# Effect of variations in sucrose consumption on salivary lactobacillus count and sucrase activity in man

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Dental students ($n = 31$) with either high salivary lactobacillus count ($>10^4$ CFU/ml) or high salivary sucrase activity ($\geq 10$ μmol/min × mg protein × $10^{-3}$), or both, were selected to participate in this dietary experiment. For 2 weeks the students avoided sucrose in their diet. Stimulated saliva samples were collected before and after the diet. An additional follow-up sample was collected after 2 weeks of normal diet. The lactobacillus count of undiluted saliva was determined by the Dentocult dip-slide technique. The sucrase activity was determined spectrophotometrically by measuring the sucrose-cleaving activity of centrifuged saliva supernatant. Both the reduction in dietary sucrose and the return to normal diet caused a significant change in these values. □ *Clinical study; dietary sucrose; saliva; sucrase*

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Nutritional counseling to improve sucrose consumption habits is an essential component in dental caries prophylaxis. However, there are few practical means of estimating the amount and frequency of the patient's sugar consumption or to verify the altered habits after counseling.

Among the salivary factors closely related to dietary sucrose consumption, the amount of lactobacilli and the level of sucrose-cleaving activity have been widely studied. The former has already been used as a clinical follow-up test in monitoring the effect of dietary counseling (1–3). It has been shown that a high lactobacillus count can be reduced significantly if the diet is entirely free of easily fermentable carbohydrates. Further, when the dietary sucrose is totally substituted with sugar alcohol, the lactobacillus counts decrease significantly (4). Vice versa, the number of lactobacilli increases when the sucrose consumption increases (4, 5). However, the amount of lactobacilli is known to be dependent on other factors in the oral cavity as well. Microbial retention sites such as open carious cavities, dentures, orthodontic bands, or partially erupted third molars are connected with high salivary lactobacillus counts (6–8). A poor salivary buffering capacity (9) and an extremely low rate of salivary flow (10) are also known to be host factors closely related to high lactobacillus counts.

The bacterial (11) enzyme sucrase catalyzes the degradation of sucrose to glucose and fructose. The term sucrase as used here covers invertase (β-D-fructofuranosidase fructohydralase, EC 3.2.1.26), fructosyl transferase (EC 2.4.1.10), glycosyl transferase (EC 2.4.1.5), and α-D-glucoside glucohydrolase (EC 3.2.1.20) as it is assumed that these enzymes may function simultaneously when using such complex enzyme preparations as oral fluid. The invertase-like sucrase activity refers to two differently located enzymes at least, one being membrane-bound and the other intracellular (12–18). Sucrase activity is induced by the presence of the substrate sucrose (14, 19, 20), and frequent consumption of sucrose is connected with high salivary sucrase activities (21). Several acidogenic species of the oral flora, including *Streptococcus mutans, S. salivarius, Actinomyces viscosus, A. naeslundii, Lactobacillus salivarius*, and *L. fermentum*, have been shown to have high sucrase activi-

ties (22). Poor oral hygiene is also known to be connected with high sucrase values (23, 24). When the sucrose consumption increases, the enzyme activity is known to increase, especially in plaque but also in saliva (21, 25). Vice versa, the enzyme activity is reduced significantly if sucrose in the diet is totally or partially substituted by sugar alcohol (21) or with fermentable sugars other than sucrose (26).

Our aim was to study the effect of a 2-week controlled sucrose restriction on these factors. We also wanted to compare the ability of the two tests to show alterations in sucrose consumption habits. Finally, the stability of these factors was tested after a 2-week period of normal diet.

## Materials and methods

### *Test subjects and dietary instructions*

A total of 31 subjects out of 101 university students, 20–23 years of age, having either high ($>10^4$/ml) salivary lactobacillus count ($n = 16$) or high ($\geq 10$ μmol/min × mg protein × $10^{-3}$) salivary sucrase activities ($n = 5$), or both ($n = 10$), participated in the present dietary experiment. The test persons were unmedicated healthy individuals who did not have any orthodontic appliances or open carious cavities during the period. No dental treatment was given to the test subjects during the test period.

Before starting the diet, the subjects received careful instructions: all foodstuffs containing added or visible sucrose should be avoided during a period of 2 weeks. If not previously regularly consumed, sugar substitutes were not supposed to be used. Otherwise, the test subjects should not alter their normal diet or oral hygiene habits.

The strict dietary experiment presupposed a high level of knowledge, motivation, and co-operation on the part of the participants. Therefore dental students, having basic knowledge of the nutritional aspects of dental caries, were selected for the present study. The stability of the achieved results was examined after the following 2-week period of normal diet. Twenty-five students out of the 31 original participants continued in this part of the study.

The test persons recorded the exact time and quality of their meals and snacks during the sucrose-free period. An additional 7-day diary of normal diet was kept by those who continued in the follow-up period, which was the 4th week from the beginning of the study. The quantity of the various foodstuffs consumed was not recorded.

### *Saliva collection*

Stimulated saliva samples were collected on three occasions: at the beginning of the test period, after the 2 weeks of sucrose-free diet, and after 4 weeks from the beginning of sucrose restriction. Saliva collections were performed at 0900 h on each occasion. The test persons brushed their teeth after breakfast and avoided all eating and drinking before the saliva collections. Saliva stimulation was performed with a piece (1 g) of sucrose-free chewing-gum base (Hellas, Turku, Finland). The collection started with a short prestimulation of 1 min during which the secreted saliva was swallowed. During the next 5 min the secreted saliva was collected via a funnel in graded test tubes. The volume of the secreted saliva was recorded with an accuracy of 0.1 ml. The buffering capacity was measured without delay, using the Dentobuff® system (Orion Diagnostica, Helsinki, Finland). The final pH of the reagent solution was determined electrometrically. The remaining saliva was chilled on crushed ice, and a part of it was used for microbial culturing. The rest was centrifuged for 10 min (12,200 *g*), and the supernatant was stored at −20° and used within 6 weeks for the enzyme activity and protein determinations. The sucrase activity of the supernatant stored at −20°C is practically the same as the activity of the samples freshly determined (27).

### *Salivary lactobacilli*

The bacterial cultivation of undiluted saliva was performed with the Dentocult® dip-slide technique (Orion Diagnostica, Helsinki, Finland). The incubation was carried

out at 35°C for 3 days. The density of the colony-forming units (CFU/ml) was determined by comparing the incubated slides with the density model charts provided by the manufacturer. After the densities had been judged freshly after incubation, all the slides were transferred to +4°C until the results from the next two cultivations were available. The three slides thus obtained per each test person were placed in chronologic order and photographed. Bacterial densities greater than $10^4$ CFU/ml were regarded as high, whereas densities of $10^4$ CFU/ml or less were considered low.

*Enzyme determinations*

Sucrase activity was determined by the method of Scheinin & Mäkinen (28) as follows: 0.2 ml of saliva supernatant was incubated at +30°C for 2 h with 0.1 M sucrose and 6.7 mM Na-acetate (pH 4.7). The total volume of the reaction mixture was 0.75 ml. The reaction was stopped by adding 0.5 ml of 1% 3,5-dinitrosalicylic acid reagent (29). The reaction mixture was heated at 100°C for 5 min, and the amount of reducing sugars—that is, glucose and fructose—was measured colorimetrically at 540 nm. Soluble protein of the saliva supernatant was measured by the method of Lowry et al. (30). Specific enzyme activity was determined as liberated μmol of glucose/min × mg protein × $10^{-3}$. Specific activities equal to 10 (μmol/min × mg protein × $10^{-3}$) or more were regarded as high sucrase activities,

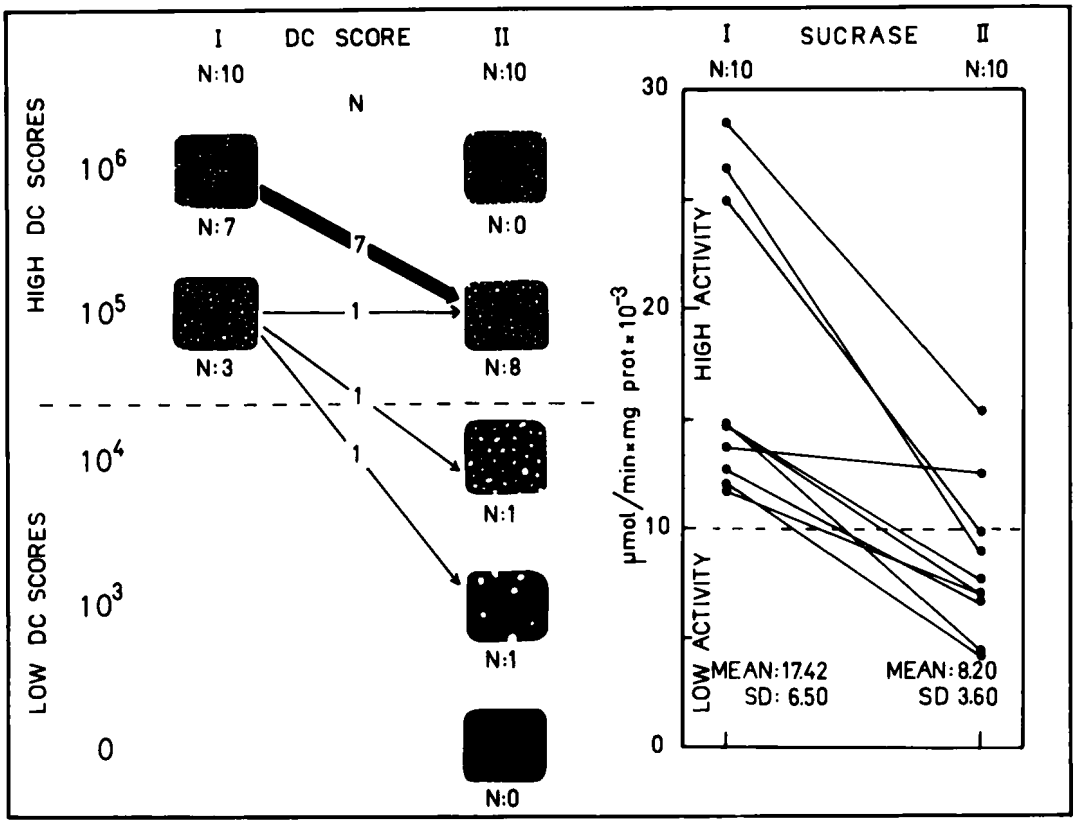


Fig. 1. The effect of the sucrose-free diet (II) on the Dentocult (DC) scores and sucrase activities of those individuals ($n$ = 10) who initially (I) had high lactobacillus counts (>$10^4$ CFU/ml) and high sucrase (≥10 μmol per min and per mg protein × $10^{-3}$) activities. The dotted quadrants describe the densities (0, $10^3$, $10^4$, $10^5$, and $10^6$ CFU/ml) of the lactobacillus growth, which correspond to the density model chart provided by the manufacturer (Orion Diagnostica).

whereas activities less than 10 were considered low sucrase activities.

*Statistical analysis*

The significance of differences in bacterial densities was tested with the non-parametric sign test, whereas the difference between the frequencies of meals and snacks, the enzyme activities, and the rate of salivary flow was tested with the paired *t* test. The non-parametric Wilcoxon matched-paired signed-ranks test was used to calculate the significance of changes in the buffering capacity.

## Results

*Diet*

The frequency values of the sucrose restriction period and those of the normal diet showed that the mean sucrose intake was 0.4 (SD, 0.2) during the sucrose restriction, whereas normally it was 3.1 (SD, 0.3).

*The effect of the sucrose restriction*

A clear reduction of both measured factors was seen in those test persons ($n = 10$) who initially had both high Dentocult (DC) scores and high sucrase activities (Fig. 1). The magnitude of bacterial reduction was one category in eight individuals and two categories in one individual. The DC score of one test person remained at the initial level (Fig. 1). The sucrase activity of these 10 test persons was reduced without exception. The reduction had the following pattern: the higher the initial activity, the greater was the reduction in enzyme activity (Fig. 1).

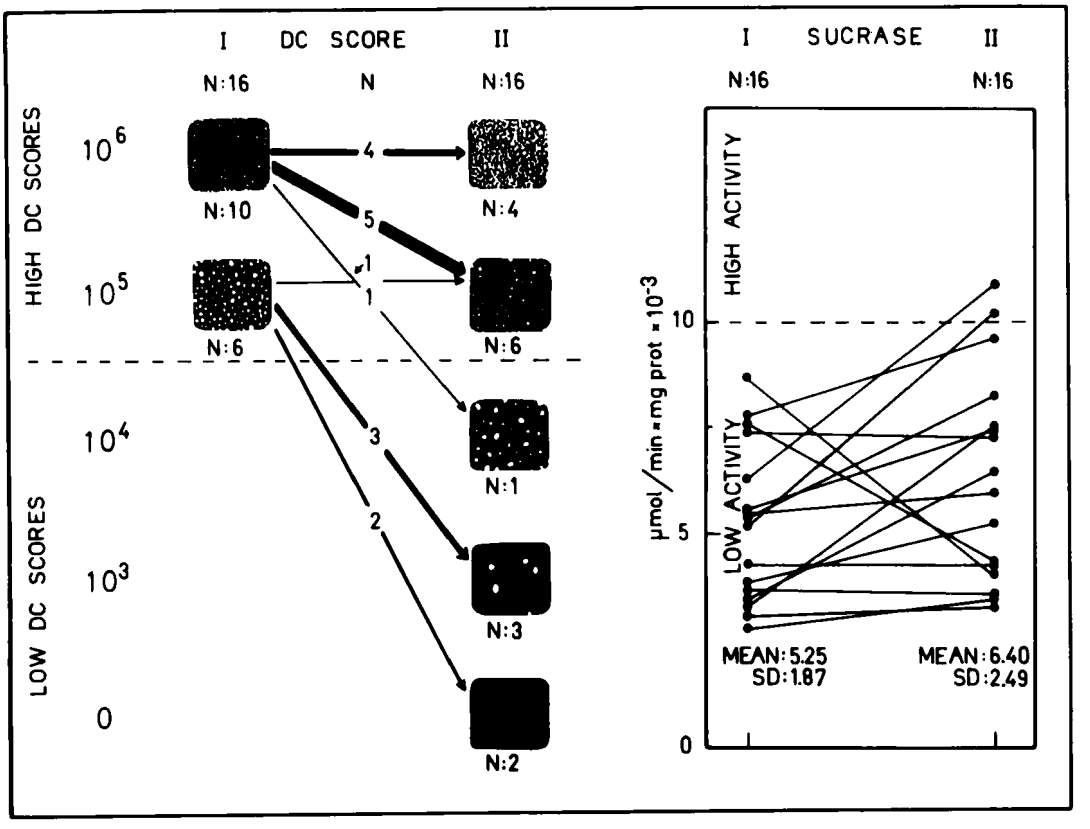


Fig. 2. The effect of the sucrose-free diet (II) on the Dentocult (DC) scores and sucrase activities of those individuals ($n = 16$) who initially (I) had high DC scores and low sucrase activities. For further explanation of abbreviations and symbols, see legend to Fig. 1.

The changes of the values in those individuals ($n = 16$) who initially had high DC scores but low enzyme activities are shown in Fig. 2. The salivary bacterial density of 11 individuals was reduced by one or more categories, whereas the DC score of 5 individuals remained at the original level. The sucrase activity of 10 individuals increased, whereas the activity of 6 test persons decreased (Fig. 2). The values of two individuals surpassed the level of high enzyme activity.

The effect of the sucrose restriction in the third group ($n = 5$) who initially had low DC scores but high sucrase activities is shown in Fig. 3. A decrease in the bacterial density was seen in three individuals, whereas the score of one subject increased but did not exceed the border value of $10^4$ CFU/ml. The lactobacillus count of one test person remained unaltered. The sucrase activity of four individuals was greatly reduced, to below the level of high activity. The reduction in two of the values was less marked (Fig. 3).

In conclusion, the 2 weeks of sucrose restriction caused a significant ($p < 0.05$) reduction of the lactobacillus counts in a majority ($n = 20$) of the subjects ($n = 26$).

The results of the salivary sucrase activities are summarized in Table 1. The sucrose-free diet reduced the enzyme activity significantly ($p < 0.005$). The values of all those individuals whose activities were initially high decreased markedly ($p < 0.001$) to below 50% of the original values. In contrast, values lower than 10 (μmol per min and per mg protein $\times 10^{-3}$) increased on an average

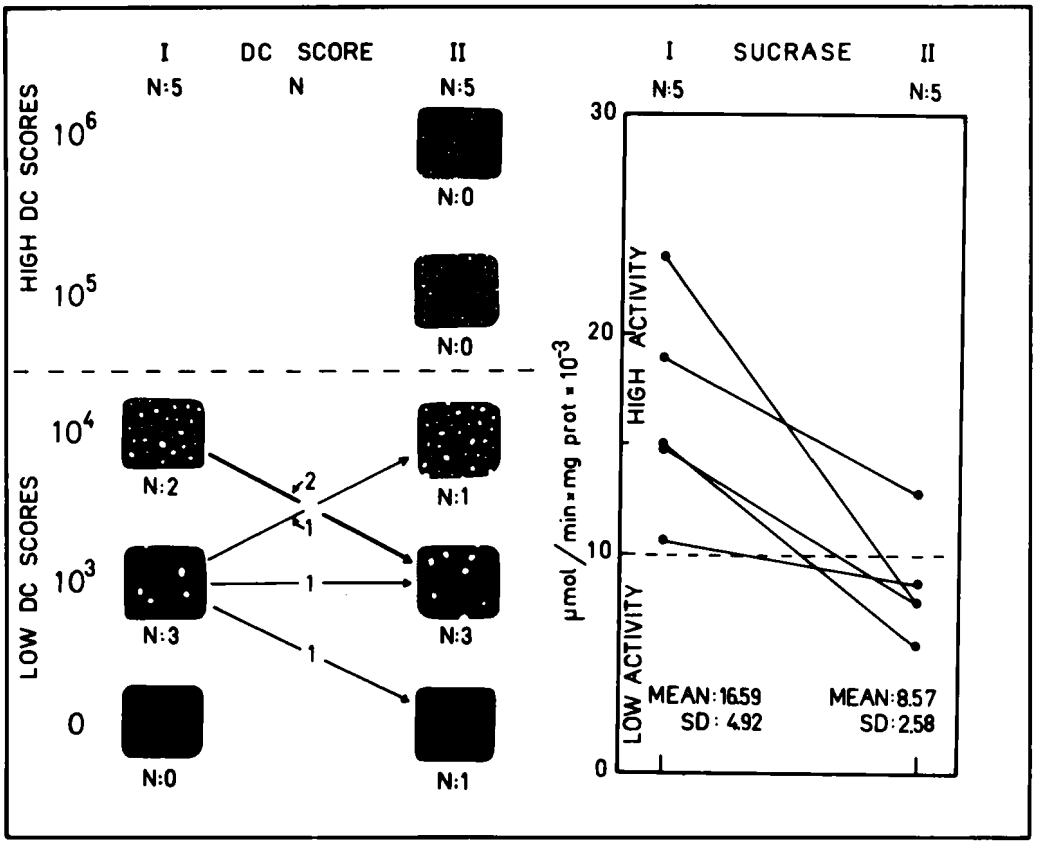


Fig. 3. The effect of the sucrose-free diet (II) on the Dentocult (DC) scores and sucrase activities of those five individuals who initially (I) had low DC scores and high sucrase activities. For further explanation of symbols and abbreviations, see legend to Fig. 1.

Table 1. The effect of variations in sucrose consumption on the salivary sucrase activities (SA). The values (μmol per min and per mg protein × $10^{-3}$) are expressed as means of standard deviations

| | Initial | | After sucrose restriction | | After normal diet | |
|---|---|---|---|---|---|---|
| Group (*n*) | Mean | SD | Mean | SD | Mean | SD |
| High* and lowt SA (25) | 11.7 | 7.9 | 7.4 | 2.8 | 8.6 | 5.2 |
| | | $p < 0.005$ | | n.s. | | |
| | | | $p < 0.01$ | | | |
| High* SA (13) | 17.6 | 6.2 | 8.1 | 3.2 | 11.4 | 5.6 |
| | | $p < 0.001$ | | $p < 0.01$ | | |
| | | | $p < 0.005$ | | | |
| Lowt SA (12) | 5.4 | 1.7 | 6.8 | 2.2 | 5.5 | 2.2 |
| | | $p < 0.05$ | | n.s. | | |
| | | | n.s. | | | |

* Sucrase activity: ≥10 μmol per min and per mg protein × $10^{-3}$.
† Sucrase activity: <10 μmol per min and per mg protein × $10^{-3}$.

($p < 0.05$). The significance of differences was similar, as described above, when the values of the whole initial study group ($n$ = 31) was tested.

*The stability of the results*

The distribution of the test persons into high and low Dentocult groups after the normalization of the diet is shown in Table 2. Further bacterial reduction appeared in 3 individuals only, whereas nearly 40% of the test subjects (9 out of 25) returned to higher DC scores (Table 2). The final density of more than half of the test persons remained at the reduced level.

After the normal diet period the average enzyme activity was significantly ($p < 0.01$) lower than the initial activity (Table 1). Yet, when the high and low values were analyzed separately, a significant increase back to higher sucrase activities ($p < 0.01$) appeared in those individuals who initially had a high activity. The final values of the test persons starting with low sucrase activities remained at the initial level (Table 1).

Table 2. The stability of the salivary lactobacillus counts. The final distribution of subjects into increased, unaltered, or decreased densities in accordance with the high (>$10^4$ CFU/ml) or low (≤$10^4$ CFU/ml) Dentocult groups obtained after the sucrose restriction period

| | Increased | Unaltered | Decreased | Total |
|---|---|---|---|---|
| High DC | 4 | 8 | 2 | 14 |
| Low DC | 5 | 5 | 1 | 11 |
| Total | 9 | 13 | 3 | 25 |

*Salivary flow and buffering capacity*

The rate of secretion (mean, 1.5 ml/min; SD, 0.6) did not alter during the diet. At the start of the study there was only one individual whose flow rate was less than 0.7 ml/min. The salivary buffering capacity was almost the same after the diet as in the beginning, varying between pH 3.2 and pH 6.5. Five of the test persons were considered to have poor buffering capacity, indicating values lower than pH 4.5.

## Discussion

A critical review (31) of earlier reports indicates that the amount of lactobacilli in saliva correlates to sugar consumption only if the oral cavity is free of open carious lesions

and other retention sites. To reduce these confounding factors to the minimum, a group of highly co-operative, well-motivated dental students with a high standard of oral hygiene were selected for this dietary experiment. The dentition of the test subjects was examined and treated yearly, excluding the possibility of open carious lesions. However, initial lesions not needing operative treatment and/or partially erupted third molars (8) may have been reasons for the initially high lactobacillus counts. On the other hand, the low flow rate of saliva and/or poor buffering capacity, known to correlate negatively with the amount of lactobacilli (9, 10), may have been other reasons for the high DC counts of some of our test subjects.

The results of this study indicate that a reduction in the dietary sucrose for 2 weeks is long enough to cause detectable reductions in the oral lactobacillus counts in most cases. The return to normal diet either had no effect ($n = 13$) or increased ($n = 9$) the DC scores.

In subjects with initially high sucrase activities variations in dietary sucrose systematically affected this factor, a finding that is in agreement with those of Mäkinen & Scheinin (21); in contrast, low enzyme activities seemed to be of less value in judging individual sucrose consumption habits. The rapid return of high enzyme values after the normalization of the diet in individuals who originally had high sucrase activities further supports the straight cause-and-effect relationship between the activity of the sucrose-splitting enzyme and its substrate. The low values again responded poorly to the reappearance of the substrate in the oral cavity.

In view of the present results we conclude that the studied factors, if initially high, both correlate with alterations in the sucrose consumption habits. However, since high lactobacillus counts easily lead to misinterpretations, a high sucrase activity may be superior in reflecting variations in sucrose consumption.

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