Long-term effects of dietary glycemic index on adiposity, energy metabolism
and physical activity in mice

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ABSTRACT

Objective: A high glycemic index (GI) diet has been shown to increase adiposity in rodents; however, the long-term metabolic effects of a low and high GI diet have not been examined.

Research Methods and Procedures: In this study, a total of 48 male 129SvPas mice were fed diets high in either rapidly absorbed carbohydrate (RAC, high GI) or slowly absorbed carbohydrate (SAC, low GI) for up to 40 weeks. Diets were controlled for macronutrient and micronutrient content, differing only in starch type. Body composition and insulin sensitivity were measured longitudinally by DEXA scan and oral glucose tolerance test, respectively. Food intake, respiratory quotient, physical activity and energy expenditure were assessed using metabolic cages.

Results: Despite having similar mean body weights, mice fed the RAC diet had 40% greater body fat by the end of the study and a mean 2.2-fold greater insulin resistance compared with mice fed the SAC diet. Respiratory quotient was higher in the RAC group, indicating comparatively less fat oxidation. Although no differences in energy expenditure were observed throughout the study, total physical activity was 45% higher for the SAC-fed mice after 38 weeks of feeding.

Discussion: We conclude that, in this animal model 1) the effect of GI on body composition is mediated by changes in substrate oxidation, not energy intake; 2) a high GI diet causes insulin resistance; 3) dietary composition can affect physical activity level.

Key Words

Glycemic index, carbohydrates, mice, physical activity, energy metabolism
Abbreviations

DEXA: dual energy X-ray absorptiometry
EE: energy expenditure
GI: glycemic index
GL: glycemic load
PA: physical activity
RAC: rapidly absorbed carbohydrate
SAC: slowly absorbed carbohydrate
INTRODUCTION

In recent decades, dietary patterns in the United States have shifted toward an increased intake of refined carbohydrate (9). This shift has paralleled a rise in the prevalence of obesity and type 2 diabetes, and epidemiological studies have demonstrated associations between carbohydrate type and body weight, adiposity or associated cardiovascular risk factors (10, 13, 20, 21, 32). Many, but not all, studies have shown that diets low in glycemic index (GI) or glycemic load (GL) have beneficial effects on weight loss and/or reduction of risk factors for chronic disease in humans (2, 6, 12-14, 18, 23, 24, 28). In two energy-restricted feeding studies, resting energy expenditure declined to a lesser extent among overweight individuals consuming lower GL/higher fat vs. higher GL/lower fat diets (1, 18).

The effects of GI on sports performance have been examined in numerous small clinical trials. Low vs. high GI meals were shown in some studies to enhance fat utilization during sustained physical activity, improve peak performance and increase endurance (8, 27, 29, 30, 33-36). A question not explored by these studies is whether GI might affect spontaneous physical activity level.

In relatively short-term nutrient-controlled rodent studies, treatment with a high GI diet resulted in increased adiposity, altered glucose homeostasis, and evidence of greater metabolic efficiency (11, 16, 17, 22). However, the sequence of physiological events relating GI to body composition and energy metabolism remains incompletely examined and the subject of controversy (4). The aim of this study was to evaluate the effects of GI on adiposity, glucose homeostasis, substrate oxidation, energy expenditure and physical activity in an animal model in which confounding dietary and environmental
factors can be controlled over the long term.

RESEARCH METHODS AND PROCEDURES

Animals

Parallel studies were performed in a total of forty-eight 129SvPas mice (Charles River Laboratories, Wilmington, MA), divided into three cohorts (n=16 each). Changes in adiposity and insulin sensitivity were studied in Cohort I over a 38-week period, and assessment of energy metabolism and physical activity was performed at Week 40 using metabolic cages. Energy metabolism and physical activity were assessed for 38 weeks in Cohort II and for 19 weeks in Cohort III. This strain of mice was chosen because of its relative resistance to diet-induced obesity, allowing us to examine the physiological effects of diet with less confounding by body weight differences.

For each cohort, five-week old male mice were acclimated to the animal facility on standard chow for two days, after which they were individually housed and placed on a low GI diet that was high in amylose, a slowly absorbed carbohydrate (SAC) for one week. After this run-in period, mice were weight-paired and randomized to either continue on the SAC diet or to receive a high GI diet that was high in amylopectin, a rapidly absorbed carbohydrate (RAC). Food and water were provided *ad libitum* throughout the study, and body weight was measured three times per week. Mice were maintained on a 12-hr dark/light cycle in standard housing, except when in metabolic cages. All animal procedures were done in accordance with protocols approved by the Institutional Animal Care and Use Committee at Children’s Hospital Boston (protocol #A04-08-114).
Diets

The two diets were of identical macronutrient composition (68% carbohydrate: 13% fat: 19% protein) and energy density (4.2 kcal/gm; measured by bomb calorimetry, presented in Table 1) and differed only in the starch component, which was 100% amylopectin for the RAC diet and 60% amylose/40% amylopectin for the SAC diet; both starches are naturally-occurring in corn. The composition of these diets has been previously described in detail (22); in the current study, they were prepared in our laboratory, color-coded using non-nutritive, lipid-soluble food coloring and provided to the mice as powdered food in aluminum dishes (Table 1). Previous studies assessing postprandial glycemic response to the two diets demonstrated significantly greater area under the curve for rats fed the RAC compared to the SAC diet (17). Glycemic response was replicated in crossover studies performed in 129SvPas mice that were separate from the current study (glucose AUC [RAC vs. SAC]: 7923 ± 1651 vs. 5627 ± 2003; p=0.049).

Body Composition

Body composition was measured in non-fasted mice between 1:00 PM and 4:00 PM during the 1-week run-in period and at indicated time-points by dual energy X-ray absorptiometry (DEXA) using the Lunar PIXIImus™ Mouse Densitometer (GE Healthcare, Madison, WI). Mice were anesthetized using a mixture of ketamine (85 mg/kg) and xylazine (8.5 mg/kg) administered intraperitoneally. Additional ketamine, to a maximum of 100 mg/kg, was administered if a mouse did not respond to the initial dosage. If a mouse could not be fully anesthetized, the DEXA scan was omitted for that animal at that time-point. Intra-mouse coefficients of variation were <5%.

Oral Glucose Tolerance Tests and Blood Collection
Glycemic index & energy metabolism in mice

Oral glucose tolerance tests (OGTT) were performed beginning two weeks after randomization and at indicated time-points. On test days, mice were placed in clean cages without food at 8:00 AM, and fasted blood was collected from the tip of the tail vein using heparinized capillary tubes between 1:00 PM and 2:00 PM. Following oral gavage of 2 mg glucose/gm body weight, blood was collected by tail-bleed for measurement of blood glucose using the One Touch® Ultra® glucometer (Lifescan, Milpitas, CA) at 30, 60 and 120 minutes. Plasma collected at 30 and 120 minutes following glucose gavage was frozen at -80°C for further analyses. Plasma insulin was measured in batches using the Rat Insulin ELISA with mouse insulin as standard (Crystal Chem Inc., Downers Grove, IL). The intra-assay and inter-assay coefficients of variation were 5.7 % and 6.3 %, respectively.

Metabolic cages

Non-protein respiratory quotient (RQ), food intake, energy expenditure (EE) and physical activity (PA) were measured using the Comprehensive Laboratory Animal Monitoring System (CLAMS) from Columbus Instruments (Columbus, OH) in individual cages without bedding. Pilot studies in our laboratory (data not shown) and the Mouse Phenome Project at the Jackson labs (Bar Harbor, Maine; http://www.jax.org/phenome) have shown that mouse behavior is altered during the first day of exposure to the CLAMS cages. Mice were therefore acclimated to the metabolic cages for 48 hrs prior to the start of data collection at all time-points. This acclimation time also allowed for close monitoring of body weight and food and water intake and to ensure the animals’ health. Data collection began at midday after 48 hrs of acclimation and continued uninterrupted for 48 hrs. Room temperature was maintained at 23°C during the CLAMS episodes based
on recommendations from the Mouse Phenome Project.

Oxygen consumption (VO₂) and carbon dioxide expiration (VCO₂) were measured by indirect calorimetry with an air flow of 0.6 L/min. RQ (VCO₂/VO₂) data are presented for the dark and light cycles and as a 48-hr average. EE was calculated using the equation, kcal/hr = \[3.815 + 1.232 \times \frac{VCO_{2}}{VO_{2}}\] x VO₂, based on the table of Lusk (15) and is presented as EE/48-hr. Total PA and ambulatory (AMB) PA were measured continuously by light beam breaks in the two dimensional, horizontal plane; total PA represents all movement recorded, whereas AMB PA is a measure of movement across the X and Y axes. PA counts were summed for each 48-hr CLAMS episode and for dark and light cycles, respectively.

**Net Energy Intake**

Food intake was measured by the CLAMS to the 0.01 gm during the 48-hr CLAMS episode and converted to total kcal intake. Previous work in our laboratory has shown differential energy malabsorption, evidenced by increased fecal energy for the SAC-fed mice (22). For the SAC group, fecal energy density was assessed from 48-hr fecal collections by bomb calorimetry at Week 2 of feeding as previously described (22). Pilot studies also showed that fecal energy density remains constant with long-term feeding in the SAC group. Thus, energy malabsorption was calculated for individual mice at each time-point based on fecal energy data from Week 2 and fecal output for each 48-hr CLAMS episode. Because of the minimal malabsorption that occurs on the RAC diet, stool output of animals on this diet was much smaller, and we had technical difficulties collecting samples of adequate volume for analysis. For this reason, we used the value obtained from our prior study, 3.062 ± 0.103 kcal/gm, in which animals of the same
Glycemic index & energy metabolism in mice

strain were fed the same RAC diet. Net energy intake was calculated for each time-point by subtracting fecal energy from total energy intake.

Exclusion criteria

During data collection in the CLAMS, if a mouse’s percent weight loss exceeded the cohort’s mean per cent weight loss + 2 standard deviations, all data for that mouse were excluded from the analyses at that time-point. In total, 4 out of 48 mice were removed from the study early. One mouse was found dead from unknown causes at Week 34, and three mice that developed severe dermatitis were removed at Weeks 21, 27 and 30, respectively.

Statistical Analyses

Differences in body weight, body composition, net energy intake, plasma insulin, blood glucose, glucose AUC, insulin resistance, PA and EE were assessed longitudinally using repeated measures ANOVA (alpha=0.05) with diet and time as factors. We did not collect baseline data for insulin. In the absence of this datapoint, ANOVA would inappropriately adjust for the difference that had occurred between groups after randomization and prior to the first available timepoint at week 14. Therefore, we analyzed insulin data by comparing the average of all available data by t-test. For RQ, we also compared the average of all available data by t-test because this endpoint appeared to change in varying ways throughout the study. For physical activity, the diet effect appeared to emerge over time, becoming greatest at the end of the study. Therefore, we used a t-test at weeks 38 to 40, including both cohorts, as a post-hoc method to characterize this effect. According to an a priori power calculation, attaining statistical power of 0.80 (alpha=0.05) required the inclusion of 16 mice per dietary group for the
detection of diet-induced differences of 3733 counts in PA and 0.44 kcal in EE. Because of necessary exclusions, our statistical power was reduced at some time-points. Analyses were performed using SAS 9.1 for Windows, and data are presented as mean ± SD.

RESULTS

Stool Analysis, Energy Intake and EE
Consistent with findings of a prior study, SAC-fed animals showed a moderate degree of malabsorption, with stool energy density of 3.996 ± 0.074 kcal/gm. Net ad libitum energy intake was measured over the course of 48 hr in the CLAMS and did not differ between the SAC and RAC groups throughout the 38-week study (Figure 1A). No differences in energy intake between groups were observed for the dark and light periods, respectively, nor in terms of length or number of feeding bouts (data not shown). Both SAC and RAC mice expended ~15% more energy during the dark vs. the light period (data not shown), but there was no effect of diet on 48-hr EE (Figure 1B) or EE during the dark and light periods throughout the study (data not shown).

Body Weight and Composition
Body weight was collected for all 48 mice, and a representative graph is shown for Cohort I (n=16) in Figure 2A. A diet x time effect was observed (p=0.002). However, differences in body weight between groups were modest throughout the study, and not significant at 38 weeks (35.2 ± 7.3 gm for RAC vs. 33.1 ± 1.4 gm for SAC; p=0.39). In contrast, body composition measured by DEXA at indicated time-points was markedly different between groups throughout the study, as shown in Figure 2B (diet x time effect: p<0.0001). By Week 38, percent body fat was 40% greater in the RAC vs. the SAC
group (30.1 ± 7.6 % vs. 21.5 ± 3.1 %; p=0.008). Results for body weight and body composition were similar for Cohorts II and III (data not shown).

**Respiratory Quotient**

Mean RQ was higher for the RAC vs. SAC group (0.91 ± 0.02 vs. 0.89 ± 0.01, p=0.0002) (**Figure 3**). The difference between groups was most pronounced for the first 8 weeks following randomization.

**Glucose Homeostasis**

Fasted blood glucose and plasma insulin were significantly higher for the RAC group compared with the SAC group (6.2 ± 0.5 vs. 5.4 ± 0.9 mmol/l, p=0.0003 and 114.5 ± 68.0 vs. 58.8 ± 22.7 pmol/l, p=0.03, respectively) (**Figure 4A and B**). Insulin resistance, as calculated by HOMA (homeostasis model assessment) index [(fasting insulin x fasting glucose)/22.5] (37), was 2.2-fold higher for the RAC vs. SAC group (5.4 ± 3.4 vs. 2.4 ± 1.1, p=0.02) (**Figure 4C**). Although there was no difference in glycemic area under the curve (AUC) following OGTT (**Figure 4D**), mean total insulin AUC was higher for the RAC vs. SAC group (108 ± 48 and 48 ± 11, p=0.02) (**Figure 4E**). Insulin at 30-min, a time point of particular interest as a measure of insulin secretion (27), was different between groups (p=0.04). Results from OGTT at Week 20 in Cohort II were similar for both glucose and insulin response (data not shown).

**Physical Activity**

Total PA was non-significantly higher (by 15%) for the SAC group compared to the RAC group, when observed as total counts/48-hr (**Figure 5A**). Results for AMB PA were similar (data not shown). At Weeks 38-40, both total PA (106347 ± 34212 vs. 73547 ± 17447, p=0.008) and AMB PA (36991 ± 11628 vs. 22725 ± 11432, p=0.004) were
significantly greater for the SAC than the RAC group (Figures 5B and 5C).

DISCUSSION

Prior research aiming to establish a relationship between GI and energy metabolism have been inconclusive, and a consensus as to whether GI directly affects substrate oxidation or energy expenditure remains elusive (4). The purpose of this study was to examine the longitudinal changes in obesity-related parameters among rodents fed diets differing in GI but controlled for macro- and micronutrients. We found the RAC vs. SAC group had lower fat oxidation, 40% greater body fat and 2.2-fold greater insulin resistance, even though net energy intake did not differ between groups. Of particular interest, the RAC group became less physically active than those fed the SAC diet.

The postprandial hyperglycemia on a high GI diet increases insulin secretion, and higher insulin levels would promote glucose uptake in insulin-sensitive tissues and inhibit lipolysis in adipose tissue. These metabolic events would acutely favor oxidation of carbohydrate rather than fat, and chronically lead to accumulation of body fat. The higher RQ in mice fed the RAC diet, especially during the initial weeks of treatment before development of more severe insulin resistance, supports this possibility.

Higher levels of physical activity are characteristically associated with greater lean body mass, a lower RQ (indicating an increased capacity to oxidize fat), and enhanced insulin sensitivity (3, 7, 19, 25, 26, 38), all characteristics observed in the SAC-fed mice. Moreover, physical activity, by virtue of its effect on energy balance, tends to promote loss of body fat. However, the question of whether dietary composition in general, and carbohydrate type in particular, might affect physical activity has not been
extensively evaluated. Indeed, attempts to study such phenomena in humans over the long term would likely be confounded by numerous dietary and environmental factors. The tendency of the SAC-fed mice to be more active throughout the study, and the significantly increased physical activity level at the end of the study, suggest a novel mechanism whereby diet may produce fat loss. The reasons for this difference in physical activity may relate to improved access to metabolic fuels or maintenance of greater lean mass on a low vs. high GI diet, though these possibilities remain speculative.

Three study limitations warrant that consideration. First, substrate oxidation and energy expenditure were assessed by indirect calorimetry which relies upon established relationships between oxygen consumption and carbon dioxide expiration. Since we did not measure urinary nitrogen excretion, we cannot determine to what degree the higher RQ on the RAC diet might have been caused by higher rates of protein oxidation (though we would presume this contribution would be relatively small, given the low protein to carbohydrate content of the diets). Second, the resistant starch content of the SAC diet was higher than that of the RAC diet. Resistant starch is carbohydrate that escapes digestion in the small intestine, thereby altering the colonic environment due to increased fermentation, subsequently influencing microbial flora composition and free fatty acid production (31). Whether these events could importantly affect fuel partitioning and energy metabolism has not been established. Recently, differences in colonic bacteria have been documented in lean and obese humans and animals (5). These differences may play a causal role in obesity through novel mechanisms, such as direct effects on fat cell metabolism. Thus, it is possible that the physiological effects of the diets were mediated not only by actions in the small intestine but also by actions in the large intestine. In order
to examine this issue, further studies employing isotopic tracers would be required. Third, the degree to which these findings apply to humans remains speculative. Humans tend to have lower rates of de novo lipogenesis than rodents, a biological difference that might attenuate the effects of diet on body composition.

In conclusion, this study illustrates that the rate of carbohydrate digestion influences substrate oxidation, body composition and insulin sensitivity independent of net energy intake, nutrient composition and other confounding factors. Our findings further suggest that a low GI diet could increase spontaneous physical activity level, a possibility that might have important implications for the prevention and treatment of obesity and promotion of physical fitness.

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REFERENCES


Table 1. Dietary Composition

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Dietary Characteristics

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aHi-Maize™, National Starch and Chemical Company, Bridgewater, NJ
bC-Gel 03420, Ceresstar USA, Inc., Hammond, IN
cHarlan Teklad, Indianapolis, IN
dCommercially available.
eCalculated from published nutrient databases.
fMeasured by bomb calorimetry, mean of two dried samples.
FIGURE LEGENDS

Figure 1. Net ad libitum energy intake and EE during the 48-hr CLAMS measurements for the RAC and SAC groups. A) Net energy intake was calculated by subtracting fecal energy from total energy intake. B) Total EE was measured by indirect calorimetry. Values are mean ± sd with 13 to 16 mice per dietary group at each time-point for Weeks 0 – 19 and 6 to 8 mice per dietary group per time-point for Weeks 20 – 38. Repeated measures ANOVA, diet x time effect: p=NS for net energy intake and EE.

CLAMS=comprehensive laboratory animal measurement system; EE=energy expenditure; RAC=rapidly absorbed carbohydrate; SAC=slowly absorbed carbohydrate.

Figure 2. Mean body weight and composition for the RAC and SAC groups throughout the study. Mean body weight (A) and % body fat (B) are presented. Values are mean ± SD with 5 to 8 mice per group at each time-point. Repeated measures ANOVA, diet x time effect was p=0.002 for body weight and p<0.0001 for % body fat. RAC=rapidly absorbed carbohydrate; SAC=slowly absorbed carbohydrate.

Figure 3. Mean RQ for RAC and SAC groups throughout the study. All mice were fed the SAC diet for a 1-week run-in period (Week 0), followed by randomization to either the RAC or SAC diet. Each time point represents a 48 hr measurement. Data are mean ± sd with 13 to 16 mice per dietary group at each time-point for Weeks 0 – 19 and 6 to 8 mice per dietary group per time-point for Weeks 20 – 38. Values, averaged across all time points, were significantly different between the RAC and SAC groups by t-test (0.91 ± 0.02 vs. 0.89 ± 0.01, p=0.0002, respectively). RAC=rapidly absorbed carbohydrate;
RQ=respiratory quotient; SAC=slowly absorbed carbohydrate.

Figure 4. Assessment of insulin sensitivity by OGTT and HOMA for RAC and SAC groups. Data for (A) fasting blood glucose (p=0.0003); (B) fasting plasma insulin (p=0.03); (C) insulin resistance calculated by HOMA (p=0.02); (D) glucose AUC during OGTT (p=0.14); and (E) insulin AUC during OGTT (p=0.02). Values are mean ± sd with n=4 to 8 per group at each time-point. Repeated measures ANOVA (diet x time effect) for panels A-D; for panel E, mean values were averaged across all time points after randomization and compared by student’s t-test. AUC=area under the curve; HOMA=homeostasis model assessment; OGTT=oral glucose tolerance test; RAC=rapidly absorbed carbohydrate; SAC=slowly absorbed carbohydrate.

Figure 5. PA during the 48-hr CLAMS measurements for RAC and SAC groups. Total PA is represented as total light beam breaks in 48 hr (A). Values are mean ± sd with 13 to 16 mice per dietary group at each time-point for Weeks 0 – 19 and 6 to 8 mice per dietary group per time-point for Weeks 20 – 38. Repeated measures ANOVA, diet x time effect: p=NS. Data for total PA and AMB PA after 38-40 weeks of feeding are shown in Panels B and C. Values are mean ± sd with 12 to 15 mice per dietary group. Student’s t-test. P-values are indicated. AMB=ambulatory. CLAMS=comprehensive laboratory animal measurement system; PA=physical activity; RAC=rapidly absorbed carbohydrate; SAC=slowly absorbed carbohydrate.
Figure 2

A

![Graph showing body weight over weeks with error bars for NAC and SAC groups.]

B

![Graph showing percentage body fat over weeks with error bars for NAC and SAC groups.]

Weeks