variation in long-term studies of serum constituents in normal subjects. III. Physiological and medical implications. Clin Chem 1970;16:1028–32.

9. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:307–10.

Plasma Total Homocysteine in Hyper- and Hypothyroid Patients before and during 12 Months of Treatment, *Bjørn G. Nedrebø,* Ottar Nygård, Per M. Ueland, and Ernst A. Lien* (LOCUS for Homocysteine and Related Vitamins, University of Bergen, 5021 Bergen, Norway; * address correspondence to this author at: Department of Internal Medicine, Haukeland University Hospital, N-5021 Bergen, Norway; fax 47-55975814, e-mail bjoern.gunnar.nedreboe@haukeland.no)

There are consistent reports demonstrating that thyroid status is an important determinant of the plasma/serum concentration of total homocysteine (tHcy) *(1–7)*, which has been established as an independent risk factor of vascular occlusive disease *(8)*. In previous studies, we showed that plasma tHcy was higher in hypothyroid than in hyperthyroid patients *(1, 2)*. Prospective studies have demonstrated normalization of tHcy after thyroid hormone replacement therapy in hypothyroid patients *(3, 4)*, and we recently observed a transient increase in tHcy during short-term iatrogenic hypothyroidism in thyroidectomized patients *(5)*.

To our knowledge, there has been no longitudinal study on tHcy in hyper- and hypothyroid patients across normalization of their thyroid status during treatment. In the present study, we investigated such patients before and during the first 12 months of treatment.

Forty consecutive patients with hyperthyroidism and 12 with hypothyroidism were enrolled. The study protocol was approved by the ethics committee of Haukeland University Hospital, and informed consent was obtained from all participants.

The diagnosis of hyperthyroidism was based on basal serum thyrotropin (TSH) values <0.3 mIU/L, and hypothyroidism on TSH >15 mIU/L. All hyperthyroid patients except one were diagnosed with Graves disease.

Thirty-one of the patients with hyperthyroidism were treated with carbimazole or propylthiouracil. Nine of the patients with hyperthyroidism received radioiodine treatment as primary treatment. All patients with hypothyroidism received thyroxine as the only thyroid hormone replacement therapy.

Blood specimens were collected at inclusion and 1, 6, and 12 months after initiation of treatment. Fasting EDTA-blood samples for tHcy determination were centrifuged within 30 min. The EDTA-plasma was stored at −20 °C until analysis. Plasma tHcy, serum cobalamin, folate, creatinine, and cholesterol were determined according to established methods *(5, 9)*. TSH and free thyroxine (FT_4) in serum were measured with the AutoDELFIA hTSH Ultra reagent set and the AutoDELFIA FT_4 reagent set from Wallac Oy. Thyroid receptor antibodies (TRAbs) were determined by radioimmunoassay from DLD Diagnostika GMBH.

Baseline characteristics of the hyper- and hypothyroid patient groups were compared by Mann–Whitney *U*-test. To investigate changes in analyte concentrations within each group, we applied analyses of covariance using an unbalanced repeated-measure design (5V module in BMDP) *(10)*. The covariance between any pairs of repeated measurements was assumed to be equal (compound symmetry). In case of missing data in the response variable (unbalanced data), the program computes and substitutes for the missing value an imputed value that is predicted from the covariate information. In the present study, missing values in the response variables varied from 0% at baseline up to 32% for tHcy in hyperthyroid patients at 12 months. The maximum likelihood of regression and covariance parameters were calculated.

The change in parameters over time was represented by a linear trend model. The time trend was coded as 0, 1, 2, and 3. Thus, the regression coefficients for time trend represent the change relative to the previous visit and are not a function of the length of follow-up. The intercept in this model represents the estimated concentration before treatment, which is close to the mean concentration at baseline. Analyses or graphic presentations based on log-transformed data, percentage of change from baseline, median values, or individual raw data gave similar results and are not presented. Essentially the same results were obtained when the analyses were restricted to patients with complete data sets (25 hyperthyroid, 9 hypothyroid). We also investigated the change in tHcy by treatment after adjustment for sex, age (years), and one additional covariate.

For patients with hyperthyroidism (n = 40) and hypothyroidism (n = 12), the median ages were, respectively, 44 years (range, 19–89 years) and 55 years (range, 35–78 years). In both groups, 75% of the patients were women. In patients with hyperthyroidism, median FT_4 was 57.4 pmol/L (24.2 to >77 pmol/L), and except for one patient with a TSH value of 0.1 mIU/L, all remaining patients had TSH <0.05 mIU/L. In hypothyroid patients, median FT_4 was 5.4 pmol/L (<5 to 8.6 pmol/L). Three hypothyroid patients had TSH values of 21.0, 28.2, and 33.5 mIU/L, respectively; all remaining patients had TSH >50 mIU/L.

At baseline, tHcy in plasma and folate, total cholesterol, HDL-cholesterol, and creatinine in serum were significantly higher in patients with hypothyroidism than in patients with hyperthyroidism, whereas cobalamin and triglycerides did not differ between the patient groups (data not shown).

In hyperthyroid patients, tHcy, creatinine, and cholesterol increased during treatment. Serum folate decreased significantly, whereas there was no significant change in cobalamin (Fig. 1 and Table 1). In hypothyroid patients, significant decreases were observed for tHcy, creatinine, and cholesterol. There were no significant changes in cobalamin and folate (Fig. 1 and Table 1). After 12 months

of treatment, plasma tHcy in the two patient groups approached the same concentrations (Fig. 1).

We determined the time-dependent changes in plasma tHcy in hyper- and hypothyroid patients with and without adjustment for one potential covariate. Inclusion of cholesterol or, to a lesser degree, creatinine in the model almost abolished the change in tHcy. This demonstrates that the changes in tHcy were strongly correlated to cholesterol and creatinine (Table 1).

This study confirms published data that tHcy is increased in hypothyroidism *(1–7)* and tends to be low in hyperthyroidism *(2, 7)*. In addition, it provides data on the time course for the normalization of tHcy and covariates such as serum cholesterol and creatinine during 12 months of treatment of patients with hypo- and hyperthyroidism, demonstrating that these blood indices gradually approached the same values (Fig. 1).

The strength of this study is the longitudinal, prospective design. Furthermore, because of the large number of hyperthyroid patients included (n = 40), the study allowed assessment of the modest increase in tHcy (median increase, 2.0 μmol/L) that occurred during treatment of these patients. The marked decrease in tHcy in hypothyroid subjects during treatment (median decrease, 5.6 μmol/L) reached significance (Table 1) in spite of the low number of subjects (n = 12).

During treatment of both patient groups, plasma tHcy showed a strong covariation with serum cholesterol and creatinine, whereas there was essentially no relation to serum folate and cobalamin (Table 1). From these observations, one may infer that changes in renal function rather than vitamin status may account for variations in plasma tHcy. This relationship may be attributable to effects of thyroid hormones on renal hemodynamics. Both animal and human studies have demonstrated that hypothyroidism is associated with low and hyperthyroidism with high glomerular filtration rate *(11, 12)*, which in turn is closely related to plasma tHcy *(13)*. Renal homocysteine excretion is negligible *(14)*, but homocysteine metabolism

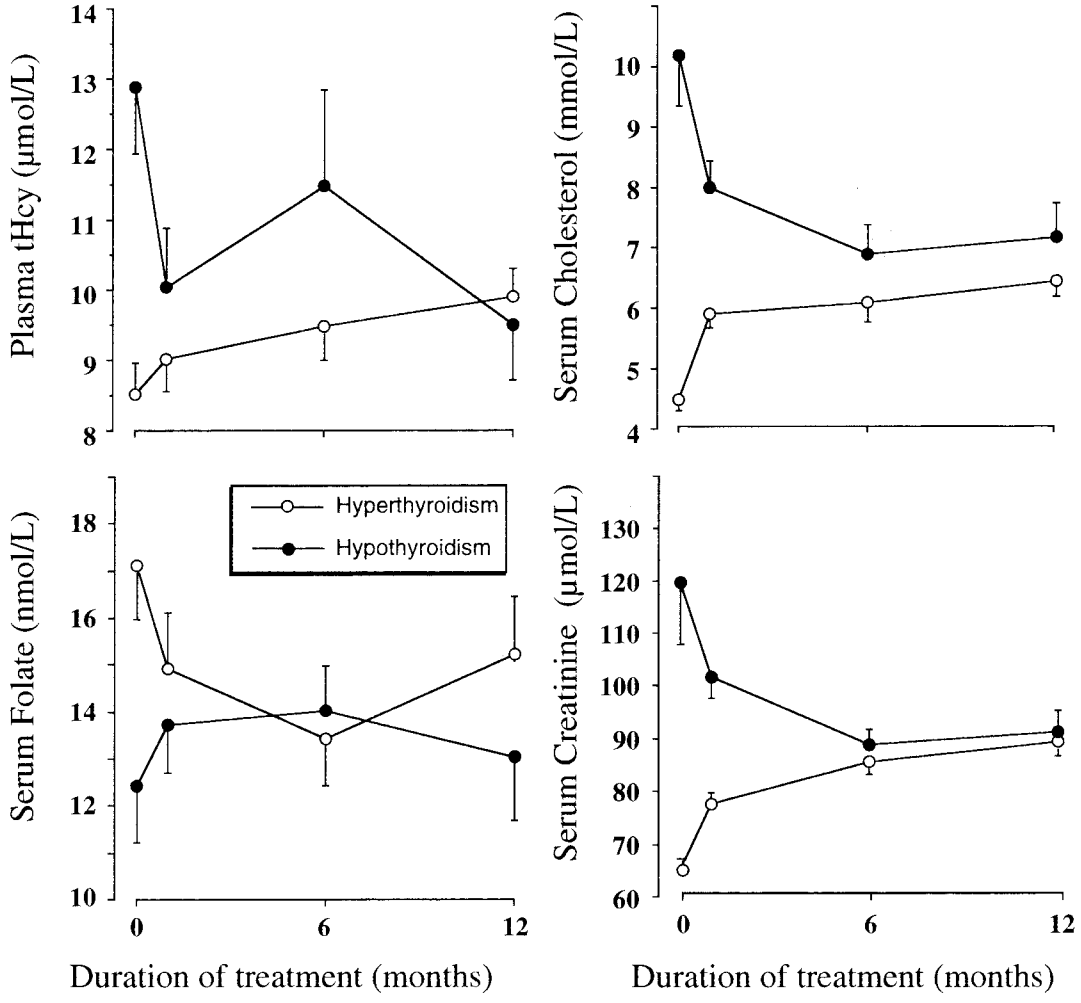

Fig. 1. Changes in plasma tHcy, serum folate, serum creatinine, and serum cholesterol before and during 12 months of treatment in hypo- and hyperthyroid patients.

Data are means and SE (*error bars*).

Table 1. Changes in parameters during treatment of patients with hyper- or hypothyroidism.[a]

	Hyperthyroidism (n = 40)			Hypothyroidism (n = 12)		
Variables	**Intercept**	**Regression coefficient**	**P^b**	**Intercept**	**Regression coefficient**	**P^b**
Unadjusted values						
Plasma tHcy, μmol/L	8.4	0.41	<0.001	12.2	−0.77	0.04
Serum folate, nmol/L	16.1	−0.82	0.04	12.6	0.29	0.49
Serum cobalamin, pmol/L	402	−8.09	0.18	383	−7.85	0.38
Serum creatinine, μmol/L	70.0	8.81	<0.001	114	−10.3	<0.001
Serum cholesterol, mmol/L	4.7	0.66	<0.001	9.5	−1.07	<0.001
Plasma tHcy values (μmol/L) adjusted for[c]						
Serum folate	9.4	0.39	0.002	14.3	−0.76	0.03
Serum cobalamin	10.3	0.36	0.003	13.3	−0.88	0.01
Serum creatinine	6.6	0.22	0.22	7.1	−0.42	0.28
Serum cholesterol	6.0	0.11	0.44	8.0	−0.37	0.36

[a] The parameters were determined before treatment and after 1, 6, and 12 months of follow-up. The intercept is an estimate of the concentration before treatment, and the coefficient is the estimated change between consecutive measurements.

[b] Test for linear trend.

[c] Adjustment for only one covariate.

in the kidneys may play a major role in homocysteine clearance *(15, 16)*.

In the present work we confirmed reports on high serum folate concentrations in hyperthyroidism and low concentrations in hypothyroidism *(2, 17)*. However, there was no covariation between folate and tHcy during treatment (Table 1), which supports our previous notion *(5)* that altered folate status may not account for changes in tHcy related to thyroid status.

We observed high serum cholesterol in hypothyroid and low concentrations in hyperthyroid subjects (Table 1), which is in agreement with previous published data *(18, 19)*. The changes in cholesterol showed a time course similar to that of tHcy (Fig. 1 and Table 1). A significant correlation between serum cholesterol and tHcy has also been demonstrated in some epidemiologic studies *(20–22)*. The mechanism behind this covariation has not been clarified *(23)*, but increases in both cholesterol and tHcy in hypothyroidism may have an interactive effect *(24)*, which may contribute to the high prevalence of arterial occlusive disease in hypothyroid patients *(25)*. Conversely, low concentrations of both factors in hyperthyroid patients may afford protection *(26)*.

In conclusion, tHcy was increased in hypothyroidism and was decreased in hyperthyroidism. During treatment, tHcy gradually approached the same concentration in these patient groups. Parallel changes in serum creatinine, but not serum folate may suggest a renal mechanism behind the tHcy response. A strong covariation between tHcy and serum cholesterol has important medical implications: (*a*) increases in both tHcy and cholesterol in hypothyroidism may increase cardiovascular risk; and (*b*) TSH should be determined in subjects with unexplained hyperhomocysteinemia and increased cholesterol, as recently suggested by Hussein et al. *(3)*.

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References

1. Nedrebø B, Ericsson U-B, Ueland PM, Refsum H, Lien EA. Plasma levels of the atherogenic amino acid homocysteine in hyper- and hypothyroid patients [Abstract]. Eur J Endocrinol 1994;130(S2):47.
2. Nedrebø BG, Ericsson U-B, Nygård O, Refsum H, Ueland PM, Aakvaag A, et al. Plasma total homocysteine levels in hyperthyroid and hypothyroid patients. Metabolism 1998;47:89–93.
3. Hussein WI, Green R, Jacobsen DW, Faiman C. Normalization of hyperhomocysteinemia with L-thyroxine in hypothyroidism. Ann Intern Med 1999; 131:348–51.
4. Catargi B, Parrot-Roulaud F, Cochet C, Ducassou D, Roger P, Tabarin A. Homocysteine, hypothyroidism, and effect of thyroid hormone replacement. Thyroid 1999;9:1163–6.
5. Lien EA, Nedrebø BG, Varhaug JE, Nygård O, Aakvaag A, Ueland PM. Plasma total homocysteine levels during short-term iatrogenic hypothyroidism. J Clin Endocrinol Metab 2000;85:1049–53.
6. Morris MS, Bostom AG, Jacques PF, Selhub J, Rosenberg IH. Hyperhomocysteinemia and hypercholesterolemia associated with hypothyroidism in the third US National Health and Nutrition Examination Survey. Atherosclerosis 2001;155:195–200.
7. Diekman MJM, van der Put NM, Blom HJ, Tijssen JGP, Wiersinga WM. Determinants of changes in plasma homocysteine in hyperthyroidism and hypothyroidism. Clin Endocrinol (Oxf) 2001;54:197–204.
8. Nygård O, Vollset SE, Refsum H, Brattström L, Ueland PM. Total homocysteine and cardiovascular disease [Review]. J Intern Med 1999;246:425–54.
9. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. Clin Chem 1993;39:263–71.
10. Dixon WJ. BMDP statistical software manual, Vol. 2. Berkeley: University of California Press, 1992:1311–52.
11. Capasso G, DeTommaso G, Pica A, Anastasio P, Capasso J, Kinne R, et al. Effects of thyroid hormones on heart and kidney functions. Miner Electrolyte Metab 1999;25:56–64.
12. Kreisman SH, Hennessey JV. Consistent reversible elevations of serum creatinine levels in severe hypothyroidism. Arch Intern Med 1999;159:79–82.
13. Wollesen F, Brattström L, Refsum H, Ueland PM, Berglund L, Berne C. Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. Kidney Int 1999;55:1028–35.
14. Refsum H, Helland S, Ueland PM. Radioenzymic determination of homocysteine in plasma and urine. Clin Chem 1985;31:624–8.
15. Bostom AG, Shemin D, Lapane KL, Miller JW, Sutherland P, Nadeau M, et al. Hyperhomocysteinemia and traditional cardiovascular disease risk factors in end-stage renal disease patients on dialysis: a case-control study. Atherosclerosis 1995;114:93–103.
16. Guttormsen AB, Ueland PM, Svarstad E, Refsum H. Kinetic basis of hyperhomocysteinemia in patients with chronic renal failure. Kidney Int 1997;52:495–502.
17. Ford HC, Carter JM, Rendle MA. Serum and red cell folate and serum vitamin B_{12} levels in hyperthyroidism. Am J Hematol 1989;31:233–6.
18. Mason RL, Hunt HM, Hurxthal L. Blood cholesterol values in hyperthyroidism

and hypothyroidism—their significance. N Engl J Med 1930;203: 1273–8.
19. Hoch F. Lipids and thyroid hormones [Review]. Prog Lipid Res 1988;27: 199–270.
20. Arnesen E, Refsum H, Bønaa KH, Ueland PM, Førde OH, Nordrehaug JE. Serum total homocysteine and coronary heart disease. Int J Epidemiol 1995;24:704–9.
21. Nygård O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, et al. Total plasma homocysteine and cardiovascular risk profile. The Hordaland homocysteine study. JAMA 1995;274:1526–33.
22. Mansoor MA, Bergmark C, Svardal AM, Lønning PE, Ueland PM. Redox status and protein binding of plasma homocysteine and other aminothiols in patients with early-onset peripheral vascular disease. Homocysteine and peripheral vascular disease. Arterioscler Thromb Vasc Biol 1995;15:232–40.
23. Karmin O, Lynn EG, Chung YH, Siow YL, Man RYK, Choy PC. Homocysteine stimulates the production and secretion of cholesterol in hepatic cells. Biochim Biophys Acta 1998;1393:317–24.
24. Graham IM, Daly LE, Refsum HM, Robinson K, Brattström LE, Ueland PM, et al. Plasma homocysteine as a risk factor for vascular disease. The European concerted action project. JAMA 1997;277:1775–81.
25. Fowler PB Ikram H, Banim SO. Serum-cholesterol, thyroid failure, and coronary-artery disease [Letter]. Lancet 1972;1:685.
26. Littman DS, Jeffers WA, Rose E. The infrequency of myocardial infarction in patients with thyrotoxicosis. Am J Med Sci 1957;233:10–5.