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Research Article

Dipeptidyl Peptidase-IV Activity and Neuropeptide Y in the Mouth: The Relationship with Body Composition and the Impact of Sucrose and Aspartame

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Abstract

Dipeptidyl peptidase-IV (DPP-IV) is an enzyme present in the saliva, and its interaction with satiety related hormones is poorly understood. The purpose of this study was to determine possible relationships between salivary DPP-IV activity and body composition. Further, the response of salivary DPP-IV activity to carbohydrates was investigated. We tested 111 people for plasma and saliva DPP-IV activity using a fluorometric assay and Neuropeptide Y₁₋₃₆ (NPY) protein using an EIA. Body composition was determined via a DEXA scan. Relationships were determined using regression analysis. In the second part, we tested 28 people on four separate occasions, where the participants either swished and spit or ingested a commercially available sucrose or aspartame sweetened beverage. Saliva and plasma were collected before and after each condition and were processed as described above. Blood glucose was also measured. No relationship was found between salivary DPP-IV activity or NPY₁₋₃₆ and any body composition measurement. For Part 2, no change in plasma DPP-IV occurred with any of the conditions, despite an increase in blood glucose with the sucrose-beverage ingestion condition ($p < 0.05$). However, salivary DPP-IV activity was attenuated with all conditions, except aspartame-beverage swish and spit. Salivary NPY₁₋₃₆ was not altered by the conditions. The unique finding from this study was that salivary DPP-IV activity was attenuated with sucrose or aspartame beverage, but plasma DPP-IV was unchanged. This result implies that satiety may be reduced when drinking sucrose or aspartame beverages.

Introduction

Dipeptidyl peptidase-IV (DPP-IV) is a widely expressed serine protease that alters the function a variety of peptides including neuropeptide Y, PYY, and glucagon-like peptide 1 (GLP-1) by cleaving the

N-terminal dipeptide [1]. DPP-IV is present in the blood and as a component of saliva in the mouth. Two possible sources of DPP-IV in the mouth are exosomes released by the salivary glands [2,3] and/or by transport from capillaries or secretion into the gingival cervical fluid [4]. Although controversial, some research posits that changes in proteins in the saliva may mirror the blood [5-8]. As a result, salivary DPP-IV may, in part, mirror the plasma activity levels

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of DPP-IV, especially at resting conditions when the modulation of its hormonal substrates is limited due to homeostatic maintenance.

While the actions of DPP-IV in the mouth are poorly understood, saliva contains the incretin hormones, neuropeptide Y and peptide YY [9,10], all of which are known substrates for DPP-IV. In particular, The Y₂ receptors are present in the basal epithelial layer of the tongue [11,12] and are sensitive to the DPP-IV-cleaved forms of Neuropeptide Y (NPY₃₋₃₆) and peptide YY (PYY₃₋₃₆) and contribute to feelings of satiety when activated [4,13,14] through its connection to the hypothalamic satiety centers [15].

Studies in humans showed that obese individuals have higher DPP-IV activity [8,16] and lower PYY and NPY levels in the plasma [17,18]. DPP-IV activity is normalized after weight loss via bariatric surgery [16], however, it is not known if these individuals had lifelong high DPP-IV activity or if they developed elevated DPP-IV activity as they gained fat mass. The effect of body fatness on salivary DPP-IV activity is not known. If saliva mirrors the plasma, then it could be hypothesized that individuals with higher amounts of fat would be more likely to have higher DPP-IV activity and thus, lower levels of salivary PYY₁₋₃₆ and NPY₁₋₃₆.

Evidence suggests that the mouth may play an important role in the physiological response to food [4,13]. It is well understood that certain foods effect the feeling of satiety [19-22], but it is unknown if salivary DPP-IV is part of this connection between the mouth and the brain. It is possible that some foods may alter the enzymatic activity of DPP-IV [23,24] and thereby change the form of NPY present in the saliva.

This study was performed in two parts. The purpose of Part 1 was to investigate the relationships between salivary DPP-IV activity, NPY₁₋₃₆, and body composition. It was hypothesized that DPP-IV activity in the saliva would be positively related to plasma DPP-IV activity, body fat composition, and salivary NPY₁₋₃₆. For Part 2, the possibility was explored that DPP-IV activity can be altered in the mouth by common dietary carbohydrates. The hypothesis was that saliva DPP-IV and NPY activity would be decreased with all of the carbohydrate

conditions presented, and plasma DPP-IV would only be decreased when the carbohydrates were ingested.

Methods

Ethical approval

All parts of the following study were reviewed and approved by the Institutional Review Board at Auburn University prior to beginning the study and conformed to the standards set by the latest revision of the Declaration of Helsinki. For this study, participants were recruited locally from the Auburn, Alabama area, and a signed informed consent was obtained from each participant prior to beginning any data collection. Participants were required to complete a health questionnaire ensuring they were apparently healthy, not prescribed any medications known to alter the metabolism of any peptides or enzymes of interest, and presented no contraindications to obtaining blood samples. Any participant with diabetes was excluded from the study.

Part 1- Determination of Plasma and Saliva DPP-IV Enzymatic Activity

A total of 111 males and females between the ages of 19 and 70 participated in the study. Body composition measures of all participants were collected during the same visit using the Lunar iDEXA (General Electric, Fairfield, CT). The information provided included relative and absolute fat mass, in addition to absolute and relative lean mass.

For at least an hour prior to coming in for data collection, participants were asked to refrain from eating and drinking [25]. Plasma samples were collected by drawing blood using standard venipuncture in the antecubital vein or by capillary draw of the finger if venipuncture was not possible. Blood was centrifuged at 1,000 g and 4°C for 10 minutes to separate plasma, which was drawn off and stored at -80°C until analysis. Saliva samples were collected by having the participant spit into a 100 mL cup until a sufficient layer was present on the bottom (~1 mL). Saliva was stored in aliquots and stored at -80°C until analysis.

Saliva samples were analyzed for DPP-IV activity by the use of a fluorometric assay developed by Scharpe et al. [26]. DPP-IV samples and assay components were brought to room temperature prior to analysis. Fifty mmol L⁻¹ Tris-HCl incubation buffer (*Sigma-Aldrich, St Louis, MO*; pH 8.3), 20 mmol L⁻¹ glycyl-L-proline-4-methoxy-2-naphthylamide (*Sigma-Aldrich, St Louis, MO*) substrate solution, and saliva sample were added to the sample wells of a white 96-well microplate. Standard wells contained 50 mmol L⁻¹ 4-methoxy-2-naphthylamine (*Bachem, Torrance, CA*) standard solution, 100 mmol L⁻¹ citrate stopping solution (*Sigma-Aldrich, St Louis, MO*; pH 4.0), substrate solution, and incubation buffer. The microplate incubated for 30 minutes on a heating block set at 37°C, and stopping solution was added to the sample wells to end the reaction. Fluorescence was excited at 340nm and measured at 435 nm in a Synergy H2 Hybrid Reader (Biotek Instruments, Winooski, VT).

Based on the availability of samples due to viable saliva that could be used for analysis, salivary NPY₁₋₃₆ protein content of 79 participants (38 males and 41 females) was determined using a peptide EIA (S-1145, Peninsula Laboratories International, San Carlos, CA, USA) per manufacturer's instructions (Protocol III for saliva analysis).

Part 1: Statistics

Results are presented as means ± standard deviation of measurements made. To determine the statistical significance of correlations between the enzymatic activity of DPP-IV in the saliva with the enzymatic activity in plasma as well as with salivary NPY₁₋₃₆ and several body composition measures, linear regression was used. In order to maximize the power, the measures obtained from the iDEXA scan were limited to the traditional measures of fat and lean mass. Results yielding a P-value less than 0.05 were considered significant. Statistics were analyzed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). A post hoc power analysis calculated the power at 0.34 using GPower 3.1 software (Universität Düsseldorf, Düsseldorf, Germany) for the relationship between plasma and DPP-IV activity in part 1.

Part 2: Response of Plasma and Saliva DPP-IV Enzymatic Activity to Carbohydrates

Thirty-five males and females, all students at Auburn University, between the ages of 19 and 35 were recruited to complete the second part of the study. Twenty-eight participants completed all four visits of the study. Those who did not complete all four visits were excluded from analysis of Part 2.

Participants were fasted overnight prior to coming into lab for each of the four visits. Four conditions were completed on individual visits to the lab. The first condition was swishing 10mL of a commercially available high carbohydrate beverage whose primary sweetener was sucrose (Sierra Mist, PepsiCo., Purchase, NY, USA), in their mouth for 30 seconds prior to spitting it out. The second condition was ingesting 8 oz. of the same carbohydrate beverage in a time of one minute or less. The third and fourth conditions were the same as the first two, except the carbohydrate beverage was replaced with the diet version of the beverage (Diet Sierra Mist, PepsiCo., Purchase, NY, USA), where aspartame was used as the primary sweetener. The order of completion for each of the four conditions was counterbalanced. Plasma and saliva samples were collected preceding the condition and 10 minutes post-condition (based on pilot data from this lab). Plasma samples were collected by capillary draw. Blood was centrifuged at 1,000 g and 4°C for 5 minutes to separate plasma, which was drawn off and stored at -80°C until analysis. Saliva samples were collected by having the participant drool into a cryovial by use of a saliva collection aid (5016.02, Salimetrics, State College, PA, USA) until 0.5mL was collected. All samples were stored at -80°C until analysis. Plasma and Salivary DPP-IV activity was determined as described in Part 1. Blood glucose measures were also collected immediately pre- and 10 minutes post-condition using a commercially available blood glucose meter and test strips (Accu-Chek Aviva Plus meter and test strips, Roche Diagnostics, Indianapolis, IN, USA). Body composition was measured during one of the four visits described above.

To further examine the salivary response to the

ingestion of the regular and diet soda, a subset of 20 participants (10 males and 10 females) were randomly selected to investigate NPY. NPY protein content in the saliva at both time points for the two conditions was determined as described above in Part 1.

Part 2: Statistics

Results are presented as mean $\pm$ standard deviation, and significant findings present mean differences and 95% confidence intervals (CIs). To determine the statistical significance of the response of plasma and saliva DPP-IV activity to carbohydrates at all time points, a two-way repeated measures ANOVA was performed. When a significant treatment or time effect was found, a one-way ANOVA along with Tukey's *t* test was performed. Results yielding a P-value less than 0.05 were considered significant. Statistics were analyzed using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA). A post hoc power analysis calculated the power at 0.79 using GPower 3.1 software (Universität Düsseldorf, Düsseldorf, Germany) for the effect of time on the salivary DPP-IV activity in part 2.

Results

Part 1: Plasma and Saliva DPP-IV Enzymatic Activity

Participant Characteristics: The mean age for the 111 participants was 26.0 ± 10.0 years, and the average BMI was $23.8 \pm 3.8 \text{ kg} \cdot \text{m}^{-2}$. According to BMI classifications, 5 participants were considered underweight ($\text{BMI} < 18.5 \text{ kg} \cdot \text{m}^{-2}$), 67 people were within normal range ($\text{BMI} 18.5\text{--}24.9 \text{ kg} \cdot \text{m}^{-2}$), 34 participants were overweight ($\text{BMI} 25\text{--}29.9 \text{ kg} \cdot \text{m}^{-2}$), and 5 participants were classified as obese ($\text{BMI} > 30 \text{ kg} \cdot \text{m}^{-2}$).

Plasma and Saliva DPP-IV Enzymatic Activity: The mean plasma DPP-IV activity for the participants was $35.9 \pm 12.3 \text{ UL}^{-1}$, and the mean salivary DPP-IV activity was $28.1 \pm 19.8 \text{ UL}^{-1}$. Figure 1A shows the relationship between salivary DPP-IV and plasma DPP-IV activity. We found no significant correlation between the two measures of DPP-IV activity.

Saliva NPY Protein Content: The mean saliva

NPY₁₋₃₆ protein content for the 79 participants was $15.2 \pm 12.8 \text{ ng} \cdot \text{mL}^{-1}$. Linear regression analysis showed there were no significant correlations between salivary NPY and either the plasma or saliva DPP-IV pools. The relationship between salivary NPY₁₋₃₆ and salivary DPP-IV activity is reported in Figure 1B. In addition, no correlation was found between any body composition measure and saliva NPY₁₋₃₆ (data not shown).

Saliva DPP-IV Activity and Body Composition: The DPP-IV activity of saliva was not significantly correlated with any measures of body composition, including BMI (Figure 2), relative fat mass ($28.3 \pm 8.8\%$), and relative lean mass ($68.8 \pm 8.1\%$; data not shown).

Part 2: Plasma and Saliva DPP-IV Activity Response to Carbohydrates

Participant Characteristics: The mean age for the 28 participants was 23.1 ± 2.8 years, and the average BMI was 24.9 ± 4.2 .

Plasma DPP-IV Activity Response: Mean plasma DPP-IV values for each challenge at pre and 10 minutes post-challenge are reported in Figure 3. Plasma DPP-IV activity levels did not significantly change across the four conditions.

Saliva DPP-IV Activity Response to Carbohydrates: Pre- and 10 minutes post-challenge mean saliva DPP-IV activities for each condition are reported in Figure 4. There was no main effect of condition. However, a significant time effect was present ($p < 0.0001$). A post-hoc analysis showed differences between the pre and post measurements for the regular soda-swish ($p = 0.0052$; Mean difference: 9.05; 95%CI 2.94–15.15), regular soda-ingest ($p < 0.0001$; Mean difference: 14.43; 95%CI=8.13–20.74), and diet soda-ingest conditions ($p = 0.0001$; Mean difference: 7.15; 95%CI=3.86–10.44).

Blood Glucose Response: The mean blood glucose values for each condition initially and 10 minutes post-challenge are reported in Figure 5. There was a significant effect of both condition ($p < 0.0001$) and time ($p < 0.0001$). Post-hoc analysis revealed

Figure 1: Relationships between saliva DPP-IV activity and plasma DPP-IV activity, age and saliva neuropeptide Y.

(A) There was no association between saliva and plasma DPP-IV activity. (B) Additionally, there was no relationship between saliva DPP-IV activity and saliva neuropeptide Y protein.

regular soda-ingest blood glucose levels at 10 minutes post challenge were significantly different from those of regular soda-swish ($p < 0.0001$; Mean difference: -17.23 ; 95% CI= -24.46 - -10.00), diet soda-swish ($p < 0.0001$; Mean difference: 17.23 ; 95% CI= 10.00 - 24.46), and diet soda-ingest ($p < 0.0001$; Mean difference: 14.77 ; 95% CI= 7.54 - 22.00) at the same time point. There were no significant differences between any of the conditions' initial blood glucose values. For the interaction, only regular soda-ingest showed a significant change in blood glucose from pre to post measures ($p < 0.0001$).

Saliva NPY Response to Carbohydrates:

Mean salivary NPY protein values at pre and 10 minutes post-challenge for the two conditions are reported in Figure 6. The NPY protein content in the saliva did not significantly change with either condition.

Discussion

This was a two part study investigating the physiology of DPP-IV in the mouth and body. For Part 1, we found that salivary DPP-IV is unrelated to plasma DPP-IV suggesting that, under basal conditions, salivary

DPP-IV is primarily from the salivary glands in people with BMI in the normal range. Further we found no relationship between salivary DPP-IV activity and body composition, suggesting that under basal conditions, there is no apparent abnormality in DPP-IV activity over a range of body fatness. In Part 2, we challenged the DPP-IV system in the mouth with a sucrose beverage or its diet counterpart, aspartame. The unique finding from this study was that salivary DPP-IV activity was attenuated with sucrose or aspartame beverage, but plasma DPP-IV was unchanged. This result suggests that some foods may alter DPP-IV activity and thus, may impact satiety.

Plasma and Saliva DPP-IV enzymatic activity

The hypothesis for this study was the enzymatic activity of DPP-IV in the plasma would correlate with the DPP-IV enzymatic activity in the saliva [7]. However, no significant correlation was found between the two pools of DPP-IV at the fasted state. This suggests that during basal conditions, DPP-IV in the mouth comes primarily from the salivary gland and little can be attributed to transport through the capillary wall into the mouth as previously suggested by Acosta et al. [4].

Figure 2: DPP-IV Activity by BMI Classification.

The BMI of the participants had no significant correlation with salivary DPP-IV activity. The mean activity for each classification is represented by the band within the box, and 95% confidence intervals are represented by the error bars.

Saliva DPP-IV Activity, body composition and age

Previous work reporting higher DPP-IV activity in the plasma of obese people [8,16] lead to the hypothesis that we may also find higher mouth DPP-IV activity in people that have higher fat masses. In contrast to this hypothesis, fasted salivary DPP-IV was not correlated to body composition. It is possible that there is a post prandial correlation in salivary DPP-IV and body composition based on the work showing impaired responses of PYY after the consumption of a meal [17,18]. Although we had a range of BMI from 17.5 to 36.3 kg · m⁻², many of the participants in this study were quite lean with an average BMI of 24 ± 4 kg · m⁻², making it possible that this relationship may be stronger in a more obese population. Chielle et al. [8] did see an increase in DPP-IV activity in the saliva of obese people compared to normal weight people, but no difference between normal weight and overweight. The majority of our participants were in the normal and overweight category, which may be the reason we did not see the same effect of obesity as Chielle et al. [8].

Saliva NPY Protein Content

The total NPY₁₋₃₆ protein content in saliva was also investigated, and it was found that it does not have a significant relationship with DPP-IV activity. This is in contrast with what was expected, as this is an important enzyme and substrate in the regulation of satiety [7]. However, we measured the total amount of full length NPY₁₋₃₆ in the saliva. It is possible that the amount of NPY₃₋₃₆, the form of NPY that has been truncated and activated by DPP-IV, is related to the amount of DPP-IV activity in the saliva. A commercial assay for the truncated form of NPY is not currently available, but it can be anticipated that future research will be able to examine the ratio of NPY₁₋₃₆ to NPY₃₋₃₆.

Plasma DPP-IV Activity Response to Carbohydrates

In part 2, the DPP-IV system in the mouth and body was challenged to examine the effects of either sucrose or aspartame on saliva and plasma DPP-IV activity. For the ingestion part of the experiment, we expected that sucrose would result in a decrease in DPP-IV activity in the blood to allow for more GLP-1 to reach the

Figure 3: The effect of ingesting or swishing different sugars on plasma DPP-IV activity.

There was no significant change in plasma DPP-IV activity with sucrose or aspartame. There also was no effect of swishing or ingesting the beverages on plasma DPP-IV activity. Each line represents an individual participant. Means and standard deviations for each condition are below the graphs. Reg Swish= sucrose beverage + swish for 30 sec and spit out; Reg Ingest= sucrose beverage + swallow; Diet Swish= aspartame beverage + swish for 30 sec and spit out; Diet Ingest= aspartame beverage + swallow.

Figure 4: The effect of ingesting or swishing different sugars on saliva DPP-IV activity.

For each condition, except Diet Swish, pre-measure was significantly different from post-measure. There was no main effect of condition. Each line represents an individual participant. Means and standard deviations for each condition are below the graphs.

Figure 5: The effect of ingesting or swishing different sugars on blood glucose.

Blood glucose was significantly elevated by ingesting the sucrose beverage (Reg Ingest). Each line represents an individual participant. Means and standard deviations for each condition are below the graphs

Figure 6: The effect of ingesting different sugars on salivary neuropeptide Y.

There was no difference in the neuropeptide Y protein sampled before or after ingesting the sucrose or aspartame beverage. Each line represents an individual participant. Means and standard deviations for each condition are below the graphs.

pancreas and release insulin [27] and reduced PYY₃₋₃₆ to signal satiety [28]. However, when presented with a carbohydrate challenge, the plasma enzymatic activity of DPP-IV was not altered, despite a significant increase in blood glucose with sucrose ingestion. It is possible that DPP-IV activity increases after a period greater than 10 minutes to return GLP-1 to baseline levels; however, a previous study showed that post-prandial plasma DPP-IV activity does not significantly change, even when the subjects were monitored 3 hours after the ingestion of a test meal [25,29]. A constant soluble DPP-IV activity in the plasma suggests that regulation of the activity level is controlled via a long-term mechanism. The mechanisms by which soluble DPP-IV in the plasma is controlled are currently unknown, but possible contributors are the muscle, fat, and T-cells [30-32].

We originally hypothesized that the mouth may communicate with the body via the binding of NPY₃₋₃₆ and PYY₃₋₃₆ to the Y₂-receptors in the mouth and signaling the ingestion of a macronutrient up to the hypothalamus. This was based on research suggesting that when food was given via the mouth, the physiological response was greater than when the person was gavage fed [10,33]. Given, in Part 1, there was no relationship between saliva and plasma DPP-IV and, in Part 2, there was no evidence that plasma DPP-IV activity changed in response to increases in blood glucose, it seems unlikely that with the consumption of sweetened beverages the mouth directly communicates with the body via the blood or vice versa under the conditions of this experiment.

Saliva DPP-IV Activity and NPY Response to Carbohydrates

The most unique finding of this study was that when a high-sucrose or a high-aspartame beverage was ingested, salivary DPP-IV activity decreased. When DPP-IV activity is decreased, hypothetically, the amount of activated NPY₃₋₃₆ or PYY₃₋₃₆ is also decreased, sending fewer signals of satiety to the brain via the Y₂ receptor [4,34]. This finding is in support of research that shows decreased feelings of satiety and increased food intake when a meal is consumed with a high carbohydrate soda [20-22]. However,

this could possibly be a response to the activation of sweet taste receptors in the mouth. Research has shown that taste receptors have different effects on an individual's satiety and caloric intake. When bitter taste receptors are stimulated, caloric intake is decreased and cholecystokinin was increased [35]. Salty taste exposure also decreased caloric intake [36].

The attenuated DPP-IV activity also occurred when the person swished the sucrose beverage for one minute, but DPP-IV was not attenuated with swishing the aspartame beverage. It is unclear why ingesting the aspartame beverage resulted in attenuation of DPP-IV activity, but swishing did not. It is possible that the mouth is more sensitive to simple sugars, like sucrose, compared to aspartame [20]. Salivary NPY was also measured, but no differences were found. As mentioned above, the assay used detected only NPY₁₋₃₆ and not NPY₃₋₃₆, therefore, it would be difficult to detect changes among the NPY products.

Conclusion

In conclusion, we found no relationship between body composition and salivary DPP-IV in people, with the majority (44/78 participants) falling in the normal BMI classification. Our most interesting finding was that plasma DPP-IV activity was resistant to change even when blood glucose was increased, but salivary DPP-IV activity was attenuated with ingestion of a sucrose or aspartame beverage. Swishing the beverage had a similar effect for the sucrose beverage, but swishing the aspartame beverage failed to attenuate DPP-IV activity. These findings suggest that the mouth may have different sensitivities to sucrose and aspartame causing different physiological changes. Further research is needed to understand how sugars attenuate DPP-IV activity and how this affects satiety.

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