Viscosity of whole saliva

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This study investigates within-subject variations and associations of salivary viscosities and flow rates in a test panel of healthy adults. After several practice sessions, unstimulated and stimulated whole saliva samples were collected 5 times daily (at 0800, 1100, 1400, 1700, and 2000 h) from 30 university students. There was a significant within-subject variation in viscosity and flow rate of unstimulated saliva ($P < 0.001$). Intra-item correlations were significantly different for salivary flow rates ($r = 0.82$ for unstimulated, $r = 0.88$ for stimulated, $P < 0.001$) and viscosity of unstimulated saliva ($r = 0.54$, $P < 0.05$), but viscosity of stimulated saliva was different in this respect. Our results indicate that there is a significant within-subject variation in viscosity of unstimulated saliva. □ *Oral physiology; salivary proteins; salivation*

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The lubricating action of saliva is very important for oral health. It facilitates the movements of the tongue and the lips during swallowing and eating and is important for clearly articulated speech (1). Several salivary glycoproteins, including the proline-rich glycoproteins and mucins, have lubricatory roles in saliva. The carbohydrate moieties of these molecules also affect their lubricating properties (2). The significance of the viscosity of saliva in general has been the subject of many studies in dentistry (3). The efficacy of saliva as a lubricant depends on its viscosity and how it changes with shear rate (1). Increased salivary viscosity may also be associated with an increase in dental caries, although it is difficult to examine flow rate and viscosity independently (4). It has been reported that the viscosity (ratio between shear rate and shear stress) of saliva depends on shear rate and on time, so that saliva can be classified as a non-newtonian fluid (5). However, there are no reports on the hourly variation of salivary viscosity of unstimulated or stimulated saliva.

Flow rate and composition of saliva vary rhythmically over 24-h periods (6). However, there are differences in timing of rhythms, as reported by various authors, and the great individual variations in salivary values have not always been taken into account.

We report here our observations about the hourly changes, within-subject variations, and correlations of apparent viscosity and salivary secretion rate of unstimulated and stimulated whole saliva in healthy adults in the course of a 12-h period. Viscosity has not been demonstrated as a clinically relevant salivary variable so far. The aim of our study was to study the variations in salivary viscosity and the possible correlation with the flow rate. The hypothesis to be tested was that within-subject variation of salivary viscosity during repeated measurements is significant and could therefore affect this important aspect of oral physiology and diagnosis.

Materials and methods

Pilot study

In a preliminary study with 18 subjects (10 men, 8 women; mean ages in years ± standard deviations (*s*): men, 25.4 ± 2.5; women, 23.5 ± 1.2; all, 24.6 ± 2.2), the viscometer was tested by calculating the apparent viscosities (η) and shear stresses (τ) at increasing shear rates for stimulated saliva and for the standard liquid. The same measurements were made from 30-min-old saliva samples to test the stability of viscosity during a short time period.

Study group

The study was performed at the Kuopio University Dental Clinic, Finland. A total of 30 young university students, 16 men and 14 women, were included. The mean ages in years ± *s* were as follows: men, 24.2 ± 2.9; women, 21.7 ± 1.5; all, 22.7 ± 2.8 years. All subjects were accepted on a voluntary basis, and only healthy subjects were recruited. Information about the subjects' health was recorded during an interview before collecting the first salivary samples in the morning. A further inclusion criterion was that baseline salivary flow rates should fall within the reference values used in our clinic for healthy adults, that is, resting saliva > 0.1 mL/min and stimulated saliva > 0.7 mL/min. The study protocol had been accepted by the ethics committee of the University of Kuopio.

Collection of saliva

The subjects were given written instructions beforehand regarding the saliva collections. The collection procedure was practiced several times with the researcher before the

collections in order to reduce the bias in repeated measurements. The subjects were told not to eat, drink, or smoke for 1 h before each sampling, but otherwise to act normally through the day. They were instructed to eat breakfast at 0630 h, and this was checked at the interview in the morning. Salivary samples were collected always in restful and quiet circumstances in our laboratory. The subject was seated comfortably, with eyes open. Unstimulated and stimulated saliva samples were collected 5 times daily, at 0800, 1100, 1400, 1700, and 2000 h. Unstimulated saliva was collected for a single 5-min period by having the subject lean forward to let the saliva drain into a graded sampling tube. Before collection the mouth was emptied by an initial swallow. Stimulated whole saliva was collected over a 3-min period, and saliva flow was stimulated by chewing a 1-g piece of paraffin wax (Orion Diagnostica, Espoo, Finland) at a constant rate (about once a second). The flow rates were evaluated visually from graded test tubes.

Viscosity measurements

A Wells Brookfield digital cone-plate viscometer, model LVTDV CP-II (Brookfield Engineering Laboratories, Stoughton, Mass., USA), was used. This instrument gives eight different rotation speeds. A cone of very shallow angle (0.8°, spindle CP-40) is allowed to rotate freely inside the testing cup. When the liquid (saliva) to be tested is introduced between the cone and plate, a resistance to the rotation of the cone is produced. A reading of the dial of the instrument gives a measure of this resistance, or shear stress (τ), of the fluid. The apparent viscosity of the fluid can be calculated from the ratio of the shear stress to the rate of shear (γ) applied.

Apparent viscosity (η) was measured immediately after collection from the fresh saliva samples. When reading the dial, the plateau-value of the viscosity was recorded. The viscometer was calibrated using a standard calibration liquid (Brookfield viscosity standard, 4.2 cP at 37°C) before the 0800 h and 1700 h collections. For each testing 1 mL of saliva was needed, and the tests were performed at a constant temperature of 37°C ($s = 0.1$°C). A constant shear rate of 90 s^{-1} was used.

Statistics

Kolmogorov-Smirnov's test was used to test the normality of distributions. Repeated measures design was used in MANOVA. These statistical tests require that data from repeated samplings correlate with each other, which was tested by correlation plotting. Initial results were computed on the basis of observed concentrations. However, viscosity of saliva can be subject to variation due to differences in flow rates between the subjects. Since flow rate seemed to correlate negatively with viscosity also in our data, we used MANOVA with covariates (flow rate) to test the within-subject variation in the repeated samplings.

To study the relationships between the variables at different time points, we used partial correlations. Partial correlation coefficients and the significance of the correlations between the variables were calculated with the aid of Pearson's formula. Flow rates were controlled for.

Correlations within subjects across samples were expressed as inter-item correlations. These correlations indicate how subjects who show low or high values for a variable remain at the low or high end of the range for each time point.

Results

Pilot study

In the preliminary study shear rates varied incrementally from 2.25 s^{-1} to 450 s^{-1}, and viscosity of stimulated saliva was determined.

The fall in viscosity with higher shear rates was typical of a non-newtonian fluid exhibiting shear-thinning. We also measured viscosities of the same samples 30 min after collection, and the results were almost the same as for fresh saliva (Fig. 1). Given the results of this preliminary study and an earlier study by Roberts (7), we decided to use a shear rate of 90 s^{-1} in repeated measurements because of its stability.

Observed data

The means of the observed viscosities and flow rates and 95% confidence intervals of different samplings are given in Figs. 2 and 3. Initial results were calculated on the basis of observed concentrations. Viscosity of unstimulated saliva was at lowest at 1700 h, and viscosity of stimulated saliva was at highest at 1700 h. However, viscosity of stimulated saliva remained stable throughout the samplings.

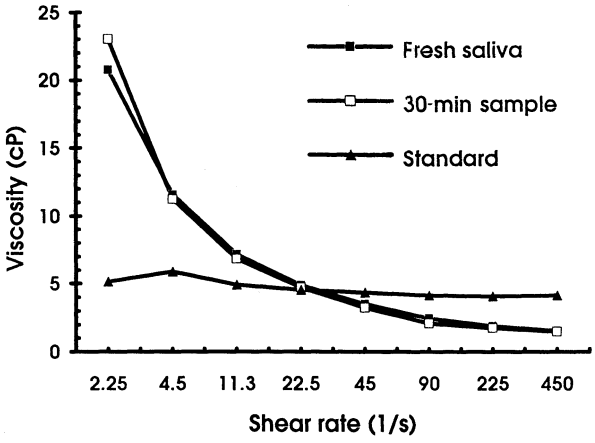

Fig. 1. Shear rate dependent viscosity of stimulated human saliva (fresh samples and after 30-min storing). All measurements with standards were made twice ($n = 18$, temperature = 37°C).

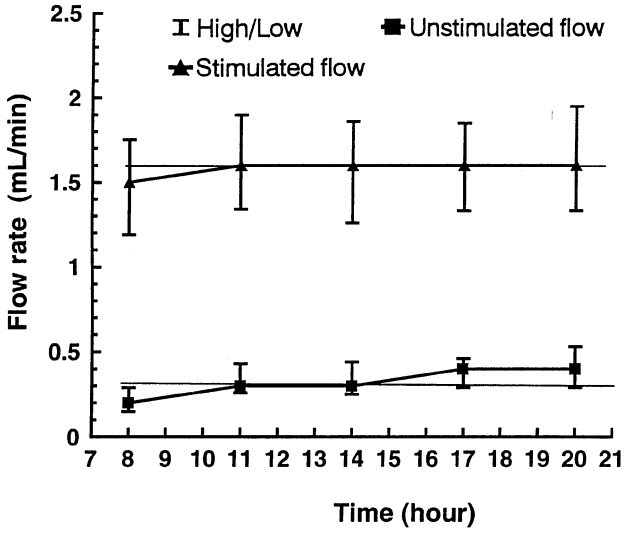

Fig. 2. Daytime means of unstimulated and stimulated flow rates with 95% confidence intervals. Straight lines represent the respective mean flow rates ($n = 30$).

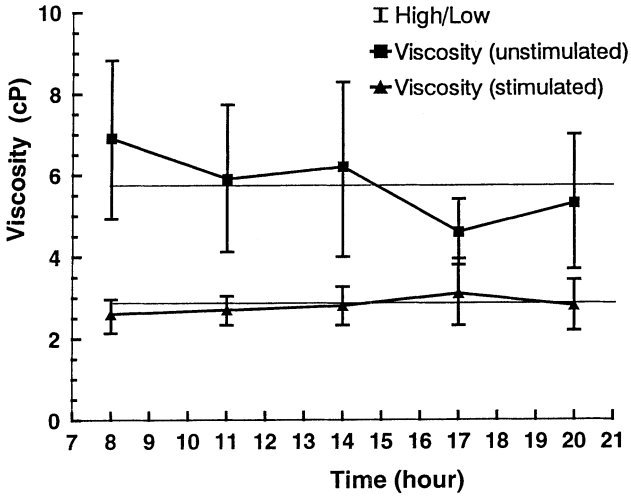

Fig. 3. Daytime means of viscosities of unstimulated and stimulated salivas with 95% confidence intervals. Straight lines represent the respective mean viscosities ($n = 30$).

Correlations of salivary viscosity

When calculating the correlation coefficients between the variables of different samplings, it was observed that the viscosities of unstimulated and stimulated saliva were correlated ($r = 0.62$, $P < 0.001$ at 1700 h). Viscosities and respective flow rates were negatively and statistically significantly correlated ($r = -0.43$, $P < 0.05$ for unstimulated saliva and $r = -0.53$, $P < 0.01$ for stimulated saliva at 1700 h). Unstimulated and stimulated salivary flow rates were clearly correlated at every sampling hour ($r = 0.70$, $P < 0.001$ at 1700 h).

Within-subject variation and intra-item correlations

The MANOVA analysis with covariates is shown in Table 1. Flow rates were used as covariates and sex was used as a between-subject factor in analysis for viscosity. Viscosity of unstimulated saliva was found to have statistically significant within-subject variation ($P < 0.001$). However, viscosity of stimulated saliva showed no significant within-subject variation across samplings. Unstimulated saliva flow rate showed statistically significant within-subject variation ($P < 0.001$). There were no differences between the sexes in this respect.

The intra-item correlations were highly significant for both flow rates ($P < 0.001$). Intra-item correlation for viscosity of unstimulated saliva was also significant ($P < 0.05$).

Discussion

Many different kinds of viscometers have been used in previous measurements. Until the 1970s most rheological measurements of saliva were made with the Ostwald-type U-tube viscometer or its modifications (1). Since then the Weissenberg rheogoniometer has been used, measuring the dynamic viscosity (η') and storage modulus (G') as functions of frequency (8). Oscillating rheometers have also been used (5). It has been reported that the apparent viscosity of saliva depends on the shear rate and time, indicating that saliva is a non-newtonian fluid (7, 8). Therefore, the viscometer used should be suitable for such systems. For this reason we used a standard Brookfield digital cone/plate microviscometer in our measurements. The apparatus gives the apparent viscosity in centipoises and shear stresses in dynes/cm^2. This kind of viscometer has been used in other salivary viscosity studies (9).

Our results from the preliminary study indicate that stimulated saliva seems to be enzymatically stable in viscosimetric respect, because there were no differences in viscosimetric properties between fresh and 30-min-old

Table 1. Within-subject variation of viscosities and flow rates and intra-item correlation coefficients (IIC)

Variable	*F*	*P*	IIC
Unstimulated flow	11.8	<0.001	0.82***
Stimulated flow	1.9	NS	0.88***
Viscosity, unstimulated†	15.7	<0.001	0.54*
Viscosity, stimulated‡	0.8	NS	0.33

† Unstimulated and stimulated flow rate were used as covariates.
‡ Stimulated flow rate was used as covariate.
For viscosity, sex was used as a between-subject factor in MANOVA.
* $P < 0.05$; *** $P < 0.001$.
NS = not significant.

saliva samples stored at room temperature. This is a surprising result, since it has been generally recommended that viscosity should be determined from fresh saliva samples (10). But it has also been shown that the viscosity of unstimulated saliva under experimental conditions is relatively stable for the first 5–8 min after collection (10).

In the present study a transverse method was applied. However, the sampling procedure was practiced several times before the collections to reduce the practice effect of repeated samplings. Another way of examining the temporal changes is the longitudinal method, in which the consistency of rhythms is studied in individual subjects. In most previous studies saliva samples have been collected over a period of several days, but from only a few subjects at a time (11).

It was interesting to find out that the within-subject variation of viscosity of unstimulated saliva in repeated samplings was significant. On the other hand, viscosity of stimulated saliva seemed to remain stable throughout the repeated samplings. We used salivary flow rates as varying covariates in our analysis (MANOVA), since viscosity of saliva can be subject to variation due to differences between the subjects' flow rates. Without the analysis of covariance, the within-subject variation of viscosity of unstimulated saliva would not have been significant. This clearly emphasizes the necessity of taking flow rates into consideration also in this respect.

Our results suggest that the viscosity of unstimulated saliva is influenced by salivary flow rate, but there could be another explanation for the within-subject variation of viscosity. One possible reason could be the changes in the salivary composition of different samplings. It is assumed that the rheological properties of human saliva may be due to the salivary glycoproteins, mainly the high-molecular-weight mucin-glycoproteins (MG1), which are secreted by the sublingual, submandibular, and palatal glands. Differences in viscoelasticity between submandibular and sublingual salivas are not due to the differences in mucin concentrations in those secretions, but rather to the mucin species (5). It seems that sublingual mucins are more elastic (η = elasticity) than submandibular and palatal mucins (5). The observed variation in he viscosity of unstimulated saliva in the present study could be an indicator of changes in salivary glycoprotein secretion.

It was also interesting that stimulated saliva remained viscosimetrically stabile over a full day. The explanation for this could be the increased proportion of parotid secretion during masticatory stimulation. On the other hand, salivary mucins, which are thought to have lubricating properties, are secreted mainly by the sublingual, submandibular, and palatal glands. This further supports the theory of association between salivary glycoproteins and salivary viscosity (5).

The intra-item correlations for salivary flow rates and viscosity of unstimulated saliva were high, indicating that rankings of subjects for these variables tended to be maintained across the samplings. However, the intra-item correlation for viscosity of stimulated saliva was not statistically significant. This means that rankings of subjects for viscosity of stimulated saliva did not maintain across the samplings, but fluctuated randomly. This observation further supports the conclusion that viscosity of stimulated saliva is not influenced by time during 12 h.

Correlations between unstimulated and stimulated flow rates were significant in our study, a finding in agreement with earlier results. In a study by Navazesh & Christensen (12), stimulation produced a fairly constant addition of saliva irrespective of the low or high individual unstimulated flow levels. Correlations between viscosities of unstimulated and stimulated salivas varied during the day, but correlation coefficients increased in later samplings.

There were no differences between the sexes in salivary viscosities, and sex did not affect the within-subject variation of salivary viscosities or flow rates. It needs to be emphasized in this context that our study group comprised healthy students of roughly the same age.

Saliva has many diagnostic uses (13), but there are a number of physiologic and methodologic factors affecting salivary diagnosis, and these must always be taken into account. One of the most important considerations is that the time of collection should be specified for diagnostic purposes. In clinical salivary diagnosis, viscosity has not yet been demonstrated as a clinically relevant variable. Our study showed that the within-subject variation of viscosity of unstimulated saliva during a 12-h period is significant. Observed variation of viscosity of unstimulated saliva could therefore be an indicator of changes in salivary glycoprotein secretion. Another interesting observation was the viscosimetric stability of stimulated whole saliva during repeated samplings. This was not what we expected, and the phenomenon should be further studied.

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