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HYPERHOMOCYSTEINEMIA IN MODERATE AND SEVERE HYPOTHYROIDISM

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Abstract

The **aim** of the study was to evaluate the prevalence of hyperhomocysteinemia in hypothyroid patients and the effect of folic acid supplementation when serum homocysteine (Hcy) was over risk level.

Patients and methods. Patients with moderate (Group1) and severe hypothyroidism (Group 2) were evaluated before any therapy and after 6 months of combined folic acid and levothyroxine substitution, versus control subjects. Hcy, folic acid, thyroid hormones and lipids were measured for all subjects. Thyroglobulin and antithyroglobulin antibodies were measured only for Group 2.

Results. Only 17 % of the cases had basal Hcy at non risk level (<10 mmol/L). Both groups had higher Hcy levels than control ($p < 0.0001$). In Group 1 basal folic acid was lower than in control and group 2 ($p<0.001$). No correlation was found between high levels of Hcy (> 12 mmol/L) and positive thyroglobulin. After 3 months of combined therapy, significant decrease of Hcy ($p<0.0001$) was observed compared with the basal level. Normalization of Hcy appears during next 3 months even with reducing the folic acid supplementation.

Conclusion. Our results report moderate hyperhomocysteinemia in hypothyroid patients. This may exacerbate the cardiovascular risk traditionally attributed to lipid changes. Six months of combined therapy (L-thyroxine and folic acid) corrected hyperhomocysteinemia excluding the additional risk.

Key words: hyperhomocysteinemia, folic acid, hypothyroidism, risk factor.

INTRODUCTION

In 1969, McCully made the clinical observation linking elevated plasma homocysteine (Hcy) concentrations with vascular disease (1). Subsequent investigations have confirmed McCully's hypothesis and it has recently become

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clear that hyperhomocysteinemia (HHcy) is an independent risk factor for atherosclerosis and atherothrombosis (3). An increased plasma Hcy level is also a strong, independent risk factor for the development of dementia and Alzheimer's disease (2). Mild HHcy (Hcy >10μmol/L) is an independent risk factor for atherosclerotic and atherothrombotic vascular diseases; 5-10 % of normal population and more than 40% of patients with cardiovascular diseases have HHcy. Additional risk factors and endocrine disorders (diabetes, hypothyroidism) may, additively or by interacting with HHcy, increase the overall risk.

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine (Fig. 1), being located in a key point in the methionine metabolism: between S–adenosylmethionone (the main methyl donor) and vitamins B12 and folate. So, plasma Hcy is a sensitive biomarker of folate or vitamin B12 deficiency.

HHcy is caused either by genetic defects in the enzymes involved in Hcy metabolism (methylenetetrahydrofolate reductase, MTHFR) or by nutritional deficiencies in vitamin cofactors (folate, vitamin B12 and vitamin B6). Epidemiologic studies revealed that the prevalence of HHcy is consistently higher in patients with cerebrovascular, peripheral or coronary artery disease than in those without such disease (3).

An elevated plasma homocysteine has been reported in some endocrine disorders: hypothyroidism, diabetes mellitus, hypoestrogenism (4), Cushing's syndrome (5). Elevated plasma homocysteine is associated with higher risk of cerebrovascular disease and lowering of homocysteine with folic acid and vitamins B6 and B12 did reduce the risk of overall stroke but not stroke severity or disability (6, 7). Deficiency of folate and vitamin B12 can lead to elevated concentrations of tHcy and disturbed methylation potential in the brain (8). Hyperhomocysteinemia

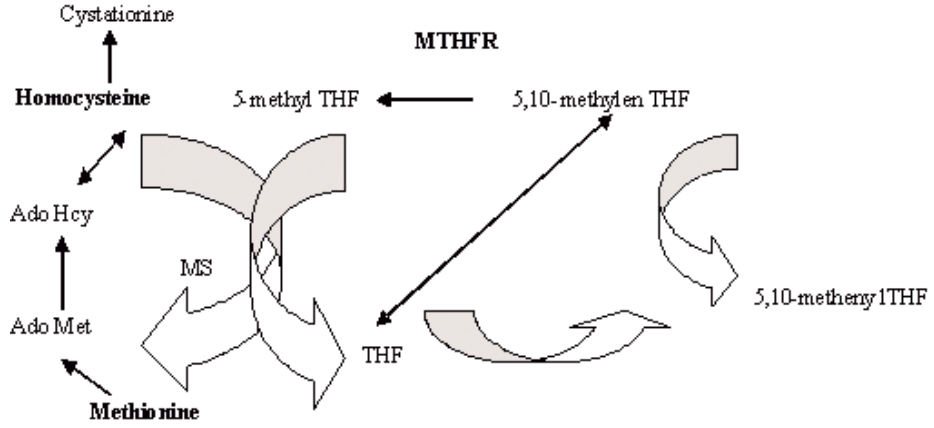

Figure 1. Homocysteine synthesis pathway.

can accelerate atherosclerosis when associated with elevated plasma lipids (9).

HHcy and dyslipidemia can occur in untreated hypothyroid patients, both of them being risk factors for atherosclerosis (10). This observation conducted us to evaluate the prevalence of HHcy and of borderline values (10-12 μmol/L) for subjects with moderate and severe hypothyroidism and also to evaluate the effect of folic acid supplementation in those patients with plasma Hcy at a risk.

PATIENTS AND METHODS

This was a prospective study for 2 groups of patients. The study protocol was approved by the local Ethical Commitee and informed consent was obtained from each patient.

Patients groups

Group 1: 36 patients with primary hypothyroidism before any therapy(t=0). Of them, only 18 patients were followed up to 6 months of therapy with Levothyroxine plus folic acid.

- inclusion criteria: patients with moderate hypothyroidism (TSH >10 μIU/ml) without substitution therapy.

- exclusion criteria: patients with primary hypothyroidism on levothyroxine.

Group 2: 18 patients with hypothyroidism after thyroidectomy for thyroid cancer. Six weeks before radioiodine therapy (t=0), they discontinued levotyroxine treatment, being in a severe hypothyroid status. Of them, only 15 subjects were followed up until 6 months of therapy with Levothyroxine plus folic acid.

- inclusion criteria: patients with severe hypothyroidism (TSH >50 μIU/ml) during discontinued levotyroxine substitution for 6 weeks

Table 1. Study groups

	n	Thyroid Status	Gender	Age	Including criteria	Therapy
Group 1	36	Moderate Hypo thyroidism	F=30 M=6	30-82	no prior therapy TSH >10 μIU/ml	n=18 Levo thyroxine: 75-150 μg/day Folic acid 0-3 month: 5 mg/day 3-6 month: 2.5 mg/day
Group 2	**18**	Severe Hypothyroidism	F=16 M=1	52-73	discontinued levotyroxine substitution during 6 weeks TSH >50 μIU/ml	n= 15 Levo thyroxine: 100-150 μg/day Folic acid 0-3 month : 5 mg/day 3-6 month: 2.5 mg/day
Control	32	euthyroid status	F=20 M=12	35-50	TSH =2.2 ±0.8	No

- exclusion criteria: patients with inappropriate TSH increase(< 30 μIU/ml) after discontinuing levotyroxine treatment

Control group: 32 spontaneously euthyroid subjects: subjects with normal TSH levels, without Levothyroxine therapy. Thyroid status, inclusion criteria and therapy design for each group are presented in Table 1.

Methods

For all the subjects before therapy and during follow-up as well as for the control group we used the following methods:

Total plasma Hcy was assayed by ELISA method (DRG-Germany) after reduction and enzymatic conversion steps. For reference values (11,12), see Table 2. Plasma samples were immediately separated after centrifugation (15 min at 3000 g) and stored at -20°C until assay.

Serum folic acid was assayed by chemiluminiscence using ACS -180, Bayer-Siemens automated system. Serum cholesterol, HDL-cholesterol, LDL-cholesterol and creatinine were assayed by colorimetry using Roche, HITACHI 912 analyzer. Serum hormones: TSH, T3, T4, were assayed by automated chemiluminiscence assay using ACS -180, Bayer- Siemens system. Thyroglobulin and antithyroglobulin antibodies were assayed by IRMA, RADIM Italia. Intima-media thickness (IMT) was measured for group 2 of subjects by a GE Ultrasound System (10 MHz transducer) in a sagittal and longitudinal view.

Statistics. When the continuous variables were normally distributed, we used parametric tests, while for non-Gaussian distributions we used non-parametric tests. Data are expressed as mean +/- SD for normally distributed variables, while for the others they are expressed as median and extremes. We used SPSS 16.0 for Windows, and Graph Pad InStat 3. A two sided $p< 0.05$ was considered significant.

RESULTS

We studied 2 groups of patients: group 1 with moderate (TSH: 35.71± 23.66 μUI/mL, TT3: 95.94 ± 33.53ng/dL, TT4: 5.85 ±3.21 μg/dL) and group 2 with severe iatrogenic hypothyroidism (TSH: 60.20± 9.3 μUI/mL, TT3: 20.54 ± 12.6 ng/dL, TT4: 1.85 ±1.21 μg/dL). The whole follow-up was of 6 months of therapy with levothyroxine and decreasing doses of folic acid.

Table 2. Reference ranges for total plasma homocysteine

Total plasma Hcy (μmol/L)	Status
< 10	safe / normal status
10-12	tolerated for normal subjects
12-30	moderate hyperhomocysteinemia
30-100	intermediate hyperhomocysteinemia
>100	severe hyperhomocysteinemia

Before any therapy: Group 1 and Group 2 versus control (euthyroid subjects)

Before starting any therapy, we compared each group with the control group (TSH: 2.2± 0.80 μUI/mL, TT3:140.54 ± 16.6 ng/dL, TT4: 8.8 ±2.20 μg/dL).

Basal levels of homocysteine, folic acid, creatinine and lipid panel for the study groups are presented in Table 3. Only 17 % of the patients from study groups had basal homocysteine at a non risk level value (Hcy < 10 μmol/L), 21% had borderline values (10-12 μmol/L), meanwhile 62 % had Hcy >12 μmol /L (Fig. 2). For both study groups, we found significant higher Hcy levels than for the control group ($p < 0.0001$). No significant difference was found between the groups (p: NS).

Table 3. Basal values of Hcy, folic acid, cholesterol, HDL- cholesterol, LDL cholesterol and creatinine in the study groups

	Hcy μmol/L	Folic Acid ng/mL	Cholesterol mg/dL	LDL cholesterol mg/dL	HDL cholesterol mg/dL	Creatinine mg/dL
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
G1 (n=36)	14.40±5.6	5.97 ± 2.70	250±69.5	156 ±52.8	52.3±14.47	0.86±0.25
G2 (n=18)	16,56±5.3	7.96±3.68	325,7±22.2	202.25±63.27	56.3±10.8	0.98±0.11
Control (n=32)	9.24±1.19	8.31 ±1.76	185±14.37	101.56 ±9.10	44±5.28	0.80±0.11

In Group 1, the basal level of serum folic acid (5.97 ±2.70 ng/mL) was significantly lower compared with control group ($p<0.001$) and compared with group 2 ($p=0.031$). It correlates with elevated plasma homocysteine values ($r =0.94$).

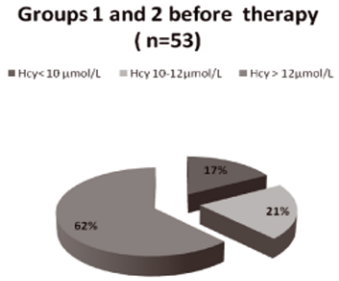

Figure 2. Basal homocysteine in study groups.

During 6 weeks of discontinued L -thyroxin substitution, patients with thyroid cancer (Group 2) are in a severe hypothyroid status (TSH >50μUI/mL) and a significant modified lipid profile versus control group. At the end of this period and before administration of radioiodine therapeutic dose, we found elevated Hcy levels (16.56 ± 3.93 μmol/L), significantly higher than for control group. Folic acid was at a normal concentration (7.96±3.68) that was significantly higher than in group 1 and not different from the control group (Fig. 3).

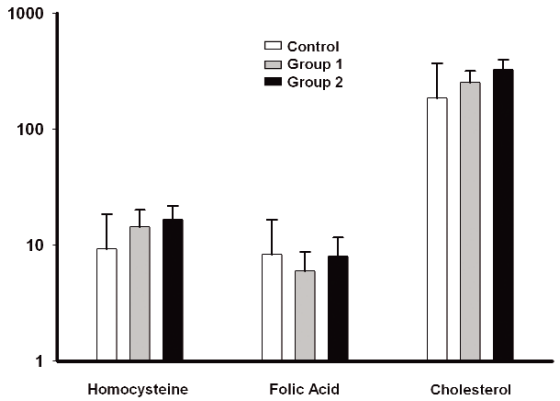

Figure 3. Folic acid concentration in the study groups.

We found higher values of cholesterol, LDL, triglycerides for subjects with severe hypothyroid status in group 2 than for those with moderate hypothyroidism ($p<0.001$). This correlates with higher concentrations of homocysteine for this group ($r=0.81$) (Fig. 3).

No correlation was found between high Hcy concentrations and positive thyroglobulin levels (>1 ng/ml) for patients with thyroid cancer (group 2).

Creatinine was within the normal range for both groups.

Results during the follow-up period

We evaluated the patients during suppression therapy at 3 and 6 months, adapting the doses of folic acid associated with levothyroxine and taking into account the normalization of Hcy plasma levels and serum levels of folate.

Group 1

Only 18 patients from the initial group 1 (n=36) were followed up and were evaluated during associated therapy: at 3 months (levothyroxine: 75-150 μg/day plus Folic acid, 5 mg/day) and respectively 6 months (levothyroxine:75-150 μg/day plus Folic acid, 2.5 mg/day). We adapted the doses of folic acid taking into account the normalization of Hcy plasma levels and serum levels of folate.

After 3 months of therapy a significant decrease of the basal Hcy levels was found ($p< 0.01$) but without a normalization of serum Hcy concentration. A normal homocysteine level (mean values =9.38 μmol/ L) was achieved only after 6 months of therapy; the decrease with 7.18 μmol/ L (43%) was significant compared with the basal concentration ($p<0.001$) and in close correlation with the increase of folic acid level and normalization of TSH ($r=0.48$). A significant decrease of cholesterol was found ($p<0.001$) but without HDL and LDL modifications (Fig. 4).

Group 2

Only 15 patients from the initial group (n=18) were followed up during 6 months. After 3 months of combined therapy, patients achieved normal value of plasma homocysteine (9.36 μmol/ L). This decrease with 7.2 μmol/ L of Hcy compared to the basal value is significant ($p<0.001$). During the next 3 month of follow-up, Hcy concentrations remain under normal limits despite the decrease of folic acid dose

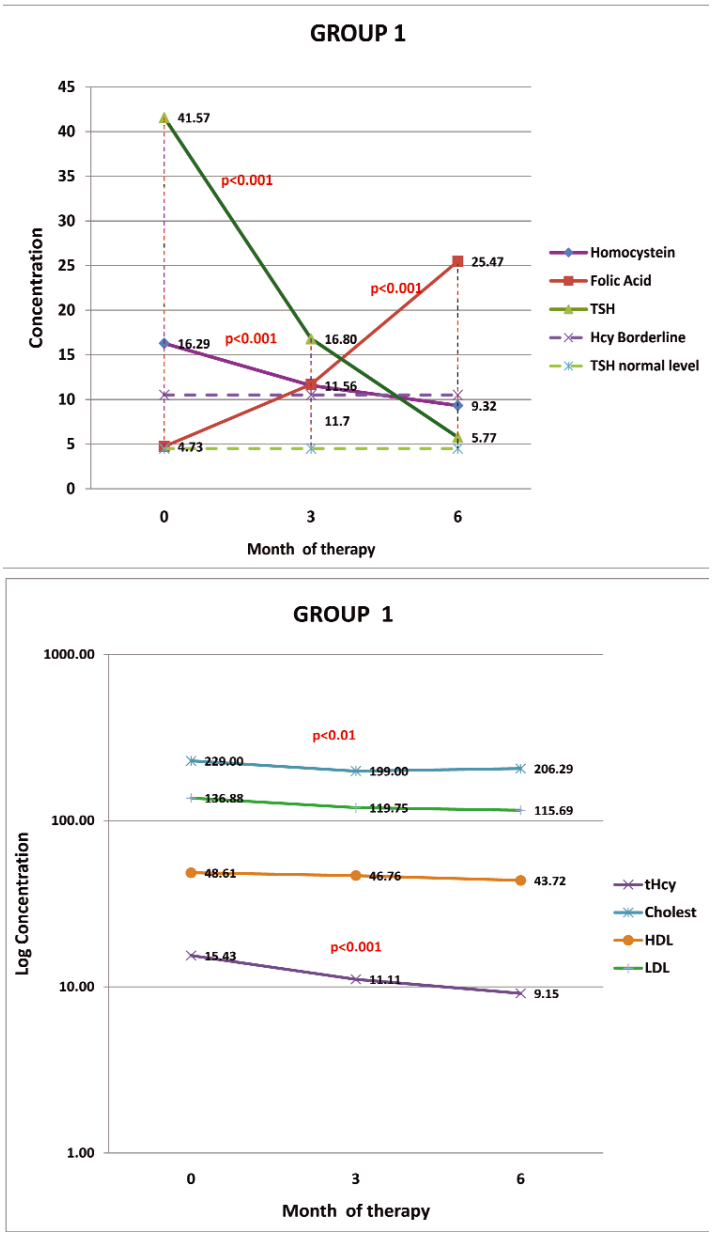

Figure 4. Follow-up in group 1.

associated with the same dose of levothyroxine. The lipid panel (cholesterol and triglycerides) improved significantly during the follow-up time (Fig. 5).

For the patients with thyroid cancer, during the follow-up period we performed the specific thyroid cancer markers: thyroglobulin and anti thyroglobulin antibodies. No correlation was found between high levels of Hcy and positive thyroglobulin levels (>1 ng/mL). IMT remained almost unchanged.

DISCUSSION

Mild hyperhomocysteinemia occurs in approximately 5 to 7% of the general population (3). Abundant epidemiologic evidence has demonstrated that the presence of mild hyperhomocysteinemia is an independent risk factor for atherosclerosis in the coronary, cerebral and peripheral vasculature (13).

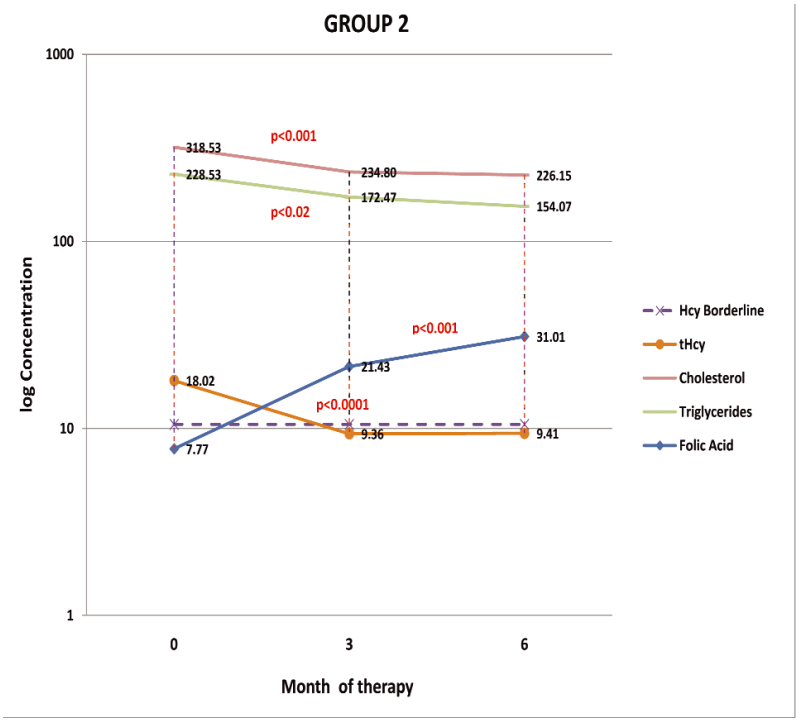

Figure 5. Follow-up in group 2.

Hyperhomocysteinemia occurs in nutritional deficiencies (folate, vitamin B12, vitamin B6, selenium), genetic deficiencies, endocrine disorders (hypothyroidism, diabetes mellitus, hypoestrogenism, Cushing's syndrome), other diseases (chronic renal failure, some malignancies), in active smokers and could be induced by some drugs (methotrexate, phenytoin, carbamazepine, metformin) (4,5, 14,15). On the other side, hyperhomocysteinemia is a risk factor of cognitive impairment ischemic stroke (2, 16, 17) and osteoporotic fractures (18).

It must be mentioned that normal reference range for plasma homocysteine has been changed over time: from 5 to 15 μmol/L (13) to 5 – 10 μmol/L. Elevated homocysteine is defined today as a serum level > 10 μmol/L (19).

Moderate hyperhomocysteinemia, a risk factor for cardiovascular disease, was frequently found in our study groups: 58% in moderate hypothyroidism (group1) and 70% in severe iatrogenic hypothyroidism (group 2). Borderline values or normal values were found only in 38 % of patients in our study groups.

Serum folate was significantly lower for patients with moderate hypothyroidism compared with euthyroid patients. Both serum cholesterol and LDL cholesterol were significantly higher in the study groups before treatment. Even serum folate was normal in group 2, homocysteine and cholesterol were significantly higher *versus* group 1. A quite possible explanation for these results is that hypothyroidism was more severe in group 2 but it was of short duration (weeks), in comparison with group G1.

Data about normalization of hyperhomocysteinemia with levothyroxine are conflicting. In a study this was obtained after 3-9 months (25) but failed after 2 months in a cohort of 14 patients study (24).

In our group 1, for untreated patients with hypothyroidism, serum folate was low. This was in accordance with the results from literature (23). In order to obtain an earlier normalization of Hcy, we decided to add folic acid to L thyroxine therapy because in a previous study the normalization of hyperhomocysteinemia after L thyroxine monotherapy failed to completely normalize Hcy (24).

For the group1, plasma homocysteine decreased significantly after 3 months of folate associated with levothyroxine therapy without reaching a normal level. This normalization was obtained after 6 months, despite the reduction of folic acid dose from 5 to 2.5 mg/day, with the same dose of levothyroxine. Probably, serum folate levels continue to increase slowly between 3-6 months of therapy by 2 mechanisms: normalization of TSH level and continuing supplementation with folic acid (Fig. 4).

In the group 2, a significant decrease of Hcy after 3 month of levothyroxine therapy plus folic acid (5 mg/day) was observed. During the next 3 month, Hcy concentration remains under normal and constant range, despite the decrease of folic acid dose associated with the same dose of levothyroxine. The level of serum folate increased slowly between 3- 6 month by continuous supplementation with folic acid.

A strong inverse relationship between homocysteine and thyroid hormones levels confirms the effect of thyroid hormones on homocysteine metabolism. Follow-up of homocysteine levels together with dyslipidemia in patients with hypothyroidism are important for a thorough evaluation of thyroid status before and during substitution therapy (10). Prolonged periods of thyroid hormones suppressive therapy interruption in patients with differentiated thyroid carcinoma could determine – via hyperhomocysteinemia – an increased vascular risk but other risks are also possible: another malignancy, osteoporotic fractures or cognitive impairment.

After 6 months of associated therapy with L–thyroxine and a stepwise decreasing dose of folic acid, plasma homocysteine levels normalised excluding the additional vascular risk factor of hyperhomocysteinemia.

Our results suggest that folic acid supplementation is beneficial in hypothyroid patients (mainly in group with moderate hypothyroidism) for the following reasons: hypothyroidism is only one of the causes of folate deficiency, normalization of serum folate (and, consequently, of serum Hcy) occurs earlier and - last but not least - an additional decrease of serum Hcy may occur even when serum folate is in the normal range.

In conclusion, thyroid status is an important determinant of plasma Hcy since

hypothyroidism decreases serum folate levels.Significantly lower serum folate in the study groups is pleading for the role of folate deficiency in hyperhomocysteinemia induced by hypothyroidism. High prevalence of hyperhomocysteinemia in hypothyroid patients represents an argument to associate folate supplementation (up to 2.5 mg/day) to levothyroxine therapy, especially in patients with other risk factors for cardiovascular disease. We recommend TSH screening of patients with unexplained hyperhomocysteinemia.

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Conflict of interest

The authors hereby declare that there is no conflict of interest that would prejudice the impartiality of the present scientific work and report.

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