

Possible Role of Inadequate Quantities of Intra-Thyroidal Cobalt, Rubidium and Zinc in the Etiology of Female Subclinical Hypothyroidism

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Abstract

Background: Subclinical hypothyroidism does affect fertility. The prevalence of subclinical hypothyroidism is 10-15 times more common in women than in men. Trace elements play important roles in thyroid function and fertility.

Objective: The aim of this exploratory study was to evaluate whether significant differences of trace element contents exists between female and male thyroids and how they can be related to the etiology of subclinical hypothyroidism.

Methods: Thyroid tissue levels of ten trace elements: silver, cobalt, chromium, iron, mercury, rubidium, antimony, scandium, selenium, and zinc were prospectively evaluated in 105 healthy persons (33 females and 72 males). Measurements were performed using instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis.

Results: It was found that content of cobaltin thyroid of females was significantly higher than that of males, while contents of rubidium and zinc were lower.

Conclusions: Inappropriate content of intra-thyroidal cobalt, rubidium and zinc can be associated with the etiology of female subclinical hypothyroidism.

Keywords: Subclinical hypothyroidism; Female Thyroid; Trace Elements; Neutron activation analysis

Abbreviations: SCH: Subclinical Hypothyroidism; TE: Trace Elements; INAA-LLR: Neutron Activation Analysis with High Resolution Spectrometry of Long-

Lived Radionuclides Fluorescent Analysis; SRM: Standard Reference Material; CRM: Certified Reference Material; BSS: Biological Synthetic Standards.

Introduction

Adequate thyroid function is important to maintain normal reproduction, because thyroid dysfunction affects fertility in various ways resulting in abnormal ovulatory cycles, luteal phase defects, high prolactin levels, and sex hormone imbalances [1,2]. Therefore, normal thyroid function is necessary for fertility, and to sustain a healthy pregnancy [2]. From large population studies, which measured thyroid function, and systematic reviews of this subject carried out in the 1990s to 2010s, it is known that untreated hypothyroidism is a common condition all over the world [2-10]. The prevalence of subclinical hypothyroidism (SCH) is between 1% and 10% in different countries [2-10] and almost everywhere it is 10-15 times more common in women than in men [2,4,9]. From such a great gender-related difference in the prevalence of SCH arises a question about a specific sensitivity of female thyroid tissue to some external and internal factors.

Although the etiology of SCH and other thyroidal disorders is unknown in detail, several risk factors including deficiency or excess of such micronutrients as iodine (I) has been well identified [11-22]. Besides I involved in thyroid function, other trace elements (TE) also play important roles such as stabilizers, structural elements, maintenance and regulation of cell function, gene regulation, enzyme cofactors, activation or inhibition of enzymatic reactions, normal peripheral utilization of thyroid hormones and regulation of cell membrane function [23]. Essential or toxic properties of TE depend on tissue-specific need or tolerance, respectively [24]. Both TE deficiencies as well as overexposures may disturb the thyroidal cell functions [24].

The reliable data on TE mass fractions in normal human thyroid separately for female and male gland is apparently extremely limited. There are a few studies regarding TE content in human thyroid, using chemical techniques and instrumental methods [25-35]. However, the majority of these data are based on measurements of processed tissue and in many studies tissue samples are ashed before analysis. In other cases, thyroid samples are treated with solvents (distilled water, ethanol etc) and then are dried at a high temperature for many hours. There is evidence that certain quantities of TE are lost as a result of such treatment [36-38]. Moreover, only a few of these studies employed quality control using certified/standard reference materials (CRM/SRM) for determination of the TE mass fractions. Sample-nondestructive technique such as instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) is good alternatives for multi-

element determination in the samples of thyroid parenchyma.

This work had three aims. The primary purpose of this study was to determine reliable values for such TE as silver (Ag), cobalt (Co), chromium (Cr), iron (Fe), mercury (Hg), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), and zinc (Zn) contents in intact (normal) thyroid gland of apparently healthy persons using INAA-LLR analysis. The second aim was to compare the levels of TE in the thyroid tissue of all females and males investigated in the study. The final aim was to compare the levels of TE in the thyroid tissue of females and males in age group 1 (≤ 40 years) and in age group 2 (> 40 years).

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Material and Methods

Samples of the human thyroid were obtained from randomly selected autopsy specimens of 33 females (European-Caucasian, aged 3.5 to 87 years) and 72 males (European-Caucasian, aged 2.0 to 80 years). All the deceased were citizens of Obninsk and had undergone routine autopsy at the Forensic Medicine Department of City Hospital, Obninsk. Age ranges for subjects were divided into two age groups, with group 1 (≤ 40 years), and group 2 (> 40 years). For females in group 1 ($n=11$) mean age (\pm standard error of mean, SEM) was 30.9 ± 3.1 years and in group 2 ($n=22$) mean age was 66.3 ± 2.7 years. For males in group 1 ($n=36$) mean age was 22.5 ± 1.4 years and in group 2 ($n=36$) mean age was 52.4 ± 2.4 years. These groups were selected to reflect the condition of thyroid tissue in the children, teenagers, young adults and first period of adult life (group 1) and in the second period of adult life as well as in old age (group 2). The available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, or other chronic disease that could affect the normal development of the thyroid. None of the subjects were receiving medications or used any supplements known to affect thyroid trace element contents. The typical causes of sudden death of most of these subjects included trauma or suicide and also acute untreated illness (cardiac insufficiency, stroke, embolism of pulmonary artery, alcohol poisoning).

All right lobes of thyroid glands were divided into two portions using a titanium scalpel [39]. One tissue portion was reviewed by an anatomical pathologist while the other was used for the ChE content determination. A histological examination was used to control the age norm conformity as well as the unavailability of microadenomatosis and latent cancer.

After the samples intended for TE analysis were weighed, they were freeze-dried and homogenized [40-42]. The pounded sample weighing about 50 mg was used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

To determine contents of the TE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [43]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) sub-samples weighing about 50 mg were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

A vertical channel of nuclear reactor was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. The quartz ampoule with thyroid samples, standards, and certified reference material was soldered, positioned in a transport aluminum container and exposed to a 24-hour neutron irradiation in a vertical channel with a neutron flux of $1.3 \cdot 10^{13}$ n·cm⁻²·s⁻¹. Ten days after irradiation samples were reweighed and repacked.

The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting

rate. The gamma spectrometer included the 100 cm³Ge (Li) detector and on-line computer-based MCA system. The spectrometer provided a resolution of 1.9 keV on the ⁶⁰Co 1332 keV line. Details of used nuclear reactions, radionuclides, and gamma-energies were presented in our earlier publications concerning the INAA chemical element contents in human prostate and scalp hair [44-48].

A dedicated computer program for INAA mode optimization was used [49]. All thyroid samples were prepared in duplicate, and mean values of TE contents were used in final calculation. Using Microsoft Office Excel software, a summary of the statistics, including, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for TE contents in thyroid tissue samples of females and males. The difference in the results between females and males (age group 1 and 2 combined), as well as between females and males separately in age group 1 and group 2 was evaluated by the parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

Results

(Table 1) depicts our data for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions in ten sub-samples of IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) certified reference material and the certified values of this material.

Element	IAEA H-4 animal muscle	This work results	IAEA HH-1 human hair	This work results
	95% confidence interval	M±SD	95% confidence interval	M±SD
Ag	-	0.033±0.008	0.19 ^b	0.18±0.05
Co	0.0027 ^b	0.0034±0.0008	5.97±0.42 ^a	5.4±1.1
Cr	0.06 ^b	0.071±0.010	0.27 ^b	≤0.3
Fe	49.1±6.5 ^a	47.0±1.0	23.7±3.1 ^a	25.1±4.3
Hg	0.014 ^b	0.015±0.004	1.70±0.09 ^a	1.54±0.14
Rb	18.7±3.5 ^a	23.7±3.7	0.94 ^b	0.89±0.17
Sb	0.0056 ^b	0.0061±0.0021	0.031 ^b	0.033±0.009
Sc	0.0059 ^b	0.0015±0.0009	-	-
Se	0.28±0.08 ^a	0.281±0.014	0.35±0.02 ^a	0.37±0.08
Zn	86.3±11.5 ^a	91±2	174±9 ^a	173±17

Table 1: Neutron activation analysis data of trace element contents in certified reference material IAEA H-4 (animal muscle) and IAEA HH-1 (human hair) compared to certified values ((mg/kg, dry mass basis).

M – arithmetical mean, SD – standard deviation, a – certified values, b – information values.

(Table 2) presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles

with 0.025 and 0.975 levels) of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in normal thyroid tissue of female and male.

Gender	Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Males n=72	Ag	0.0156	0.0155	0.0021	0.0017	0.08	0.0104	0.0018	0.0661
	Co	0.0352	0.0234	0.0031	0.0046	0.124	0.0302	0.0113	0.101
	Cr	0.52	0.286	0.041	0.13	1.3	0.414	0.152	0.98
	Fe	222	96	12	51	487	221	76.1	432
	Hg	0.0461	0.0391	0.0053	0.0091	0.18	0.0324	0.0102	0.15
	Rb	7.89	4.56	0.58	2.24	29.4	6.86	2.73	18.2
	Sb	0.108	0.076	0.01	0.0047	0.308	0.0965	0.0095	0.291
	Sc	0.0051	0.0036	0.0012	0.0005	0.0118	0.0044	0.0007	0.0112
	Se	2.36	1.34	0.17	0.53	5.8	1.96	0.804	5.7
Zn	103	43	5.5	34	221	94.6	40.5	200	
Females n=33	Ag	0.014	0.0093	0.002	0.0012	0.0331	0.013	0.0021	0.0321
	Co	0.0505	0.0322	0.0064	0.017	0.14	0.0405	0.0183	0.13
	Cr	0.573	0.246	0.049	0.29	1.22	0.488	0.303	1.11
	Fe	232	112	22	63	512	199	64.8	480
	Hg	0.0329	0.0246	0.0051	0.0065	0.1	0.0263	0.0079	0.1
	Rb	6.16	2.42	0.48	1.11	12.8	6.3	2.38	10.8
	Sb	0.116	0.063	0.012	0.0115	0.248	0.108	0.0183	0.247
	Sc	0.0042	0.004	0.0012	0.0002	0.0143	0.0032	0.0003	0.0124
	Se	2.22	1.19	0.23	0.439	5.32	2.07	0.773	4.85
Zn	85.7	38	7.44	8.1	166	83	22.9	156	

Table 2: Some statistical parameters of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions (mg/kg, dry mass basis) in normal thyroid tissue of females and males.

M – arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – minimum value, Max – maximum value, P 0.025 – percentile with 0.025 level, P 0.975 – percentile with 0.975 level.

The comparison of our results with published data in normal human thyroid is shown in Table 3. for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction

Element	This work	Published data [Reference]		
	Males and females M±SD	Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M or M±SD, (n)**
Ag	0.015±0.014	0.25 (12)	0.000784 (16) [26]	1.20±1.24(105) [27]
Co	0.040±0.027	0.34 (17)	0.026±0.031 (46) [28]	70.4±40.8 (14) [29]
Cr	0.54±0.27	0.69 (17)	0.105 (18) [30]	24.8±2.4 (4) [31]
Fe	225±100	252 (21)	56 (120) [32]	2444±700 (14) [29]
Hg	0.042±0.036	0.08(13)	0.0008±0.0002(10) [33]	396±40 (4) [31]
Rb	7.4±4.1	12.3 (9)	≤0.85 (29) [33]	294±191 (14) [29]
Sb	0.111±0.072	0.105 (10)	0.040±0.003(-) [34]	4.0 (-) [35]
Sc	0.0046±0.0038	0.009 (4)	0.0018±0.0003(17) [36]	0.0135±0.0045 (10) [33]
Se	2.32±1.29	2.61 (17)	0.95±0.08(29) [33]	756±680 (14) [29]
Zn	97.8±42.3	118 (51)	32(120) [32]	820±204 (14) [29]

Table 3: Median, minimum and maximum value of means of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in normal thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis).

M – arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** – number of samples.

The ratios of means and the difference between mean values Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions in normal thyroid of females and males are presented in Table 4. Because, in our previous studies

age-dependents of many TE in thyroid gland was found [50-53], the comparison between TE contents in thyroid of females and males separately in age group 1 and in age group 2 was also performed (Tables 4-6).

Element	Thyroid tissue				Ratio
	Males 2.0-80 years n=72	Females 3.5-87 years n=33	Student's t-test p≤	U-test p	Females to Males
Ag	0.0156±0.0021	0.0140±0.0020	0.586	>0.05	0.9
Co	0.0352±0.0031	0.0505±0.0064	0.039	≤0.01	1.43
Cr	0.520±0.041	0.573±0.049	0.411	>0.05	1.1
Fe	222±12	232±22	0.692	>0.05	1.05
Hg	0.0461±0.0053	0.0329±0.0051	0.079	>0.05	0.71
Rb	7.89±0.58	6.16±0.48	0.024	≤0.01	0.78
Sb	0.108±0.010	0.116±0.012	0.649	>0.05	1.07
Sc	0.0051±0.0012	0.0042±0.0012	0.59	>0.05	0.82
Se	2.36±0.17	2.22±0.23	0.633	>0.05	0.94
Zn	103±5.5	85.7±7.4	0.069	≤0.05	0.83

Table 4: Differences between mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid tissue of males and females.

M – arithmetic mean, SEM – standard error of mean, t-test - Student's t-test, U-test - Wilcoxon-Mann-Whitney U-test, Statistically significant values are in **bold**.

Element	Thyroid tissue				Ratio
	Males (MG1) n=44	Females (FG1) n=11	Student's t-test p≤	U-test p	FG1/MG1
Ag	0.0160±0.0032	0.0143±0.0032	0.7	>0.05	0.89
Co	0.0374±0.0046	0.0328±0.0042	0.467	>0.05	0.88
Cr	0.502±0.051	0.567±0.065	0.441	>0.05	1.13
Fe	224±16	172±22	0.078	≤0.05	0.77
Hg	0.0439±0.0071	0.0275±0.0046	0.058	>0.05	0.63
Rb	7.96±0.61	4.95±0.58	0.0011	≤0.01	0.62
Sb	0.109±0.012	0.0880±0.0096	0.174	>0.05	0.81
Sc	0.0052±0.0012	0.0026±0.0017	0.32	>0.05	0.5
Se	2.05±0.18	1.86±0.27	0.565	>0.05	0.91
Zn	102±6.6	59.8±8.7	0.0008	≤0.01	0.59

Table 5: Differences between mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid tissue of males and females aged ≤40 years.

M – arithmetic mean, SEM – standard error of mean, t-test - Student's t-test, U-test - Wilcoxon-Mann-Whitney U-test, Statistically significant values are in **bold**.

Element	Thyroid tissue				Ratio
	Males (MG2) n=28	Females (FG2) n=22	Student's t-test p≤	U-test p	FG2/MG2
Ag	0.0148±0.0019	0.0138±0.0027	0.753	>0.05	0.93
Co	0.0317±0.0031	0.0644±0.0096	0.005	≤0.01	2.03
Cr	0.548±0.070	0.578±0.073	0.767	>0.05	1.05
Fe	218±19	279±31	0.105	>0.05	1.28
Hg	0.0491±0.0082	0.0370±0.0084	0.311	>0.05	0.75
Rb	7.75±1.25	7.05±0.63	0.62	>0.05	0.91
Sb	0.107±0.018	0.136±0.019	0.276	>0.05	1.27
Sc	0.0049±0.0024	0.0045±0.0014	0.868	>0.05	0.92
Se	2.89±0.32	2.48±0.34	0.396	>0.05	0.86
Zn	104±10	104.7±8.4	0.98	>0.05	1.01

Table 6: Differences between mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid tissue of males and females aged >40 years.

M – arithmetic mean, SEM – standard error of mean, t-test - Student's t-test, U-test - Wilcoxon-Mann-Whitney U-test, Statistically significant values are in **bold**.

Discussion

Precision and Accuracy of Results

Good agreement of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents analyzed by INAA-LLR with the certified data of CRM IAEA H-4 and IAEA HH-1 (Table 1) indicates an acceptable accuracy of the results obtained in the study of TE of the thyroid presented in Tables 2–6.

The mean values and all selected statistical parameters were calculated for ten TE (Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn) mass fractions in thyroid of female and male (Table 2).

Comparison with Published Data

Values obtained for Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in the normal human thyroid (Table 3) agree well with median of mean values reported by other researches [25-35]. The obtained means for Ag and Co were almost one order of magnitude lower median of previously reported means but inside the range of means (Table 3). Data cited in Table 3 also includes samples obtained from patients who died from different non-endocrine diseases. A number of values for TE mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using published data for water (75%) and ash (4.16% on dry mass basis) contents in thyroid of adults [27,54].

The range of means of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn level reported in the literature for normal human thyroid vary widely (Table 3). This can be explained by a dependence of TE content on many factors, including the region of the thyroid, from which the sample was taken, age, gender, ethnicity, and mass of the gland. Not all these factors were strictly controlled in cited studies. Another and, in our opinion, leading cause of inter-observer variability can be attributed to the accuracy of the analytical techniques, sample preparation methods, and insufficient quality control of results in these studies.

Gender-Related Differences

Strongly pronounced differences in Co, Rb, and Zn mass fraction were observed between female and male thyroid (Table 4). The mean Co mass fraction in female thyroids was almost 1.4 times higher while the means of Rb and Zn mass fractions were respectively 22% and 17% lower than in male thyroids. During the first 40 years of life (Age group 1) the situation with TE contents in female thyroids was some different than that for older females. In Age group 1 no statistically significant difference between the Co content in female

and male thyroids was found, but differences between their Rb and Zn contents were detected (Table 5). In Age group 1 of females with mean age 30.9 years the Rb and Zn contents in thyroid were respectively 38% and 41% lower than in thyroid of males from the same age group. Moreover, in this age group a modest but statistically significant reduced level of Fe mass fraction in female thyroids was observed using the non-parametric Wilcoxon-Mann-Whitney *U*-test. For ages over 40 years (Age group 2) a statistically significant difference between the Co content in female and male thyroids was observed and the mean Co content in female thyroids was 2 times higher than that in male thyroids. In Age group 2 differences between the Fe, Rb and Zn contents in thyroids of females and males, previously found in the Age group 1, was no longer evident.

Because the prevalence of SCH is 10-15 times more greater in women than in men, we can accept that the levels and relationships of TE mass fractions in male thyroids as more suitable (perhaps optimal) for normal function of the gland [3,9]. If so, we have to conclude that up to age 40 years there is a significant deficiency of Rb and Zn contents in female thyroid parenchyma, accompanied by a modest deficiency of Fe. In age over 40 deficiencies of Fe, Rb and Zn contents in female thyroid disappear and an excess of Co is now seen.

Cobalt: Co is widely used in a bijouterie production. It may be one of the reasons of the higher level of this TE content in female thyroids than in that in male thyroids. Health effects of high Co occupational, environmental, dietary and medical exposure are characterized by a complex clinical syndrome, mainly including neurological, cardiovascular and endocrine deficits, including hypothyroidism [55,56]. Moreover, Co is genotoxic and carcinogenic, mainly caused by oxidative DNA damage by reactive oxygen species, perhaps combined with inhibition of DNA repair [57]. Therefore, an excessive Co level in the thyroid of elderly females might inhibit thyroid hormonal synthesis.

Iron: The low Fe level in the thyroid of young women compared with men can be attributed to physiological characteristics of the female body related to reproduction and pre-menopausal physiology [45].

Rubidium: As for Rb, there is very little information about its effects in organisms. No negative environmental effects have been reported. Rb is only slightly toxic on an acute toxicological basis and would pose an acute health hazard only when ingested in large quantities [58]. Rb has some function in immune response, probably by supporting cell differentiation [59,60]. Both potassium (K) and Rb are in the first group of the periodic table. Rb, like K, seems to be

concentrated in the intracellular space and transferred through membrane by the Na⁺K⁺-ATPase pump. Thus, the low Rb level in the thyroid of women compared with men might reflect the reduced ratio "Volume of thyroid cells / Volume of follicular colloid" in the female thyroid. Thyroid function depends in part on the total volume of active thyroid cells. From this it might be concluded that the reduced level of active cells in the thyroids of women compared to men increases risk of hypothyroidism.

Zinc: Zn is a most essential TE for humans. Today more than 300 proteins and over 100 DNA-binding proteins that require Zn have been classified. Zn is required for the synthesis of thyroid hormones, and deficiency of this TE can result in hypothyroidism [61,62]. Thus, a Zn deficiency in female thyroid parenchyma observed in the present study may be one of the reasons for the higher incidence of SCH in females in comparison with males.

Conclusion

Our data indicate that there is a statistically significant gender-related difference between TE levels in thyroid tissue of females and males. The Co mass fraction is higher while the Rb and Zn mass fractions are lower in female thyroids compared with those in male thyroids. Subclinical hypothyroidism is a multietiological and multifactorial complex condition. The complete understanding of the role of inadequate levels of some TE in thyroid parenchyma in the etiology of SCH requires a global vision of their different mechanisms of action, which is not yet possible with the present state of knowledge. However, from the results of our study it follows that an involvement of inadequate contents of intra-thyroidal Co, Rb and Zn in the etiology of female SCH may be assumed.

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Conflict of Interest

There is no any financial interest or any conflict of interest.

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