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ORIGINAL CONTRIBUTION

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Concentrations of thyroid hormones in serum and activity of hepatic 5′monodeiodinase in copper-deficient rats

Konzentrationen der Schilddrüsenhormone im Serum und Aktivität der 5′Monodeiodase in der Leber von Kupfermangelratten

Summary The aim of the present study was to investigate the effect of copper deficiency on thyroid hormone metabolism in rats. Therefore, an experiment with growing male Sprague-Dawley rats was carried out, consisting of two groups of rats fed either a copper-deficient (0.06 mg Cu/kg) or a copper-adequate diet (16 mg Cu/kg). Both groups of rats were fed identical quantities of diet by pair-feeding. Copper deficiency decreased the final body weight of the rats by 5 % compared to copper-adequate control rats. A severe copper-deficient state in the rats fed the copper-deficient diet was proved by a large decrease of ceruloplasmin activity in serum (by 97 %) and hematological changes.

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For estimation of thyroid hormone metabolism, the concentrations of total and free thyroxine (T_4) and triiodothyronine (T_3) in serum and the activity of hepatic 5′monodeiodinase (5′D) were determined. Copper-deficient rats had an increased concentration of T_3 in serum, whereas the concentrations of total and free T_4 as well as the activity of hepatic 5′D were not different compared with copper-adequate control rats. Therefore, the study shows that copper deficiency has only slight effects on thyroid hormone metabolism in growing rats.

Zusammenfassung Ziel der vorliegenden Arbeit war es, den Einfluß von Kupfermangel auf den Stoffwechsel der Schilddrüsenhormone bei der Ratte zu untersuchen. Dazu wurde ein Versuch mit männlichen wachsenden Sprague-Dawley-Ratten durchgeführt. Der Versuch beinhaltete zwei Gruppen, die entweder eine Kupfermangeldiät (0,06 mg Cu/kg) oder eine Kontrolldiät (16 mg Cu/kg) erhielten. Die Ratten beider Gruppen erhielten dabei durch ein Pair-feeding Regime identische Futtermengen.

Die Tiere der Kupfermangelgruppe waren zu Versuchsende um 5 % leichter als die der Kontrollgruppe. Die Kupfermangeltiere hatten darüber hinaus eine stark verminderte Aktivität des Ceruloplasmins im Serum (um 97 %) sowie veränderte hämatologische Parameter, was einen starken Kupfermangel bei diesen Tieren belegte.

Zur Abschätzung des Stoffwechsels der Schilddrüsenhormone wurden die Konzentrationen des gesamten und des freien Thyroxins (T_4) und des Triiodthyronins (T_3) im Serum sowie die Aktivität der 5′Monodeiodase (5′D) in der Leber bestimmt. Die Kupfermangeltiere hatten eine erhöhte Konzentration an T_3 im Serum, während sowohl die Konzentration an freiem und am gesamten T_4 als auch die Aktivität der 5′D in der Leber nicht verändert waren. Insgesamt zeigt die vorliegende Untersuchung, daß auch sehr starker Kupfermangel nur geringfügige Änderungen des Stoffwechsels der Schilddrüsenhormone bei wachsenden Ratten verursacht.

Key words Copper deficiency – rat – thyroxine – triiodothyronine – 5′Monodeiodinase

Schlüsselwörter Kupfermangel – Ratte – Thyroxin – Triiodthyronin – 5′Monodeiodase

Abbreviation index BSA = Bovine serum albumin · *Exp.* = experiment · f T_4 = free thyroxine · 5′D = 5′Monodeiodinase · *T4* = Thyroxine · T_3 = Triiodothyronine

Introduction

Trace elements have important metabolic functions, particularly as components of enzymes and hormones (16). Relationships between trace elements and thyroid hormone metabolism have been reported for iodine, which is an integral component of thyroxine (T_4) and triiodothyronine (T_3) (14, 22) and for selenium as a component of type-I-deiodinase (1, 2, 23). In contrast, there is less information about possible functions of other trace elements in thyroid hormone metabolism. Therefore, in a series of experiments, we have examined the effects of iron, zinc and manganese deficiency on thyroid hormone metabolism in rats (8, 17–19). Additionally, we have carried out an unpublished experiment to investigate the effect of copper deficiency on thyroid hormone metabolism. In that experiment, copper deficient rats had significant reduced concentrations of thyroxine in serum as well as a reduced activity of hepatic deiodinase. However, copper-deficient rats also had reduced food intake and reduced body weight gain compared with copper-adequate controls. Since reduced energy intake markedly affects thyroid hormone metabolism (5, 12, 15, 26), it cannot be elucidated whether those effects were due to copper deficiency *per se* or to reduced energy intake. The aim of the present study was to investigate the effects of copper deficiency on thyroid hormone metabolism in rats without the confounding effect of reduced energy and nutrient intake. Therefore, copper-deficient and copper-adequate control rats were pair-fed identical quantities of diet. In order to assess the physiological state of the rats, apart from parameters of thyroid hormone metabolism, also hematological and some clinical-chemical parameters were determined.

Material and methods

Twenty-four male specific-pathogen free Sprague-Dawley rats with an average body weight of 40 ± 2 g (SAVO GmbH, Kisslegg, Germany) were divided into two groups of 12 animals each. Both groups were fed a semisynthetic diet with casein as protein source (Table 1). The copper concentration of the basal diet was 0.06 mg/kg. The copper-adequate diet was supplemented with $CuSO_4 \cdot 5H_2O$ to give a copper concentration of 16 mg/kg. In order to guarantee identical food intake, in a pair-feeding regime the rats of the control group were fed the amount consumed by copper-deficient rats the day before. All animals had free access to water (deionized water adjusted by the addition of 0.014 % NaCl suprapure to the average osmolarity of tap water).

The rats were housed individually in Macrolon cages in a room maintained at 23 °C with a humidity of 60 % and a 12-h light-dark cycle. After the experimental period of 40 days, all the animals were killed by decapitation after a light anesthesia with diethyl ether, and blood was collected into polyethylene tubes. Serum and liver samples were stored at -80 °C until analyzed. An aliquot of the blood samples was immediately analyzed for hemoglobin concentration, erythrocyte count, hematocrit and mean corpuscular volume (MCV) using a Coulter Counter and a Coulter Hemoglobinometer (Coulter Electronics, Krefeld, Germany). Clinical-chemical parameters (concentrations of albumin, creatinin, total protein, urea, uric acid and activity of alkaline phosphatase) in serum were measured with an automatic analyzer (Model 704, Hitachi, Tokyo, Japan) and commercially available test kits (Boehringer-Mannheim, Germany).

Table 1 Composition of the basal experimental diet

Component	Amount (g/kg)
Casein (EDTA purified)	200
Corn starch	328
Sucrose	300
Fiber (cellulose)	30
Coconut oil	65
Safflower oil	15
Mineral mixture*	40
Vitamin mixture*	20
DL methionine	2

* Minerals and vitamins were added in sufficient amounts as described by Eder et al. (7) with the exception of copper (see text)

For estimation of the copper status of the rats, activity of ceruloplasmin was determined using the method of Curzon and Speyer (4) as modified by Graßmann (10). For determination of copper concentrations in the diets, aliquots of the diets were wet ashed and copper concentrations thereafter were determined by flame atomic absorption spectrophotometry (AAS; Perkin-Elmer, Überlingen, Germany).

Concentrations of T_3, T_4 and free T_4 (fT_4) in serum were determined using commercially available ELISA kits (Boehringer-Mannheim, Germany). Activity of hepatic 5'D was determined by measuring the release of T_3 from T_4 during an incubation period. The determination was based on a method by Visser et al. (27). 3.0 g liver tissue and 9 mL bovine serum albumin solution (BSA, 7 %) were homogenized with a Potter-Elvehjem-homogenizer (B. Braun, Melsungen, Germany) at 1 200 rpm for 30 s. BSA as a constituent of the homogenizing solution was necessary for complete recovery (> 98 %) in the measurement of thyroid hormones by ELISA. 2 mL homogenate and 20 µL T_4 solution (100 mg thyroxine/L, Merck, Darmstadt, Germany, in 5 mM NaOH) were incubated at 37 °C for 10 min. The reaction was stopped by heat denaturation of the protein at a temperature of 65 °C. After centrifugation (1 100 g, 4 °C, 10 min) the supernatant was used to determine T_3 concentration. Each sample was run in duplicate. The results were cor-

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rected for origin T_3 and non enzymatic conversion of T_4 to T_3.

For statistical evaluation of the data, means were compared using Student's *t*-test. The results in the tables are given as means ± standard deviations of individual values.

Results

At the end of the experiment, copper-deficient rats had a lower body weight than copper-adequate control rats (287 ± 12 g vs. 300 ± 16 g, $p < 0.05$), whereas the food intake was identical within both groups of rats (568 g) meaning that copper-deficient rats had a slightly lower food conversion (0.44 ± 0.03 vs. 0.46 ± 0.03 g body weight gain/g food).

Hematological and clinical-chemical parameters, including the activity of ceruloplasmin are shown in Table 2. Rats fed the copper-deficient diets had a largely reduced activity of ceruloplasmin in serum (-97 %) proving their copper-deficient state. Copper-deficient rats also developed an anemia characterized by reduced hemoglobin concentration, hematocrit and erythrocyte count, whereas the mean corpuscular volume of erythrocytes was not different between the two groups of rats. Regarding clinical chemical parameters determined in serum, copper deficiency influenced merely the concentration of creatinine, whereas the concentrations of total protein, albumin, urea and uric acid as well as the activity of alkaline phosphatase were not influenced by copper deficiency.

Concentrations of thyroid hormones and activity of hepatic 5'D are shown in Table 3. Copper deficiency increased the concentration of T_3. In contrast, the concentrations of T_4 and fT_4 as well as the activity of 5'D were not influenced by copper deficiency.

Table 2 Hematological and clinical-chemical parameters of copper-adequate and copper-deficient rats*

Parameter	Cu-adequate	Cu-deficient
Hematological parameters		
Hemoglobin (g/dL)	10.3 ± 0.8	8.1 ± 1.0+
Hematocrit (%)	32.2 ± 2.9	26.8 ± 3.9+
Erythrocyte count (10^{12}/L)	6.6 ± 0.4	5.3 ± 0.6+
Mean corpuscular volume (μm^3)	48.6 ± 1.4	50.6 ± 2.7
Clinical-chemical parameters		
Ceruloplasmin ($\Delta E \cdot mL^{-1} \cdot h^{-1}$)	19.2 ± 5.2	0.5 ± 0.3+
Alkaline phosphatase (U/L)	650 ± 157	572 ± 191
Total protein (g/dL)	6.05 ± 0.22	6.25 ± 0.19
Albumin (g/dL)	3.62 ± 0.17	3.66 ± 0.16
Urea (mg/dL)	37.2 ± 6.6	42.1 ± 8.0
Uric acid (mg/dL)	2.03 ± 0.35	2.25 ± 0.28
Creatinine (mg/dL)	0.51 ± 0.02	0.47 ± 0.02+

*Results are means ± STD; +Significant ($p < 0.05$) different means

Table 3 Concentrations of thyroid hormones in serum and activity of hepatic 5'monodeiodinase (5'D) in liver of copper-adequate and copper-deficient rats*

Parameter	Cu-adequate	Cu-deficient
T_3 (nmol/L)	1.41 ± 0.25	1.97 ± 0.22+
T_4 (nmol/L)	25.7 ± 2.8	28.1 ± 7.7
fT_4 (pmol/L)	43.8 ± 5.5	41.4 ± 5.0
5'D (pg $T_3 \cdot$ mg protein$^{-1} \cdot$ min^{-1})	51.5 ± 29.7	51.4 ± 23.1

*Results are means ± STD; +Significant ($p < 0.05$) different means

Discussion

In a pre-study, we observed markedly reduced concentrations of total and free T_4 as well as a largely reduced activity of hepatic 5'D in growing severe copper-deficient rats. However, the copper-deficient rats of that study consumed less diet than the *ad libitum*-fed copper-adequate control rats. Since the energy intake markedly influences the metabolism of thyroid hormones, particularly concentration of T_3 (5, 26) and deiodination of T_4 to T_3 (12, 15), it cannot be elucidicated whether the effects were due to copper deficiency *per se* or to reduced energy intake in copper-deficient rats. In order to guarantee identical food intake, in the present study copper-deficient and copper-adequate control rats were fed identical quantities of diet by pair-feeding, and therefore the effects of copper-deficiency could be investigated without the distortion by reduced food intake. The severity of copper deficiency was confirmed by a large decrease in the activity of ceruloplasmin which is a copper-containing enzyme (6) and an anemia which is a typical symptom of severe copper deficiency (9, 11, 13). In contrast to hematological parameters, clinical-chemical parameters were only slightly affected by copper deficiency, and remained within the physiological range given for growing rats (24).

The present study clearly reveals that copper deficiency has only slight effects on thyroid hormone metabolism in growing rats. The only effect of copper deficiency was an increased concentration of T_3 in serum, whereas concentrations of total and free T_4 were not influenced by copper deficiency. An increased concentration of T_3 might be of physiological significance because T_3 is the most active thyroid hormone (4). More than 80 % of circulating T_3 are formed by the action of deiodinase (20). Since the activity of hepatic deiodinase was not altered by copper deficiency, it is likely that copper disturbs the catabolism of T_3 rather than its formation by deiodination. It is well known that copper deficiency affects iron metabolism (11). Iron deficiency also has been shown to influence thyroid hormone metabolism (3, 17, 25). However, it is unlikely that the increased concentration of T_3 in copper-deficient rats is due to an

interaction with iron, because iron deficiency does not increase but lowers the concentration of T_3, and additionally markedly influences the concentrations of total and free T_4 as well as the activity of hepatic 5'D (17).

In clear contradiction with the present study, Lukaski et al. (21) observed in both severe and marginal copper-deficient adult male rats reduced concentrations of T_3 and T_4 as well as reduced activity of deiodase in liver and brown adipose tissue. In that experiment, the effect was due to copper deficiency, because copper-deficient and -adequate rats had similar food intake and final body weights. Since the severity of copper deficiency, regarding the copper concentrations of the diets and the reduction of coeruloplasmin, was larger in the present study than in the study of Lukaski et al. (21), this contradiction can be explained only by the age of the animals.

In conclusion, the present study shows that even severe copper deficiency has only slight effects on thyroid hormone metabolism in growing rats.

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