Alteration of Platelet Aggregation in Patients With Thyroid Disorders

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To determine whether Graves' disease or primary hypothyroidism influence platelet function, we evaluated platelet aggregation in the platelet-rich plasma (PRP) from such patients. Platelet aggregation induced by adenosine diphosphate (ADP) in blood obtained from patients with untreated Graves' disease was significantly lower than normal, whereas that in patients with untreated primary hypothyroidism was relatively increased. The magnitude of platelet aggregation induced by collagen in both groups of patients resembled that induced by ADP. However, significant differences were evident between the two diseases ($P < .05$). In addition, we observed a significant inverse correlation between the extent of platelet aggregation and plasma levels of thyroid hormones (triiodothyronine [T_3], thyroxine [T_4], and free T_3). Platelet aggregation returned to normal when the euthyroid condition was obtained in the patients following administration of antithyroid drugs or thyroid hormone. The findings are consistent with the possibility that thyroid hormones influence platelet aggregation partly via inhibition of myosin light-chain kinase (MLCK).

ALTHOUGH THYROID HORMONES act mainly on nuclear receptors, they also directly affect extranuclear cytosolic elements such as the mitochondria and various enzymes.[1] Platelet dysfunction in patients with thyroid disease has rarely been reported.[2,3] Although platelets lack nuclei, the parent megakaryocytes possess nuclei; the effects observed in platelets may therefore be the consequence of the actions of thyroid hormone on the nuclei of megakaryocytes. It has also been reported that platelet function is regulated in part by myosin light-chain kinase (MLCK) activity and its phosphorylation.[4]

Mamiya et al[4] reported that thyroid hormones inhibit MLCK contained in platelets. This enzyme catalyzes the Ca^{2+}-calmodulin–dependent phosphorylation of the myosin light chain, subsequently affecting the contractile mechanism of the platelets. Masaki et al[5] showed that reverse triiodothyronine (rT_3) also inhibits phosphorylation of a 20-kd protein in platelets in vitro, and this process affects platelet aggregation.

Accordingly, we evaluated the status of platelet aggregation in hyperthyroid and hypothyroid states using platelets obtained from patients with Graves' disease and patients with primary hypothyroidism. We sought to define the putative effects of thyroid hormone levels on platelet function.

SUBJECTS AND METHODS

Platelet-rich plasma (PRP) was separated from blood samples obtained from the cubital vein of 29 fasted patients with thyroid disorders (14 with Graves' disease, 12 with Hashimoto's thyroiditis, and three with idiopathic primary hypothyroidism; Table 1). Thyroid function in these patients is shown in Table 2. Antithyroid antibodies were present in 78% of Graves' disease patients, 100% of Hashimoto's thyroiditis patients, and none of the idiopathic primary hypothyroidism patients. These patients were not taking any potential platelet function inhibitors such as aspirin or indomethacin during the study.

Immediately after collection, blood samples were centrifuged with 3.8% sodium citrate at 250 × *g* for 10 minutes at room temperature. These supernatants were used as the PRP samples that were subsequently adjusted by platelet-free plasma to contain 25 to 30 × 10^4 platelets/μL plasma. A volume of 200 μL of the adjusted PRP was preincubated at 37°C for 5 minutes. Then, platelet aggregation in response to the agonists, collagen (Hormone Chemi, Stuttgart, Germany) and adenosine diphosphate (ADP) (Sigma, St Louis, MO) was measured by adding either 50 μg collagen or 2 × 10^{-6} mol/L ADP. These concentrations were previously determined to be adequate for the nephelometric measurements used.

Platelet aggregation was calculated as the turbidity of these solutions using a four-channel aggregometer (Nikko Bioscience, Tokyo, Japan).

In Graves' disease the ADP-induced aggregation curve demonstrated biphasic kinetics for aggregation, but in hypothyroidism and euthyroidism the curve continually increased. Aggregation was expressed as the maximum change in turbidity in all cases and as the initial change in light transmission in Graves' disease patients (Fig 1).

Normal values for platelet aggregation were obtained for each assay by testing blood obtained from 20 euthyroid volunteers (13 men and seven women aged 25 to 50 years).

Platelet aggregation was not affected by antithyroid drugs in vitro (data not shown).

Serum triiodothyronine (T_3) and thyroxine (T_4) levels were measured using an immunochemiluminescence method as described by the manufacturer (Amersham International, Amersham, Bucks, UK).

Statistical Analysis

Data are expressed as the mean ± SE or mean ± SD. Differences between data sets were evaluated by one-factor ANOVA for paired or nonpaired values or by Spearman rank correlation. P less than .05 was accepted as statistically significant.

RESULTS

Platelet aggregation data for all groups of patients are summarized in Table 3. In the group with Graves' disease, ADP-induced aggregation exhibited a biphasic pattern, whereas in hypothyroid and euthyroid groups, a continuous increase in aggregation was observed. Relative to the values in euthyroid control subjects, platelet aggregation induced by ADP in patients with untreated Graves' disease was significantly decreased both initially and upon completion of the assay. Conversely, a nonsignificant increase in platelet aggregation was found in untreated primary hypothyroidism patients compared with euthyroid controls. In addition, statistically significant differences were observed between aggregation levels obtained from untreated Graves' disease patients and primary hypothyroidism patients.

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Table 1. Patient Population

	Graves' Disease		Hypothyroidism	
	Overall	M/F	Overall	M/F
No. of patients	14	8/6	15	0/15
Age (yr)	43 ± 3	39 ± 4/48 ± 5	54 ± 4	—/54 ± 4

NOTE. Data are expressed as the mean ± SE.
Abbreviations: M, male; F, female.

Table 2. Thyroid Function Data

Variable	Hyperthyroid (n = 14)	Euthyroid (n = 20)	Hypothyroid (n = 15)
T_3 (ng/mL)	3.4 ± 0.4	1.1 ± 0.1	0.7 ± 0.2
T_4 (μg/dL)	18.9 ± 1.5	8.9 ± 0.1	3.4 ± 0.7
Thyrotropin (μU/mL)	0.06 ± 0.01	2.38 ± 0.95	87.9 ± 18.6
Free T_3 (ng/dL)	9.1 ± 1.2	3.7 ± 0.9	1.8 ± 0.4
Free T_4 (pg/mL)	5.6 ± 1.7	1.4 ± 0.1	0.4 ± 0.1

NOTE. Data are expressed as the mean ± SE. Each value in hyperthyroid or hypothyroid patients is statistically significant ($P < .05$) compared with those in euthyroid volunteers. Hyperthyroid, Graves' disease; hypothyroid, Hashimoto's thyroiditis and idiopathic primary hypothyroidism; euthyroid, healthy volunteers.

Table 3. Platelet Aggregation in Patients With Graves' Disease or Primary Hypothyroidism

Agonist	Percent Change	Graves' Disease	Euthyroid	Hypothyroid
ADP	Initial	13.9 ± 4.2	40.8 ± 7.2	ND
		<.05*		
	Total	26.3 ± 2.9	45.7 ± 7.7	57.8 ± 5.9
		<.05*	NS	
		<.05*		
Collagen	Total	54.4 ± 4.7	69.6 ± 4.3	71.4 ± 3.1
		<.05*	NS	
		<.05*		

NOTE. Data are expressed as the mean ± SE. The number of patients is the same as in Table 2. Values at the initial change in euthyroid volunteers were measured at the time the initial change appeared in Graves' disease.
Abbreviation: ND, not determined.
*Statistical significance between data connected with brackets, using ANOVA for nonpaired values.

Table 4. Platelet Aggregation Before and After Treatment With Antithyroid Drugs (methimazole or propylthiouracil) or Thyroid Hormone (L-thyroxine)

	Graves' Disease		Hypothyroidism	
	Before	After	Before	After
ADP-induced (%)	24.7 ± 10.5	35.7 ± 9.2†	49.2 ± 16.2	32.6 ± 17.5†
Collagen-induced (%)	38.2 ± 7.9	54.6 ± 15.0*	62.7 ± 25.9	46.3 ± 27.0*

NOTE. Data are expressed as the mean ± SD for 9 patients with Graves' disease or 9 patients with primary hypothyroidism (7 Hashimoto's thyroiditis and 2 idiopathic primary hypothyroidism).
*$P < .05$, †$P < .01$: v before treatment by ANOVA for paired values.

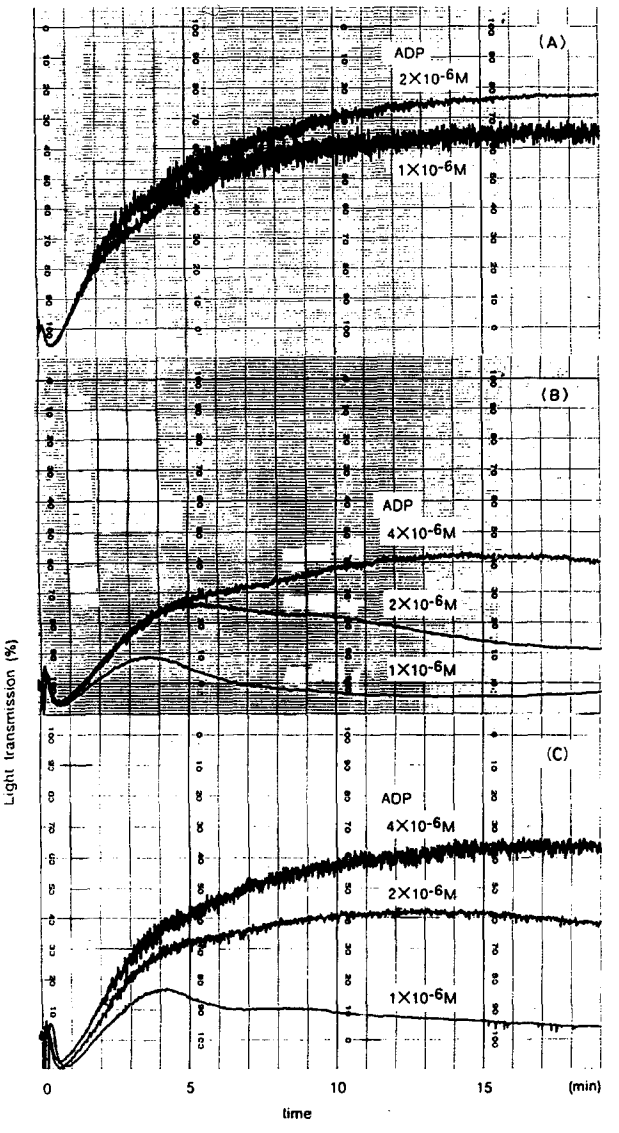

Fig 1. Platelet aggregation assay. PRP adjusted to 25 to 30 × 10^4 platelets/μL was prepared. Increasing amounts of ADP were added to samples obtained from (A) hypothyroid patients, (B) Graves' disease patients, and (C) euthyroid subjects. Aggregation was then monitored as a function of light transmission (%).

The ADP-induced platelet aggregation measured in untreated Graves' disease and primary hypothyroidism patients and patients with euthyroidism induced by treatment with antithyroid drugs or thyroid hormone exhibited strong inverse correlations with plasma T_3, T_4, and free T_3 levels (Fig 2). However, free T_3 levels were not measurable in all patients. The collagen-induced platelet aggregation in these patients showed a pattern similar to that for ADP-induced aggregation, but the correlation between aggregation levels and hormone levels was significant only for free T_3 levels (Fig 3).

The levels of ADP- and collagen-induced platelet aggregation significantly improved after thyroid function was normalized following oral administration of antithyroid drugs or thyroid hormone (Table 4).

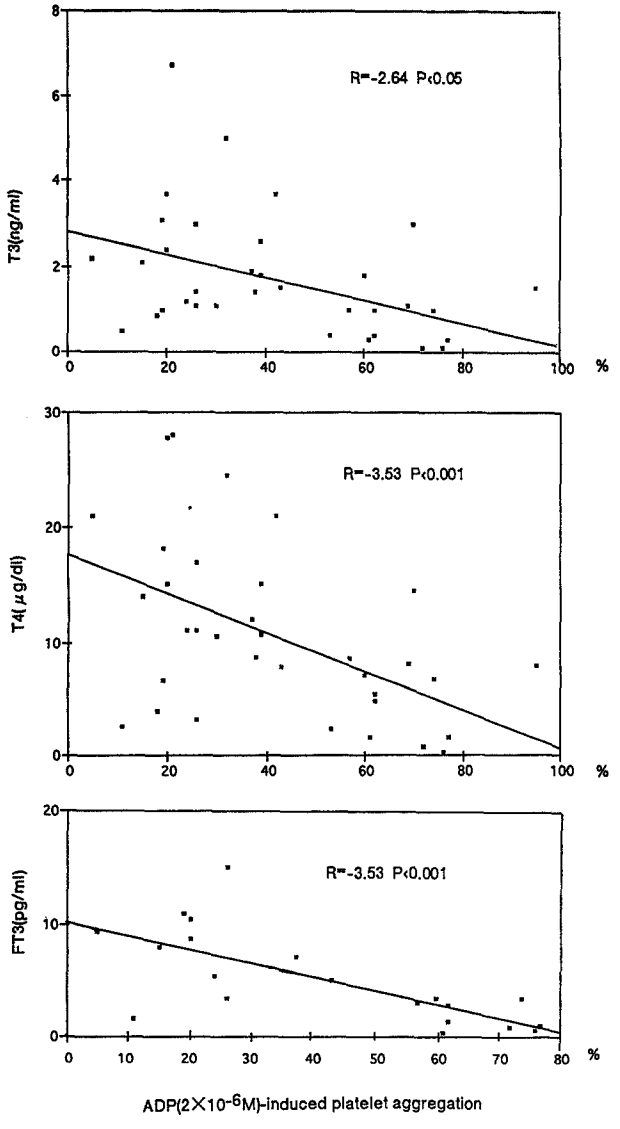

Fig 2. Relationship between platelet aggregation induced by ADP and plasma levels of thyroid hormones. In 5 cases of Graves' disease, data after treatment were additionally used. The number of data for free T_3 was less than for the other thyroid hormones because serum free T_3 concentrations were unable to be measured in all patients.

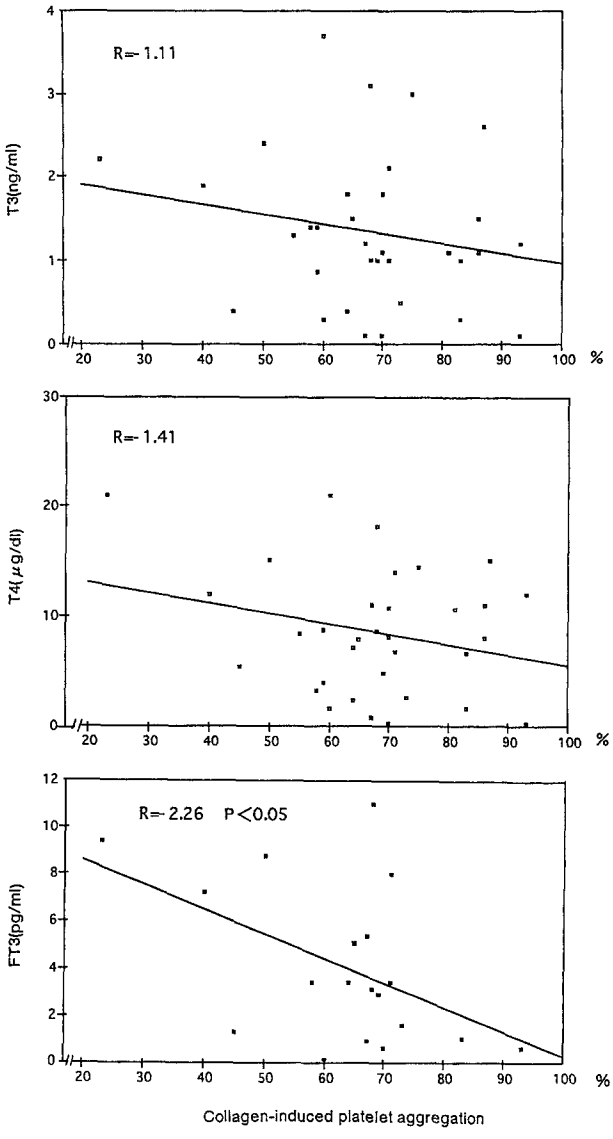

Fig 3. Relationship between collagen-induced platelet aggregation and plasma thyroid hormone levels. For 4 cases, data before and after treatment were used. The number of data for free T_3 is the same as in Fig 2.

DISCUSSION

Previous studies have not clarified the detailed effects of thyroid hormones on platelet function,[6-8] particularly platelet aggregation. However, recent studies by Mamiya et al[4] and Masaki et al[5] have confirmed the direct action of thyroid hormones on human platelet aggregation in vitro by demonstrating inhibition of the phosphorylation of a 20-kd protein, as well as inhibition of MLCK activity.

In this study, we confirmed the inhibitory effect of thyroid hormones on platelet aggregation in vivo by evaluating platelet function in patients with thyroid disorders. The finding that the rate of platelet aggregation in these patients was inversely correlated with serum concentrations of thyroid hormones is consistent with the results of in vitro studies by Mamiya et al[4] and Masaki et al.[5] Mamiya et al also confirmed that thyroid hormones act as competitive inhibitors of MLCK toward calmodulin, binding directly to MLCK.[4] We believe this interaction between thyroid hormones and MLCK is partly responsible for the abnormal function of platelets obtained from our patients.

Correlation coefficients for the aggregation were more significant with ADP induction than with collagen induction. This suggests that platelets may be more sensitive to aggregation induced by ADP.

Serum levels of epidermal growth factor[9] and thrombomodulin[10] are closely correlated with serum levels of thyroid hormones, suggesting that thyroid hormones may influence the metabolism of endothelial cells in patients with thyroid disease.

In treating patients with thyroid disorders, particularly those with hypothyroidism, who are at risk for atherosclerosis, platelet function should be periodically evaluated.

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REFERENCES

1. Werner SC, Ingbar SH (eds): The Thyroid (ed 6). Hagerstown, MD, Harper & Row, 1991, pp 138-148

2. Lamberg BA, Kivikangas V, Pelkonen R, et al: Thrombocytopenia and decreased life-span of thrombocytes in hyperthyroidism. Ann Clin Res 3:98-102, 1971

3. Hymes K, Blum M, Lackner H, et al: Easy bruising, thrombocytopenia, and elevated platelet immunoglobulin G in Graves' disease and Hashimoto's thyroiditis. Ann Intern Med 94:27-30, 1981

4. Mamiya S, Hagiwara M, Inoue S, et al: Thyroid hormones inhibit platelet function and myosin light chain kinase. J Biol Chem 264:8575-8579, 1989

5. Masaki H, Nishikawa M, Urakami M, et al: 3,3′5′-Triiodothyronine inhibits collagen-induced human platelet aggregation. J Clin Endocrinol Metab 75:721-725, 1992

6. Golde DW, Bersch N, Chopra IJ, et al: Potentiation of erythropoiesis in vitro by thyroid hormones. Clin Res 24:309A, 1976 (abstr)

7. Marshall JS, Weisberger AS, Levy RP, et al: Coexistent idiopathic thrombocytopenic purpura and hyperthyroidism. Ann Intern Med 67:411-414, 1967

8. Myrup B, Bregengård C, Faber J: Primary haemostasis in thyroid disease. J Intern Med 238:59-63, 1995

9. Kung AW, Hui WM, Ng ES: Serum and plasma epidermal growth factor in thyroid disorders. Acta Endocrinol (Copenh) 127:52-57, 1992

10. Morikawa Y, Morikawa A, Makino I: Relationship of thyroid states and serum thrombomodulin (TM) levels in patients with Graves' disease: TM, a possible new marker of the peripheral activity of thyroid hormones. J Clin Endocrinol Metab 76:609-614, 1993