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Effects of hyperthyroidism and radioactive iodine given to ablate the thyroid on the composition of whole stimulated saliva

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Summary

OBJECTIVE For many years there has been speculation about possible damage to the salivary glands following administration of ablative doses of radioactive iodine for treatment of hyperthyroidism. We have investigated the changes that occur in the composition of saliva in hyperthyroidism and after the administration of an ablative dose of radioactive iodine to hyperthyroid subjects.

DESIGN The study consisted of two parts: first, a comparison of a group of hyperthyroid patients with a group of normal subjects with regard to the concentration or activity of 10 constituents of saliva; second, measurement of those constituents 3–42 weeks after administration of 370 MBq of radioactive iodine to a group of hyperthyroid subjects.

PATIENTS Saliva specimens from 38 untreated outpatients with hyperthyroidism due to Graves' disease or toxic nodular goitre were studied to evaluate the effects of hyperthyroidism and the results were compared with a group of 93 normal subjects. Seventy-one samples of saliva from 26 patients with persistent hyperthyroidism were collected and analysed 3–42 weeks after radioactive iodine administration.

MEASUREMENTS The flow rate; the concentrations of total protein, iodine, calcium, urate, phosphate, potassium and immunoglobulin A; and the activities of *N*-acetylglucosaminidase, lysozyme and lactate dehydrogenase were measured.

RESULTS In hyperthyroidism the salivary flow rate and the concentrations of urate and potassium were significantly ($P < 0{\cdot}05$) increased and the concentrations of total protein, calcium and lactate dehydrogenase activity significantly decreased compared to the control group. After radioactive iodine was administered, significant positive trends were observed in the concentrations of total protein, *N*-acetylglucosaminidase and immunoglobulin A. These trends were independent of the free T3 levels obtained from the same specimens.

CONCLUSIONS Hyperthyroidism leads to a number of changes in salivary composition. For most of the salivary components measured no significant changes were observed 3–42 weeks after administration of 370 MBq of radioactive iodine to patients with persistent hyperthyroidism. The relatively small positive trends in the concentrations of total protein, *N*-acetylglucosamidase activity and immunoglobulin A may have been due either to changes in thyroid status or to the effects of radiation on the salivary glands, or both.

The salivary glands trap circulating iodine by a mechanism very similar to that present in the thyroid gland (Bowen-Grant, 1961). For many years there has been speculation and concern about possible damage to the salivary glands following administration of ablative doses of radioactive iodine for treatment of hyperthyroidism (Ingbar & Woeber, 1974); however, no extensive investigation into the possibility of salivary gland damage has been published, to our knowledge. We decided to undertake such an investigation using stimulated whole saliva in order to monitor the function of all salivary glands. Since amelioration of hyperthyroidism usually follows radioactive iodine treatment, it was necessary to determine the effects on salivary composition of hyperthyroidism alone before investigating possible effects of radioactive iodine.

Materials and methods

The study group of hyperthyroid subjects consisted of patients who came to the Endocrine Department with suspected hyperthyroidism. The diagnosis was confirmed by clinical examination, thyroid function test results and ^{99m}Tc scan and

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Table 1 Within-day and within-month variability of the flow rate and concentration of constituents of saliva from 10 normal subjects

		Variability	
	Median (range)	Within-day	Within-month
Flow rate (ml/min)	1·3 (0·5–4·4)	1·2	2·2
Total protein (g/l)	0·57 (0·24–1·54)	1·27	0·95
NAG (units/l)	1018 (449–3942)	2623	3065
Lysozyme (units/1)	26 (9–76)	65	98
Iodide (μmol/l)	2·98 (0·32–8·16)	2·57	4·15
Calcium (mmol/l)	0·68 (0·07–3·19)	0·99	1·59
Urate (mmol/l)	0·12 (0·02–0·46)	0·26	0·37
Phosphate (mmol/l)	3·87 (2·19–5·85)	1·42	1·67
Potassium (mmol/l)	18·0 (5·0–26·4)	7·9	14·1
LDH (units/l)	122 (4–463)	181	431
IgA (g/l)	0·40 (0·10–2·10)	1·14	1·60

Within-person variabilities were calculated as the reference changes in volume and analyte concentration (Harris & Yasaka, 1983; Albert & Harris, 1987). Changes larger than the reference changes were expected to occur less than 5% of the time in 95% of individuals.

uptake studies. The patients were treated with carbimazole for 6–18 months; no patient was taking carbimazole when hyperthyroid saliva specimens were obtained. If after discontinuation of carbimazole hyperthyroidism recurred, the patient was given 370 MBq of ^{131}I as a single oral dose. The number of saliva specimens obtained after radioactive iodine administration and their timing varied among the patients. The normal subjects were drawn non-selectively from employees at Wellington Hospital. No personal details were obtained. The study was approved by the Wellington Regional Ethical Committee.

Variability was investigated in two circumstances: over a day and between months. Ten subjects gave a salivary specimen 3 times daily for one day at monthly intervals for 3 months. Results from these specimens were not included in the euthyroid group (Table 1). The data obtained were log-transformed where necessary to obtain a normal distribution. Because of an outlier in the logged distribution of calcium and lysozyme, the variances for these analytes were estimated by removing the highest and lowest individual variances (Healy, 1979). The within-person variabilities were calculated as the reference changes in volume and analyte concentration (Harris & Yasaka, 1983; Albert & Harris, 1987). By definition, changes larger than the reference changes were expected to occur less than 5% of the time in 95% of the individuals. These changes were estimated by calculating the mean and variance of within-person variances and the mean serial correlation between an individual's values (Harris & Yasaka, 1983; Albert & Harris, 1987). For the variability between consecutive samples on the same day, within-person variances were calculated allowing for a different mean on each day, that is, the weighted sum of the individual day variances. The within-person serial correlations were estimated from the correlation between the first and second and the second and third sample values on each day. To estimate the variability between months, within-person variances were calculated for each time allowing for a different mean for each time of day, that is, the weighted sum of the individual time variances. The serial correlation between months was assumed to be zero.

Saliva was collected over a 5-minute period during which time the subject chewed raw gum (chicle) and spat into a plastic container. Lysozyme activity and total protein concentration were determined on fresh specimens that had been left at 5°C overnight. Other assays were performed on specimens that had been frozen at −70°C for variable periods up to 3 weeks. For iodine determination, 1 drop of concentrated HCl was added to each 2·5 ml of saliva, the mixture was swirled and centrifuged (3000 r.p.m., 10 minute, room temperature) and the supernatant assayed (Garry *et al*., 1973). β-*N*-Acetyl-D-glucosaminidase (NAG) and lysozyme activities were determined in duplicate as previously described (Fouda *et al*., 1987). Total protein was measured with protein assay kits (Bio-Rad Laboratories, Richmond, CA) based on the method of Bradford (1976). Bovine serum albumin was used as the standard. Assays of lactate dehydrogenase (LDH) activity and of the concentrations of calcium, phosphate, potassium, urate and immunoglobulin A (IgA) were carried out with a Hitachi 717 random access analyser after appropriate dilutions of the specimens. The assay methods were those routinely used for human serum and urine specimens.

Free T4 (fT4) and free T3 (fT3) were assayed in serum using kits obtained from Amersham International (Buckinghamshire, UK) and thyroid-stimulating hormone (TSH) with immunoradiometric sensitive RAI-gnost hTSH kits from Behring (Marburg, Germany).

Table 2 Effect of hyperthyroidism on the flow rate and concentration of constituents of stimulated whole saliva

	n	Median	Range
Flow rate (ml/min)			
Hyperthyroid	38	1·4*	0·30–3·7
Euthyroid	93	1·2	0·02–4·5
Total protein (g/l)			
Hyperthyroid	36	0·69**	0·20–2·24
Euthyroid	93	0·98	0·32–2·20
NAG (units/l)			
Hyperthyroid	36	1187	5–4285
Euthyroid	93	1315	152–5388
Lysozyme (units/l)			
Hyperthyroid	34	20·9	2·5–746
Euthyroid	92	18·5	0–80
Iodide (μmol/l)			
Hyperthyroid	34	4·83	1·45–26·0
Euthyroid	88	4·15	0·25–18·2
Calcium (mmol/l)			
Hyperthyroid	37	0·38**	0·19–1·80
Euthyroid	91	0·53	0·21–2·95
Urate (mmol/l)			
Hyperthyroid	37	0·17**	0·05–0·38
Euthyroid	91	0·11	0·01–0·40
Phosphate (mmol/l)			
Hyperthyroid	37	4·44	2·37–8·55
Euthyroid	90	3·89	1·71–7·23
Potassium (mmol/l)			
Hyperthyroid	37	24·3***	15·4–47·7
Euthyroid	90	20·0	11·4–36·6
LDH (units/l)			
Hyperthyroid	37	21***	0–372
Euthyroid	90	134	8–720
IgA (g/l)			
Hyperthyroid	37	0·72	0–1·90
Euthyroid	91	0·65	0·15–4·60

* $P < 0·05$; ** $P < 0·02$; *** $P < 0·01$.

The concentrations of constituents of saliva in hyperthyroidism were compared with euthyroid individuals with Wilcoxon rank sum tests. Trends in salivary flow rate and levels of constituents were estimated by generalized least-squares regression with transformations that assumed that the measurements on the same person were correlated with a common correlation (Fuller & Battese, 1973). P values <0·05 were considered significant.

Results

The within-day and within-month variabilities of the flow rate and concentration of constituents of saliva from 10 normal subjects are given in Table 1.

In hyperthyroidism the salivary flow rate was significantly increased when compared with the euthyroid population, the concentrations of urate and potassium were significantly increased, and the concentrations of total protein, calcium and LDH activity significantly reduced (Table 2).

In studies from 3 to 42 weeks after radioactive iodine administration to 26 hyperthyroid patients, significantly increasing trends were observed in the salivary concentrations of total protein, NAG activity and IgA when all specimens were included in the analysis (Table 3). Because changes in thyroid status occurred over the same time period, we analysed a subgroup consisting of 54–58 specimens (depending on the salivary constituent) for which fT3 values were recorded at the time the specimens were obtained (Table 3). Contrary to what was observed in the larger group of patients, no significant trends were observed in the concentrations of total protein and NAG activity in the smaller subgroup whether or not the data were adjusted for the associated fT3 values (Table 3). On the other hand, the significant positive trend in IgA concentration that was observed in the larger group of patients was also observed in the smaller group whether or not the fT3 values were taken into account. A significant negative trend in urate concentration and a significant positive trend in LDH activity were observed in the smaller group, but not when all patients were included.

In the group of samples obtained between 3 and 7 weeks after radioactive iodine administration, a significant increase in flow rate (P <0·01) and in LDH activity ($P < 0·03$) and a significant decrease in NAG activity ($P < 0·02$) were observed (data not shown).

Discussion

Stimulated whole saliva was used in our investigations because it is convenient to collect, it represents the function of all salivary glands, the volume that can be collected in a short time is sufficient for multiple assays, and circadian variation in flow rate and composition is slight (Ferguson & Botchway, 1980). It is recognized, however, that there are limitations to the use of whole saliva. Food debris, bacteria and other particulate matter present in the oral cavity may have affected the assay results of our studies and may have contributed to the wide variability that was observed. Furthermore, there is evidence that the parotid glands are the most radiosensitive of the salivary glands (Kashima *et al*., 1965) and minor changes in their function might have been obscured.

Comparable previous studies in humans of the effects of hyperthyroidism on salivary flow and composition are not available, to our knowledge. In rodents, a variety of results have been reported after thyroid hormone administration which reflect the differing experimental conditions under which the studies were conducted (Takuma *et al*., 1977; Johnson *et al*.,

Table 3 Effects of radioactive iodine (RAI) and changes in thyroid function on the flow rate and concentration of constituents of stimulated whole saliva 3–42 weeks after RAI administration to 26 hyperthyroid subjects

	All specimens		Specimens with fT3 values		
				Change/week	
	n	Change/week	*n*	Not adjusting for fT3	Adjusting for fT3
Flow rate (%)†	71	−3·1	58	−3·8	−3·8
Total protein (g/l)	69	0·0085***	57	0·0055	0·0055
NAG (%)†	70	0·7***	58	0·5	0·5
Lysozyme (%)†	64	−0·3	56	0·1	0·1
Iodide (%)†	68	−1·1	55	0·6	0·7
Calcium (mmol/l)	70	0·004	57	0·002	0·002
Urate (%)†	70	−0·8	57	−0·9**	−0·9**
Phosphate (mmol/l)	67	−0·0004	54	0·0057	0·0058
Potassium (mmol/l)	68	−0·046	55	−0·003	−0·005
LDH (%)†	68	1·9	54	2·8*	2·5
IgA (g/l)	70	0·008***	57	0·008*	0·008*

† Because a multiplicative model was used after the data were normalized by log transformation, the change/week is a percentage of the previous week's value.
* $P < 0{\cdot}05$; ** $P < 0{\cdot}03$; *** $P < 0{\cdot}01$.

1987; Sagulin & Roomans, 1989). Aside from the direct effects of thyroid hormone excess on salivary gland function, certain of the changes that we observed during hyperthyroidism may have been secondary to effects of thyrotoxicosis on other organs and tissues. For example, the mean plasma urate concentration was found to be higher in a group of patients when hyperthyroid than when euthyroid (Ford *et al*., 1989) and this difference may explain the higher mean concentration of urate in the saliva of the hyperthyroid subjects. It is unlikely that the differences in analyte concentrations that we observed between normal and hyperthyroid subjects were the result of the increased flow rate in hyperthyroidism since the differences were, for the most part, inconsistent with those observed in normal subjects when salivary flow rate is increased (Mason *et al*., 1966; Mandel, 1979). Furthermore, the increase in flow rate in hyperthyroidism, although significant, was slight. Finally, since the control and hyperthyroid populations were not specifically matched, it is possible that unrecognized differences between the two groups contributed to the different results that were observed.

It is well recognized that large doses of radioactive iodine (> 2590 MBq) may damage the salivary glands (Maxon & Smith, 1990). Acute and chronic sialadenitis (Allweiss *et al*., 1984), a decline in flow rate (Laufender *et al*., 1976; Laupa *et al*., 1993) and an increased concentration of sodium, protein and amylase activity (Maier & Bihl, 1987) have been described. To our knowledge, no study of the effects of smaller doses of radioactive iodine on salivary composition and flow have been published in full, although a preliminary communication many years ago suggested that as little as 296 MBq may reversibly suppress salivary amylase activity (Schneyer *et al*., 1953) and others (Delprat *et al*., 1983) found no abnormal ^{99m}Tc scintigraphic patterns of the salivary glands of patients who had received 185 MBq or 1850–2590 MBq of radioactive iodine.

Our study showed significant changes in the salivary concentration of total protein, NAG activity and IgA 3–42 weeks after administration of 370 MBq of radioactive iodine to patients with recurrent hyperthyroidism. Since amelioration of the hyperthyroidism occurred over the same period of observation in most patients, it was not possible to attribute the changes observed unequivocally to direct effects of the radioactivity on the salivary glands. In particular, the significant positive trend in total protein concentration that was seen in the group of all specimens after radioactive iodine administration may have reflected correction of the significantly lower level that was observed in hyperthyroidism. The same explanation may apply to the significant positive trend in NAG activity after radioiodine treatment. In this case the very wide range of values encountered in both the hyperthyroid and normal groups may have obscured a lower level in hyperthyroidism. On the other hand, it may be argued that for both total protein and NAG activity it is unlikely that the significant positive trends observed in the larger group after radioactive iodine treatment can be explained by changes in fT3 values since in the corresponding subgroups with fT3 values there was little difference between the estimated trends whether or not the fT3

levels were taken into account. Similar arguments can be adduced for and against a direct effect of radioactive iodine administration on salivary IgA concentration. The significant trends in urate concentration and LDH activity observed after radioactive iodine administration in the subgroup with fT3 values, but not the larger group, may be attributed to the different samples with the larger group yielding the more reliable results.

The changes observed between 3 and 7 weeks were not observed in the larger group of subjects over the 3–42-week period and apparently represented transient changes. Of particular interest in this regard was NAG activity which decreased in the early period but increased overall. It is possible, however, that individual variation in the timing and extent of the effects of radioactive iodine on thyroid and salivary gland function may have obscured significant changes in our study.

It should be emphasized that for most of the salivary components measured no significant changes were observed 3–42 weeks after administration of 370 MBq of radioactive iodine to patients with persistent hyperthyroidism; and that the changes in total protein, NAG activity and IgA, although statistically significant, were small in relation to their concentrations. It seems unlikely that clinically significant salivary gland dysfunction was associated with the minor changes that were observed; however, further study is required to assess the possible long-term consequences as well as possible unusual reactions in certain individuals.

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