CLINICAL STUDY

Thyroid hormone increases mannan-binding lectin levels

Anne Lene Dalkjær Riis, Troels Krarup Hansen[2], Steffen Thiel[4], Claus Højbjerg Gravholt, Signe Gjedde[1], Lars Christian Gormsen, Jens Otto Lunde Jørgensen, Jørgen Weeke and Niels Møller[3]

Medical Department M, [1]Medical Department C, and [2]Immunoendocrine Research Unit, Medical Department M, Aarhus University Hospital, Aarhus, Denmark, [3]Institute of Clinical Medicine, and [4]Institute of Medical Microbiology and Immunology, Aarhus University, 8000 Aarhus, Denmark

(Correspondence should be addressed to Anne Lene D Riis; Email: anne.lene.riis@ki.au.dk)

Abstract

Background: Recent studies have indicated the existence of causal links between the endocrine and immune systems and cardiovascular disease. Mannan-binding lectin (MBL), a protein of the innate immune system, may constitute a connection between these fields.

Methods: To test whether thyroid hormone regulates MBL levels, we studied eight patients with Graves' hyperthyroidism before and after methimazole therapy, eight healthy subjects before and after short-term experimental hyperthyroidism, and eight hypothyroid patients with chronic auto-immune thyroiditis before and after L-thyroxine substitution.

Results: In all hyperthyroid patients, MBL levels were increased – median (range), 1886 ng/ml (1478–7344) – before treatment and decreased to 954 ng/ml (312–3222) after treatment ($P = 0.01$, paired comparison: Wilcoxon's signed ranks test). The healthy subjects had MBL levels of 1081 ng/ml (312–1578). Administration of thyroid hormones to these persons induced mild hyperthyroidism and increased MBL levels significantly to 1714 ng/ml (356–2488) ($P = 0.01$). Two of the eight hypothyroid patients had undetectably low levels of MBL both before and after L-thyroxine substitution. The other six hypothyroid patients had decreased levels of MBL of 145 ng/ml (20–457) compared with 979 ng/ml (214–1533) after L-thyroxine substitution ($P = 0.03$, paired comparison: Wilcoxon's signed ranks test).

Conclusion: Our data show that thyroid hormone increases levels of MBL. MBL is part of the inflammatory complement system, and this modulation of complement activation may play a role in the pathogenesis of a number of key components of thyroid diseases.

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Introduction

A number of studies have indicated the existence of causal links between the endocrine and the immune system and cardiovascular disease; for instance, insulin resistance and elevation of inflammatory markers are well-known cardiovascular risk factors (1, 2). Mannan-binding lectin (MBL) is one possible connection between these three fields, and it has attracted considerable interest. MBL is a phylogenetically old, innate, immune-defense plasma protein synthesized in the liver. It is a molecule that may bind to specific, repetitive carbohydrate structures on microbial surfaces, and subsequently activates the complement cascade through MBL-associated serine proteases (MASP-1, MASP-2 and MASP-3) (3, 4) – the so-called MBL pathway of complement activation. MBL participates in the pathology of infectious, autoimmune and cardiovascular diseases (5–11). A number of MBL haplotypes exist, and combinations of these influence the levels of MBL in the blood (12). Recent studies indicate that MBL levels are also under the influence of growth hormone and insulin (13–15). With respect to cardiovascular diseases, it appears that low levels of MBL may entail an increased risk of arterial atherosclerosis and thrombosis (7, 9, 11, 16), whereas high levels entail an increased risk of diabetic angiopathy (6) and endothelial ischemia/reperfusion injury of the myocardium (8, 10).

Thyroid hormone has many effects on the cardiovascular system, and patients with hyper- and hypothyroidism are prone to cardiovascular diseases. Hyperthyroidism increases heart rate and cardiac output and may aggravate heart failure, and hypothyroidism decreases heart rate and cardiac output and causes hypertension and hypercholesterolemia (17, 18). In addition, elevated triiodothyronine (T_3) levels are associated with a threefold increased risk of coronary events (19), and cardiovascular mortality is substantially increased in individuals with subclinical hyperthyroidism (20) and in patients previously treated with radioiodine (21). Hypothyroidism is also associated with a higher risk of cardiovascular disease (22), and subclinical hypothyroidism increases the

risk of atherosclerosis and myocardial infarction by roughly twofold (23).

The aims of the present study were to test whether thyroid hormone influences MBL concentrations in the blood, and, if so, whether this relates to thyroid hormone *per se* or the autoimmune process of thyroid disease. Consequently, we studied patients with hypo- and hyperthyroidism before and after therapy and compared the findings to those from a group of healthy subjects without medication and during mild, short-term hyperthyroidism induced by experimental thyroid hormone administration.

Subjects and methods

Subjects and experimental design

Protocol 1 Eight hyperthyroid women, aged 26–49 years, with newly diagnosed Graves' disease were consecutively recruited and studied before and after 3 months of medical treatment with methimazol. All patients exhibited anti-thyroid-stimulating hormone (TSH)-receptor antibodies (Trab > 2 IU/l). Other results from this protocol have been published earlier (24).

Protocol 2 In a single-blind, randomized design, eight healthy women (24–46 years) were studied twice with an interval of 3 months. Once after 6 days' placebo tablet administration and once after 6 days' administration of thyroid hormones: L-thyroxine (T_4) 50 µg once a day and T_3 0.67 µg/kg per day divided into four daily doses. The placebo situation served as control group for the patients in protocols 1 and 3.

Protocol 3 Eight hypothyroid patients with newly diagnosed chronic autoimmune thyroiditis (i.e. Hashimoto's thyroiditis or atrophic thyroiditis – HT/AT), two men and six women, aged 24– 65 years, with TSH greater than 20 µmol/ml, were studied before and after 6 months' substitution therapy with T_4. Anti-thyroperoxidase-antibodies (anti-TPO-antibodies) were elevated in all patients.

All blood samples were drawn after an overnight fast. The participants gave their written, informed consent after receiving oral and written information concerning the studies according to the Declaration of Helsinki II. The Aarhus County Ethical Scientific Committee approved the studies. The patients did not suffer from any associated diseases, and neither the patients nor the healthy control subjects were taking any other medication.

Analytic methods

Serum MBL concentrations were measured by an in-house, time-resolved monoclonal immunofluorometric assay as previously described (25). In brief, microtiter wells were coated with mannan, followed by incubation with diluted samples. After washing, europium-labeled monoclonal anti-MBL antibody (131−I, State Serum Institute, Copenhagen, Denmark) was added; and, after incubation, the binding of labeled antibody was assessed by time-resolved fluorometry (Delphia; Perkin Elmer, Turku, Finland). Serum MASP-2 concentrations were measured by a previously described procedure (26). In brief, microtiter wells were coated with monoclonal anti-MASP-2 antibodies, followed by incubation with sample; subsequently, the wells were incubated with labeled monoclonal anti-MASP-2 antibody. Serum concentrations of C-reactive protein (CRP) were measured at the Department of Clinical Biochemistry, Aarhus University Hospital, by the standard ultra-sensitive latex-enhanced immunotechnique (Cobas Integra 700; Hoffman-LaRoche Inc., Basel, Switzerland). Thyroid hormones (total T_3 and total T_4) and TSH were measured by immunofluorescent methods (Immulite, DPC, Los Angeles, CA, USA). Our laboratory reference range for total T_3 is 1.1−2.6 nmol/l; for total T_4, 58−161 nmol/l; and for TSH, 0.3−4.0 µmol/ml. Free thyroid hormones thyroxin (fT_4) and triiodothyronine (fT_3) were measured by ultrafiltration and RIA (18). The laboratory reference ranges are 3.7−9.5 pmol/l for free T_3 and 12−33 pmol/l for free T_4.

Statistical methods

Statistical calculations were done with SPSS for Windows, Version 10.0 (SPSS, Chicago, IL, USA). The data were tested for normal distribution. MBL, TSH and thyroid hormones in hypothyroid patients did not comply with normal distribution, and when comparing these parameters Wilcoxon's signed rank test for paired comparisons or Mann−Whitney's *U*-test for unpaired comparisons was used. Data corresponding to serum MBL, TSH and thyroid hormones in hypothyroid patients are given as medians and ranges. With all other variables, Student's two-tailed *t*-test for paired or *t*-test for unpaired data was used to evaluate the differences as appropriate. The results are expressed as mean±S.E. Correlation analysis was performed with Spearman's rho analysis.

Results

In the hyperthyroid state, the Graves' disease patients in protocol 1 had two- to fivefold increased plasma total T_3 and free T_3, compared with after treatment, after which T_3 decreased to normal levels (see Table 1 for thyroid hormones). Upon admission, the patients were clinically thyrotoxic, and during the restoration of euthyroidism, body weight increased 5 kg. The experimental thyroid hormone administration to the healthy subjects (protocol 2) resulted in mild hyperthyroidism, raising the total T_3 levels 250% above baseline values and free T_3 240% above baseline values. TSH levels were suppressed, but T_4 remained constant (Table 1).

Table 1 Thyroid hormones and thyroid–stimulating hormone in eight patients with Graves' disease before (hyperthyroid) and after (euthyroid) treatment with methimazole, eight healthy subjects studied once after placebo tablet administration (placebo) and once after 6 days' administration of thyroid hormones (experimental hyperthyroidism, exp. hypert.) (see text), and eight hypothyroid patients with chronic autoimmune thyroiditis before (hypothyroid) and after treatment with L-thyroxine (T$_4$-substituted).

Paired comparisons P-values	Patients with Graves' disease			Healthy subjects			Patients with chronic autoimmune hypothyroidism		
	Hyperthyroid	Euthyroid	Hyperthyr. vs euthyr. (P-values)	Placebo	Experimental hyperthyroidism	Placebo vs exp. hypert. (P-values)	Hypothyroid	L-thyroxine substituted	Hypothyr. vs T$_4$ subst. (P-values)
Total T$_3$ (1.1–2.6 nmol/l)	6.89±0.77	1.76±0.15	0.0004	1.29±0.07	3.3±0.2	<0.0001	0.88 (<0.616–1.7)	1.6±0.1	0.04
Total T$_4$ (58–161 nmol/l)	235±13	84±8	<0.0001	76.6±5.2	75.8±4.6	0.2	16.1 (12.9–32.5)	100±9	0.04
Free T$_3$ (3.7–9.5 pmol/l)	40±4	9.0±0.9	0.0006	6.25±0.25	14.9±0.9	<0.0001	1.8 (1.0–3.6)	5.3±1.0	0.04
Free T$_4$ (12–33 pmol/l)	139±13	28±2	0.0001	22±1	23.0±1.7	0.3	7 (3–9)	25.8±7.8	0.04
TSH (μmol/ml)	<0.002	0.0019 (<0.002–0.07)	0.07	1.5 (0.02–2.58)	0.031±0.004	0.003	75 (51.8–>75)	1.9±2.1	0.04

Data are given as means±S.D., except for TSH and thyroid hormones in hypothyroid patients that do not exhibit normal distribution and are listed as medians and ranges. For thyroid hormones, normal reference values for our laboratory are given in parentheses. Unpaired comparisons between Graves' disease patients and healthy control subjects who were administered placebo: hyperthyroid patients had significantly higher levels of thyroid hormones than healthy control subjects (P < 0.001), while comparisons of thyroid hormone levels between euthyroid patients and healthy control subjects were not significant.

Unpaired comparisons between patients with chronic autoimmune thyroiditis and healthy control subjects who were administered placebo: hypothyroid patients had significantly lower levels of thyroid hormones than healthy control subjects (P < 0.01), while comparisons between T$_4$-substituted patients with chronic autoimmune thyroiditis and healthy control persons were not significant.

The hypothyroid patients had considerably elevated TSH levels and were clinically hypothyroid.

In all hyperthyroid patients, MBL levels were increased 228–915% (median 383%), compared with after treatment (P = 0.01), as shown in Fig. 1. Median (range) levels of MBL were 1886 ng/ml (1478–7344) before treatment and decreased to 954 ng/ml (312–3222) after treatment when the patients were euthyroid. The healthy subjects had MBL levels at a median of 1081 ng/ml (312–1578), significantly lower than hyperthyroid patients (P = 0.01) and not significantly different from euthyroid patients after treatment. MBL levels were thus normalized after treatment. Administration of thyroid hormones to the healthy persons for 6 days, to induce a slight, experimental hyperthyroidism, increased MBL levels in all subjects to a median of 1714 ng/ml (356–2488) (P = 0.01). The relative increase in MBL levels was 142%±30% (mean±S.D.).

Two of the eight hypothyroid patients had undetectably low levels of MBL, both before and after T$_4$ substitution. The other six hypothyroid patients had decreased levels of MBL, median (range) 301 ng/ml (20–1889) compared with after T$_4$ substitution: 1390 ng/ml (214–2287); P = 0.028 (Fig. 2). The relative increase in MBL levels after T$_4$ substitution was 21–189%, median 34%. MBL levels were normalized after T$_4$ substitution, the difference between T$_4$-substituted patients and healthy controls being nonsignificant.

For all the participating subjects, the relative change in MBL levels in percentage of the particular person's euthyroid level correlated significantly with the simultaneously occurring change in thyroid hormone levels (Fig. 2).
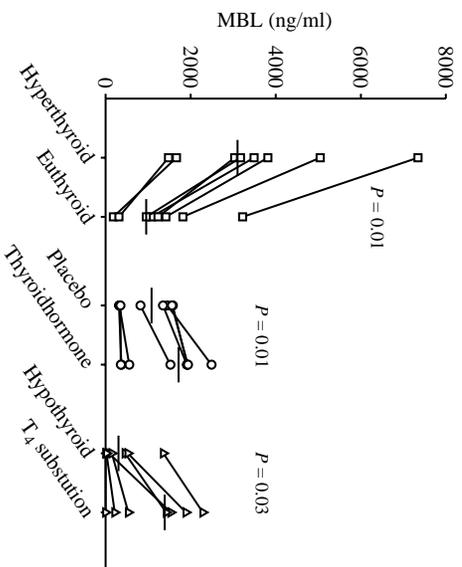

Figure 1 MBL levels in (□) hyperthyroid patients before and after treatment, (○) healthy controls before and after experimental thyroid hormone administration, (△) hypothyroid patients before and after L-thyroxine (T$_4$) substitution. The open symbols connected with a line represent the same individual before and after treatment. Horizontal lines represent medians. P-values represent paired comparisons.

MBL (ng/ml)

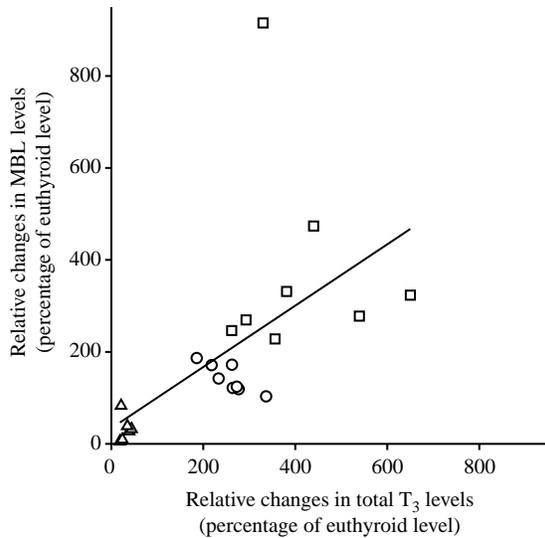*Thyroid hormone and mannan-binding lectin* 645

Figure 2 Relative changes in total T_3 levels (percentage of euthyroid level) vs relative changes in MBL levels (percentage of euthyroid level). □, Hyperthyroid patients before and after treatment; ○, healthy controls before and after experimental thyroid hormone administration; △, hypothyroid patients before and after LT_4 substitution. The two subjects with undetectably low levels of MBL both before and after treatment are not depicted. Results of the regression: Spearman's rho (correlation coefficient) = 0.731; $P = 0.0001$.

Changes in MASP-2 levels (Table 2), although less dramatic, mirrored the observed changes in MBL with increased levels in hyperthyroidism as compared with after treatment, increased levels after thyroid hormone administration to healthy persons, and increased levels after T_4 substitution of hypothyroid patients. Levels of CRP were within the normal range in all study groups, excluding the possibility that intercurrent disease influenced the results. The levels of CRP, however, tended to follow the changes in MBL and MASP-2.

Discussion

The present study was undertaken to test whether thyroid hormone participates in the regulation of the MBL pathway of complement activation. Our results show that thyroid hormone increases circulating levels of MBL and MASP-2 in hyperthyroid, euthyroid and hypothyroid subjects. This novel finding adds to our understanding of the regulation of the activity of the MBL/MASP system, and the abnormalities of the system may influence morbidity and mortality from cardiovascular disease in hyper- and hypothyroidism.

We observed a minor but highly significant increase in MBL, when small amounts of thyroid hormone were given to normal subjects, a 3.4-fold increase in MBL, when hypothyroid patients were treated with T_4 and a 3.8-fold decrease after treatment of the hyperthyroid state in patients with Graves' disease. Although not of

Table 2 MBL-associated serine proteases (MASP)-2 C-reactive protein (CRP) in eight patients with Graves' disease before (hyperthyroid) and after (euthyroid) treatment with methimazole, eight healthy subjects studied once after placebo tablet administration (placebo) and once after 6 days' administration of thyroid hormones (experimental hyperthyroidism, exp. hypert.) (see text), and eight hypothyroid patients with chronic autoimmune thyroiditis before (hypothyroid) and after treatment with L-thyroxine (T_4-substituted). Data are given as means±S.D.

Paired comparisons P-values	Patients with Graves' disease			Healthy subjects			Patients with chronic autoimmune hypothyroidism		
	Hyperthyroid	Euthyroid	Hyperthyr. vs euthyr. (P-values)	Placebo	Experimental hyperthyroidism	Placebo vs exp. hypert (P-values)	Hypothyroid	L-thyroxine substituted	Hypothyr. vs T$_4$ subst. (P-values)
MASP-2 (ng/ml)	252±69	191±57	0.002	252±134	280±146	0.02	384±100	465±139	0.1
CRP (nmol/l)	17.8±17.3	10.1±2.6	0.2	9.5±8.1	13.4±9.9	0.12	15.9±13.5	23.7±17.4	0.06

the same magnitude, circulating levels of MASP-2 showed the same pattern.

The basal concentration of MBL in human plasma is genetically determined. Because of the high frequency of three mutant MBL alleles, as well as mutations in the promoter region of the gene, very large inter-individual differences in MBL concentrations exist and the presence of MBL deficiency (i.e. below 100 ng/ml) among 10% of the population makes it the most frequent immunodeficiency described (5). This makes comparison between groups difficult and in general means that large numbers of subjects need to be studied. The paired design of the present study eliminates inter-individual variation and therefore allows paired comparisons in three small groups of subjects with different levels of thyroid hormone. The fact that thyroid hormone level greatly influences MBL levels, suggests that thyroid hormone regulates the MBL levels within subjects, and implies that in future studies of MBL thyroid hormone levels should be stated.

Our finding that thyroid hormone regulates the MBL system could relate to a generalized hepatic or systemic inflammatory response. In parallel to MASP-2 and MBL, the levels of the acute phase reactant and inflammatory marker CRP tended to be increased by thyroid hormone, although this did not reach statistical significance. In other studies, CRP are reported to be increased in hypothyroidism (27, 28), whereas others have found no evidence of elevated CRP levels in hyperthyroidism (29, 30). Both Graves' disease and chronic autoimmune hypothyroidism are autoimmune diseases that share many immunologic features, including high serum concentrations of antibodies against thyroglobulin, thyroid peroxidase and possibly the sodium–iodide cotransporter in thyroid tissue (31). Graves' disease hyperthyroidism is caused by stimulating IgG antibodies against the thyrotropin receptor, which bind to and activate the thyrotropin receptor on thyroid cells, stimulating thyroid hormone secretion (32). In hyperthyroidism due to Graves' disease or multinodular goiter, plasma levels of cytokines, especially interleukin-6, are elevated (33–35) and fall to normal levels after treatment, indicating that the aberrations result from the chronic effects of excess thyroid hormone rather than the autoimmune inflammatory condition in Graves' disease. On the other hand, a recent study shows that adipose tissue secretion of interleukin-6 is elevated in Graves' disease (36) even after restoration of euthyroidism. Some authors have found elevated cytokine concentrations in autoimmune hypothyroidism (37, 38), as opposed to our finding of decreased MBL levels. In parallel to our findings, it has been shown that serum sIL-2R levels are positively modulated by thyroid hormone, mostly independently of thyroid autoimmunity (39–41). A complex picture emerges, with cytokine abnormalities to some extent related to the auto-immune process rather than thyroid function. In this background, our data suggest that the MBL system plays a distinct role in thyroid disease with decreased levels in hypothyroidism and increased levels in hyperthyroidism. These results suggest that the changes in MBL concentration are mainly caused by thyroid hormone levels per se, rather than autoimmunity. This suggestion is further supported by the fact that small doses of thyroid hormone given to normal volunteers increase both MBL and MASP-2.

The mechanisms whereby thyroid hormone increases blood concentrations of MBL are uncertain. Changes in creatinine clearance and the alterations in the degradation rate of MBL are not likely to explain our findings. Clearance of creatinine, insulin and other proteins is increased in hyperthyroidism and decreased in hypothyroidism (42, 43). MBL is internally degraded and the half-life of circulating MBL is about 3 days (44). Presumably, increased levels of thyroid hormone activate hepatic MBL synthesis via transcriptional regulation of target genes (45). *In vitro* studies show that stimulating cultured hepatocytes with T_3 increases the synthesis of MBL by four- to eightfold (46). It has been shown that growth hormone (GH) increases and intensive insulin therapy decreases MBL concentrations (6, 13–15), so it is possible that increased GH levels contribute to the increased MBL levels in hyperthyroidism. The changes in MBL levels with changes in thyroid hormone status, observed in the present study, are greater than those previously described under the influence of GH, estrogen and insulin (13–15).

Our findings identify a new peripheral effect of thyroid hormone and demonstrate another link between the endocrine and the immune systems, of which the physiologic and pathologic implications remain to be clarified. Whether the finding that two out of eight hypothyroid patients have MBL deficiency, as compared with none of the healthy controls or patients with Graves' disease, is due to a genetically determined coexistence of autoimmune hypothyroidism and MBL deficiency needs further study.

In conclusion, we clearly show that thyroid hormone increases levels of MBL in hyperthyroid, euthyroid and hypothyroid subjects. In this way, thyroid hormone may modulate complement activation, and immunologic abnormalities may play a role in the pathogenesis of a number of key components of thyroid diseases.

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