Plasma Total Homocysteine Levels in Hyperthyroid and Hypothyroid Patients

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We found a higher plasma concentration of total homocysteine (tHcy), an independent risk factor for cardiovascular disease, in patients with hypothyroidism (mean, 16.3 μmol/L; 95% confidence interval [CI], 14.7 to 17.9 μmol/L) than in healthy controls (mean, 10.5 μmol/L; 95% CI, 10.1 to 10.9 μmol/L). The tHcy level of hyperthyroid patients did not differ significantly from that of the controls. Serum creatinine was higher in hypothyroid patients and lower in hyperthyroid patients than in controls, whereas serum folate was higher in hyperthyroid patients compared with the two other groups. In multivariate analysis, these differences did not explain the higher tHcy concentration in hypothyroidism. We confirmed the observation of elevated serum cholesterol in hypothyroidism, which together with the hyperhomocysteinemia may contribute to an accelerated atherogenesis in these patients.

HYPOTHYROIDISM is associated with an increased risk for cardiovascular disease, which is in accordance with autopsy studies showing that the atherosclerotic process is increased in hypothyroidism[1] and decreased in hyperthyroidism.[2]

The increased cardiovascular morbidity in hypothyroid patients has been related to elevated low-density lipoprotein cholesterol.[3-6] Treatment with thyroid hormones normalizes the blood lipid profile.[7] However, lipid abnormalities in hypothyroid patients do not fully account for the accelerated atherogenesis, and other pathogenic factors may be involved.[8-10]

The plasma concentration of total homocysteine (tHcy) is another independent risk factor for cardiovascular disease.[11] The plasma tHcy level is partly a genetic trait, but several acquired factors like folate and cobalamin deficiency and renal function also determine it.[12] Its relation to vitamin deficiencies is explained by the requirement for folate and cobalamin in the methionine synthase reaction that remethylates homocysteine to methionine.[13]

The present study was undertaken to investigate whether plasma tHcy levels are related to thyroid function. We therefore studied patients with hypothyroidism and hyperthyroidism and compared their tHcy levels against those in a healthy control population.

SUBJECTS AND METHODS

Patients

Patients with hyperthyroidism or hypothyroidism were included in the study, and the diagnoses were based on basal plasma thyrotropin (TSH) values less than 0.3 mIU/L and greater than 15 mIU/L, respectively. Diagnosis of borderline cases was ascertained by determination of total thyroxine (T_4), total triiodothyronine (T_3), or free T_4.

Sixty-four patients with hyperthyroidism and 45 with hypothyroidism were enrolled. One group contained patients seen in general practice ([GP] n = 53) by primary physicians, who sent blood samples to the hormone laboratory at Haukeland University Hospital in Bergen, Norway, in 1989 (n = 27) or the hormone laboratory at the University Hospital in Malmø, Sweden, from 1983 to 1989 (n = 26) for assessment of the thyroid status. The physicians received no information on the optimal procedure for sample collection and handling, and after hormone analyses, the samples were stored at −20°C until tHcy analysis.

Another group consisted of 56 consecutive outpatients of the endocrinological outpatient clinics at Haukeland University Hospital in Bergen, Norway, or the University Hospital in Malmø, Sweden, from 1991 to 1992. Fasting blood samples (10 mL in EDTA vacutainer tubes) were obtained by venipuncture and centrifuged within 30 minutes at 3,000 × *g* for 5 minutes. The plasma fraction was stored at −20°C until analysis.

The median age (62 years) of hypothyroid patients was 14 years higher than that of hyperthyroidism patients, with an overrepresentation of women in both groups. Six outpatients with hyperthyroidism received medication for other conditions, ie, diabetes, asthma, angina, nephritis, or cystic renal disease. Three outpatients with hypothyroidism received drugs for ischemic heart disease. For the GP patients, we did not have detailed information concerning other diseases or drug intake.

In the County of Hordaland, in which the city of Bergen is located, a large population-based study of tHcy has been conducted.[14,15] A subpopulation of 329 men and women aged 40 to 67 years were used as a control group in the present study.

tHcy and thyroid hormones were determined in all patients, whereas serum levels of folate, cobalamin, creatinine, and cholesterol were determined in patient samples with sufficient serum for analysis. In more than 93% of the control sera, folate, cobalamin and cholesterol were determined.

Tables 1 and 2 summarize demographic and laboratory features of the patients and controls.

Biochemical Methods

Plasma tHcy levels were determined by a modification[16] of an automated procedure developed for determination of tHcy in plasma. The method involves treatment of the plasma with sodium borohydride, which quantitatively reduces all Hcy species into the sulfhydryl form that is derivatized with monobromobimane, and finally quantified with high-performance liquid chromatography and fluorescence detection. The between-day precision (coefficient of variation) of the method is less than 3%.

Serum cobalamin was determined with a microparticle enzyme intrinsic factor assay on an IMx system from Abbott (Abbott Park, IL). Serum and blood folate were assayed using the Quantaphase folate radioassay from Bio-Rad (Hercules, CA). Cholesterol and creatinine were determined using the Technicon Chem 1 system (Technicon Instruments, Terrytown, NY).

Thyroid function parameters were assayed by routine methods of the laboratories.

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Table 1. Sex and Age Characteristics of the Patients and Controls

Characteristic	Hyperthyroidism	Hypothyroidism	Control
Total			
No.	64	45	329
Women (%)	78.1	68.9	51.4
Age (yr)			
Median	48	62	53
Range	19-89	35-85	40-67
Outpatients			
No.	43	13	
Women (%)	74.4	69.2	
Age (yr)			
Median	45	55	
Range	18-89	35-78	
GP patients			
No.	21	32	
Women (%)	85.7	68.7	
Age (yr)			
Median	56	66	
Range	24-73	43-85	

Statistical Analyses

Adjusted mean concentrations were estimated using analysis of covariance. Since the distribution of plasma tHcy was markedly skewed and the other biochemical variables showed a normal or only mildly skewed distribution, the analyses were performed with the logarithm of tHcy and with nontransformed values of the other variables. Thus, the geometric mean for tHcy and the arithmetic mean for the other variables are presented.

Univariate relations between plasma tHcy and the other variables are presented as Spearman rank correlations. To assess the simultaneous relation among the various predictors of tHcy in the combined group of patients and control subjects and to provide effect estimates adjusted for the other factors, multiple linear regression models were used. The analysis was initially performed with log-tHcy as the dependent variable, but was repeated with nontransformed values for tHcy. Since the coefficients for the various predictors of tHcy differed only slightly between these methods, the arithmetic values from these analyses are presented for simplicity. Biochemical parameters are represented as continuous variables in these models.

Hypothyroidism was a strong determinant of plasma tHcy and serum total cholesterol in linear regression. In the combined group of hypothyroid patients and control subjects, we therefore performed logistic regressions with hypothyroidism as the binary dependent variable, and plasma tHcy or total cholesterol was represented in the model as an indicator variable denoting membership in one of three groups defined according to tertile levels in the control subjects. Thus, the odds ratio for each of the two highest categories approximates the adjusted risk for hypothyroidism, relative to the lowest category.

The analyses were performed with the BMDP statistical package.[17] All tests were two-tailed, and P values less than .05 were considered significant.

RESULTS

tHcy and Other Blood Parameters

The age- and sex-adjusted mean levels for blood parameters are presented for the hyperthyroid and hypothyroid patients and control subjects in Table 2. Plasma tHcy levels were higher in patients with hypothyroidism than in patients with hyperthyroidism. However, only in patients with hypothyroidism were tHcy levels significantly different versus the control subjects.

tHcy levels were higher in GP patients with hyperthyroidism and hypothyroidism compared with the corresponding outpatients and the control subjects.

Serum folate was significantly higher in patients with hyperthyroidism compared with controls. In hypothyroid cases, serum folate values did not differ from values in the controls (Table 2). Both serum creatinine and cholesterol levels were higher in hypothyroid patients than in control subjects. The mean value for serum cobalamin was higher in GP patients compared with outpatients and controls, but showed no relation to thyroid status. The difference in cobalamin values between outpatients and GP patients was significant only in hypothyroid patients.

Table 2. Biochemical Parameters in Patients With Hyperthyroidism and Hypothyroidism and Control Subjects

Biochemical Parameter	Patient Group	Hyperthyroidism			Hypothyroidism			Control Subjects		
		No.	Mean	95% CI	No.	Mean	95% CI	No.	Mean	95% CI
Plasma tHcy (μmol/L)	Total	64	9.6	8.8-10.4	45	16.3	14.7-17.9	329	10.5	10.1-10.9
	Outpatients	43	8.1	7.4-8.8	13	11.7	10.0-13.7			
	GP patients	21	11.7	10.2-13.4	32	18.6	16.6-20.9			
Serum folate (nmol/L)	Total	49	16.5	14.7-18.1	33	12.3	10.4-14.2	323	11.1	10.6-11.7
	Outpatients	41	17.4	15.3-19.4	12	12.1	8.3-15.9			
	GP patients	8	13.1	9.4-16.8	21	12.5	10.2-14.9			
Serum cobalamin (pmol/L)	Total	51	426	374-478	34	535	474-596	326	376	356-395
	Outpatients	43	393	349-437	13	381	298-463			
	GP patients	8	541	373-669	21	631	551-712			
Serum creatinine (μmol/L)	Total	49	70.3	66.8-73.8	19	111	106-117	307	89.8	88.5-91.2
	Outpatients	41	65.5	60.8-70.2	12	110	101-119			
	GP patients	8	74.6	68.5-82.5	7	109	100-117			
Serum total cholesterol (mmol/L)	Total	52	4.6	4.2-5.0	39	8.3	7.9-8.8	329	6.2	6.0-6.3
	Outpatients	42	4.6	4.1-5.0	12	9.4	8.6-10.2			
	GP patients	10	4.4	3.8-5.3	27	7.8	7.2-8.3			
Serum total T_4 (nmol/L)	Total	64	224	212-236	39	43.3	28.5-58.0			
	Outpatients	43	231	216-246	13	37.4	19.4-65.4			
	GP patients	21	212	196-228	26	43.8	29.8-57.8			

NOTE. The geometric mean is shown for plasma tHcy; the arithmetic mean is shown for other parameters. Data are adjusted for differences in sex and age between patients with hyperthyroidism and hypothyroidism and controls (if measured).

Table 3. Correlations Between Plasma tHcy and Other Parameters in Hypothyroid and Hyperthyroid Patients and Controls

Parameter	Hypothyroidism			Hyperthyroidism			Control		
	No. Assessable	r	P*	No. Assessable	r	P*	No. Assessable	r	P*
Sex†	45	−.13	.41	64	−.07	.58	329	.29	<.001
Age (yr)	45	.44	.003	64	.41	.001	329	.22	<.001
Serum folate	33	−.33	.06	49	−.37	.008	323	−.41	.008
Serum cobalamin	34	.10	.59	51	−.08	.58	326	−.27	<.001
Serum creatinine	17	.15	.54	49	.01	.94	307	.33	<.001
Serum cholesterol	38	−.06	.73	52	.18	.19	329	.08	.12
Serum total T_4	39	−.14	.40	64	−.05	.67	ND		
Serum total T_3‡	12	−.15	.63	41	.09	.59	ND		

Abbreviation: not determined.

*Spearman rank correlation.

†1:women, 2:men.

‡Values outside the range of the assay were excluded.

Simple Correlations Between Plasma tHcy and Other Blood Parameters

Serum folate was related to tHcy in hyperthyroid patients ($P = .008$) and in hypothyroid patients ($P = .06$). Cobalamin and creatinine correlated with tHcy in the controls, but not in the patient groups (Table 3).

No significant correlations were observed between tHcy and total T_4 or total T_3 in the separate patient groups (Table 3). Of 45 patients with hypothyroidism, 29 had TSH values higher than the upper limit of the assay, and 13 (of 18 analyzed) had free T_4 values less than detection limits. All patients with hyperthyroidism had TSH levels less than the detection limit. Due to a low number of exact estimations of TSH and free T_4 levels, correlations between these hormones and tHcy were not evaluated.

Multivariate Analysis

Hypothyroidism, but not hyperthyroidism, was significantly related to plasma tHcy. The relation persisted after adjustment for serum folate and serum cobalamin (Table 4), and further adjustment for serum creatinine also did not change the relation (data not shown).

Because hypothyroidism was a strong determinant of plasma tHcy, we determined the risk, compared with the controls, for hypothyroidism according to plasma tHcy and serum cholesterol. For the highest tertile versus the lowest, the odds ratio was 14.9 for tHcy and 3.87 for cholesterol. The predictive effect of tHcy or cholesterol was not weakened by additional adjustment for folate, cobalamin, and creatinine serum levels (data not shown).

DISCUSSION

In the present study, we found that the geometric mean tHcy was 5.8 μmol/L higher in patients with hypothyroidism compared with a control population. The mean level of plasma tHcy in subjects with hyperthyroidism was lower than in the controls, but this difference was not significant. The increase in tHcy observed in hypothyroid patients may contribute to a high cardiovascular risk, since a recent study indicates that an increase of plasma tHcy of 4 μmol/L confers a 40% increase in the risk for coronary heart disease compared with healthy controls.[11,14]

In both outpatients and GP patients, hypothyroid patients had higher tHcy than hyperthyroid patients (Table 2). However, tHcy levels in both patient groups were higher in GP patients than in outpatients. This difference was more pronounced in hypothyroid (6.9 μmol/L) than in hyperthyroid (3.6 μmol/L) patients, and therefore cannot be fully explained by inappropri-

Table 4. Predictors of Plasma tHcy in Multivariate Analysis

Predictor	Model I			Model II		
	β Coefficient	SE	P	β Coefficient	SE	P
Hypothyroid patients and controls		(n = 356)			(n = 355)	
Intercept	−3.57			0.49		
Sex (women *v* men)	−0.94	0.62	.13	−0.73	0.54	.18
Age (yr)	0.11	0.036	<.005	0.11	0.031	<.005
Hypothyroidism	11.0	1.19	<.005	11.4	1.27	<.005
Serum folate (nmol/L)				−0.23	0.052	<.005
Serum cobalamin (pmol/L)				−0.0046	0.0015	<.005
Hyperthyroid patients and controls		(n = 394)			(n = 372)	
Intercept	5.94			9.28		
Sex (women *v* men)	−1.37	0.043	<.005	−1.08	0.37	<.005
Age (yr)	0.067	0.021	<.005	0.075	0.019	<.005
Hyperthyroidism	0.47	0.92	.61	1.21	1.28	.35
Serum folate (nmol/L)				−0.20	0.036	<.005
Serum cobalamin (pmol/L)				−0.0037	0.0012	<.005

ate blood sampling in GP patients leading to Hcy export from blood cells.[16] Differences in disease severity or metabolic status in GP patients versus outpatients are not supported by biochemical indices (Table 3), but the duration of disease is probably longer in GP patients. This possibility is supported by the higher age of GP patients (Table 1), but also by different indications for determining the thyroid hormone status in a hospital versus a GP's office.

In line with previous studies,[4-6] we found that serum cholesterol was higher in patients with hypothyroidism and lower in patients with hyperthyroidism than in a population-based control group (Table 2).

We observed that patients with hyperthyroidism had higher serum folate than controls and patients with hypothyroidism. Similar observations have been made in some[18] but not all[19] studies. Furthermore, the documented inverse relation between tHcy and serum folate[20] was confirmed in controls and was found in both hyperthyroid and hypothyroid patients, demonstrating that folate status is a determinant of tHcy in both groups of patients.

There are numerous reports on experimental studies showing that T_4 and hyperthyroidism affect folate metabolism and the enzymes involved. The observations that methylenetetrahydrofolate reductase is increased in hyperthyroidism and decreased in hypothyroidism[21] may be relevant for the relation between the tHcy level and thyroid status. This enzyme is responsible for the formation of 5-methyltetrahydrofolate, which functions as methyl donor during remethylation of homocysteine to methionine.[13] During endemic goiter in humans, plasma tHcy increases, whereas most other amino acids except for methionine decrease.[22] The investigators suggest an impairment of cystathionine β-synthase function as a possible explanation.

The serum cobalamin level did not differ in outpatients with hyperthyroidism or hypothyroidism versus the controls. However, the levels in GP patients with hypothyroidism were higher than in the controls. We do not have any explanation for this observation. Results from previous studies of hyperthyroid and hypothyroid patients suggest that the abnormalities of thyroid function per se do not alter serum cobalamin levels.[19]

Compared with the controls, serum creatinine was higher in hypothyroid patients and lower in hyperthyroid patients. Such relations between serum creatinine and thyroid function have been reported by others. In hyperthyroidism, serum creatinine is often reduced and the glomerular filtration rate (GFR) is increased,[23] whereas GFR decreases and serum creatinine increases during hypothyroidism.[24] There are consistent reports on a positive relation between tHcy and serum creatinine concentrations.[25] This may partly be explained by Hcy production in conjunction with creatine-creatinine synthesis, which is related to muscle mass.[26] tHcy shows a negative relation to the GFR, and patients with renal failure have hyperhomocysteinemia attributed to low tHcy clearance, possibly due to impaired renal Hcy metabolism.[25,27-29] These data point to the possibility that thyroid function may modulate Hcy metabolism by affecting creatine-creatinine synthesis and/or Hcy metabolism in the kidneys. Thus, thyroid hormones may influence the tHcy plasma level both through effects on Hcy formation and its elimination from plasma.

An influence of thyroid status on the tHcy level was still observed in multivariate models including serum levels of cobalamin, folate, and creatinine. This suggests that changes in these parameters do not fully account for the high tHcy observed in hypothyroid patients.

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