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Kinematic viscosity of unstimulated whole saliva in healthy young adults

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Abstract. – OBJECTIVE: To analyze kinematic viscosity and pH of unstimulated whole saliva, evaluate possible variations after sampling, identify any gender differences and detect possible correlations between them.

SUBJECTS AND METHODS: The sample consisted of sixty-four healthy young adults (37 females and 27 males, mean age 25.2 years). Saliva was collected using the spitting method at 11:00 am. Kinematic viscosity was determined with a capillary viscometer (ViscoClock, Schott-Geräte Mainz, Germany) equipped with a micro-Ubbelohde capillary. Viscosity and pH were measured at a temperature of 36°C in a thermostatic bath. Viscosity and pH data were evaluated almost simultaneously at six different times after sampling in order to identify any variations due to aging. The data were statistically analyzed using Student's t test and Wilcoxon-Mann-Whitney test.

RESULTS: In total sample kinematic viscosity was 1.40 cSt (SD = 0.39; RSD % = 27.81), in the male and female groups was 1.33 cSt (SD = 0.35, RSD% = 26.31) and 1.45 cSt (SD = 0.41, RSD % = 28.45) respectively; the difference was not statistically significant. Viscosity decreased exponentially as a function of time after sampling then reaching a plateau around 1.12 cSt, while the pH values increased linearly. There was a trend of pH to increase while viscosity decreases.

CONCLUSIONS: Kinematic viscometry could be a valid tool to evaluate salivary viscosity. Degradation of saliva after sampling affects viscosity and slightly pH. The use of capillary viscometer to evaluate salivary aging needs more improvements. Further studies are required to investigate and explain the effects of different techniques to reduce the film forming on the air/liquid interface during measurement.

Key Words:
Kinematic viscosity, Salivary pH, Capillary viscometry, Human whole saliva.

Introduction

Whole saliva is a watery complex mixture of proteins and other molecules secreted principally by the salivary glands. Saliva contributes to numerous functions in oral cavity such as speech and swallowing of foods, maintenance of oral health, protection of mucosa from bacterial attack and fungal growth, prevention of demineralization of teeth and lubrication of oral cavity. The latter is one of the most important functions of saliva, which provides lubrication of the oral, pharyngeal and other hard (teeth) and soft (mucosal) oral tissues[1-4]. Lubrication is generated by the viscous resistance to motion of saliva between surfaces and viscosity is the most representative parameter of this function[1]. The most important macromolecules that contribute largely to the rheological properties and to the protective action of saliva are mucins[5-8]. Rheologically saliva is a non-Newtonian pseudoplastic fluid (i.e. dynamic viscosity decreases upon increasing shear rate)[9]. As show in Table I different protocols were used to determine salivary viscosity[1,5-7,10-17]. Therefore non-standardized methodologies employed for saliva analysis can affect the variability of salivary viscosity data (e.g. it was noted that centrifugation did not affect the rheological properties whereas other authors found a viscosity decrease)[9]. Moreover, the decrease of saliva viscosity due to its aging[9,10,13] and the relationship with pH are controversial. Is reported that *in-vitro* increasing or decreasing of saliva pH causes precipitation of mucins and consequently decrease in viscosity[1,9]. Veerman et al[9] found an increase of viscosity at *in-vitro* lowered pH (4.5) due to the possible mucin association.

Consequently, it is possible that physiological or pathological changing of pH can affect salivary viscosity. Nowadays there is a lack in understanding how saliva viscosity values are influenced by pH *in-vivo* situation[9]. The aims of this study were: (1) to determine pH and kinematic viscosity of unstimulated whole saliva (U.W.S.) in a cohort of healthy young volun-

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Table I. Reported viscosities of U.W.S. saliva.

Authors	Cohort	Timetable collection	Time for sampling/ analysis	Instrument	Shear rate (s^{-1})	Temperature analysis (°C)	Viscosity (mPa·s)
Roberts BJ (1977)	N = 20 Y = 56.5 H = 20	9:00-11:00	Just after collection	Cone plate microviscometer (Brookfield)	11.5; 230	37	< 12 × < 15; < 2 × < 5
Vissink A et al (1984)	N = 2 Y = n.s. H = n.s.	9:00	Just after collection	Low shear rheometer (Contraves)	94.5	35	2.5
Nordbo H et al (1984)	N = 1 Y = n.s. H = n.s.	n.s.	n.s.	Capillary rheometer (Cannon)	n.s.	26	< 1.26 × < 1.30
Waterman HA et al (1988)	N = 7 Y = 23-48 H = 7	9:00-11:00	Just after collection	Couette-type (Contraves)	70*	25	1.1
Veerman ECI et al (1989)	N = n.s. Y = n.s. H = n.s.	n.s.	Freshly unhomogenized	Low-shear rheometer (Contraves)	20.4	37	< 4.20 × < 4.25
Van der Reijden WA et al (1994)	N = 7 Y = n.s. H = n.s.	n.s.	5 minutes of centrifugation	Oscillating capillary rheometer (Vilastic)	100	23	< 1.5 × < 2.0
Rantonen PJF et al (1998)	N = 30 Y = 22.7 ± 2.8 H = 30	8:00	Just after collection	Cone plate viscometer (Brookfield)	90	37	< 6 × < 7
Preetha A et al (2005)	N = n.s. Y = n.s H = n.s	n.s.	n.s.	Rotational co-axial viscometer (Contraves)	0.5-94.5	37	15.5- 2.8
Park MS et al (2007)	N = 20 Y = 22- 35 H = 20	9:00-11:00	Centrifuged 10 min at 4°C	Cone plate viscometer (Brookfield)	90	37	2.52 ± 0.59
Mehravaran N et al (2008)	N = 10 Y = 24-60 H = 10	n.s.	30 minutes	Cone plate viscometer (Brookfield)	n.s.	25	20.16
Inoue H et al (2008)	N = 40 Y = n.s. H = 40	14:00-18:00	Just after collection	Round vibration viscometer (CBC Materials).	n.s.	n.s.	1.09 ± 0.11
Sajewicz E (2009)	N = 14 Y = 24-53 H = 14	6:00-7:00	5 hours	Cone plate viscometer (Brookfield)	450	20	2.33 ± 1.03
Actual research	N = 64 Y = 25.2 ± 8.7 H = 64	11:00	Just after collection	Capillary viscometer (Schott-Geräte)	533.78	36	1.40 ± 0.39

N = number subjects; Y = age; H = healthy subjects; n.s.= not specified; *Measurement unit Hz.

teers; (2) to assess possible variations in pH and kinematic viscosity due to the degradation of saliva; (3) to investigate possible correlations between viscosity and pH. In order to increase robustness of the experimental data, the authors paid attention to: (1) reduce time between sampling and analysis and duration of the latter; (2) avoid any pre-treatment of saliva; (3) restrict interpersonal variation with a strict selection of the subjects.

Subjects and Methods

The study initially involved 68 Dental School students: 38 females and 30 males with a mean age of 25.8 years (range 18.6-29.4). They were informed of the purpose of the study, which was approved by the local Ethic Committee (n° RQ3210), and enrolled after their signed informed consent was obtained. All subjects answered an anamnestic form in order to exclude

those with systemic diseases, that could decrease saliva productions, or with symptoms such as dry mouth or oral burning syndrome, those taking drugs (except estrogens contraceptives) and women that could be pregnant. All enrolled subjects each year undergo a medical examination, including electrocardiogram, blood and urine tests for admission to the school attendance. An identification code consisting of a letter and a number was assigned to the subjects, and each of them was submitted to an oral examination during which particular attention was given to the condition of the mucous membrane in order to exclude subjects with oral diseases, wearing any intraoral appliances or having a poor oral hygiene. The same dentist, expert in oral medicine and trained in salivary testing, performed the oral examinations. Four subjects were excluded during the preliminary selection: one had oral mucosa pathology, two provided a saliva sample volume less than the minimum required for the measurement, and one did not respect the behavioural norms. The final sample consisted of 64 subjects with a mean age of 25.2 years: 37 females (57.8%), and 27 males (42.2%).

The enrolled subjects were submitted to a rigid protocol of behavioural norms, already validated in a previous study[18]: in the two weeks preceding the saliva collection, they had to avoid consumption of chewing gum; in the day before the collection they had to be relaxed and not to practice sports activity. In the sampling day, participants had to be free from symptoms of fever and/or cold; if they were hungry or thirsty, they could eat or drink water, but later immediately they had to clean their teeth with a provided toothpaste; during the last hour before the salivary collection, it was not permitted them to eat, drink or smoke[18]. All subjects were experienced, during the test, in the Province of Novara (Italy) or surrounding areas. U.W.S. was collected at 11:00 a.m., under controlled temperature (22-24°C) and humidity conditions (75% ± 5%), in order to minimize variations induced by these variables, using the spitting method[31]. U.W.S. was collected in a 5 minutes time span. The undisturbed subject, sitting in a comfortable position, swallowed residual saliva present in the mouth before the beginning of the collection and then, with the head down and mouth slightly open, saliva was allowed to drip from the lower lip into a weighed, dried and sterile plastic test tube. In the last few seconds of the 5 minutes interval, saliva accumulated in the mouth was spat out into the plastic funnel. No other conscious movements of the oral musculature were allowed during the collection. A portable pH meter (HI 9026, Hanna Instruments, Burlington, VT, USA) with a special 5 mm diameter electrode was used to measure pH[18]. Viscosity was determined by a capillary viscometer equipment (Visco Clock and micro Ubbelohde capillary series 357/10, Schott-Geräte Mainz, Germany), which measures kinematic viscosity (i.e. the resistance to flow of a stream under the sole influence of gravity)[5,15,19-21]. The choice of this micro-capillary was dictated by the very small volume of sample to be measured. Kinematic viscosity was calculated in centiStokes (cSt) by multiplying the flow time showed by ViscoClock by the capillary constant (0.01003 mm^2/s^2). Viscosity and pH were always measured at 36°C temperature in a thermostatic bath. At the end of a single collection, part of the sample was transferred into the capillary to measure viscosity without any preliminary treatment, taking care to avoid to transfer bubbles and/or solid particles. The tube containing the residual sample was maintained in a thermostatic bath. Approximately 20 seconds before the end of a viscosity measurement, the tube was taken from the thermostatic bath, shaken, and pH was measured. Six successive viscosity and pH measurements were carried out on the same sample. This procedure made it possible to measure viscosity and pH almost simultaneously at six different times, and the values were plotted against time in order to identify any variations.

Statistical Analysis

The data were statistically analysed using R software 2.12.1. The values were descriptively analysed, including their relative standard deviation (RSD). To assess the normal distribution of quantitative variables Shapiro-Wilk normality test was used. Variables with a normal distribution were compared by means of a Student's t test for independent samples; Wilcoxon's non-parametric test was used to compare variables without normal distribution. A $p \leq 0.05$ was considered statistically significant in all tests. Correlation coefficient R between pH and viscosity was evaluated for both the total cohort and the two gender groups.

Results

pH

The pH increased linearly over time, with a mean variation of 0.03 points/min (Figure 1).

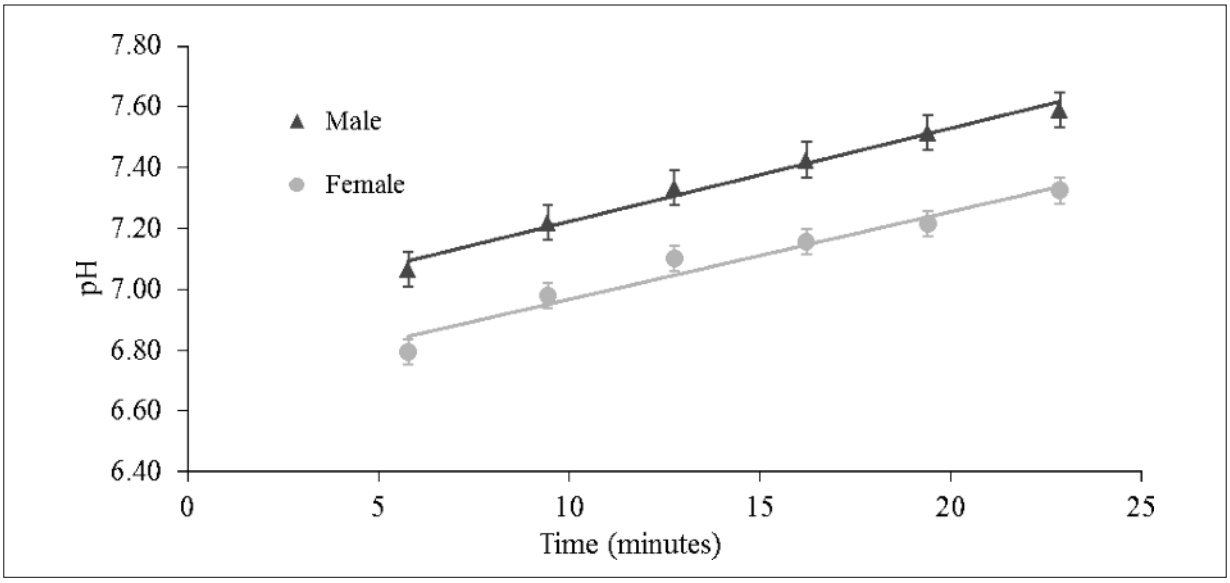

Figure 1. pH of saliva as a function of time (error bar as standard error).

The first value of each sample was considered the most representative of the situation in oral cavity; consequently, only the first one was considered for the statistical analysis. The pH values of the cohort ranged from 7.50 to 6.28 (mean 6.84, SD = 0.25, RSD% = 3.62) and were normally distributed. In male and female groups maximum and minimum values were 7.37-6.49 and 7.50-6.28 respectively; the corresponding figures were: mean 6.93, SD = 0.20, RSD% = 2.89 and mean 6.78, SD = 0.26, RSD% = 3.83. The difference between the two groups was statistically significant ($p = 0.009$).

Viscosity

Viscosity showed the tendency to decrease in a short time, reaching a constant value of about 1.12 cSt (Figure 2). The decrease ranged widely from subject to subject; the mean difference between the first and second measurement was

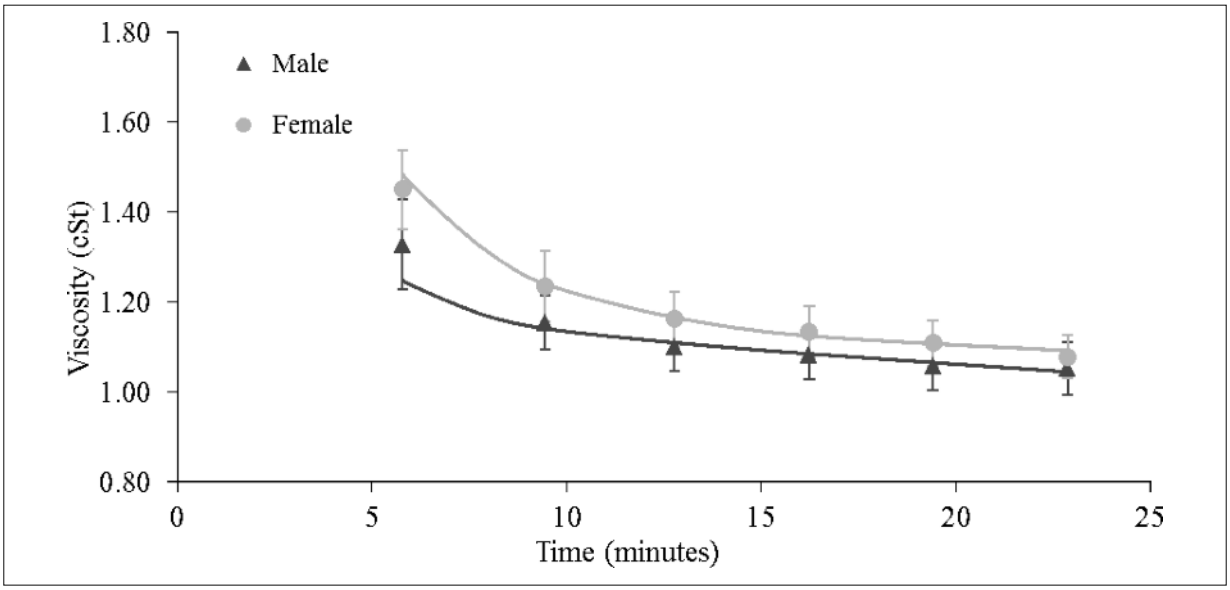

Figure 2. Viscosity of saliva as a function of time (error bar as standard error).

12% but, as the maximum was 42% and the minimum 1%, only the first value of each sample was used for the statistical analysis. The viscosity value in total sample ranged from 2.91 to 0.90 cSt (mean 1.40 cSt, SD = 0.39, RSD% = 27.81) and were not normally distributed ($p < 0.05$). The average viscosity was 1.33 cSt (SD = 0.35, RSD% = 26.31) in the male and 1.45 cSt (SD = 0.41, RSD% = 28.45) in the female group respectively; no significant difference between gender was found ($p = 0.153$). During analysis a shear rate of 533.78 s^{-1} was generated (calculated by using Weissenberg-Rabinowitsch equation[22]). Correlation coefficient between pH and viscosity in the sample as a whole was 0.35. Analyzing the data separately for gender, higher R value for female subject was found (R = 0.46), while no correlation was observed for male group (R = 0.04, Figure 3).

Discussion

It is know that saliva pH slowly rise due to the continuous loss of CO_2 from saliva exposed to air[23]. This increasing could be described by linear regression (Figure 1); moreover no differences occur in this rate between gender. This trend revealed how the accuracy of pH measurements depends on the time interval between collection and analysis. If a high precision is required, the saliva should be tested immediately after collection. Our pH results are similar to that reported by Fenoll-Palomares et al[24] and in our previous work[18], but not to that others authors: the difference may be due to subjects' age, ethnic group, and to the time intervening between saliva collection and pH measurement[18,24]. In this study kinematic viscosity was measured using capillary viscometer previously adopted by Nordbo et al [1]; it is not the most suited in viscosity determination of saliva because is impossible to set the shear rate during the measure[25]. However, a review of the literature[12,13] indicated that saliva shows a Newtonian behavior at shear rates higher than 90 s^{-1}. Usually, in micro-capillary viscometer and with low viscosity fluids, like saliva, high shear rate are generated[25] (in our case 533.78 s^{-1}); therefore accurate salivary viscosity values could be obtained with a micro-capillary tool like that used in our work. Moreover, we think that the easiness of use of capillary viscometer could be a great advantage in salivary studies. Although a rotational viscometer allows to perform a more detailed rheological analysis, capillary viscometer is preferred when rapid testing is required[5], because it allows reducing the time for loading the sample and the aging of the material. With capillary viscometer it is not necessary to let the sample recover from the shear-induced effects (typically with rotational viscometer), reducing the time of measure. With capillary viscometer it is not necessary to apply pre-treatment to the saliva; filtration, centrifugation, or addition of surfactant are required when viscosity is determined by

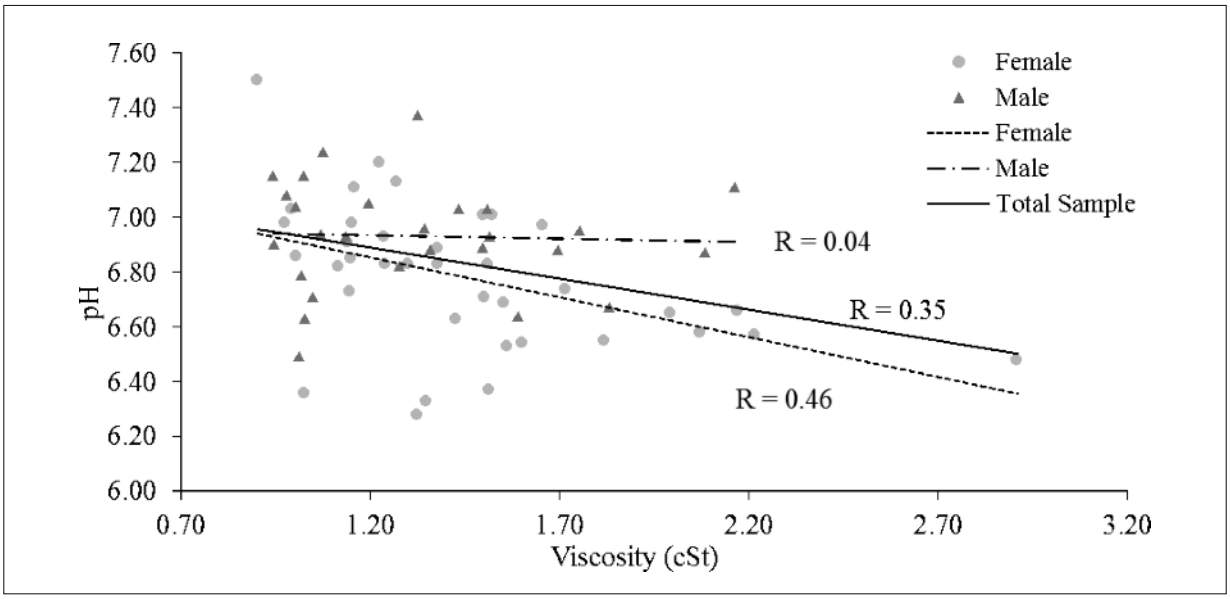

Figure 3. Viscosity vs pH of saliva.

other types of viscometer[9]. Saliva is a biologic fluid that underwent several chemical and biochemical reactions after collection (i.e. become aged). However, there is a lack in understanding how aging affects viscosity value of saliva. Bongaerts et al[26] demonstrated that 24 hours aging of saliva results in a dramatic decrease of viscosity. Rantonen et al[13] found that stimulated saliva viscosity remains almost constant during the first 30 minutes after collection. Other authors affirmed that viscosity of stimulated saliva decreases within few hours[9]; otherwise unstimulated saliva seems to be less stable (first 5-8 minutes)[10]. Sajewicz[17] revealed that unstimulated saliva viscosity starts to decrease immediately after collection and reaches a plateau value within 5 hours[17]. Therefore, the exponential trend of viscosity found in our measurements could be associated to a rapid aging of saliva due to several physico-chemical phenomena, among them the most signifcant been absorption at air/liquid interface of mucins[7]. Capillary viscometer could be a valid tool for the evaluation of salivary viscosity, especially because it is rapid and easier to use than other types of viscometer. Nevertheless, repeated measures on the same sample damage saliva, and only the first run may give a correct rheological measure. Taking in account that saliva has a specific gravity of 1.00[13,14,27,28], and its shear rate was 533.78 s^{-1} (as calculated from our data), it is possible to compare our values with dynamic viscosity values obtained by other authors. Unfortunately, some data cannot be fully compared because several authors did not report all the necessary information for the comparison. In other cases different instruments, shear rate or temperature were adopted during measurements, moreover different time between sampling and analysis occur in several works (Table I). Like Rantonen et al[13], we did not find any significant differences between genders. Furthermore, despite the accurate selection of donors, our viscosity values showed relatively high variations (2.91 to 0.90 cSt), that are comparable to those reported in the majority of the cited studies (Table I). This finding could be due to a high interpersonal variability of viscosity values. It is known that UWS viscosity is provided mainly by its content of high weight molecular glycoproteins (i.e. mucins) that are sensitive to environmental changes such pH that induce modifications of conformation and interactions of these macromolecules[1,6]. However, in *in-vitro* experiments, only with a great deviation from physiological value of pH significant modification in viscosity values occurs[6]. This consideration could explain why in our donor population (young healthy subjects) pH and viscosity are not well correlated. It is possible that using a broad donor population, including pathological subjects (i.e. with pH lower than 6) correlation between pH and viscosity could become more evident.

Conclusions

Understanding saliva rheology and the principles that affect his properties are clinically important for people with a compromised or altered function or production of saliva. It is known that viscosity changing has been associated with development of oral disease in human model studies[29]. Saliva is intrinsically inhomogeneous, as it simultaneously consists of a liquid-gaseous and a gel phase[9]. The complexity of this system reduce the accuracy in the evaluation of its characteristics and in particular of viscosity. The use of different methods for saliva viscosity determination make difficult to compare the results of different studies; therefore there is a need to create a protocol that would standardise the evaluation of salivary viscosity. Within the limitations of this study, we can say that capillary viscometry can be a valid method to evaluate this property. Although, the capillary viscometry for the evaluation of salivary aging needs more improvements. The modification of saliva after sampling significantly affects the pH value in a linear way, while it is not possible to estimate how aging affects viscosity values. Further studies are required to investigate and explain the interpersonal variations in viscosity, and the effects of different techniques in reducing the film formation on the surface of viscometer during the measure.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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