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Cystic fibrosis-like changes in saliva of healthy persons subjected to anaerobic exercise

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Summary

The biochemical composition of saliva secreted by healthy persons and by heterozygotes and homozygotes of cystic fibrosis at rest and by healthy persons subjected to aerobic or anaerobic effort were compared.

In the saliva from cystic fibrosis homozygotes at rest substantial increases of the activity of ribonuclease ($p < 0.001$) and of the concentrations of protein ($p < 0.001$), lactate ($p < 0.001$), sodium ($p < 0.001$), potassium ($p < 0.01$) and calcium ($p < 0.05$) were found in comparison with saliva from healthy persons at rest. In the saliva from cystic fibrosis heterozygotes at rest similar but less pronounced changes were seen. After anaerobic exercise these biochemical parameters were increased in the saliva of healthy persons and mimicked the values of cystic fibrosis saliva. However, after aerobic effort no changes other than a slightly increased ribonuclease activity were seen in the saliva of healthy persons.

This indicates that salivary glands of cystic fibrosis patients, at rest, are in the same state of lactate acidosis and energy depletion as these glands are in healthy persons after anaerobic work.

Introduction

Cystic fibrosis (CF) is the most common recessively inherited congenital disease in the Caucasian population. It mainly affects the exocrine glands with primary symptoms from the lungs (recurrent bronchitis and pneumonias) and the digestive tract (pancreatic insufficiency and malnutrition). The sweat glands also are affected

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and the sweat contains increased concentrations of electrolytes [1]. The basic genetic defect is still unknown and thus only symptomatic treatment can be given.

We and others have shown an increased activity of ribonuclease (RNAase) and an increased concentration of protein in the saliva from both homozygotes and heterozygotes for CF as compared to age-matched controls (see e.g. [2,3]). Studies of the biochemical changes in healthy persons after anaerobic physical exercise have shown an increased release of RNAase from skeletal muscle into the blood to be associated with acidosis [4]. A higher concentration of lactate has been shown in the sweat and blood of CF-patients at rest [5]. Increased concentration of electrolytes in sweat has been demonstrated in healthy persons after anaerobic physical exercise [6]. Increased amounts of mucus and higher concentration of sodium have been obtained in rat submaxillary saliva after partial arterial occlusion of the gland [7]. All these results indicate that anaerobic physical exercise as well as the decrease of oxygen supply — which both lead to lactate acidosis — can cause CF-like changes in the secretions from healthy persons.

The purpose of this study was to verify this. Thus, we measured RNAase activity, concentrations of protein, lactate, sodium, potassium and calcium in the saliva of healthy individuals before and after aerobic (submaximal) and anaerobic (maximal) effort, and in the saliva of CF-patients and their parents at rest.

Material and methods

CF-patients: children (6 months–14 years old; $n = 16$) with typical clinical symptoms and positive sweat tests.

CF-heterozygotes: healthy parents of these children (20–47 years old; $n = 8$).

Normal controls: physically well-trained males (18–22 years old; $n = 29$) with known V_{O_2}max-values, were divided into two groups. The first group ($n = 10$) worked at 60% of their maximal capacity (aerobic exercise) for 30 min. The second group ($n = 19$) performed pulsating (anaerobic) exercise for 8–10 min, i.e. alternating 30 s of maximal work and 30 s of work at 70% of the maximal capacity. Both groups did their exercise on a bicycle-ergometer in the morning hours at least 2 h after a breakfast containing little sugar.

Whole mixed saliva was collected, without stimulation, after rinsing of the oral cavity with water. Typically 2–3 ml of saliva was obtained. Collection of saliva was done at least 2 h after the last meal.

Capillary blood was collected from the finger tip for lactate estimations.

RNAase activity was assayed by incubating 10 μl of the supernatant from centrifuged saliva ($2000 \times g$, 10 min) in a mixture containing: 50 μl of 0.2 mol/l Tris-HCl buffer, pH 8.2, 20 μl of 0.5 mol/l glucosamine HCl, 100 μl of distilled water and 20 μl of 1% RNA, for 15 min at 37°C. The reaction was terminated by the addition of 200 μl of 1 mol/l HCl in 76% ethanol at −20°C. The sample was kept on ice for 15 min and the resulting precipitate was removed by centrifugation ($2000 \times g$, 7 min). The supernatant was diluted 6-fold in water and the increase in

absorption at 260 nm was measured relative to a blank with the saliva added after addition of HCl-ethanol. If the absorption was above 0.3–0.4 the assay was redone with a smaller volume of saliva, since the reaction is not linear at higher absorption. The RNAase activity is expressed as mmol acid-soluble products formed per hour and litre of saliva. The ribonucleic acid used was a highly polymerized, sodium salt, from yeast (British Drug Houses, Poole, Dorset, UK).

Protein was estimated with the method of Lowry with crystalline bovine albumin as standard.

Lactate was estimated as described by Ström [8].

Electrolytes were assayed with a Perkin-Elmer atom-absorbtion spectrophotometer.

Statistical analyses were performed by the method of Morrison [9] for CF-homozygotes/CF-heterozygotes, and Brownlee [10] for the comparison of results before and after work and with a one-way analysis of variance for CF versus controls. All results are presented as mean ± SEM.

Results

At rest CF-patients have significantly increased activity of RNAase ($p < 0.001$) and concentrations of lactate ($p < 0.001$), protein ($p < 0.001$), sodium ($p < 0.001$), potassium ($p < 0.01$) and calcium ($p < 0.05$) in comparison with healthy controls (Fig. 1, Table I). Heterozygote values are as a rule in between the values of the other two genotypes. Only the lactate concentration and the RNAase activity are significantly higher ($p < 0.05$ for both variables) in CF-homozygotes compared to CF-heterozygotes (Fig. 1, Table I).

After aerobic exercise there were no significant changes in any of these parameters in saliva (Fig. 1, Table II). In blood there was a moderate increase in lactate concentration which is a typical result found after aerobic effort.

Five minutes after intensive (anaerobic) exercise there were significant increases in the concentrations of lactate ($p < 0.001$), sodium ($p < 0.001$) and protein ($p < 0.001$) and in the activity of RNAase ($p < 0.01$) (Fig. 1, Table III). Thus, the biochemical composition of saliva from healthy persons 5 min after anaerobic work

TABLE I

Biochemical composition of resting saliva from normal controls and from heterozygotes and homozygotes for cystic fibrosis

Mean ± SEM (number of subjects). Control values are the pooled resting values from Tables II and III.

	Controls	Heterozygotes	Homozygotes
Lactate (mmol/l)	0.3 ± 0.06 (29)	0.6 ± 0.10 (8)	5.6 ± 1.25 (16)
RNAase (mmol·h^{-1}·l^{-1})	11.5 ± 1.13 (29)	24.3 ± 6.07 (8)	58.1 ± 10.52 (16)
Protein (g/l)	1.3 ± 0.07 (29)	2.6 ± 0.48 (8)	2.9 ± 0.42 (16)
Na^+ (mmol/l)	7.4 ± 0.54 (29)	12.0 ± 0.62 (8)	47.6 ± 17.46 (9)
K^+ (mmol/l)	18.1 ± 0.69 (29)	33.6 ± 3.72 (8)	25.3 ± 2.77 (9)
Ca^{2+} (mmol/l)	0.9 ± 0.06 (29)	0.9 ± 0.18 (8)	2.4 ± 0.99 (9)

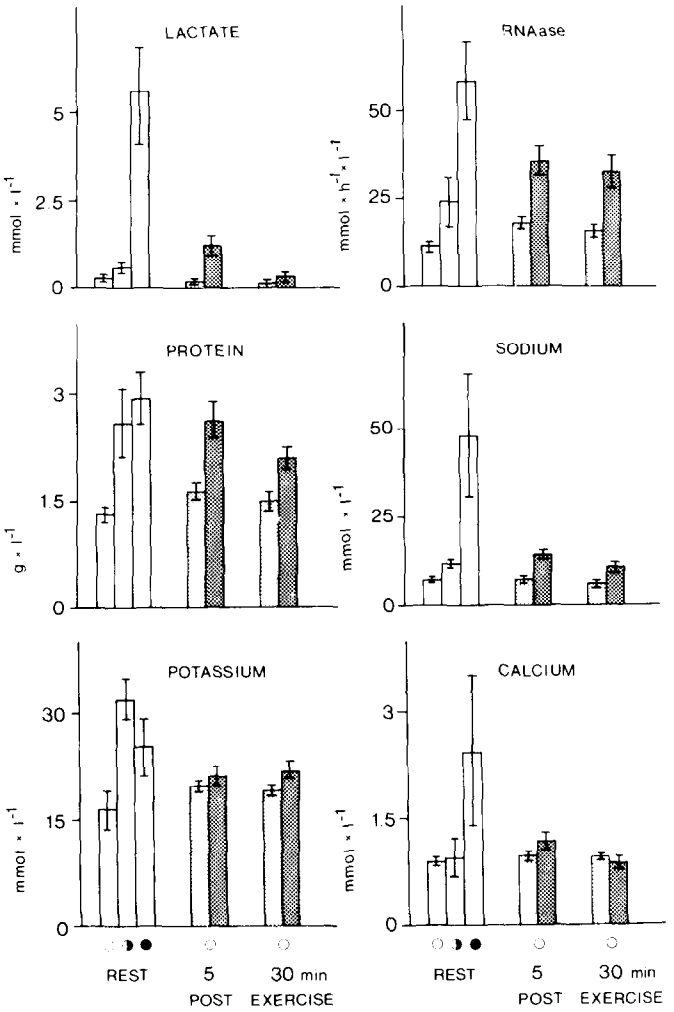

Fig. 1. The biochemical composition of saliva from healthy persons (○), CF-heterozygotes (◑) and CF-homozygotes (●) at rest (open columns) and from healthy persons 5 and 30 min after aerobic (light columns) or anaerobic (dark columns) effort. Mean ± SEM.

TABLE II

Biochemical changes in blood and saliva of healthy persons subjected to aerobic exercise
Mean ± SEM, $n = 10$.

	Rest	Post exercise	
		5 min	30 min
	Blood		
Lactate (mmol/l)	1.4 ± 0.10	3.6 ± 0.32	1.3 ± 0.17
	Saliva		
	0.3 ± 0.07	0.2 ± 0.05	0.2 ± 0.07
RNAase ($mmol \cdot h^{-1} \cdot l^{-1}$)	12.9 ± 2.53	18.7 ± 1.39	17.5 ± 1.80
Protein (g/l)	1.4 ± 0.13	1.7 ± 0.14	1.6 ± 0.16
Na^+ (mmol/l)	7.6 ± 0.60	7.8 ± 0.44	6.3 ± 0.70
K^+ (mmol/l)	17.8 ± 1.14	19.4 ± 0.88	18.5 ± 0.85
Ca^{2+} (mmol/l)	0.9 ± 0.03	1.0 ± 0.06	1.0 ± 0.03

TABLE III

Biochemical changes in blood and saliva of healthy persons subjected to an anaerobic exercise
Mean ± SEM, $n = 19$.

	Rest	Post exercise	
		5 min	30 min
	Blood		
Lactate (mmol/l)	1.8 ± 0.11	17.1 ± 0.28	5.2 ± 0.37
	Saliva		
	0.2 ± 0.10	1.2 ± 0.22	0.3 ± 0.10
RNAase ($mmol \cdot h^{-1} \cdot l^{-1}$)	10.8 ± 1.51	35.5 ± 4.34	32.6 ± 4.36
Protein (g/l)	1.2 ± 0.11	2.6 ± 0.25	2.0 ± 0.23
Na^+ (mmol/l)	7.3 ± 0.60	14.4 ± 1.24	10.4 ± 1.31
K^+ (mmol/l)	18.3 ± 0.92	21.1 ± 1.19	21.7 ± 1.49
Ca^{2+} (mmol/l)	0.9 ± 0.11	1.2 ± 0.10	0.9 ± 0.08

became more like the composition of CF-saliva; 30 min after work most of the values had returned to (or nearly returned to) the resting values (Fig. 1, Table III). In blood highly increased lactate concentrations were found which is a typical result found after anaerobic effort.

Discussion

The present study shows that CF-like changes can be obtained in the saliva of healthy persons subjected to intensive (anaerobic) physical exercise. It is important to underline that only anaerobic and not aerobic work causes these CF-like secretions in the salivary glands (compare Tables II and III). This is in concordance with earlier studies on the sweat [6,11].

The existence of an analogy of the examined biochemical parameters, after anaerobic work and in CF, indicates that the metabolic pathways in salivary glands in CF at rest are altered in the same manner as in healthy persons during anaerobic work. Under these conditions the carbohydrates are metabolised mainly by the glycolytic pathway. This anaerobic pathway is an uneconomic way to produce energy and causes lactate acidosis inside and outside the cell.

In this investigation an increased concentration of lactate has been shown in saliva from CF-children and their parents at rest (Table I). Others have shown increased lactate concentration in sweat and blood from CF-children [5]. This indicates that salivary glands of CF-patients at rest are in a state of lactate acidosis.

Lactate acidosis has been shown to activate the biosynthesis of some acid hydrolases [12], to cause release of lysozomal enzymes [12], to disturb calcium metabolism [13] and to change the permeability of cell membranes [14]. Similar changes of these parameters have also been found in CF [1–3,15–17]. Further, it has been shown that decreased intracellular pH and a decreased ATP level will cause an increased release of proteins from the tissues into the body fluids [14,18], which is in concordance with our results.

Lactate acidosis during anaerobic work is a consequence of accumulation of pyruvate due to its decreased oxidative metabolism [19]. We suggest that this is also the cause for the changes found in CF saliva. We are at present investigating the effect of anaerobic work on the secretions from CF patients and their parents.

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