Northumbria Research Link

The effect of dietary intake, physical activity and posture on pepsin concentrations detected in the saliva of free-living, healthy individuals

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Introduction

The reflux/aspiration of gastric contents into the aerodigestive tract has been associated with a range of proximal gastrointestinal and airways conditions, particularly gastro-oesophageal reflux disease (GORD) [1; 2]. Outside of disease manifestation, the occurrence of reflux events may not be overt[3]. Appropriate detection of the occurrence of reflux in a non-invasive manner could benefit treatment strategies for the wide spectrum of reflux-associated disease.

Clinical assessment of reflux occurrence (by intraluminal impedance or pHmetry) is well-validated. However, such measurements techniques are invasive and time-consuming. They may also not allow for assessment of reflux up the oesophagus and beyond by aerosolisation or microaspiration. Assessment of biomarkers of reflux provides a means of developing non-invasive monitoring tools. Pepsin is the main proteolytic enzyme secreted by the stomach. Within gastric juice, it occurs up to concentrations approaching up to 1 mg/ml. It should only occur in upper GI and airways secretion either as a result of reflux, or at very low levels 1 ng/ml or less) as a result of circulating pepsinogen exudation from plasma. Pepsin therefore represents a rational biomarker molecule for assessment of reflux occurrence in aerodigestive secretions[4].

Diet, physical activity and posture are widely accepted to be major factors that could contribute to the occurrence of reflux and the exacerbation of reflux-associated diseases, although the link between food intake/physical activity and reflux events is not well characterised.

The current study aimed to assess whether dietary intake, physical activity and posture were factors that could influence the occurrence of gastric reflux. Pepsin concentrations in saliva were assessed using a previously described, microwell plate ELISA[5].

Methods

Participants and sampling

These studies were approved ethically by Sheffield Hallam University Ethics Committee and Newcastle University Science, Agriculture and Engineering Faculty Ethics Committee. Participants were recruited in two centres at different times (Sheffield, UK in 2011 and Singapore in 2012). Participants were eligible for the study if they were aged 18 or above, non-smokers and were not currently suffering from any chronic or acute respiratory conditions or bloodborne diseases. Individuals were excluded from participation if they had previously been diagnosed with reflux disease.

Saliva samples were collected in 30 ml Universals with a small amount (50 mg) of citric acid to act as a preservative. Samples were refrigerated as soon as possible (< 24 h) after collection. The Sheffield cohort of participants (n = 12) provided saliva samples before (< 30 min) and after (< 1 h) meals and physical activity bouts over a 5-day period. The Singaporean cohort of participants (n = 39) provided saliva samples before going to bed (after brushing their teeth) and just after waking up (as soon as possible) each day for a seven day period.

Baseline reflux symptoms were assessed by the validated Reflux Symptom Index (RSI) questionnaire [6].

Sample handling

Participants were asked to provide approximately 1 ml of saliva at each sampling point. Samples were centrifuged (500 rpm for 5 min at 4^{o}C) to remove particulate matter and cell debris. The resulting supernatant was isolated and stored at 4^{o}C prior to analysis.

Pepsin ELISA

Samples were diluted 1 in 5 for the previously described pepsin ELISA[5]. A commercially available antipepsin antibody was used as the primary antibody (W59117G raised in goat, Meridian Life Sciences, Abingdon, UK). Paired samples (i.e. those taken before and after each meal, bout of physical activity or sleep) were always analysed on the same microplate. Each microplate included a standard curve (using isolated porcine pepsin, Sigma, US).

Statistical analysis

All statistical analysis was carried out using Prism 6 software (Graphpad, San Diego, CA). Wilcoxon matched pairs rank sign tests were performed on samples collected before and after each meal, bout of physical activity or sleep. Unpaired samples were not analysed for pepsin content.

Results

Fifty-seven paired pre-and post-meal, 48 paired pre- and post-physical activity samples and 168 pre- and post-sleep samples were analysed. The majority of saliva samples (92% of the Sheffield samples and 88% of the Singaporean samples) analysed had detectable concentrations of pepsin present. Mean (± standard deviation) pepsin concentrations in saliva were significantly higher (P=0.037) in the pre-meal samples (44.2 (± 42.2) ng/ml) than the post-meal samples (32.8 (± 29.6) ng/ml), as illustrated in Figure 1A.

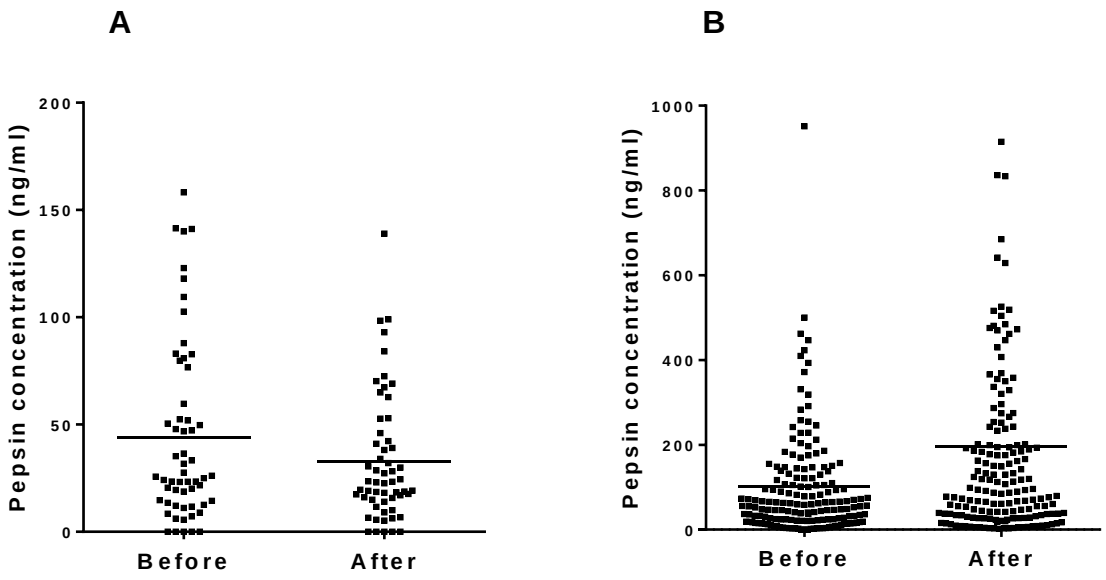

Figure 1: A - Pepsin concentration of saliva samples before and after meal consumption. Pepsin concentration was significantly higher before meal than after(P = 0.037). B - Pepsin concentration of saliva samples before and after sleep. Pepsin concentration was significantly higher after sleep than before (P < 0.001). Five data points with values above 1000 ng/ml pepsin (2 for "Before" and 3 for "After" have been removed for scale clarity.

Post-sleep pepsin concentrations (196.4 (± 323.4) ng/ml) were significantly higher (P<0.001) than pre-sleep (102.3 (± 152.8) ng/ml – see Figure 1B). There was no significant difference (P=0.491) between pre- (45.2 (± 56.8) ng/ml) and post- (40.8 (± 38.6) ng/ml) physical activity saliva samples (data not shown).

Discussion

Perhaps the most striking observation from the current pilot study was that pepsin was frequently present (approximately 90% of the time) in quantifiable levels in the saliva of healthy individuals with no previous or current respiratory or reflux-related complaints. Previous clinical data would suggest that bulk reflux events do occur within normal participants. Guidelines have been developed to consider the number of events in a day that would be considered normal or abnormal due to careful clinical validation. Inferences taken from repeated measures in relatively small groups of individuals (i.e. the physical activity and meal-associated reflux data) should be considered with extreme caution. These data suggest that microreflux events could be very common physiological events within a healthy population. Further consideration as to the timing of sampling and concentrations of pepsin detected are necessary to elucidate better consideration of what might be considered normal and abnormal amounts of pepsin for clinical evaluation. Nonetheless, a saliva sample positive for pepsin would highlight that reflux could be a problem and that further clinical investigation is warranted.

Any pepsin occurring in saliva is likely to only occur transiently within the oral cavity before it is swallowed or diluted by subsequent saliva production. Saliva sampling is the most

minimally invasive, least time-consuming method of collection of aerodigestive tract secretions. Further work in different patient groups and healthy populations is also required in order to assess whether saliva concentrations of pepsin correlate with those found in other airways clinical samples, particularly where the reflux-associated symptoms may be relatively site-specific, such asthma or sinusitis[7-9]. Assessment of how dietary and lifestyle factors may affect pepsin levels in airway is also of an area of general interest to understanding the circadian causes of reflux events.

Within the Sheffield cohort, bouts of physical activity were generally of low intensity, most of which were walking. Previous data would suggest that high intensity physical activities may be more likely to cause reflux, with both posture during activity (e.g. lying down flat on one's stomach) and changes in plane of acceleration (for example, the "bouncing" of gastric contents caused by the action of jogging) also being key factors that might drive reflux[10; 11].

The results of this study suggest that, following further validation, in normal and patient populations, pepsin detection in saliva may form the basis of a non-invasive pre-screening prior to standard oesophageal monitoring methods.

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References

1. Enzinger PC & Mayer RJ (2003) Esophageal Cancer. *New England Journal of Medicine* **349**, 2241-2252.
2. Dent J, El-Serag HB, Wallander MA *et al.* (2005) Epidemiology of gastro-oesophageal reflux disease: A systematic review. *Gut* **54**, 710-717.
3. DiMango E, Holbrook JT, Simpson E *et al.* (2009) Effects of asymptomatic proximal and distal gastroesophageal reflux on asthma severity. *American Journal of Respiratory and Critical Care Medicine* **180**, 809-816.
4. Pearson JP & Parikh S (2011) Review article: Nature and properties of gastro-oesophageal and extra-oesophageal refluxate. *Alimentary Pharmacology and Therapeutics* **33**, 2-7.
5. Stovold R, Forrest IA, Corris PA *et al.* (2007) Pepsin, a biomarker of gastric aspiration in lung allografts: A putative association with rejection. *American Journal of Respiratory and Critical Care Medicine* **175**, 1298-1303.
6. Belafsky PC, Postma GN & Koufman JA (2001) Laryngopharyngeal reflux symptoms improve before changes in physical findings. *Laryngoscope* **111**, 979-981.
7. Harding SM, Richter JE, Guzzo MR *et al.* (1996) Asthma and gastroesophageal reflux: Acid suppressive therapy improves asthma outcome. *American Journal of Medicine* **100**, 395-405.
8. Pincus RL, Kim HH, Silvers S *et al.* (2006) A study of the link between gastric reflux and chronic sinusitis in adults. *Ear, Nose and Throat Journal* **85**, 174-178.
9. Liu L, Teague WG, Erzurum S *et al.* (2011) Determinants of exhaled breath condensate pH in a large population with asthma. *Chest* **139**, 328-336.
10. Emerenziani S, Zhang X, Blondeau K *et al.* (2005) Gastric fullness, physical activity, and proximal extent of gastroesophageal reflux. *American Journal of Gastroenterology* **100**, 1251-1256.
11. Jóźków P, Waśko-Czopnik D, Dunajska K *et al.* (2007) The relationship between gastroesophageal reflux disease and the level of physical activity. *Swiss Medical Weekly* **137**, 465-470.