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Mucosal Maltase-Glucoamylase Plays a Crucial Role in Starch Digestion and Prandial Glucose Homeostasis of Mice[1–3]

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Abstract

Starch is the major source of food glucose and its digestion requires small intestinal α-glucosidic activities provided by the 2 soluble amylases and 4 enzymes bound to the mucosal surface of enterocytes. Two of these mucosal activities are associated with sucrase-isomaltase complex, while another 2 are named maltase-glucoamylase (Mgam) in mice. Because the role of Mgam in α-glucogenic digestion of starch is not well understood, the Mgam gene was ablated in mice to determine its role in the digestion of diets with a high content of normal corn starch (CS) and resulting glucose homeostasis. Four days of unrestricted ingestion of CS increased intestinal α-glucosidic activities in wild-type (WT) mice but did not affect the activities of Mgam-null mice. The blood glucose responses to CS ingestion did not differ between null and WT mice; however, insulinemic responses elicited in WT mice by CS consumption were undetectable in null mice. Studies of the metabolic route followed by glucose derived from intestinal digestion of ^{13}C-labeled and amylase-predigested algal starch performed by gastric infusion showed that, in null mice, the capacity for starch digestion and its contribution to blood glucose was reduced by 40% compared with WT mice. The reduced α-glucogenesis of null mice was most probably compensated for by increased hepatic gluconeogenesis, maintaining prandial glucose concentration and total flux at levels comparable to those of WT mice. In conclusion, mucosal α-glucogenic activity of Mgam plays a crucial role in the regulation of prandial glucose homeostasis. J. Nutr. 139: 684–690, 2009.

Introduction

Starches are the major food source of glucose. In humans, starch α-glucogenic digestion requires a consortium of 6 enzymes. Two are soluble salivary and pancreatic amylases, 2 are associated with the small intestinal brush border membrane protein sucrase-isomaltase complex [(SI)[8] in humans], and the last 2 with the intestinal brush border membrane protein maltase-glucoamylase complex (MGAM) in humans. We recently documented the roles of MGAM and SI for in vitro starch digestion in mouse and human small intestinal mucosa (1,2). Human MGAM is 10 times more active in vitro than SI but is inhibited by the products of amylase starch hydrolysis (1). Human SI is present at a 20 times higher concentration than MGAM and is not inhibited by lumenal starch-derived oligomers (1). Salivary and pancreatic amylases act as synergistic amplifiers of the 4 mucosal

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[3] Supplemental Figures 1–4 and Supplemental Tables 1–3 are available with the online posting of this paper at jn.nutrition.org.
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[8] Abbreviations used: AMG, amyloglucosidase; AMY, amylase; CS, normal corn starch; DW, dry weight; E, plateau tracer:tracee ratio; EE, energy expenditure; F, fractional appearance rate; GLM, general linear model; LDx, α-limit dextrin; MDx, maltodextrin; MGAM, human maltase-glucoamylase; Mgam, mouse maltase-glucoamylase; MPE, mol% of isotope enrichment at steady state; RAG, rapidly appearing glucose; RER, respiratory exchange ratio; RS, resistant starch; SAG, slowly appearing glucose; SI, human sucrase-isomaltase; Si, mouse sucrase-isomaltase; TS, total starch; V_{CO2}, carbon dioxide production; V_{O2}, oxygen consumption; WT, wild-type mice.

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α-glucosidases by producing shorter soluble glucose oligomer substrates (1,2).

The apparent redundancies of the mucosal α-glucosidase activities of SI and MGAM have resulted in persisting confusion about their individual roles and their in vivo contributions to the production of free glucose absorbable and available for subsequent metabolism by energy-related pathways. We have been studying the roles and relative contribution of these enzymes to starch digestion using mouse models with naturally occurring or artificial deficiencies of sucrase-isomaltase (Si in mice) and maltase-glucoamylase (Mgam in mice). Our general hypothesis is that apparent redundancy among mucosal α-glucosidases provides a broader spectrum of activities enabling the digestion of a wider botanical variety of starches and assuring the continuous flow of exogenous glucose for the energetic requirements of organisms.

In a previous publication, starch consumption of CBA/CaJ mice (3), displaying a naturally occurring deficiency of Mgam and partial deficiency of Si, did not affect their prandial blood glucose concentration compared with mice expressing normal levels of Mgam and Si. However, we have more recently shown that Sv/129 mice whose Mgam gene was ablated to produce Mgam null mice had a lower rate of CO_2 production derived from ingested starch metabolism than wild-type (WT) mice (1). The apparent paradox of normal blood glucose levels with lower metabolism of exogenous glucose led us to hypothesize that deficiency of Mgam or Si, naturally occurring or experimentally induced, also induced changes in liver glucose metabolism. Hepatic gluconeogenesis and glycogenolysis metabolic pathways play a central role in glucose homeostasis during starvation (4). About 95% of intestinally produced and absorbed glucose is transported to the liver (4,5), where it is stored as glycogen and subsequently mobilized by glycogenolysis during episodes of food deprivation. In prolonged starvation or ingestion of low-carbohydrate diets, glucose production from gluconeogenesis may contribute >90% of the circulating glucose. In the prandial state, both elevated glucose and insulin levels inhibit hepatic gluconeogenesis (4). In this study, we evaluated the role of small intestinal Mgam in starch digestion to glucose and its effects on prandial glucose homeostasis and energy metabolism using Sv/129 Mgam null and WT mice.

Methods

Mutation of Mgam by homologous recombination and mice genotyping. The method of ablation of the Mgam gene in Sv/129 mice has been reported (1). Briefly, a targeting vector containing the Lac Z -MC1neo selection marker and the flanking thymidine kinase genes was designed to replace parts of exon 2. The construct was electroporated into strain Sv/129 stem cells. Positive stem cell clones were identified by Southern analysis and were implanted in prepared foster dams. The F1 generation was back-crossed with the same Sv/129 strain of mice and heterozygotes identified by Southern analysis. After germ line transmission, the Mgam genotypes were identified by quantitative PCR of tail DNA. Mgam genotypes were confirmed by quantitative PCR and by Western blot using purified specific rabbit polyclonal antibodies prepared against a peptide coded by exon 25 of the C-terminus subunit of Mgam. All mouse experiments were approved under the Baylor College of Medicine Institutional Animal Care and Use Committee protocol AN-1577.

Phenotyping of mucosa by α-glucogenic assays. Sucrase assays were those described by Dahlqvist (6–8) at 16 mmol/L sucrose concentrations and 60-min incubations at 37°C. The α-glucosidase assay was a modification previously described (1,2) using 20 g/L maltodextrin (MDx) as substrate. Activity was reported as international enzyme units (U/g protein; 1 U = 1 μmol glucose released/min reaction). Both mid-jejunum and mid-ileum segments were assayed.

Experimental diets. The standard pelleted mouse diet was Pico Lab Rodent Diet 20 (5053) (Purina Mills) with a gross energy of 16.74 kJ/g (4 kcal/g) containing 34% corn starch (CS) and 19% sucrose by dry weight (DW). Experimental diets formulated and processed as pellets by Harlan TEKLAD are summarized in **Table 1**. A low-^{13}C diet was formulated from rice starch and beet sugar, plants with a C3 photosynthesis pathway that depletes the ^{13}C, which is normally enriched by the C4 pathway of CS and cane sugar. The CS diets contained 53% normal CS with 12% corn-derived MDx. A control diet was formulated to contain 65% cane sucrose that replaced the starch and MDx. The remainder of each experimental diet approximated the Pico diet and composition with (g/kg) 200 casein, 3 DL-methionine, 50 soybean oil, 49.49 cellulose, 2.5 choline bitartartrate, 35 AIN-93G-MX mineral mix, 10 AIN-93-VX vitamin mix, and 0.01 TBHQ antioxidant. The detailed compositions of these diets are reported (**Supplemental Tables 1–3**).

The total starch (TS) in the diets (Table 1) was measured by the weight of dry starch converted to free glucose by the *Aspergilous niger* AMG and *Bacillus lichiniformis* α-amylase method (Megazyme Int.; K-TSTA) as previously reported (9–12). Englyst assays (13) fractionated the starch by digestibility using AMG, *Saccharomyces cerevisiae* invertase, and porcine pancreatic α-amylase enzymes (VI-B; Sigma Chemical). Released glucose was assayed by the glucose oxidase-peroxide reaction method (Megazyme Int.; K-GLUC) (9,10). Rapidly appearing glucose (RAG, also called RDS) is the quantity of free glucose measured at 20 min and slowly appearing glucose (SAG or SDS) is the remaining glucose appearing at 120 min. Resistant starch (RS) is the residual glucose equivalent remaining after 120 min.

Prandial energy metabolism. Energy expenditure (EE) of the mice was measured in an open-circuit indirect calorimeter (Oxymax, Columbus Instruments) designed to measure oxygen consumption (V_{O2}), carbon dioxide production (V_{CO2}), and the respiratory exchange ratio (RER) of mice. The system has separate chambers that permit measurements on 16 mice by sampling each chamber every 43 min. At the end of each cycle, the system samples inlet air. V_{O2} and V_{CO2} were calculated using equations derived from mass balance across the chamber. The V_{O2} and V_{CO2} sensors and flow meters were calibrated before each cycle through the chambers. We used the Weir equation (14) to calculate EE adjusted for mouse body weight and RER from V_{O2} and V_{CO2}. A total of

TABLE 1 Starch content and properties of experimental diets[1]

Diet	Supplier	TS	RAG	SAG	RS
		% DW[2]		%TS[3]	
MDx (Polycose)	Abbott Nutrition	100	88	7	5
Pico 5053	Purina	32.6 ± 1.1	58.7 ± 4.7	43.7 ± 3.1	−2.4
Low-^{13}C Pico variant	Harlan no. 06089	24.2 ± 0.3	53.4 ± 3.8	44.0 ± 2.0	2.6
53% CS	Harlan no. 01629	51.8 ± 0.6	74.0 ± 4.8	26.9 ± 5.4	−0.9
65% sucrose	Harlan no. 01631	0.0 ± 0.0	32.2	32.7	–

[1] Values are means ± SD of triplicate determinations.
[2] Amyloglucosidase/bacterial α-amylase method.
[3] Englyst assay: (amyloglucosidase/invertase/pancreatic α-amylase method); see details of diet composition in Supplemental Tables 1–3.

8 male mice from each the null and WT strains were tested in each experiment over the same 2-d protocol. All mice were conditioned in individual chambers with the specified diet for >96 h prior to onset of the calorimetry experiment. The calorimetry data collections started between 0800 and 1000 (d 1) and continued for 2 consecutive 24-h periods that were divided into 12-h cycles of light (0600–1800) and dark (1800–0600). Data were recorded over 1 period of light (d 2) and 2 of dark (nights 1 and 2). The mice were continually watered and fed with mouse food pellets placed in the chambers. Body weights were measured before the experiments to express EE as kJ/(g·h).

Prandial blood glucose and insulin levels. We studied groups of 4 WT and 4 null mice at 1200 ± 1 h during food deprivation or at 2300 ± 1 h during unrestricted consumption. The food deprivation period was 30 h long to reduce luminal contents in the small intestine. The fed state was associated with visible food content in the stomach and small intestine. Each mouse was killed by decapitation and blood collected by flow from the carotids into a chilled tube. Serum was removed and conserved at −70°C for analysis. The abdomen was opened and the small intestine removed by sharp dissection. The lumen was immediately flushed with 4°C 0.01% $CaCl_3$ in PBS (10 mmol/L phosphate, 0.15 mol/L NaCl, pH 6.8) to cleanse the lumen and chill the tissue. The intestine was then arranged on a glass plate that was chilled on ice and segments were removed for analyses. Mid-jejunal and mid-ileal segments were placed in capped tubes and snap-frozen in liquid nitrogen and conserved at −70°C until assayed. These intestinal segments were assayed for enzyme activity (see above) and the serum samples were analyzed in duplicate for glucose by analyzer and insulin concentrations as described below.

In vivo starch digestion to glucose. Starch-digesting phenotypes were investigated in null and WT mice by assaying the extent of conversion of fed ^{13}C-starch substrate to ^{13}C-glucose levels in blood. Universally labeled $^{13}C_6$-starch of algal source (Sigma-Aldrich, hydrolyzed glucose units = M+6) was exhaustively digested with porcine pancreatic amylase (AMY) (type VI-B, Sigma-Aldrich) to make U-$^{13}C_6$-labeled limit dextrin (LDx), which is insensitive to further AMY digestion. The soluble fraction of ^{13}C-LDx was freeze-dried and used for i.g. infusion to measure rates of starch digestion to glucose ($F_{i.g.}$). Based on exhaustive AMG digestion followed by the glucose oxidase reaction, the ^{13}C-LDx solution contained 3.48 ± 0.12 g/L total glucose equivalents, only 0.21 ± 0.01 g/L (6%) of which was free glucose. Filter sterilized D-glucose-C-d7 (M+7) solution (7.5 g/L in PBS, pH 7.4) was also infused i.v. to measure the fractional appearance rate ($F_{i.v.}$) of total plasma glucose (Isotec T82–30011; lot IM1322–4).

Sixteen genotyped mice were fed a low-^{13}C diet for 2 wk before the experiment to achieve a constant ^{13}C-glucose baseline (Table 1). The mice were then food deprived for 30 h to reduce liver glycogen stores (15) and tail vein and gastric cannulas were inserted under brief fluorothane anesthesia (16). The cannulas were infused at 100 μL/h. AMG-hydrolyzed i.g. infusion contained 28.27 mmol/L of available $^{13}C_6$-glucose as LDx and the i.v. infusion 33.8 mmol/L free $^{2}H_7$-glucose. The i.v. infusion contained 25 kIU heparin/L to facilitate terminal blood collections. After 4 h of constant infusion, blood was obtained by percutaneous puncture of the submandibular sinus and collected into sodium fluoride-containing tubes. The tubes were chilled, centrifuged (2000 × *g*; 5 min), and plasma frozen until assays. Total plasma glucose was measured with the hexokinase reaction (Worthington Biochemical; HKQS) and insulin concentration was determined by RIA (Linco Research; SRI-13K), both in duplicate samples at the Vanderbilt Mouse Metabolic Phenotyping Center.

Eight isotopomers of plasma glucose were measured in duplicate on a Hewlett Packard 9890A gas chromatograph quadrupole mass spectrometer by positive chemical ionization GC-MS as penta-acetate derivatives, monitoring ions from a mass:charge ratio of 330–337 for glucose (17,18). Baseline enrichments were obtained from noninfused littermates receiving the same diet. The calculation of individual glucose fluxes used the following steady-state equation:

$$Ra = F/E, \qquad \text{(Eq.1)}$$

where Ra = glucose flux ($\mu mol \cdot kg^{-1} \cdot m^{-1}$), F = constant isotope infusion rate ($\mu mol \cdot kg^{-1} \cdot m^{-1}$), and E = mol% (moles labeled glucose × 100/total moles plasma glucose) of isotope enrichment of plasma glucose at steady state (MPE). To calculate individual intestinal starch digestion to glucose, we used the i.g. (M+6) and i.v. (M+7) fluxes derived from Eq. 1. At isotopic steady state, the fraction of the i.g. tracer that enters systemic plasma (sampled compartment) would be given by the ratio of the plasma tracer:tracee ratio of the i.g. (M+6) tracer to the tracer:tracee ratio of the i.v. (M+7) tracer normalized for their infusion rates (5,17). This intestinal digestion fraction is given by:

$$\text{Intestinal digestion} = [E_{i.g.}(M+6)/\text{Ei.v.}(M+7) \times (F_{i.v.}(M+7)/F_{i.g.}(M+6)], \qquad \text{(Eq.2)}$$

where $E_{i.g.}$ (M+6) is the plateau tracer:tracee ratio of the i.g. tracer U-$^{13}C_6$-LDx-derived glucose, $E_{i.v.}$(M+7) is the plateau tracer:tracee ratio of the i.v. tracer $^{2}H_7$, $F_{i.v.}$(M+7) is the rate of the i.v. tracer infusion ($\mu mol \cdot kg^{-1} \cdot m^{-1}$), and $F_{i.g.}$(M+6) is the rate of the i.g. tracer infusion ($\mu mol \cdot kg^{-1} \cdot m^{-1}$) (21,22). Thus, $F_{i.g.}$(M+6) glucose equivalents not digested from the LDx tracer but retained by the intestine will be:

$$\text{Intestinal retention} = [1 - E_{i.g.}(M+6)/E_{i.v.}(M+7) \times F_{i.v.}(M+7)/F_{i.g.}(M+6)]. \qquad \text{(Eq.3)}$$

Means ± SD intestinal retention values are summarized (Table 5).

Statistical procedures. The effects of mouse genotype, diet, and segment of the small intestine on the levels of α-glucosidase or sucrase activities were analyzed using a 3-factor (genotype, diet, intestinal segment) general linear model (GLM) design. Because significant effects on the enzyme activities were observed for all 3 factors, the model was transformed into a 1-way ANOVA using a code built with the 3 factors (genotype × diet × intestinal segment) as individual factors; the individual pairs of relevant data groups with significant differences were determined by Tukey's test (**Table 2**). The effect of genotype, diet, and time (day/night) on RER or EE were analyzed using a 3-factor (genotype × diet × time) GLM design; in both RER and EE, only diet had a significant effect and therefore no further test was performed (**Table 3**). The effects of genotype and feeding status on blood glucose and insulin concentration were determined by a 2-way ANOVA. Because only feeding status affected blood glucose concentration, no additional test was performed; for blood insulin concentration, genotype, feeding status, and their interactions had a significant effect and the differences between individual treatment groups were determined by Tukey's test using coded genotype × feeding status as classifying factor (**Table 4**). Differences between genotypes in total blood glucose concentration, glucose isotopomer enrichment ($E_{i.g.}$ and $E_{i.v.}$), rates of fractional appearance of glucose ($F_{i.g.}$ and $F_{i.v.}$), rates of glucose flow ($Ra_{i.g.}$ and $Ra_{i.v.}$), and percent of intestinal retention were evaluated using unbalanced 2-tailed *t* tests (**Tables 5** and **6**). In all cases with significant differences between samples, the power of the *t* test was ≥0.95 with $n = 6$ and pooled SD of each individual parameter. Limits for significance were set at $P \leq 0.05$ in all cases. Only significant differences are noted in the text and tables. Statistical tests were performed using the specialized software Minitab or SAS/STAT (SAS Institute).

TABLE 2 Effects of starch consumption on mucosal enzyme activities of Mgam null and WT mice[1]

	α-Glucosidase		Sucrase	
Feeding	Null	WT	Null	WT
Jejunum	*U/g protein*[2]			
Food deprived	90.7 ± 22.6[a]	180.4 ± 16.7	153.4 ± 35.2	116.7 ± 13.6
CS	87.1 ± 18.8[a]	230.7 ± 51.0	104.6 ± 7.5[b]	145.2 ± 37.4
Sucrose	92.3 ± 11.4[a]	187.5 ± 16.3	142.6 ± 7.7	158.2 ± 13.7[b]
Ileum				
Food deprived	89.3 ± 32.5	114.6 ± 18.7[c]	128.8 ± 47.5[a]	51.5 ± 12.5[c]
CS	105.6 ± 16.7[a]	182.4 ± 24.4[b]	108.7 ± 6.2[a,b]	80.8 ± 8.7[c]
Sucrose	55.6 ± 20.5[a]	123.4 ± 22.9[c]	84.2 ± 32.3	65.0 ± 6.4[c]

[1] Values are means ± SD, $n = 4$. Letters indicate significant differences ($P \leq 0.05$): [a]vs. corresponding WT; [b]vs. corresponding food deprived; [c]vs. corresponding jejunum.
[2] U/g protein; 1 U = 1 μmol of glucose released per minute of reaction.

TABLE 3 Twelve-hour calorimetric measurements of Mgam null and WT mice receiving CS or sucrose diets[1]

	Night 1		Day 2		Night 2	
Feeding	Null	WT	Null	WT	Null	WT
RER						
CS	1.02 ± 0.05	0.98 ± 0.07	0.92 ± 0.01	0.84 ± 0.08	1.03 ± 0.03	1.03 ± 0.03
Sucrose[2]	1.07 ± 0.03	1.06 ± 0.03	0.88 ± 0.04	0.88 ± 0.04	1.10 ± 0.04	1.09 ± 0.03
EE, *kJ/(g·h)*						
CS	2.22 ± 0.21	2.34 ± 0.13	2.05 ± 0.21	2.05 ± 0.21	2.34 ± 0.25	2.38 ± 0.17
Sucrose[2]	2.43 ± 0.25	2.38 ± 0.29	2.13 ± 0.13	2.09 ± 0.21	2.55 ± 0.17	2.51 ± 0.21

[1] Values are means ± SD, $n = 8$ (see Supplemental Figs. 1–4).
[2] Significant effect of fed diet ($P \leq 0.05$) was determined by a 3-factor (genotype × diet × day/night) GLM design for each independent RER and EE parameter.

Results

Characterization of starches in experimental diets. Using corn MDx as a reference of starch content (100%), the 2 standard diets (normal Pico 5053 and low-^{13}C 6089) had 25–33% of DW as measurable starch. In contrast, experimental diets (CS 1629 and sucrose 01631) had the expected wide variation in TS content ranging from 0 to 52%. The proportions of RAG and SAG in standard diets were ~60 and 40%, respectively. CS diet and MDx had a considerably higher proportion of RAG ranging from 75 to 90% and the remaining proportion consisted primarily of SAG (Table 1). Due to the presence of invertase in the Englyst assay, 32% of the available glucose derived from the sucrose diet was detected as RAG and the same proportion was detected as SAG. All diets contained <5% of RS. In summary, experimental diets had the expected glucogenic variation necessary for the experimental procedures and standard and experimental diets maintained characteristics of high digestibility in vitro.

α-Glucosidase and sucrase activities of WT and null mice. The α-glucosidase activity of small intestinal mucosa can be modulated by dietary carbohydrates. Therefore, the changes of α-glucosidase enzyme activity as a response to carbohydrate consumption were investigated in 70-wk-old null and WT mice before or after ad libitum consumption of standard and experimental diets for 4 d. Due to the inverted circadian cycle of mice, sampling of baseline small intestine was performed at noon (after 30 h of food deprivation) and the experimental small intestine was sampled at midnight (during the food consumption period).

Jejunal α-glucosidase activity in null mice fed a regular diet followed by 30 h of food deprivation was ~90 U/g, significantly lower than the 180 U/g in WT mice (Table 2). In contrast, although in food-deprived mice, jejunal sucrase activity tended to be ~30% higher in null mice than in WT mice ($P = 0.1$), high within-group variability precluded detecting statistical significance. In ileum of food-deprived null mice, α-glucosidase activity was similar to that in jejunum, whereas in WT mice, ileal activity (115 U/g) was lower than jejunal activity but was ~30% higher than that of null mice (Table 2). Sucrase activity of food-deprived null mice did not differ between the jejunum and ileum; however, in the ileum of WT mice, it was ~50% lower than in jejunum and corresponded to only 40% of the activity in the ileum of null mice ($P = <0.05$) (Table 2). These results agree with those reported previously for the same WT and null mice (1) and are consistent with our earlier observation with genetic Mgam-deficient CBA/CaJ mice (3).

Induction of α-glucosidase and sucrase activities by dietary starch. Feeding WT mice the starch diet for 4 d induced a significant increment in α-glucosidase activities of jejunum and ileum of ~25 and 60%, respectively; however, a significant increment of almost 60% in sucrase activity was observed only for the ileal segment (Table 2). In contrast, the intestinal α-glucosidase activity in either the jejunum or ileum did not increase in null mice fed the CS diet and sucrase activity tended to be lower in the ileal segment ($P = 0.1$) (Table 2).

Feeding mice the control sucrose diet resulted in a much lower response of α-glucosidase and sucrase activities than the feeding the CS diet. A significant response to the sucrose diet was observed only for the jejunal sucrase activity of WT mice (Table 2).

In summary, adaptive responses to carbohydrate consumption of jejunal and ileal activities of null mice significantly differed from those of WT mice (Table 2). Food-deprived null mice had higher sucrase activity than WT mice; the jejunal sucrase activity in null mice was lowered by the CS diet, whereas ileal sucrase activity

TABLE 4 Fasting and prandial blood glucose and insulin responses of Mgam null and WT mice to CS consumption[1]

	Null	WT
Plasma glucose, *mmol/L*		
Food deprived	2.6 ± 1.0	2.9 ± 1.3
CS consumption	8.2 ± 1.6[a]	9.6 ± 3.2
Plasma insulin, *fmol/L*		
Food deprived	0.022 ± 0.036	0.026 ± 0.028
CS consumption	0.043 ± 0.017	0.284 ± 0.138[b]

[1] Values are means ± SD, $n = 4$. Letters indicate significant differences ($P \leq 0.05$): [a]vs. food deprived; [b]vs. all other groups.

TABLE 5 Effect of carbohydrate infusion and Mgam mouse genotype on total blood glucose isotopomer enrichments[1]

	Uninfused,[2] $n = 4$	Null infused, $n = 7$	WT infused, $n = 5$
		mol%	
$E_{i.g.}$ (M+6) 337	0 ± 0	3.12 ± 0.79[a]	5.31 ± 1.14[a]
$E_{i.v.}$ (M+7) 338	0 ± 0	5.54 ± 1.88	6.00 ± 0.59

[1] Values are means ± SD of Penta-acetate-glucose isotopomer MPE. [a]Different between genotypes, $P \leq 0.05$.
[2] Two Mgam null and 2 WT mice on low-^{13}C diet; no differences by genotype.

TABLE 6 Effect of Mgam mouse genotype on steady-state total plasma glucose isotopomer enrichments and homeostasis[1]

Parameter	Null	WT
Total glucose, *mmol/L*	3.73 ± 1.28 (7)	4.84 ± 0.59 (5)
$E_{i.g.}$ (M+6) 337, *mol %*	3.12[a] ± 0.79 (7)	5.31[a] ± 1.14 (5)
$E_{i.v.}$ (M+7) 338, *mol %*	5.54 ± 1.88 (7)	6.00 ± 0.59 (5)
$F_{i.g.}$ (M+6), $\mu mol \cdot kg^{-1} \cdot min^{-1}$	1.81[a] ± 0.18 (6)	1.67[a] ± 0.18 (5)
$F_{i.v.}$ (M+7), $\mu mol \cdot kg^{-1} \cdot min^{-1}$	2.16 ± 0.22 (6)	2.00 ± 0.22 (5)
$Ra_{i.g.}$ (M+6), $\mu mol \cdot kg^{-1} \cdot min^{-1}$	620[a] ± 160 (6)	330[a] ± 90 (5)
$Ra_{i.v.}$ (M+7), $\mu mol \cdot kg^{-1} \cdot min^{-1}$	380 ± 100 (6)	340 ± 70 (5)
Intestinal retention, *%*	37.6[a] ± 5.4 (6)	5.8[a] ± 19.8 (5)

[1] Values are means ± SD, $n = 5$–7. [a]Differences between MPE by genotypes using 2-sample t test ($P \leq 0.05$).

remained unchanged. α-Glucosidase activity of null mice was noninducible by the experimental diets, whereas that in WT mice was significantly induced proximally and distally by the CS diet.

Influence of genotype and experimental diets on energy metabolism. RER and EE measured during 2 nocturnal and 1 intervening light cycle on each diet are summarized (Table 3). Mean RER and EE by genotype, light cycle, and diet are illustrated (**Supplemental Figs. 1–4**). About 85% of consumption activity occurred during the dark cycle. These nocturnal cycles thus represent the in vivo RER and EE prandial responses and the light cycles relative to food-deprived states. We used the GLM test to evaluate the possible differences in RER or EE dependent on genotype or the dark/light cycle. Surprisingly, RER and EE Mgam null and WT mice did not differ during the 2 dark periods of activity and 1 light period of resting. However, the type of diet significantly affected RER and EE values in both WT and null mice; the sucrose diet caused higher RER and EE than the CS diet (Table 1). In summary, whereas consumption and type of carbohydrate significantly modulated prandial RER and EE responses, Mgam genotype, WT or null, did not affect these metabolic characteristics.

Effects of genotype and diet on prandial blood glucose and insulin levels. Given the reductions of mucosal α-glucosidase activity in Mgam null knockout mice and the lack of effect on energy metabolism, it is logical to examine the blood glucose levels of food-deprived and fed mice in detail. We previously reported that Mgam-deficient CBA/CaJ mice had normal postprandial blood glucose concentrations (3); thus, it was important to establish the similarities between Sv/129 Mgam null and CBA/CaJ mice in this respect. WT and null mice had similar concentrations of blood glucose after food deprivation (Table 4). Similarly, in both groups of mice, being fed the CS diet induced a significant increase of prandial blood glucose concentration; however, the 2 groups did not differ in the attained concentration of glucose (Table 4). In summary, the prandial blood glucose responses were independent of the mucosal α-glucosidase activities. These results confirmed the conserved energy metabolism in the null mice and revealed homology to CBA/CaJ mouse prandial blood glucose responses.

We measured serum insulin levels to obtain information about the hormonal control of glucose metabolism in null mice compared with WT mice (Table 4). WT mice had a blood insulin (serum insulin in Table 4 and plasma insulin in Table 6) response with a mean concentration of 0.284 ± 0.138 fmol/L after being fed the CS diet. In contrast, null mice had a blood insulin concentration of 0.043 ± 0.017 fmol/L after being fed the CS diet, significantly lower than that of WT mice. In summary, null mice did not have an insulin response to CS consumption.

Effect of genotype on exogenous glucogenesis and endogenous glucose fluxes. In a previous publication, we reported that in vivo starch digestion, as measured by enrichment of $^{13}CO_2$ in breath after an i.g. bolus of ^{13}C-LDx, was reduced by 40% in null mice (1). To trace the fate of glucose produced from digestion of ^{13}C-LDx and its contribution to blood glucose concentration in WT and null mice, we used an i.g. infusion protocol and sampled steady-state total and isotopomer-specific blood glucose (M+6) enrichment after 4 h of constant infusion. Constant i.v. infusion of a different glucose isotopomer (M+7) was added to measure total glucose flux (Table 5). Baseline isotopomer concentrations were determined in uninfused mice. Final total blood glucose concentrations reached after the infusion procedures were not significantly different between WT and null mice (Tables 5 and 6); similarly, plasma glucose enrichments of the i.v.-infused M+7 did not differ between null and WT mice. In contrast with the above observations, the M+6 glucose enrichment in null mice derived from digestion of i.g.-infused LDx was 40% lower than in WT mice. The assay of plasma insulin revealed that variation was greatly reduced after 4 h of constant infusion and that its concentration in null mice was 0.031 ± 0.005 fmol/L, whereas in WT mice, it was significantly higher at 0.062 ± 0.017 fmol/L. These infused mouse plasma insulin concentrations were within the range observed in food-deprived mice (Tables 3 and 5). In summary, whereas glucose concentration and flux remained unchanged in null mice compared with WT mice, the fraction of glucose flux arising from digestion of LDx was reduced by 40% and insulin levels were significantly lower. In addition, 38% of the LDx-infused i.g. in null mice remained undigested, sequestered in the small intestine, and did not appear as blood glucose This confirmed and explained the reduction in $^{13}CO_2$ production from ingested LDx reported in our previous work (1).

Discussion

In our previous article on the features of Mgam null mice (1), we showed that intestinal α-glucosidase activity, measured with MDx as substrate, is substantially lower compared with WT mice. In this article, we have corroborated that observation and found that in null mice, this activity is not inducible after 4 d of starch consumption, whereas in WT mice, the activity increased by 25% with the same dietary regimen. We also observed previously that the enzyme activities of Si are upregulated in null mice (1). Here we confirmed that observation and found that whereas in the ileum of WT mice, sucrase activity and Mgam activity is inducible by starch consumption, these activities in null mice cannot be further upregulated but, instead, can suffer some degree of suppression, particularly in the jejunal segment. The mechanisms involved in the regulation of Si and Mgam activities appeared to be complex, substrate-specific, and different along the intestinal longitudinal axis, because differences in the inductive capacity of the CS and sucrose diets were observed among groups of mice and among the jejunal and ileal segments of the intestine. We also found that that Mgam null and WT mice did not differ in concentration of total circulating blood glucose and its response to feeding carbohydrate-rich diets, which agreed with our original observation obtained in CBA/CaJ mice (3).

Effect of Mgam ablation and diet on energy metabolism. It was expected that a deficiency in activity of intestinal Mgam would lead to a deficiency in energy intake derived from starch digestion. After food deprivation, this energy intake deficiency could be responsible for the lower production of starch-derived respiratory CO_2 in our previous article (1) and could influence the EE of the affected mice. Thus, one of our hypotheses was that Mgam null mice could have altered respiratory and energetic management compared with normal WT mice. Such a concept has been observed in humans, where respiratory parameters and energy management were adapted or conditioned upon training, displaying substantial differences from untrained subjects after food deprivation and low intensity exercise (19). The complexity of the starch digestion led us to use the selected diets (Table 1) with the expectancy of enhancing any possible difference in respiratory and EE metabolism. Importantly, none of these diets caused differences in RER or EE of the Mgam null mice from the WT mice (Table 3), suggesting that the overall energetic management and respiratory efficiency was not significantly modified in Mgam null mice. Thus, the previous finding of a lower CO_2 production from exogenous starch could only be ascribed to a decreased availability of exogenous fuels for EE.

Role of Mgam on hepatic glucose production. The apparent paradox between the low mucosal α-glucoamylase activity of Mgam null mice and their normal prandial blood glucose levels and RER (Tables 2–4) was reconciled by the i.g. and i.v. infusion studies (Tables 5 and 6). Intestinal glucose flux from starch was measured by digestion of i.g.-infused ^{13}C-LDx to blood glucose and total glucose flux measured by i.v.-infused $^{2}H_7$ glucose. Although, during these experiments, infusions did not substantially perturb total blood glucose concentration in null or WT mice, enrichment of the intestinally derived ^{13}C-glucose (^{13}C-glucose enrichment) was markedly lower in null mice than in WT mice, demonstrating a diminished contribution of starch-derived glucose to the total circulating glucose pool in these mice. These differences reflected the ability of each group of mice to digest LDx, because in WT mice expressing both Mgam and Si α-glucosidases there was a virtually complete digestion of LDx with intestinal retention of -0.06 ± 0.2 (Table 6) and 89% of the i.g.-infused ^{13}C- found as blood circulating glucose. This is consistent with reports that >90% of $F_{i.g.}$ glucose appears in the circulation (5,17). In contrast, in Mgam null mice with only Si α-glucosidase, there was an intestinal retention of 0.38 ± 0.05 ($P = 0.009$) and only 48% of the LDx was observed as circulating glucose. Thus, the most probable source of the glucose necessary to preserve the normal blood glucose concentration was gluconeogenesis, because the infused mice were food deprived for 30 h to deplete glycogen (20) and thus the contribution of glycogenolysis was probably insignificant.

Assuming that total blood glucose results from the contribution of intestinal glucose input and hepatic gluconeogenesis, the experiments suggested that Mgam plays a crucial role in regulation of prandial glycemia through a reciprocal interaction with hepatic gluconeogenesis sufficient to maintain prandial glucose homeostasis (4,21).

What are the mechanisms for the regulation of hepatic gluconeogenesis? The regulatory function of insulin on gluconeogenesis and the molecular mechanisms involved have been widely studied (22). The marked differences in prandial serum insulin between WT and null mice suggests that the rapid Mgam α-glucogenic activity releasing relatively large amounts of absorbable glucose triggered the release of the hormone suppressing gluconeogenesis; in contrast, slow α-glucogenesis by Si cannot trigger the release of insulin and suppression of hepatic gluconeogenesis. The low concentration of insulin in starch-infused null mice supports this conclusion. An additional consideration is that, despite the different abilities to generate prandial glucose in WT and Mgam null mice, the prandial blood glucose flux, instead of differing between groups, was identical for WT and null mice. This suggests that normal prandial blood glucose concentration (normoglycemia) is a set point determining the activation or inactivation of synthetic/degradative metabolic pathways for glucose. Changes by adaptation of this set point could lead to changes in the status of glucose metabolism. Our conclusion is that Mgam activity, by regulation of exogenous glucogenesis, is crucial for complementary regulation of endogenous gluconeogenesis and prandial glucose homeostasis.

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