Effect of caffeine on zinc absorption and Zn concentration in rat tissue

BY MAGDALENA J. ROSSOWSKA AND TETSUO NAKAMOTO

Laboratory of Perinatal Nutrition and Metabolism, Department of Physiology, Louisiana State University Medical Center, New Orleans, LA 70119, USA

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The purposes of the present study were to determine whether caffeine has an effect on zinc absorption and tissue levels of Zn. Under anaesthesia, one side of the intestine of female rats was connected to infusion pumps and an infused solution (first caffeine and later Zn solution) was collected from the other side of the intestine using either 300 mm or the whole small intestine to determine Zn absorption. In a further study, different doses of caffeine solution were injected directly into the femoral vein and a saline or Zn solution was infused into the intestine to measure Zn absorption and tissue Zn concentrations. The results consistently showed that the caffeine solution infused into the intestine did not affect intestinal Zn absorption nor was absorption affected by the direct injection of caffeine into the vein. In contrast, injection of different doses of the caffeine solution significantly decreased Zn tissue levels for the heart only. Calcium concentrations in the heart tissue were also decreased, but not magnesium concentrations. Tissue Zn levels recovered immediately on infusion of a Zn solution into the intestine.

Caffeine: Zinc: Absorption: Intestine: Rat

Caffeine (1,3,7-trimethylxanthine) is contained in beverages such as coffee, tea, cola, other carbonated soft drinks and over-the-counter medications. Because of the widespread use of caffeine in our daily life, it is practically impossible to avoid its intake. A number of studies have described the effects of caffeine on various organs of the body. Chronic caffeine ingestion by growing rats through drinking water is reported to affect the zinc content of the testicle (Friedman *et al.* 1979) but not of the brain (Haydel *et al.* 1986). Zn is known to play an important role in growth and development of animals and humans (Dreosti, 1982), and its absorption from the small intestine greatly increases during the latter part of pregnancy and lactation (Davies & Williams, 1977). On the other hand, Southon *et al.* (1989) have shown that Zn absorption increases in pregnant rats only at low intakes of Zn.

Recently a close interaction was observed between the maternal dietary caffeine intake and Zn content of certain rat tissues such as the brain (Nakamoto *et al.* 1989*b*) and bone (Nakamoto *et al.* 1989*a*) of their offspring. Because there was a decrease in Zn content in these tissues when caffeine was added to the maternal diet, the question raised was whether caffeine could impair intestinal Zn absorption. If impairment of maternal Zn absorption occurs, then it is possible that the tissue levels of Zn in the offspring might be decreased. It is also conceivable that caffeine could directly affect the uptake or release of Zn at the tissue level. Therefore, using adult animal models, we determined whether caffeine affects intestinal Zn absorption and Zn concentrations in various tissues.

MATERIALS AND METHODS

Adult female Sprague-Dawley rats (Holtzman strain, Holtzman Co., Madison, WI) (230–250 g) were used for all experiments. The rats were kept in cages in a room with controlled lighting (12 h/d), constant temperature (22°) and relative humidity (65 %). Rats were fed on a commercial chow diet containing 70 mg Zn/kg (Rodent chow no. 5001,

Table 1. *Effect of caffeine on the zinc uptake by proximal and whole small intestine of the rat after 60 min infusion of 0·1 mM zinc chloride in physiological saline (9 g sodium chloride/l) at a flow-rate of 0·5 ml/min**

(Mean values with their standard errors for four rats)

		Zn uptake (μg)
Proximal intestine (300 mm)	Control	
	Mean	26·9
	SEM	2·5
	Caffeine†	
	Mean	30·4
	SEM	2·8
Whole small intestine (950 mm)	Control	
	Mean	79·4
	SEM	1·4
	Caffeine†	
	Mean	77·5
	SEM	5·1

* For details of procedures, see p. 554.

† Concentration of caffeine solution was 10 μg/ml; infusion continued for 15 min with a flow-rate of 0·5 ml/min.

Purina Mills, St Louis, MO). Animals were fasted for 3–4 h before the experiments. Rats were anaesthetized with pentobarbital (60 mg/kg body-weight) by the intraperitoneal route. The abdomen was opened by a midline incision and the stomach and small intestine were carefully exposed; approximately 5 mm from the pyloric sphincter of the stomach toward the duodenum was tied firmly and cut. A polyethylene cannula (Tygon, S-50-HL Tubing; Norton Co., Akron, OH) was then inserted into the duodenum and secured by ligature. Special care was taken not to disturb nerves and blood supply to the small intestine. The cannula was then connected to a syringe pump (model 255-1; Sage Instrument Co., White Plains, N.Y.).

For the study of Zn absorption in the upper intestine, 300 mm intestine were precisely measured using a 300 mm string from the incision of the duodenum and cut. A second cannula was then introduced into the small intestine. For the study of Zn absorption by the whole intestine, a second cannula was inserted 50 mm from the ileo-cecal junction and secured by ligature. The distal part of the intestine was closed and secured by a ligature in both studies.

The small intestine was gently washed with physiological saline (9 g sodium chloride/l) at 37° with a constant flow rate of 3 ml/min. After a 10 min stabilization period, caffeine (Sigma Chemical Corp, St Louis, MO) dissolved in saline (10 μg caffeine/ml) was infused into the small intestine and infusion was continued for 15 min with a constant flow-rate of 0·5 ml/min. In the control group, saline replaced the caffeine solution. Subsequently an infusion of 0·1 mM-zinc chloride in saline was started at a constant flow-rate of 0·5 ml/min and was continued for 60 min. The perfusate was collected at 5 and 10 min and every 10 min thereafter. The volume was measured and samples were prepared for determination of Zn content.

In order to study the effects of different doses of caffeine on Zn absorption and Zn uptake in various tissues, animals were prepared as described previously, and the femoral vein was exposed for intravenous injection. Rats of the control group were injected with 0·2 ml saline. In the caffeine group, 0·2 ml saline containing 0·02, 0·1 or 2 mg caffeine was injected.

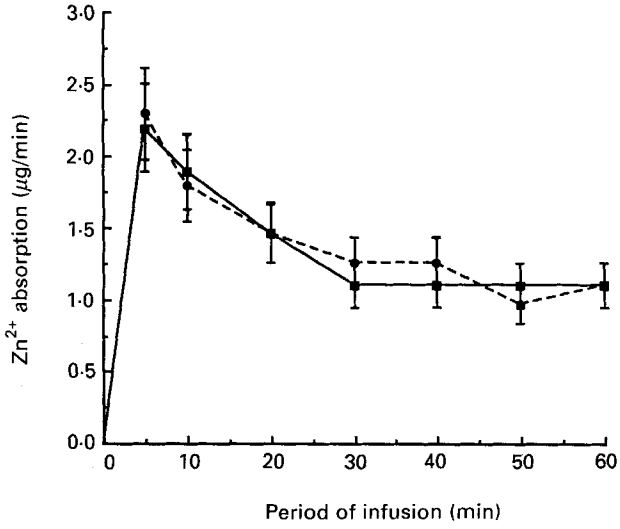

Fig. 1. The rate of zinc absorption in rat whole small intestine infused with caffeine (10 μg/ml) in physiological saline (9 g sodium chloride/l) (– – –) or saline alone (——). Values are means with their standard errors represented by vertical bars. For details of infusion procedures, see p. 554.

After 15 min, saline or 0·1 mM-$ZnCl_2$ in saline was infused into the intestine at the rate of 0·5 ml/min for 30 min.

At the end of the infusion period, the blood was collected into heparinized tubes by heart puncture and the animals were killed. Liver, heart, kidney and spleen were removed and weighed. Tissues were cut into small pieces and homogenized in double-distilled deionized water (DDDW) in the proportions 1 g tissue/5 ml DDDW.

Concentrated nitric acid (1 ml) was added to 1 ml homogenate or 1 ml whole blood in a tightly capped tube and samples were left at room temperature for 24 h followed by digestion at 100° overnight. Digested samples were diluted in DDDW and Zn concentration was measured using atomic absorption flame emission spectrophotometry (Model 280, Fisher Scientific Co., Fairlawn, N.J.).

In addition, total calcium and magnesium concentrations in the heart and liver of the non-Zn group were measured. Homogenate (1 ml) was placed into a porcelain crucible and dried at 60° for several hours. This was then ashed at 600° overnight. Ashed samples were dissolved in concentrated hydrochloric acid and diluted with DDDW. Ca and Mg contents were measured using atomic absorption spectrophotometry. Protein concentration was determined according to Lowry *et al.* (1951) using bovine serum albumin as a standard.

Statistical analyses were performed using analysis of variance and a multiple comparison *post hoc* test (Student–Newman-Keuls) or the Student's *t* test, with $P < 0·05$ considered significant.

RESULTS

Zn absorption from both the proximal and whole length of the small intestine showed no differences in the caffeine plus (+) Zn-infused group compared with the saline + Zn-infused control group (Table 1). Fig. 1 shows the rate of Zn absorption in the caffeine + Zn-infused group compared with the saline + Zn-infused control group over a period of 1 h using the whole small intestine. The rate of Zn absorption also showed no difference at any point between these groups. Zn absorption was extremely rapid and increased with time over

Table 2. *Effect of different doses of caffeine injected into the femoral vein on zinc uptake by the whole small intestine of the rat*

(Mean values with their standard errors; no. of animals in parentheses. 0·2 ml physiological saline (9 g sodium chloride/l) or 0·2 ml saline containing caffeine at 0·02, 0·1 or 2·0 mg was injected into the femoral vein. After 15 min, 0·1 mM-zinc chloride in saline was infused into the intestine at the rate of 0·5 ml/min for 30 min)

	Zn uptake (μg)
Control	
Mean	46·60
SEM	2·50 (4)
Caffeine (mg)	
0·02	
Mean	51·90
SEM	9·18 (3)
0·10	
Mean	51·03
SEM	7·16 (4)
2·00	
Mean	44·77
SEM	3·68 (3)

Table 3. *Effect of caffeine on zinc concentration in various tissues. Each value was obtained 30 min after infusing physiological saline (9 g sodium chloride/l) with (+) or without (−) 0·1 mM-zinc chloride into the whole small intestine of rats*

(Mean values with their standard errors; no. of measurements in parentheses. 0·2 ml saline containing caffeine at 0·02, 0·1 or 2·0 mg was injected into the femoral vein)

			Caffeine (mg)		
Tissues	Zn	Control	0·02	0·10	2·00
Whole blood (μg Zn/ml)	− Mean	5·26	4·72	5·90	4·75
	SEM	0·37 (5)	0·45 (3)	0·06 (3)	0·05 (4)
	+ Mean	6·34	6·20	6·54	5·66
	SEM	0·45 (5)	0·69 (4)	0·19 (5)	0·40 (3)
Heart (μg Zn/mg protein)	− Mean	0·134	0·113*	0·103*	0·107*
	SEM	0·006 (6)	0·004 (3)	0·002 (3)	0·005 (4)
	+ Mean	0·121	0·132	0·142†	0·119
	SEM	0·004 (3)	0·007 (4)	0·011 (6)	0·005 (3)
Liver (μg Zn/mg protein)	− Mean	0·130	0·153	0·146	0·145
	SEM	0·006 (4)	0·002 (3)	0·006 (4)	0·003 (4)
	+ Mean	0·154†	0·147	0·166	0·130
	SEM	0·006 (4)	0·006 (4)	0·012 (6)	0·007 (3)
Kidney (μg Zn/mg protein)	− Mean	0·112	0·123	0·110	0·128
	SEM	0·012 (6)	0·021 (3)	0·006 (3)	0·009 (4)
	+ Mean	0·111	0·104	0·102	0·126
	SEM	0·012 (4)	0·015 (4)	0·007 (6)	0·011 (3)
Spleen (μg Zn/mg protein)	− Mean	0·104	0·111	0·113	0·114
	SEM	0·005 (6)	0·019 (3)	0·010 (3)	0·011 (4)
	+ Mean	0·117	0·116	0·116	0·133
	SEM	0·006 (4)	0·007 (3)	0·006 (6)	0·026 (3)

* Mean values were significantly different from the controls ($P < 0·05$).
† Mean values were significantly different from non-Zn group ($P < 0·05$).

Table 4. *Effect of caffeine on calcium and magnesium concentrations (μg/mg protein) in the tissues of rats*

(Mean values with their standard errors; no. of measurements in parentheses. 0·2 ml physiological saline (9 g sodium chloride/l) containing caffeine at 0·02, 0·1 or 2·0 mg was injected into the femoral vein. After 15 min saline was infused into the intestine at the rate of 0·5 ml/min for 30 min)

Tissues			Control	Caffeine (mg) 0·02	0·10	2·00
Heart	Ca	Mean	0·231	0·140*	0·147*	0·145*
		SEM	0·016 (6)	0·010 (3)	0·010 (3)	0·018 (6)
	Mg	Mean	0·988	0·970	0·825	1·075
		SEM	0·064 (4)	0·080 (4)	0·160 (3)	0·036 (4)
Liver	Ca	Mean	0·126	0·126	0·115	0·093
		SEM	0·010 (5)	0·030 (5)	0·010 (6)	0·010 (6)
	Mg	Mean	0·604	0·635	0·643	0·546
		SEM	0·055 (4)	0·109 (4)	0·054 (5)	0·053 (5)

* Mean values were significantly different from the controls ($P < 0·05$).

5 min, after which a decline in rate was observed and a steady-state was achieved within the initial 25 min of infusion (Fig. 1).

Intravenous injection of caffeine at different doses did not change the Zn absorption compared with that of the controls (Table 2).

Zn levels in the blood, liver, kidney and spleen of rats receiving doses of 0·02, 0·1 or 2 mg caffeine showed no statistically significant differences among the groups either with or without Zn infusion (Table 3). However, the Zn concentration in the heart of the rats receiving the various doses of caffeine was significantly lower than that in the controls (non-Zn group). There were no differences between the caffeine-injected groups. With Zn infusion, the differences between the groups disappeared.

Tissue Zn concentrations in the heart of animals receiving a caffeine injection of 0·1 mg with infusion of Zn was higher than that of the group without Zn infusion, but there were no differences when compared with the Zn-control group (Table 3). The tissue Zn concentration of the liver in the control group with Zn infusion was greater than the control group without Zn infusion.

The total heart Ca concentrations after the rats had received 0·02, 0·1 or 2 mg caffeine were lower than those of the controls (Table 4). There were no differences in the heart Ca concentration between the groups receiving different doses of caffeine. In contrast, Mg concentrations of the heart and liver and Ca concentrations of the liver showed no differences between caffeine and control groups.

DISCUSSION

Caffeine is reported to be absorbed from the duodenum, ileum and jejunum (Chvasta & Cooke, 1971; Blanchard & Sawers, 1983). Zn absorption is believed to be limited primarily to the duodenum and ileum (Davies, 1980; Seal & Heaton, 1983). Zn uptake across the brush-border surface may occur partly by a regulated carrier-mediated diffusion mechanism (Davies, 1980; Menhard & Cousins, 1983; Steel & Cousins, 1985) and may be energy dependent (Kowarski *et al.* 1974). The velocity of Zn uptake by brush-border membrane vesicles is increased in Zn-deficient rats, compared with controls (Cotzias & Papavasilion, 1964; Seal & Heaton, 1983), suggesting that there is a brush-border membrane that responds homeostatically to the dietary Zn supply.

If Zn absorption is regulated by the amount of Zn that is bound to the inducible

intestinal Zn-binding protein, metallothionein (Richards & Cousins, 1975; Cousins *et al.* 1978; Richards, 1989), then we may conclude that the presence of caffeine in the intestine did not affect the ability to bind to metallothionein, although a decrease of tissue Zn levels was reported (Nakamoto *et al.* 1989*b*).

The rate of Zn absorption was extremely rapid in the first 5 min. Similarly, rapid Zn absorption has been demonstrated using ligated loops of rat duodenum (Davies, 1980). The rate of Zn absorption was practically identical for caffeine-infused and saline-infused groups, suggesting that direct contact between the caffeine solution and the intestinal brush-border membrane did not influence the rate of Zn absorption.

Caffeine solution was injected directly into the femoral vein to determine whether blood caffeine levels might affect Zn absorption. Because no differences were observed, in spite of the different concentrations of caffeine injected, we can conclude, also, that blood caffeine levels did not influence Zn absorption.

If intestinal Zn absorption was not affected by caffeine, we can hypothesize that caffeine could directly affect Zn at the tissue level. Because of the short period of time from the start of caffeine injection to the end of the study, sampling of brains and bones was excluded. The heart was the only organ which consistently showed a decrease in tissue Zn level. Even if the amount of caffeine was increased 100 times, the decrease in tissue Zn level was about the same as that with lower doses. On the other hand, tissue Zn levels in the heart returned to control levels quickly with infusion of Zn solution to the intestine, suggesting that the tissue Zn level in the heart could recover in a relatively short period of time. Since there was no alteration in Zn levels of whole blood under different experimental conditions, the variation in tissues was seemingly not the result of blood contamination.

Because the presence of caffeine in the blood caused a rapid release of Zn from the heart, further studies were conducted to determine the heart Ca and Mg levels. Effects of caffeine on heart Ca content were observed. Several studies *in vitro* of Ca and caffeine-induced Ca release from heavy sarcoplasmic reticulum (SR) vesicles under different conditions suggest that Ca and caffeine can interact with a common receptor of the Ca-release channels (Rubtsov & Murphy, 1988; Rousseau & Meissner, 1989). Caffeine can induce a rapid Ca efflux from skinned fibres (Stephenson, 1981) and skeletal (Thore, 1973; Ikemoto *et al.* 1985; Meissner *et al.* 1986) and cardiac (Thore, 1973; Meissner & Henderson, 1987) SR membrane fractions. These studies suggest that caffeine acts on the SR Ca-release channel. However, the mechanism of action of caffeine on heart Zn is unknown at the present time, and cellular storage of Zn is not well documented. In the rat myometrium and electric organ of *Electrophorus electricus* (Bettger & O'Dell, 1981), intracellular Zn was predominantly concentrated in the microsomal fraction. On the other hand there is also some evidence to suggest that Zn may be stored in the cytoplasmic protein metallothionein (Cousins, 1985). The binding of Zn by skeletal muscle SR has shown that Zn competes with Ca for the same binding sites (Carvalho, 1968). Thus, we propose that Zn release from heart tissue as a result of caffeine intake plays a critical role in the functioning of the heart (Temples *et al.* 1985). Furthermore, in view of the fact that both Zn and Ca in the heart were decreased within a short period of time, Zn may influence the functions of contraction and relaxation of the heart.

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