Association of antiepileptic drug usage, trace elements and thyroid hormone status

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Abstract

Background: Levels of thyroid hormone (TH) and trace elements (copper (Cu) and selenium (Se)) are important for development and function of the brain. Anti-epileptic drugs (AEDs) can influence serum TH and trace element levels. As the relationship between AEDs, THs, and trace elements has not yet been studied directly, we explored these interactions. **Method:** In total 898 participants, from the Thyroid Origin of Psychomotor Retardation study designed to investigate thyroid parameters in subjects with intellectual disability (ID), had data available on serum Se, Cu, thyroid stimulating hormone (TSH), free thyroxine (FT₄), tri-iodothyronine (T₃), reverse T₃, T₄, and thyroxine-binding globulin (TBG); 401 subjects were on AED treatment. Differences in trace elements according to medication usage was investigated using ANOVA, and associations between trace elements and thyroid parameters were analysed using (non-) linear regression models. **Results:** Study participants were not deficient in any of the trace elements analyzed. AED (carbamazepine, valproate and phenytoin) usage was negatively associated with serum Se and showed compound-specific associations with Cu levels. After correction for drug usage, Se was positively associated with TSH levels, negatively associated with FT₄ levels, and positively with T₃ levels. Cu was positively associated with T₄, T₃, and rT₃, which was largely dependent on TBG levels. **Conclusion:** The subjects with ID did not display profound deficiencies in trace element levels. AEDs were associated with serum Se and Cu levels, while serum Se and Cu were also associated with thyroid parameters. Further studies on the underlying mechanisms and potential clinical importance are warranted.

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Introduction

Thyroid hormone (TH) is important for the normal development and energy metabolism of almost all tissues (1). The thyroid gland pre-dominantly secretes the inactive pro-hormone thyroxine (T₄), which is locally converted to the active form tri-iodothyronine (T₃) by selenium (Se)-dependent enzymes of the family of iodothyronine deiodinases (2). T₃ mediates its major effects by binding to nuclear TH receptors (TRs) which regulate the transcription of target genes (3). Abnormalities in TH status are commonly found

among endocrine disorders (4) at varying prevalences according to the group of patients that is being studied (5, 6).

Essential trace elements, like Se and copper (Cu), are micronutrients that are present in low concentrations in the human body and are dependent on a sufficiently high dietary intake. Trace elements are essential for many enzymatic reactions and are therefore important for proper functioning of biochemical pathways and the endocrine system (7).

Sufficient levels of both TH and trace elements are important for normal development of the brain (8). Abnormalities in TH signaling can give rise to neurocognitive impairment, as illustrated by subjects with mutations in the gene encoding the TH transporter monocarboxylate transporter 8 (*MCT8*) (9, 10) or in the TR *THRA* (encoding TR α) (11, 12, 13, 14). Adequate levels of Cu are also important for normal development of the brain, as Cu is important in energy metabolism, antioxidative defense, and the production of neurotransmitters (15). Also, it was shown that TH or Cu deficiencies resulted in similar defects during rodent cerebral cortical development (16). The brain appears to preserve Se levels during Se shortage, as rats on a Se deficient diet did not display any neurological symptoms, because of only slightly reduced brain Se levels (17). However, mice lacking selenoprotein P (SelP), which is an important Se carrier, showed very low brain Se levels together with movement disorders and occasional seizures (18).

Only limited data on the relationship between TH and Se and Cu are currently available. It was shown in rats that adequate Se in nutrition supports TH synthesis and metabolism and protects the thyroid gland from damage by excessive exposure to reactive chemicals (19). Furthermore, in humans, high levels of Cu were associated with higher levels of both T₃ and T₄ (20). As a corollary, Bastian *et al.* (16, 21, 22) showed in different rat studies that fetal deficiency of Cu resulted in impaired TH-regulated brain gene expression. While these studies suggested an interaction of the trace elements Se and Cu and TH during brain development, its relevance for humans is unknown.

In the Thyroid Origin of Psychomotor Retardation (TOP-R) study, thyroid parameters of subjects with intellectual disability (ID) were extensively profiled (23). In this cohort, it has been shown that TH profiles in subjects without anti-epileptic drugs (AEDs) were comparable with the general population. However, AEDs were strongly associated with decreased T₄, free T₄ (FT₄), T₃, and rT₃ levels which is in agreement with other studies (24, 25, 26). It has been speculated that the changed thyroid parameters in patients that use AEDs can be explained by an influence of AEDs on binding proteins (27), a stimulation of hepatic degradation or conjugation of TH (28) or an altered peripheral deiodinase activity (23). Interestingly, AEDs have been associated with changes in serum levels of Se and Cu (23, 29, 30, 31, 32, 33). However, the complex relationship between AEDs, THs, Se, and Cu has never been directly studied.

The aim of this study was to analyze the associations between AEDs, Se, Cu, and thyroid parameters using the TOP-R cohort.

Subjects and methods

TOP-R study

The TOP-R study is a nation-wide cohort study in The Netherlands, which was designed to investigate thyroid parameters in subjects with intellectual disability (ID). The study population has been described in detail before (23). In short, subjects were excluded if the aetiology of the ID was known. Information about other medication was unavailable. Mean age was 47.9 ± 0.5 years, and 47% were women. The study was approved by the medical ethics committee of the Erasmus University Medical Centre. Written informed consent was obtained from the legally authorized representatives. Of the 946 eligible individuals, data on TH and trace element serum levels were available in 898 subjects, and information about thyroid medication (anti-thyroid drugs and l-T₄ replacement therapy) was available in 806 subjects and about AEDs in 786 subjects. Full case analyses according to this data did not change the results (data not shown). After exclusion of subjects that used thyroid-interfering medication ($n=40$) and/or thyroid peroxidase antibodies (TPOAb) positive individuals (cut-off > 60 IU/ml; $n=24$), 834 subjects were included in one or more analyses. After exclusion, 372 of 725 eligible patients used AEDs (51.3%).

Measurements of thyroid status, Se, and Cu

Serum samples were stored frozen at -20°C . Serum T₄, FT₄, and thyroid-stimulating hormone (TSH) were measured by chemiluminescence assays (Vitros ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Inc., Rochester, NY, USA). T₃ was measured using an in-house RIA and rT₃ using a commercial RIA (Immunodiagnostic Systems, Scottsdale, AZ, USA). Thyroxine-binding globulin (TBG) and TPO antibodies were determined by immunoassay (Immulite 2000, Siemens, Breda, The Netherlands). Se and Cu concentrations were determined by total reflection X-ray fluorescence spectroscopy (34). The method was validated with a Seronorm standard (Sero AS, Billingstad, Norway). Briefly, all samples were diluted 1:1 in a gallium-containing solvent for standardization. The analysis was performed in duplicate, and the results of each sample differed by $<20\%$ for both Se and Cu. In every measurement run,

a human control serum was included, allowing the calculation of an intra-assay coefficient of variation of 7% (Se) and 8% (Cu) and an inter-assay coefficient of variation of 12% (Se) and 18% (Cu) respectively.

Statistical analysis

To satisfy model assumptions, TSH levels were logarithmically transformed. The association between trace elements and thyroid parameters was investigated using ordinary least squares linear regression models with restricted cubic splines utilizing three to five knots. Figures show back-transformed axis values for TSH, and all associations were adjusted for sex, age, and relevant medication usage (covariates set to mean levels or most appearing category). The analyses were adjusted for medication usage when a drug was associated with a thyroid function parameter in a backward linear regression analysis, utilizing a cut-off of $P < 0.15$. In addition, a variable for missing data on AED usage was added but did not reach the threshold for any thyroid parameters.

We investigated differences in the ratio of T_3 to rT_3 using linear regression analyses with a product term of the independent thyroid function parameter and trace element. Subsequently, to allow for non-linear associations/interactions, a sensitivity analysis was performed by adding quadratic terms and/or a product term of the trace element or thyroid function variables; these were maintained according to P values or changes in R^2 . Interaction figures show the associations between thyroid function parameters according to low (red line with 95% CI) or high (blue line with 95% CI) trace element values (rounded number of 10th percentile or 90th percentile respectively).

All statistical analyses were performed using R Statistical Software v 3.03 (1) (package rms or visreg) or SPSS version 21.0 for Windows.

Results

Descriptive statistics of the study population are shown in [Supplementary Table 1](#), see section on [supplementary data](#) given at the end of this article. First, we studied the association between AED usage and serum Se and Cu levels ([Fig. 1](#)).

None of the study participants were deficient in Se or Cu, whether they were on AED treatment or not. Carbamazepine or valproate usage was associated with lower serum Se levels ([Fig. 1A and B](#)). Usage of carbamazepine or phenytoin was associated with higher serum Cu levels and valproate usage with lower serum Cu levels

Figure 1

The association between AED usage and serum Se or Cu and as predicted mean (gray dot) and 95% CI (gray line). In addition, there was no dose-dependent effect of AED usage for A, B, C, D and E ($P = 0.16$; $P = 0.32$; $P = 0.57$; $P = 0.40$; and $P = 0.62$ respectively). All analyses were performed in subjects with AED usage data available after exclusion of subjects with thyroid medication usage or TPOAb positivity and were adjusted for sex and age.

([Fig. 1C, D and E](#)). Other AED usage was not associated with serum Se or Cu levels, and there was no association between the daily dosage of AEDs and Se, Cu, or TH levels (data not shown). Thus, commonly prescribed AEDs affect serum Se and Cu levels in a compound-specific way.

Next, we studied associations between serum Se and Cu levels and thyroid parameters. The associations between serum Se levels and thyroid parameters are shown in [Fig. 2](#). After correction for drug usage, Se was positively associated with TSH levels ($\beta \pm \text{s.e.m.}$; 0.0008 ± 0.0003 ; $P = 0.03$), negatively associated with FT_4 levels (linearly for Se below 125: -0.0212 ± 0.0065 ; $P = 0.001$), and positively with T_3 levels (linearly for Se below 125:

Figure 2

Graphs show the association between serum selenium and thyroid function parameters, as predicted mean (black line) and 95% CI (grey area). All analyses were performed after exclusion of subjects with thyroid medication usage or TPOAb positivity and were adjusted for sex, age, and antiepileptic drug usage and TBG levels.

0.0028 ± 0.0005 ; $P < 0.001$; Fig. 2B and C). Since all three deiodinases are selenoproteins, we also investigated possible effects of trace elements on peripheral deiodinase activity. As a proxy for peripheral TH deiodination, we studied the association between serum Se, Cu, and T₃/rT₃ ratio. Serum Se was positively associated with the T₃/rT₃ ratio (0.0122 ± 0.0036 ; $P < 0.001$; Supplementary Figure 1A, see section on supplementary data given at the end of this article).

Next, the associations between serum Cu levels and thyroid parameters were studied. Cu levels were positively associated with T₃, rT₃, and T₄ levels (Fig. 3A, B and C). Also, a particularly strong association was observed between Cu and TBG (0.0022 ± 0.0002 ; $P < 0.001$; Fig. 3F). As estrogens may influence TBG levels, we also investigated

sex differences. The association between Cu and TBG was stronger amongst women, as compared to men (P interaction = 0.0007; Supplementary Figure 2, see section on supplementary data given at the end of this article). After correction for TBG, the positive associations between serum Cu and T₃, rT₃, and T₄ levels disappeared (Supplementary Figure 3). Serum Cu levels were not associated with changes in the T₃/rT₃ ratio (Supplementary Figure 1B).

Discussion

TH and the trace elements Se and Cu are important for normal neurocognitive development, and abnormal brain development may increase AED usage. The relationship

Figure 3

Graphs show the association between serum Cu and thyroid function parameters, as predicted mean (black line) and 95% CI (grey area). All analyses were performed after exclusion of subjects with thyroid medication usage or TPOAb positivity and were adjusted for sex, age, and antiepileptic drug usage, but not for TBG levels.

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between TH, Se, Cu, and AED is currently unclear. In this study, we analyzed this relationship in the TOP-R cohort, in which Se and Cu and thyroid parameters were determined. Our analyses indicated that serum Se levels were associated with TSH, FT₄, and T₃ levels, while serum Cu levels were associated with T₃, rT₃, and T₄, via changes in TBG levels.

In this cohort of patients with ID, Se levels were significantly lower in patients that use valproate or carbamazepine, whereas phenytoin and carbamazepine usage was associated with increased Cu levels (Fig. 1). Unfortunately, these measurements were performed during treatment with AEDs; therefore, no data is available on pre- or post-treatment levels. Many studies described the effects of AEDs on trace element levels like Se and Cu, although the effects of different classes of AEDs were neither consistent in magnitude nor in direction (29, 30, 31, 32, 35, 36, 37). There are several potential reasons for these inconsistencies, ranging from dosage and duration of AED usage, age, health, and nutritional status of the patients to baseline trace element concentrations which differ profoundly in different geographical areas as well as the small number of subjects studied (38). Due to the observational nature of this study, it is very difficult to draw any conclusions on the causative or mechanistic features of these results. It is very well possible, although speculative, that AEDs interfere with the transport, metabolism, or excretion of trace elements, as is described for TH (23, 24, 25, 26, 27, 28). Furthermore, although the generalizability of our findings may be limited, it is important to be aware of confounders if Se or Cu levels are analyzed in subjects on commonly prescribed AEDs. Depending on the baseline level of the population, this may cause a physiologically meaningful disbalance.

Se is a trace element that is incorporated in seleno-cysteine, which is required for the normal production of selenoproteins (39, 40, 41). In the last decades, accumulating evidence has shown that Se plays an essential role in TH biosynthesis and metabolism as well as in normal thyroid function (42, 43). The thyroid gland contains the highest Se concentration among human tissues due to the expression of several selenoproteins that are important in the maintenance of normal TH metabolism (deiodinases) and the protection of thyroid cells against oxidative damage such as glutathione peroxidases (43, 44). If Se is limiting, lower levels of selenoproteins are synthesized which may potentially disturb peroxide-dependent iodination of thyroglobulin in the thyroid, thyrocyte defence systems, and TH activation and inactivation (19, 41, 45). In this study, we showed that serum Se was positively

associated with TSH, inversely associated with FT₄, and positively associated with T₃ levels and the T₃/rT₃ ratio, as a proxy for peripheral deiodination (Fig. 2 and Supplementary Figure 1). This is also reminiscent of the constellation of critical illness, where a Se deficit often parallels a low T₃ syndrome. These results are in line with several studies in rats, in which Se deficiency decreased liver and kidney D1 activities in combination with modest alterations in thyroid parameters (increased T₄ and decreased T₃ levels) (46, 47, 48, 49, 50). However, the majority of studies with human subjects on the interaction of Se and TH yielded inconsistent results (51). Our results indicated that even low normal Se levels may already impact negatively on the TH state. However, net effects of limiting Se availability for the expression of the three deiodinase isoenzymes in different human tissues are difficult to predict, as hierarchical principles control the expression of the selenoenzymes with organ-specific preferences (52).

Cu is necessary in many metabolic processes and plays an important role in endogenous anti-oxidative defense mechanisms (53). Studies of Bastian *et al.* (16, 21, 22) in mice showed that low levels of Cu were associated with a decreased TH state, which interfered with normal brain development. Similarly, serum ceruloplasmin and Cu levels have recently been proposed as direct biomarkers of TH signaling (54). In line with these studies, we observed strong positive associations between serum Cu and T₃, rT₃, and T₄ levels (Fig. 3). However, we were first to describe that these effects are totally driven by TBG (Supplementary Figure 3). Indeed, FT₄ levels are not affected by Cu state. After correction for TBG, TH parameters were not significantly associated with serum Cu anymore, suggesting that the effects of Cu on thyroid function are mediated via changes in TBG. This may be explained by the fact that Cu may directly affect hepatic TBG expression, secretion, or turnover. TBG is the main transport protein for TH in serum, and many substances are known to influence TBG concentrations, such as estrogens, androgens, glucocorticoids, and heroin (55, 56). Most extensively studied are estrogens, which increased TBG levels by slowing its clearance via the liver, therefore increasing its half-life (55). In addition, estrogens have also been shown to increase serum Cu levels, which are most likely driven by an increase in ceruloplasmin, the transport protein of Cu (57, 58, 59, 60, 61). The association of Cu with TBG was stronger in women, which is consistent with a positive effect of Cu on TBG levels via estrogens, although this did not fully explain our observations.

Our study has strengths and limitations. The strength of this study is that the TOP-R cohort contains one of the largest numbers of subjects with available data on serum Se, Cu, extensive profiling of TH parameters, and commonly prescribed AEDs. It is important to note that the generalizability of this study is limited, as the patients in the cohort are all diagnosed with unexplained ID. Another potential limitation is the observational nature of the study, which precludes the analysis of causality of the detected associations. Still, many of our findings itself, and the direction of causality, are supported by results from *in vivo* animal studies.

Together, our analyses indicate that the commonly prescribed AEDs carbamazepine and valproate affect serum Se and Cu levels. Furthermore, while Se levels may partially affect TH signaling via modifying the expression of deiodinases, the effects of Cu on thyroid parameters may be primarily driven by its effects on TBG. Future research is needed to explore the underlying mechanisms of the observed associations in the current study and to investigate to what extent trace elements are risk factors for the development of thyroid diseases.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-15-1081>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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