Plaque pH and oral retention after consumption of starchy snack products at normal and low salivary secretion rate

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The effect of plain potato chips, sugar-free cheeze doodles, and sweetened crackers on plaque pH and oral retention was tested in 10 volunteers and compared with 5% starch and 5% sucrose, during both normal and low salivary secretion rate. The first 30 min 5% sucrose gave the most and 5% starch the least attenuated pH drop, but the three snack products reached or even passed the level seen by sucrose during the second 30-min phase. All products resulted in greater pH falls and remained at a low level for a longer period during low secretion rate. There were no differences in concentration of carbohydrates in saliva after consumption of potato chips, cheese doodles, and a cracker. However, low secretion rate increased the oral retention for all three products. To conclude, this study showed that low salivary secretion rate accentuated the pH decrease in dental plaque and prolonged the oral retention of carbohydrates. □ *Cariogenic potential; dry mouth; oral retention; plaque pH; salivary flow; starchy food*

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Sucrose has for long been considered to be the most cariogenic carbohydrate of the human diet (1, 2). It has, however, been discussed whether starch may also play an active role in the caries process (3). The cariogenic potential of starch, evaluated as the plaque pH response, is influenced by the heat and processing conditions used during manufacturing (4–8). Several studies have shown that cooked, and thereby gelatinized, wheat starch is far more caries-inducing than raw starch in rats (9, 10). Starch may therefore vary from having a low to a relatively high 'cariogenicity', depending on the degree of gelatinization. This is of importance because the consumption of processed starch-containing between-meal products, such as potato chips and cheeze doodles, has increased dramatically in many industrialized countries during the past years (3). The 1991 per capita consumption in Sweden, for example, was for this type of snack products around 6.8 g/day, of which potato chips made up around 50% (Estrella AB, personal communication).

The cariogenic potential of any fermentable product can be expected to increase in people with low salivary secretion rate (11–14). Starchy food products, which often have a high retention to the teeth and the oral mucosa, give rise to prolonged acid production in dental plaque compared with foods that are more easily removed from the oral cavity (15–17). This is of special importance in individuals with exposed root surfaces, which have lower resistance against demineralization than enamel (18, 19).

The aim of the present investigation was to study the influence of salivary secretion rate on plaque pH and oral retention after consumption of various starchy snack food products and to compare two areas of the dentition known to be at risk for caries—that is, buccal and approximal sites.

Materials and methods

Subjects

Ten healthy volunteers, 30–71 years old (mean, 47 years), with more than 5×10^5 mutans streptococci per milliliter of saliva, were selected for the study. They had a mean of 27 teeth of their own (range, 18–31), of which 12 showed exposed root surfaces both in the upper and lower jaw (range, 5–19 surfaces). The DMFS varied between 47 and 103 (mean, 75). The secretion rate for stimulated whole saliva was 2.11 ± 1.20 ml/min (mean ± SD), and the buffer capacity 5.7 ± 0.8 (final pH), collected and measured by the method of Heintze et al. (20). The number of mutans streptococci in saliva was 6.44 ± 0.86 log colony-forming units (CFU)/ml (mean ± SD), and the number of lactobacilli 3.83 ± 1.75 log CFU/ml, determined on selective agar media (21).

Study design and test products

All tests were carried out twice—that is, both at 'normal' and at 'low' salivary secretion rate. To induce a dry mouth, methylscopolamine nitrate was used (Skopyl®, 0.5 mg/ml, Apoteksbolaget, Stockholm, Sweden) as described earlier (13, 22). Methylscopolamine, 0.4 ml, was injected submucosally in the labial sulcus. Reduced secretion rate was established within 30 min and lasted for about 1 h, after which the salivary flow gradually recovered over approximately 8 h. The experiments at low secretion rate were always carried out 1 h after the injection.

During each test session one of the following five products was tested: 1) 5 g of potato chips, 2) 5 g of cheeze doodles, 3) 5 g of a cracker, 4) 10 ml of 5% soluble starch 'nach Zulkowsky' (wt/vol), boiled and cooled to room temperature just before the test, and 5) 10 ml of 5% (wt/vol) sucrose. The potato chips were a plain unsweetened product (Potatis chips, Estrella AB, Angered, Sweden), the cheeze doodles a sugar-free product (Ostbågar, Estrella AB), and the cracker a sucrose-containing product (Mariekex, Göteborgs Kex AB, Kungälv, Sweden). High-pressure liquid chromatography (HPLC) of low-molecular-weight carbohydrates (23) showed that the potato chips contained 0.3% sucrose; the cheeze doodles 0.1% sucrose, 0.3% glucose, and 2.9% lactose; and the cracker 15% sucrose, 2% glucose, and 2% fructose. The subjects were asked to rinse with the sucrose and starch solutions for 2 min, whereas for the solid test products (nos. 1–3) time was given for consumption. The 10 test sessions (5 products × 2 conditions) were administered

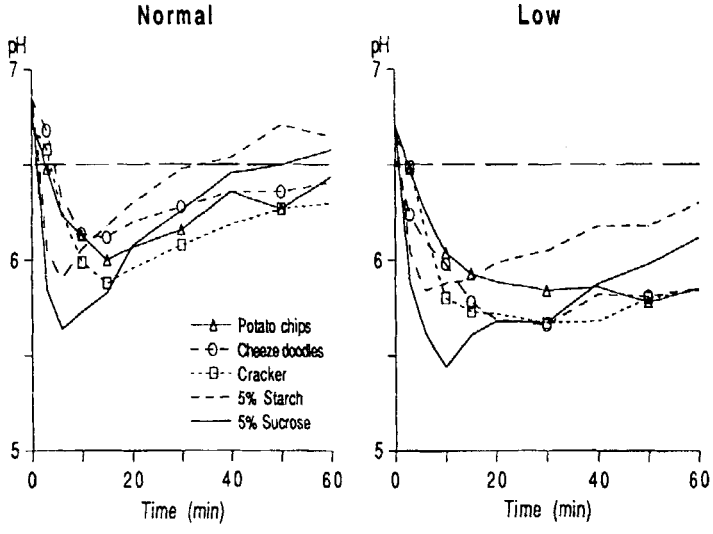

Fig. 1. Changes in pH of human dental plaque after consumption of 5 g potato chips, cheeze doodles, or cracker and after a mouthrinse with 10 ml 5% starch and 5% sucrose at normal (to the left) and low (to the right) salivary secretion rate. Mean values of 10 subjects. pH 6.5 is indicated as a broken line.

in randomized order with regard to both the products and the secretion rates (normal and low).

Plaque pH

The subjects refrained from toothbrushing for 3 days and came to the laboratory without eating or drinking anything, except water, for the last 2 h before each test. pH measurements were made at two approximal areas—that is, in the right upper jaw (A1) and in the right lower jaw (A2)—and at two buccal areas—that is, in the right upper jaw (B1) and in either the right upper ($n = 8$) or lower jaw ($n = 2$) (B2). Thus, four sites were measured at each time point. All buccal sites were located on exposed root surfaces, whereas the approximal sites covered both enamel and dentin. The pH was measured before (0 min) and at 3, 6, 10, 15, 20, 30, 40, 50, and 60 min after the start of the consumption/rinsing. Time zero was set when the product was put into the mouth.

A palladium touch microelectrode with a diameter of 0.1 mm (Beetrode MEPH-1, W.P. Instruments Inc., New Haven, Conn., USA) was inserted into the approximal and buccal areas. The electrode was connected to an Orion SA 720 pH/ISE Meter (Orion Research Inc., Boston, Mass., USA), equipped with a porous glass reference electrode (MERE 1, W.P. Instruments Inc.). A salt bridge was created in a 3 M KCl solution between the reference electrode and a finger of the subject. The electrodes were calibrated before the reading of each test value in accordance with Scheie et al. (24).

Oral retention

The concentration of carbohydrates was measured, during both the normal and low secretion rate, at two different sites in the oral cavity after consumption of the potato chips, cheeze doodles, and cracker, and after rinsing with the starch solution (that is, four of the five test products). The sampling method of Hase et al. (25) was used. Immediately after the pH measurements at 3, 6, 10, 15, 20, and 30 min—that is, on six occasions—two circular paper discs (diameter, 4.0 mm), punched from filter paper (Millipore AP 25, Millipore Corp., Bedford, Mass., USA) capable of absorbing in mean 20 µl of saliva (26), were placed in the mouth, one in the oral vestibule near the upper right first premolar and one in the oral vestibule near the lower right first premolar, close to the two sites for the approximal pH measurements (A1 and A2). After 10 sec the discs were removed from the mouth and transferred to two test tubes, each containing 1.0 ml of distilled water. The tubes were immediately placed in a boiling water bath for 5 min, after which they were shaken vigorously for 15 sec. The water extracts were stored frozen until analyzed.

Table 1. Minimum pH, final pH, and area under the pH response curve ($\times 10^3$) below pH 6.5 ($AUC_{6.5}$) for 0–60 min and 30–60 min after consumption of potato chips, cheeze doodles, and a cracker and after a mouthrinse with 5% starch and 5% sucrose at normal and low salivary secretion rate. Mean values ± SD of 10 subjects and 4 sites per subject. Degree of significance is shown (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

Variable/Product	Normal		Low
Minimum pH			
Potato chips	5.80 ± 0.41		5.64 ± 0.20
Cheeze doodles	5.93 ± 0.48	**	5.50 ± 0.41
Cracker	5.75 ± 0.44	*	5.46 ± 0.26
5% starch	5.83 ± 0.32	*	5.66 ± 0.37
5% sucrose	5.53 ± 0.33		5.26 ± 0.55
Final pH (60 min)			
Potato chips	6.44 ± 0.56	**	5.80 ± 0.24
Cheeze doodles	6.41 ± 0.59	*	5.85 ± 0.43
Cracker	6.31 ± 0.36	***	5.84 ± 0.41
5% starch	6.66 ± 0.30	**	6.30 ± 0.28
5% sucrose	6.59 ± 0.39	*	6.12 ± 0.51
$AUC_{6.5}$ 0–60 min			
Potato chips	27.2 ± 26.0		45.2 ± 11.7
Cheeze doodles	24.8 ± 25.8	**	52.3 ± 24.4
Cracker	32.4 ± 20.6	***	55.3 ± 18.2
5% starch	16.7 ± 12.2	*	34.4 ± 22.2
5% sucrose	29.4 ± 19.5	**	56.4 ± 35.6
$AUC_{6.5}$ 30–60 min			
Potato chips	12.0 ± 14.9	*	26.2 ± 7.0
Cheeze doodles	11.7 ± 15.3	**	27.8 ± 14.1
Cracker	13.6 ± 9.4	***	30.5 ± 12.3
5% starch	3.5 ± 3.4	**	13.2 ± 10.2
5% sucrose	7.2 ± 8.2	*	20.4 ± 18.9

For comparisons of $AUC_{6.5}$ 0–60 min *between* the various products, see Results.

After thawing, the concentration of starch in the samples was analyzed enzymatically as described by Holm et al. (27). To each test tube, 3 µl alpha-amylase (Termamyl 300L, thermostable; Nordisk Novo A/S, Bagsvaerd, Denmark) was added, and the suspension mixed on a magnetic stirrer. The test tube was then placed in a boiling water bath for 15 min with mixing every 5 min. After cooling to room temperature, 5 µl amyloglucosidase (cat. no. 208469, Boehringer Mannheim Scandinavia AB, Bromma, Sweden) and 1 ml 0.1 M sodium acetate buffer were added, and the sample incubated at 60°C for 30 min with frequent mixing. A portion of this solution (0.5 ml) was mixed with 1 ml of a glucose oxidase–peroxidase reagent (Glox) and incubated at 37°C for 30 min. The absorbance of the supernatant was measured in a spectrophotometer at 450 nm. A standard curve was prepared using glucose, and the amount of starch in saliva was expressed as millimoles of glucose per milliliter.

Statistical methods

The mean pH value of the four sites at each time point was calculated. The mean oral glucose retention values were calculated for the two sites, using both raw and logarithmic values. The total area of the pH response curve below pH 6.5 ($AUC_{6.5}$), for time 0 to 30 min, 30 to 60 min, and 0 to 60

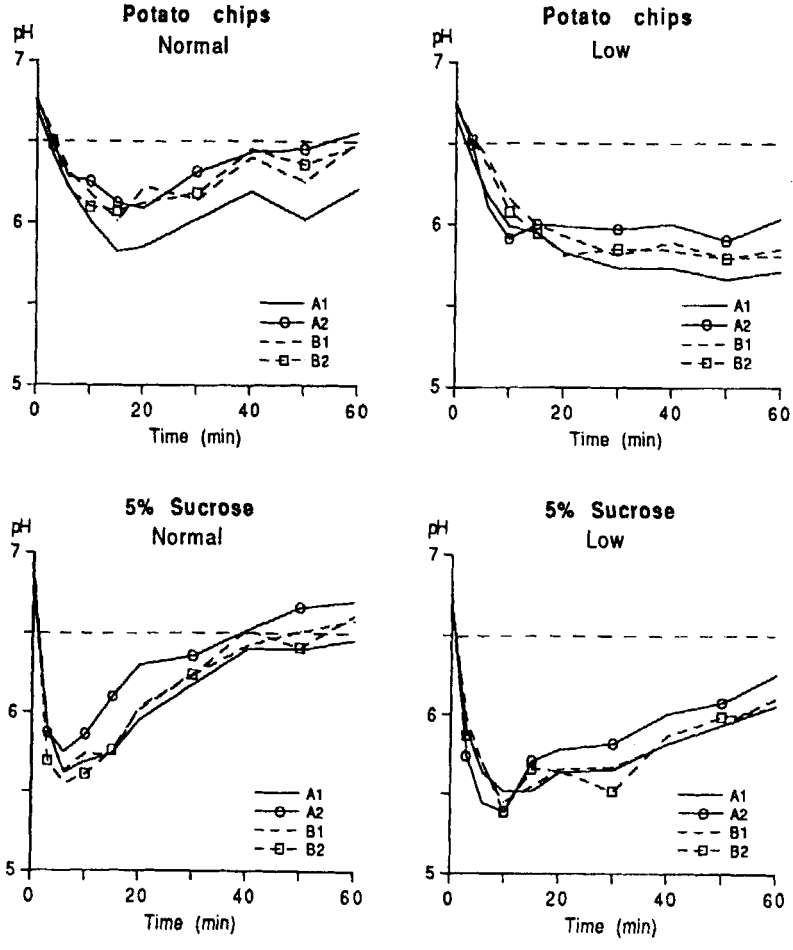

Fig. 2. Changes in pH of human dental plaque at four different sites: approximal area in the upper jaw (A1), approximal area in the lower jaw (A2), buccal area in the upper jaw (B1), and buccal area in either the upper or lower jaw (B2), after consumption of 5 g potato chips and after a mouthrinse with 10 ml 5% sucrose. Data both for normal and low salivary secretion rate are shown. Mean values of 10 subjects. pH 6.5 is indicated as a broken line.

min, was measured in a computer for each pH curve, and the AUC value of each individual carbohydrate clearance curve over the base line (0 mM) for time 3 to 30 min. Student's paired t test (two-tailed) was used to compare the plaque pH and oral retention variables. $P < 0.05$ was considered statistically significant.

Results

Salivary secretion rate and time of consumption

The 'normal' salivary secretion rate for all five test sessions was 2.10 ± 1.04 ml/min, and the 'low' salivary secretion rate 0.35 ± 0.22 ml/min (mean ± SD; $n = 5 \times 10$). The time taken to chew the potato chips, cheeze doodles, and cracker was during normal secretion rate 2.1 ± 1.2, 3.1 ± 1.8, and 2.0 ± 1.0 min, respectively, and during low secretion rate 3.9 ± 2.7, 3.8 ± 2.6, and 4.8 ± 3.0 min, respectively (mean ± SD; $n = 10$). The chewing time between normal and low secretion rate, when all three products were taken together ($n = 30$), was statistically significant ($p < 0.01$).

Plaque pH

The results at normal salivary secretion rate for the five test products are shown in Fig. 1 (to the left). The pH curves represent the mean of the 4 sites and of the 10 individuals. The most pronounced pH fall was seen with the 5% sucrose solution, followed by the cracker.

The results when the products were tested at low salivary secretion rate are shown in Fig. 1 (to the right). They came out in about the same order as at normal secretion rate. However, the plaque pH was about 0.5 pH units lower for all five test products. The starch and sucrose solutions showed a faster pH recovery than seen with the potato chips, cheeze doodles, and cracker, both at low and normal salivary flow.

The minimum pH, final pH, and $AUC_{6.5}$ 0–60 min and $AUC_{6.5}$ 30–60 min, at both normal and low secretion rate, are shown in Table 1. Since the differences between the approximal and buccal sites were small, only the mean values of all four sites are given. For all plaque pH variables, significantly higher values were found for most products at low than at normal salivary secretion rate. The $AUC_{6.5}$ 0–30 values (data not shown) differed significantly between normal and low secretion rate for cheeze doodles ($p < 0.05$) and the cracker ($p < 0.05$). A later occurrence (calculated as number of minutes; data not shown) of minimum pH was seen for all products at low secretion rate; most of these differences were statistically significant ($p < 0.05$).

Comparison of $AUC_{6.5}$ 0–60 min between the five test products showed the following significant differences: for normal secretion rate, 5% starch versus 5% sucrose ($p < 0.01$), and for low secretion rate, cheeze doodles versus 5% starch ($p < 0.05$), cracker versus 5% starch ($p < 0.01$), and 5% starch versus 5% sucrose ($p < 0.05$).

Fig. 2 shows the mean plaque pH for each of the four sites for two of the products, potato chips and 5% sucrose, at normal and low secretion rate. A similar pattern for both pH fall and pH recovery was noted for all sites. When the two approximal sites were compared, a more pronounced pH drop was found in the upper (A1) than in the lower

Table 2. Comparison between the area under the pH response curve below pH 6.5 ($AUC_{6.5}$ 0–60 min) for the four sites (A1, A2, B1, and B2) at normal and low secretion rate. All products and individuals are combined ($n = 50$)

Secretion rate/site	Difference	p*
Normal		
A1 versus A2	12.40 ± 18.60	<0.001
A1 versus B1	6.80 ± 12.90	<0.001
A1 versus B2	5.56 ± 15.60	<0.05
A2 versus B1	−5.60 ± 15.90	<0.01
A2 versus B2	−6.88 ± 15.80	<0.01
B1 versus B2	−1.24 ± 8.43	NS
Low		
A1 versus A2	8.99 ± 17.20	<0.001
A1 versus B1	4.37 ± 10.00	<0.01
A1 versus B2	2.62 ± 13.00	NS
A2 versus B1	−4.62 ± 13.40	<0.05
A2 versus B2	−6.37 ± 16.60	<0.01
B1 versus B2	−1.75 ± 10.40	NS

* NS = not significant.

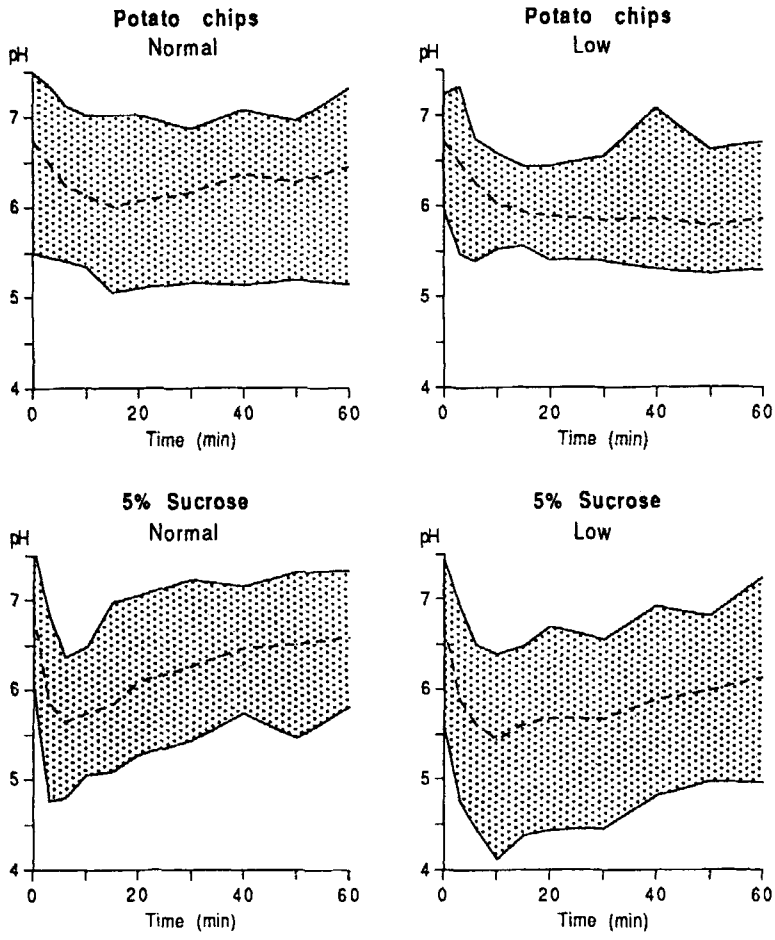

Fig. 3. Individual variation of plaque pH (mean value of 4 sites/individual) for the 10 subjects during normal and low salivary secretion rate after consumption of 5 g potato chips and after a mouthrinse with 10 ml 5% sucrose. The mean values are indicated as a broken line.

jaw (A2). The two buccal sites (B1 and B2) gave almost identical pH values at all time points. Statistical comparisons between the four sites, when all products are combined ($n = 50$), are shown in Table 2. There were significant differences between the two approximal sites and between the approximal and the buccal sites. No statistically significant differences were, however, found between the two buccal sites.

A great individual variation in plaque pH was observed at both secretion rates for all products. Fig. 3 illustrates the range of pH values for two of the products, potato chips and 5% sucrose. A difference of more than 2 pH units was observed at some time points.

Oral retention

The mean salivary carbohydrate concentrations for the upper jaw (both in absolute and logarithmic figures) are shown in Fig. 4. The cheeze doodles and cracker gave the highest retention values both at normal and at low salivary secretion rate. Only small differences were found between the three solid products, whereas the solution was cleared faster.

When the two salivary flow rates were compared, the low secretion gave higher carbohydrate values than the normal secretion for the three solid foods, but not for the starch solution. The mean AUC values of

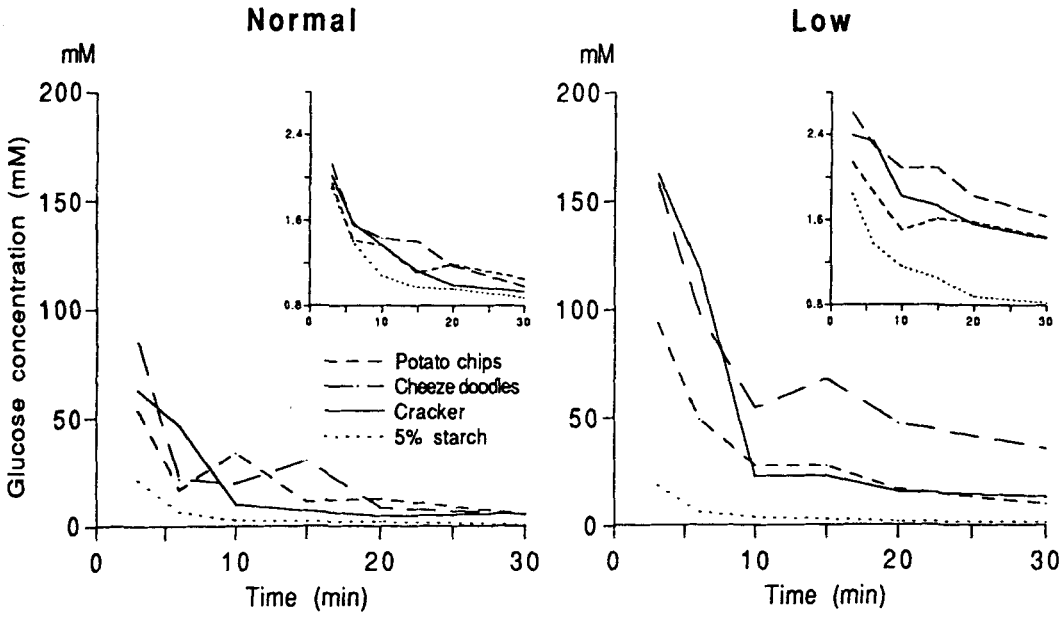

Fig. 4. Salivary carbohydrate concentration for the upper jaw (both as absolute and logarithmic figures) at normal (to the left) and low (to the right) salivary secretion rate after consumption of 5 g potato chips, cheeze doodles, or cracker and after a mouthrinse with 10 ml 5% starch. Mean values of 10 subjects. Inset shows logarithmic values.

the oral retention curves are shown in Table 3. There were statistically significant differences between the two secretion rates for potato chips, cheeze doodles, and cracker.

Comparison of the AUC values between the different products showed the following statistically significant differences at low secretion rate: for the upper jaw, potato chips versus cheeze doodles ($p < 0.05$), cheeze doodles versus 5% starch ($p < 0.05$), cracker versus 5% starch ($p < 0.05$), and for the lower jaw, potato chips versus cracker ($p < 0.05$), potato chips versus 5% starch ($p < 0.05$), and cracker versus 5% starch ($p < 0.05$). No significant differences were seen at normal secretion rate.

Discussion

The most striking results from the present study were that a lowering of the secretion rate increased the pH drop and, especially, delayed the pH recovery in dental plaque. This trend was similar for both approximal and buccal sites. When comparing, for example, potato chips, the final pH at 60 min was 0.7–0.8 pH units lower and the $AUC_{6.5}$ during the later phase (30–60 min) twice as high at low as at normal secretion rate. The delayed pH recovery probably depends on the fact that the oral retention of carbohydrates was prolonged, but also on the fact that the dilution and buffering effect of saliva was impaired. This is in agreement with other reports (12, 13, 28, 29), which have shown a dramatic effect of dry mouth on pH changes in dental plaque.

There were great individual variations in the pH response to the various products, as illustrated for the potato chips and the sucrose solution in Fig. 3. Furthermore, the comparison between the four sites showed that plaque pH was rather site-specific and that in some cases there were great differences in the pH fall within one and the same individual. This trend seemed to be independent of which type of product was

Table 3. Mean area under the curve ($n = 10$) of the salivary carbohydrate curve (mean ± SD) for potato chips, cheeze doodles, cracker, and 5% starch in the upper and lower jaw, both at normal and low salivary secretion rate. Degree of significance is shown ($^* p < 0.05$)

	Normal		Low	
Product	Upper jaw	Lower jaw	Upper jaw	Lower jaw
Potato chips	30.4 ± 62.5	16.9 ± 24.8	42.6 ± 59.9	29.9 ± 27.5
Cheeze doodles	37.5 ± 56.6	33.8 ± 63.2	107.9 ± 122.5	78.6 ± 111.5
Cracker	24.6 ± 41.3	15.0 ± 11.0	73.2 ± 68.1	73.9 ± 68.7
5% starch	7.0 ± 1.6	5.4 ± 1.6	6.8 ± 2.6	5.8 ± 1.4

For comparison *between* the various products, see Results.

tested. A high reproducibility and a relatively small weekly variation of the plaque pH at various sites have previously been reported (30, 31). Our measurements were all made in the premolar–molar region on the one side of the oral cavity, since it has been shown that the pH response using the microtouch method is similar on the right and left side (31). When the two approximal areas were compared in the present study, a lower plaque pH was seen in the maxilla than in the mandible. This variation in acidogenic response in the interproximal areas between the two jaws has also been found in other studies (11, 30–32). The reason for this difference is probably the higher access to saliva in the lower jaw.

We could not find any differences in the pH response between the two buccal sites B1 and B2, of which B1 was always located in the upper jaw, whereas B2 was located both in the upper and lower jaw. Thus, we were unable to make a clear distinction between the two buccal areas, owing to difficulties in subject selection. An interesting observation was, however, the fact that the buccally oriented root surfaces gave pH minima and AUC values similar to the approximal enamel–dentin sites. It has been found that the minimum pH for approximal sites is in mean 0.7 pH units lower than for buccal surfaces after a sucrose rinse (33). On the other hand, several studies have confirmed that even on buccal sites, the plaque pH measured with the telemetric method can reach values below pH 5.0 (33, 34). The reason no difference was found between the buccal and approximal sites in the present investigation may be that we selected exposed root surfaces strictly for the buccal areas. Some of these also showed a tendency to be caries-active, which is known to accentuate the plaque pH drop (30, 32, 35).

Relatively small differences were found between the oral retention values for the three snack products at normal salivary secretion rate. This is in agreement with other studies (36, 37). The observation that solid starch food products give high retention values compared with solutions is in accordance with earlier observations (17, 38). At reduced salivary flow, the oral retention increased, especially for the cheeze doodles and the cracker. These two products were considered to be more difficult to chew and swallow than the potato chips. The importance of salivary flow rate for oral clearance and retention of carbohydrates, using the same experimental design as in the present study (with injection of methylscopolamine), has recently been demonstrated by Hase & Birkhed (13). We found in this study higher oral retention values, even though not statistically significant, in the upper than in the lower jaw. The fact that the clearance rate varies considerably, not only between prod-

ucts but also from site to site in the oral cavity, is in agreement with other studies (39, 40).

The cariogenic potential of starch compared with sucrose has long been discussed in the dental literature (for a review, see Ref. 3). The snack products included in the present study all had a high degree of gelatinized starch, which is more easily hydrolyzed by salivary alpha-amylase than raw and uncooked starch (6–8). It must be remembered, however, that the potato chips and cheeze doodles contained small amounts of low-molecular-weight carbohydrates, whereas the crackers contained up to 19% mono- and di-saccharides, which all are easily fermented by oral microorganisms. Besides measuring the plaque pH response, we also evaluated the oral carbohydrate retention, which provides information about the availability of substrate. By combining these two methods, one may conclude that all three starchy snack products studied—potato chips, cheeze doodles, and cracker—have a rather high 'cariogenicity'. For individuals with exposed root surfaces and with a low salivary secretion rate, the risk of dental caries when eating such products should not be underestimated.

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