Trace elements and electrolytes in human resting mixed saliva after exercise

J L Chicharro, V Serrano, R Ureña, A M Gutierrez, A Carvajal, P Fernández-Hernando, A Lucía

Abstract

Objectives—Exercise is known to cause changes in the concentration of salivary components such as amylase, Na, and Cl. The aim of this investigation was to evaluate the effect of physical exercise on the levels of trace elements and electrolytes in whole (mixed) saliva.

Methods—Forty subjects performed a maximal exercise test on a cycle ergometer. Samples of saliva were obtained before and immediately after the exercise test. Sample concentrations of Fe, Mg, Sc, Cr, Mn, Co, Cu, Zn, Se, Sr, Ag, Sb, Cs, and Hg were determined by inductively coupled plasma mass spectrometry and concentrations of Ca and Na by atomic absorption spectrometry.

Results—After exercise, Mg and Na levels showed a significant increase (p<0.05) while Mn levels fell (p<0.05). Zn/Cu molar ratios were unaffected by exercise.

Conclusions—Intense physical exercise induced changes in the concentrations of only three (Na, Mg, and Mn) of the 16 elements analysed in the saliva samples. Further research is needed to assess the clinical implications of these findings.

Keywords: trace elements; electrolytes; saliva; exercise

The determination of trace elements in saliva is important in the study of its amylase activity and role in the maintenance of the functional integrity of several tissues of the oral cavity. The significant role of saliva in the maintenance of the structure of teeth, gums, and different epithelial tissues of the mouth are well known. Analysis of changes in the composition of saliva may help us to understand abnormalities in oral functions such as taste and smell. Several diseases that affect the oral cavity have also been shown to be related to changes in salivary composition. Few investigations have analysed the trace element composition of saliva and its relation to incidence of dental caries. Borella et al found evidence that the Zn/Cu molar ratios in whole saliva were significantly reduced in subjects with more than three decayed teeth compared with those with no caries. Duggal et al evaluated the relation between changes in trace element levels and incidence of caries in children. They found that the elements Cu and Fe showed a consistent inverse relation to caries experience. Zaichik and Bagirov analysed 21 elements in non-stimulated mixed saliva in subjects with different periodontal diseases. Significant changes in the levels of many of the elements were related to the degree of periodontal tissue involvement.

The study of the physiological responses of salivary glands to exercise has gained attention in recent years. The autonomic nervous system controls the physiological activity of the salivary glands. Both parasympathetic and sympathetic activity stimulates the secretion of saliva. More specifically, sympathetic stimulation induces changes in the secretion and reabsorption of electrolytes. It is well documented that sympathetic adrenal activity increases during submaximal incremental exercise. Increased sympathetic activity (estimated by plasma levels of catecholamines) during exercise has been previously associated with an increase in saliva concentrations of Na and Cl. These findings were later confirmed by a field test. Changes in the concentration of salivary amylase during exercise have recently been related to attainment of the anaerobic threshold which was associated with increased adrenal sympathetic activity.

Whether intense physical exercise produces changes in the levels of trace elements and electrolytes present in saliva has not been evaluated to date. Besides the fact that changes in the chemical composition of saliva could be hypothetically associated with different periodontal diseases and dental caries, the study of exercise induced changes in salivary composition is of physiological interest per se.

The aim of this investigation was to evaluate the effect of incremental physical exercise on the concentration of 16 trace elements and electrolytes in whole (mixed) saliva.

Methods

SUBJECTS

Forty healthy young men of different physical characteristics were selected as subjects for the study. Mean (SD) age was 31 (8) years, height 176.54 (7.96) cm, and body weight 74.16 (9.06) kg. Before being tested, each subject signed an informed consent form in accordance with the regulations of the Complutense University. The participants were also required to complete a medical questionnaire and undergo a physical examination (including a 12 lead electrocardiogram and examination of the oral cavity). Subjects were caries free and pain free, they had healthy gums, and regularly visited the dentist. No subject took vitamin or mineral supplements or medication of any type. All of them followed a similar type of diet, mainly based on complex carbohydrates with a lower content of proteins and lipids.
EXERCISE TEST
Each subject performed an incremental exercise test to exhaustion to determine maximal oxygen uptake (VO_{2MAX}) and other ergospirometric variables using an automated breath by breath system (CPX; Medical Graphics, St Paul, Minnesota, USA). The test was performed on a cycle ergometer (Ergoline 90; Ergometrics, Barcelona, Spain) at the same time of day (1000–1200 h) after an overnight fast. All the subjects were instructed to follow the same type of carbohydrate-rich diet the day before testing. The exercise consisted of a ramp protocol which started at 0 W with workload increments of 25 W/min at a pedal frequency of 60–80 rpm. All the exercise tests were performed to volitional fatigue or fulfilment of test termination criteria. Capillary blood samples (25 µl) were obtained from fingertips before and immediately after the termination of tests for monitoring of blood lactate concentration and immediately after the termination of tests (25 µl) were obtained from fingertips before exercise. The time of sample collection was chosen to minimise the influence of circadian variations on salivary flow and was chosen to minimise the influence of circadian variations on salivary flow and composition. Each subject had been instructed to floss his teeth and thoroughly clean the oral cavity the night before saliva collection. Thirty minutes before the exercise test, each subject was given 400 ml of water to ensure adequate body hydration. The mouth was rinsed with deionised water immediately before saliva collection (resting or after exercise). In addition, each subject was instructed to empty his mouth of “old” saliva before spitting into a sterile container. Samples were kept at 4°C and transported to the laboratory in three hours. Once in the laboratory, samples were centrifuged and the supernatants separated and stored at −80°C for 15 days before preparation. Each sample was diluted 1:250 (40 µl to 10 ml) with Milli-Q water and stored at 4°C for 30 days until the time of analysis. Concentrations of Fe, Mg, Sc, Cr, Mn, Co, Cu, Zn, Se, Sr, Ag, Sb, Cs, and Hg were determined by inductively coupled plasma mass spectrometry using a model PQ3 ICP-MS (VG Elemental, Winsford CW7 3BX, UK). Nebuliser gas flow rate, ion lens voltages, quadrupole, resolution, and pole bias were optimised using a standard solution containing elements across the mass range from beryllium to uranium at a concentration of 10 µg/l. Ca and Na concentrations were determined using a Perkin-Elmer 2380 atomic absorption spectrometer (Norwalk, Connecticut, USA) equipped with a calcium or sodium hollow cathode lamp and an air/acetylene flame. These elements were determined at wavelengths of 422.7 and 589.0 nm respectively. The spectral band pass was 0.7 nm. Results are expressed in mg/l (ppm).

As all the reagents were of analytical grade, high purity Milli-Q water (Millipore, Bedford, Massachusetts, USA) was used in each experiment. Element standard solutions (Spectrosoil; BDH, Poole, Dorset, UK) were prepared daily by appropriate dilution of a stock solution (1000 µg/ml). Indium solution (10 µg/ml) was used as the internal standard for the inductively coupled plasma mass spectrometry determinations.

Limits of detection, defined as three times the standard deviation (95% confidence level) of ten replicate blank determinations, were calculated for the elements analysed. These values were as follows (µg/l): 1.5 for Mg, Fe, and Zn; 0.2 for Cr, Se, Hg, Sr, Mn, and Sc; 0.03 for Co, Sb, Cs, and Ag; 150 for Ca and 18 for Na. Precision, expressed as relative standard deviation, was less than 3.5% except in the case of Se (9.3%) and Cu (8.4%).

DATA ANALYSIS
Once the Kolmogorov-Smirnov test had been applied to establish a Gaussian distribution of results, rest and maximal exercise sample means were compared using Student’s t test for paired data. When data for each element did not show a normal distribution, the Wilcoxon test was applied. Results were expressed as mean (SD) and significance was set at p<0.05.

Results
Table 1 shows the maximal ergospirometric values of the subjects.

Table 2 shows the concentrations of the trace elements in saliva at rest and after maximal exercise. Only Na, Mg, and Mn concentrations showed variation in response to exercise. The levels of Na and Mg increased from 376.32 (340.18) to 836.17 (520.34) ppm (p<0.01) and from 9.41 (6.19) to 11.78 (7.49) ppm respectively. The spectral band pass was 0.7 nm. Results are expressed in mg/l (ppm).

Table 1 Maximal ergospirometric values of the subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO_{2} (l/min)</td>
<td>4.0 (0.9)</td>
</tr>
<tr>
<td>VO_{2} (ml/kg/min)</td>
<td>56.1 (9.9)</td>
</tr>
<tr>
<td>RER</td>
<td>1.3 (0.1)</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>187 (15)</td>
</tr>
<tr>
<td>BLa (mM/l)</td>
<td>13.5 (3.3)</td>
</tr>
<tr>
<td>Power output (W)</td>
<td>358.6 (67.9)</td>
</tr>
</tbody>
</table>

Values are mean (SD).

Abbreviations: VO_{2}, pulmonary ventilation; VO_{2MAX}, oxygen uptake; RER, respiratory exchange ratio; HR, heart rate; BLa, blood lactate.

Table 2 Concentrations (µg/l) of trace elements and electrolytes in whole saliva

<table>
<thead>
<tr>
<th>Element</th>
<th>Rest</th>
<th>Post-exercise</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>129.92 (98.22)</td>
<td>110.28 (59.82)</td>
<td>NS</td>
</tr>
<tr>
<td>Fe</td>
<td>4.73 (2.64)</td>
<td>4.51 (2.13)</td>
<td>NS</td>
</tr>
<tr>
<td>Mg</td>
<td>9.41 (6.19)</td>
<td>11.78 (7.49)</td>
<td>0.05</td>
</tr>
<tr>
<td>Na</td>
<td>376.32 (340.18)</td>
<td>836.17 (520.34)</td>
<td>0.01</td>
</tr>
<tr>
<td>Sc</td>
<td>0.12 (0.12)</td>
<td>0.16 (0.32)</td>
<td>NS</td>
</tr>
<tr>
<td>Cr</td>
<td>0.23 (0.20)</td>
<td>0.23 (0.27)</td>
<td>NS</td>
</tr>
<tr>
<td>Mn</td>
<td>1.99 (2.04)</td>
<td>1.40 (1.69)</td>
<td>0.05</td>
</tr>
<tr>
<td>Co</td>
<td>0.03 (0.03)</td>
<td>0.02 (0.03)</td>
<td>NS</td>
</tr>
<tr>
<td>Cu</td>
<td>0.18 (0.28)</td>
<td>0.19 (0.31)</td>
<td>NS</td>
</tr>
<tr>
<td>Zn</td>
<td>8.72 (7.55)</td>
<td>8.11 (13.91)</td>
<td>NS</td>
</tr>
<tr>
<td>Se</td>
<td>0.91 (1.34)</td>
<td>0.64 (0.84)</td>
<td>NS</td>
</tr>
<tr>
<td>Sr</td>
<td>1.35 (1.45)</td>
<td>1.15 (2.08)</td>
<td>NS</td>
</tr>
<tr>
<td>Ag</td>
<td>0.04 (0.06)</td>
<td>0.04 (0.05)</td>
<td>NS</td>
</tr>
<tr>
<td>Sb</td>
<td>0.05 (0.04)</td>
<td>0.05 (0.05)</td>
<td>NS</td>
</tr>
<tr>
<td>Cs</td>
<td>0.02 (0.02)</td>
<td>0.01 (0.01)</td>
<td>NS</td>
</tr>
<tr>
<td>Hg</td>
<td>1.36 (2.46)</td>
<td>1.07 (1.33)</td>
<td>NS</td>
</tr>
<tr>
<td>Zn/Cu ratio</td>
<td>50.33 (12.86)</td>
<td>60.56 (46.28)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SD).
activity of the di- 

plasma but that it also reflects the metabolic 

saliva is not simply a transudate of blood 

Na and Mg reabsorption. It is well known that 

- adrenoceptor dependent ductal regulation of 

\( \alpha \)-trace element in resting saliva reported here has 

induced changes in salivary composition. 

The change in concentration of some of the 

trace elements in saliva as a consequence of 

exercise induced decrease in salivary flow rate 

that is, from the parotid gland—may be 

altered with exertion \(^{22}\) and could, at least 

partially, account for some of the exercise 

induced changes in salivary composition. 

Several authors \(^{8-11}\) have associated changes 

in the concentrations of trace elements in 

saliva with the incidence of periodontal disease 

dental caries in different population groups. Borella et al \(^{9}\) showed during at least 

one year of follow up that Zn/Cu molar ratios 

were lower in subjects with decayed teeth. In 

the present investigation, exercise did not 

produce significant modifications to this ratio 

in the short term. Whether continuous exer-

cise over the year would induce significant 

changes in Zn and Cu levels and/or a greater 

incidence of caries in subjects who exercise 

regularly is not known. Zaichk and Bagirov \(^{11}\) 

found that adults with a greater incidence of 

periodontal disease showed increased sali-

vary concentrations of Fe, Sc, Mn, Cr, Co, 

Cu, Se, Ag, and Hg and reduced concentra-

tions of Zn. Based on the present increase in 

Na and Mg, and decrease in Mn levels, it 

seems that physical exercise does not produce 

a short term change in the composition of 

saliva with, in turn, no greater risk of 

periodontal disease or caries. Whether regular 

training produces significant changes in sali-

vary composition requires investigation. Dug-

gal et al \(^{9}\) found that the concentrations of Cu 

and F showed a consistent inverse relation to 

the incidence of caries in children, but the ele-

ments Zn, Fe, and Mn did not. In this study, 

there was no short term change in Cu concen-

tration after exercise. Loss of tooth mineral \(^{16}\) 

has been related to a decrease in the pH of 

saliva, although some authors \(^{21,22}\) report no 

changes in pH attributable to exercise. How-

ever, the effect of exercise on pH was not stud-

ied in this investigation. 

It may be concluded that intense physical 

exercise produced significant change in the 

saliva concentrations of only three of the 16 

elements analysed. It is felt that future inves-

tigations should focus on whether such modi-

fication in salivary composition takes place 

in subjects who exercise regularly and 

whether these putative changes could lead to a 

greater incidence of periodontal disease, caries, 

and/or disorders of oral function.

Discussion

The change in concentration of some of the 

trace elements in saliva as a consequence of 

incremental physical exercise was the most sig-

nificant finding. After the exercise test, Na and 

Mg concentrations showed a significant in-

crease and Mn levels fell. Ergospirometric data 

defined that the exercise performed by sub-

jects was intense and that criteria for \(VO_2 \text{MAX}\) \(^{22}\) 

had been met. 

Although Zn, Cu, Fe, and Mn concentra-

tions before the exercise test were comparable 

with those observed by Dugall et al \(^{9}\) in children 

with dental caries, the resting levels of most of 

the salivary trace elements were in overall 

agreement with those reported for the healthy 

adult. \(^{23}\) Nevertheless, the high degree of 

variability between subjects in the levels of 

trace element in resting saliva reported here 

has also been previously described. \(^{10}\) 

The increase in Na concentration attributed 

to physical exercise is in accordance with the 

findings of several authors \(^{7,8,18-21}\) and may be 

related to increased sympathetic activity during 

exercise. Indeed, sympathetic stimulation of 

salivary glands induces a decrease in salivary 

flow and an increase in the concentration of 

some of its constituents such as Na. \(^{2}\) There 

are no directly comparable reports, however, to 

confirm the increase in Mg and decrease in Mn 

levels or the behaviour of the other trace 

elements in saliva after exercise. Furthermore, 

the possible explanation for the reported 

changes in Mg and Mn concentrations are 

speculative, as little is known about the site 

of secretion of either of these trace elements in 

the salivary glands. It may be hypothesised that 

the exercise induced decrease in salivary flow rate 

may lead to a low supply of capillary blood to 

the salivary glands which, in turn, may alter 

their reabsorptive and secretory mechanisms. 

Such phenomena could be partly responsible 

for the increases in salivary concentrations of 

Na and Mg that may occur during exhaustive 

exercise. \(^{22}\) Further, the increased levels of these 

two trace elements could be indicative of the 

\(\beta\)-adrenoceptor dependent ductal regulation of 

Na and Mg reabsorption. It is well known that 
saliva is not simply a transudate of blood 

plasma but that it also reflects the metabolic 

activity of the different salivary glands. \(^{3}\) The 

use of non-stimulated mixed saliva does not 

permit specification of the changes occurring 
in each salivary gland in response to physical 

exercise. Furthermore, interpretation of our 

findings is limited by the fact that we did not 

measure salivary flow rate. Indeed, salivary 

flow—that is, from the parotid gland—may be 

altered with exertion \(^{22}\) and could, at least 

partially, account for some of the exercise 

induced changes in salivary composition.

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1. Orstavik D, Kraus FW. The acquired pellicle: immunofluo-

rescent demonstration of specific proteins. J Oral Pathol 


2. Rolla G, Ciardi JE, Bowen WH. Identification of IgA, IgG, 

lysozyme, albumin, alpha-amylase and glucosyltransferase 

in the protein layer adsorbed to hydroxyapatite from whole 

saliva. Scandinavian Journal of Dental Research 1981;91: 

186–90.


hypoguesia with dysgeusia, hyposmia, and dysosmia. A new 


5. Shatzman AR, Henkin RI. Gustin concentration changes 

relative to salivary zinc and taste in humans. Proc Natl Acad 


7. Dreizen S, Levy BM. Comparative concentrations of 

selected trace metals in human and mammoset saliva. Arch 


experience and the mineral content of plaque in a primitive 


9. Duggal MS, Chawla HS, Curzon ME. A study of the 

transmission of the manuscript.
Take home message

Intense physical exercise—that is, incremental test to exhaustion—induces acute changes in the salivary concentrations of only a few trace elements/electrolytes such as Na, Mg, and Mn. Further research may determine whether salivary composition is chronically modified in subjects who exercise regularly.

Commentaries

Few studies have investigated the effect that exercise has on saliva and thus on general dental health in sports people. This paper found no change in resting whole salivary concentrations in 13 out of 16 trace elements before and after strenuous exercise. The subjects did not regularly engage in sports and the authors rightly point out that the results could alter with regular training. The increase in Na and Mg needs careful interpretation as flow rates were not measured or controlled for and the relative contribution to whole saliva from different glands may be altered by exercise. The current literature on the influence of exercise on salivary Na is thus conflicting, although different methods could account for the different results. Perhaps of greater interest for future research is the bicarbonate concentration before, during, and after exercise as this will influence both caries and acid erosion.

A MILOSEVIC
Liverpool

The object of this paper is to establish a relation between trace element concentration of human mixed saliva and physical exercise. The authors have justified the aims on the basis of possible implications for dental disease, namely dental caries and periodontal disease. The study is worth while because it has established the concentrations of a large number of trace elements in saliva, which would be a useful reference for other researchers. However, guarded conclusions should be drawn about its clinical relevance from a dental perspective. Although most of the discussion is based on the possible relation between the trace elements and dental disease, it should be remembered that the study was a snapshot in time of the concentration of these elements related to exercise. To establish clinical significance, the trace element concentrations should be studied in subjects who exercise on a regular basis, such as athletes, and related to the prevalence of dental disease in that population.

M S DUGGAL
Leeds
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