Salivary amylase and adiponectin as potential non-invasive markers of glycaemic control in Malaysian type 2 diabetes mellitus participants

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Abstract

Introduction

Serum amylase and adiponectin levels have shown promise as markers of cardio-metabolic diseases. However, the levels of these markers in saliva and their association with glycaemic management in diabetes mellitus (DM) are not well documented. Therefore, we investigated the correlation of salivary amylase and adiponectin concentrations with measures of glycaemic control in type 2 diabetes mellitus (T2DM) participants.

Methods

We conducted a cross-sectional study involving 80 T2DM participants of Indian and Malay ethnicity. Saliva was collected, and salivary amylase and adiponectin concentrations were analysed. Recent fasting blood sugar and HbA1c of the participants was obtained form their medical records. The correlations of salivary amylase and adiponectin with fasting blood sugar and HbA1c were calculated using Spearman's correlation.

Results

There was a weak positive correlation between salivary adiponectin and HbA1c (rho = 0.221, p = 0.051). The salivary adiponectin levels was significnalty lower among participants with good glycaemic control (HbA1c ≤ 7.0%) compared to those with poor glycaemic control (HbA1c > 7.0%,) (1.13 (1.75) vs. 2.34 (3.54) ng/ml, p = 0.039).

Conclusion

Salivary adiponectin weakly correlated with HbA1c, while salivary amylase showed no correlation with the glycaemic parameters studied. Therefore, salivary adiponectin may warrant further investigation as a potential non-invasive biomarker of T2DM.

Key words: Type 2 diabetes mellitus (T2DM); salivary adiponectin; salivary amylase; haemoglobin A1c (HbA1c)

Introduction

Diabetes mellitus (DM) is a metabolic disorder, which happens when the pancreas does not produce adequate insulin, or when the body cannot effectively use the insulin produced leading to hyperglycaemia.[1] Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance and insulin deficiency, where insulin secreted by the body is inadequate to overcome insulin resistance.[2]

Proper glycaemic control through frequent monitoring of fasting blood sugar (FBS) and HbA1c is crucial in preventing severe complications.[3] Hence, a monitoring tool that is less invasive than HbA1c and fasting blood glucose tests, which are the current standard tests, would make monitoring of glycaemic control in diabetics more patient-friendly. Furthermore, the venepuncture procedure may be avoided, as some patients find it painful. This may potentially lead to poorer detection, management and increase in complications of the disease. There is a need to develop non-invasive biomarkers to diagnose and monitor T2DM; and saliva contains the most promising biomarkers that can be used for early diagnosis, prognostic and post-therapeutic monitoring and management of T2DM.[4] Salivary amylase and adiponectin are two such potential biomarkers.[5,6] From studies investigating salivary composition, it was found that salivary flow rate in diabetics was significantly reduced when compared with non-diabetic controls.[7] Saliva contains mucous secretions MG1 and MG2 which have the ability to form large complexes with proline-rich proteins, statherin, amylase and other proteins.[8] Serum amylase has been purported to be useful as a biomarker for T2DM as it was found to be inversely associated with most cardio-metabolic risk factors.[9] Furthermore,

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in participants with low serum amylase, the severity of metabolic abnormalities worsened over 5-years.[10] Low serum amylase was associated with a high level of insulin resistance in asymptomatic T2DM participants who were not receiving treatment.[11] In agreement with these findings, lower salivary amylase levels have been observed in diabetic patients when compared to normal participants;[10] indicating that salivary amylase may be useful in diagnosing T2DM.

Saliva also contains adiponectin, an adipokine, which has anti-inflammatory and insulin-sensitizing properties.[12] Reduced adiponectin levels have been associated with numerous conditions such as insulin resistance, atherosclerosis and dyslipidemia.[13] The few studies reported appear to suggest that the risk of developing T2DM decreases with increased plasma adiponectin levels.[14-16] To date, the association between salivary adiponectin and indicators of glycaemic control in T2DM has not been established. However, studies have found that salivary adiponectin had a correlation with plasma adiponectin.[17]

Thus, the objective of this study is to evaluate the associations between salivary amylase and adiponectin levels with measures of glycaemic control in Malaysian T2DM patients of Indian and Malay ethnicities.

Materials and Methods

Study design: A cross sectional study was carried out in 2015 to investigate the associations between salivary amylase and adiponectin levels with measures of glycaemic control in Malaysian T2DM patients of Indian and Malay ethnicities.

Setting: The experimental procedures gained approval from the International Medical University, Kuala Lumpur Research and Ethics committee BMS I-1/2015 (04). This study was also registered with the Malaysian National Medical Research Register (NMRR) with the registration number NMRR-15-733-24909. Participants were recruited from the venepuncture room in a Health Clinic (Klinik Kesihatan) in Seremban during April to June 2015.

Participants: 83 volunteers were screened for inclusion and exclusion criteria of this study (Table 1). Of these, 80 volunteers were found to be eligible and were recruited once they gave informed written consent to participate in the study. The study enrolled participants of Malay and Indian ethnicities as these groups were predominantly represented in the Malaysian diabetic population.[18]

Data sources/measurement

Fasting blood sugar and HbA1c Measurements

Participants recruited had phlebotomy done on the same day as the saliva samples were taken. FBS and HbA1c results were obtained directly from the clinic laboratory. Thus, FBS and HbA1c results were obtained from the laboratory of the health clinic in Seremban on the same day the saliva samples were collected. Further participant details such as diabetic medications, blood pressure, fasting lipid levels were obtained from the clinic records when available.

Saliva collection

Participants were requested to fast overnight for a minimum of 8 hours. Saliva collection was carried out within 7.30 am to 9 am for all participants to prevent variability of the saliva secretory rate, which follows a circadian rhythm[5,17] Sitting comfortably upright and calm, the participants' unstimulated saliva samples were collected in 15 mL polypropylene tubes by the passive drool method for 2 minutes.[17] The participant was

seated upright, and their head leaned forward over the polypropylene tube, allowing their saliva to drain into the tube.[17] The collection tubes of unstimulated saliva samples were weighed before and after sample collection for the determination of salivary flow rate. The samples in the 15 mL polypropylene tubes were centrifuged at *2000g* for 10 minutes at 4°C, then stored in aliquots of 100 µL at -80°C for future analysis. The salivary samples were analyzed using suitable commercial kits at the research laboratories at the International Medical University (IMU).

Salivary amylase analysis

A commercial colorimetric kit [Amylase Activity Colorimetric Assay Kit (K711-100; BioVision, USA)] was used to determine the activity of salivary amylase in the saliva samples. The assay was performed as recommended by the manufacturer.

Salivary adiponectin analysis

The activity of salivary adiponectin in the saliva samples was determined using a commercial adiponectin enzyme-linked immunosorbent assay (ELISA) kit [Human Adiponectin Platinum ELISA (BMS2032)] using the manufacturer recommended protocol (eBioscience, USA).

Statistical methods

Study size: Sample size calculation showed that a minimum of 67 participants were required to detect a correlation of 0.3 between the variables of interest. This number was calculated using the correlation coefficient formula where power=0.8 and alpha (α)=0.05. However, to account for missing data, an additional 20% was recruited with a target of 80 participants.

Statistical analysis: Descriptive statistics was done for all important variables (e.g. participants' characteristics). With respect to non-normal variables, non-parametric statistical tests were applied. Spearman's test was performed to assess the correlation between amylase, adiponectin, FBS and HbA1c levels. To compare the salivary flow rate, salivary amylase and adiponectin concentrations between the two ethnic groups, Mann-Whitney U test was used. The statistical significance was set at 0.05 and SPSS software (Version 20, IBM) was used for statistical analysis

Results

Participants: The demographic profile of the participants involved in this study is presented in Table 2. Majority of the participants were females (66.3%) of Indian ethnicity (61.3 %) with the median age of 55 (IQR: 7.75) years. On average, their duration of illness with T2DM was 8.2 (±7.8) years.

Blood biochemical tests: Only 50 out of the 80 participants had their FBS levels available and this information was obtained from the Seremban Health Clinic's laboratory records. The FBS levels for these 50 participants were found to range from 4.2 to 22.5 mmol/l (Figure 1). The HbA1c levels of 78 out of the 80 participants were also obtained from the laboratory records of the same health clinic. The HbA1c levels were found to range from 5.4% to 15.8% (Figure 2).

Salivary profile: The unstimulated flow rates ranged from 0.11 ml/min to 1.57 ml/min (Figure 3). The salivary amylase activities in the unstimulated saliva samples of the 80 participants ranged from 1.55 U/ml to 95.43 U/ml (Figure 4). The salivary adiponectin concentrations in the unstimulated saliva samples of the 80 subjects ranged from 0 ng/ml (undetectable amounts) to 24.16 ng/ml (Figure 5).

Correlations between blood parameters and saliva: There was no correlation between blood glycemic parameters and salivary amylase or adiponectin (Table 3). However, a weak positive correlation was observed between HbA1c levels and salivary adiponectin concentrations, which almost reached statistical significance. Since there were both participants with poor (HbA1c > 7.0%) and good glycaemic control (HbA1c ≤ 7.0%), the salivary markers were statistically compared between the groups. Only salivary adiponectin concentrations were found to be significantly different between those with poor and good glycemic control (Table 4).

As there were two ethnic groups involved in this study, a secondary analysis was carried out to compare the salivary flow rates, amylase activity and adiponectin concentrations between participants of Indian and Malay ethnicity. The salivary flow rate was significantly ($p < 0.05$) higher among Indians when compared to those of Malay ethnicity; with the Indians having twice the flow rate compared to the Malays (Table 5).

Discussion

This study evaluated the associations between salivary amylase and adiponectin with glycemic control among 80 men and women of Indian and Malay ethnicities with T2DM. From data collected by the National Diabetes Registry of Malaysia (NDR) in 2011, Malays make up the majority of T2DM patients at 58.9% followed by Chinese and Indians at 21.4% and 15.3%, respectively.[18] However, Indians are over-represented in the Malaysian NDR.[18] It was noted that the current study had a higher representation of female Indian participants due to the characteristics of the population attending the clinic used for recruitment. In terms of HbA1c levels, the current study showed a slightly higher average HbA1c level at 8.64%, as compared to the Malaysian national average of 8.1%.[18]

Salivary flow rate

The unstimulated salivary flow rate in two different populations was measured in two earlier studies, by Panchbhai in 2010[10] on a population in India (0.18±0.12 ml/min), and Lasisi[19] in 2012 in Nigeria (0.52ml/min). The current study measured the average unstimulated salivary flow rate to be 0.51 (0.35) ml/min. Relative to the previous two studies, the average salivary flow rate of T2DM Malaysian subjects is higher than the Indian participants (0.18 ± 0.12 ml/min)[10] but compares well with the Nigerian participants (0.52 ml/min)[19].

Salivary amylase activity

As for the salivary amylase activity, Panchbhai's study in India recorded an average unstimulated salivary amylase activity of 108.48 ± 6.37 U/ml in the uncontrolled T2DM group and 100.83 ± 60.77 U/ml in the controlled T2DM group.[10] Another small study conducted in India reported the salivary amylase activity was 2.74 U/ml.[5] A third study, also in India had an unstimulated salivary amylase activity on 19.2±1.8 U/ml.[20] In the current study among Malaysian T2DM subjects the average unstimulated salivary amylase activity was 22.5 (18.3) U/ml and falls within the range of the earlier studies.[5,10,20] The value of unstimulated salivary amylase activity in the current study (22.5 (18.3) U/ml) was most similar to that of Prathiba's research (19.2±1.8 U/ml) as similar methods of analysing salivary amylase with colorimetrical kits were utilised.[20] In comparison, the studies by Malathi and Panchbai were done with kinetic enzyme essays under the direct substrate method.[5,10] Therefore, this could explain the variation in values obtained.

Salivary adiponectin concentration

With respect to salivary adiponectin concentrations, Thanakun in 2014 reported in the Thai population a median value of 2780 (1050-6480) ng/ml.[17] In the current study, the median value for salivary adiponectin concentration was 1.99 (3.22) ng/ml which was significantly lower than the Thai study. The results from the present study are more in line with the salivary adiponectin range of 0.37 to 6.42 ng/ml reported among 188 Japanese males.[21] It is interesting to note these studies[17,21] including the current study were performed with ELISA kits, indicating that there could be other factors influencing the salivary adiponectin concentration.

While variations in salivary amylase have been linked to dietary composition, adiponectin levels are known to show ethnicity related differences.[22] Firstly, salivary amylase has also been found to vary in relation to the carbohydrate content of diets with subjects with high carbohydrate consumption having higher salivary amylase levels.[23] Secondly, ethnicity may be an important determinant of salivary adiponectin levels independent of lifestyle factors.[22] The previous Asian study on salivary adiponectin concentration focused on Thai metabolic syndrome patients and not T2DM patients.[17] Variations in genetic encoding, dietary habits and pathophysiology of disease could explain the variations in the salivary amylase reported among these populations.[24-25] None of the studies so far, however, have looked into salivary amylase and adiponectin within the Malaysian diabetic population, whose daily carbohydrate intake varies significantly from the Western population.

It is interesting to note that the Indian population in the current study showed higher salivary adiponectin concentration than the Malay population. It has been reported among Saudi Arabian diabetic patients, females have statistically significant higher levels of serum adiponectin when compared to males.[26] In the current study a higher representation of the female gender among the Indian diabetic participants, may explain the higher salivary adiponectin levels seen in this ethnicity.

Associations between glycaemic control and salivary flow rate, amylase and adiponectin concentrations

We hypothesized that fasting blood sugar and HbA1c would correlate negatively with salivary amylase activity and adiponectin concentrations. However, findings revealed that salivary amylase activity was not correlated to either fasting blood sugar or HbA1c in Malaysian T2DM subjects. Similarly, there was no significant correlation between fasting blood sugar and salivary adiponectin, however, a trend for weak positive correlation was seen between HbA1c and salivary adiponectin concentration. This finding supports a previous study conducted on plasma adiponectin by Nonogaki, where it was revealed that elevations in HbA1c were associated with elevations in plasma adiponectin in T2DM subjects.[27] However, this lack of correlation between fasting blood glucose and adiponectin is unexpected given that in the healthy population, plasma adiponectin is inversely linked with the risk of developing T2DM.[12,14,28]

It is generally expected that the salivary flow rate would be decreased in T2DM subjects when compared to normal subjects.[10,20] The overall range of salivary flow rate was narrow among the diabetic subjects in this study and was found to be independent of the glycaemic parameters or salivary parameters. Additionally, a study by Arhakis and associates indicated a negative correlation between salivary flow rate and amylase activity, where the flow rate was one of the factors which affected the secretion of salivary amylase in healthy adults.[29] Together, these

findings indicate a physiological adaptation wherein increased salivary flow rate could compensate for the lowered amylase activity and adiponectin levels and this compensation occurs probably prior to the onset of overt diabetes. The current study found a significant difference in the salivary flow rate between that of the Indian and Malay ethnicity with the Indian participants having double the value of salivary flow rate when compared to the Malay population. It is unknown whether this is an indication of the above adaptation. The lack of established literature in these areas precludes any conclusions, given the small sample size and preliminary nature of the current study. In addition, when the participants in the present study were stratified according to their glycemic control, as per the cut-off values prescribed by the American Diabetes Association,[30] there were no significant differences seen in the salivary flow rate and amylase activity between the two groups. These findings agree with those of Panchbhai's study who found no significant differences in salivary flow rate and amylase activity between subjects with controlled and uncontrolled T2DM.[10]

However, it is interesting to note that subjects with poor glycemic control had double the salivary adiponectin concentration than those with good glycemic control and this difference was statistically significant. These findings are in agreement with Nonogaki's study on plasma adiponectin, which indicated that elevations in HbA1c were associated with elevations in plasma adiponectin.[27] These findings contradict previous reports, which showed that plasma adiponectin was decreased in those with metabolic syndrome and salivary adiponectin was not significantly different in healthy and metabolic syndrome subjects.[17] Furthermore, there were more female participants than males in the poor glycaemic control group, posing a question how gender based variations could influence salivary adiponectin concentrations.

Study Limitations

This study is among the preliminary studies to investigate the associations between glycaemic parameters and salivary amylase and adiponectin in Malaysian T2DM patients. However, it is acknowledged that generalizing the results of this preliminary study is limited by its small sample size, lack of representation of Chinese ethnicity. Furthermore, the cross sectional nature of the study prevents assumptions of cause and effect. The choice of study population also does not permit confirmative evaluation regarding the utility of saliva adiponectin for diabetes detection as no non-diabetic control group was included in this pilot study. Validation of a novel biomarker is best done in a prospective study with repeated measurements.[31] This would allow for comparison against "gold standard" markers of disease control and progression and elucidate mechanistic pathways of involvement, rather than mere correlation.[31]

Conclusions

The findings suggest that salivary adiponectin may have a potential role as a non-invasive biomarker of T2DM. To substantiate the findings, future well- designed larger studies including other ethnic groups are recommended.

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Conflicting interest

The authors declare no conflicting interest.

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Table 1: Inclusion and Exclusion Criteria for Participant Recruitment

	INCLUSION CRITERIA	EXCLUSION CRITERIA
Age	• 30 to 60 years old	• Below 30 or above 60 years old
Ethnicity	• Malay and Indians	• Chinese and other ethnicities
Diabetic status	• Diagnosed T2DM patients	• Non T2DM patients or T1DM patients
Pregnancy status	• Not pregnant	• Pregnant
Smoking status	• Non-smokers or former smokers	• Daily or current smokers
Others	• None	• Any reported illness or use of medication known to affect salivary flow
		• Reported severe co-morbidities

Table 2: Demographic Data of Type 2 Diabetes Patients Enrolled In This Study

DEMOGRAPHIC DATA					NUMBER (Total = 80)	PERCENTAGE OF TOTAL (%)
GENDER	Male				27	33.7
	Female				53	66.3
AGE	Median:	55	Min	30		
	IQR	7.75	Max	60		
Number of years with T2DM	Mean ± SE:	8.2±1.03	Min	1		
	SD	7.79	Max	39		
RACE	Malay	31	Male	12		39.7
			Female	19		
	Indian	49	Male	15		61.3
			Female	34		

F: *Female;* ***M:*** *Male;* ***Max:*** *Maximum;* ***Min:*** *Minimum;* ***SE:*** *Standard Error;* ***SD:*** *Standard Deviation;* ***IQR:*** *Interquartile range;* ***T2DM:*** *Type 2 diabetes mellitus*

Table 3: Spearman's Correlation Between Salivary Markers and Measures of Glycemic Control

	Correlation coefficient (rho)	*p-value*
Fasting blood sugar vs. salivary amylase activity	-0.066	0.650
HbA1c vs. salivary amylase activity	-0.007	0.952
Fasting blood sugar vs. salivary adiponectin concentration	0.192	0.182
HbA1c vs. salivary adiponectin concentration	0.221	0.051

Table 4: Comparison Between Salivary Parameters Among Participants with Poor and Good Glycemic Control

	HbA1c ≤ 7.0%, (n=20)	***HbA1c > 7.0%, (n=58)***	***p-value***
Salivary flow rate (ml/min)	0.35 (0.43)	0.49 (0.37)	0.308
Salivary amylase activity (U/ml)	19.8 (16.9)	20.1 (19.2)	0.221
Salivary adiponectin concentration (ng/ml)	1.13 (1.75)	2.34 (3.54)	0.039

Values are presented as Median (Interquartile range)

Table 5: Ethnic Differences In Salivary Parameters

	Indian (N=49)	***Malay (N = 31)***	***p-value***
Salivary flow rate (ml/min)	0.63 (0.43)	0.31 (0.32)	<0.001
Salivary amylase activity (U/ml)	18.9 (16.3)	23.1 (18.6)	0.206
Salivary adiponectin concentration (ng/ml)	1.83 (3.19)	1.53 (2.20)	0.251

Values are presented as Median (Interquartile range)

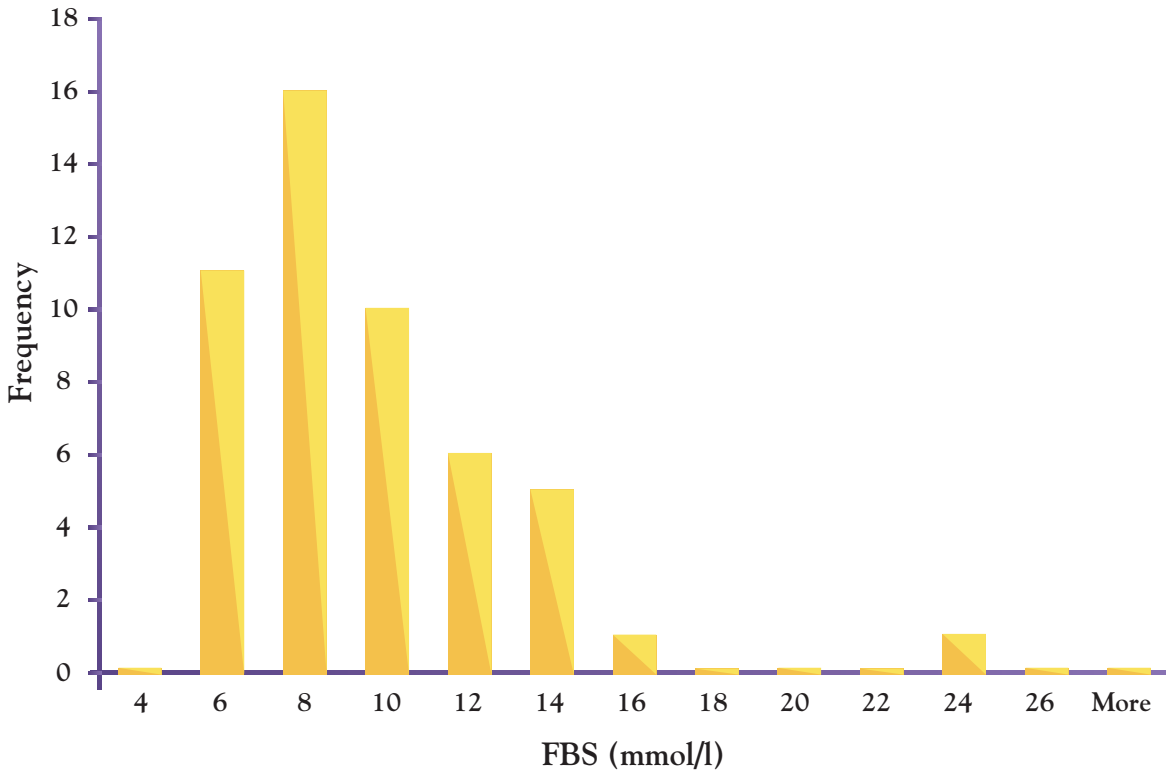

Figure 1: This figure shows the frequency distribution of fasting blood sugar levels with a median of 7.8 mmol/l and IQR of 4 mmol/l.(n=50)

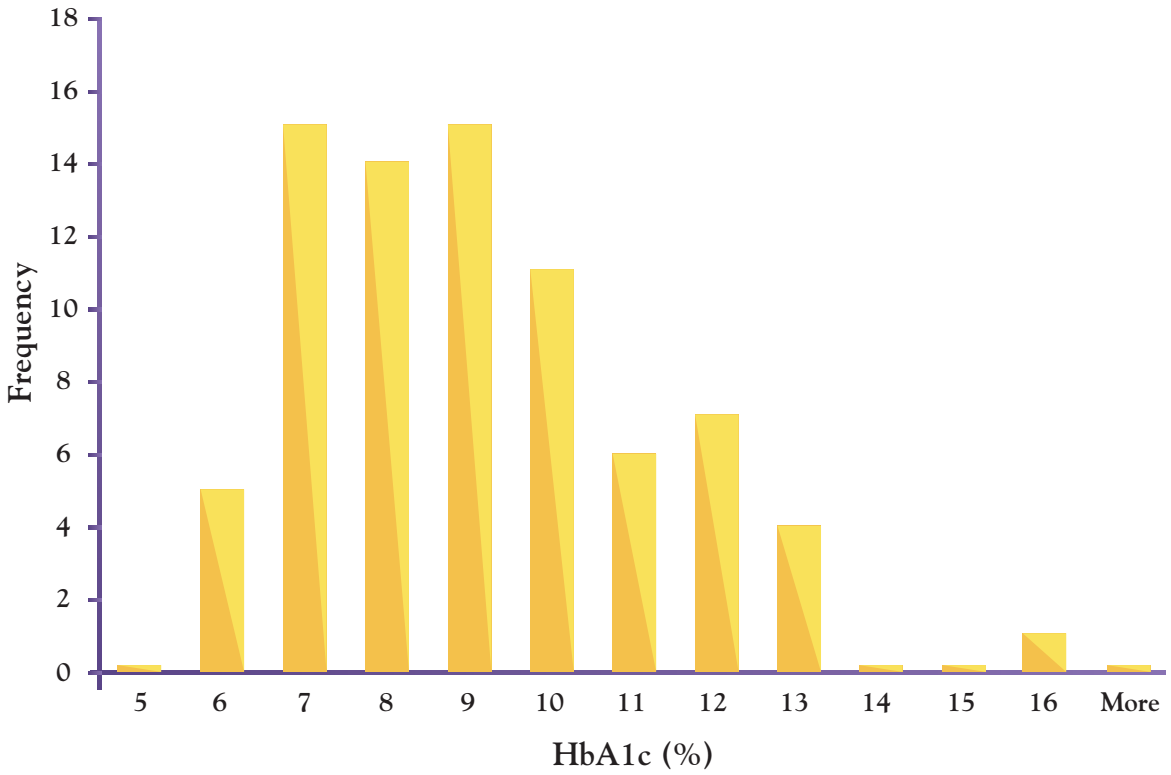

Figure 2: This figure shows the frequency distribution of HbA1c levels with a median of 8.45% and IQR of 2.85%.(n=78)

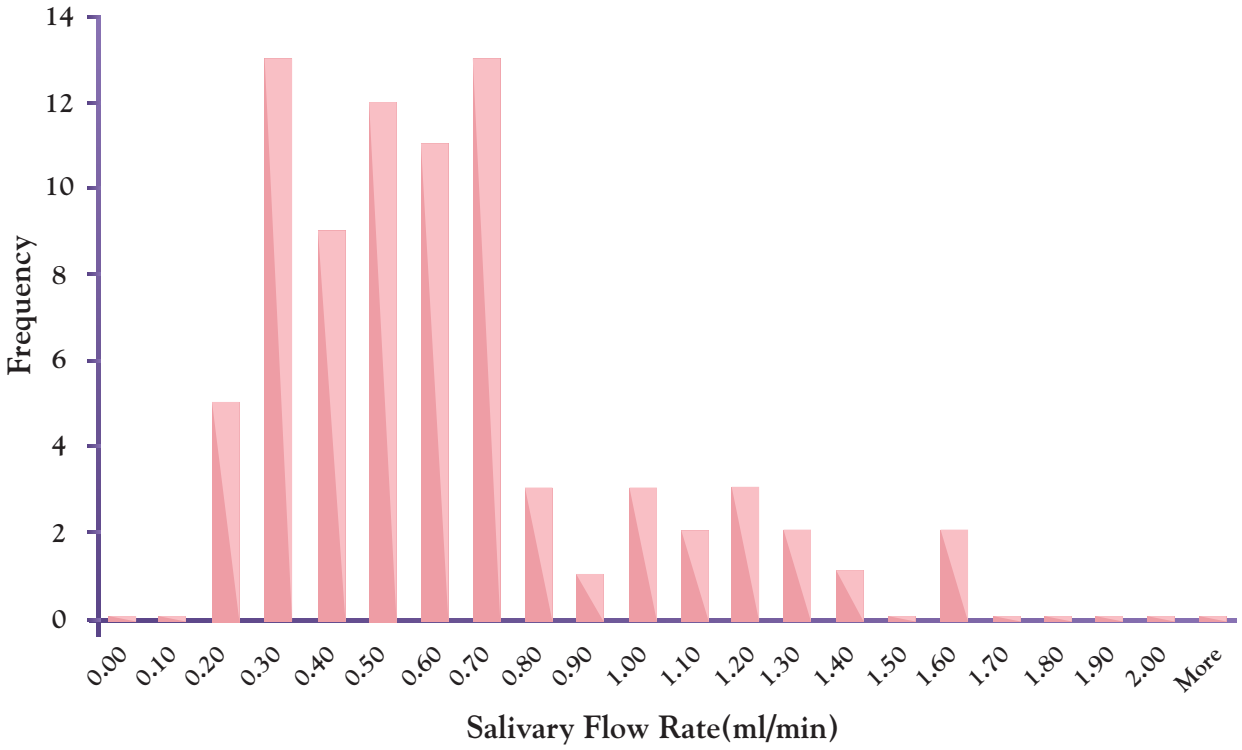

Figure 3: This figure shows the frequency distribution of unstimulated salivary flow rate with a median of 0.51 ml/min and IQR of 0.34 ml/min (n=80)

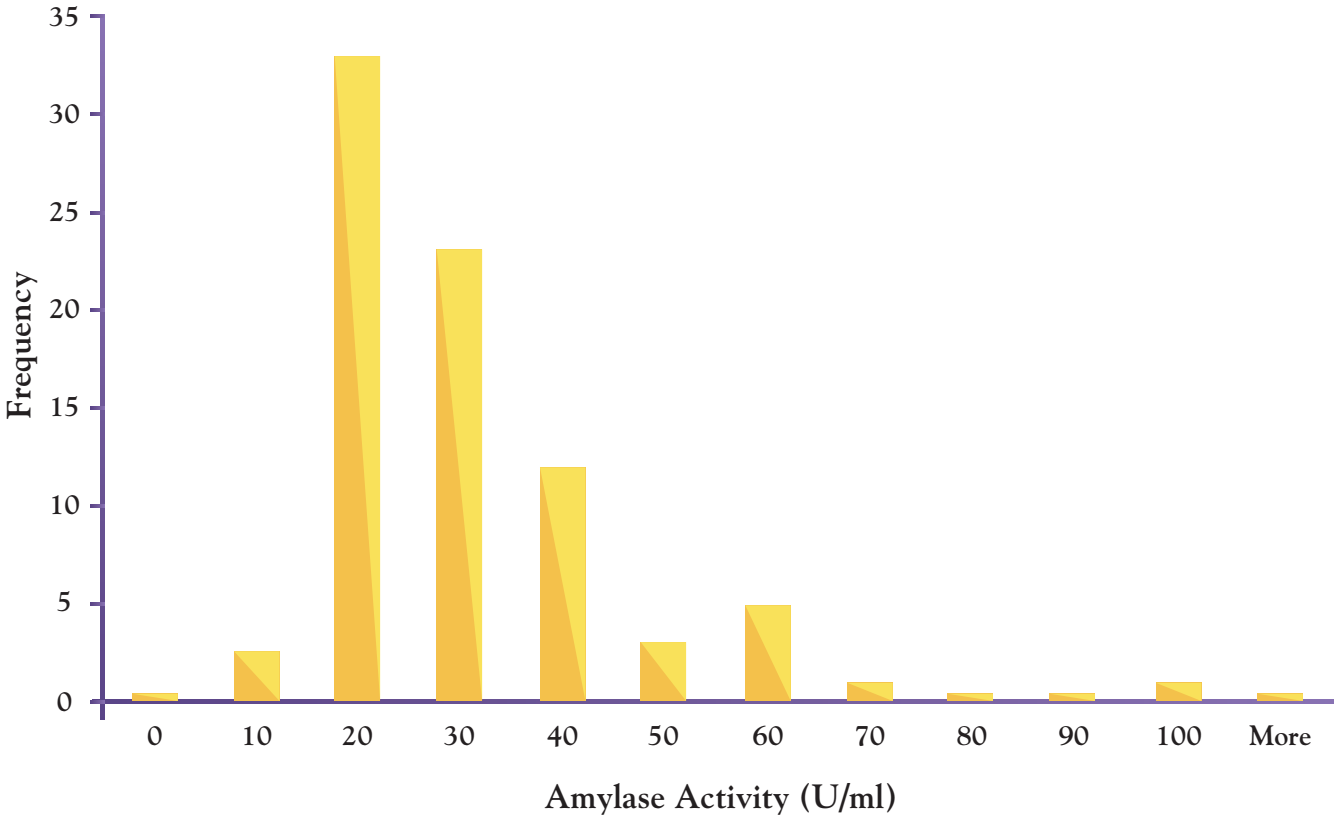

Figure 4: This figure shows the frequency distribution of unstimulated salivary amylase activity with a median of 22.5 U/ml and IQR of 17.2 U/ml.(n=80)

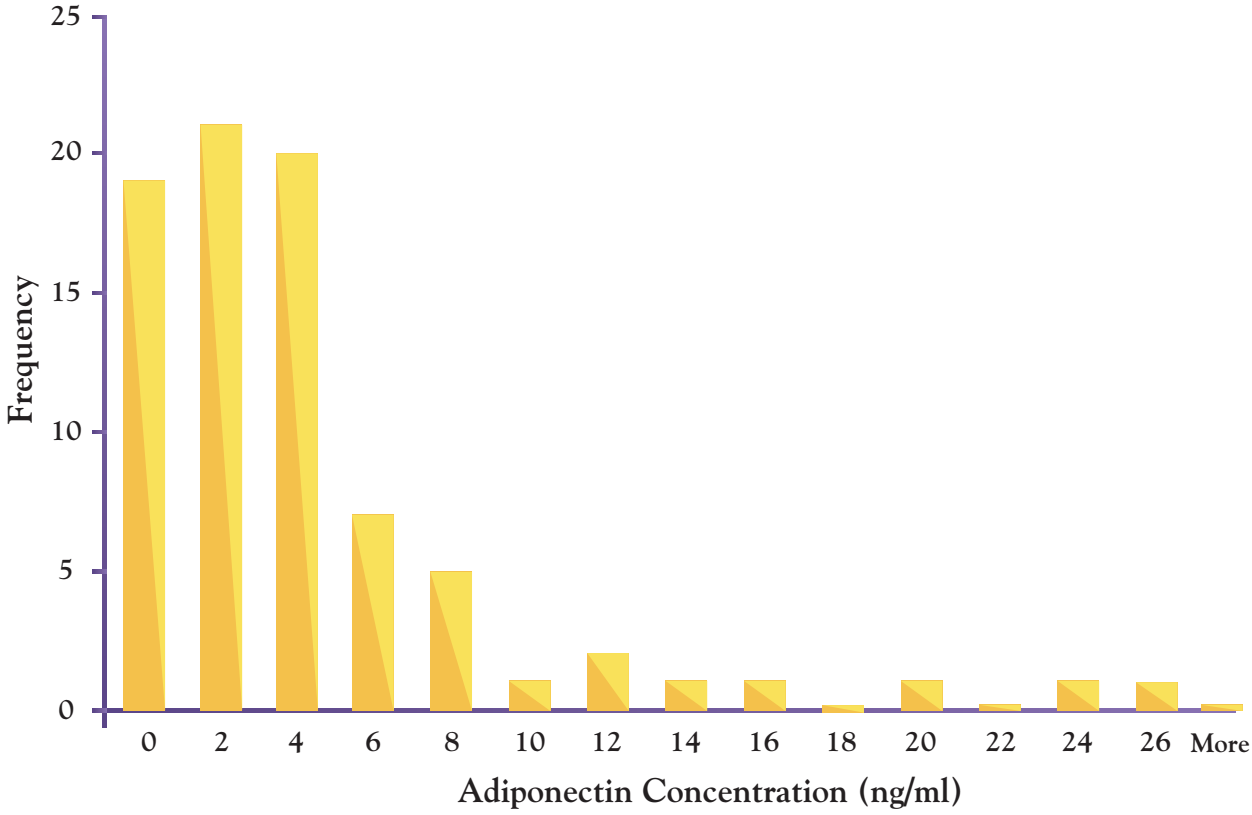

Figure 5: This figure shows the frequency distribution of unstimulated salivary adiponectin concentration with a median of 1.99 ng/ml and IQR of 3.04 ng/ml.(n=80)