Low- vs. high-glycemic load diet: effects on postprandial plasma free fatty acid levels

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Abstract

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Introduction: Low-glycemic load diets are commonly prescribed for blood glucose management. Currently, however, it is not clear as to whether the diet has any implications, positive or negative, on cardiovascular health in healthy individuals.

Methods: Using data collected from the Carbohydrates and Related Biomarkers (CARB) Study, conducted at the Fred Hutchinson Cancer Research Center, we analyzed the effects of a high- vs. low-glycemic load diet on fasting lipids and postprandial plasma free fatty acid levels in healthy, non-insulin resistant, adults. Participants were randomized to complete either a high- or low-glycemic load diet for 28 days, followed by a washout period of 28 days, and then complete the opposite diet for 28 days.

Results: We found that a high- vs. low-glycemic load diet did not have any significant effect on plasma free fatty acid levels in the eight hour time period following a test breakfast.

Conclusions: Glycemic load does not appear to affect cardiovascular health in the short-term, however, further research is needed to determine any long-term implications. Based on our findings, we cannot support a recommendation that a low-GL diet is protective against cardiovascular disease in healthy individuals.

INTRODUCTION AND BACKGROUND

GLYCEMIC INDEX AND GLYCEMIC LOAD

GLYCEMIC INDEX

The concept of glycemic index (GI) was first introduced as a method to classify carbohydrate-containing foods by their ability to raise blood glucose levels.[29,62] GI is calculated as the change in blood glucose after a carbohydrate-containing food item is eaten, divided by the change in blood glucose after an equal mass of bread is eaten, multiplied by 100.[6,30] GI has been standardized to be the comparison of the 2-hour post-prandial glucose response of 50g of a carbohydrate food with that of 50g of white bread or glucose, and is represented by the area under the glucose response curve.[32,53] Thus, GI is an indicator of the intestinal absorption of carbohydrates; low-GI foods are absorbed more slowly and induce a slower glycemic response than high-GI foods, which are rapidly absorbed in the intestines.[10,23,32] When carbohydrate-containing foods of differing GI are mixed together in a meal, the GI of the meal is a relative median of the individual carbohydrate food GIs.[65]

GLYCEMIC LOAD

Glycemic load, a concept related to GI, is a representation of both the quantity and the quality of carbohydrate consumed. GL is calculated as the product of the food's carbohydrate content and its GI value.[39,64] GL is a way to summarize the combined effects of total carbohydrate intake and overall dietary GI.[38] A low-GL diet does not

necessarily mean that low-GI foods were consumed.[38] Since GL is dependent upon both the amount of carbohydrate and the GI of the foods consumed, either decreased consumption of total carbohydrate or improved quality of carbohydrate, GI, can lower GL of the diet.[68] Thus, the long-term health implications of a reduced GL diet may be different depending on whether the lower GL is due to reduced carbohydrate intake, reduced GI, or a combination of the two.

BIOLOGICAL RESPONSES TO GI/GL

Traditionally, low GI foods or low GL diets have been recommended in the setting of glycemic control, as low GI food consumption is thought to result in lower and more stable blood glucose and insulin levels.[16] Total meal GL has been shown to, moderately well, predict serum glucose and insulin responses postprandially.[24] A positive association between meal GL levels and serum glucose and insulin responses has been demonstrated, indicating that, for foods with equal GIs, as the amount of carbohydrate increases, there will be a proportionally constant increase in the blood glucose and insulin responses.[24]

However, current evidence supporting a link between dyslipidemia and GL is lacking. Some studies have found that GL has no effect on plasma lipid levels,[16,24,32,64] some have found an inverse relationship between GL and circulating plasma lipids,[53,60] and some have found improved lipid status with low GL diets.[13,39,40,56]

INSULIN

EFFECTS OF GI/GL

There is epidemiological and clinical evidence supporting that diet significantly influences insulin sensitivity in obese and/or insulin resistant individuals;[23] specifically, that a low GL diet increases insulin sensitivity.[15] Other previous studies have found similar results, and further concluded that high GL diets, over time, contribute a state of pancreatic β-cell dysfunction and a defective insulin response.[10,60,68] A diet with a high composition of carbohydrate foods induces compensatory hyperinsulinemia.[64,72] However, if a heightened insulin response is required over an extended period of time, the ability of β-cells to produce and secrete insulin declines. Chronically high glucose intake, paired with decreasing insulin secretion, ultimately results in β-cell death and reduced pancreatic cell mass. The long-term consequence is glucose intolerance and diabetes.[28] The FAO/WHO has made a recommendation, based on evidence supporting the efficacy of a low GI or GL diet in improving glycemic control,[10] to reduce dietary GI for the prevention and management of metabolic disease.[19] However, some studies have not found conclusive evidence that differences in GI consumption lead to differences in insulin response.[16,32] It has been suggested that GI/GL may be most useful in its ability to regulate glucose responses postprandially, not necessarily the associated insulin response.[16]

INCRETIN EFFECT

The incretin effect is a phenomenon in which oral glucose administration has been demonstrated to elicit a greater insulin response than intravenous glucose administration.[48] This has recently been explained by the activity of insulin-mediating molecules secreted by the gut.

Incretins are peptide hormones that are produced entero-endocrine cells in the small intestinal mucosa.[48] The incretins glucose-independent insulinotropic polypeptide (GIP) and glucagon-like peptide (GLP-1) are secreted in response to nutrient ingestion and act on pancreatic β-cells for insulin secretion.[2,28] GIP and GLP-1 have low basal plasma concentrations and have an insulin secretory response only at levels that correlate to a certain level of hyperglycemia; they are thus ineffective at eliciting an insulin response with low amounts of dietary glucose.[48] This ultimately serves to maintain a certain blood glucose level despite ingestion of a wide range of carbohydrate loads.[2] The insulin response to these hormones accounts for approximately 70% of postprandial insulin secretion.[28] This suggests that the effect of GI on insulin responses is additional to the effect of incretins; the postprandial insulin response is not entirely dependent upon the GI of the food consumed.

The incretin effect has been demonstrated to be defect or completely absent in individuals with impaired glucose tolerance, hyperglycemia, and diabetes.[48] In one study, plasma levels of incretins do not appear to differ between healthy and

diabetic persons, implying that a defect or absence of the incretin effect is a marker of beta cell dysfunction and the reduced secretion of insulin.[2] This hypothesis has been confirmed by other recent investigations.[28,49] One of these studies, however, has also concluded that obese subjects with normal glucose tolerance also have an impaired incretin effect, likely due to reduced GLP-1 secretion in the gut.[28] This supports current literature that obesity contributes to insulin resistance, but with additional data regarding the mechanisms.

SHORT-TERM BIOLOGICAL EFFECTS: SERUM FREE FATTY ACIDS

It is well known that insulin has a significant effect on the activities of lipases that regulate FFA availability: lipoprotein lipase found in adipose tissue, hormone-sensitive lipase, and adipose triglyceride lipase.[60] In the presence of high levels of insulin, when lipoprotein lipase activity is increased and hormone-sensitive lipase and adipose triglyceride lipase activities are suppressed, the combined effect is improved clearance of FFAs from the bloodstream.[31]

Lipoprotein lipase hydrolyzes triglycerides from circulating lipoproteins, either chylomicrons from the intestines or very low-density lipoproteins (VLDL) from the liver. This results in the production of fatty acids. In adipose tissue, insulin increases lipoprotein lipase activity and thus increases the production of fatty acids, which are either oxidized for energy or repackaged as triglycerides for storage.[26] Conversely, insulin as an inhibitory effect on the lipoprotein lipase found in skeletal muscle, serving to direct fatty acids to adipose tissue for storage. The hormone glucagon,

released during times of low available blood glucose for energy, is responsible for increasing lipoprotein lipase activity in muscle tissue.[20] The result is the increased ability of muscle tissue to mobilize fatty acids for energy production when the preferred substrate, glucose, is unavailable. The ultimate effect of increased lipoprotein lipase activity is favorable uptake of FFAs from the circulation.[60]

Hormone-sensitive lipase and adipose triglyceride lipase are found in adipose tissue and both serve to hydrolyze stored triglycerides into FFA for release into the circulation.[35,72] While it was thought for quite some time that hormone-sensitive lipase was the primary enzyme in the hydrolysis of triglycerides in adipose tissue, it has been discovered that adipose triglyceride lipase is in fact the catalyst for the reaction and has the more significant effect on the overall rate of fatty acid production.[65] Adipose triglyceride lipase is highly expressed in adipose tissue and highly specific for triglycerides. It removes the first fatty acid from a triglyceride molecule, initiating its catabolism.[73] Insulin suppresses the activities of these molecules, although through two different mechanisms. Adipose triglyceride lipase is regulated by insulin at the transcriptional level; high levels of insulin reduce the production of the lipase.[33] Insulin does not, however, halt production of hormone-sensitive lipase, it simply decreases its level of activity.[63] Increased levels of insulin cause the combined effects of hormone-sensitive lipase and adipose triglyceride lipase: reduction of triglyceride hydrolysis and decreased liberation of FFA into the circulation.

INSULIN SIGNALING AND LIPID METABOLISM

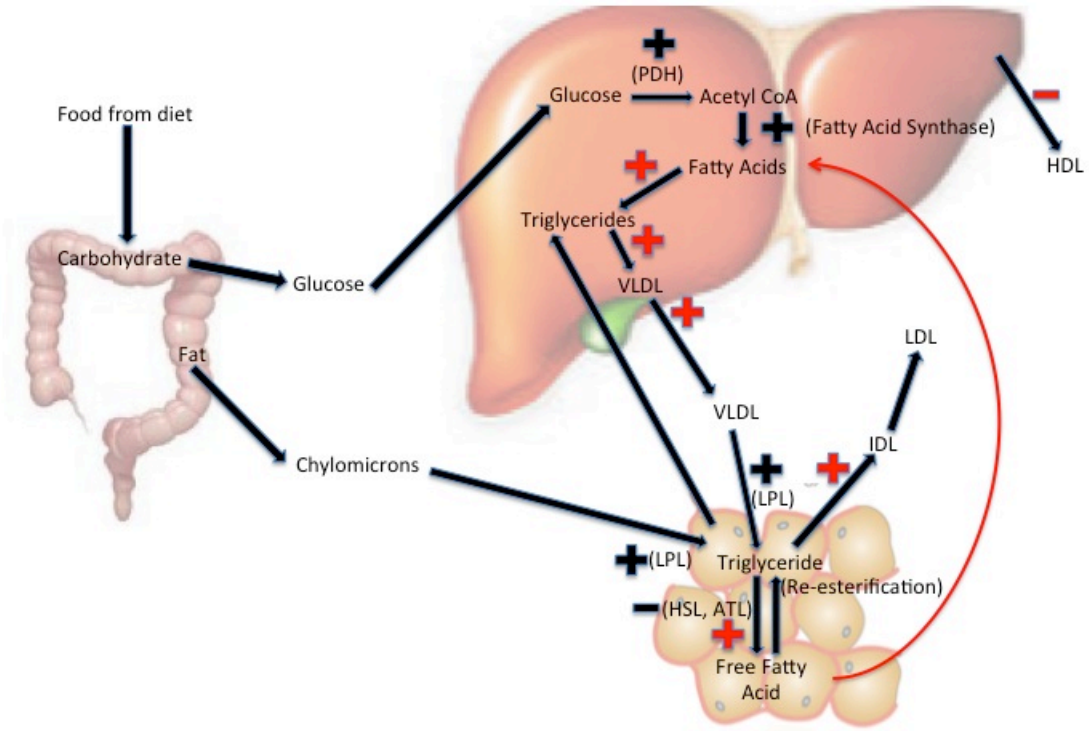

Figure 1. Normal lipid metabolism (black) vs. lipid metabolism in a state of insulin resistance (red). Insulin has inhibitory (-) and enhancing (+) effects on many stages of lipid metabolism.

Insulin plays an important role in nutrient uptake in the postprandial period. Dietary carbohydrates are absorbed in the intestines and transported through the bloodstream as glucose. While glucose uptake by the liver is independent of insulin action, the conversion of glucose to fatty acids is insulin-dependent. Glucose is converted to pyruvate through glycolysis, and then to acetyl-coA through the insulin-stimulated action of pyruvate dehydrogenase.[18] Acetyl-coA is then converted into fatty acids, in which the enzyme fatty acid synthase is upregulated by insulin. Fatty acids are subsequently esterified into triglycerides and packaged into very

low-density lipoprotein (VLDL) for transport through the circulation system.[18] VLDL is carried to adipose tissue for deposition of fatty acids via the insulin-stimulated action of lipoprotein lipase (LPL).[6] Extraction of the fatty acids leaves a VLDL remnant, which then becomes an intermediate-density lipoprotein (IDL). These IDL molecules lose triglycerides in the bloodstream and form low-density lipoproteins (LDL).[44] The rate-limiting step of LDL synthesis is indirectly regulated by insulin. 3-hydroxy-3-methylglutaryl-coA reductase is the responsible enzyme and is most active when blood glucose levels are high.[48] Increased insulin clearance of blood glucose decreases the enzyme's activity and thus cholesterol production.

Dietary fats, on the other hand, are emulsified in the intestines for absorption, and then packaged into chylomicrons for transport through the circulation before being deposited in adipose tissue. Again through the actions of LPL, stimulated by insulin, triglycerides are extracted from the chylomicrons.[6] In adipose tissue, deposited triglycerides are hydrolyzed into free fatty acids, a process inhibited by the action of insulin. Additionally, the fatty acid-triglyceride cycle ensures conservation of triglyceride storage in adipose tissue through the consistent re-esterification of free fatty acids to triglycerides.[6,69]

In a state of insulin resistance, serum lipid levels are negatively altered. HSL activity is increased in adipocytes, increasing triglyceride hydrolysis and build-up of free fatty acids.[6] These are then transported to the liver, where the increased availability of fatty acids for triglyceride synthesis increases the production and secretion of

VLDL. When the threshold for serum VLDL is reached, formation of HDL by the liver decreases while formation of LDL increases. The end result is dyslipidemia.[69]

FREE FATTY ACIDS

RELATIONSHIP TO GI/GL

As demonstrated in the figure above, FFAs enter the circulation through the hydrolysis of both triglycerides in adipose tissue and glucose in the liver. However, high circulating levels of FFAs have yet to be definitively linked to a diet high in carbohydrates or glycemic load. The current evidence describing a relationship between GI or GL and FFA levels varies widely. Some studies determining that plasma FFA levels were increased by a low GL diet,[13,35] some have found no effect of GI or GL on FFA levels,[16,24] and still others have found that FFA concentrations are suppressed by high GI meals.[41,53]

As discussed previously, insulin acts to both increase lipoprotein lipase activity for the uptake of FFA to adipose from the circulation and decrease the activities of hormone-sensitive lipase and adipose triglyceride lipase for the reduction of FFA liberation from adipose tissue into the circulation. Through these actions, an increased insulin response due to a high glycemic load meal could likely mediate the FFA response so that there is either no difference in plasma levels or very little difference postprandially.[56] However, in the late postprandial period (approximately four to six hours after the meal), low blood glucose levels trigger a counter-regulatory response and glucagon is released. Glucagon stimulates

gluconeogenic and glycogenolytic pathways in order to produce glucose and restore normal blood levels, concurrently elevating FFA levels.[41] In this way, a high-GL meal may actually cause FFA levels to rise versus the more attenuated hormone response elicited by a low-GL meal. Again, this result would only be seen in the later hours after a test meal.

EFFECTS ON CARDIOVASCULAR HEALTH

There are several mechanisms by which high FFA levels might impact cardiovascular health. An increased amount of FFA is available to the liver for increased triglyceride production and VLDL secretion.[39,60,70] High serum triglyceride level as been discovered to be an independent risk factor for coronary heart disease (CHD), after adjustment for LDL and HDL cholesterol levels, age, blood pressure, smoking, diabetes, family history of myocardial infarction, and angina pectoris.[1,12,42] While it is not necessarily the triglyceride itself that leads to heart disease, high levels of triglycerides indicate high levels of high cholesterol-containing remnant lipoproteins that then deposit that cholesterol into arterial walls.[51,75]

High levels of FFAs are also believed to contribute to insulin resistance.[55] FFA levels surpassing storage capacity in adipose tissue are deposited in other tissues, like skeletal muscle and liver,[35,58] through the inhibition of insulin signaling. FFAs complete with glucose as an energy substrate in skeletal muscle, altering the partitioning of fat between adipocytes and muscle.[58] The exact mechanism by which

FFAs affect glucose transport and utilization has yet to be clearly identified.[8] Insulin resistance itself is associated with high rates of cholesterol synthesis.[54]

Lastly, high levels of FFAs may themselves predict cardiovascular mortality.[55] The myocardium appears to be negatively affected by high levels of FFAs, as increased use as a metabolic substrate appears to have unfavorable alterations at the cellular level, resulting in inefficient glucose metabolism.[47] This has been demonstrated to have proarrhythmic effects, increase ischemic damage, and contribute to the development of heart failure.[27,52]

GLYCEMIC LOAD AND CARDIOVASCULAR HEALTH

CURRENT EVIDENCE

As of yet, the literature is unable to determine what effects, if any, glycemic load has on cardiovascular health. While low-GI diets are frequently recommended to diabetic patients with the intent of improving glycemic control and insulin sensitivity, there is little evidence to support improvement of lipid profiles. There is even fewer data to describe the effects of this diet on non-diabetic individuals.[9]

Some studies have found that GL has no effect on plasma lipid levels,[13,24,32,64] some have found an inverse relationship between GL and circulating plasma lipids,[50,57] and some have found improved lipid status with low GL diets.[13,40,42,62]

Study results have varied based upon gender, nationality, age, and BMI classification. In middle-aged and older Swedish, dietary GI/GL had no significant

association with cardiovascular illness.[67] Another study done in Italy with overweight, older men and women found a positive association between dietary GI and incidence of myocardial infarction, but no association between dietary GI/GL and incidence of cardiovascular disease or its risk factors.[37] A meta-analysis evaluated studies of the associations of GI and GL with coronary heart disease events and found discrepancies between nationalities and sex; Danish and Black American men demonstrated positive benefits with consumption of lower GI foods, and overall only women, not men, showed an increase in relative risk of coronary heart disease with the consumption of high GI foods/a high GL diet.[66]

However, there have been studies showing a significant positive association between dietary GL and the risk of cardiovascular disease. Various studies have shown that low-GI diets result in lower triglyceride and lipoprotein cholesterol levels, as well as a lower ratio of total cholesterol to high-density lipoprotein levels, indicators of cardiovascular health.[41] One of the first studies regarding GI indicated that it might have a beneficial impact on blood lipids.[30] Increased dietary GL was shown to be associated with and increase in the cardiovascular disease risk factor high-density lipoprotein (HDL) in Brazilian middle-aged men.[16] In US women, GI was found to be a strong indicator of coronary heart disease risk, independent of carbohydrate complexity.[40]

A Cochrane review assessing the prescription of low GI diets for cardiovascular health did not find any evidence from randomized controlled trials to show an effect

of low GI diets on coronary heart disease. Some weak evidence for minor effects on some CHD risk factors was found; there was some evidence of a reduction in total cholesterol with low GI diets and borderline support for a decrease in LDL with low GI diets.[32] Obesity was identified as a known risk factor for coronary heart disease, therefore energy-restricted diets based on low GI foods might produce the greatest weight loss and thus the greatest cardiovascular protection. These diets also may improve insulin sensitivity in obese individuals, thereby improving cholesterol profiles.[46]

There is a need for well-designed, adequately powered, randomized controlled trials of significant duration to better understand the full effects of GI or GL on lipid profiles and cardiovascular health. Currently, there is insufficient evidence that healthcare providers should recommend a low-GL diet over other therapeutic diets for the prevention of heart disease in healthy, non-insulin resistant individuals

METHODS

STUDY POPULATION

Participants in the Carbohydrates and Related Biomarkers (CARB) study, conducted at the Fred Hutchinson Cancer Research Center, (FHCRC) included healthy, non-smoking men and women between the ages of 18 and 45 years old from the Seattle area. Special recruitment efforts were given for African American and Hispanic populations. Prior to enrollment, potential participants were given an eligibility questionnaire, excluding those who: (1) had physician-diagnosed diseases requiring dietary restrictions or modifications; (2) had a BMI between 25 and 28, in order to demonstrate any significant differences in outcomes between healthy weight and obese individuals; (3) were currently pregnant or lactating, or had plans to become pregnant; (4) regularly took hormones, anti-inflammatory medications, or other medications that might interfere with outcome measures; (5) used tobacco or consumed >2 drinks per day; (6) restricted their eating or had food allergies; and (7) had impaired fasting glucose, defined as fasting blood glucose levels ≥ 5.6 mmol/L. Enrolled participants were also asked to discontinue the use of any dietary supplements. Approval was obtained from the Institutional Review Board and the Clinical Trials Office of the FHCRC and all participants provided written, informed consent.[50]

RESEARCH DESIGN

89 participants were enrolled in the study between June 2006 and July 2009. Participants were block randomized by the BMI group (18.5-24.9kg/m2 or 28.0-40.0kg/m2) and sex to the order of experimental diets.[61] Participants were assigned to both a low- and high-GL diet, in a cross-over design, for 28 consecutive days each, with a 28 day washout period in between diets during which participants consumed their habitual diets (Figure 2).[62] A subset of CARB study participants was chosen to participate in a postprandial study (CARB-PPL). This included 20 participants who consumed a standardized test breakfast and remained at the facility for post-prandial testing.[61] It is the CARB-PPL samples that were used in this analysis.

Participants were instructed to consume only the food and beverages provided during the feeding periods; coffee and tea (creamers and sweeteners were provided by the study) were permitted at stable continuous levels.[61] Participants ate one meal each day, Monday through Friday, at the Human Nutrition Lab at FHCRC under supervision by study staff. After the evening meal, participants were provided with breakfast, lunch, and snack for the following day. Following the evening meal on Friday, participants were provided with all food to be consumed over the weekend as well as Monday's breakfast and lunch. All food that remained unconsumed was returned to the lab where staff weighed and recorded it. Participants also completed a daily checklist confirming consumption of the day's foods, along with consumption of non-study foods.[60]

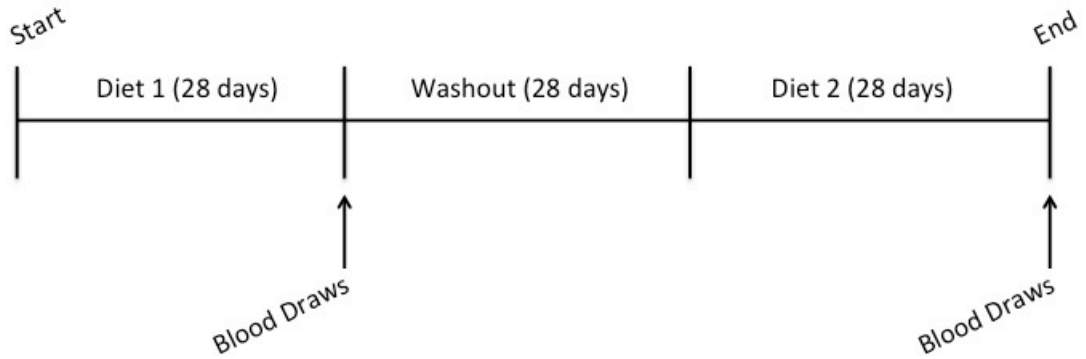

Figure 2. Cross-over design of study diets.

STUDY DIETS

Both high- and low-GL diets were designed to be weight-maintaining. 3-day diet records were obtained from participants in order to estimate habitual energy intake. This data was used in conjunction with the Mifflin equation to estimate energy needs.[50] A 7-day menu rotation for each diet was generated using ProNutra (Version 3.2, Viocare). Macronutrient content was identical between each diet, with 15% of daily energy coming from protein, 30% of daily energy coming from fat, and 55% of daily energy coming from carbohydrates. Fiber content differed between the two diets with 55g/day in the low GL diet versus 28g/day in the high GL diet. The low GL diet was defined at GL 125, and high was 250.[61] Participant weights were taken three times per week and energy adjustments were made in 200 kcal increments as necessary to maintain baseline weight.[62] The test breakfasts for the postprandial study were also designed to be similar to the 28-day diets in overall macronutrient content and match the GL profile of the diet treatment.[61]

SAMPLE COLLECTION AND ANALYSIS

Sample collections were done in the mornings of the first and last days of the diet periods.[50] On day one a 12-hour fasting blood draw was taken and on day 28, from the 20 postprandial study participants, a postprandial blood draw was collected following breakfast, at 0, 30, 60, 120, 180, 240, 270, 300, 330, 360, 420, and 480 minutes after the start of the meal (Figure 3). After being allowed to clot for approximately 30-45 minutes, both serum and plasma were spun and aliquoted. Samples were immediately stored at -20°C, and placed for storage in a -80°C freezer by the end of the day. The samples provided by the CARB-PPL study for this lipid analysis had not been previously thawed. Plasma samples were analyzed at the University of Washington's Nutrition Obesity Research Center analytical core and lipid panels were generated.

FFAs were measured on a Roche Cobas Mira Plus Chemistry Analyzer using reagent from Genzyme/Wako. After being allowed to thaw for approximately 30 minutes, samples were analyzed at room temperature.

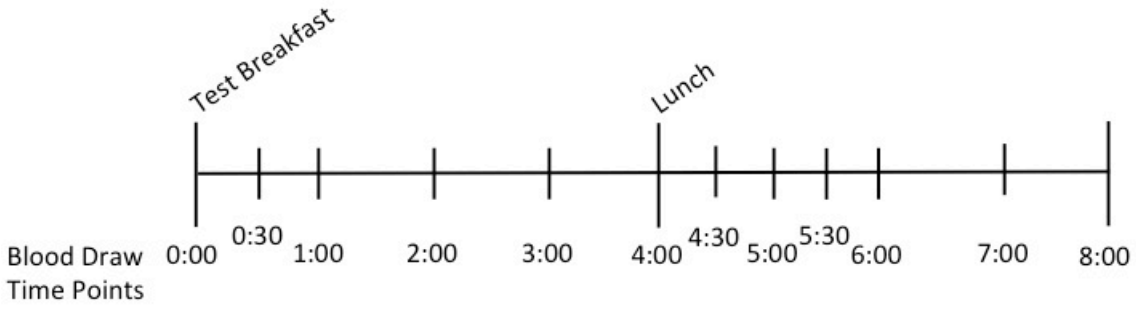

Figure 3. Times of postprandial blood draws.

STATISTICAL ANALYSIS

The overall goal of this study was to test the effects of a 28-day low- vs. high-GL diet intervention on the postprandial responses of serum lipids, specifically free fatty acids. We calculated area under the curve (AUC) of post-prandial FFA measurements using trapezoidal approximation. In 30 and 60 minute time periods, according to when blood draws were taken, we calculated the AUC for the postprandial periods after breakfast and after lunch. We repeated the AUC analysis separately for each the normal weight and overweight/obese groups for the total time period, before lunch, and after lunch. A paired t-test was done for each of the AUC analyses in order to determine if a significant p-value existed between the diet sets.

Fasting lipid panels drawn on the 28th day of each diet period were analyzed using a 2-way paired ANOVA.

RESULTS

Table 1. Characteristics of study participants

n=20	
Age range (y)	19-44
Male/Female (n)	10, 10
BMI (n)	
18.5-24.9 kg/m^2 ("Normal" weight group)	10
28-40 kg/m^2 ("Overweight/obese" weight group)	10
Ethnicity (self-reported)	
Non-Hispanic white	8
Hispanic	7
African American	4
Asian/Pacific Islander/Native American	1

Block randomization of the participants ensured that the participant group was composed of equal parts males and females and normal weight and overweight/obese weight groups. There were five women and five men comprising both the normal and overweight/obese weight groups. On average, the overweight/obese weight group was slightly older than the normal weight group ($P < 0.001$). Ethnicity did not vary across weight groups. Based upon self-reported data from the daily food check-off forms and returned food, it appeared that 97% of participants consumed more than 90% of the provided foods. No significant difference in adherence by study diet was determined.

Table 2. Mean energy and macronutrient content of 2400kcal high- and low-GL reference diets[a]

	High-GL diet n=7 days	*Low-GL diet n=7 days*
Dietary descriptor	*mean ± SD*	*mean ± SD*
Energy (kcal/day)[b]	2398 ± 8	2396 ± 6
Protein (g/day)	90 ± 1	90 ± 0
Energy from protein (% using total carb)	15 ± 0.2*	14 ± 0.4*
Energy from protein (% using available carb)[c]	15 ± 0.2	16 ± 0.2
Fat (g/day)	80 ± 0	81 ± 1
Energy from fat (using % total carb)	30 ± 0.3*	29 ± 0.8*
Energy from fat (using % available carb)	31 ± 0.2	31 ± 0.6
Total carbohydrate (g/day)	341 ± 6*	361 ± 15*
Energy from total carbohydrate (%)	56 ± 0.5*	57 ± 1.1*
Energy from available carbohydrate (%)	54 ± 0.8	53 ± 0.8
Dietary fiber (g/day)	24 ± 5*	49 ± 8*
GI/day	78 ± 5*	34 ± 1*
GL/day	244 ± 14*	117 ± 3*

[a]Data from ProNutra: Metabolic Diet Study Management System (Viocare Technologies) and/or the Nutrition Data System for Research (version 2005, Nutrition Coordinating Center, University of Minnesota)
[b]Total energy calculated as sums of 4kcal/g protein, 9kcal/g fat, and 4kcal/g total carbohydrate.
[c]Total energy calculated using available carbohydrate.
Asterisk (*) next to *P*-value, 0.05 for paired t-tests comparing high- and low-GL diets.

Table 2 shows the planned daily mean macronutrient content, dietary GI, dietary GL, and distribution of energy for both high- and low-GL 2400-kcal reference diets. All diets at each energy level were similar to the reference diet shown. Fiber content was higher in the low-GL diet. Increased fiber intake has been shown to reduce total cholesterol and apoB levels relative to HDL-cholesterol and ApoA1 levels, thus reducing the risk of coronary heart disease. (29) The percent available carbohydrate does not factor the fiber content into energy calculations, as fiber is only available for energy after fermentation. Available carbohydrate is recommended by the FAO as a useful concept for energy evaluation. Necessary energy adjustments for weight

maintenance throughout the study did not significantly differ by diet type or feeding period. The test breakfast consumed for postprandial analysis was constructed to be composed of macronutrient ratios consistent with the study diets.

Table 3. End of diet period fasting lipid panel results (n=20)

	High-GL	Low-GL
mg/dL	*Mean ± SD*	*Mean ± SD*
Cholesterol	142 ± 29	140 ± 33
Triglyceride	84 ± 51	93 ± 73
HDL	41 ± 10	40 ± 9
LDL	85 ± 23	81 ± 24
ApoA1	130 ± 21	130 ± 20
ApoB	73 ± 19	73 ± 21

Fasting blood samples from 20 participants were drawn on day 28 of each diet period, before consumption of the test breakfast. There was no effect of GL on fasting total cholesterol, HDL cholesterol, LDL cholesterol, ApoA1, and ApoB levels.

Table 4. Total participant (n=20) area under the curve, plasma free fatty acids

	Before Lunch mEq/L	*After lunch mEq/L*	*Total Time mEq/L*
High-GL	1.00±0.28	1.34±0.41	2.33±0.54
Low-GL	1.02±0.36	1.48±0.52*	2.53±0.79

The “before lunch” time period is defined as time points 0:00 through 4:00, and the “after lunch” time period as time points 4:00 through 8:00.

At specific time periods, the high- vs. low-GL diet did not have any significant effect on plasma FFA levels.

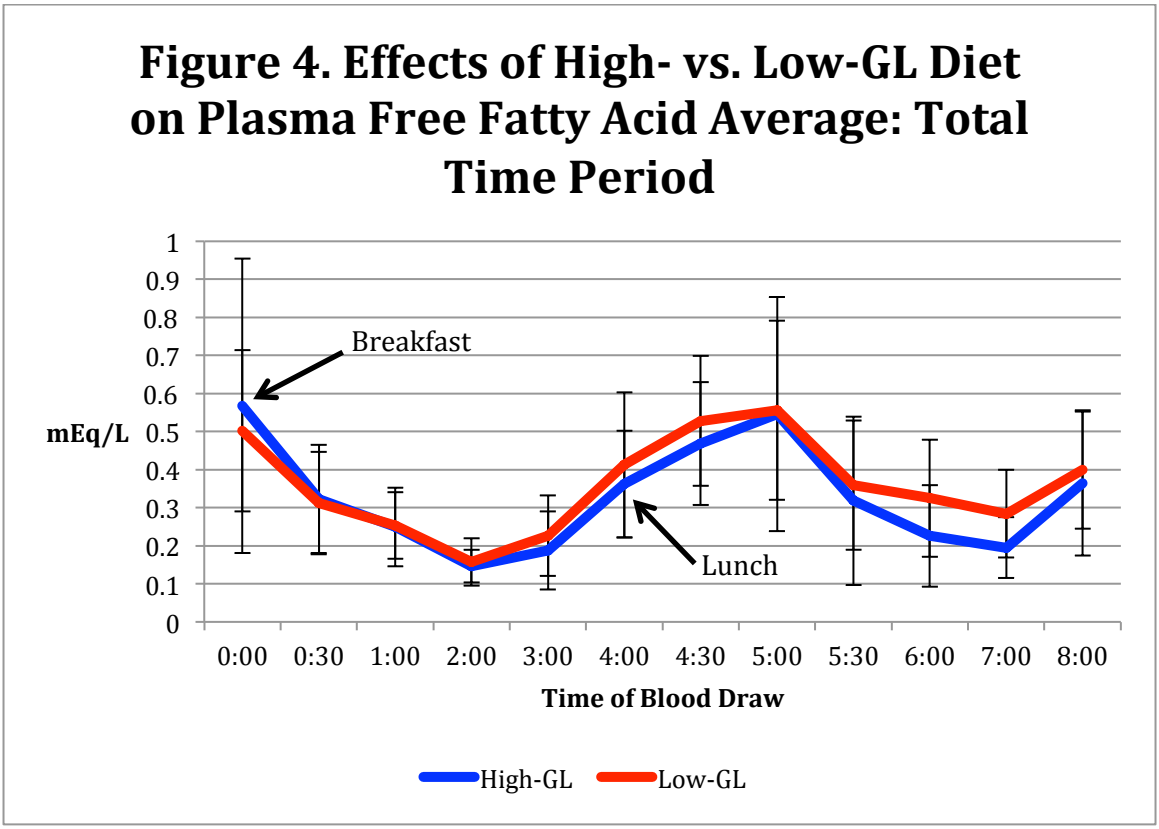

Figure 1. Mean ± SD FFA levels (mEq/L) of the CARB-PPL participants at the time of each blood draw. Between the high-GL (solid blue line) and the low-GL (solid red line) diets, there is not a significance difference in circulating FFA levels across the eight hour time period.

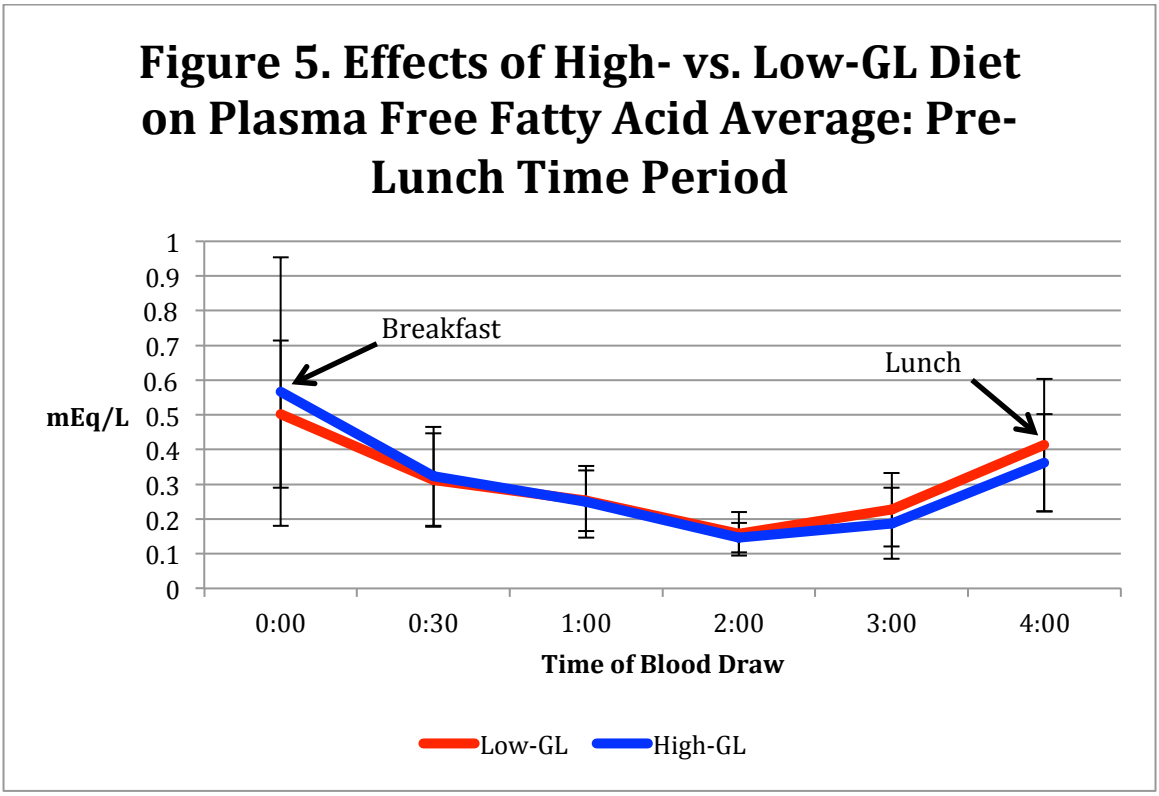

Figure 2. Mean ± SD FFA levels (mEq/L) of the CARB-PPL participants at the time of each blood draw in the time period before a lunch meal is consumed (0:00 to 4:00). Trapezoidal approximations did not determine significance in the difference between FFA levels for the two study diets, high-GL (solid blue line) and low-GL (solid red line).

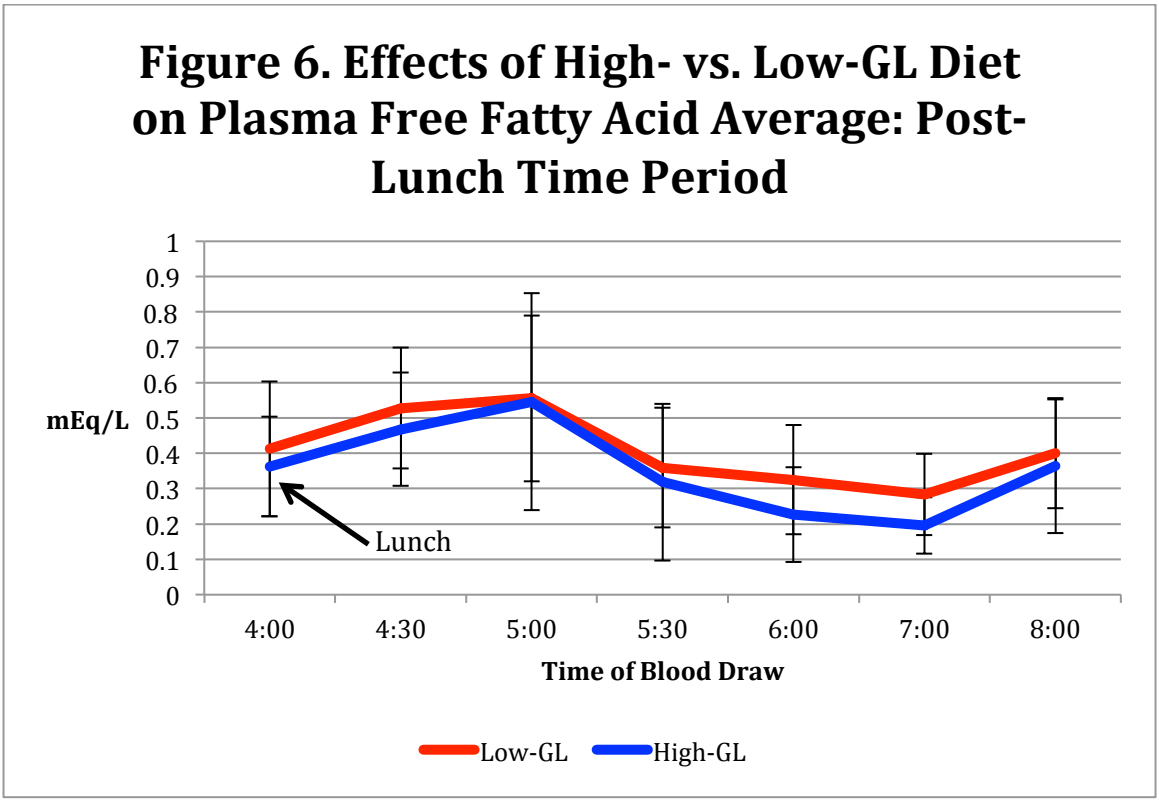

Figure 3. Mean ±SD FFA levels (mEq/L) of the CARB-PPL participants at the time of each blood draw in the time period after a lunch meal is consumed (4:00 to 8:00). Trapezoidal approximations did not identify significance in the difference between FFA levels for the two study diets, high-GL (solid blue line) and low-GL (solid red line) at any of the individual time points.

Table 5. Area under the curve, FFA: normal vs. overweight/obese participants

n=10	*Pre-Lunch (mEq/L)*	*Post-Lunch (mEq/L)*	*Total Time Period (mEq/L)*
Normal Weight BMI 18.5-24.9 kg/m^2			
High-GL	1.31±0.28	2.29±0.92	3.57±1.0
Low-GL	1.25±0.37	2.26±1.00	3.51±1.30
Overweight/Obese BMI 25-40 kg/m^2			
High-GL	1.40±0.50	1.98±0.50	3.38±0.78
Low-GL	1.45±0.57	2.34±0.72	3.29±1.18

We analyzed the FFA response of the two weight groups, normal and overweight/obese, for the two study diets separately because of the potential impact that adipose biology may have on FFA clearance. There was no effect of GL on postprandial FFA levels between the two weight groups.

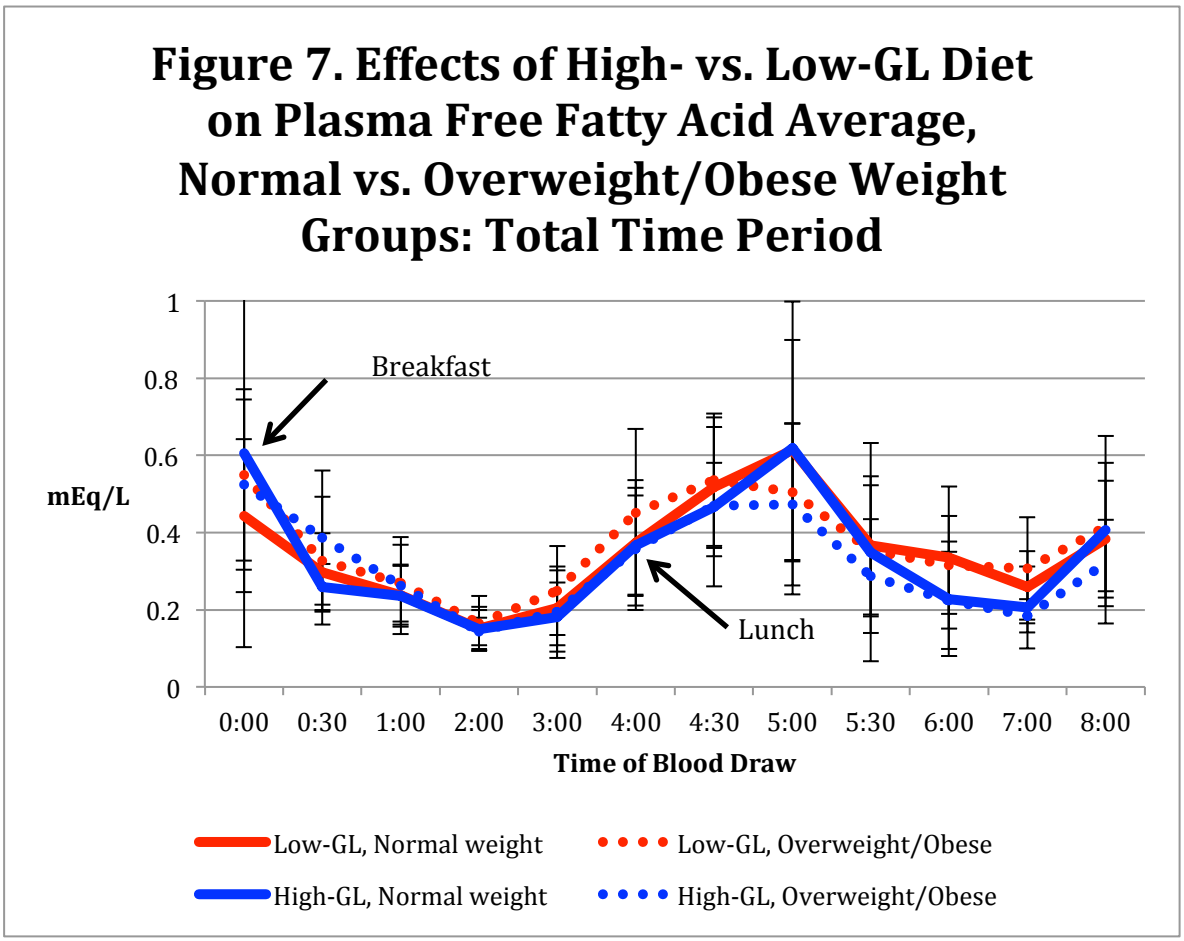

Figure 4. Mean ± SD FFA levels (mEq/L) of the CARB-PPL participants at the time of each blood draw in the eight-hour period following the test breakfast.

DISCUSSION

This randomized, cross-over, controlled feeding study tested the effect of high-GL vs. low-GL experimental diets on post-prandial plasma FFA levels. The results of our analysis showed that a high- vs. low-GL diet did not have any significant effect on fasting lipids or post-prandial plasma FFA levels in the eight hours following a test breakfast meal. However, there is a complex relationship between dietary carbohydrates and blood lipid levels. While GL does not appear to affect blood lipid levels, and by extension cardiovascular health, in the short-term, further research is needed to determine any long-term implications. The current state of research does not support the recommendation of a low-GL diet for cardiovascular disease prevention over the recommendation of other, proven therapeutic diets.

A previously published study investigating the effects of GL on insulin response found that a high-GL test meal resulted in an increased insulin response as compared to a low-GL test meal (Figure 8).[61]

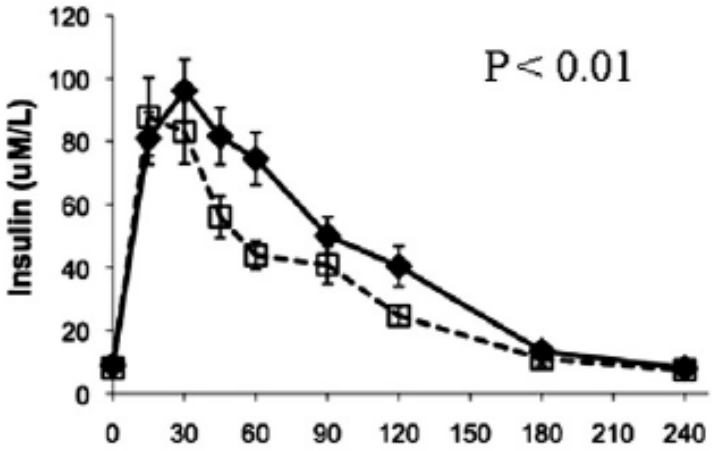

Figure 8. Mean concentrations (±SD) of fasting and post-prandial plasma insulin in healthy and overweight-obese participants who were fasting (time 0) and then consumed a high-(solid line with closed diamonds) and low-(dotted with open squares) glycemic load meal. Taken from Runchey et al, 2013.

It could be expected that a higher insulin response would mediate the FFA response; the FFA response to a high-GL meal would thus be the same or lower than that to a low-GL meal. FFA uptake from the circulation into adipose tissue is insulin-dependent through the action of lipoprotein lipase.[25] The storage of FFA in adipose tissue as triglycerides is also insulin-dependent through the actions of hormone-sensitive lipase and adipose triglyceride lipase.[35,72] However, while the action of insulin on hormone-sensitive lipase is immediate, its action on the more crucial enzyme, adipose triglyceride lipase, is transcriptive.[33] Thus, the true effects of insulin on plasma FFA levels would not be visible in the early postprandial period. The four hour postprandial time period monitored in the insulin study is inadequate in in determining any carryover effect of GL on insulin, also making it impossible to compare the observed post-lunch plasma FFA levels of this study to an insulin response.

We also explored the possible difference in FFA response between the normal weight and overweight/obese groups. We did not find that GL had a different effect on postprandial plasma FFA levels between the two weight groups. We can then postulate that adiposity alone does not have an effect on plasma FFA levels. The participants of this study were not insulin resistant, therefore their insulin response was the same as their normal weight counterparts. The insulin study similarly did not observe effects of adiposity on insulin response.[59]

STRENGTHS AND LIMITATIONS

This study was a crossover design with two 28-day controlled diet interventions differing substantially in GL. It was also unique in that the diets were individualized to be realistic, isocaloric, and weigh-maintaining despite being high- or low-GL.

There were several significant limitations to this study. There was a small sample size for the postprandial arm of the study; the sample size was even smaller when participants were divided by weight group. Participants were also sampled from a population group that met very specific enrollment criteria. All participants were healthy and insulin-responsive, with normal glycemic control. This was not a population-based participant group, thus results are likely not generalizable. Because volunteers with insulin resistance were not included in this study, we were unable to explore the effects of GL on blood lipids in those individuals. There was no difference in pancreatic function between our weight groups, and therefore no expected, or observed, difference in insulin response. Our participants were also limited to the normal and very overweight or obese. Being overweight is thought to be protective against many chronic diseases and their risk factors, yet we were unable to explore differences in FFA response among these individuals compared to the other weight groups.

The diet length of 28 days only allowed us to interpret the short-term effects of GL on postprandial FFA levels. Insulin resistance developing due to a chronically high carbohydrate intake and stress on the pancreatic β-cells is not discernable in a four-

week span. A study of longer duration would be needed to determine if GL directly contributes to the development of insulin resistance, contributing indirectly to unfavorable lipid profiles and the development of cardiovascular disease.

FUTURE DIRECTIONS

There is a need for a randomized controlled trial of duration longer than 12 weeks to determine the effects of GL on blood lipids, specifically plasma FFA levels, and the risk of cardiovascular disease. There is currently little, if any, evidence to support that a low-GL diet could be protective against the development of cardiovascular disease.

Studies exploring the effects of GL on blood lipid profiles between individuals with insulin resistance are necessary to determine heart health implications. We could then more appropriately determine whether a low-GL diet is beneficial to both insulin resistant and health individuals, or only the former. We would also be able to explore the effects of GL versus another therapeutic diet that perhaps provides protective benefits inclusive of but not limited to GL.

CLINICAL IMPLICATIONS

Based on this study and the current state of the literature, it cannot be recommended that healthcare providers counsel a low-GL diet for the prevention of cardiovascular disease in a healthy patient population over other proven therapeutic diets. This study does not support a recommendation for the use of GL

in preventing cardiovascular disease in healthy individuals as neither low- nor high-GL diets were shown to be protective against coronary heart disease in regards to FFA.

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