.004	0.18	$p < 0.01$
Cystamine	Taurine and hypotaurine metabolism	Amino acid metabolite	+	0.77	0.004	0.18	
Proline	Arginine and proline metabolism	Amino acid	+	1.11	0.010	0.29	
Acetylcholine	Glycerophospholipid metabolism	Neurotransmitter; in the periphery it activates skeletal muscles; inhibits cardiac muscles to slow heart rate	+	0.86	0.014	0.29	
Hydroxyproline	Arginine and proline metabolism	Component of collagen	+	0.83	0.016	0.29	
Creatine	Glycine, serine, threonine metabolism; Arginine and proline metabolism	Energy production	+	0.83	0.016	0.29	
Trimethylamine *N*-oxide	Gut bacterial metabolite	Osmolyte	+	1.37	0.025	0.39	$p > 0.01$ and $p < 0.05$
Carnitine	Fatty acid metabolism	Energy production via beta oxidation	+	1.11	0.030	0.42	
Homovanillate	Tyrosine metabolism	Catecholamine/dopamine metabolite	-	1.18	0.036	0.42	
Lysine	Biotin metabolism;Carnitine synthesis	Amino acid	+	1.05	0.039	0.42	
Nitrotyrosine	Product of reactive nitrogen species	Marker of oxidative stress	-	1.12	0.042	0.42	
Niacinamide	Vitamin B3	Coenzyme in oxidation-reduction reactions	+	0.85	0.046	0.42	
Dimethylguanosine	Nucleoside	RNA/DNA synthesis	+	1.10	0.047	0.42	

[1] Geometric mean within-person ratio of plasma metabolite concentrations comparing LGL diet versus HGL diet at Day 28; a ratio >1 means that the metabolite was higher in the LGL diet relative to the HGL diet, whereas a ratio <1 means that the metabolite was lower

[2] Paired t-test *P* value

[3] False discovery rate (Benjamini-Hochberg);

* Significant after Bonferroni correction ($P<4\times10^{-4}$)

HGL, high glycemic load; LGL, low glycemic load; NMDA, *N*-methyl-D-aspartate