The Saliva/Serum Iodide Ratio as an Index of Sodium Iodide Symporter Efficiency

by Guy E. Abraham, MD, David Brownstein, MD, and JD Flechas, MD

Introduction

The essential element iodine is present in every organ and tissue of the human body, not just the thyroid gland.[1] Several cells beside the thyrocyte concentrate peripheral iodide against a gradient. So far, the list of these iodide concentrating cells besides the thyrocyte has increased to include: white blood cells, salivary and lacrimal glands, the ciliary body of the eye, the renal cortex, the pancreas, the liver, gastric, small, and large intestinal mucosa, the nasopharynx, the choroid plexus, skin, the adrenal cortex, mammary glands, placenta, uterus and ovaries.[2-6 and vide infra] In the target cells studied, the mechanism used to concentrate peripheral iodide involved an energy-dependant transport of one atom of iodide sandwiched between two atoms of sodium across the cell membrane.[7]

Recently, a second mechanism for the cellular transport of iodine has been reported by several investigators in the thyroid, the mammary gland, and the renal cortex, namely a chloride/iodide transporter identified as pendrin.[8-13] The iodine transporter, pendrin, was speculated to function at the apical membrane of the cell. Rodriguez, *et al*[14] identified a third human protein, homologous to NIS at the apical membrane of the human thyrocyte. This new protein does not catalyze the accumulation of iodide like NIS, but mediates its passive transfer. It was designated as human apical iodide transporter (hAIT).

In the thyrocyte, the sodium/iodide symporter (NIS) is located in the basolateral membrane. The peripheral iodide enters the thyrocyte via the symporter in the basal membrane and crosses the thyrocyte as iodide to exit the thyrocyte via the apical membrane transporter just prior to oxidation and organification. In the case of the symporter, iodide must bind to a site called the halide symporter binding site before cellular uptake. Other substances compete with iodide for these binding sites. These competing substances are called goitrogens, because they sometimes cause goiter by creating a relative iodide deficiency in the thyroid gland. These substances also interfere with iodide transport and utilization in several organs besides the thyroid gland, and a better term would be "iodide transport inhibitors" or "iodide utilization inhibitors" instead of goitrogens, depending on whether the inhibition is at the cell membrane transport system or at intracellular sites of iodide oxidation and utilization.

Iodine deficiency caused by these inhibitors is sometimes relative because peripheral iodide levels may be adequate, but the target cells are starving for iodide because these iodide transport inhibitors prevent the transfer of peripheral iodide across the cell membrane of the target cell for iodide utilization. Some iodide inhibitors, like fluoride and perchlorate, bind to the halide site of the symporter without being symported inside the cells. They cause oxidative damage to the halide binding site. Fluoride and perchlorate are iodide transport inhibitors. Bromide and thiocyanate not only attach to the halide binding site but are also transported inside the target cells, causing further damage by preventing oxidation and organification of iodide. Bromide and thiocyanate are both iodide transport and iodide utilization inhibitors.[2]

A simple test to assess the efficiency of the iodide cellular transport system is greatly needed in order to quantify the degree of defect/damage/inhibition of that system in patients with decreased efficiency of the iodide transport system. The saliva radioiodide/serum radioiodide ratio is used in neonates with elevated TSH and low thyroid hormone levels in order to confirm a congenital iodide symporter defect.[15] The procedure involves injecting radioactive iodide into the neonate and measuring the ratio of radioactivity between saliva and serum. According to Viljder and Vulsma, a ratio >10 is considered normal, 3-10 — borderline, and <3 — abnormal. "Partial iodide transport defect is an ill-defined condition; if it exists, the diagnostic test results depend entirely on the iodine intake, which varies greatly worldwide."[15]

Stable iodide, instead of radioactive iodide, as the means to assess the efficiency of the iodide transport system has not been previously reported because of technical difficulties in measuring low levels of iodine in biological fluids. Measurement of stable iodide in serum and saliva under standardized conditions seems the ideal procedure for fine tuning the assessment of the iodide transport efficiency, and it is the least invasive way to assess response of the symporter function following intervention. This approach would obviate the need to expose

(Continued on next page)

the patient to radioactive iodide. A ratio near unity would indicate a severe defect/damage/inhibition of the symporter function. An increase in the ratio following intervention would reflect an improvement in the symporter function. The data presented in this preliminary communication suggest that the saliva/serum iodide ratio, using a stable iodide load, may be of value in assessing iodide transport efficiency prior to, and following, nutritional intervention.

Theoretical Considerations

The expected serum inorganic iodide level under steady state conditions would be equal to the average daily intake of elemental iodine divided by the renal clearance rate of iodide, that is 43.5 L/day as we previously reported.[16] With an average daily intake of iodine at the level of the US RDA of 0.15 mg/day, the expected serum level of iodide would be 0.0034 mg/L (0.15 mg/L divided by 43.5 L/day). This level is below the sensitivity of our assay: 0.006 mg/L.[16,17] To measure accurately the concentration of iodide in serum and saliva, a load of 50 mg iodine is ingested and the samples of biological fluids are collected 24 hours later. Based on our published data,[17] the levels of serum inorganic iodide 24 hours following 50 mg of iodine/iodide were markedly above the sensitivity of our assay in normal subjects under conditions of pre- and post-orthoiodosupplementation.

The following information summarizes our findings on the 24-hour post-load serum iodide levels. Serum inorganic iodide levels were measured serially for 24 hours following the ingestion of four tablets of Iodoral® (50 mg) in six normal women with normal body weight prior to orthoiodosupplementation. The serum levels of inorganic iodide 24-hour post-load were 0.45±0.051 mg/L (mean ± SD).[17] Following three months of orthoiodosupplementation at 50 mg/day in eight normal subjects (three males and five females) who achieved whole body sufficiency for iodine, the 24-hour post-load serum inorganic iodide were 1.1±0.18 mg/L.[16]

The Procedure

The subject ingests 50 mg of elemental iodine. For the loading test, the amount of the load excreted in the following 24-hour urine collection is measured. Whole body sufficiency for iodine is achieved when 90% or more of the load is recovered in the 24-hour urine collection.[18] For the iodide transport efficiency test, 24±2 hours following the intake of the 50 mg load, a serum sample is collected concomitant with a sample of mixed saliva. Iodide levels in the serum and saliva samples are measured using the ion-selective electrode procedure we described previously.[2,18]

Assessing whole body iodine sufficiency and iodide symporter function can be performed following the same loading test. A 24-hour urine collection is initiated after 50 mg iodine is ingested. Twenty-four hours after the load, serum and saliva samples are obtained. For assessing whole body iodine sufficiency, the percentage of the load recovered in the 24-hour urine collection is measured in an aliquot of urine. Using one sample each of urine, serum, and saliva 24 hours following the iodine/iodide load, whole body iodine sufficiency, as well as the efficiency of the iodide transport system in the whole human body, can be evaluated.

Results

In order to test the validity of the saliva/serum iodide ratio as an index of the efficiency of iodide transport mechanism, we compared the ratios obtained in normal subjects who achieved whole body sufficiency for iodine with patients prior to and post-orthoiodosupplementation. We first established a normal range of values based on data obtained in 14 normal subjects (five males and nine females) who achieved whole body sufficiency for iodine. The mean ± SD for the saliva/serum ratios of iodide in these 14 subjects was 44.2±13.7 with a range of 28-74. The mean ± SD for the serum iodide levels 24 hours post-load in the same subjects were 0.76±0.17 mg/L.

In patients with an inefficient cellular iodide transport mechanism, the absorbed iodine/iodide is rapidly excreted in the urine resulting in low serum iodide levels 24 hours following a load of 50 mg iodine/iodide.[19] In seven patients not on orthoiodosupplementation and with 24-hour post-load serum iodide levels below 0.1 mg/L (normal is 0.45±0.051 mg/L), the mean ± SD of serum/saliva iodide ratio was 4.1±1.5.

In one female patient with breast cancer and elevated serum bromide level, following six weeks on orthoiodosupplementation at 100 mg iodine/day, serum bromide levels dropped 10-fold and saliva/serum ratio increased three-fold. Although this patient was taking iodine prior to this study, we do not have data on serum bromide and saliva/serum iodide ratio previous to this intervention. At the beginning of this study, her serum bromide level was 121 mg/L (normal range 3-12 mg/L) and her saliva/serum ratio was 22. Following six weeks at 100 mg iodine/day, serum bromide was 12.7 mg/L and the saliva/serum ratio increased to 61.

In another female patient with breast cancer on orthoiodosupplementation for several months at 50-75 mg iodine, the saliva/serum iodide ratio was 48. After de-

(Continued on next page)

creasing the iodine intake to 25 mg/day for six weeks, the ratio dropped to 24. In a normal male volunteer on orthoiodosupplementation at 12.5 mg/day, for several months, the serum bromide level was elevated at 127 mg/L and the saliva/serum iodide ratio was 9.3. After six weeks at 50 mg/day, the serum bromide decreased to 37.3 mg/L, and the ratio increased five time (48.6). In a female patient with elevated serum fluoride level at 0.32 mg/L (normal range: 0.001-0.048 mg/L), and a saliva/serum iodide ratio of 1.1, orthoiodosupplementation at 50 mg/day and vitamin C at 3 g/day for six weeks resulted in a decrease in serum fluoride at 0.13 mg/L, and the saliva/serum iodide ratio increased to 47.

We previously reported a case of iodide transport defect with elevated bromide levels that responded to low dose iodine (half tablet of Iodoral®/day) and vitamin C at 3 g/day.[19] This patient was hypothyroid on Synthroid at 50 µg/day. Following the nutritional intervention, she was able to stop the thyroid hormone. Following three months off all nutritional supplementation, her saliva/serum iodide ratio was 3.5. We do not have data on saliva/serum ratios prior to this current study. We plan to repeat this ratio following reinstatement of the nutritional program. The negative correlation between serum bromide levels and the saliva/serum iodide ratios observed in the patients reported in this article strongly suggests that bromide interferes with the cellular uptake of iodide. More research is needed to identify the lowest serum bromide levels that result in significant interference with the iodide transport system.

Discussion

The saliva/serum iodide ratio is currently used to diagnose congenital iodide transport defect in newborns with elevated TSH and low thyroid hormone levels.[15] However, radioiodide is used in this test, not stable iodide. Congenital NIS defect as a cause of congenital hypothyroidism is extremely rare, with only 38 cases reported worldwide.[2] In one case of homozygous missense mutation of NIS, a saliva/serum radioiodide ratio of 1.6 was reported.[20] This patient responded to potassium iodide therapy. There is no consensus in the literature on the normal range of the saliva/serum iodide ratio. Viljder and Vulsma[15] stated that a value above 10 is considered normal, whereas Miki, *et al*[20] think a normal ratio should be above 20. Although congenital hypothyroidism due to sodium/iodide symporter defect is extremely rare, milder forms of iodine/iodide transport defect/damage throughout the whole body may be more common and undetected.[2]

We have decided to investigate the potential diagnostic and prognostic value of a standardized procedure for assessing the saliva/serum iodide ratio, using the stable form of iodine instead of radioiodide. Our first choice was to collect parotid saliva through a parotid gland canula. However, this would require a special setup which is not commonly available. Mixed saliva was then used and collected by spitting into a container after rinsing the mouth with water. The preliminary results obtained with this procedure suggest that the saliva/serum stable iodide ratio is a very sensitive index of iodide symporter function pre- and post-nutritional intervention.

Published data suggest that iodide symporter malfunction may play a role in several clinical conditions. Sera from breast cancer patients block the cellular uptake of radioiodide using an *in vitro* system.[21] The concentration of iodine in breast tissue from breast cancer is four times lower than breast tissue with benign breast disease.[21] A significant difference in spot urine iodide levels was observed between smokers and non-smokers in women attending a university breast center.[22] Because of the increased serum thiocyanate levels in smokers,[23,24] a decreased efficiency of the symporter system would be expected. This can now be confirmed with the saliva/serum iodide ratio.

The serum of 15% of patients with Hashimoto's thyroiditis and 84% of patients with Graves' disease have antibodies against NIS.[25] Using fluorescence excitation analysis (FEA) to measure the amount of stable (non-radioactive) iodine in the thyroid gland, very low levels of thyroidal iodine have been reported in Hashimoto's thyroiditis. Palmer, *et al*[26] reported a mean ± SD of 10.3±2.2 mg in 15 normal men, 8.2±2.5 mg in 43 normal women and 4.4±3.1 mg in 30 cases of Hashimoto's thyroiditis. Okerlund,[27] likewise, published values of 9.9±4.5 mg in 26 men and 9.2±4.3 mg in 41 women. In cases of Hashimoto's thyroiditis, euthyroid patients had a mean ± SD of 4.8±3.7 mg and hypothyroid patients 2.3±2 mg.

The ideal set-up for the evaluation and follow-up of patients with iodide transport defect on nutritional intervention should include measurement of stable iodide in the thyroid gland by FEA. This would require the availability of equipment for performing FEA. Unfortunately, there is a moratorium in the US on the use of FEA to measure thyroidal stable iodine content, probably because it exposes the harmful effect of radioiodide and thyroid hormones in depleting the thyroid gland of iodine in patients not supplemented with iodine.[2] In a recent textbook on thyroid cancer,[28] published in 2000, Wartofsky wrote: "Fluorescent thyroid scanning offers

(Continued on next page)

special advantages in childhood and pregnancy due to minimal radiation exposure. The procedure has been said to be nearly 100% sensitive but only 64% specific when cold areas are taken as positive results. The procedure employs 241Am, which has the ability to excite thyroidal iodine causing release of X-rays that quantitatively correlate with iodine content of the imaged tissue. Unfortunately, the required equipment is not widely available and accumulated data remain too limited to recommend standard use of this technique."

From a review of the literature, Wartofsky's statement is true for the US but not Western Europe, Japan, and Russia. Although US physicians are totally unaware of this technology, which was invented in the US by Hoffer in 1971,[29] it is routinely used in Europe. Jonckheer and Deconinck[30] wrote in 1982: "After a preliminary investigative period, XRF scintigraphies are done routinely in our department, and the purpose of this review is to share our experience of more than 2,000 such determinations with those that might be interested."

Published data on the safe and useful applications of FEA for both scanning of the thyroid gland and quantification of intrathyroidal iodine suggest that it is safer and more informative than radioisotope scanning. Some quotes follow.

"Twenty-one patients in this series demonstrated single or multiple 'cold' nodules on either the fluorescent or the radioisotope scan. No 'cold' nodule seen on the radioisotope scan was missed by the fluorescent scan. However, the fluorescent scan did pick up one nodule missed on the original isotope scan."[29]

"XRF measurement of ITI is an innocuous, easy-to-perform procedure, readily accepted by the patients, that probes an important parameter of thyroid function which had to be neglected so far because of lack of appropriate means. It opens up a new field of investigations in thyroid physiopathology but has already reached, to our mind, the stage of a routine clinical method."[30]

"The introduction of fluorescent thyroid scanning by Hoffer and associates in 1968, and further technical developments over the ensuing years have presented us with a new tool of potential use in thyroid diagnosis and research … Fluorescent thyroid scans may be preferable to conventional scans when the radiation dose needs to be kept as low as possible (as in children and pregnant women), when an expanded iodine pool reduces the uptake of the radioactive tracer, and when a patient is on short-term thyroid-stimulating hormone-suppressing therapy with thyroid hormones … The technique allows us, however, to follow changes in iodine content of the thyroid in thyroid disease, such as Hashimoto's thyroiditis, in which this may have some prognostic implications."[31]

"The detection of iodine in a thyroid nodule by X-ray fluorescence pre-operatively would significantly decrease the probability of malignancy and the need for surgical excision."[32]

Pavoni,[33] as well as Reiners, *et al*,[34] used FEA to assess response of thyroidal iodine after administration of iodine supplementation. This cannot be performed with radioisotope scanning. Since US thyroidologists and radiologists would not have the incentive to set up FEA in their practice, a grassroots effort is needed, via proper education of physicians about the clinical importance of FEA in medical practice. We are planning to evaluate the saliva/serum iodide ratio in several clinical conditions prior to and following nutritional intervention. We are evaluating the possibility of setting up the FEA technology *in situ* in order to correlate the saliva/serum ratio with the thyroidal iodine content.

About the Authors

Guy E. Abraham, MD, is a former Professor of Obstetrics, Gynecology, and Endocrinology at the UCLA School of Medicine. Some 35 years ago, he pioneered the development of assays to measure minute quantities of steroid hormones in biological fluids. He has been honored as follows: General Diagnostic Award from the Canadian Association of Clinical Chemists, 1974; the Medaille d'Honneur from the University of Liege, Belgium, 1976; the Senior Investigator Award of Pharmacia, Sweden, 1980.

The applications of Dr. Abraham's techniques to a variety of female disorders have brought a notable improvement to the understanding and management of these disorders. Twenty-five years ago, Dr. Abraham developed nutritional programs for women with premenstrual tension syndrome and post-menopausal osteoporosis. They are now the most commonly used dietary programs by American obstetricians and gynecologists. Dr. Abraham's current research interests include the development of assays for the measurement of iodide and the other halides in biological fluids and their applications to the implementation of orthoiodosupplementation in medical practice.

David Brownstein, MD, is a family physician who utilizes the best of conventional and alternative therapies.

(Continued on next page)

He is the Medical Director for the Center of Holistic Medicine in West Bloomfield, Michigan. A graduate of the University of Michigan and Wayne State University School of Medicine, Dr. Brownstein is board certified by the American Academy of Family Physicians. He is a member of the American Academy of Family Physicians and the American College for the Advancement in Medicine. Over the past 2 1/2 years, he has had extensive experience in the use of orthoidosupplementation in his practice.

Jorge D. Flechas, MD MPH, is the medical director of Flechas Family Practice in Hendersonville, North Carolina, specializing in hormonal therapy for treatment of fibromyalgia and chronic fatigue and immune dysfunction syndrome (CFIDS) since the late 1980s. He also specializes in iodine therapy for hypothyroidism and fibrocystic breast disease.

REFERENCES

1) Broekhuysen J, Laruel R, and Sion R. "Recherches dans la serie des benzofurannes XXXVII. Etude comparee du transit et du metabolisme de l'amiodarone chez diverses especes animals et chez l'homme." *Arch Int Pharmacodyn*, 1969; 177(2):340-359.
2) Abraham GE. "The historical background of the Iodine Project." *The Original Internist*, 2005; 12(2):57-66.
3) Slebodzinski AB. "Ovarian iodide uptake and triiodothyronine generation in follicular fluid. The enigma of the thyroid ovary interaction." *Domest Anim Endocrinol*, 2005; 29(1):97-103.
4) Carrasco N. "Iodide transport in the thyroid gland." *Biochimica et Biophysica Acta*, 1993; 1154:65-82.
5) Brown-Grant K. "Extrathyroidal iodide concentrating mechanisms." *Physiol Rev*, 1961; 41:189-213.
6) Spitzweg C, Joba W, Schriever K, *et al*. "Analysis of human sodium iodide symporter immunoreactivity in human exocrine glands." *J Clin Endocrinol & Metab*, 1999; 84:4178-4184.
7) Carrasco N. "Thyroid synthesis and secretion." In: *Werner & Ingbar's The Thyroid*. Braverman LE and Utiger RD, editors. Lippincott, Williams & Wilkins, 2000; 52-61.
8) Everett LA, Glaser B, Beck JC, *et al*. "Pendred syndrome is caused by mutation in a putative sulphate transporter gene (PDS)." *Nat Genet*, 1997; 17:411-422.
9) Van Hauwe P, Everett LA, Coucke P, *et al*. "Two frequent missense mutations in Pendred syndrome." *Human Molecular Genetics*, 1998; 7(7):1099-1104.
10) Soleimani M, *et al*. "Pendrin: an apical Cl-/OH-/HCO3- exchanger in the kidney cortex." *Am J Physiol Renal Physiol*, 2001; 280:F356-F364.
11) Rillema JA and Hill MA. "Prolactin regulation of the pendrin-iodide transporter in the mammary gland." *Am J Physiol Endocrinol Metab*, 2003; 284:E25-E28.
12) Scott DA, Wang R, Kreman TM, *et al*. "The Pendred syndrome gene encodes a chloride-iodide transport protein." *Nat Genet*, 1999; 21:440-443.
13) Fugazzola L, Cerutti N, Mannavola D, *et al*. "The role of pendrin in iodide regulation." *Exp Clin Endocrinol Diabetes*, 2001; 109:18-22.
14) Rodriguez AM, Perron B, Lacroix L, *et al*. "Identification and characterization of a putative human iodide transporter located at the apical membrane of thyrocytes." *J Clin Enocr & Metab*, 2002; 87(7):3500-3503.
15) Viljder JJM and Vulsma T. "Hereditary metabolic disorders causing hypothyroidism." In: *Werner & Ingbar's The Thyroid*. Braverman LE and Utiger RD, editors. Lippincott, Williams & Wilkins, 2000; 733-742.
16) Abraham GE. "The concept of orthoiodosupplementation and its clinical implications." *The Original Internist*, 2004; 11(2):29-38.
17) Abraham GE. "Serum inorganic iodide levels following ingestion of a tablet form of Lugol solution: Evidence for an enterohepatic circulation of iodine." *The Original Internist*, 2004; 11 (3):29-34.
18) Abraham GE. "The safe and effective implementation of orthoiodosupplementation in medical practice." *The Original Internist*, 2004; 11(1):17-36.
19) Abraham GE and Brownstein D. "Evidence that the administration of vitamin C improves a defective cellular transport mechanism for iodine: A case report." *The Original Internist*, 2005; 12 (3):125-130.
20) Miki K, *et al*. "Congenital hypothyroidism caused by a mutation in the Na+/I- symporter." *Nat Genet*, 1997; 16:124-125.
21) Kilbane MT, Ajjan RA, Weetman AP, *et al*. "Tissue iodine content and serum-mediated 125I uptake-blocking activity in breast cancer." *J Clin Endocr Metab*, 2000; 85:1245-1250.
22) Eskin BA, Anjum W, Abraham GE, *et al*. "Identification of breast cancer by differences in urinary iodine." *Proceeding of the Am Assoc of Cancer Res*, 2005; 46:504(2150).
23) Foss OP and Lund-Larsen PG. "Serum thiocyanate and smoking: Interpretation of serum thiocyanate levels observed in a large health study." *Scand J Clin Lab Invest*, 1986; 46:245-251.
24) Tonacchera M, Pinchera A, Dimida A, *et al*. "Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter." *Thyroid*, 2004; 14:1012-1019.
25) Endo T, Kaneshiga M, Nakazato M, *et al*. "Autoantibody against thyroid iodide transporter in the acra from patients with Hashimoto's thyroiditis possesses iodide transport inhibitory activity." *Biochem Biophys Res Commun*, 1996; 228:199-202.
26) Palmer DW, Deconinck F, and Swann SJ. "Low cost intrathyroidal iodine quantification with a fluorescent scanner." *Radiology*, 1976; 119:733-735.
27) Okerlund MD. "The clinical utility of fluorescent scanning of the thyroid." In: Medical Applications of Fluorescent Excitation Analysis. Kaufman L and Price DC, editors. CRC Press, Boca Raton, FL, 1979; 149-160.
28) Wartofsky L. "The thyroid nodule." In: *Thyroid Cancer: A Comprehensive Guide to Clinical Management*. Wartofsky L, editor. Humana Press, Totowa, NJ, 2000; 41.
29) Hoffer PB and Gottschalk A. "Fluorescent thyroid scanning: Scanning without radioisotopes." *Radiology*, 1971; 99:117-123.
30) Jonckheer MH and Deconinck F. "X-ray-fluorescence determination of stable iodine in the thyroid gland. A review." *Acta Clinica Belgica*, 1982; 37(1):31-40.
31) Wahner HW, Sweet RA, McConahey WM, *et al*. "Fluorescent thyroid scanning. A method based on stable iodine measurements." *Mayo Clin Proc*, 1978; 53(3):151-156.
32) Tadros TG, Malsey MN, Fui T, *et al*. "The iodine concentration in benign and malignant thyroid nodules measured by x-ray fluorescence." *British J Radiology*, 1981; 54:626-629.
33) Pavoni P. "Radioisotope x-ray fluorescence technique in the dynamic study of thyroid behaviour under iodine load." *Eur J Nucl Med*, 1976; 1:117-122.
34) Reiners Chr, Hanscheid H, Lassmann M, *et al*. "X-ray fluorescence analysis (XFA) of thyroidal iodine content (TIC) with an improved measuring system." *Exp Clin Endocr Diabetes*, 1998; 106:S31-S33. u