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Positive correlation of thyroid hormones and serum copper in children with congenital hypothyroidism

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ABSTRACT

Thyroid hormones are of central relevance for growth and development. However, the underlying molecular mechanisms are still not fully understood. Recent studies in humans and mice have demonstrated that serum levels of selenium (Se) and copper (Cu) are positively affected by thyroid hormones. Given the importance of these trace elements for many biochemical processes, we tested whether this interaction is found in children at risk for hypothyroidism, potentially providing a novel factor contributing to the disturbed development observed in congenital hypothyroidism (CH). We conducted a cross-sectional analysis of 84 children diagnosed with CH displaying a wide range of thyroid hormone concentrations. Serum Se and Cu concentrations were measured by total reflection X-ray fluorescence. Data for thyrotropin (TSH) were available in all, thyroxine (T4) and free thyroxine (fT4) in the majority and triiodothyronine (T3) in 29 of the children. Spearman rank analyzes were performed. Cu and thyroid hormones showed a strong positive correlation (Cu/T4, rho = 0.5241, P = 0.0003; Cu/T3, rho = 0.6003, P = 0.0006). Unlike in adults, no associations were found between Se and any of the thyroid hormones. Our data highlight that serum Cu and thyroid hormones are strongly associated already in early postnatal life. Severely hypothyroid children are thus at risk of developing a Cu deficiency if not adequately nourished or supplemented. This finding needs to be verified in larger groups of children in order not to miss an easily-avoidable risk factor for poor development.

1. Introduction

Thyroid hormones (TH) are of central importance for the development of almost all tissues, including lung, bone, and brain [1]. This has become most evident in congenital hypothyroidism (CH). If left untreated, CH will cause severe growth impairment, neurocognitive defects, hypotonia, bradycardia and other systemic disturbances [2–4]. Despite the strong phenotype of CH, however, the molecular mechanisms underlying these TH-dependent developmental defects have remained largely enigmatic.

Our recent studies in humans with inherited defects in TH signalling (RTH, resistance to thyroid hormones) and corresponding rodent models have indicated that TH affect the metabolism of the trace elements selenium (Se) and copper (Cu) [5,6]. Both trace elements are important during postnatal development, especially in the control of metabolism and defence against oxidative damage. Low Se levels in premature newborns have been associated with pulmonary dysplasia [7] and retinopathy [8]. Accordingly, Se supplementation is discussed as a meaningful supportive adjuvant treatment in preterm neonates [9].

Se deficiency has also been implicated in general growth retardation and bone maturation defects accompanied by lower growth hormone (GH) and insulin-like growth factor-1 (IGF1) levels in both rodents and humans [10,11]. This finding was corroborated in studies with transgenic mice lacking the Se-transport protein selenoprotein P (SePP) that develop a strong growth phenotype [12]. Similarly, children with defects in selenoprotein biosynthesis and reduced SePP levels present with delayed bone maturation [13,14]. Accordingly, SePP receptors were identified in bone [15], and both Se and SePP levels are associated with bone quality and bone turnover in healthy elderly women [16].

Cu is another essential trace element with high importance for growth and development. Cu is a component of several enzymes implicated in fundamental biochemical pathways, e.g. oxidative

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phosphorylation, protection against oxidative stress and collagen cross-linking. Among the prominent Cu-dependent enzymes are mitochondrial cytochrome C oxidase, superoxide dismutase, lysyl oxidase, and dopamine beta-hydroxylase [17]. Cu deficiency may thus result in developmental defects including anaemia, neutropenia, infection susceptibility and growth defects with alterations in bone structure [18,19]. Consequently, Cu is included into the group of essential vitamins and trace elements considered for active supplementation in premature infants [20].

Given the potential regulation of Se and Cu metabolism by TH, and the remarkably overlapping phenotypes of CH with developmental Se or Cu deficiency, we speculated that these two trace elements may constitute TH targets already in early postnatal life, probably involved in the devastating developmental effects of CH. Hence, we studied a group of children diagnosed with CH displaying a relatively wide range of TH status, and compared their TH concentrations with serum levels of Se and Cu. Surprisingly, only Cu, but not Se displayed a strong association with TH concentrations.

2. Subjects and methods

2.1. Children

The children included in this study have all been diagnosed with primary CH and thyroid dysgenesis in the newborn screening program. All received L-thyroxine supplementation therapy. Written informed consent was obtained from the children's parents. All patient data were collected during routine care at the Department for Paediatric Endocrinology, Charité—Universitaetsmedizin Berlin, Germany. Data and blood sample collection complied with the guidelines laid down in the Declaration of Helsinki. The study was approved by the local Ethics Committee of the Charité (number EA2/132/11), functioning according to the 3rd edition of the Guidelines on the Practice of Ethical Committees in Medical Research issued by the Royal College of Physicians of London.

2.2. Hormone and trace element analyses

TSH, T4, free T4 (fT4) and T3 concentrations were determined in the routine analytical laboratory of Charité-Berlin using Elecsys assays (Roche Diagnostics, Mannheim, Germany) and children-specific reference intervals [21]. Total Se and Cu were determined by Total Reflection X-Ray Fluorescence (TXRF) analysis using a benchtop TXRF device (PicoFox S2, Bruker nano, Berlin, Germany) as described earlier [6]. Briefly, serum samples were diluted with ultrapure H_2O, supplemented with an external Gallium standard solution, and applied to glass carriers for TXRF analysis. A human reference serum (Sero, Billingstad, Norway) was used as quality control. Intra-assay CV was below 10% for both the Se and Cu determinations during the analyses.

2.3. Statistical analyses

Statistical Package for Social Sciences (SPSS, Version 17.0, SPSS Inc., Chicago, IL, USA) and Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) were used for statistical analysis and data presentation. Normal distribution was tested by the Shapiro-Wilk test. As normal distributions were not given for all the parameters analysed, interactions were determined by Spearman rank-order correlation.

3. Results

A total of 84 serum samples from children with CH in the age range of 3–17 years were available for analysis (Table 1). About two thirds of the patients were girls (n = 60). This is in line with the incidence of CH which is approximately twice as high in girls than in boys [22]. TSH levels were available for all subjects; T4 and fT4 values had been determined in roughly half, and data on T3 were available in one third of the children.

Table 1
Anthropometrics, trace elements and thyroid hormones.

	N	Minimum	Maximum	mean (SD)
age [years]	84	3.0	17.0	11.3 (±3.9)
height [cm]	84	87.5	187.8	150.6 (±34.1)
weight [kg]	84	11.6	127.9	35.1 (±25.6)
Se [μg/l]	84	33.0	148.0	69.2 (±17.4)
Cu [μg/l]	84	747.8	2587.8	1384.2 (±388.8)
Cu/Se ratio	84	8.3	46.4	21.0 (±7.5)
TSH basal [mU/l]	84	0.0	285.3	8.9 (±32.1)
T4 [μg/dl]	44	0.8	24.9	11.3 (±3.6)
fT4 [ng/dl]	44	0.4	2.3	1.4 (±0.4)
T3 [μg/l]	29	0.5	2.3	1.5 (±0.3)
T4/T3 ratio	27	0.5	10.6	7.5 (±2.0)

Se levels ranged from 33 to 148 μg/l (Table 1). There are no accepted reference ranges for Se concentrations in children. As judged by criteria for Se adequacy [23], concentrations of >20 μg/l are considered as sufficient to protect from endemic Se deficiency disease risks described in the severely Se-deficient areas of Asia. From studies in adults, it is established that concentrations of 80–95 μg/l are needed for the full expression of the circulating selenoproteins glutathione peroxidase-3 and selenoprotein P [23]. According to a published reference interval for children, Se concentrations have been considered normal in the range of 70–150 μg/l for children 1–10 y old, and 95–165 μg/l for children older than or equal to 11 y old [24]. We decided to consider Se concentrations of <70 μg/l as an indicator of Se deficiency, and <20 μg/l as severely Se-deficient. None of our children had to be classified as severely Se-deficient, but half of them displayed Se concentrations below 70 μg/l.

In accordance with the study aim, several children displayed highly elevated TSH concentrations, while a normal TSH was present in the majority of children as they are routinely treated by T4 substitution (Fig. 1A). The strongly elevated TSH values observed in some of the patients were obviously due to insufficient compliance with the therapy.

High TSH was accompanied by low fT4 indicative of a normal thyroid feedback axis in the children under analysis (Fig. 1B).

Se concentrations were unrelated to age (Fig. 2A). In comparison, serum Cu was negatively associated with age, in accordance with a large study from China of >4000 children [25]. This large study reported a reference interval for blood Cu of 11.8–39.3 μM (corresponding to 749–2496 μg/l), indicating a similar pattern of Cu status in the Chinese and German children. In CH, the Cu concentrations ranged from 748 to 2588 μg/l, with some subjects displaying particularly high values in comparison to a prior analysis of German children (Fig. 2B) [26]. With respect to a case-control study on Cu concentrations in children with Wilson's disease [27], who accumulate Cu in liver and show low Cu concentrations in serum (515 ± 14 μg/l), none of our children has to be considered as severely Cu-deficient. The Cu/Se ratio was negatively associated with age, but the relation was not more significant as compared to Cu and age alone (Fig. 2C).

In experimental animals [5] and humans [6], we have previously reported on a direct association of TH with serum Se concentrations. Testing this association in our group of children with CH, no significant associations of serum Se with T3 (Fig. 3A), T4 (Fig. 3B), TSH, fT4, or the T4/T3 ratio (not shown) were observed when analysed by Spearmen rank correlation. However, a strong positive correlation of TH with serum Cu levels was evident, both for the active hormone