# THE EFFECT OF VARIOUS DIETARY CALCIUM CONCENTRATIONS ON THE BIOLOGICAL INTERACTIONS OF ZINC AND CALCIUM IN RATS

## Ratlarda kalsiyum ve çinkonun biyolojik etkileşimine diyetteki farklı kalsiyum konsantrasyonlarının etkisi

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***Summarty:*** *The purpose of this study was to clarify the influence of calcium on zinc status. The animals were divided into four groups consisting of five rats in each group. The control group was fed on basal diet with adequate levels of zinc and calcium while the experimental diets containing different levels of calcium ad libutum for fifteen days. Low calcium significantly increased the absorption percentage but there was an decrease in high and very high calcium groups . The retention percentage changes were found parallel to the changes in the absorption percentage curve. Zinc and Zn-65 distributions of duodenum, jejunum and ileum increased in low calcium group while they decreased in the others. However, these changes were significant only for duodenum. When the zinc distribution expressed per gram of dry tissue, an increase in the kidney zinc content in the low calcium group but a decrease in the high and very high calcium groups was observed. These results suggest that calcium affects zinc status in rats.*

***Key Words*** *: Calcium, Zinc, Zn-65, Zinc absorption, Mineral-mineral interactions*

***Özet:*** *Bu çalışmanın amacı, çinko konsantrasyonuna kalsiyumun etkisini araştırmaktır.Her bir grupta 5 rat olacak şekilde hayvanlar 4 gruba ayrılmıştır.Kontrol grup çinkosu normal olan bazal diyetle , deney grupları ise içerisinde farklı oranlarda kalsiyum bulunan diyetlerle ad libitum olarak beslenmişlerdir.Düşük kalsiyum alanlarda apsorbsiyonda önemli bir artış görülürken,yüksek ve çok yüksek kalsiyum alanlarda bir azalma meydana gelmiştir. Retansiyon eğrisindeki değişiklikler de apsorbsiyon eğrisindekilere benzemektedir.Çinko ve Zn-65 'in duedenum,jejunum,ve ileum seviyeleri düşük kalsiyum alanlarda artmış diğer gruplarda ise azalmıştır.Bu değişikliklerden sadece duedenumdaki önemli bulunmuştur.Kuru dokunun gramı başına düşen çinko ölçüldüğünde ise ,düşük kalsiyum alan grubun böbrek çinko muhteviyatında bir artış , yüksek ve çok yüksek kalsiyum alan gruplarda bir azalma gözlenmiştir. Bu sonuçlar, vücut çinko konsantrasyonuna diyetle alınan kalsiyum miktarının etkili olduğunu göstermektedir.*

***Anahtar Kelimeler:*** *Kalsiyum, Çinko, Zn-65, Çinko absorbsiyonu, Mineral-mineral etkileşimi*

Recognition of an antagonism of calcium to zinc has been made by various investigators. The initial reports (2,3,9,10) showed that on low zinc rations, an increase of dietary calcium concentration decreases weight gain and increases the severity of parakeratosis both of which could be overcome by increasing the zinc content of the diet (12). Other investigators, however, have not demonstrated a significant degree of interaction between calcium and zinc in the development of symptoms of parakeratosis (3,13). The number of more direct attempts to determine the action of calcium on zinc by using radio active Zinc-65 were limited and gave conflicting results. One of the most important reasons of this apparent confusion is most likely due to the different experimental conditions under which the studies were done. This makes it extremely difficult to evaluate the literature and reach a conclusion regarding the effect of calcium on zinc absorption.

In light of these facts, the objectives of this investigation were (i) to determine the effect of

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low, high and very high calcium concentrations on zinc absorption by measuring radioactive Zn-65 contents of all samples, (ii) to determine the possible changes in Zinc distribution of the organs and (iii) to find out where this interaction could take place in the intestinal segment.

## METHODS

In the experiments, 20 weaning Swiss albino rats, weighing 160±27 g, were used. The rats each (three experimental groups and one control group) and maintained in stainless steel cages. The control group was fed a diet containing 10.53 g Ca H PO4/Kg and ferric ammonium citrate of 150 mg/Kg. The diets of low calcium, high calcium and very high calcium groups, on the other hand, were supplemented by calcium carbonate of 10.53 mg/Kg, 20.81 g/Kg and 30.92 g/Kg, respectively. Experimental groups were given free access to the prepared diet and deionized water. The rats in both experimental and the control groups were fed for a period of 15 days and were injected with 2 μ Ci Zinc-65 (Zinc chloride, Amersham) in 0.2 ml sterile saline solution intramuscularly in the m.gastroknemius muscle of the right rear leg. After the Zinc-65 injection, each of the rats were transferred to a deionized stainless steel metabolism cage where food and water consumption were measured and urine and feces were collected quantitatively for a period of four days. All the rats were made to fast at the end of the fourth day for a period of 18 hours, then were slightly anesthetized to take whole blood from their carotid-arteries. In addition, their livers, kidneys, the three areas of their intestinal segment (duodenum, jejunum and ileum) were dissected and the wet-weight of these organs was measured. All the organs, feces and urine were stored under -20 C until measurement day.

*Determination of Zinc:* The zinc concentration in feces and tissues was determined by atomic absorption spectrophotometry (Hittachi Z-8000 Model) (5). Standard solutions were prepared with deionized water from a certified stock zinc solution containing 1 g Zn/l. Standard solutions produced identical standard curves.

After dried tissues and feces were reweighed, concentrated nitric acid (0.5 ml),hydrochloric acid (0.5 ml) and tridistilated deionized water (1 ml) were added to these tissues and feces of 100 mg. The zinc was determined by flame atomic absorption spectrophotometry after destruction of organic matter by alternately heating for 2 days at 70°C. The amount of zinc in dried feces and tissues was calculated from the formula.

*Determination of Radioactive Zn-65:* The radioactivity in tissues, feces, urine and whole blood was determined in an automatic gamma-well sintillation counter set at the photopeak for Zn-65. The radioactivity values were corrected for background and decay and then converted to nCi Zinc-65. The activity in tissues was expressed as a percentage of the injected Zn-65 dose per gram of tissue

*Calculations:* The true absorption of dietary zinc was calculated by the isotope dilution technique described by Weigand and Kirchgessner (11,12). The percentage absorption (A) of zinc was computed from the following formula :

$A= D- F + [F (sf/sm)] /D$

Where D= zinc i take per day; F= total fecal zinc excretion per day; sf= specific radioactivity of zinc in the feces; sm=specific radioactivity of zinc endogenous origin. In these experiments, the average value of the specific activities in the kidneys was taken as sm.

The daily absorption of zinc was computed from the formula;

$A(mg/day)= D-f + [F(sf/sm)]$

The daily zinc balance in the rats was calculated by subtracting the amount of zinc in the feces from the amount of zinc in the diet.

Zinc balance(mg/day)=D-E

The daily dietary zinc intake was used in calculating the apparent absorption of zinc.

Apparent absorption(%)=Zinc balance/D

The experimental data were analyzed by student's t-test and the values were given as - standard error

**Table 1.** The effect of dietary calcium on the body weight

| Groups | Control (n=5) | Low-Ca (n=5) | High-Ca (n=5) | Very High-Ca (n=5) |
|---|---|---|---|---|
| Ca/Zn ratio | 111/1 | 0.1/1 | 231/1 | 343/1 |
| Body weight at start (g) | 167±11.89 | 174±8.71 | 162±9.16 | 163±10.90 |
| Body weight on the 15th day (g) | 176±11.81 | 190±8.28 | 150±8.55 | 149±10.70 |

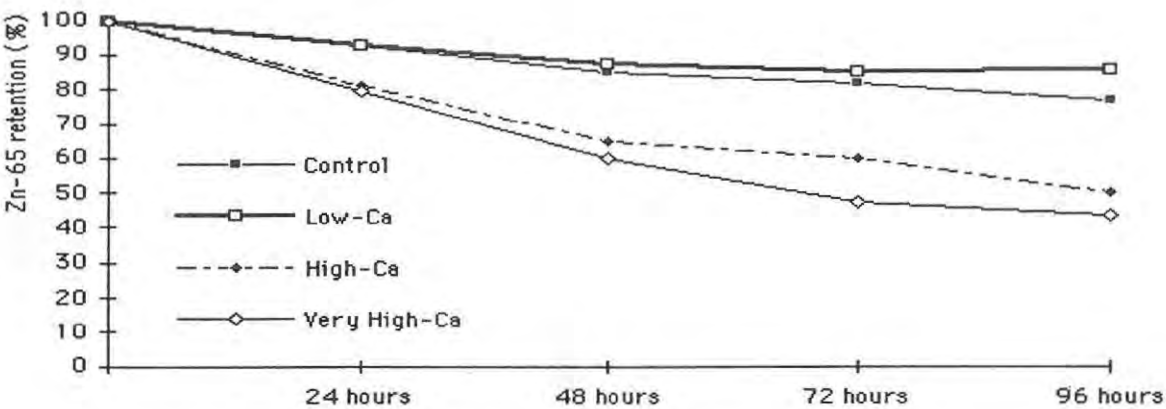


**Figure 1.** The effect of calcium on the zinc retention

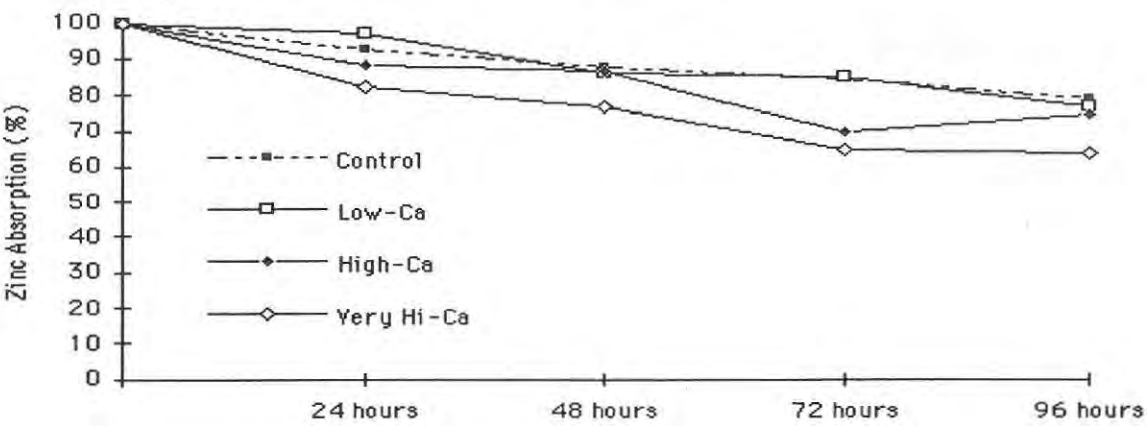


**Figure 2.** The effect of calcium on zinc absorption

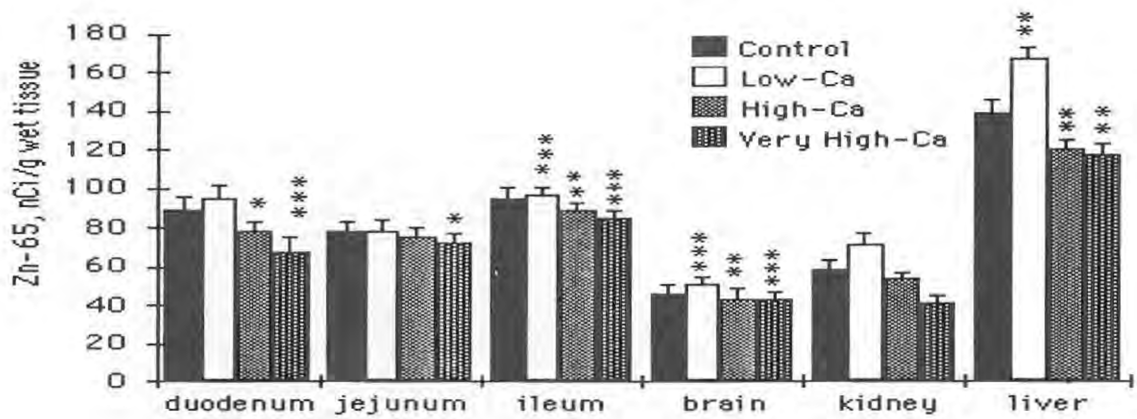


**Figure 3.** Zn-65 concentrations in tissues of rats (*p<0.02, **p<0.05, ***p<0.001)

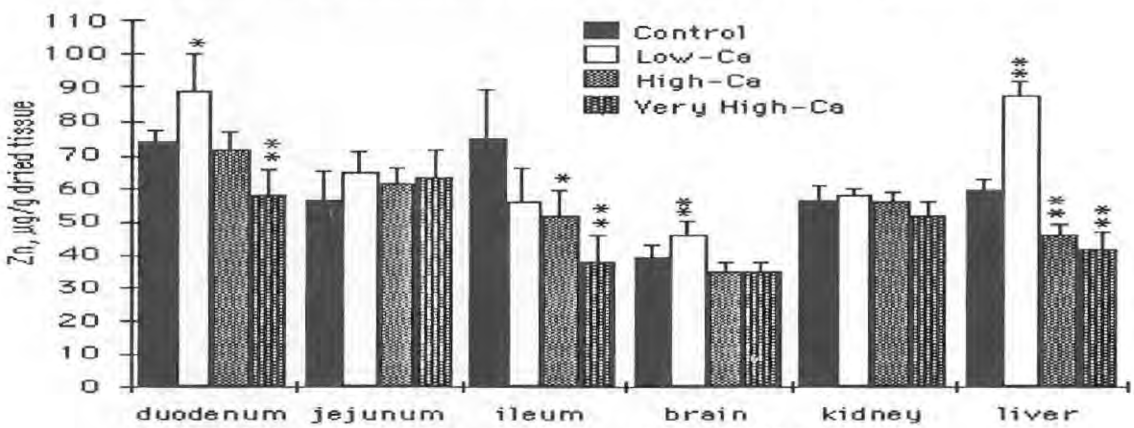


**Figure 4.** Zinc concentrations in tissues of rats (*p<0.05, **p<0.001)

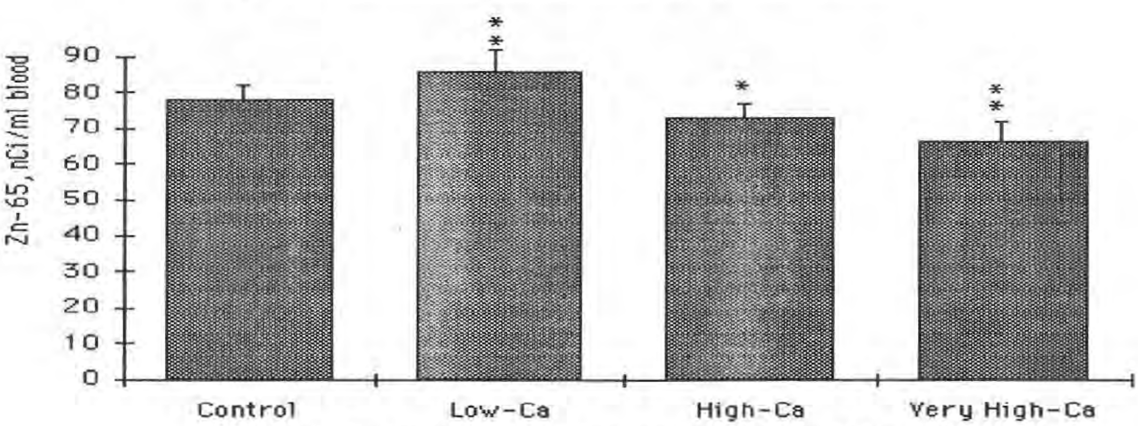


**Figure 5.** Zinc concentrations in blood of rats (*p<0.05, **p<0.001)

## RESULTS

*The effect of dietary calcium on the body weight:* When the means of the groups weights before and after dietary treatment were compared, it was seen that control and low calcium groups gained weight while high and very high calcium groups lost weight (Table 1).

*The effect of calcium on the absorption and retention of Zinc:* In the study, the effects of the three dietary levels of calcium on the retention, absorption and excretion of Zn- 65 in rats were determined ( Figure 1). It was observed that absorption of Zn-65 as a percentage was significantly increased in low calcium group while absorption of Zinc-65 was significantly reduced in the first four days. The retention curve was obtained by subtracting the amount of Zn-65 injected into the rats, the amount which was accepted as 100. Comparing the control, the decrease in the excretion of Zn-65 in the low calcium group and the increase in the zinc excretion of Zn-65 in the other groups were statistically significant (Figure 2).

*The effect of calcium to the Zn and Zn-65 distribution in tissues:* The distribution of Zn and Zn-65 in intestine areas like duodenum, jejunum and ileum, reflects the differences in absorption and retention it was found that Zinc amount per gram of dry tissue and Zn-65 amount per gram of wet tissue in low-calcium group increase,while they decrease in high-calcium group (Figure 3,4).The distribution of zinc and Zn-65 in soft tissues like brain, kidney, liver has changed according to diet with calcium. The most significant change from statistical perspective has been observed in the liver and brain. Also, while the Zn- 65 amount in blood, increases substantially in low-calcium group, it decreased in high and very high calcium groups. These changes are also statistically significant (Figure 5).

## DISCUSSION

When the average weight of study groups before and after the diet was compared, it was observed that average weight increases in control and low-calcium groups, while a loss in average weight was observed in the other two groups taking high calcium. In calculating the zinc absorption, Weigand and Kircgessner's isotope dilution technique was used (11). According to this method, theoretical or observed absorption percentage is found by looking at the zinc amount taken daily and the specific activities of feces and tissue (kidney). Farbes and Yohe's (6) balance study on rats shows that increasing the amount of calcium mineral does not reduce the zinc absorption. In another study, it was found that Zn-65 body retention after gavage feeding decreases 3 days after the Zn-65 giving. Heth and Hoekstra (7) also came up with a retention graph similar to ours, if 0.60 and 1.76 percent calcium is added to basal diet.

However, since in these studies, the effect of the low dietary calcium was not studied, it is impossible to compare our results on the low dietary calcium to their results. In another study, when the percentage retention of Zn-65 was studied in those given 1/3 percent calcium with low zinc, while any statistically significant change was not seen in those given in another study. At the end of the fourth day, it was observed that the percentage Zn-65 retention showed a statistically significant decrease in those fed a zinc deficient and high calcium diet, while the high calcium diet markedly decreased Zn-65 absorption in rats fed a zinc-deficient diet but not in those fed a control diet (8).

In the studies on rats done by Heth and Hoekstra (7), it was shown that 0.6 and 1.76 percent calcium added to basal diet significantly increased carcass Zn-65 in the femur and decreased it in liver, kidney and muscle. On the other hand, it was found that Zn-65 activity per one gram tissue caused a significant change in only kidney and bone. Adham and Song (1) showed that dietary calcium reduced the Zn-65 activities in pancreas, liver, kidney, heart, spleen, intestine, muscle and testis. Berry et al. (4) studied Zn-65 activities in blood collected 3, 6, 12 and 24-hour intervals until the the 144-hour collection period was completed. They found that calcium added to a low-zinc ration decreased blood, plasma and total tissue Zn

65 levels. They also observed that calcium added to a zinc supplemented ration increased blood and total tissue Zn-65 activity. In the same study, it was found that increased dietary zinc decreased retention of Zn-65 in blood and tissues. As a result, changes in dietary Ca/Zn rations have important effects on zinc absorption and its distribution in tissues.

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