Relationship of Starchy Snack Consumption with Salivary Amylase Activity, Measured via the “Pudding Assay”

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ABSTRACT
People vary dramatically in salivary amylase, which alters the oral texture of and chewing requirements for foods. Salivary amylase might also influence how quickly glucose is released early in the small intestines. Our research study is investigating whether dietary intake of starch causes changes in salivary amylase activity. Here, we present the initial data on a unique assay, which can be adapted remotely, to determine salivary amylase activity and its relationship to dietary modifications. Participants were given kits that included the saliva testing supplies and snacking items for one week. Two different sets of snacks were provided: one set higher in starch content and the other set lower in starch content. The kit also included a guide and survey link for the salivary amylase activity test—the “Pudding Assay.” For this assay, participants chewed wax for 30s, collecting all saliva generated. Volume of saliva was measured, and then the saliva was added to a container of starch-thickened pudding. The viscosity of the pudding/saliva mixture was measured by observing its flow rate through a 10mL syringe, a technique adapted from the International Dysphagia Diet Standardization Initiative. Measurements of the pudding/saliva mixtures’ viscosity were taken at 40s and 240s after adding saliva to the pudding. Thinner, less viscous pudding/saliva mixtures flow through the syringe more quickly and indicate higher salivary amylase activity. Here, we will present the initial findings from our first wave of participants, observing whether the dietary additions influenced their salivary amylase activity as measured through the Pudding Assay.

METHODS
An overview of the study is shown in figure 1. Participants were recruited and completed 3 days of diet records (to be analyzed later). Participants were randomly assigned to either consume the lower starch snacks the first intervention week, and the higher starch snack the second intervention week, or vice versa. At the beginning and end of each intervention week, we conducted taste and smell acuity tests, sensory ratings of a variety of snacks used in the study, and the Pudding Assay. For this presentation, we focus on the Pudding Assay for our first 12 participants. Other data will be analyzed as more participants complete the study.

The Pudding Assay
Saliva is collected while chewing wax for 30 seconds. Volume of saliva is measured.

Figure 2: Saliva collected into a cuvette during experiment.

Figure 3: All saliva mixed with Pudding (Snack Pack, Chocolate flavor, upper left image). Pudding and saliva is stirred. At 40s and again 240s, 10mL of pudding/saliva mixture is extracted from the cup using a 10mL disposable syringe (right image).

Figure 4: Old syringe is used to fill new syringe (no plunger on new syringe, fill from top with old syringe). A finger is kept at the opening of the new syringe to prevent mixture from leaking out.

Figure 5: Finger is removed from the bottom of the newly filled syringe (still without plunger). Gravity forces the pudding/saliva through the tip. Finger is replaced over the type of the syringe at 10s, and the amount of Pudding/Saliva mixture remaining in the syringe is recorded.

RESULTS
Analysis
We used linear mixed models with subjects as a repeated measure in SAS OnDemand through Jupyter Lab. We analyzed whether the amount of pudding remaining in the syringe was related to: which snack week the participant was on ("Intervention"), whether it was the beginning or the end of the week ("Visit"), or the saliva volume collected from that participant (Model 1). We also analyzed whether the difference in amount of pudding remaining in the syringe at 40s compared to 240s changed due to these factors (Model 2).

Model 1 analysis indicated:
- Saliva volume had a negative effect on amount of pudding remaining. (p<0.013). This means people who had higher salivary flow, and thus more saliva to add to the pudding, had less pudding left in the syringe.
- No other effects were significant. In particular, there is no effect looking at the baseline to end of the week. So in Figure 6, none of the pairs of light/dark boxes are significantly different.

Model 2 analysis indicated:
- Greater change from 40s to 240s at the end of either intervention type compared to the beginning. (p=0.0036 overall effect, specific effect for each intervention type shown in Figure 7).
- No more effect of saliva volume.

INTERPRETATION, FUTURE DIRECTIONS
While Model 1 showed no effect, this could be due to small sample size (12 participants compete). As more people finish the study, we might see significant effects of the interventions on pudding flow rate/saliva amylase activity comparing the baseline to end visits.

Model 2 indicates a change in rate may occur due to our interventions (both high or low starch). While we expected the high starch to induce greater changes in saliva amylase activity, its possible our low starch intervention is causing effects too. The foods for the high starch week are high in starch but easier to chew (oat bars), while the foods for the low starch week are low in starch but harder (almond bars). Further analysis of the sensory data from these experiments, which asks about texture of the snack products, may help shed light on why both the high and low starch intervention may be influencing saliva amylase.

Overall, the Pudding Assay shows great potential to allow remote participants to assess salivary amylase activity, which could be applied in many other research settings or study designs.

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