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High Endogenous Salivary Amylase Activity Is Associated with Improved Glycemic Homeostasis following Starch Ingestion in Adults[1–3]

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Abstract

In the current study, we determined whether increased digestion of starch by high salivary amylase concentrations predicted postprandial blood glucose following starch ingestion. Healthy, nonobese individuals were prescreened for salivary amylase activity and classified as high (HA) or low amylase (LA) if their activity levels per minute fell 1 SD higher or lower than the group mean, respectively. Fasting HA (n = 7) and LA (n = 7) individuals participated in 2 sessions during which they ingested either a starch (experimental) or glucose solution (control) on separate days. Blood samples were collected before, during, and after the participants drank each solution. The samples were analyzed for plasma glucose and insulin concentrations as well as diploid *AMY1* gene copy number. HA individuals had significantly more *AMY1* gene copies within their genomes than did the LA individuals. We found that following starch ingestion, HA individuals had significantly lower postprandial blood glucose concentrations at 45, 60, and 75 min, as well as significantly lower AUC and peak blood glucose concentrations than the LA individuals. Plasma insulin concentrations in the HA group were significantly higher than baseline early in the testing session, whereas insulin concentrations in the LA group did not increase at this time. Following ingestion of the glucose solution, however, blood glucose and insulin concentrations did not differ between the groups. These observations are interpreted to suggest that HA individuals may be better adapted to ingest starches, whereas LA individuals may be at greater risk for insulin resistance and diabetes if chronically ingesting starch-rich diets. J. Nutr. 142: 853–858, 2012.

Introduction

Saliva plays a vital role in maintaining the health of the oral cavity and gastrointestinal tract by aiding in lubrication, inhibiting potentially harmful microbes, and promoting oral tissue healing (1). Whether saliva also plays an important role in the digestion and metabolism of food is currently unknown. The presence of high concentrations of the enzyme α-amylase, however, has led to the hypothesis that saliva could be important for the digestion of complex carbohydrates (2–4).

Amylase is a digestive enzyme produced by the salivary glands and pancreas that cleaves the glycosidic linkages in starch molecules to produce smaller saccharides, such as maltotriose, maltose, and small amounts of glucose (5). Salivary amylase can account for up to 50% of total salivary protein in some individuals (6), whereas others produce barely detectable concentrations. Such substantial variation in amylase production is due to both environmental [e.g., stress (7)] and genetic factors, such as copy number variation (CNV)[6] in *AMY1*, the gene that codes for salivary amylase. Copy number is positively correlated with salivary amylase concentrations (8,9). Individuals can carry anywhere from 1 to 15 diploid copies of the *AMY1* gene in their genome.

Salivary amylase has been extensively studied since its discovery almost 200 y ago (10). Nevertheless, the fundamental question of whether the enzyme contributes to overall starch digestion and metabolism remains unanswered. Because food is only in the mouth for a few seconds, oral amylolytic "predigestion" is often assumed to be of minimal importance, particularly given the presence of pancreatic amylase within the gastrointestinal tract. However, there are hints that salivary amylase could be of practical and clinical importance. For example, we know that considerable starch hydrolysis occurs within seconds in the oral cavity (11) and can also continue after swallowing, because partially digested starch protects salivary amylase from acid inactivation (12). In vivo digestion studies demonstrate that delivery of starch directly into the small intestine, thereby skipping the oral digestion stage, results in substantially less starch digestion and glucose absorption (13). In addition, postprandial blood glucose concentrations following ingestion of starchy foods, such as rice and potatoes, are lower when the food is swallowed whole, rather than chewed first, mixed with saliva, and then swallowed (14).

Recent evidence suggests that populations who historically relied on starch for dietary energy have higher copy numbers of the

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[3] Supplemental Figures 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[6] Abbreviations used: CNV, copy number variation; HA, high amylase group; LA, low amylase group; PIR, preabsorptive insulin release.
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AMY1 gene, with correspondingly higher concentrations of salivary amylase, than populations who consumed a high-protein diet (8). CNV of *AMY1* may have evolved independently in diverse populations across the globe (8). This suggests that evolutionary nutritional pressures increased the number of *AMY1* copies in select human populations, thereby facilitating the digestion and metabolism of starch. In contrast, pancreatic amylase, produced by the gene *AMY2*, has not undergone similar genetic repetition (15) even though the vast majority of starch digestion occurs in the small intestine via pancreatic amylase (16). These observations collectively suggest that salivary amylase plays a critical role in the metabolism of complex carbohydrates.

Salivary amylase enables rapid cleavage of starch glycosidic linkages to produce smaller saccharides (5). We therefore surmised that individuals who produce more salivary amylase (group HA) would have faster and more substantial postprandial blood glucose responses following starch ingestion, due to more rapid starch breakdown, than individuals who produce less salivary amylase (group LA). We utilized a glucose solution, equivalent in energy to the starch solution, as a negative control. Because salivary amylase plays no role in glucose digestion, the 2 amylase groups should not differ in postprandial response.

Participants and Methods

Participant selection. Adult volunteers were recruited from the surrounding area of Philadelphia and were of mixed ethnicity. Individuals initially underwent a screening by phone or email to assess eligibility; they were asked about height and weight, medical history, and cigarette use. Individuals with a BMI <25 kg/m^2 who reported no illness nor use of cigarettes or medications known to affect salivary flow were invited to participate further. Height and weight were verified in the laboratory and participants (n = 48) were asked to provide a timed, stimulated saliva sample, which was analyzed for salivary amylase activity and flow rate. Participants were classified as either high or low amylase producers if their enzyme concentrations per minute (as calculated by salivary flow rate) fell 1 SD higher or lower than the group mean, respectively. Ten high amylase (HA) and 9 low amylase (LA) individuals (14 female, 5 male) participated in this study. Procedures were approved in accordance with the ethical standards of the Office of Regulatory Affairs at the University of Pennsylvania and all participants gave informed consent for participation on an approved form.

Experimental protocol. Participants visited the laboratory for 2 separate morning sessions and had no food or beverages other than water since midnight of the previous night. The 2 sessions were at the same time on each day. Each individual participated in the experimental condition in which they consumed 50 g (10% solution) of a corn starch hydrolysate solution (M40; Grain Processing Corporation) and the control condition, in which they ingested 50 g (10% solution) of a glucose solution (Sigma Aldrich). The 2 solutions were equal in terms of energy provided. The starch hydrolysate was used in order to have a solution that did not noticeably differ in viscosity from the glucose solution. The glucose solution was prepared 24 h in advance to allow for complete mutarotation of the glucose tautomers. Participants were instructed to drink each solution at a constant rate over the course of 20 min and their rate of intake was monitored and timed. They were also instructed to swish every sip of solution around their mouth "like they would for mouthwash" for ~5 s before swallowing in order to fully mix the solution with saliva.

Before consuming the solutions, each individual gave a stimulated, whole saliva sample by chewing on a 4-cm square of parafilm for 90 s and expectorating into a 15-mL polypropylene tube. The tube was weighed before and after sample collection to calculate salivary flow rate (mL/min). The tube was vortexed, centrifuged at 2000 $\times$ g at 4°C for 10 min, and the saliva aliquoted and frozen at −80°C for future analysis.

For blood sampling, a butterfly needle was inserted into an antecubital vein and secured to the arm for the full duration of the study by a certified phlebotomist. All blood samples were collected into EDTA-coated tubes. Baseline blood samples were collected at −5 and 0 min. Following collection of the second baseline, the participant started to drink the carbohydrate solution. Blood samples were obtained every 3 min for the first 15 min and then every 15 min up to 2 h. The line was flushed with saline between samples to prevent clogging. Samples were immediately centrifuged and the plasma was aliquoted and frozen at −80°C.

For genotyping, ~5 mL of blood was collected from each individual into a tube coated with EDTA to prevent coagulation. The tubes were inverted gently 10 times and then frozen at −80°C for future use.

FFQ. To assess dietary intake, participants completed a computerized Block 2005 FFQ (NutritionQuest). This validated questionnaire estimates the usual intake for a wide variety of foods and provides an analysis of overall carbohydrate, protein, and fat intakes. The data were also specifically analyzed for intake of starch-rich foods, including pasta, rice, bread, potatoes, etc. An intake frequency $\times$ quantity score was calculated for each food and the scores summed (FxQ Starchy Food Intake Score) to determine overall intake of starch-rich foods for each individual.

Biochemical measures. Plasma glucose was analyzed in duplicate by a glucose oxidase method using a 2300 STAT Plus laboratory glucometer (YSI). Plasma insulin was analyzed in duplicate using a commercially available human insulin-specific RIA (HI-14K; Millipore). The assay was performed by the Diabetes Research Center of the University of Pennsylvania. Technicians were unaware of the conditions of the experiment.

Enzymatic activity assay for salivary amylase. Upon thawing, saliva samples were centrifuged once more to ensure that solids were removed from suspension. Salivary amylase activity was determined using a chromogenic kinetic reaction assay kit (1–1902; Salimetrics), according to a previously described method (9).

qPCR for the AMY1 gene. DNA was extracted from whole blood using the Gentra PureGene DNA extraction kit (Qiagen) and quantitated using a NanoDrop 2000C (Thermo Scientific). The diploid *AMY1* gene copy number was determined using a Taqman Copy Number Assay for *AMY1* (Assay ID Hs07226362_cn; Applied Biosystems),with a standard curve constructed from a reference DNA sample (NA18972; Coriell), as previously described (9).

Data analysis. Participants were excluded from analysis if they exhibited resting blood glucose >6.1 mmol/L, resting insulin concentrations >140 pmol/L, or a peak blood glucose or insulin concentration more than twice the group mean on either study day.

Statistical analyses were performed using Statistica 9.0 software (Statsoft). Relationships between data sets were analyzed using the Pearson correlation coefficient. To determine between-group and between-treatment effects, incremental AUC was calculated for blood glucose and insulin as net change from baseline concentration (mean of 2 baseline samples) using the trapezoidal method. Glycemic index values were calculated as (starch AUC/glucose AUC) $\times$ 100. AUC values were compared using t tests. Peak blood glucose and insulin concentrations and other biological variables (age, BMI, salivary flow, amylase concentrations, and dietary intake) were also compared using t tests. CNV medians were compared using the nonparametric Mann-Whitney U test. Additionally, repeated-measures ANOVA was used to determine whether there were significant differences between the 2 groups or treatments during the blood sampling period. Repeated-measures ANOVA was also used to determine if there were significant differences within participants between baseline plasma glucose or insulin concentrations and subsequent measurements. For both tests, Tukey's HSD post hoc pairwise analysis was used when significant interactions were found to determine which individual time points were significantly different from each other.

A 2-tailed $P < 0.05$ was considered significant. All results are presented as mean ± SE.

Results

HA and LA groups. Five individuals were removed from the analysis based on the exclusion criteria described in the

"Methods." This exclusion left 7 participants in the HA group and 7 in the LA group (**Table 1**). The groups did not significantly differ in age or BMI. The salivary flow rate for the entire group (mean of 2 study days) was 1.58 ± 0.25 mL/min. The amylase concentration was 120 ± 24 kU/L and the amylase activity level was 202 ± 50 U/min. The HA group had greater salivary flow rate ($P < 0.05$) and amylase levels in terms of both concentration ($P < 0.05$) and rate ($P < 0.01$). This result confirms that the groups were properly sorted by amylase concentrations. Enzyme concentrations were analyzed by amount and activity to ensure that the difference between the groups was not simply due to differences in salivary flow rate. There was a positive relationship between an individual's amylase concentration (mean of the 2 study days) and their number of *AMY1* gene copies ($r = 0.90$; $P < 0.0001$) (**Supplemental Fig. 1**). The HA group had more *AMY1* gene copies than the LA group ($P < 0.05$) (Table 1).

Plasma glucose and insulin responses following carbohydrate ingestion. Following starch ingestion, plasma glucose concentrations differed over time between the 2 groups ($P < 0.01$). Specifically, the HA group had lower postprandial glycemic responses at 45 ($P < 0.01$), 60 ($P < 0.001$), and 75 ($P < 0.01$) min (**Fig. 1**). The HA group also had lower incremental AUC (89 ± 21 vs. 244 ± 55 mmol/L · 120 min; $P < 0.05$) and peak blood glucose concentrations (9.56 ± 0.43 vs. 7.57 ± 0.35 mmol/L; $P < 0.01$) than the LA group. The 2 groups did not differ in their resting blood glucose concentrations (LA = 5.02 ± 0.13 mmol/L; HA = 4.99 ± 0.18 mmol/L). In both groups, blood glucose concentrations had risen above each group baseline within 15 min ($P < 0.05$).

Plasma insulin concentrations following starch ingestion did not significantly differ at any time point between the HA and LA groups when the curves were analyzed over the entire testing session (**Fig. 2***A*). Because differences between low insulin concentrations during the preabsorptive period (before glucose absorption begins) may be masked by high concentrations later in the session, insulin concentrations for the first 9 min of the testing session were analyzed separately. The 2 groups differed during this period ($P < 0.05$). The HA group had higher insulin concentrations at 9 min compared to their group baseline ($P < 0.01$), whereas insulin concentrations for the LA group did not increase above their baseline at this time (Fig. 2*B*). The HA group also had higher insulin AUC values than the LA group for the 0 to 9-min period (144 ± 71.8 vs. −76.9 ± 20.9 pmol/L · 9 min; $P < 0.01$). There was a positive correlation between insulin production for the 0 to 9-min period (AUC) and the amount of oral amylase produced per minute ($r = 0.70$; $P < 0.01$) (**Supplemental Fig. 2**).

TABLE 1 Biological characteristics and dietary starch intake of healthy adult participants by salivary amylase activity[1]

	LA group	HA group	*P* value[2]
Age, *y*	23.4 ± 0.7	27.3 ± 2.6	0.18
BMI, *kg/m²*	21.4 ± 0.8	21.8 ± 0.5	0.67
Salivary flow rate,[3] *mL/min*	1.03 ± 0.23	2.13 ± 0.33	0.02
Salivary amylase,[3]			
kU/L	64 ± 12	176 ± 36	0.01
U/min	64 ± 16	341 ± 66	0.002
CNV[4]	4 (2–4)	6 (4–11)	0.01
Carbohydrate intake, %	50 ± 9.7	46.6 ± 7.9	0.43
FxQ intake score[5]	163 ± 48	192 ± 46	0.30

[1] Values are mean ± SE or median (range), $n = 7$ (5 females, 2 males). CNV, copy number variation; HA, high amylase group; LA, low amylase group.
[2] Data were compared using *t* tests.
[3] Mean of 2 study days.
[4] Data were compared using the nonparametric Mann-Whitney U test.
[5] Frequency × quantity score for intake of starchy foods, calculated from FFQ data.

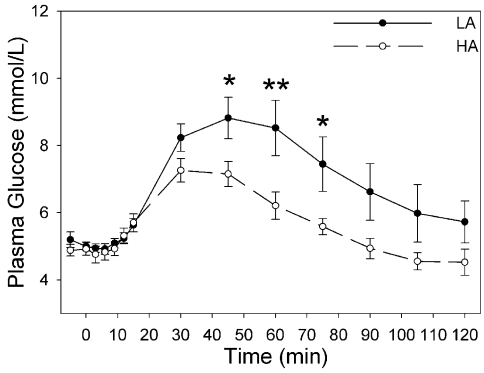

FIGURE 1 Postprandial plasma glucose concentrations in healthy, normal-weight adults by salivary amylase activity after ingestion of a 50-g starch solution. *Values are mean ± SE, $n = 7$. Asterisks indicate different from HA: *$P < 0.01$; ** $P < 0.001$. HA, high amylase group; LA, low amylase group.

Glycemic responses following ingestion of the control glucose load did not differ between the amylase groups at any time point (**Fig. 3***A*), nor were there differences in AUC or peak blood glucose concentrations (data not shown). Furthermore, plasma insulin response did not differ between the 2 groups either overall or in the first 9 min (Fig. 3*B*). Notably, both groups had insulin concentrations higher than baseline within 9 min, indicating that both groups were capable of preabsorptive insulin responses to the glucose solution (HA, $P < 0.01$; LA, $P < 0.05$) (Fig. 3*C*).

Within-participant comparisons. Blood glucose concentrations following starch and glucose ingestion did not differ within each group (**Supplemental Fig. 3**). However, the LA group had a larger AUC following starch ingestion (244 ± 55 mmol/L · 120 min) compared to the glucose load condition (152 ± 48 mmol/L · 120 min) (not shown; $P < 0.005$). Accordingly, the LA group (111 ± 7) had a significantly higher glycemic index for the starch solution than the HA group (94 ± 3) ($P < 0.05$).

Dietary intake of carbohydrates. Analysis of the FFQ data for each participant demonstrated that the groups did not significantly differ in terms of overall carbohydrate intake or intake of high-starch foods (Table 1).

Discussion

In the current study, we tested whether high salivary amylase concentrations altered blood glucose responses following starch ingestion. We hypothesized that because starch is cleaved into simple sugars by salivary amylase, people possessing high salivary amylase concentrations (group HA) might thus be expected to have higher postprandial blood glucose following starch ingestion relative to participants with lower salivary amylase concentrations (group LA). Instead, we found the opposite occurred: compared with LA individuals, HA individuals had significantly lower postprandial blood glucose responses following starch ingestion. This difference was apparently mediated by the increased plasma insulin concentrations in the HA

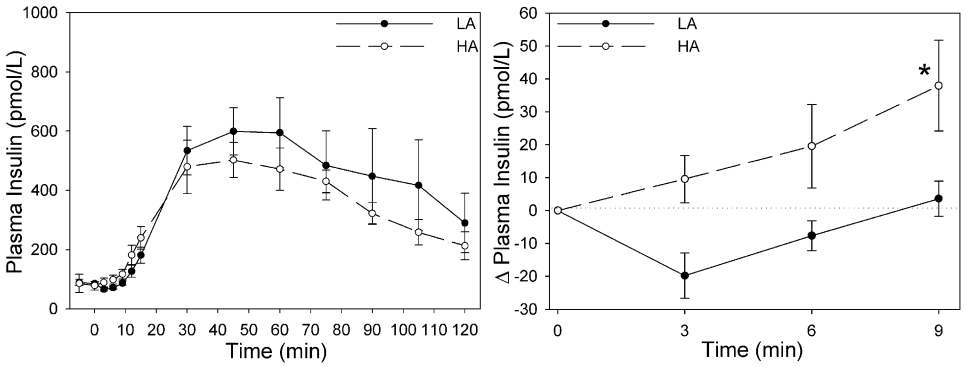

FIGURE 2 Postprandial plasma insulin concentrations in normal-weight individuals by salivary amylase activity after consumption of a 50-g starch solution over the entire testing session (*A*) and during the preabsorptive period (0–9 min) (*B*). For *B*, each group was compared against their own baseline. The data are portrayed as change from baseline (Δ) in order to highlight the differences between the groups. Values are mean ± SE, $n = 7$. *Significantly different from baseline, $P < 0.01$. HA, high amylase group; LA, low amylase group.

group observed early in the testing session. Nevertheless, both groups had similar plasma glucose and insulin responses following glucose ingestion. Thus, it is unlikely that group differences were due to innate differences either in their ability to produce insulin or in their capacity for insulin-mediated glucose disposal.

Plasma glucose concentrations following starch ingestion did not begin to rise in either group until 15 min into the session and, therefore, the early insulin release described above can be termed preabsorptive (occurring during the preintestinal absorption period). It has been known since the work of Ivan Pavlov more than 100 y ago that the flavor of food or food ingestion can stimulate anticipatory digestive and metabolic responses, prior to nutrient absorption, that result in the increased secretion of saliva (17), gastric acid (17,18), and pancreatic secretions (17,19). Such responses presumably prepare the digestive system to digest food, as well as absorb and metabolize nutrients (20). This strategy increases the efficiency of digestion and metabolism and also enables better maintenance of homeostasis (20,21).

Preabsorptive insulin release, also known as cephalic phase insulin release (PIR) is one such anticipatory response to eating (22). Though it is a relatively minor component of total insulin secretion, PIR is an extremely important determinant of overall glucose tolerance (23). Studies in both laboratory animals and humans have demonstrated that loss of this response leads to impaired glucose tolerance (24,25). For example, i.g. administration of glucose in rats, which bypasses the oral cavity, leads to delayed insulin release and much higher blood glucose concentrations than when the same amount of glucose is orally ingested (24). Similarly, the LA group in the current study did not exhibit PIR in response to starch and consequently had a higher glycemic response. After ingesting the glucose solution, however, both groups exhibited PIR, which indicates that such a response can be elicited in the LA group.

Though the specific process by which salivary amylase stimulates PIR and affects glucose homeostasis remains unclear, we offer several possibilities. One possibility is that the production of glucose and/or maltose through amylolytic activity in the oral cavity signals the body to prepare for incoming starch and the ensuing glucose. The sugars would bind lingual T1R2-T1R3 sweet taste receptors (26) and/or glucose transporters in taste receptor cells (27). Because the amount of glucose produced by salivary amylase is too low to be consciously tasted and maltose is only weakly sweet tasting, the stimulation of these taste receptors would not be expected to activate perceptible sweet taste (28). Second, the mechanism may also involve binding of short-chain oligosaccharides by the putative polysaccharide receptor, hypothesized to enable identification of starch-rich foods (29). Finally, it is also possible that hormones or incretins (e.g., glucagon-like peptide-1) are peripherally released by lingual taste cells into the blood stream in response to carbohydrates, stimulating insulin release from the pancreas during the PIR period.

With the advent of agriculture and the domestication of cereals such as barley, wheat, maize, and rice, the reliance on starches for dietary energy dramatically increased in many regions of the world. Evolutionarily, increased *AMY1* copy number and salivary amylase concentrations would provide a considerable nutritional advantage following this dietary change. Efficient starch digestion would have been of immense benefit, providing rapid replenishment of blood glucose following periods of intense energy expenditure, such as during farming, active hunting, or episodes of lower gastrointestinal malaise or toxicosis.

In today's society, starches contribute over one-half of the total carbohydrate energy consumed in the US (30). More than 85% of these starches are highly processed and refined (31), similar to the starch solution in the current study. "Dietary globalization" has led to widespread availability of these highly refined, starch-rich foods and therefore it is perhaps not surprising that we did not find any differences in carbohydrate intake between the HA and LA groups. However, although these 2 groups eat similar foods, our data suggest that they experience different glycemic responses to them. This has potential implications for the calculation of glycemic indices for starch-rich foods because the current method does not take into account individual differences in starch digestion. It may, therefore, be necessary to calculate different glycemic indices for individuals with different amylase concentrations.

The imbalance between genetic background and evolutionary optimized diet may also have potential implications for the development of noninsulin dependent diabetes and obesity. The reasons why some individuals develop these conditions while others do not are not currently understood. In light of our current findings, we suggest that *AMY1* gene copy number may play a role in the development of insulin resistance and diabetes. Both high and low amylase individuals in this study were young and healthy, with a mean BMI <22 kg/m^2, yet the groups had different glycemic responses following starch ingestion. Although overall insulin concentrations did not differ between the groups, it is possible that chronic high blood glucose concentrations induced by high starch intake may elicit a number of hormonal, receptor, and physiological changes that will

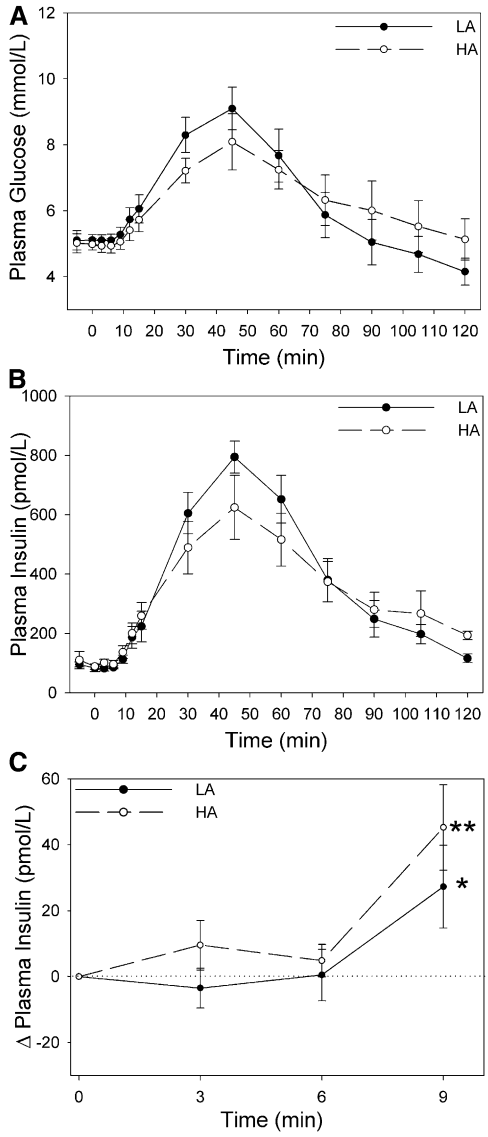

FIGURE 3 Postprandial plasma glucose (*A*) and insulin (*B,C*) concentrations after consumption of a 50-g glucose solution. For *C*, each group was compared against their own baseline. The data are portrayed as change from baseline (Δ). Values are mean ± SE, $n = 7$. Asterisks indicate significantly different from baseline: $*P < 0.05$, $**P < 0.01$. HA, high amylase group; LA, low amylase group.

eventually result in the development of insulin resistance and diabetes. We suggest that it may, therefore, be useful to begin testing individuals for low *AMY1* gene copy number and salivary amylase concentrations to help assess risk for these conditions.

One potential limitation of this study was our use of a liquid starch hydrolysate solution for our experimental condition. A previous study involving the mastication of more complex starch-rich foods found that blood glucose concentrations were higher if the food was first chewed and then swallowed rather than swallowed whole (14). It will be necessary to verify our findings with future studies of more complex starch-rich foods.

To our knowledge, this is the first report demonstrating that salivary amylase interacts with certain ingested complex carbohydrates to affect insulin and blood glucose concentrations. This research provides a possible explanation for the benefits of the oral predigestion of starch as well as the benefits of high *AMY1* gene copy number and salivary amylase production. Our results indicate that individual differences in salivary amylase may considerably contribute to overall nutritional status.

Acknowledgments

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