Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.elsevier.com/locate/aob

A tentative model for D-glucose turnover in human saliva

Sibel Cetik *, *Ying Zhang*, *Emeline Hupkens*, *Cedric Jurysta*, *Willy J. Malaisse*, *Abdullah Sener*

Laboratory of Experimental Hormonology, Université Libre de Bruxelles, Brussels, Belgium

ARTICLE INFO

Article history:
Accepted 17 July 2013

Keywords:
Salivary flow
Saliva glucose concentration
Glycolysis rate in oral bacteria
Salivary ATP

ABSTRACT

Objective: The aim of the present study is to propose a tentative model for D-glucose turnover in human saliva. The whole saliva and the saliva from parotid and submandibular/sublingual glands were collected by use of the Salivette™.

Results: The saliva glucose concentration was measured by the hexokinase method, saliva bacteria glycolysis by use of D-[5-^{3}H] glucose, and the saliva ATP content by the luciferase method. The concentration of glucose amounted to 43.9 ± 6.3 (n = 29), 197.5 ± 17.3 (n = 29), 104.0 ± 12.4 (n = 27) μM in whole saliva, parotid saliva and submandibular/sublingual saliva, respectively. The rate of D-glucose utilization by oral bacteria at a physiological concentration of D-glucose in saliva (50 μM) was estimated at 0.047 ± 0.003 (n = 11) nmol/min per 10^6 bacteria. Unstimulated salivary D-glucose turnover rate, as calculated from the amount of glucose secreted in saliva which comes from parotid and submandibular and sublingual glands represented 214.6 ± 19.1%/min. In order for salivary D-glucose production to match bacterial utilization of the hexose, the total number of oral bacteria was estimated at about 2.0×10^9 bacteria, in fair agreement with previously published data.

Conclusion: This study thus provides support for a tentative model for D-glucose turnover in human saliva.

1. Introduction

The D-glucose concentration in human unstimulated saliva ranges from 25 to 100 μM.[1–12] This large variation is probably due to various factors such as the secretion of glucose from different salivary glands, the oral retention of alimentary carbohydrates,[13,14] the glucose utilization by oral bacteria,[15] the release of carbohydrates from salivary glycoproteins[16,17] and the contamination of saliva by a large outflow of crevicular fluid in patients with a poor gingival status.[18–20]

The aim of the present study is to propose a tentative model for D-glucose turnover in human saliva, taking into account such variables as the production of D-glucose by salivary glands, the number of bacteria found in either salivary samples or the entire oral cavity[21] and the rate of D-glucose utilization by oral bacteria.

2. Materials and methods

2.1. Subjects

The present study was conducted in 82 normal subjects, in 3 independent studies concerning the salivary glucose concentration (n = 39), the salivary D-glucose turnover rate (n = 29) and the salivary glucose utilization by oral bacteria (n = 14), including males and females with mean ages (± SEM) of

* *Corresponding author at*: Laboratory of Experimental Hormonology, Université Libre de Bruxelles, 808 Route de Lennik, B-1070 Brussels, Belgium. Tel.: +32 2 555 82 37/+32 2 555 61 74; fax: +32 2 555 61 86.
E-mail address: Sibel.Cetik@ulb.ac.be (S. Cetik).

0003–9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.archoralbio.2013.07.004

22 ± 2 years. The present research was conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki and the ethical regulations stated by the Ethical Board of the Université Libre de Bruxelles. The experiments were undertaken with the understanding and written consent of each subject and according to the above-mentioned ethical principles.

2.2. Saliva sample collection

To collect saliva, a standardized tube Salivette™ (Starstedt, Nümbrecht, Germany) with two compartments was provided. Both the cotton and two-compartment tube were obtained from the same manufacturer. The upper part of the tube containing the cotton presented a hole, so that, after centrifugation, the saliva was recovered in the lower part and became available for analysis.

Saliva was collected from subjects (a short questionnaire indicated they are healthy and that they did not eat within the two hours of making saliva collection) by means of cotton kept in the oral cavity for 1 to 3 min either in the resting state or during mastication (stimulated saliva). Two cottons were applied in front of each parotid and a third cotton rested under the tongue. The cotton was transferred in the upper part of the tube. Salivary flow was determined by weighing the device with the cotton before and after saliva collection, assuming that 1 g of saliva corresponds to 1 ml. Centrifugation of the device at 2000 g for 5 min allowed the saliva adsorbed to the cotton to pass through the orifice into the lower compartment of the device. NaF (10.0 mM) in final concentration was added to the saliva which was then immediately frozen at −20 °C, after centrifugation.

Although salivary flux could be affected by use of the Salivette™, the latter was used to standardize the collection of saliva, both for hygiene reasons and to remove particles from the saliva.

2.3. Saliva collection for metabolic study and saliva ATP assay

Saliva, without stimulation, was accumulated in the oral cavity during 3 min, transferred into a Falcon tube (NJ, USA) and then filtered through Cell Strainer nylon (BD Falcon, NJ, USA) of 0.45 μm to avoid the presence of detached or desquamated epithelial cells. Saliva, after filtration, was directly used for the study of D-glucose metabolism and measurement of ATP concentration. It is important to underline that the saliva collected for metabolic study (glycolysis) did not contain NaF.

2.4. Salivary glucose assay

Salivary D-glucose concentration was determined by the hexokinase method adapted from the literature.[22,23] Centrifuged saliva (100 μl) was mixed with 95 μl of reagent medium containing 2.0 mM $MgCl_2$, 0.5 mM ATP (Sigma-Aldrich, St. Louis, Missouri, USA), 0.5 mM $NADP^+$ (Sigma-Aldrich), 0.06 units of yeast glucose 6-phosphate dehydrogenase (Roche, Mannheim, Germany) in TRIS-HCl buffer (200 mM, pH 8.1). After a first reading of the absorbance at 340 nm, the reaction was started by addition of 5 μl yeast hexokinase in reagent medium (0.06 units). The absorbance at 340 nm was recorded after 30-min incubation at room temperature (22 °C). The assay was simultaneously conducted on glucose standards (final concentration comprised between 5 and 250 μM). The results were calculated as nmoles of glucose/ml saliva after the subtraction of the reading in the absence of hexokinase and taking into account glucose standards and saliva volume. The coefficient of variation in the glucose assay technique is 1.44% ($n = 30$). The recovery of the internal standard of glucose (5 nmol) was 91.4 ± 4.1 per cent ($n = 6$).

2.5. Salivary ATP assay

The salivary ATP concentration was determined by mixing 50 μl luciferine/luciferase reagent (Promega, Madison, WI, USA) with 50 μl filtered saliva and the luminescent signal was read on a luminometer (GloMax® Multi Detection System, Promega, Madison, WI, USA). The saliva ATP concentration was calculated from a standard curve for each luciferase assay using a serial dilution of ATP standard (0.5 to 4.0 pmol ATP/assay). A typical standard curve of ATP is illustrated in Fig. 1. Under our experimental conditions, the relationship between the two variables is linear with a correlation coefficient amounting to 0.9989 ($p < 0.0001$).

2.6. Salivary glucose utilization by oral bacteria

The method for measurement of D-[5-^{3}H] glucose utilization was described previously.[24] In a first series of D-glucose dose-related experiments, 50 μl of unstimulated filtered saliva was mixed with 50 μl of a bicarbonate-buffered medium containing 200–800 μM D-glucose mixed with a trace amount of D-[5-^{3}H] glucose (5 μCi/ml). In a second series of experiments, the final D-glucose concentration was adjusted to 400 μM. After 120 min incubation at 37 °C, the metabolism of glucose was stopped by the addition of 100 μl of citrate buffer (400 mM, pH 4.9) containing metabolic poisons (NaF 10 mM and KCN 5 mM). The ^{3}HOH formed during incubation was recovered over 20 h

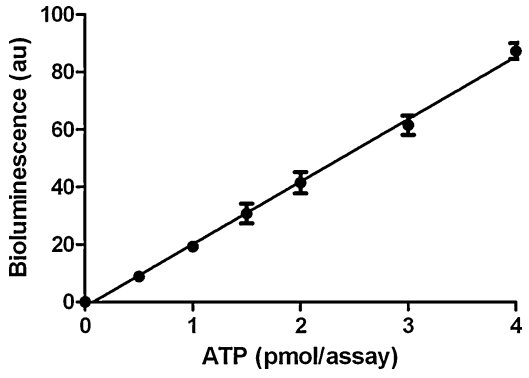

Fig. 1 – Typical standard calibration of ATP. This figure shows the calibration between the luminescence intensity (relative luminescence unit: arbitrary unit, au) versus the amount of ATP. Each ATP value represents the average of 3 independent normalized series, each measurement being made in duplicate.