

## The Effects of Oral Levothyroxine Sodium Application on Serum Copper Concentration in Rabbits

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### Summary

This study was carried out to determine the effect of oral Levothyroxine Sodium (T<sub>4</sub>) applications with oral CuSO<sub>4</sub> (0.1%) on serum copper (Cu) concentration. Forty male New Zealand rabbits (6 month-old, weighing 2.29±0.06 kg) were allocated to four groups. All groups received 0.1% CuSO<sub>4</sub> (\*5H<sub>2</sub>O) (Copper (II) sulfate pentahydrate M102787) in drinking water with the beginning of the study. T<sub>4</sub> was applied orally to three experimental groups at the doses of 1.67, 3.33 and 6.67 µg/kg-BW/day except control group. Serum Cu measurements were performed by Atomic Absorption Spectrometer equipped with Flame system. Serum Cu levels were increased on the following days and correlated with applied dose of T<sub>4</sub> (r=0.832, P<0.001). The T<sub>4</sub> affected serum Cu level with minimum r<sup>2</sup>=56.9% and P<0.001. The effect of time was found significant on serum Cu levels (P<0.001). There was also an interaction between time and groups on serum Cu levels (P<0.001). Consequently, T<sub>4</sub> caused an increase in serum Cu level at second day and this increase continued on the following days.

**Keywords:** *Thyroxine, Serum, Cu, Experimental treatment, Rabbit*

## Tavşanlarda Oral Levotiroksin Sodyum Uygulamasının Serum Bakır Konsantrasyonu Üzerine Etkileri

### Özet


Bu çalışma oral %0.1'lik CuSO<sub>4</sub> ile birlikte Levotiroksin Sodyum (T<sub>4</sub>) uygulamalarının serum Cu konsantrasyonu üzerindeki etkisinin tespit edilmesi için yapılmıştır. Kırk adet erkek Yeni Zelanda ırkı tavşan (6 aylık, ortalama ağırlıkları 2.29±0.06 kg) dört gruba ayrıldı. Bütün grupların içme sularına %0.1 CuSO<sub>4</sub> (\*5H<sub>2</sub>O) çalışmanın başından itibaren eklendi. Üç çalışma grubuna, kontrol grubu hariç 1.67, 3.33 ve 6.67 µg/kg-CA/gün dozunda T<sub>4</sub> oral olarak uygulandı. Serum Cu ölçümleri Alev sistemli Atomic Absorbsiyon Spektrofotometre ile yapıldı. Takibenden günlerde serum Cu düzeyleri arttı ve uygulanan T<sub>4</sub> miktarı ile serum Cu düzeyi arasında korelasyon tespit edildi (r=0.832, P<0.001). T<sub>4</sub>, serum Cu düzeyini en küçük r<sup>2</sup>=56.9% ve P<0.001 düzeyinde etkiledi. Serum Cu düzeyleri üzerinde zamanın etkisi önemli bulundu (P<0.001). Aynı zamanda serum Cu düzeyleri üzerinde zaman ve gruplar arasında etkileşim vardı (P<0.001). Sonuç olarak, T<sub>4</sub> serum Cu düzeyinde ikinci gün artışa sebep oldu ve artan bu düzey T<sub>4</sub> uygulamalarının devamı ile birlikte sürdü.


**Anahtar sözcükler:** *Tiroksin, Serum, Cu, Deneysel uygulama, Tavşan*


### INTRODUCTION

Diseases or stressful conditions induce fluctuations on serum Cu level<sup>1-6</sup>. Territories with low Cu in soil and grass also cause animals to suffer from Cu deficiency<sup>7-10</sup>. Similarly, different time periods of the year may be effective on serum Cu levels<sup>7,11,12</sup>. Cu is involved in Cu/Zn-SOD and ceruloplasmin in human, and Cu deficiency may cause poor activation of these enzymes<sup>13-15</sup>. And this organism may need oral Cu supplementation<sup>7,16-18</sup>.

Serum Cu levels are affected by intestinal absorption, urinary excretion and metabolism rate. It is reported that the intestinal absorption of Cu is limited. Absorption rate of Cu in diet is known as 5-10% for adult animals and 15-30% for young<sup>7,19</sup>. Therefore, improved Cu absorption gained importance. Active transport mechanism is important for intestinal Cu absorption. This mechanism needs energy and is related to the

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thyroidal hormones (TH) <sup>20,21</sup>.

TH status influences a number of functions in the body including energy metabolism <sup>21-26</sup>. Thyroxin is a relatively inactive form of thyroidal hormones and used in the clinical practice in patient with hypothyroidism or extirpation of thyroidal glands <sup>21,25,27</sup>.

Intestinal Cu absorption increases as the Cu requirements also increase <sup>19</sup>. Increased serum Cu levels in a short time are desired for effective therapy. Copper deficiency usually results from malabsorptive disorders <sup>28</sup>. Thyroidal hormones can affect absorption, excretion and metabolism of serum Cu. Therefore the aim of this study was to investigate the effect of Levothyroxine sodium with oral CuSO<sub>4</sub> (0.1%).

## MATERIAL and METHODS

### Animals and Procedures

The study was approved Ethics Committee of University of Kafkas (Approval No. 2009-21). Clinically healthy 40 male New Zealand Rabbits, aged 6 months and weighing 2.29±0.06 kg were used in this study. The animals were divided into four equal experimental groups. Levotiroksin Sodyum (T<sub>4</sub>) (Levotiron tablet®) orally applied and treatment groups were as follows; Group A was kept as control and received tap water only, Group B received T<sub>4</sub> at 1.67 µg/kg-BW/day, Group C: 3.33 µg/kg-BW/day and Group D: 6.67 µg/kg-BW/day in tap water for four days. T<sub>4</sub> applications were given at ten o'clock daily at a single dose per day. 0.1% Copper (II) sulfate pentahydrate (CuSO<sub>4</sub>\*5H<sub>2</sub>O, Merck 102787) in drinking water were given *ad libitum* to all groups including group A. The applications of T<sub>4</sub> and Cu were given simultaneously at the same time.

The animals were fed with a commercial animal food. The food and water were given *ad libitum* before and during the experiment. Copper content of food and water was determined by flame system Atomic Absorption Spectrometer (FAAS) (Thermo Elemental S4, Thermo Electron Corporation, Cambridge, UK) <sup>29,30</sup> and the results were as follows 12.13 mg/kg in dry matter (DM) of food and 0.013 mg/L of tap water.

The commercial food was purchased from Bayramoğlu Yem ve Un San. Tic. A.Ş. ISO 9001:2000, ISO 22000:2005 and the composition is given in [Table 1](#).

### Blood Collections and Laboratory Analyses

Two milliliters of the blood was collected via

**Table 1.** Ingredients of diet given to rabbits

**Tablo 1.** Tavşanlara verilen rasyon içeriği

Diet composition	Units
Dry matter (%)	88
Crude protein (%)	17
Crude cellulose (%)	12
Crude ash (%)	10
Acid insoluble ash (%)	1
Calcium (%)	1.5
Phosphorus (%)	0.75
NaCl (%)	0.6
Vitamin A (IU/kg)	5000
Vitamin D3 (IU/kg)	600
Vitamin E (mg/kg)	25
Metabolic energy (kcal/kg)	2600

*Raw-materials for this composition: Barly, corn, corn chaff, corn glutelin, wheat, rye, craff, cottonseed meal, sunflower meal, dicalcium phosphate, vitamin, mineral*

\*:  $P < 0.05$

cardiac puncture under the ether anesthesia. Serum was separated by centrifugation at 3500 rpm for 15 min and serum Cu contents were also determined by FAAS <sup>29,30</sup>. Standard solutions for Cu were purchased from Fluka Chemie GmbH, Switzerland (Fluka 61147).

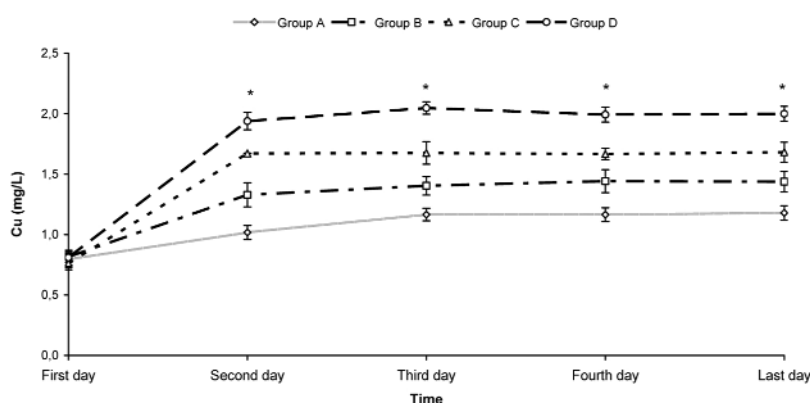
Accuracy Control of FAAS was performed using a previously known standard solution for Cu measurement. This solution was aspirated for 6 times per 10 samples during analyses and the Cu levels were measured. Coefficient of variation (CV) for Cu was calculated from these measured levels. CV for Cu was found to be 3.47% <sup>29,30</sup>. All lab-ware used were made of PTFE material in the laboratory.

### Statistical Analysis

The effect of time and interactions between time and T<sub>4</sub> dose on serum Cu level analyzed by repeated measurements ANOVA. One-Way ANOVA were performed for comparisons of days in each T<sub>4</sub> groups (groups A, B, C and D). One-Way ANOVA were also used to compare the serum Cu levels between groups in days (for first, second, third, fourth and last days). Duncan test for all ANOVA analyses was employed for Post Hoc Multiple comparisons between groups <sup>31</sup>. Pearson correlation test was used to determine the relationship between the dose of T<sub>4</sub> and individual average values of serum Cu levels of days. Correlations between groups receiving four different doses of T<sub>4</sub> (group A, B, C and D) were also analyzed by Pearson correlation test. Linear regression analyses were employed for the effect of T<sub>4</sub> on serum Cu levels. Statistical analyses were performed using SPSS statistical software version 10.0.1 <sup>32</sup>. Data were presented as means ± S.E.M.

## RESULTS

The serum Cu levels of groups according to time are illustrated in *Fig 1*. The Cu levels of all groups increased on second day and maintained their levels on the following days. The increase of serum Cu levels were correlated with the dose of T<sub>4</sub> applied ( $r=0.832$ ,  $P<0.001$ ) (*Fig 2*). ANOVA test with multiple comparisons employed for analyses of differences between days (groups for days of first-last) for each T<sub>4</sub> groups. Except for first day ( $P<0.001$ ), there were no significant difference among the days for each T<sub>4</sub> group ( $P>0.05$ ) (*Fig 1*).



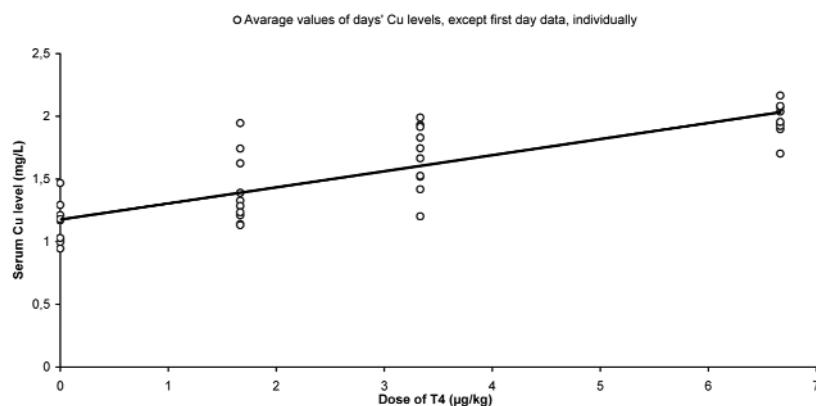
**Fig 1.** The serum Cu levels of groups according to time

**Şekil 1.** Grupların zamana göre serum Cu düzeyleri

\*: The differences between the values in the same day are significant ( $P<0.05$ )

**Fig 2.** Relationship between T<sub>4</sub> amount applied and serum Cu levels ( $r=0.832$ ,  $P<0.001$ )

**Şekil 2.** Uygulanan T<sub>4</sub> miktarı ile serum Cu düzeyleri arasındaki ilişki ( $r=0.832$ ,  $P<0.001$ )



**Table 2.** Regression analyses results to display effect of T<sub>4</sub> amount ( $\mu\text{g}/\text{kg}$ ) on serum Cu level

**Tablo 2.** T<sub>4</sub> miktarının ( $\mu\text{g}/\text{kg}$ ) serum Cu düzeyi üzerine etkisini ortaya koymak için regresyon analiz sonuçları

Response	Regression Equations	r <sup>2</sup> (%)
First day serum Cu level (mg/L)	First day = 0.795 - 0.0006 Applied T <sub>4</sub>	0.0
Second day serum Cu level (mg/L)	Second day = 1.09 + 0.137 Applied T <sub>4</sub>	56.9*
Third day serum Cu level (mg/L)	Third day = 1.19 + 0.132 Applied T <sub>4</sub>	70.2*
Fourth day serum Cu level (mg/L)	Fourth day = 1.21 + 0.122 Applied T <sub>4</sub>	61.4*
Last day serum Cu level (mg/L)	Last day = 1.22 + 0.122 Applied T <sub>4</sub>	66.4*

\*:  $P<0.001$ , Predictor was amount of T<sub>4</sub> ( $\mu\text{g}/\text{kg}$  BW)

Serum Cu levels among groups (A-D) within particular days (first-last days) were significantly different ( $P < 0.05$ ), except for first day ( $P > 0.05$ ) (Fig 1). There were correlations between T<sub>4</sub> groups during experimental days (Table 3).

**Table 3.** Correlation test results of T<sub>4</sub> groups (r), n=50 (10 animals for each groups and for 5 days)

**Table 3.** T<sub>4</sub> gruplarına göre korelasyon testi sonuçları (r), n=50 (5 gün için her bir gurup 10 hayvan)

Group	Group A	Group B	Group C
Group B	0.387**		
Group C	0.550**	0.338*	
Group D	0.537**	0.596**	0.767**

\*:  $P < 0.05$ , \*\*:  $P < 0.01$

## DISCUSSION

Thyroxine is an important factor for energy metabolism in the body<sup>22-26</sup>. Intestinal Cu absorption requires energy for active transport<sup>20</sup>. Levothyroxine sodium (T<sub>4</sub>) applications can affect the intestinal Cu absorption. The aim of this study was to investigate the effect of orally applied T<sub>4</sub> on serum Cu levels.

Several literatures indicated that intestinal Cu absorption could be increased by additives or some manipulations<sup>33-35</sup>. The Cu level was increased in group A which is control group taking only CuSO<sub>4</sub> without T<sub>4</sub>. In our opinion, these results could indicate that 5-30% of dietary Cu is readily absorbed during oral CuSO<sub>4</sub> treatment<sup>19</sup>. However, application of increasing T<sub>4</sub> elevates serum Cu levels. Increased serum levels of Cu observed in groups B, C and D could be associated with the effect of T<sub>4</sub> on intestinal Cu absorption via improving active transport of Cu<sup>19,20,22-26</sup>.

It was reported that low level thyroidal hormones (TH), induced by thyroparathyroidectomy (TPYT), cause increased serum Cu levels<sup>34</sup>. Contrarily, findings of this study showed that T<sub>4</sub> fortifications increased serum Cu level. Leblondel et al.<sup>34</sup> reported that, T<sub>4</sub> administration, partially decreased the serum Cu level. The possible cause of this difference between the current and the previous study may be due to purge of all thyroidal and para-thyroidal hormones performed in previous study. Other hormones may also affect the serum Cu levels. In addition extra CuSO<sub>4</sub> application in drinking water in our study could induce the serum Cu level elevation.

Liver is a major storage and also a central organ for

Cu metabolism. Therefore, mineral content of liver tissue is a good indicator for the mineral status of the organism such as serum levels<sup>19,36</sup>. In a similar study, Swick et al.<sup>35</sup> reported that addition of 5% Senecio jacobaea into a diet containing 100 µg/g Cu and 100 µg/g Zn cause an increase in Cu and decrease in Zn levels of liver tissue in the rabbit. Senecio jacobaea is a very common wild flower in the family of Asteraceae that is found throughout Europe. In our study, findings showed a similar course for serum Cu level. Serum Cu level was increased with concomitant supplementation of CuSO<sub>4</sub> and T<sub>4</sub> in the rabbit, as the results of fortified diet with Cu and Senecio jacobaea<sup>35</sup>.

Interactions between minerals are well known<sup>19,37,38</sup>. T<sub>4</sub> may be effective on other minerals and the levels of these minerals affect the serum Cu level. In this relation, Domellöf et al.<sup>39</sup> reported that Fe supplements are recommended for therapy or fortification. It was also known that Fe supplementation may have inhibitory effect on Cu absorption. However, Dolmellöf et al.<sup>39</sup> reported that no significant effect of Fe supplementation on intestinal Cu absorption was observed. Similarly, the effect of aluminum chloride on tissue Cu level was studied. The results showed that oral aluminum chloride treatment did not affect tissue Cu levels of stomach, intestinal mucosa, kidney and bone<sup>33</sup>. Our findings apparently showed that CuSO<sub>4</sub> supplementation with or without T<sub>4</sub>, increased the serum Cu level, but interaction between other minerals is possible. Therefore, further investigations are needed to clarify this condition.

The applications of both CuSO<sub>4</sub> and T<sub>4</sub> were started together on first day in this study. As expected there was no statistically significant difference in Cu levels for the first day. After day one, serum Cu levels were increased in all groups, but these increases were correlated to dose of oral T<sub>4</sub> applications. In our opinion, the possible cause of this correlation was due to the effect of T<sub>4</sub> on active transport system for intestinal Cu absorption<sup>25,26</sup>.

Average plane courses for all T<sub>4</sub> groups were continued forward until the last day. Excretion and absorption of Cu might be balanced by the dose of T<sub>4</sub> during treatment days. Because urinary excretion is also under the effect of thyroidal hormones (TH). The effect of T<sub>4</sub> on renal system raises the urinary out put by affecting the cardiovascular system<sup>21-24</sup>.

As the results of this study, oral T<sub>4</sub> with CuSO<sub>4</sub> in drinking water increased serum Cu level in a dose-

dependent manner. In our opinion, probable consequences of this situation as follows;

- T<sub>4</sub> is widely used for the therapy of hipo-thyroidial functional disorders. During this cure, the diet can include high or normal levels of Cu. T<sub>4</sub> may elevate the serum Cu level and cause Cu intoxication.

- T<sub>4</sub> may be used for the therapy of Cu deficiency in a short time.

- Furthermore, in some cases, Cu deficiencies may be a result of thyroxine hormone insufficiency and the fortification of T<sub>4</sub> may help to heal serum Cu disorders. In these situations cases, thyroxine hormone measurements can also be helpful for the Cu insufficiency, diagnosis and therapy.

Further investigations are needed to clarify these conditions.

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