Platelet Na^+,K^+-Adenosine Triphosphatase as a Tissue Marker of Hyperthyroidism

A.Y.W. Chan, R. Shinde, C.C. Chow, C.S. Cockram, and R. Swaminathan

Platelet Na^+,K^+-adenosine triphosphatase (ATPase) activity was measured in 34 (15 males, 19 females) healthy subjects, 89 (35 males, 54 females) hyperthyroid patients, and 34 (7 males, 27 females) treated hyperthyroid patients to assess the potential of this measurement as a tissue marker and diagnostic test for hyperthyroidism. Platelet Na^+,K^+-ATPase activity was measured in platelet lysates by the rate of release of phosphate from adenosine triphosphate (ATP) in the presence and absence of ouabain. Platelet Na^+,K^+-ATPase activity (median and range) in the hyperthyroid group (271, 169 to 821 pmol/h/g protein) was significantly higher compared with the healthy group (125, 74 to 185 μmol/h/g protein, $P < .001$ by Mann-Whitney U test). The treated hyperthyroid group had slightly, but significantly higher, free triiodothyronine (FT_3) and free thyroxine (FT_4), as well as platelet Na^+,K^+-ATPase activity (147, 98 to 246 μmol/h/g protein, $P < .05$). If a platelet Na^+,K^+-ATPase activity of 190 μmol/h/g protein was used as a cut off value, the specificity and sensitivity were 90% and 93%, respectively. We conclude that platelet Na^+,K^+-ATPase may be a useful tissue marker of hyperthyroidism.

DISORDERS OF THE THYROID gland are the most common endocrine diseases in clinical practice, and over the years, better and more sensitive tests of thyroid function have been introduced to help in the diagnosis and management. These assays use more specific antibodies, show improved analytical performance, and they can be automated.[1] However, it is well documented that these thyroid function tests have limitations.[2] Furthermore, the variation of serum thyroid hormones in an individual is small,[3] and a value within the population reference range may be abnormal for a given individual.[4] Thus, these tests do not always give a reliable index of tissue hyperthyroidism,[5] and there is poor correlation between clinical assessment of disease activity and serum concentrations of thyroid hormones.[6] The presentation of patients with thyroid disorders can be very heterogeneous, and atypical presentations are not uncommon. Plasma thyroid hormone concentrations could be modestly elevated when the patient exhibits typical symptoms and vice versa,[7] and this is sometimes explained by differences in sensitivity to thyroid hormones.[8] Theoretically, hyperthyroidism should be assessed by measurement of tissue responses rather than by serum thyroid hormone concentrations, which are several steps removed from the site of thyroid hormone action. However, most of the markers of tissue effects of thyroid hormones, such as basal metabolic rate, are not suitable for routine clinical practice. Many of them are nonspecific, require labor-intensive methodology, and there is overlap between disease and control groups.[9] Of these markers, red blood cell zinc[10-12] and neutrophil alkaline phosphatase[13] have been suggested to be good markers. While conventional thyroid function tests have a dominant role in the routine clinical practice, selective tissue markers of thyroid status may continue to have a minor, but important role.

Sodium pump activity is known to be affected by hyperthyroidism. The activity is increased in muscle[14] and leukocytes,[15,16] but is decreased in erythrocytes.[16,17] As far as we are aware, sodium pump activity has not been evaluated as a tissue marker of hyperthyroidism in routine clinical practice. In this study, we have examined platelet Na^+,K^+-adenosine triphosphatase (ATPase) as a tissue marker of hyperthyroidism.

MATERIALS AND METHODS

Subjects

Three groups of subjects were studied: 34 healthy individuals, none of whom had any known illness or were taking any medication; 89 thyrotoxic patients, attending the thyroid clinic who had clinical and biochemical evidence of hyperthyroidism at the time of the study; and 34 treated thyrotoxic patients, attending the thyroid clinic who had been treated for hyperthyroidism with antithyroid drugs and at the time of the study were clinically euthyroid.

In all subjects, venous blood samples were taken in the morning, at least 3 to 4 hours after the last meal or after an overnight fast, into trisodium citrate (9 mL of blood to 1 mL of citrate) and heparinized tubes. Citrated blood was used for platelet studies, and plasma from heparinized blood was used for the measurement of thyroid hormones and thyrotropin (TSH).

Plasma TSH was measured by an immunochemiluminometric assay (Ciba Corning, Medfield, MA) with a detection limit of 0.02 mU/L. Plasma free thyroxine (FT_3) and free triiodothyronine (FT_3) were determined by commercial immunoassay kits (Amerlex-M; Amersham Corp, Arlington Heights, IL for FT_4). The analytical coefficient of variation (CV) (%) of methods was 6.4, 4.8, and 7.5 for TSH, FT_3, and FT_4, respectively.

Platelet Na^+,K^+-ATPase Activity

Na^+,K^+-ATPase activity was measured with a method based on the rate of release of phosphate (Pi) by hydrolysis of adenosine triphosphate (ATP) in the presence and absence of ouabain as described by Baron and Khan.[18] Platelet-rich plasma was prepared as described by Turaihi et al.[19] The citrated blood was centrifuged at 160 $\times$ *g* for 15 minutes at room temperature, and platelet-rich plasma was removed

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Table 1. Age and Thyroid Function Tests in Healthy Subjects, Euthyroid Patients, and Hyperthyroid Patients

	Sex M/F	Age (mean/range)	FT_3 (pmol/L) (3.3-8.2)*	FT_4 (pmol/L) (7.0-21.5)*	TSH (mIU/L) (0.3-8.2)*
Healthy subjects	15/19	38 (21-89)	4.6 ± 1.4 (3.5-8.1)	10.8 ± 3.6 (7.2-20.3)	2.1 ± 0.9 (0.7-3.8)*
Thyrotoxic	35/54	34 (15-69)	23.6 ± 9.7†‡ (8.5-43.3)	39.4 ± 10.4†‡ (24-57.0)	0.03 ± 0.04 (0.02-0.20)
Treated thyrotoxic	7/27	34 (18-66)	6.2 ± 1.4§ (3.2-8.8)	13.5 ± 3.8§ (8.3-21.4)	1.9 ± 1.4§ (0.5-8.3)

NOTE. Results are given as mean ± SD. Range is given in parentheses.

* Reference ranges.

† $P < .001$ compared with healthy subjects.

‡ $P < .001$ compared with treated thyrotoxic group (Mann-Whitney *U* test).

§ $P < .05$ compared with healthy subjects.

and centrifuged again. The platelet pellet was then washed twice with ice cold magnesium (Mg) chloride to remove any trapped plasma. The platelet pellet was stored at -70°C and the Na^+,K^+-ATPase activity measured within 24 hours. The platelets were resuspended in a fixed volume of distilled water and lysed by freezing and thawing followed by the addition of an equal volume of 0.1% (wt/vol) of saponin solution containing 2 mmol/L EDTA and 50 mmol/L Imidasol buffer, pH 7.6. A total of 0.1 mL of platelet lysate was added to 0.1 mL of incubation buffer containing (in mmol/L) Na 100, K 15, ATP 5, Mg 7, EDTA 1, and Tris-HCl 50, and incubated for 60 minutes at 37°C in a shaking water bath. Another aliquot was incubated similarly with the addition of 1 mmol/L ouabain. After 60 minutes, the reaction was stopped by the addition of 0.1 mL 10% trichloroacetic acid (TCA), and the tubes were then centrifuged and the phosphate content of the supernatant measured as described previously.[20] The Na^+,K^+-ATPase activity was calculated from the difference in the phosphate content in the presence and absence of ouabain. Protein content of the platelet lysate was determined by the method of Lowry et al,[21] and the Na^+,K^+-ATPase activity was expressed at μmol Pi/h/g protein. Precision (CV) of the assay determined by analysis of 20 samples in duplicate was 6.4%.

Results were compared by the Mann-Whitney *U* test, and a *P* value less than .05 was considered significant. Correlation between parameters was performed by using linear regression analysis.

RESULTS

Results of thyroid hormones of the 3 groups are summarized in Table 1. All thyrotoxic subjects had increased FT_4 and FT_3, whereas the TSH concentrations were suppressed. In the treated thyrotoxic group, the FT_4 and FT_3 concentrations were significantly higher than that of the healthy group.

Results of the platelet Na^+,K^+-ATPase activity for all patient groups are shown in Fig 1. There was no significant difference between sexes ($P > .5$) within each group. The enzyme activity of the thyrotoxic group (median, 271; range, 169 to 821 μmol Pi/h/g protein) was almost twice that of the treated hyperthyroid (147, 98 to 246 μmol Pi/h/g protein) and control groups (125; 74 to 185 μmol/Pi/h/g protein). The treated thyrotoxic group had a slight, but significantly higher, platelet Na^+,K^+-ATPase activity when compared with healthy subjects ($P < .05$). There was good correlation between platelet ATPase activity and FT_3 ($r = .706$ and $P < .001$) and FT_4 ($r = .696$ and $P < .001$) (Fig 2).

The performance of this test was evaluated by plotting receiver operator characteristic (ROC) curve and highest sensitivity (93%), and specificity (90%) was seen at a cut-off of 190 μmol Pi/h/g protein.

DISCUSSION

Techniques for measurement of thyroid hormones in serum have improved dramatically in the last decade making diagnosis of hyper and hypothyroidism easier. However, even these have some limitations,[2] and it is well recognized that in a minority of patients, a tissue marker of thyroid hormone is desirable.[14] Although serum thyroid hormones may lie within the reference range, patients may not necessarily be euthyroid. This may be due to differences in tissue sensitivity to thyroid hormones between people and/or due to very small intraindividual variation compared with interindividual variation.[3] The small intraindividual variation is explained by the observation that genetic factors are important in determining serum thyroid hormone concentration[22] (Swaminathan, Macgregor, and Spector, unpublished observations). A large number of tests have been examined as potential tissue markers of thyroid hormone status.[9] Of these, leukocyte alkaline phosphatase has been found to be useful in hypothyroidism.[13] We have previously shown that red blood cell zinc, an indirect measure carbonic anhydrase isoenzyme I, is a good marker for thyroid status.[10] This was recently confirmed by others.[11,23,24] However, as the half life of red blood cell is 120 days, red blood cell zinc reflects the integrated thyroid hormone levels over the previous few months.[10,24] This property is useful in distinguishing transient hyperthyroid states, such as thyroiditis[11,12,25] or hyperthyroidism of hyperemesis gravidarum.[26,27] In this study, we have examined another effect of thyroid hormone as a tissue

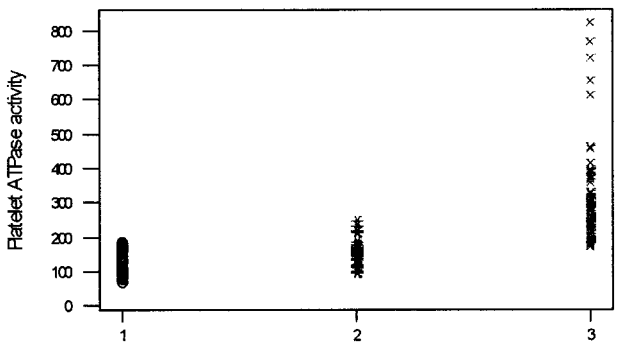

Fig 1. The distribution of platelet Na^+,K^+-ATPase activity in different groups. Group 1, healthy subjects; group 2, treated thyrotoxic patients; and group 3, untreated thyrotoxic patients.

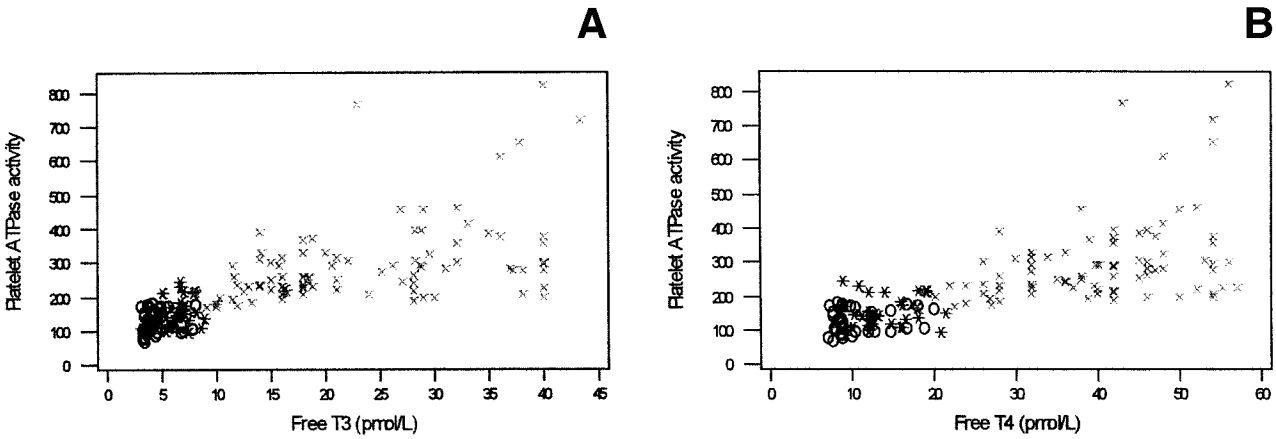

Fig 2. Correlation between platelet Na^+,K^+-ATPase activity and (A) plasma FT_4 and (B) plasma FT_3 concentration. Healthy subjects (○); treated thyrotoxic patients (*); and untreated thyrotoxic patients (x).

marker. Thyrotoxicosis is characterized by changes in tissue sodium pump number and activity,[28] although the response is not uniform over different body tissues. It has been shown in both animal and human studies that the number and activity of sodium pump in most tissues are increased by thyroid hormone.[15,16,28] However, there is no detectable change in brain tissue,[28] and erythrocytes showed a decrease.[16,17] The differences between tissues may be due to different isoforms of Na^+,K^+-ATPase,[28] and in the case of red blood cells, it is due to stimulation of the ATP-dependent proteolytic pathway.[29]

The potential usefulness of platelet Na^+,K^+-ATPase as a tissue marker of hyperthyroidism has been clearly demonstrated in this study. In the thyrotoxic group, Na^+, K^+-ATPase activity was 2.2 times that of the control group. The overlap between the 2 groups was very small (Fig 1) with a sensitivity of 93%, a specificity of 90% at a cut-off value of 190 μmol Pi/h/g protein.

Potential problems in using platelet Na^+,K^+-ATPase are the effects of food intake, drugs, and other illnesses. We have previously shown that platelet Na^+, K^+-ATPase is higher 1 hour after a standard 75-g oral glucose load,[30] and the value returns to baseline after 3 hours. Hence, blood samples were taken 3 to 4 hours after a meal or in the fasting state. The effect of drugs, such as analgesics and diuretics is not known. Digoxin is a known inhibitor of Na^+, K^+-ATPase, and in subjects taking digoxin or other cardiac glycosides, platelet Na^+,K^+-ATPase may not be high even in the presence of hyperthyroidism. Diabetes[31] and allergic disorders[32] have been reported to decrease the activity of platelet Na^+,K^+-ATPase. In patients with these disorders, caution is required in the interpretation of platelet Na^+,K^+-ATPase.

The determination of platelet Na^+,K^+-ATPase activity required a small blood sample volume, and the sample preparation was not demanding. Both the protein and the phosphate measurements could be automated, and it is possible to automate the ATPase assay.[20,33]

Treated patients had lower platelet Na^+,K^+-ATPase. However, this was still significantly higher than in healthy subjects, suggesting that although the serum thyroid hormone concentrations have fallen within the reference range, these patients may be slightly hyperthyroid. Adjusting the treatment according to the tissue markers may be a potential benefit in protecting other organs, such as heart and bone.[34]

There was a good correlation between platelet Na^+,K^+-ATPase activity and FT_3 and FT_4. However, it is interesting to note that at any given concentration of FT_3 or FT_4, there was wide variation in ATPase activity, and this variation was greater at higher FT_4 and FT_3 concentrations (Fig 1). This possibly reflects different tissue sensitivity among individual subjects. Such individual variation might explain the variable presenting symptoms and signs of thyrotoxicosis. A detailed clinical study correlating clinical score (a combination of signs and symptoms) with platelet Na^+,K^+-ATPase is required to test this hypothesis.

We conclude that platelet Na^+,K^+-ATPase is a good tissue marker of hyperthyroidism and may be a useful test in patients with diagnostic difficulties.

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REFERENCES

1. Kricka LJ: Ultrasensitive immunoassay techniques. Clin Biochem 26:325-331, 1993

2. Liewendahl K: Thyroid function tests: Performance and limitations of current methodologies. Scand J Clin Lab Invest 52:435-445, 1992

3. Fraser CG, Petersen PH, Ricos C, et al: Proposed quality specifications for the imprecision and inaccuracy of analytical systems for clinical chemistry. Eur J Clin Chem Clin Biochem 30:311-317, 1992

4. Sauvin CT: Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease. Washington, DC, National Academy of Clinical Biochemistry, 1996

5. Trzepacz PT, Klien I, Robert M, et al: Grave's disease: An analysis of thyroid hormone levels and hyperthyroid signs and symptoms. Am J Med 87:558-61, 1989

6. Braverman LE, Utiger RD: Introduction to thyrotoxicosis, in Braverman LE, Utiger RD (eds): The Thyroid: A Fundamental and Clinical Text, ed 6. Philadelphia, PA, Lippincott, 1991, pp 645-647

7. Borst GC, Eil C, Burman KD: Euthyroid hyperthyroxinemia. Ann Intern Med 98:366-378, 1983

8. Spaulding SW, Lippes H: Hyperthyroidism causes, clinical features and diagnosis. Med Clin North Am 69:937-951, 1985

9. Kaplan MM: Clinical and laboratory assessment of thyroid abnormalities. Med Clin North Am 69:863-881, 1985

10. Swaminathan R, Segall NH, Chapman C, et al: Red blood cell composition in thyroid disease. Lancet 2:1382-1395, 1976

11. Yoshida K, Kiso Y, Watanabe T, et al: Clinical utility of red blood cell. Carbonic anhydrase I and zinc concentrations in patients with thyroid diseases. Metabolism 40:1048-1051, 1991

12. Chan AYW, Mak YT, Shek CC, et al: Changes in erythrocyte zinc in a case of transient thyrotoxicosis. Ann Clin Biochem 28:524-525, 1991

13. Barsono CP, Angulo M, Burke SF, et al: Leucocyte alkaline phosphatase in hypothyroidism and hyperthyroidism. Response to initiation of thyroxine replacement therapy. Metabolism 38:311-314, 1989

14. Sherman SI, Ladenson PW: Thyroid gland, In Noe DA, Rock RC (eds): Laboratory Medicine. Baltimore, MD, Williams & Wilkins, 1994, pp 629-645

15. Arnott RD, White R, Jerums G: Effect of thyroid status on ouabain binding to the human lymphocyte. J Clin Endocrinol Metab 54:1150-1156, 1982

16. Khan FA, Baron DN: Ion flux and Na^+,K^+-ATPase activity of erythrocytes and leucocytes in thyroid disease. Clin Sci 72:171-179, 1987

17. Arumanayagam M, MacDonald D, Cockram CS, et al: Erythrocyte sodium fluxes, ouabain binding sites and Na^+,K^+-ATPase activity in hyperthyroidism. Metabolism 39:952-757, 1990

18. Baron DA, Khan FA: Optimal conditions for measurement of Na^+,K^+-ATPase activity of human leucocytes. Clin Sci 68:143-149, 1985

19. Turaihi K, Khokher MA, Barradas MA, et al: Rb(K) influx and ^{3}[H]Ouabain binding by human platelets: Evidence for β-adrenergic stimulation of Na^+,K^+-ATPase activity. Metabolism 38:773-776, 1989

20. Chan ELP, Swaminathan R: A rapid assay for the measurement of Na^+,K^+-ATPase inhibitors. Clin Biochem 25:15-19, 1992

21. Lowry OH, Rosebrough NJ, Farr AL, et al: Protein measurement with the Folin-phenol reagent. J Biol Chem 193:265-275, 1951

22. Meikle AW, Stringham JD, Woodward MC, et al: Hereditary and environmental influences on the variation of thyroid hormones in normal and male twins. J Clin Endocrinol Metab 66:588-592, 1988

23. Kiso Y, Yoshida K, Kaise K, et al: Erythrocyte carbonic anhydrase I concentrations in patients with Grave's disease and subacute thyroiditis reflect integrated thyroid hormone levels over the previous few months. J Clin Endocrinol Metab 72:515-518, 1991

24. Yoshida K, Kiso Y, Watanabe T, et al: Erythrocyte zinc in hyperthyroidism: Reflection of integrated thyroid hormone levels over the previous few months. Metabolism 39:182-186, 1990

25. Chow CC, Shek CC, Mak YT, et al: Erythrocyte zinc concentrations in subacute (De Quervain's) thyroiditis. BMJ 299:1505-1506, 1989

26. Lao TTH, Chin RKH, Swaminathan R, et al: Erythrocyte zinc in differential diagnosis of hyperthyroidism in pregnancy: A preliminary report. BMJ 294:1064-1065, 1987

27. Swaminathan R, Chin RKH, Lao TTH, et al: Thyroid function in hyperemesis gravidarum. Acta Endocrinol 120:155-160, 1989

28. Gick GG, Ismail-Beigi F, Edelman IS: Hormonal regulation of NaK ATPase, in Skou JC, Norby JG, Maunsbach AB et al (eds): Progress in Clinical and Biological Research, vol 268B: The Na^+,K^+-ATPase. New York, NY, Liss, 1988, pp 277-295

29. Arymananayagam M, Swaminathan R: Induction of the ATP-dependent proteolytic system in guinea pig reticulocyte lysates by triiodothyronine. Life Sci 19:1369-1376, 1991

30. Chan A, Shinde R, Chow CC, et al: Hyperinsulinaemia and Na^+,K^+-ATPase activity in thyrotoxic periodic paralysis. Clin Endocrinol 41:213-216, 1999

31. Mazzanti L, Mutus B: Diabetes-induced alterations in platelet metabolism. Clin Biochem 30:509-515, 1997

32. Gentile DA, Skoner DP: The relationship between air way hyperactivity (AHR) and sodium, potassium adenosine triphosphatase (Na^+,K^+-ATPase) enzyme inhibition. J Allergy Clin Immunol 99:367-373, 1997

33. Li PWC, Ho CS, Swaminathan R: The effect of high salt intake on Na^+,K^+-ATPase activity of tissues in the rat. Clin Exp Hypertens 16:327-340, 1994

34. Grant DJ, McMurdo ME, Mole PA, et al: Is previous hyperthyroidism still a risk factor for osteoporosis in post-menopausal women? Clin Endocrinol 43:339-345, 1995