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Oral glucose clearance in 12-year-old South Africans

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SUMMARY

A portable glucometer method was used to measure clearance of salivary glucose in 31 black and in 28 white 12-year-old school children. The aims of this study were to evaluate a readily available portable glucometer method and to investigate salivary glucose clearance as a marker of caries risk. Following the initial evaluation, we adapted the portable adapted glucometer method and found it reproducible and suitable for the measurement of salivary glucose clearance. Black children had almost double the salivary clearance rates of white children. No significant differences in salivary glucose clearance were seen between caries free (DMFT=0) and caries active (DMFT ≥3) children. Salivary glucose clearance is not suitable as a single caries risk predictor.

OPSOMMING

'n Draagbare glukometer metode is gebruik om die suiweringstempo van speekselglukose in 31 swart en 28 blanke 12-jarige skoliere te meet. Met hierdie studie is beoog om 'n draagbare glukometermetode vir die meting van bloedglukose te evalueer en aan te pas vir die bepaling van speekselglukose vlakke sodat vasgestel kon word of die suiweringstempo van speekselglukose 'n nuttige merker is vir kariesrisiko. Die aangepaste glukometermetode is herhaalbaar en geskik om die suiweringstempo van glukose te meet. Die suiweringstempo in swart kinders is bykans twee maal hoër as in wit kinders. Die suiweringstempos in kariesvrye (DMFT=0) en kariesaktiewe (DMFT ≥3) kinders het nie merkbaar verskil nie. Die suiweringstempo van speekselglukose is nie geskik as 'n enkele bepaler van kariesrisiko nie.

INTRODUCTION

Sixty-five to eighty per cent of the South African population, estimated at 40 million (Steenkamp, 1994), rely on the government for oral health care. Manpower and the associated funding are insufficient for the nation's oral health requirements unless resources can be concentrated on those most in need. This could be achieved by implementing a caries prediction model such as the University of North Carolina Caries Risk Assessment study (Stamm *et al.,* 1993). Saliva, which is readily available and its collection non-invasive, is a possible material for prediction of caries risk. Examples of the salivary predictors used are buffering capacity (Granath *et al.,* 1991), mutans streptococci counts (Klock *et al.,* 1989) lactobacilli counts (Russel *et al.*, 1991), IgA levels (Widerström, 1994) and flow rate (Sundin, Granath and Birkhed, 1992). Opinions on the usefulness of these methods vary from author to author.

In an extensive study of dental caries and associated causative variables in young South African children, stimulated saliva flow rate in black children was double that of their white compatriots, indicating a potential for inter-ethnic differences in other salivary variables (Grossman *et al.,* 1991). Linked to flow rate is salivary clearance which is the reduction in concentration of a substance introduced into the mouth by a route other than via saliva until it reaches resting concentration. Individuals show characteristic fast or slow clearance times (Goulet and Brudervold, 1984; Sreebny, Chatterjee and Kleinberg, 1985).

The purpose of the present study was to examine the potential relationship between salivary glucose clearance and caries activity. The aims were to develop a simple method suitable for use either in the field or in dental surgeries to measure glucose concentration in saliva following a glucose mouth rinse, and to compare glucose clearance between caries-free and caries-active children from two ethnic groups.

MATERIALS AND METHODS

Analytical methods

An appropriate method for South African conditions is to use readily available equipment with relatively simple and inexpensive tests. The portable glucometer (Glucometer® II, Ames Diagnostics, Bayer-Mills (Pty) Ltd, South Africa), a reflectance colorimeter with a digital display in the range 0,6 to 22,0 mM was used with glucose oxidase test strips (Dextrostix®, Ames Diagnostics). A laboratory evaluation was done according to the protocol of Lloyd (1978) to see if this method which is used by diabetics to measure their blood sugar levels, is suitable to measure salivary glucose.

Intra-assay variation was tested by making 10 replicate readings with each of the two calibration chips, 2,8 mM and 16,5 mM, according to the manufacturer's instructions. Coef-

ficients of variation were 1,8 per cent for the 2,8 mM chip and 0,8 per cent for the 16,5 mM chip, indicating that the test glucometer was working to high precision.

The glucose oxidase test strips are designed to measure glucose in whole blood, so two groups of solutions less viscous than whole blood were used to evaluate the accuracy of the glucose measurement. In the first group glucose solutions of 5, 10 or 15 mM in isotonic saline were prepared gravimetrically using analytical grade reagents. These isotonic glucose solutions were used to prepare the test saliva samples — 100 µL glucose solution was added to 900 µL resting saliva to produce 0, 0,50, 1,0 and 1,5 mM glucose in saliva. It was expected that the peak salivary glucose concentration would exceed the upper limit of detection, i.e., 22 mM and that salivary samples should be diluted to a concentration within the analytical range. In the second group the effect of dilution was determined on a resting saliva sample, diluted 1:5 in saline and containing 1,0 mM glucose. After initial analysis, the aliquot containing 1,5 mM was kept on ice and analysed 6 hours later to observe the stability of glucose in saliva under these storage conditions. Linearity was assessed by preparing salivary samples containing 0, 0,5, 1,5, 3,0 and 5,0 mM glucose.

Since the test strip package insert outlined several interfering and limiting factors of this dipstick method, it was decided to minimize their effects by pipetting a standard amount of 20 µL of test solution or saliva onto the strip and to stop the reaction with a 3,0 mL of 0,5 per cent sodium fluoride solution after one minute. The reagent pad was gently blotted before it was placed into the glucometer and the reading recorded. Calibration with the manufacturer's calibration chips was done before each work session and after every 20 readings. Ten replicates were obtained for the 5, 10 and 15 mM glucose solutions in isotonic saline as well as for the salivary samples containing 0, 0,5, 1,0 and 1,5 mM glucose and the 1:5 diluted salivary sample. After 6 hours 4 readings were obtained for the 1,5 mM sample stored on ice. Three readings were obtained for each sample over the linearity range 0,5 to 5 mM.

Study sample

Before commencing the clinical study the protocol was approved by the University's Committee for Research on Human Subjects (Clearance 930623) and informed consent was obtained from all participants and from their parents. Children who were 12 years old at their previous birthday and who attended schools receiving community dental services from one author (LGL), were examined using WHO caries diagnostic criteria (WHO, 1987). The study design required 30 caries-free children (DMFT=0) and 30 caries-prone children (DMFT $\geq$ 3, including an untreated DT$\geq$3), half of each group from a white community and half from a black community. These target numbers were not completely met; the numbers studied were 31 black children and 28 white children. The lower number in the white group was due to fewer caries free children in the school. Both groups received water from the same source with a fluoride content of 0,21 to 0,33 ppm (Grobler, 1992).

Saliva Collection

Unstimulated saliva was collected at 09:30 each morning, school having begun at 07:45 so each child had not eaten for at least 105 minutes before sampling. After careful explanation and demonstration, subjects swallowed the saliva in their mouths, accumulated saliva for 30 seconds then drooled the saliva into a collection tube which was baseline and immediately sealed and termed resting sample (time 0). Each subject then rinsed with 10 mL of 10 per cent glucose for 30 seconds and swallowed. Saliva samples, accumulated for 30 seconds each time, were collected at 1 minute, 5 minutes and 9 minutes after the rinse. Finally, as a caries preventive measure each child rinsed with a fluoride solution. A replicate experiment was performed for each child during a second school visit 2 weeks after the first. The saliva samples were transported to the laboratory in insulated carrier boxes containing crushed ice and glucose concentrations were determined not later than 8 hours after collection. The long period of measurement was due to the number of samples. If the upper limit of the glucometer was exceeded, 1:1, 1:4 or 1:5 dilutions of samples with normal saline were made.

Statistical analysis

The study results were analysed in a Sun SPARCcentre 200 computer with GraphPad Prism (1995) and SAS (1989). For each test, area under the curve, rate/min of ascent [(minute 1 result-baseline)/1 minute] and rate/min of descent [(minute 1 result — minute 9 result)/8 minutes] were calculated. A 3-way general linear model analysis was used with area under the curve, rate of ascent and rate of descent as the dependent variables and ethnic group, caries group and test visit as the independent variables.

RESULTS

The 10 and 15 mM glucose in isotonic saline solutions gave readings exceeding the 22 mM limit of detection of the glucometer as had been expected. For the 5 mM solution the glucose concentration was 15,5 (95 per cent CI [Confidence Interval] 12,8-18,2) mM, with a coefficient of variation of 24,5 per cent. This almost five time greater measurement was similar to the pattern seen when the linearity of detection was determined (Fig. 1) in saliva samples.

Precision in glucose detection is shown in Table I. Coefficients of variation were low, except for the diluted saliva sample, indicating that the method was sufficiently reproducible for the study. However, the absolute glucose concentrations measured were some 4 times higher than they should be. These two facts indicated that only relative changes could be measured which is in keeping with the purpose of this study.

For each child there was a rapid rise in salivary glucose concentration from the resting saliva concentration (time 0) to the peak at 1 minute after the glucose rinse, followed by a slower drop in concentration but baseline values had not been reached by the 9-minute sample. The pattern is shown through mean values in Table II and typical curves of mean concentrations are shown in Figure 2. The values for black

Table I: *Observed precision in glucose detection.*

	(CI = confidence interval) saliva solutions (mM) immediate				after 6h	diluted saliva
	0	0,5	1,0	1,5	1,5	1,0
	0,9	1,8	3,8	6,8	8,3	4,8
95% CI lower	0,86	1,7	3,5	6,2	7,5	3,6
upper	0,94	1,9	4,1	7,4	9,1	6,0
coefficient of variation (%)	5,7	10,8	9,3	11,5	6,1	35,9

Table II: *Salivary glucose concentrations by ethnic group (me = median).*

	black children (n=31)			white children (n=28)		
Time	$\bar{x}$	sd mmol/L	me	$\bar{x}$	sd mmol/L	me
baseline	0,8	0,2	0,8	0,8	0,2	0,8
1	72,3	20,0	72,5	44,3	28,5	34,4
5	18,3	15,6	15,3	11,3	6,6	9,6
9	4,2	4,7	4,6	2,5	2,6	1,5

Table III: *Details of statistical analysis of area under the curve, rate of ascent, rate of descent and general linear models analysis.*

Level	n	mean	sd	95% confidence interval	F	P
Area under the curve						
black	62	264,9	89,0	242,3 – 287,5	46,54	0,0001
white	54	156,9	78,1	135,7 – 178,3		
caries-free	50	218,4	90,0	192,8 – 244,0	0,01	0,9073
caries-active	66	211,7	107,0	185,4 – 238,0		
visit 1	58	215,9	104,8	188,3 – 243,5	0,02	0,8759
visit 2	58	213,4	95,1	188,4 – 238,4		
Rate of ascent (mM/min)						
black	62	71,5	20,0	66,4 – 76,6	37,41	0,0001
white	54	43,6	28,5	35,8 – 51,4		
caries-free	50	60,4	26,8	52,8 – 68,0	0,02	0,6484
caries-active	66	57,1	28,9	50,0 – 64,2		
visit 1	58	57,4	30,0	49,5 – 65,3	0,02	0,8759
visit 2	58	59,6	25,9	62,8 – 66,4		
Rate of descent (mM/min)						
black	62	8,5	2,6	7,8 – 9,2	31,91	0,0001
white	54	5,2	3,6	4,2 – 6,2		
caries-free	50	7,2	3,3	6,3 – 8.1	0,1	0,7482
caries-active	66	6,8	3,7	5,9 – 7,7		
visit 1	58	6,9	3,8	5,9 – 7,9	0,18	0,6685
visit 2	58	7,1	3,2	6,3 – 7,9		

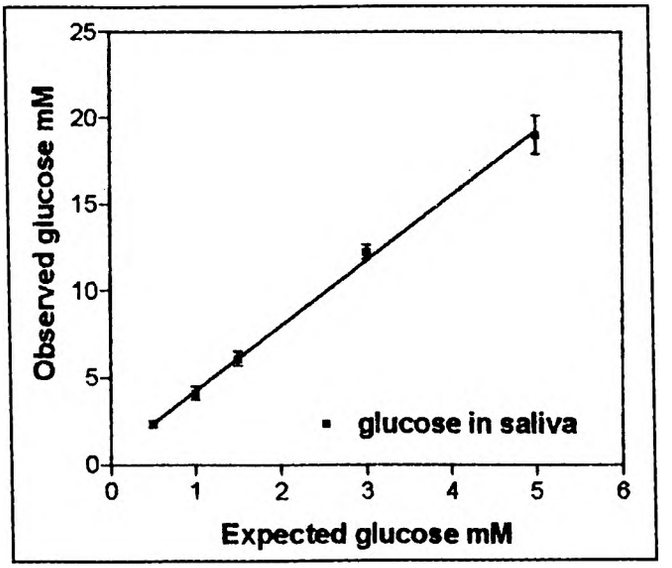

Fig. 1: **Linearity of detection of glucose in saliva. Mean values (95 per cent confidence intervals) are shown.**

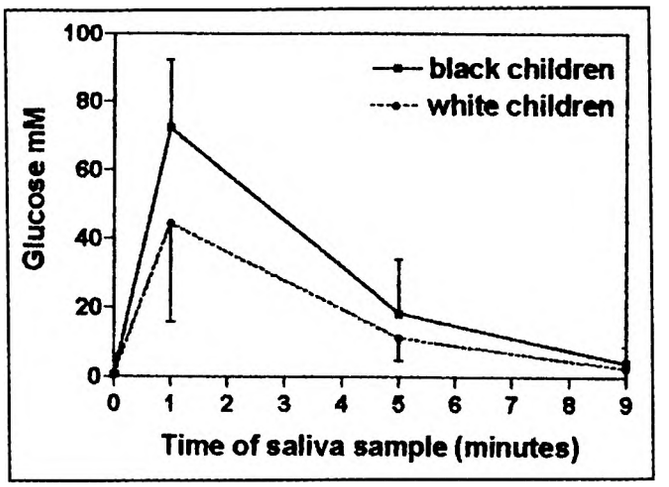

Fig. 2: **Mean (95 per cent confidence intervals) for black and for white children.**

children are clearly higher than that for the white group. Absolute values for area under the curve and rates of ascent and descent are listed in Table III. In this table the numbers per group are for both visits combined as used in the general linear models analysis. This analysis showed significant effects for ethnic group but not for caries grouping as well as for visit. The values within caries grouping as well as within visit showed little difference but values between the black and the white children were markedly different.

DISCUSSION

Because the coefficients of variation were low and storage on ice for 6 hours had minimal effect on the glucose concentration, the methods used in this study were suitable for field work or the dental surgery. A proviso is that relative change, not absolute values must be used since the observed glucose concentrations were four to five times higher than the standard solution.

Most studies in which salivary sugar levels have been determined have used complex methods of measurement requiring multiple steps and expensive reagents and equipment. Typical examples are a ß-glucose analyser (Goulet and Brudervold, 1984), complex anthrone method (Sreebny, Chatterjee and Kleinberg, 1985) and protein precipitation plus enzymatic method (Britse and Lagerhöf, 1987). A much less complex method in which reagent indicator strips are used has been described by two research groups. Adorjan and Stack (1976) used Clinistix®, a dipstick method to test for urinary glucose, to determine salivary sugar clearance but they did not detail their method. In contrast, Miura *et al.*, (1991) described, in great detail, self-prepared strips. Their

results were consistent with studies using more complex analytical techniques. Because of ease of use and suitability for field work, the reagent strip method was chosen for the current project. No references, however, were found where ready prepared and widely available reagent strips were used, so the laboratory evaluation was done before the main investigation. This showed, convincingly, that reproducibility of the method is high but that relative rather than absolute values must be used.

Clearance of substances from saliva may be slow or fast. Slow clearance may be beneficial for example, if fluoride is in contact with teeth for a longer period (Oliveby *et al.*, 1990). It may be harmful if, for example, fermentable carbohydrate is in contact with teeth for a prolonged period (Dawes, 1983). A feature of clearance is that individuals show characteristic fast or slow clearance times (Goulet and Brudervold, 1984; Sreebny *et al.*, 1985). The clearance may be aided by dilution, by swallowing, by absorption, by transferral into other compartments such as plaque, by metabolism, by evaporation or by binding to oral tissues (Lagerhöf and Dawes, 1985).

Sugar clearance from the mouth is biphasic, an initial rapid reduction is followed by a less rapid reduction, and may be mathematically modelled (Dawes, 1983). The glucose clearance seen in the current study followed this typical pattern. While several mechanisms operate, gustatory stimulation of saliva flow predominates (Watanabe and Dawes, 1988). The faster rate of clearance seen in black children in the current study may be due to a greater salivary flow rate, something seen for stimulated saliva in younger children (Grossman *et al.*, 1991) but more research is needed to confirm this. Why the rate of increase in glucose was also faster we do not know.

Dental plaque pH is influenced by salivary clearance (Dawes, 1983). In theory therefore low salivary sugars clearance should be associated with increased caries susceptibility. There is surprisingly little direct examination of this in the dental literature. In one study an increase in sugar clearance time paralleled an increase in DMFT in boys but not in girls (Britse and Lagerhöf, 1987). In contrast 2 other studies found no correlation (Watanabe and Dawes, 1988; Widerström, 1994). Critical examination of the latter three studies showed weaknesses in standardization of the experiments. Our study rectified these by using clear caries diagnostic criteria and a single age group.

A benefit of our study was the development of a useful field method to study salivary glucose clearance. Our results showed no significant association between glucose clearance and amount of caries in 12-year-old children. We accept that caries is multifactorial in origin and that for prediction multiple predictors are required (Stamm *et al.*, 1993). The association between glucose clearance and caries in our sample was so weak that it is unsuitable as a single caries predictor. Additional support for this conclusion is that our sample size was large enough to show a statistically significant effect of ethnic group. The role of glucose clearance in a multiple predictor model in South African populations has yet to be determined.

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