Comparing the responsiveness of ghrelin concentrations in saliva compared with blood draws by fingerprick

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Abstract

Ghrelin is a hormone secreted by the stomach that influences hunger. Blood draws are used to measure ghrelin levels. A less invasive method to detect ghrelin concentration could simplify research. A ghrelin ELISA assay was performed on five individuals before and after consumption of a meal to determine the effect of a meal on concentrations of ghrelin in saliva and blood. No statistical difference in the pre and post consumption ghrelin concentration in saliva and blood was observed.

Keywords: Ghrelin, Saliva, Fingerprick

Introduction

Ghrelin is a hormone produced by the lining of the stomach that plays a major role in influencing hunger (Wren et al. 2001, Leite-Moreira et al. 2007, Foster-Schubert et al. 2008, and Remington 2013). Ghrelin expression is suppressed after eating, with the different type of food reported to affect the degree of suppression (Wren et al. 2001, Akhavan and Anderson 2007, Foster-Schubert et al. 2008, Garin et al. 2013, Remington 2013). Several studies have investigated the effects of ghrelin on various diseases and medical conditions including cancer (Lanfranco et al. 2007), cystic fibrosis (Cohen et al. 2008), eating disorders (Troisi et al. 2005), and in the elderly (Akamizu et al. 2006). Ghrelin has a short half-life of about eight minutes (Hosoda and Kangawa 2012), which allows the body to respond quickly to environmental changes but makes assays challenging (Akhavan and Anderson 2007, Satou and Sugimoto 2012 and Sato et al. 2012). Venous blood draws are typically used to test for ghrelin (Troisi et al. 2005, Akhavan and Anderson 2007, Cohen et al. 2008, Foster-Schubert et al. 2008 and Hosoda and Kangawa 2012), but these can be traumatic, require a licensed phlebotomist, are prone to infection, and can easily cause bruising (Ranasinghe and Harrison 2000 and Ramos 2014). To minimize the invasiveness of the testing, saliva ghrelin concentrations have been compared with those of venous blood (Aydin et al. 2005 and Li et al. 2011), and, while ghrelin can be detected in saliva, concerns have arisen as to whether or not these concentrations may be less responsive
to changes than are those obtained from venous blood (Aydin et al. 2005 and Li et al. 2011). The purpose of this study was to investigate the concentrations of ghrelin obtained by fingerstick, compared with those obtained in saliva samples, for the detection of acute changes in ghrelin concentrations.

**Methods**

The experimental protocol was approved by the Brigham Young University-Hawaii Institutional Review Board. A group of five volunteers, three males and two females, (ages 18-25) participated in this study. Participants were instructed to fast eight hours prior to sample collection. Saliva samples were collected by having participants collect their saliva in a 1.5 ml micro-centrifuge tube. Using a microcentrifuge tube 60 µL of saliva was collected from each sample. A blood sample was obtained by having the participants disinfect their finger with an alcohol wipe and then prick their own finger using a 1.5 mm contact activated lancet (BD Microtainer, ref 366592). From the site of the prick 60 µL of blood was collected. Pefabloc was added to a final concentration of 1 mg/mL. The samples were incubated at room temperature for 30 minutes allowing the blood to clot. The samples were centrifuged at 2,500 rpm for 15 minutes at 4°C; the clarified serum was transferred to clean tubes and HCl was added to a final concentration of 0.05 N, where upon they were stored on ice. After the fasting samples were collected, volunteers consumed a small breakfast meal (780 calories, 23 g of protein, 52 g of fat, and 55 g of carbohydrate), which they were instructed to consume within 15 minutes. Blood and saliva samples were again collected 15, 30 and 45 minutes post consumption. All samples were analyzed in duplicate for ghrelin concentration using the Human Ghrelin (Active) ELISA kit (Millipore, cat. #EZGRA-88K) and the absorbance at 450 nm and 590 nm was determined and
was compared to a standard curve to estimate ghrelin concentrations. Replicates were averaged, and a repeated measures ANOVA test was used to determine significance.

Results

The ghrelin concentration of each sample was calculated and compared (Figures 1, 2, and 3). Concentrations of ghrelin in blood were found to range from 88.563 to 1565.8 pg/mL. Ghrelin concentrations in saliva ranged from 45.75 to 397.75 pg/mL. An average decrease in blood concentration was 226.52 pg/mL, while the average decrease in saliva was 76.81 pg/mL. The greatest individual concentration difference in the blood sample was a 1457.8 pg/mL decrease while the greatest individual concentration difference in saliva sample was a 235.44 pg/mL decrease. However statistical analysis using a repeated measures ANOVA test found the data were insignificant (p > 0.05).

![Figure 1: The ghrelin concentrations over time in the blood samples of 5 individuals. The first collection was performed pre-consumption, followed by collections at 15, 30, and 45 minutes post-consumption.](image)
Figure 2: The ghrelin concentrations over time in the saliva samples of 5 individuals. The first collection was performed pre-consumption, followed by collections at 15, 30, and 45 minutes post-consumption.

Figure 3: A comparison of ghrelin concentrations in blood and saliva, by individual. B1 is the pre-consumption blood ghrelin concentration, B3 is the 30 minute post-consumption ghrelin concentration in blood; S1 is the pre-consumption saliva ghrelin concentration, S3 is the 30 minute post-consumption ghrelin concentration in saliva.
Discussion

Varying levels of ghrelin concentrations were observed, but no statistical significance was determined. While ghrelin concentrations are measured and are known to decrease after eating, fingerpricks and saliva samples do not yield an accurate portrayal of short term changes in ghrelin concentrations. Small volumes of saliva and blood yield inaccurate results in performing and ELISA assay. The small volumes used in this experiment did not give reliable results. Other methods, such as Radioimmunoassay (Aydin et. al, 2005), could be used for future studies to detect ghrelin accurately in small volumes of sample.

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Works Cited


