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Adiponectin: Serum-saliva associations and relations with oral and systemic markers of inflammation

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ABSTRACT

This study addresses gaps in our understanding about the validity and utility of using salivary adiponectin to index serum adiponectin levels. Matched blood and saliva samples were collected on a single occasion from healthy adults (n = 99; age 18–36 years, 53% male). Serum and saliva was assayed for adiponectin and inflammatory cytokines (IL-1β, IL-6, IL-8, TNFα), and saliva was also assayed for markers of blood contamination (transferrin), total protein (salivary flow rate) and matrix metalloproteinase-8 (MMP-8). We examined the extent to which salivary adiponectin was associated with serum adiponectin, and the influence of potential confounders on the serum-saliva correlation, including age, sex, body mass index, and markers of inflammation, oral health, salivary blood contamination, and flow rate. Findings revealed a modest serum-saliva association for adiponectin, and strong positive associations between salivary adiponectin and salivary levels of inflammatory cytokines, MMP-8, transferrin, and total protein. By contrast, salivary adiponectin was not related to serum levels of inflammatory activity. The magnitude of the serum-saliva association was strengthened when controlling for total protein in saliva, blood leakage into oral fluid, salivary inflammatory cytokines, and MMP-8. The pattern of findings extends our understanding of salivary adiponectin and its potential use as an index of circulating adiponectin levels.

1. Introduction

Adipose tissue secretes bioactive proteins, known as adipocytokines, into circulation. Among the important members of the adipocytokine family is adiponectin [1]. Adiponectin modulates several metabolic processes including glucose and lipid metabolism in insulin-sensitive tissues. Studies suggest that adiponectin has anti-atherogenic and anti-inflammatory properties, and adiponectin levels are associated with a range of cardiovascular and metabolic diseases [2–4]. Adiponectin levels are lower in patients with essential hypertension and type-2 diabetes, and adiponectin is associated with insulin resistance, triglycerides and HDL cholesterol levels [5–7]. Given its role in metabolic diseases, adiponectin may be important in tracking and diagnosing some of the most rapidly growing and costly diseases in the United States (e.g., prediabetes/insulin resistance, metabolic syndrome, and vascular disease) [8–10].

Adiponectin is also produced locally in the oral cavity by salivary glands [11]. As such, adiponectin plays a role in oral inflammation and the oral immune response. Salivary adiponectin has potent beneficial functions on the maintenance and homeostasis of periodontal health [12,13]. The relationship between protein levels in oral fluids and plasma makes oral fluid an attractive biospecimen that could be used as an alternative to blood in tests measuring adiponectin. Many studies suggest that the measurement of adiponectin in saliva by immunoassay

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is feasible, minimally-invasive, and may be an acceptable alternative to plasma sampling [14,15]. However, more research on the relationship between adiponectin levels in serum and saliva is needed as some studies report low levels of correspondence in serum and saliva [16,17]. To the best of our knowledge, studies also have yet to account for a host of potential confounding factors when assessing the serum-saliva relations of adiponectin (for one exception, see [18]).

One of the most important factors potentially confounding the adiponectin serum-saliva correlation is inflammation in the oral mucosal immune compartment, as the extent of adiponectin activity in saliva might be strongly associated with oral health, rather than metabolic processes. In addition to oral inflammation, poor oral health or injury (e.g., open wounds, abrasion) may also cause blood leakage into saliva which can confound salivary determinations of adiponectin. Furthermore, variation in local oral mucosal immune responses is largely confined to that particular compartment and relatively independent of the more general systemic level of immune activation (e.g., [19]). Correspondingly, in contrast to the strong serum-saliva associations for many endocrine markers, the serum-saliva correlations for immune markers has been shown to be small to modest (e.g., [20,21]). Thus, characteristics of oral health and inflammation may significantly compromise the possibility of using salivary measures of immune-sensitive analytes, such as adiponectin, as an alternative to blood measures.

1.1. Present study

We investigated the utility of salivary adiponectin as an index of circulating adiponectin and relations between adiponectin and oral and systemic inflammatory activity. More specifically, using adiponectin assayed from matched serum and saliva samples from healthy young adults, we: 1) compare adiponectin levels in serum and saliva, (2) examine the extent to which salivary adiponectin is associated with oral health using both self-reported measures of oral hygiene practices and a known biomarker of oral health (matrix metalloproteinase-8 (MMP-8); 3) examine the associations between adiponectin and inflammatory markers both within and across biospecimens; 4) examine the associations between salivary adiponectin levels and blood contamination and salivary total protein; 5) explore relations between adiponectin levels in serum and saliva and age, sex, and body mass index; and 6) examine the serum-saliva correlation for adiponectin.

To further interrogate the relation between serum and salivary adiponectin, we also examine serum-saliva correlations controlling for salivary indictors of leakage of serum into the oral mucosal compartment and salivary total protein. Furthermore, based on previous studies [21], we hypothesize that salivary adiponectin is positively associated with measures of oral inflammatory activity and oral health, and, therefore, we also examine the serum-saliva correlation for adiponectin when controlling for measures of oral inflammatory activity and oral health. We hypothesize that the serum-saliva correlation will become stronger when we control for indicators of oral inflammation and oral health.

2. Methods

2.1. Participants

The sample consisted of 99 adults ranging between 18 and 36 years old. Participants were mostly white (n = 45; 45%), 18% were Black/African American, 5% were South East Indian, 5% were Native Hawaiian, and 27% were of other or mixed race/ethnicities. To be eligible, participants had to be 18–36 years old, not currently under a physician's care for any acute or chronic medical conditions, not taking prescription or over the counter medication (not including oral contraceptives), without open wounds and sores in their mouths, and have had no recent dental surgery.

2.2. Procedures

Data were collected in 2012–2013. All participants provided written informed consent and the Johns Hopkins University Bloomberg School of Public Health Institutional Review Board approved all study procedures. During a single 45-min assessment, participants completed a demographic survey and provided blood and saliva samples. Participants were compensated $50 for their time.

2.3. Blood and saliva collection

Blood was drawn by venipuncture into 2 mL lavender/EDTA tubes. Additionally, a SST Tiger serum separator tube (BD, Becton-Dickenson) of blood was drawn for serum isolation. EDTA/whole blood was mixed well by inversion and spun at 900 × g for 15 min. The top plasma layer was transferred into 4 × 1 mL aliquots which were snap frozen and stored at −80 °C. Serum was mixed well by inversion and allowed to clot at room temperature for 30 min but not longer than two hours. Serum was aliquoted into 4 × 1 mL aliquots and subsequently stored at −80 °C until assayed.

Following Granger and colleagues [22], whole unstimulated saliva was collected by passive drool and aliquoted into 2 mL cryogenic vials. Saliva samples were snap frozen and stored at −80 °C until assayed.

2.4. Measures

Health and Demographic Information. Participants reported their age, sex, race/ethnicity, height and weight. Body mass index (BMI) was calculated for each participant using the Centers for Disease Control and Prevention's BMI formula (CDC, 2015). Participants also reported on their current oral hygiene practices. Specifically, participants indicated the number of times they flossed per week, the number of times they brushed their teeth per day, and whether or not their saliva had a red/pinkish color when they brushed their teeth (yes/no).

2.4.1. Determination of salivary and circulating biomarkers

2.4.1.1. Adiponectin. Salivary adiponectin was measured using a multiplex electrochemiluminescence assay (Meso Scale Discovery, Gaithersburg, MD, USA). The assay had a test volume of 10 μL test and a range of sensitivity from 0.00529 to 200 ng/mL. The inter- and intra-assay coefficients of variation (CVs) were less than 5%. Serum adiponectin was measured using a commercial kit (EMD Millipore Corporation, cat# EZHADP-61K) with no modification to the manufacturer's recommended protocol. The assay had a test volume of 10 μL (diluted 1:500) and a lower limit of detection (LLD) of 0.2 ng/μL. The intra- and inter-assay CVs were 10.3% and 2.1%, respectively.

2.4.1.2. Cytokines. Salivary and serum cytokines were used to index oral and systemic inflammatory activity. Cytokines were measured using a 96-well format multiplex (9-plex) electrochemiluminescence immunoassays manufactured by Meso Scale Discovery (MSD, Gaithersburg, MD). Each well of a 96-well plate was coated with capture-antibodies specific to nine cytokines (granulocyte macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFNγ), TNFα, interleukin (IL)-1β, IL-2, IL-6, IL-8, IL-10 and IL-12p70). Detection antibodies were coupled to SULFOTAG™ labels that emit light when electrochemically stimulated via carbon-coated electrodes in the bottom each microwell. The assay was run following the manufacturer's recommended protocol without modification and using standard diluent (MSD # R51BB). Cytokine concentrations were determined with MSD Discovery Workbench Software (v. 3.0.17) using curve fit models (4-PL with a weighting function option of $1/y^2$). GM-CSF, IL-2, IFNγ, IL-10 and IL-12p70 were not examined in the current analyses, because these analytes were either not available in serum (GM-CSF, IL-2) or had high rates of censored data in saliva (> 20% of determinations below the LLD). Lower limits of detection were: IL-1β