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EVALUATING THE CARIOGENIC POTENTIAL OF DIETARY BISCUITS AND NUTRITIVE BAR – A RANDOMIZED FIELD TRIAL

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Introduction

Dental caries is an oral disease of multifactorial origin affecting the permanent teeth of 2.3 million and primary teeth of 530 million people.[1] This biofilm mediated and sugar driven disease, causes phasic demineralization and remineralization of the teeth structure.[2] Diet rich in extrinsic sugars plays an integral role in inception and progression of dental caries.

The ultra – processed snack products like biscuits and chocolates are reportedly rich in extrinsic sugars and other unhealthy constituents.[3] These nutritionally imbalanced snack products when overtly consumed contribute to NCD's like obesity, diabetes, dental caries and cardiovascular disorders.[4] Evidence states that overt consumption might be associated with external factors like availability of the type of food and time of consumption.[5] So, necessary measures must be taken to control these external factors.

Concerning dental caries, the cariogenic potentiality of these ultra – processed snack products can be evidently reflected in the saliva with decline in pH and flow rate along with reduced oral clearance rate of the food constituent like glucose.[6] The physical state and texture of these snack products matters as they

contribute to food retention on tooth surfaces. These retentive food substances are metabolized by the oral bacterium by releasing acidic by-products.[7] These by-products in turn causes demineralisation of organic matter and dissolution of inorganic matter of the teeth causing dental caries.[8]

Conversely, alternative snack products with balanced nutrient levels and zero added sugars have been marketed as healthy counterparts in the recent years.[9] These commercially regarded healthy snacks includes grain based dietary biscuits and nutritive bars. Generally, these healthy snackable items are a source fibre, nutrients, complex carbohydrates, vitamins and protein. Compared to the ultra – processed snack products, the grain based biscuits and nutritive bars are an healthy option.[10] But, their impact on the dental tissues with regard to cariogenic potentiality is still a query.

India is claimed to experience a "nutrition transition".[11] It is estimated that 6 percent of the household spending is on packaged, processed foods.[11] The availability of these products, accessibility to stores and rigorous marketing strategies have urged the general public to purchase these unhealthy snack products. A recent meta-analysis revealed that the mean dmft for 5 and 12 years old was 2.36 (49%), for 15 years old was 1.95 (60%), for 35-44 years old was3.31 (78%) and for 65-74 years old was 7.01 (84%).[12] The past caries experience is the best predictor of future caries susceptibility. The association between diet and dental caries addresses the need for limited sugar consumption. Therefore, to control the urge for snacking, people could opt for healthy snack products like grain based dietary biscuits and nutritive bars that are available in the local market.

Thus, a field trial was designed to assess and compare the cariogenic potential of commercially available dietary biscuits and nutritive bars among young adults in Chennai city.

Materials & Methods

A cross-over trial was conducted espousing Latin square design among 18 – 25 year old volunteers. Ethical clearance was obtained from the institutional review board of MAHER. The study protocol was registered with the Clinical Trial Registry of India (CTRI) bearing the registration number: CTRI/2020/12/029715. The study participants were recruited based on the inclusion and exclusion criteria.

Inclusion Criteria

- Subjects of 18 – 25 years of age.
- Subjects with no carious lesion.
- Subjects who give voluntary informed consent.

Exclusion Criteria

- Subjects with any dental prosthesis / under fixed orthodontics.
- Subjects with systemic disorder and under medication that alters the salivary flow rate and glucose metabolism.
- Subjects allergic to nuts & seeds..

Study Setting

The trial was executed in a at the department of Public Health Dentistry and at the Central Research Laboratory, Meenakshi Ammal Dental College and Hospital.

Outcome of the Study

The primary outcome were detectable changes in salivary pH, salivary flow rate and salivary glucose clearance rate after consuming the intervention products. The secondary outcome was to assess the food retentiveness on the occlusal surfaces of the teeth after consumption of the intervention products.

Sampling and Sample size

A Criteria – based purposive sampling was adopted. Fourteen volunteers who satisfied the eligibility criteria were recruited in the present trial.

Block Randomization & Latin Square Design

A Computer generated block randomization was performed. As the study followed a Latin square design, the study participants (n = 14) were randomly allocated within 7 blocks with 2 participants per block.

Experimental Interventions

The seven experimental interventions included the multigrain fibre biscuits, cracker biscuits, choco – nutritive bar, nuts & seed nutritive bar (test products), , unfilled biscuits and chocolate (positive controls) and orthodontic elastics (negative control). On day 1, the intervention products were randomly allocated to each block and on the subsequent days the products were based on the provisions of the Latin square design. On all the days of the trial, the study subjects were asked to have an early breakfast and report to the department of Public Health Dentistry for examination. The weight(15 grams) of a multigrain biscuit was kept as a reference for all other test products and positive controls'. Each product was individually packed and offered to the participant. The snack products were individually consumed by the principal investigator, it took around two minutes to eat every snack product. Thus, a standard time of 2 minutes was allotted for the study subjects.

Salivary Sample Collection

The "passive method" was adopted as the method for salivary sample collection. The salivary samples were collected in a sterile container. The unstimulated salivary samples were collected to assess the basal salivary pH; salivary flow rate and salivary glucose clearance rate.

Stephan's curve has been endorsed in caries research to describe the carcinogenicity of a food product. After the consumption of any food product, the pH is susceptible to variations at the 5^{th} minute, 20^{th} minute, 45^{th} minute and 60^{th} minute. A drop in pH levels below the level of 5.5 (i.e.) below the critical pH marks caries potentiality of the food product. Therefore, in the present study upon consuming the intervention snack products and / chewing the orthodontic elastics, the stimulated salivary samples were collected at the end of 5^{th} minute, 20^{th} minute, 45^{th} minute and 60^{th} minute (as per the Stephan's curve) to assess changes in the salivary pH; salivary flow rate and salivary glucose clearance rate.(Picture 1 and 2)

Salivary pH Assessment (Pre and Post Intervention)

A calibrated digital pH meter was used to measure the salivary pH not later than 10 minutes after the salivary sample collection. (Picture 3)

Salivary Flow Rate Assessment (Pre and Post Intervention)

Salivary Flow Rate was assessed by pipetting the salivary samples into calibrated test tubes for measuring amount (ml) of saliva collected. (Picture 4)

Salivary Glucose Clearance Rate Assessment (Pre and Post Intervention)

To begin, the salivary sample of 0.5ml was pipetted into an Eppendorf (Small test tube used for salivary collection). Secondly, the Eppendorf's containing salivary sample was centrifuged at 3000 rpm for 3 minutes. Upon centrifugation, glucose reagent was added to the salivary sample. Then, the salivary sample was incubated at 32 degree Celsius for 15 minutes. Finally, after incubation the level of glucose in the salivary sample was assessed using a biosensor. The appropriate glucose concentration was recorded. Notably, salivary samples with high level of glucose concentration displayed an evident pink hue. (Picture 6-9)

Food Retention (Post Intervention)

At the end of 60^{th} minute post consumption of the intervention product, ADA Type III examination was performed to assess the food retentiveness on the occlusal surfaces of the teeth. The modified occlusal plaque index by (Addy et al) was conceptualized for assessing the food retention. The average value was calculated based on the clinical examination and relevant modified descriptions. (Table. 1, Picture 5)

Statistical Analysis

Statistical analysis was carried out using SPSS software (IBM Statistics 20). Intra group comparison was done using Friedman test. Inter group comparison was carried out using Kruskall Wallis followed by Post hoc analysis.

The modified occlusal plaque index / Food Retention Index (Table 1)

CODE	DESCRIPTION
0	No food debris or discrete food particles in the fissure pattern
1	Line of food debris in fissure pattern but not outlining whole fissure system
2	Fissure system completely outlined by food debris
3	Food debris beginning to extend out of the fissure system at some sites with < 1/3 coverage
4	Food debris extending out of the fissure system with 1/3 to 2/3 of the coverage
5	Food debris extending to cover > 2/3 of the surface

Results

Comparison of Salivary pH changes within and between the groups at measured time intervals: (Table 2)

Intra-group comparison:

The salivary pH level at the end of 5th minute, declined for the multigrain biscuit, nut & seed nutritive bar; increased for the unfilled biscuit and choco nut nutritive bar, chocolate bar, orthodontic elastics and remained unchanged for cracker biscuit compared to its baseline. At the end of 20th, 45th and 60th minute, the salivary pH levels exhibited a variation of increase or decrease. But, at no time point did the salivary pH fall below the critical level of 5.5 for any of the intervention product used.

For the unfilled biscuit, the rise in pH sustained till the end of 45th minute, following a dip at 60th minute. For the nut & seed nutritive bar, the rise in pH was noted at the 5th minute and immediately started to dip at the end of 20th, 45th and 60th minute respectively. For the choco nut nutritive bar, a rise in pH was evident at the 5th minute with constant dip at the end of the 20th, 45th and 60th minute. The differences were found to be statistically significant with regard to unfilled biscuit (p – value < 0.022), nut & seed nutritive bar(p – value

< 0.005)and choco nut nutritive bar(p – value < 0.006). At no time point did the salivary pH fall below the critical level of 5.5 indicative of diminished chances of demineralization.

Inter-group comparison:

At the end of 5th minute, the salivary pH levels of all the test products were lesser than the orthodontic elastics. At the end of 20th minute, except the nut & seed nutritive bar, the other test products showed a decline in pH levels compared to the orthodontic elastics. At the end of 45th minute, all the test products had less pH levels compared to orthodontic elastics except the cracker biscuit, unfilled biscuit and chocolate bar. At the end of 60th minute, a decline a in salivary pH levels were noted in comparison to the orthodontic elastics. None of these differences were statistically significant (p – value > 0.05).

Comparison of Salivary Flow rate (ml/min) within and between the groups at measured time intervals: (Table 3)

Intra-group comparison:

Following the consumption of the test products, an increase in the salivary flow rate was noted at the end of 5th minute for all the products compared to their baseline values. A constant increase in the flow rate was noted at the end of 20th, 45th and 60th minute, for cracker biscuit, unfilled biscuit and the choco nut nutritive bar. The statistical significant difference was noted only among the unfilled biscuit (p – value: 0.001) and the choco nut nutritive bar(p – value: . 0.006). Though a rise was noted at the end of 5th minute and 20th minute, a decline was evident at the 45th minute and by the end of 60th minute the flow rate returned to that of the baseline values in the chocolate bar and this difference was found to be statistically significant (p – value: 0.001). There was gradual decline in the flow rate at the end of 20th, 45th for the nut & seed nutritive bar and by the end of the 60th minute the flow rate levels were found to be similar to their baseline values. These differences was found to be statistically significant (p – value: 0.001). The Salivary flow rate after consumption of all the test products did not decline below the level of 1.1mL/min.

Inter-group comparison:

There was a rise and drop in the salivary flow rate of all the test products compared to the orthodontic elastics at varied time points (i.e) at the end of 5th, 20th, 45th and 60th minute. But, none of the differences were found to be statistically significant (p – value > 0.05).

Comparison of Oral Glucose Clearance rate within and between the groups at measured time intervals: (Table 4)

Intra-group comparison:

The oral glucose level was higher at the end of 5th minute for all the test products except the orthodontic elastics. A gradual and steady oral glucose clearance rate was noted only in the cracker biscuit and the differences were found to be statistically significant (p – value: < 0.001). The other test products had a slow oral glucose clearance rate at end of the 20th, 45th and 60th minute. A statistical significant difference was noted in the multigrain biscuit (p – value: < 0.001), unfilled biscuit (p – value: < 0.001), nut & seed nutritive bar (p – value: < 0.001), choco nut nutritive bar (p – value: < 0.001) and chocolate bar (p – value: < 0.001).

Inter-group comparison:

Among all the test products post consumption, the oral glucose level experienced a rise at the end of the 5th minute among the unfilled biscuits, followed by the cracker biscuit, chocolate bar, multigrain biscuit, choco nut nutritive bar and nut & seed nutritive bar compared to their respective baseline values. The differences were found to be statistically significant (p– value: <0.001). By the end of 45th minute, the oral glucose level declined for all test products compared to the 5th minute value and the differences were found to be statistically significant (p – value: 0.009), indicative of oral glucose clearance.

Comparison of Food Retentiveness among the groups at the end of 60th minute: (Figure 1)

In comparison to the orthodontic elastics, the nut & seed nutritive bar, choco nut nutritive bar, unfilled biscuit and cracker biscuit exhibited retention at the end of 60th minute post consumption. But, as the extent of retention was score is < 1, the retention of the above stated test products were deemed negligible.

Table 2: Intra and inter group comparison of salivary pH

Groups	Baseline (Median ± IQR)	5th minute (Median ± IQR)	20th minute (Median ± IQR)	45th minute (Median ± IQR)	60th minute (Median ± IQR)	P – Value (#)
Multigrain Biscuit	7.25±0.53	7.20±0.42	7.20±0.22	7.20±0.45	7.35±0.45	(0.417)
Cracker Biscuit	7.35±1.05	7.35±0.77	7.30±0.77	7.30±0.6	7.25±0.7	(0.706)
Unfilled Biscuit	7.40±0.45	7.45±0.7	7.30±0.67	7.45±0.6	7.25±0.85	**(0.022)***
Nut & seed Nutritive Bar	7.35±0.55	7.30±0.57	7.25±0.5	7.20±0.48	7.15±0.45	**(0.005)***
Choco nut Nutritive Bar	7.25±0.55	7.35±0.42	7.10±0.32	7.15±0.55	7.15±0.53	**(0.006)***
Chocolate Bar	7.20±0.65	7.35±0.55	7.15±0.6	7.25±0.88	7.05±0.82	(0.073)
Orthodontic Elastics	7.15±0.8	7.50±0.48	7.25±0.42	7.25±0.73	7.40±0.95	(0.063)
P – Value (##)	(0.994)	(0.398)	(0.060)	(0.320)	(0.290)	

(#) Intra group comparison – Friedman Test

(##) Inter group comparison – Kruskal Wallis Test

*P – value ≤ 0.05 is considered to be statistically significant.

Table 3: Intra and inter group comparison of salivary flow rate (ml/min)

Groups	Baseline (Median ± IQR)	5th minute (Median ± IQR)	20th minute (Median ± IQR)	45th minute (Median ± IQR)	60th minute (Median ± IQR)	P – Value (#)
Multigrain Biscuit	1± 0.78	1.36 ± 1.20	1.07 ± 0.83	1.53 ± 1.45	1.10 ± 1.02	(0.188)
Cracker Biscuit	1.07 ± 0.67	2.11 ± 2.56	1.5 ± 1.96	1.46 ± 1.42	1.53 ± 1.42	(0.845)
Unfilled Biscuit	1.25 ± 1.10	2.50 ± 2.85	1.39 ± 1.04	1.36 ± 1.48	1.57 ± 2.03	**(0.001)***
Nut & seed Nutritive Bar	1.22 ± 1.12	2.28 ± 2.12	1.34 ± 10.73	1.21 ± 3.02	1.14 ± 3.04	**(0.001)***
Choco nut Nutritive Bar	0.86 ± 0.60	1.46 ± 0.86	1.8 ± 1.99	1.96 ± 2.52	1.32 ± 1.47	**(0.006)***
Chocolate Bar	0.96 ± 0.66	1.55 ± 1.28	1.56 ± 1.09	1.14 ± 0.77	0.96 ± 0.77	**(0.001)***
Orthodontic Elastics	1.49 ± 1.75	2.15 ± 2.95	1.69 ± 2.63	1.50 ± 2.25	1.39 ± 1.98	**(0.013)***
P – Value (##)	(0.874)	(0.837)	(0.320)	(0.863)	(0.907)	

(#) Intra group comparison – Friedman Test

(##) Inter group comparison – Kruskal Wallis Test

*P – value ≤ 0.05 is considered to be statistically significant.

Table 4: Intra and inter group comparison of oral glucose clearance rate

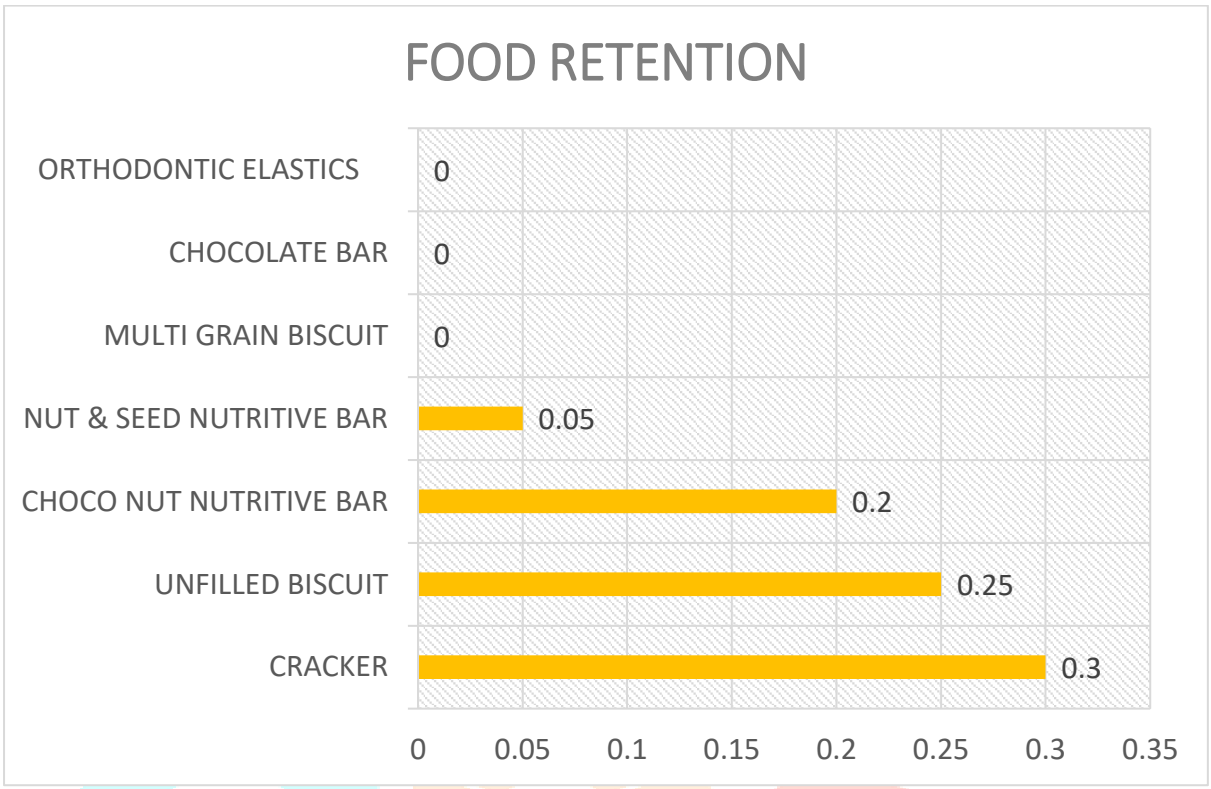
Groups	**Baseline** (Median ± IQR)	**5th minute** (Median ± IQR)	**20th minute** (Median ± IQR)	**45th minute** (Median ± IQR)	**60th minute** (Median ± IQR)	**P – Value (#)**
Multigrain Biscuit	19.56 ± 4.45	75.17 ± 64.42	24.72 ± 8.74	22.72 ± 3.66	23.58 ± 6.41	**(< 0.001)****
Cracker Biscuit	22.27 ± 18.96	91.71 ± 55.06	26.61 ± 13.65	21.34 ± 3.76	20.82 ± 3.07	**(< 0.001)****
Unfilled Biscuit	19.95 ±3.80	131.07 ± 72.80	30.79 ± 19.02	21.66 ± 2.42	22.94 ± 2.99	**(< 0.001)****
Nut & seed Nutritive Bar	21.87 ± 5.15	62.72 ± 47	25.86 ± 10.73	23.23 ± 3.02	23.29 ± 3.05	**(< 0.001)****
Choco nut Nutritive Bar	19.79 ± 3.29	75.02 ± 92.02	22.96 ± 5.40	20.54 ± 3.58	22.60 ± 7.81	**(< 0.001)****
Chocolate Bar	21.22 ± 4.70	77.43 ± 99.96	28.98 ± 12.04	22.73 ± 5.18	21.95 ± 2.91	**(< 0.001)****
Orthodontic Elastics	20.52 ± 1.96	21.27 ± 3.40	20.89 ± 3.59	20.18 ± 1.73	21.03 ± 1.60	(0.10)
P – Value (##)	(0.143)	**(<0.001)****	(0.088)	**(0.009)***	(0.145)	

(#) Intra group comparison – Friedman Test

(##) Inter group comparison – Kruskal Wallis Test

*P – value ≤ 0.05 is considered to be statistically significant.

Figure 5: Evaluation of Food Retention at the end of 60th minute



Discussion

The present study has adopted William's Latin square design in such a way that the same subject receives all the interventions in different phases of the trial and thus, acts as his / her own control. Moreover, Latin square design eliminates any biological differences which exists between the groups and the difference among any observed groups can be totally attributed to the intervention per se.[13] This design can also be considered as an extension of a cross – over trial and hence, is a highly efficient study design requiring a very small sample size when compared to a concurrent parallel design.[13] Healthy 18 – 21 years old, volunteers were recruited in the present study as they more often indulge in snacking and were likely to have less wear of occlusal fissures compared to the adult population.

The study included test products and positive controls commonly available at local shops and super markets. They are commercially regarded as healthy snacks compared to the other ultra-processed snack products rich in extrinsic sugars. Therefore, the study aimed to evaluate the cariogenic potential of dietary biscuits and nutritive bar with no restriction in the study subjects' oral hygiene maintenance and regular dietary habits.

The present study undertook salivary parameters like Salivary pH, flow rate and oral glucose clearance rate, food retentiveness on tooth surfaces as indicative factors for cariogenic potential of the consumed test product

and positive control. Scientific literature reports that these salivary parameters tend to change on consuming cariogenic foods.[14]

The change was noted post consumption of the test products and the positive controls, by the end of the 5th, 20th, 45th and 60th minute as per the Stephan's curve.[15] Though plaque pH is the best parameter to indicate caries inception, the study participant's objection to refrain from brushing for two days was the reason to choose the salivary pH as a pseudo marker for plaque pH. The salivary flow rate is essential to determine the availability of food substrate in the oral cavity. The complete oral clearance of food products have been reported to take place by around 60 minutes post consumption. The retentiveness of the food product can potentiate the chances of caries inception. So, the need for assessment of salivary flow rate, glucose clearance rate and food retentiveness is justified..

Studies conducted by Pallepati et al[16] and Kumar et[17] al had justified that the assessment of salivary pH to assess the cariogenic influence of dietary snacks and sugars due to their demineralising ability over the enamel and dentin. In the current trial, none of the intervention products at any point of time had a drop in salivary pH below the critical pH indicating a very low cariogenic potentiality with respect to salivary pH.

On consuming any food product the sense of its taste, smell and texture increases the Salivary flow rate.[18] As expected immediately after the consumption of the food substances there was a drastic increase in the salivary flow rate at the end of 5th minute and by the 20th minute the salivary flow rate began to gradually reduces and by the 60th minute it was very close to the baseline values.

Though the test products are marketed as containing no added sugars, the carbohydrate content in them gets metabolized by the salivary enzyme (amylase) action producing a drastic increase in the salivary glucose level.[19] This has been reflected in the results. There was a drastic increase at the 5th minute and subsequently by the 20th minute oral glucose clearance rate started and reached normalcy post consumption of the snack products and this was consistent with the trial conducted by Kumar et al. The food retention was negligible.

The personal factors, social factors cultural factors, environmental factors and policy factors sculpts the human behavior.[20] An individual's knowledge about the food he/she eats, family and social norms, easy accessibility to unhealthy, cheap foods and lack of fiscal policy in raising taxes for sugar products are possible reasons to choose the nutrient-poor products.[21] Thus necessary interventions at the upstream, downstream and midstream levels are required. At the upstream level, policy makers can introduce "Tooth friendly" labels

on snack products making it as a wiser choice of consumption across the nation and impose heavy taxes on unhealthy food products. At the midstream level, future research can be conducted on other commercially regarded healthy snacks and beverages. At the downstream level, health education on harmfulness of free sugars should be conveyed to the public using a common risk factor approach.

Strength of the study

- It is the first study to document the cariogenic potential of the commercially advertised dietary snacks (Nutritive bars) available in the Indian market.

Limitation

- As it is a preliminary investigation, further studies need to be carried out with larger sample size.
- The assessment of plaque pH was not feasible and if recorded could have been better indicated the cariogenic potentiality. Future studies with assessment of plaque pH is recommended.

Conclusion

It could be concluded that though the test products were in par with each other and the positive controls with regard to the parameters like the salivary flow rate and pH, when considering the oral glucose clearance rate, it could be healthier to consume choco nut nutritive bar and cracker biscuit.

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