ANALYSIS OF THE STIMULATED WHOLE SALIVA IN OVERWEIGHT AND OBESE SCHOOL CHILDREN

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SUMMARY
Objective. To determine if some stimulated whole saliva parameters are influenced by an increase of Body Mass Index.
Methods. Controlled cross-sectional study involving 90 school children of both genders between 7 and 10 years of age, from Bragança Paulista - SP. Three groups were formed: overweight, obese and control. Body Mass Index and diet intake by the Food Register method were evaluated. The salivary pH, flow rate, buffer capacity, protein, phosphate, calcium, fluoride, total and free sialic acid, and peroxidase activity were determined.
Results. The overweight and obese groups showed greater energy and lipid intake ($P < 0.001$) than the control group. There was no difference in the saliva flow rate between groups, however only the control group showed a mean value considered normal. In the overweight and obese groups a decrease in both the concentration of phosphate ($P < 0.001$) and peroxidase activity ($P < 0.001$) was observed. In the obese group an increase in the concentrations of free sialic acid ($P = 0.004$) and protein ($P = 0.003$) occurred.
Conclusion. Overweight and obese children show alterations in the concentrations of phosphate, free sialic acid and proteins, and in the peroxidase activity that are favorable conditions for dental caries.

Key words: Saliva.Obesity.Child.

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Introduction

Saliva plays an important role in oral health. Based upon its constituents, saliva adopts properties such as a lubricant, clearance of unwanted substances, digestion, neutralization of acids or bases, protection against demineralization and anti-microbial role. For relative protection against dental cavities, flow rate, buffer capacity, calcium, phosphate and fluoride concentrations are essentials[1]. Metabolic alterations may influence synthesis, composition and secretion of saliva. Thus, hypofunction of the salivary glands, and a consequent reduced flow rate, may be present in some situations such as irradiation[2], Down syndrome[3], and diabetes mellitus[4]. Obesity, currently a rapidly growing form of malnutrition, is common in children, adolescents and adults and is a, challenging, contemporary health problem. Its prevalence is rapidly increasing throughout the world[5].

Excess body fat mass arises from energy imbalance caused by taking in too much energy and expending too little. The increase in body fat mass during childhood and adolescence is frequently associated with the cardiovascular, respiratory, skeletal, gastrointestinal, and endocrine systems [6]. Adipocytes do not merely store fat, acting upon the cells when they secrete or bring about the secretion of hormones while at the same time being a target[7]. Hormone alterations are present in obese children and adolescents.

For these reasons, it is valid to surmise that inadequate nutritional status may influence saliva composition, which in turn will have a greater or lesser effective participation, in the process of dental demineralization, by altering the protective role of mouth structures. For treatment of obesity, it is clear that all repercussions should be considered including odontological issues, all of which play a role in overall health.

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To the best of the authors' knowledge no study focusing on the association of overweight and obesity with saliva composition has been performed. Therefore this study was conducted to determine if stimulated whole saliva parameters as pH, flow rate, buffer capacity, concentrations of protein, phosphate, calcium and sialic acid and the peroxidase activity are influenced by an increased body mass index (overweight and obese) in school children.

Methods

This was a cross-sectional study involving 90 children, 49 males, of which 30 children, 16 males, comprised the control group, with ages from 7 to 10 years, who attended primary school in the town of Bragança Paulista, SP, Brazil. The study was approved by the Ethics Research Committee of the Federal University of Sao Paulo., The nature of the study was reviewed with the parents or guardians of each child, prior to their inclusion and written consent forms were obtained.

Inclusion criteria

Ratio of Weight to Height (W/H) > 110% of the 50 percentile of the National Center for Health Statistics (NCHS)[8]; Body Mass Index (BMI) $\geq$ to the 85 percentile[9].

Exclusion criteria

Presence of salivary gland inflammation, epidemic parotiditis, gingivostomatitis, oral ulcers, tonsillitis, epiglotitis, foreign bodies, dehydration, diabetes, fever, tuberculosis, sarcoidosis, Sjögren and Mikulicz syndromes, and treatment with mercury compounds, iodine, histamine, polycarpine, acetylcholine, organophosphates, atropine, anti-hystaminics, amphetamines and phenothiazidics.

Sample determination

Selection of the schools was carried out by a randomized electronic draw, by which 10% of the total number of schools in the urban area of Bragança Paulista were obtained [10], that is one state, two municipal and two private schools. The number of children corresponded to all of the allotted school children who fulfilled the criteria of age and inclusion, taking into account that there had been no refusal to participate by either a parent or the child.

Control group

For the control group, inclusion criteria were a weight to height ratio Ibetween 90% and 110% of the 50 percentile of the NCHS [8] and BMI $\geq$ 15 percentile and < 85 percentile [9].

Three groups were formed, according to nutritional status: control, overweight and obese.

Anthropometric evaluation

Weights and heights were collected in accordance with predetermined and recognized techniques [11], and the body mass index (BMI= Kg/height^2) was calculated .

Biochemical analysis

Collection of saliva: Stimulated whole saliva was collected in the school between 2 and 3 p.m., some 2 hours after the lunch meal. Before collection, the children rinsed their mouth with distilled water and 15 minutes afterward saliva was collected by chewing a piece of parafilm. Saliva produced in the first 2 minutes was discarded, then saliva was collected for exactly 5 minutes in a graduated cylinder, so that the flow rate (mL/min) could be calculated. During the collection period, children remained comfortably seated in a ventilated and well illuminated room.

pH and buffer capacity determinations: Soon after collection a portion of saliva was used for pH determination with a portable pH-meter (Orion 410A, Orion Research, Boston, Mass, USA). The buffer capacity was determined by titration using 1 mL of saliva, with 0.01 M HCl and after each addition of acid the change in pH was monitored up to pH 5.0. The buffer capacity was analyzed by ranges of pH. The volume of acid added to the saliva was calculated for each interval considered: initial pH-7.0, pH 6.9-6.0, and pH 5.9-5.0. For practical purposes the buffer capacity was expressed in volume (mL) of the acid added to 1 mL of saliva in the pH range considered, instead of equivalents of H. The remaining saliva was frozen in dry ice, transported to the laboratory, and stored at - 80°C until analyses could be performed.

Protein concentration: Determination of protein concentration was carried out using Folin's phenol reagent with bovine serum albumin as the standard [12].

Calcium, phosphate, and fluoride concentrations: Calcium[13] and phosphate[14] were determined by colorimetric testing. The fluoride concentration was determined using a fluoride selective electrode (Orion Research, Boston, Mass, USA) and calculated from a calibration curve between 0.1 to1.0 ppm of fluoride[15].

Free and total sialic acid: The sialic acid levels (free and total) were determined by the thiobarbituric acid assay[16], using N-acetyl-neuraminic as a standard.

Activity of peroxidase: The peroxidase activity was determined and interference of pseudoperoxidase activity was eliminated by carrying out duplicate assays in the presence of 10 mM3amino1,2 triazole, an inhibitor for peroxidase activity [17].

Statistical analysis: Statistical analysis was initially performed considering the genders. As no differences were observed, the parameters relating to different genders were combined for analysis. The Anderson-Darling normality test was first used to evaluate normality of the samples. When a normal distribution was found, a one way variance analysis was used for comparing the studied saliva variables between the three groups (control, overweight and obese). When necessary, this analysis was complemented by the Student-Newman Keuls test. Peroxidase activity and fluoride concentration did not show normal distribution. Consequently, the Kruskal-Wallis test was used in these cases. An $\alpha < 0.05$ was adopted .

Results

The overweight group showed a higher pH value for stimulated whole saliva than the control group, while the obese group was not different from the other two groups ($P < 0.001$). No difference in the saliva flow rate between the groups was noted, however only the control group showed a mean value considered normal (Table 1)

Both salivary calcium and fluoride concentrations did not show differences when the groups were compared. On the other

hand, the concentration of phosphate from overweight and obese children was lower than the control group, with no difference between them (P< 0.001) (Table 1).

Free sialic acid in stimulated whole saliva from the obese group was higher than the control and overweight results (P= 0.004), but no difference in the total sialic acid was observed among the three groups (P= 0.171) (Table 1).

While the protein content in saliva for obese children showed a higher value than the control and overweight children (P= 0.003), the peroxidase activity was reduced in both overweight and obese groups when compared with the control group (P< 0.001) (Table 1).

Considering the pH intervals analyzed, the buffer capacity showed no difference between the groups either in the initial interval pH - 7.0 or pH 6.9-6.0. In the interval pH 5.9-5.0 the overweight group showed a higher value than the control group (P= 0.005) (Table 2).

DISCUSSION

The mean saliva pH values of the 3 groups were normal, although the overweight group was statistically higher than the control group. In literature results are conflicting with respect to saliva pH. Factors such as collection methods and determination, the ages, geographic location and diet can influence results[3].

In relation to saliva flow rate, the overweight and obese groups had a mean < 1mL/min, namely 13 and 10 children respectively, and presented values of 0.7mL/min, which is considered the flow rate at risk for xerostomia. Individuals with an accentuated reduction of the saliva flow rate demonstrate oral mucosa irritation, difficulty in swallowing and speaking, diminished taste acuity, and a high tendency towards dental cavities[18]. Values below 0.7mL/min are considered very low[19] and 11 children

Table 1 - Mean values of the studied parameters of stimulated whole saliva in control, overweight and obese school children.

	Control	Overweight	Obese	*P*
pH	7.51 ± 0.22[a] (30)	7.80 ± 0.29[b] (30)	7.66 ± 0.27[a,b] (30)	<0.001[1]
Flow rate [mL/min]	1.06 ± 0.52[a] (30)	0.89 ± 0.54[a] (30)	0.95 + 0.47[a] (30)	0.432[1]
Phosphate [ug/mL]	0.44 ± 0.15[a] (26)	0.32 ± 0.07[b] (30)	0.32 ± 0.07[b] (26)	<0.001[1]
Calcium [mg/dL]	17.25 ± 7,96[a] (24)	18.79 ± 6.24[a] (29)	15.39 ± 6.89[a] (29)	0.193[1]
Fluoride [ppm]	0.019 ± 0.020[a] (30)	0.019 ± 0.018[a] (30)	0.017 + 0.010[a] (30)	0.798[2]
Protein [mg/mL]	1.22 ± 0.30[a] (30)	1.32 ± 0.41[a] (30)	1.54 ± 0.35[b] (30)	0.003[1]
Total sialic acid [ug/mL]	55.3 ± 14.6[a] (22)	59.3 ± 21.0[a] (24)	65.7 ± 19.4[a] (24)	0.17[1']
Free sialic acid [ug/mL]	18.4 ± 9.3[a] (23)	17.9 ± 8.9[a] (27)	26.6 ± 10.8[b] (23)	0.004[1]
Peroxidase activity [ug/mg prot]	13.22 ± 7.88[a] (26)	8.21 ± 4.00[b] (28)	4.66 ± 1.84[c] (28)	<0.001[2]

( ) number or children in each group
[1] *P* descriptive levei or ODe ",ay analysis or variance, complemented by Ihe Student~Newman~Keu I s test
[2] *P* descriptive levei of Kruskal-Wallis test
Lines with different superscript leUers: with statistical difference: P<0.OS

Table 2 - Mean buffer capacity in stimulated whole saliva in control, overweight and obese school children.

Buffer capacity (mL acid/mL saliva)	Control	Overweight	Obese	*P*
pHi - 7.0	0.65 ± 0.33[a] (29)	0.56 ±0.24[a] (28)	0.55 ± 0.29[a] (30)	0.377
pH 6.9 - pH 6.0	1.25 ± 0.43[a] (27)	1.23 ± 0.33[a] (29)	1.13 ± 0.30[a] (30)	0.370
pH 5.9 - pH 5.0	0.60 ± 0.14[a] (27)	0.75 ± 0.15[b] (29)	0.70 ± 0.21[a,b] (30)	0.005

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presented a value below the lower limit (0.5mL/min), a flow rate comparable to xerostomia. Among the salivary protective factors, the clearance of the saliva flow rate is one of the most important, not only because it removes endogenic/exogenic microorganisms, and their products, but also because a constant amount of saliva guarantees the presence of antimicrobials, immunological and non-immunological factors in the mouth[20]. Clearance of the saliva shows a direct correlation with the flow rate[21]therefore, individuals with reduced saliva flow rate show a greater quantity of Lactobacilli and *S. mutans* than those with a normal saliva flow rate[22], which can increase the risk of mouth diseases.

The phosphate concentration showed a reduction of about 27% in the overweight and obese children. On the one hand, peroxidase activity showed a greater reduction, of about 40 % for the overweight group and about 65 % for the obese group. Peroxidase is an enzyme with antimicrobial properties, and in the mouth, it is secreted by salivary glands and catalyzes the oxidation of thiocyanate by hydrogen peroxide to produce on oxidized form of thiocyanate[23]. The product of the reaction catalyzed by peroxidase inhibits bacterial growth, and in addition, by consuming hydrogen peroxidet prevents the accumulation of this toxic substance[24]. On the other hand, an association between reduction in saliva phosphate concentration and increase in dental caries has been reported[25]. The decrease of phosphate concentration, saliva flow rate, and peroxidase activity observed in this investigation may be linked to the increase of dental caries with obesity, as described by other authors[26].

In saliva, the calcium binds to the protein, to the phosphate, citrate, and lactate and only half is found in free form[27]. The degree of bonded calcium depends upon the saliva pH. Although atomic absorption spectrophotometry can be conveniently used to determine the total calcium in saliva, formation of precipitates rich in calcium can interfere with this analysis.

Fluoriude concentration in saliva varies with the amount and concentration of fluoride ingested, by way of systemic or topic use. Paez, Dapaz[28] found a great variation in the saliva fluoride content of individuals residing in regions with different concentrations of fluoride in the tap water. Thus, water containing 0.1 or 1.0 ppm of fluoride did not cause differences in the saliva fluoride content. But when the level of fluoride in the water varied from 2.8 to 8.0 ppm, the concentration of saliva fluoride rose to 0.132 ppm. The fluoride level in the water supplied to the town of Bragança Paulista varied between 0.7 and 1.2 ppm and did not affect fluoride concentration in the saliva samples analyzed. Therefore, confirming previous results, the intake of fluoride contributes to determining its saliva concentration, which is not influenced by the nutritional status[29].

The concentration of saliva protein was 26.2% higher in the obese group than in the control group. According to Rudney et al.[30] a high protein concentration in the saliva contributes to greater adherence of *S. mutans*, the first inhabitant of dental plaque.

The obese children showed a higher free sialic acid mean value than the control group. Sialic acid is an important component of salivary glycoprotein and enhances bacterial aggregation and participates in the formation of acquired pellicle, and dental plaque[31]. In a comparative oxidative stress study-obesity and diabetes[32], it was reported that oxidative stress may be a link between type 2 diabetes and obesity. In this study the oxidative stress was not analyzed, however, based on the preceding papers cited, perhaps an increase in the free sialic acid may be linked to the oxidative stress in obesity.

In the range of pH 6.9 -6.0 the buffer capacity of saliva of the three groups was no different. In fact, the range pH 6.9-6.0 constitutes the most important pH interval related to dental cavity formation, since in this range two pKs of two buffer systems are found, namely, the bicarbonate/carbonate system with a pK around 6.1 and the phosphate buffer system with a pK around 6.8. The presence of these two buffer systems in this range is the cause of the higher acid consumption in this pH interval.

Conclusion

The results obtained allow us to conclude that overweight and obese children in relation to control ones show alterations in the salivary concentrations of phosphate, free sialic acid and proteins, and in the peroxidase activity,all of which are favorable conditions for dental caries.

Conflict of interest: none

Resumo

Análise da saliva total estimulada em escolares com sobrepeso e obesos

Objetivo. Verificar se alguns parâmetros da saliva total estimulada são influenciados pelo aumento do Índice de Massa Corporal.

Métodos: Estudo transversal controlado com 90 escolares, de ambos os sexos, de 7 a 10 anos incompletos de Bragança Paulista, SP, formando três grupos: sobrepeso, obeso e controle. Avaliou-se o Índice de Massa Corporal (P/E2) e a ingestão dietética pelo registro alimentar. Na saliva foram avaliados o fluxo, pH, capacidade tampão e concentrações de proteína, fósforo, cálcio, flúor, ácido siálico livre e total e atividade da peroxidase.

Resultados. Nos grupos sobrepeso e obeso houve maior consumo de energia e lipídios (P<0,001). Não houve diferença no fluxo salivar entre todos os grupos, mas somente o controle mostrou valor médio considerado normal. O pH salivar do grupo sobrepeso foi maior do que o do controle (P<0,001). Nos grupos sobrepeso e obeso houve decréscimo na concentração de fosfato (P<0,001) e na atividade da peroxidase (P<0,001). No grupo obeso houve aumento nas concentrações de ácido siálico livre (P= 0,004) e proteína (P= 0,003).

Conclusão. Crianças com sobrepeso e obesas apresentam alterações nas concentrações salivares de fosfato, ácido siálico livre e proteínas e na atividade da peroxidade, as quais favorecem a formação de cárie dentária. [Rev Assoc Med Bras 2010; 56(1): 32-6]

Unitermos: Saliva.Obesidade.Criança.

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