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Effect of Dietary Cadmium on Calcium Metabolism in the Rat During Late Gestation[1] (40258)

B. J. KELMAN, B. K. WALTER, G. E. JARBOE, AND L. B. SASSER

Comparative Animal Research Laboratory, Oak Ridge, Tennessee 37830

Toxic effects of Cd on adult animals have been reported, but data explaining the relationship between dietary Cd and fetal development are limited. High concentrations of cadmium injected into the dam are toxic to the developing fetus (1); little information is available, however, concerning the effect of lower levels of Cd on fetal metabolism. While relatively high levels of dietary Cd (200 ppm in drinking water) decrease fetal Fe and increase fetal Ca (2), the physiological significance and origins of these changes are not known. The purpose of the present study was to determine the effects of lower levels of dietary Cd supplied continuously throughout gestation on tissue Ca in the developing fetus.

Materials and methods. Forty-six pregnant Sprague-Dawley rats, each approximately 130 days old and weighing 200 g, were divided into nearly equal groups and given drinking water containing one of three Cd levels (control, 10 ppm, and 25 ppm) beginning on day 0 of gestation. The control water and the diet contained less than 20 ppb Cd as determined by atomic absorption analysis (3). The composition of the diet, administered *ad libitum* to all three groups, is shown in Table I. On day 20 of gestation, each dam was injected iv with 16 μCi of high specific activity $^{45}CaCl_2$ (16.3 μCi/μg) in a saline vehicle; the dam was killed with Metophane (Pitman-Moore) one day later. Maternal and fetal hematocrits were measured and recorded.

Maternal plasma, bone (femur), kidney, and liver were analyzed for stable and radiocalcium. Three fetuses were analyzed from each litter, acid digested, and aliquots of the pooled digest analyzed for stable and radiocalcium. Fetal plasma, bone (femur), kidney, liver, and carcass (remaining tissues from each fetus) from five additional fetuses of each litter were pooled and analyzed for stable and radiocalcium. Placentas associated with the same five fetuses were also collected. All maternal tissues and fetal carcasses were acid digested. Fetal tissues except carcasses were digested in Unisol (Isolab). Tissue ^{45}Ca was determined by chelating radiocalcium in digests with EDTA and adding a scintillation cocktail, Unisol-Compliment (Isolab). Samples were counted for not less than 10,000 net counts on a Mark III Liquid Scintillation Counter (Searle). Stable calcium in tissues was obtained on an atomic absorption spectrophotometer (Perkin-Elmer Model 303) by diluting digests 1/100 with a solution of 1% La_2O_3 in 5% HCl.

Data were examined by analysis of variance; differences between means were tested by Student's *t* test.

Results. No changes in total or radiocalcium content resulting from Cd treatment were found in whole fetuses.

There were significant increases in stable and radiocalcium in fetal bone as a result of maternal ingestion of cadmium (Table II). Stable calcium found in bone increased by 33% over the control level at 25 ppm Cd. Although this trend seemed to be present at 10 ppm, the size of the variance precluded detection of a significant difference from the control measurements. Radiocalcium in whole fetal femurs increased 24% after the 10 ppm Cd treatment with no further change detected at 25 ppm Cd. The concentration of radiocalcium in fetal bone appeared to increase with Cd treatment, as was evident in the measurements of stable calcium and whole fetal bone radiocalcium, but the variance was too large to detect an increase at any treatment level. However, the values listed for radiocalcium concentration (% dose/g) in Table II, along with the changes seen in stable calcium and radiocalcium from whole bones, make it appear likely that a significant increase in radiocalcium concen-

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tration would be detected with larger sample sizes.

While changes in fetal liver radiocalcium were not detected, stable Ca in the fetal liver decreased by 25% at 10 ppm and by an additional 15% at 25 ppm compared to controls. Stable Ca in the fetal kidney decreased by 30% at 10 ppm; no further change occurred at 25 ppm. No significant changes in calcium were observed in other fetal tissues measured (Table II).

Fetal hematocrits (Table II) were suppressed by Cd treatment to a greater extent than were maternal hematocrits (Table III), decreasing by 12% in animals administered 10 ppm Cd compared to controls. No further decrease was detected in fetuses from mothers receiving 25 ppm Cd.

Placental stable Ca decreased by 12% between 10 and 25 ppm with no associated decrease in radiocalcium (Table III).

No significant changes in stable or radiocalcium occurred in any maternal tissues. A slight increase in maternal plasma stable calcium was observed at 10 ppm (Table III). Since this increase was significant only at $P < 0.05$ at 10 ppm but not at the higher dose, it is likely to be a spurious result. A 7% decrease in maternal hematocrit was observed at 10 ppm with no further decrease between 10 and 25 ppm (Table III).

No changes in litter size (Table III), rate of conception, or number of gross fetal malformations were observed at Cd levels adminis-

TABLE I. DIET COMPOSITION.

	%
Casein (vitamin-free)	25.0
DL methionine	0.3
Sucrose	28.0
Corn starch	30.5
Vitamin mix (ICN* "Vitamin Diet Fortification Mixture")	2.2
Salt mix (ICN No. 4164)	4.0
Ca	0.37
Fe	0.0066
Cu	0.00010
Zn	0.00050
Vegetable oil	10.0

* ICN Pharmaceuticals, Inc., 121 Express St., Plainview, NY.

TABLE II. EFFECT OF DIETARY CADMIUM ADMINISTERED IN DRINKING WATER ON FETAL CALCIUM AND HEMATOCRIT (MEAN ± 1 SE).

	Control	10 ppm	25 ppm
Bone:			
Stable calcium (mg/100 g)	84.6 ± 6.5^a	96.7 ± 10.6^a	112.2 ± 7.4^b
Radiocalcium (% dose/g)	18.2 ± 1.3	28.5 ± 6.1	26.5 ± 3.8
Radiocalcium (% dose × 10^{-2})	6.23 ± 0.27^c	7.70 ± 0.47^d	7.01 ± 0.27^d
Liver:			
Stable calcium (mg/100 g)	5.48± 0.44^e	4.11 ± 0.14^f	3.27 ± 0.15^g
Radiocalcium (% dose/kg)	19.3 ± 4.3	19.3 ± 3.5	19.0 ± 5.3
Kidney:			
Stable calcium (mg/100 g)	6.69 ± 0.66^h	4.70 ± 0.13^i	5.12 ± 0.16^i
Radiocalcium (% dose/kg)	20.1 ± 1.1	22.2 ± 1.3	22.4 ± 2.1
Plasma:			
Stable calcium (mg/dl)	7.26 ± 0.27	7.30 ± 0.26	7.16 ± 0.25
Radiocalcium (% dose/l)	31.7 ± 1.3	32.2 ± 1.7	32.7 ± <0.1
Hematocrit	24.3 ± 0.9^j	21.5 ± 0.8^k	19.9 ± 1.0^k

$^{a-k}$ Different superscripts denote means which are statistically different across Cd treatments ($P < 0.025$).

TABLE III. EFFECT OF DIETARY CADMIUM ADMINISTERED IN DRINKING WATER ON PLACENTAL CALCIUM, MATERNAL PLASMA CALCIUM, LITTER SIZE, AND MATERNAL HEMATOCRIT (MEAN ± 1 SE).

	Control	10 ppm	25 ppm
Placenta:			
Stable calcium (mg/100 g)	5.03 ± 0.13^a	4.88 ± 0.32^a	4.28 ± 0.27^b
Radiocalcium (% dose/kg)	50.0 ± 2.4	50.4 ± 0.6	53.3 ± 6.4
Plasma:			
Stable calcium (mg/dl)	7.68 ± 0.20^c	8.12 ± 0.15^d	7.67 ± 0.25^c
Radiocalcium (% dose/l)	20.9 ± 0.9	20.9 ± 0.8	21.5 ± 2.3
Litter size	12.8 ± 1.0	12.4 ± 1.0	13.2 ± 0.8
Hematocrit	30.9 ± 0.5^e	28.7 ± 1.2^f	29.0 ± 1.1^f

$^{a-f}$ Different superscripts denote means which are statistically different across Cd treatments ($P < 0.05$).

tered in this study.

Discussion. Our results indicate that Cd in drinking water at concentrations as low as 10 ppm causes significant changes in fetal Ca metabolism without inducing gross morphological changes. Most reviews of cadmium toxicology support the concept of a strong placental barrier to cadmium (4–8) even though there are little data from direct studies on the placenta. Recently, we have shown that Cd crosses the placenta of the guinea pig with relative ease (9). Further, Pond (2) reported an increase in Cd concentration in rat pups when Cd was administered to dams at 200 ppm in drinking water, indicating that there is significant placental transfer and fetal accumulation of Cd in the rat.

Measurements of radiocalcium administered 24 hr before tissue sampling are an indication of the exchangeable calcium pool for that period. Stable calcium measurements at the three treatment levels reflect changes which occur throughout gestation. Since no changes in total or radiocalcium content were found in whole fetuses as a result of Cd treatment, the increased Ca found in bone therefore undoubtedly resulted from a redistribution of Ca within the fetal body as opposed to a change in the amount of Ca transported across the placenta.

The increases in fetal bone Ca were especially interesting. Since three treatment levels were used, it is difficult to interpret differences in dose-responses for stable and radiocalcium. Our results seem to indicate, however, that stable bone calcium increased linearly up to 25 ppm Cd ($r = 0.55$, $P < 0.001$ for the regression of stable Ca on dietary Cd) while the bone radiocalcium tended to reach a maximum at 10 ppm Cd. These differences between stable and radiocalcium responses to Cd in fetal bone make it tempting to hypothesize that a process exists in fetal rat bone between 20 and 21 days of gestation which is especially sensitive to dietary cadmium and not apparent from measurements of stable calcium in this study. If there is a dose-effect relationship for low concentrations of Cd which approaches a maximum in fetal bone radiocalcium, then the lack of a significant difference between effects at the 10 ppm and 25 ppm treatments indicates that a half-maximal effect is reached at some Cd concentration below 10 ppm.

Decreases in fetal and maternal hematocrits were most likely due at least in part to reductions in fetal and maternal iron. Reductions in fetal and maternal liver iron have been reported in Cd-fed rats at 200 ppm dietary Cd (2). The decreases in hematocrits tend to confirm the decreases in iron reported at 200 ppm Cd (2) and indicate that they occur at least as low as 10 ppm Cd. Similar decreases were seen in fetal and maternal hematocrits. While the decrease in fetal hematocrit was larger, both hematocrits showed no further decrease between 10 and 25 ppm. As in the case of the changes seen in fetal bone radiocalcium, the lack of an increased effect between 10 and 25 ppm makes it tempting to hypothesize that effects will be seen at Cd concentrations below 10 ppm. The relationship between the changes in fetal and maternal hematocrits and those in tissue Ca distribution is not readily apparent.

A significant linear relationship was present between fetal liver stable calcium and Cd ($r = -0.62$, $P < 0.001$ for the regression of fetal liver stable calcium on dietary Cd concentration) over the range of Cd concentrations studied, while fetal kidney stable calcium decreased maximally between control and 10 ppm Cd. Placental tissue decreased maximally between 10 and 25 ppm Cd levels. A lack of similar effects in the corresponding radiocalcium measurement shows that effects due to Cd on Ca metabolism in these tissues most likely occurred before 20 days gestational age. The physiological significance of changes in soft organ Ca is poorly understood.

Summary. Sprague-Dawley rats were given access to water containing control, 10, or 25 ppm Cd beginning on day 0 of gestation. On day 21 of gestation, fetal and maternal tissues were collected from dams which had been dosed 24 hr previously with 16 μCi of $^{45}CaCl_2$. Maternal and fetal hematocrits decreased, indicating a possible interference with iron metabolism. No changes in total or radiocalcium found in fetal body were detected as a function of Cd treatment. Fetal bone stable and radiocalcium increased by as much as 33% at 25 ppm while fetal liver and kidney stable Ca

decreased compared to controls.

It is evident that low Cd exposures lead to changes in fetal Ca metabolism rather than causing changes in the amount of Ca transported across the placenta.

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