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.

Keywords: saliva; serum; adiponectin; cytokines; inflammation; oral health

Introduction

Adipose tissue secretes bioactive proteins, known as adipocytokines, into circulation. Among the important members of the adipocytokine family is adiponectin (Scherer, Williams, Fogliano, Baldini, & Lodish, 1995). 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 (Fasshauer & Bluher, 2015; Li & Wu, 2012; Robinson, Prins, & Venkatesh, 2011). 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 (Lihn, Pedersen, & Richelsen, 2005; Nigro et al., 2014; Vaiopoulos, Marinou, Christodoulides, & Koutsilieris, 2012). 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; Ebrahimi-Mamaeghani, Mohammadi, Arefhosseini, Fallah, & Bazi, 2015; Lopez-Jaramillo et al., 2014; Stojanović, Ilić, Ilić, Petrović, & Djukić, 2015).

Adiponectin is also produced locally in the oral cavity by salivary glands (Katsiougiannis, Kapsogeorgou, Manoussakis, & Skopouli, 2006). 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

(Iwayama et al., 2012; Kraus et al., 2012). 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 is feasible, minimally-invasive, and may be an acceptable alternative to plasma sampling (Desai & Mathews, 2014; Nigro et al., 2015). 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 (Mamali et al., 2012; Thanakun, Watanabe, Thaweboon, & Izumi, 2013). 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 Akuailou, Vijayagopal, Imrhan, & Prasad, 2013).

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., Kindt, Osborne, & Goldsby, 2006). 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., Nishanian, Aziz, Chung, Detels, & Fahey, 1998; Riis et al., 2013). 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.

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 (Riis et al., 2013), 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.

Methods

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 to 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.

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-minute assessment, participants completed a demographic survey and provided blood and saliva samples. Participants were compensated $50 for their time.

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 x*g* for 15 minutes. The top plasma layer was transferred into 4x1 mL aliquots which were snap frozen and stored at -80℃. Serum was mixed well by inversion and allowed to clot at room temperature for 30 minutes but not longer than two hours. Serum was aliquoted into 4x1 mL aliquots and subsequently stored at -80℃ until assayed.

Following Granger and colleagues (Granger et al., 2007), whole unstimulated saliva was collected by passive drool and aliquoted into 2 mL cryogenic vials. Saliva samples were snap frozen and stored at -80℃ until assayed.

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).

Determination of Salivary and Circulating Biomarkers

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.

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^{TM} 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 (0.08 pg/mL), IL-6 (0.08 pg/mL), IL-8 (0.20 pg/mL), and TNFα (0.18 pg/mL). Intra-assay CVs for saliva were: IL-1β (2.4%), IL-6 (4.0%), IL-8 (2.0%), and TNFα (3.4%). Inter-assay CVs for saliva were: IL-1β (1.9%), IL-6 (3.8%), IL-8 (5.8%), and TNFα (7.1%). Intra-assay CVs for serum were: IL-1β (3.1%), IL-6 (2.3%), IL-8 (4.9%), and TNFα (4.8%). Inter-assay CVs serum were: IL-1β (3.9%), IL-6 (2.4%), IL-8 (6.1%), and TNFα (5.0%).

Matrix Metalloproteinase-8. Salivary MMP-8 was assessed as a biomarker for oral health (Sorsa, Tjaderhane, & Salo, 2004). Levels were tested using a commercially available development kit (DuoSet ELISA, R&D Systems, cat# DY908) and following manufacturer's guidelines. Samples were diluted 1:50 in Phosphate-buffered saline (PBS) containing 1% BSA Fraction5 (Akron Cat#11103090148) followed by a PBS/ 0.05% Tween® (Sigma-Aldrich 129K00412) wash. The test volume was 10 µL. The range of sensitivity for the assay was 62.5 to 4000 pg/mL. The inter- and intra-assay were 5.2% and 3.7%, respectively.

Salivary Transferrin. Following Kivlighan and colleagues (Kivlighan et al., 2004), saliva samples were assayed for blood contamination using a commercially available enzyme immunoassay kit for transferrin (Salimetrics, State College, PA). The test volume was 20 µl, and the range of sensitivity was 0.08 to 6.6 mg/dL. The inter- and intra-assay CVs were 4.7% and 2.9%, respectively.

Salivary Total Protein. Salivary total protein was examined as an indirect indicator of salivary flow rate. Total protein was determined using a commercial protein

assay kit (Pierce™ BCA Catalog # 23225, ThermoScientific) and following the manufacturer's guidelines for the microplate procedure. The test volume was 25 µl and the range of sensitivity was 20 to 2000 µg/mL. Saliva samples were assayed neat (undiluted). After incubation, the 96-well plate was read in an ELx800 absorbance reader (BioTek) at 562 nm and analyzed in Gen5 software using a four-parameter logistical curve fit against an albumin standard (cat# 23209 Pierce™) prepared by serial dilution in PBS.

Analytic Plan

The quality of the serum and salivary analyte data was first examined, including the level of censoring due to undetectable concentrations and the distribution of the data for each analyte. Determinations falling below the LLD were replaced as missing. All analyte data were positively skewed. Data were Winsorized and log-transformed to improve the normality of the distributions. Winsorization was performed by analyte to bring all concentrations within four standard deviations of the mean. Raw data for all analytes were first examined, then Winsorized and log-transformed data were used in all subsequent statistical analyses.

Levels of adiponectin in serum and saliva were compared using paired samples *t*-tests. The association between salivary adiponectin and oral health was examined using both self-reported and biologic measures of oral hygiene practices and health. First, Pearson correlations examined variation in salivary adiponectin by the number of times participants reported flossing per week. A Kruskal-Wallis test examined differences in

salivary adiponectin by the frequency of brushing per day, and an independent samples *t*-test examined whether salivary adiponectin levels were different for participants who reported red/pink tinted saliva when brushing and those that did not. The relation between salivary adiponectin and oral health was also examined using Pearson correlation with salivary adiponectin and salivary MMP-8.

The association between adiponectin and inflammatory activity was examined within and across biospecimens using Pearson correlations and serum and salivary determinations of adiponectin, IL-1β, IL-6, IL-8, and TNFα. Further, a principal components analysis (PCA) was conducted to assess the correlation structure of the inflammatory biomarkers in saliva, and to determine whether a composite score for salivary inflammatory activity could be constructed. A PCA of the four salivary inflammatory biomarkers (IL-1β, IL-6, IL-8, and TNFα) pointed to one underlying component explaining approximately 76% of the variance in inflammatory biomarker levels. The single factor PCA solution resulted in high component loadings for all four analytes ranging from 0.83-0.92. A composite score for the oral inflammatory markers was derived from the PCA using the regression method. The associations between the composite oral inflammatory activity score and salivary and serum adiponectin was assessed using Pearson correlations.

The influence of blood contamination and salivary flow rate on salivary adiponectin was examined using Pearson correlations between salivary adiponectin and salivary transferrin and total protein (separately). The associations between both serum

and salivary adiponectin and participant age and BMI were assessed with bivariate correlations. Independent samples *t*-tests were used to examine sex difference in serum and salivary adiponectin.

The serum-saliva correlation for adiponectin was examined using Pearson correlation. Partial Pearson correlations were conducted to examine the serum-saliva associations for adiponectin controlling for serum leakage into the oral mucosal compartment (transferrin), salivary flow rate (total protein), oral inflammatory activity (cytokines) and oral health (MMP-8). All analyses were conducted using SPSS 22.

Results

Participants

Participants were about half male (53% male) and the mean age was 23.76 years old (SD= 4.57). Most participants were of a healthy weight, with 61% of participants falling in the "normal" range for BMI (mean BMI= 23.56, SD= 3.67, range= 15.78, 36.96).

On self-reported measures of oral hygiene practices, participants flossed, on average, 2.07 times a week (SD= 2.29, range= 0-7 times a week), and brushed their teeth between one and three times a day (M= 1.98, SD= 0.36). Twenty-seven percent of participants reported bleeding gums.

Descriptive Statistics of all Analytes

Table 1 displays descriptive statistics for all analytes using raw data (censored determinations were replaced with zero for descriptive purposes in this table). Across all

analytes 0-2% of concentrations were Winsorized.

Table 1. *Descriptive Statistics of Raw Data for all Analytes in Saliva and Serum (N=99).*

	Number of undetectable samples	Number of samples CV > 15%	Mean	*SD*	Minimum	Maximum
Saliva						
Adiponectin (ng/mL)	0	0	9.87	10.75	.33	59.22
MMP-8 (pg/mL)	0	6	63822.42	45251.89	957.16	203033.28
IL-1β (pg/mL)	0	0	264.04	255.85	9.10	1591.18
IL-6 (pg/mL)	0	0	10.09	16.72	.35	120.93
IL-8 (pg/mL)	0	0	935.77	714.76	109.11	3374.14
TNFα (pg/mL)	0	0	5.13	11.95	.16	110.67
Total Protein (mg/mL)	0	0	700.76	364.09	148.50	1911.20
Transferrin (mg/dL)	3	0	.72	.85	0.00	4.69
Serum						
Adiponectin (µg/mL)	0	0	11.06	5.07	2.82	32.61
IL-1β (pg/mL)	0	0	1.20	.77	.28	6.23
IL-6 (pg/mL)	0	0	1.25	2.45	.17	18.90
IL-8 (pg/mL)	0	0	15.43	33.29	2.26	246.20
TNFα (pg/mL)	0	0	4.77	12.55	.93	111.72

Note. Samples that were not detectable because they were below the assay lower limit of detection were set to 0 for the purpose of descriptive statistics only. CV=coefficients of variation; SD=Standard deviation; MMP-8= matrix metalloproteinase-8; IL-1β=interleukin-1β; IL-6=interleukin-6; IL-8=interleukin-8; TNFα=tumor necrosis factor alpha.

Comparison of Adiponectin in Serum and Saliva

Levels of adiponectin in serum were higher than in saliva (Table 1; $t(95)= 79.58$, $p < .01$).

Salivary Adiponectin and Oral Health

Salivary adiponectin was not significantly related to the frequency of flossing per week nor the frequency of daily brushing. There were also no significant differences in salivary adiponectin levels for individuals who reported bleeding gums compared to those who did not. Salivary adiponectin was, however, significantly positively associated with salivary MMP-8 (Table 2).

Table 2. Correlations Between Adiponectin and Other Analytes in Saliva and Serum

	Salivary Adiponectin		Serum Adiponectin	
	N	Pearson's *r*	N	Pearson's *r*
Salivary Analytes				
MMP-8	87	.65*	90	.04
IL-1β	96	.62*	99	-.06
IL-6	96	.74*	99	.13
IL-8	96	.74*	99	.18
TNFα	96	.56*	99	-.01
Composite Oral Inflammatory Activity Score	96	.76*	84	.07
Total Protein	96	.47*	99	
Transferrin	93	.79*	96	
Serum Analytes				
IL-1β	96	-.19	99	-.02
IL-6	96	.06	99	.04
IL-8	96	.06	99	.15
TNFα	96	-.15	99	-.03

* $p<.01$. MMP-8= matrix metalloproteinase-8; IL-1β=interleukin-1β; IL-6=interleukin-6; IL-8=interleukin-8; TNFα=tumor necrosis factor alpha.

Adiponectin and Inflammatory Activity

Salivary adiponectin was positively associated with all salivary inflammatory cytokines and with the composite oral inflammatory activity score (Table 2; Figure 1). In contrast, there were no significant associations between salivary adiponectin and serum

inflammatory cytokines (Table 2). Serum adiponectin was not significantly associated with any inflammatory cytokine determination in serum or saliva (Table 2).

Figure 1. Correlation between Salivary Adiponectin and Oral Inflammatory Activity in Healthy Young Adults (*N*=96)

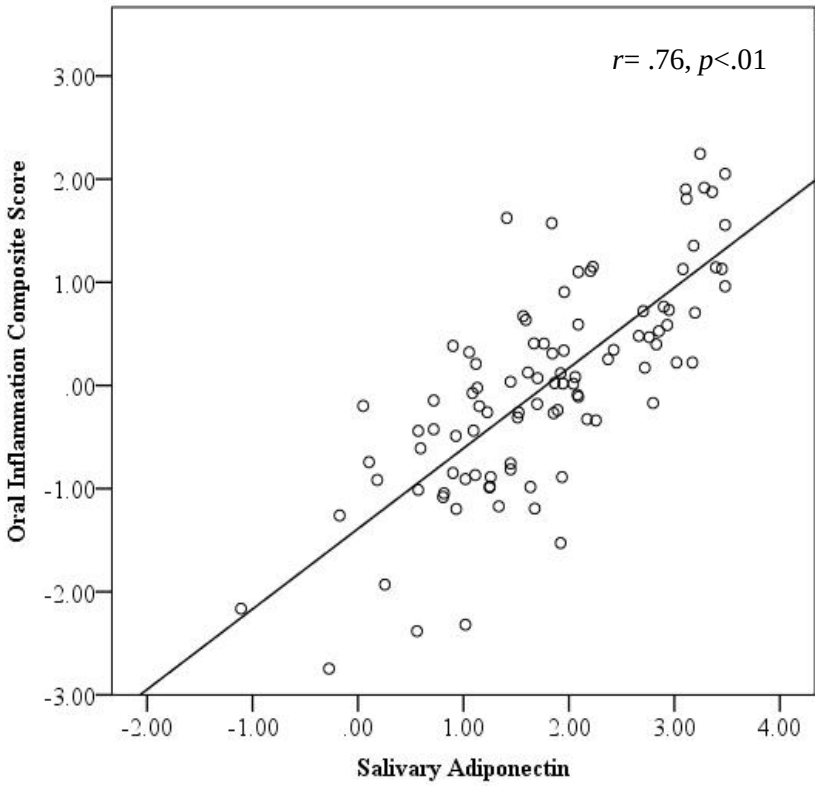

Figure Caption: Salivary adiponectin was significantly positively associated with oral inflammatory activity.

Note: Oral Inflammation Composite Score is a composite of salivary IL-1β, IL-6, IL-8 and TNFα determinations.
Winsorized and log-transformed data were used in all analyses.

Salivary Adiponectin, Blood Contamination, and Total Protein

Salivary adiponectin was positively correlated with salivary transferrin and with salivary total protein (Table 2).

Adiponectin with Age, Sex and Body Mass Index

Neither serum nor salivary adiponectin was associated with participant age or BMI. Females had significantly higher levels of serum adiponectin (M= 12.71 µg/ml, SD= 5.013 µg/ml) than males (M= 9.76 µg/ml, SD= 4.79 µg/ml), $t(93)= 3.21$, $p< .01$). However, there were no sex differences in salivary adiponectin.

Serum-saliva Correlation for Adiponectin

Figure 2 illustrates the bivariate and partial serum-saliva correlations for adiponectin. Serum and salivary adiponectin were positively correlated (r= .39, p<.01; d= .85). To adjust for variations in blood contamination and salivary flow rate, serum-saliva correlations were performed controlling for salivary transferrin and total protein. When controlling for transferrin, there was a significant positive partial correlation between salivary and serum adiponectin (n= 92; r= .43, p< .01; d= .95). Similarly, when controlling for salivary total protein, the serum saliva correlation for adiponectin was positive and significant (n= 95; r= .47, p< .01; d= 1.06). Further, given the significant associations between salivary adiponectin and markers of oral inflammatory activity, partial correlations were conducted for serum and salivary adiponectin controlling for inflammatory cytokines in saliva. Controlling for the composite oral inflammatory activity score, the strength of the serum-saliva correlation for adiponectin increased (n=

Figure 2. Serum-saliva Correlations for Adiponectin in Healthy Young Adults.

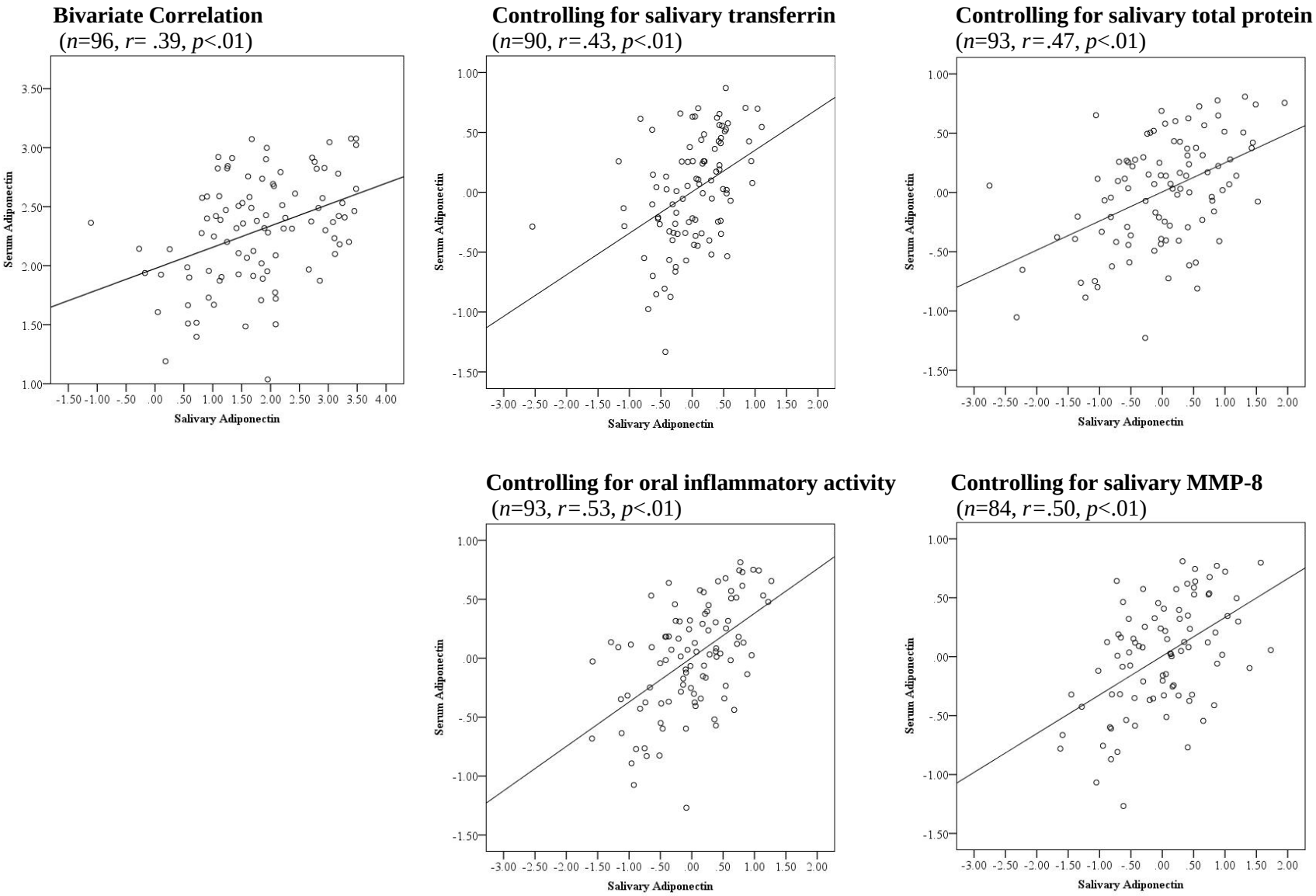

Figure Caption: Serum-saliva correlations for adiponectin were stronger when controlling for potential confounders.
Note: Oral inflammatory activity is a composite of salivary IL-1β, IL-6, IL-8 and TNFα determinations. Winsorized and log-transformed data were used in all analyses. MMP-8= matrix metalloproteinase-8.

95; r= .53, p< .01; d= 1.25). Similarly, the strength of the serum-saliva adiponectin partial correlation increased when controlling for salivary MMP-8 (n= 80; r=.50, p<.01; d= 1.15).

Discussion

This study both confirms previous findings related to adiponectin and provides new insight into the characteristics and correlates of adiponectin measured in serum and saliva in healthy young adults. In particular, the study findings provide novel information about the utility of salivary adiponectin as a marker for serum adiponectin activity.

Serum and Saliva Adiponectin and Associations with Age, Sex and Body Mass Index

Our findings are consistent with prior literature showing that adiponectin levels are considerably lower in saliva compared to serum (Lin, Maeda, Fukuhara, Shimomura, & Ito, 2014). Also consistent with previous studies, we found that serum adiponectin levels were higher in females compared to males (Robinson et al., 2011), while salivary adiponectin did not vary by sex (Thanakun et al., 2013). There were also no differences in adiponectin (in serum nor saliva) related to age.

In our sample of healthy young adults, the majority of whom were of a healthy weight, we did not find any associations between adiponectin (in serum or saliva) and BMI. While serum adiponectin has consistently been associated with BMI and obesity (Lihn et al., 2005), the link between salivary adiponectin and BMI is not as well supported (Mamali et al., 2012; Nigro et al., 2015). The overall health and narrow range

of BMI in our sample may have made it more difficult to see an adiponectin-BMI association. The relation between BMI and adiponectin may be most pronounced in overweight or at-risk populations where metabolic-related diseases are more likely.

Adiponectin and Inflammatory Activity and Oral Health

The findings confirmed our hypothesis that salivary adiponectin is strongly associated with measures of oral inflammatory activity and oral health. We found strong positive associations between salivary adiponectin and indices of oral inflammatory activity and oral health (IL-1β, IL-6, IL-8, TNFα and MMP-8). While consistent with findings from a previous study of healthy adolescent girls (Riis et al., 2013), these findings are contrary to findings from *in vitro* studies of adiponectin's effect on oral epithelial cells, primary gingival fibroblasts, and periodontal ligament cells (Iwayama et al., 2012; Kraus et al., 2012). Together, the findings demonstrate the importance of assessing adiponectin's relations with inflammation and health using various approaches and populations; the function and proprieties of adiponectin may vary based on characteristics of the hormone, its receptors and the health of the individual (Almabouada et al., 2013; Li & Wu, 2012).

Despite strong adiponectin-immune relations in the oral cavity, salivary adiponectin was unrelated to self-reported oral hygiene practices. This suggests that these self-reported oral health indices are insufficient measures of the oral immune environment. The oral hygiene practices questions used in the current study are commonly used by salivary bioscience researchers to assess oral health in their analyses.

Our results, however, suggest that these indices do not adequately capture the impact of the oral immune environment on salivary adiponectin. To better control for the oral health and the oral immune environment, researchers should assess salivary immunologic markers.

We found no evidence of the systemic anti-inflammatory effects of adiponectin in our sample. While the anti-inflammatory effects of adiponectin are well-documented (Fasshauer & Bluher, 2015; Lihn et al., 2005; Robinson et al., 2011), the influence of adiponectin on the surrounding cells likely depends on the health of the individual, and on the availably of different isoforms and receptor types (Almabouada et al., 2013; Li & Wu, 2012; D. Liu, Luo, & Li, 2015). While it remains unclear why we found no significant relations between adiponectin and serum cytokines, it is possible that the overall health and homogeneity of our sample made it more difficult to see adiponectin-immune relations that may be more pronounced under conditions of stress, disease or acute inflammation.

Salivary Confounders of Adiponectin Levels

The validity of salivary analyte measures as indices of systemic analyte activity depends on several factors– most notably the level of blood contamination in the saliva and salivary flow rate. Blood in saliva introduces the possibility that salivary analyte concentrations represent greater than normal contributions of serum analyte levels. Blood leakage into saliva can also be considered an index of oral health. The positive association observed between salivary adiponectin and transferrin may reflect

adiponectin's association with the oral immune environment and/or the effects of blood contamination which may elevate measured adiponectin levels as increased adiponectin is introduced via blood.

The effect of salivary flow rate on analyte levels depends on several characteristics of the analyte, in particular, its molecular weight and solubility, and the mechanism by which the analyte enters saliva. We found that total protein was positively associated with adiponectin levels. These findings suggest that flow rate and blood contamination are two important factors that should be adjusted for when studying salivary adiponectin.

Adiponectin Serum-Saliva Relations

Similar to prior investigations, we found a significant, but modest, positive association between serum and salivary adiponectin levels (Mamali et al., 2012; Thanakun et al., 2013). Our findings further demonstrate that the magnitude of the serum-saliva association is slightly strengthened when controlling for measures of flow rate and for blood contamination. In contrast, controlling for oral health and inflammatory markers considerably strengthened the association between salivary and serum adiponectin. This, along with strong associations between salivary inflammatory biomarkers and salivary adiponectin, suggests that salivary adiponectin is strongly associated with immune functioning within the oral cavity, and that adjusting for the oral immune environment may improve the utility of salivary adiponectin as a marker of systemic adiponectin activity.

Strengths and Limitations

Our data were drawn from a large sample of young adults. The findings are strengthened by a wealth of analytic data and matched samples which allowed us to directly examine serum-saliva relations controlling for a number of possible confounding factors. Despite the large sample size, the homogeneity of the sample limits the generalizability of the findings to young individuals with relatively good oral and physical health. Additional research is needed to explore the nature of adiponectin levels in serum and saliva in populations with acute and chronic oral and systemic diseases, and under conditions of stress and acute inflammation. The homogeneity of the sample also prohibited us from examining race and ethnicity differences in adiponectin levels. Race and ethnicity differences in adiponectin have been found in prior studies (e.g., Hulver, Saleh, MacDonald, Pories, & Barakat, 2004; Lopez-Jaramillo et al., 2014; Ohman-Hanson et al., 2016) and future study of these differences may help elucidate mechanisms underlying health disparities in cardiometabolic conditions.

Adiponectin circulates in three different molecular weight complexes: a trimer (low molecular weight), hexamer (medium molecular weight), and a high molecular weight oligomer (HMW; Liu & Liu, 2014). Studies suggest that the primary species of adiponectin in oral fluid is even more complex, a super HMW oligomer (Nigro et al., 2015). Antibodies in the adiponectin assays may not necessarily recognize each species with the same affinity and may differ in avidity effects with the various complexes of adiponectin. Our anecdotal observations suggest that the levels of adiponectin measured

in saliva using modifications of assays produced by different manufacturers, although highly intercorrelated, yield very different adiponectin levels. This may have restricted the magnitude of the observed serum-saliva association. Our assessment of overall adiponectin, rather than isolated adiponectin isoforms, may have also complicated our examination of the BMI-adiponectin relationship as the different isoforms have been found to correlate differently with changes in weight (Engl et al., 2007).

Moreover, while total adiponectin in the present study was associated with oral inflammatory activity, the actual function of adiponectin in the oral environment remains a matter of debate and likely depends on the roles of different isoforms and possibly different patterns of expression of adiponectin receptors (Almabouada et al., 2013). For example, a positive effect of total adiponectin on skin keratinocyte proliferation has been reported in mice where adiponectin was directly associated with cutaneous wound healing (Shibata et al., 2012), and this effect was confirmed in human periodontal ligament (PDL) cells (Nokhbehsaim et al., 2014). However, a study in human gingival keratinocytes (GK) found no effect of total adiponectin alone on cell proliferation and an inhibitive effect in combination with *P. gingivalis* LPS (Kraus et al., 2012). Likewise, in separate studies, neither MMP-1, IL-6 nor IL-8 protein expression was affected by total or globular adiponectin in human GK, gingival fibroblasts (GF) or PDL cells (Iwayama et al., 2012; Kraus et al., 2012; Park et al., 2011). Yet, combined stimulation of human GK with *P. gingivalis* LPS and adiponectin, or of GFs with IL-1β and adiponectin, resulted in

decreased IL-6 and IL-8 protein expression compared to IL-1β /LPS stimulation alone (Iwayama et al., 2012; Kraus et al., 2012).

Conclusion

Our findings suggest that salivary adiponectin is strongly related to the oral immune environment. Future research should further examine salivary adiponectin as a marker of oral health risks, such as gingivitis and periodontal disease, in the general population. The utility of systemic adiponectin levels as a marker of disease risk has been the focus of many studies. Promising findings suggest that tracking serum adiponectin levels may help in the early detection and disease monitoring of several of the most prevalent and costly diseases, such as metabolic syndrome and type-2 diabetes, and rheumatoid arthritis (Fasshauer & Bluher, 2015; D. Liu et al., 2015). While additional research is needed, particularly with at-risk and diseased participants, our findings suggest that salivary adiponectin, when assessed along with the oral immune environment, may offer a minimally-invasive and less expensive assessment of systemic adiponectin levels. Future research should continue to explore ways to maximize the validity and reliability of salivary adiponectin as a measure of systemic adiponectin activity.

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