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CORTICAL INDEX OF THE FEMUR IN RATS EXPOSED TO SOME TOXIC METALS AND ASH FROM COAL GASIFICATION

M. BLANUŠA, K. KOSTIAL, V. MATKOVIĆ and M. LANDEKA
Institute for Medical Research and Occupational Health, Zagreb, Yugoslavia

ABSTRACT

The effect of ash from coal gasification on bone metabolism and its interaction with cadmium, manganese or mercury were studied. Cortical indices of rat femur were measured after chronic oral exposure to ash and/or toxic metals. Ash was given in food (0.5, 1 and 5 per cent) and toxic metals in water in concentrations of 100 ppm of cadmium, 2000 ppm of manganese and 50 ppm of mercury.

The diet with a high ash (5%) content significantly reduced bone mass. The applied doses of toxic metals did not significantly affect the same bone parameters in male and female rats. There was no synergistic effect of toxic elements and ash either.

The aim of this study was to determine the effect of ash from coal gasification and toxic metals cadmium, mercury and manganese on bone metabolism.

Coal gasification is a technological procedure which is likely to find large use in energy production in the near future. The health effects of solid and liquid wastes from coal gasification are mostly unknown. The solid waste (ash) contains a mixture of elements (toxic and essential) some of which could influence calcium metabolism. They might also potentiate the effect of other toxic metals in the environment, which are known to affect the bone tissue.

The most extensive experimental studies of the influence of toxic metals on calcium metabolism are related to cadmium[5,8,9]. A thinning of the cortical osseous tissue and an increased osteoclastic activity were found after a long exposure of rats to cadmium in food. It was suggested that the effect of cadmium on bone tissue is secondary to an effect on the renal tubules[10,13]. Nutritional factors, such as calcium and vitamin D deficiency were found to be of importance for the bone development of cadmium – exposed animals[4,9,16]. The kinetic study of calcium metabolism during chronic cadmium poisoning showed a decrease of intestinal absorption of calcium and a decrease of calcium compartments, exchange rates and osteogenesis[3]. The toxic effect of manganese and mercury on bone has not been reported. Only skeletal abnormalities were described in manganese deficiency[17].

In our experiment in rats exposed to ash in diet and/or toxic metals (cadmium, mercury, manganese) in drinking water we determined the effect on bone by measuring the cortical index of the rat femur. The addition of metals had no influence on bone but the addition of large amounts of ash caused a reduction in bone density which was not potentiated by simultaneous exposure to both ash and toxic metals.

METHODS

Six-week-old male and female rats were divided according to treatment into 16 groups of 14 animals each. They received ash, toxic metals or both orally during four or eight months. Toxic metals were given in drinking water in the following quantities: 100 ppm of cadmium as $CdCl_2$, 2000 ppm of manganese as $MnCl_2$ and 50 ppm of mercury as $HgCl_2$. Coal ash was mixed in various percentages with our standard rat diet (0.5, 1 and 5 per cent) according to the procedure described in another paper[15] and it was given to rats *ad libitum*. The experiment was performed by combining three levels of ash in food and one level of each metal in drinking water as shown in Tables 1 and 2. Body weights of the animals at the beginning of the experiment were 126 g for males and 110 g for females. The weight was also determined after four and eight months of specific dietary treatment. After the first period of four months seven animals from each group were killed, and right femurs were dissected. The same was done after eight months of exposure with other seven animals from each group.

After dissection of right femurs contact X-rays were made. Total midshaft width (T) and medullary midshaft width (M) were measured under magnification. From these measurements cortical area (CA), total area (TA) and cortical index (CA/TA) were calculated according to Garn and co-workers[6] from expressions:

$$CA = (T^2 - M^2)\ \frac{\pi}{4};\quad TA = T^2\ \frac{\pi}{4};\quad \frac{CA}{TA} = \frac{T^2 - M^2}{T^2}$$

The cortical index is considered as a good estimate of bone density i.e. bone mass corrected for bone volume.

All the results were subjected to the analysis of variance as 2×4 factorial experiment, for each sex and each toxic element separately[7].

RESULTS AND DISCUSSION

In the present experiment the lowest body weights (Tables 1 and 2) were recorded in most groups of rats on the diet with five per cent ash. The reduction was always higher in males than in females.

The analysis of variance of cortical indices of the first and second interval of chronic exposure to various combinations of toxic elements and/or ash showed a significant influence of ash on bone mass (Tables 5 and 6). Cortical indices were significantly lower after both intervals of exposure (Tables 3 and 4). Our

TABLE 1

Body weights (g) of 5.5 month-old rats, measured after four months of exposure to coal ash and/or toxic elements. Results are presented as arithmetic means of seven animals in each group ± S.E.M.

Rats	Ash in diet (%)	Toxic elements in drinking water			
		None	Cadmium (100 ppm)	Manganese (2 000 ppm)	Mercury (50 ppm)
Females	0	202.1 ± 5.3	207.1 ± 4.7	217.1 ± 7.1	221.4 ± 5.5
	0.5	213.4 ± 3.2	199.3 ± 4.7	208.6 ± 7.2	218.6 ± 4.3
	1	240.0 ± 10.9	205.0 ± 5.2	215.8 ± 5.8	219.3 ± 8.9
	5	197.1 ± 2.9	170.7 ± 4.6	205.0 ± 6.2	209.2 ± 7.8
Males	0	337.8 ± 10.1	322.9 ± 7.2	325.7 ± 11.5	324.3 ± 10.1
	0.5	337.1 ± 7.4	317.9 ± 7.9	324.3 ± 11.4	314.3 ± 8.3
	1	357.1 ± 9.7	304.2 ± 13.8	341.4 ± 9.7	328.6 ± 6.3
	5	323.6 ± 9.9	240.0 ± 8.1	282.5 ± 5.3	291.4 ± 12.2

TABLE 2

Body weights (g) of 9.5 month-old rats, measured after eight months of exposure to coal ash and/or toxic elements. Results are presented as arithmetic means of seven animals in each group ± S.E.M.

Rats	Ash in diet (%)	Toxic elements in drinking water			
		None	Cadmium (100 ppm)	Manganese (2 000 ppm)	Mercury (50 ppm)
Females	0	218.0 ± 9.0	223.6 ± 4.3	220.7 ± 8.9	232.1 ± 3.8
	0.5	217.1 ± 7.9	217.0 ± 2.4	232.1 ± 10.9	227.9 ± 6.9
	1	223.6 ± 3.4	220.7 ± 10.9	235.8 ± 13.2	230.0 ± 10.1
	5	232.1 ± 7.6	196.4 ± 8.9	221.0 ± 8.4	209.3 ± 8.1
Males	0	400.7 ± 13.9	342.9 ± 10.5	380.0 ± 9.7	351.4 ± 12.5
	0.5	370.7 ± 9.7	360.0 ± 6.9	374.3 ± 19.3	352.1 ± 11.9
	1	395.7 ± 4.4	354.3 ± 12.3	387.1 ± 15.2	376.1 ± 5.7
	5	331.4 ± 6.9	282.1 ± 17.4	330.0 ± 11.8	308.6 ± 5.5

preliminary experiments[2] indicate that a higher per cent of ash in the diet (10%) causes even more drastic changes in cortical indices. When absolute values of indices between the first and the second interval of exposure are compared, it is noticed that all male values irrelevant of the dietary treatment are lower after a longer exposure. A possible explanation for such effect could be advanced age of the animals, although previous results[1] indicate that male animals between 5–16 months of age have an almost constant value of the cortical index.

The influence of cadmium and mercury on bone was significant only after the first interval of exposure and only in female rats. This might be due to higher intestinal cadmium absorption in females than in males[11]. These changes were

TABLE 3

Cortical index CA/TA of rat femur measured after four months of exposure to coal ash and/or toxic elements. Results are presented as arithmetic means of seven animals in each group ± S.E.M.

Rats	Ash in diet (%)	Toxic element in drinking water			
		None	Cadmium (100 ppm)	Manganese (2000 ppm)	Mercury (50 ppm)
Females	0	0.674 ± 0.017	0.656 ± 0.005	0.666 ± 0.013	0.640 ± 0.012
	0.5	0.630 ± 0.010	0.647 ± 0.014	0.626 ± 0.018	0.641 ± 0.010
	1	0.650 ± 0.009	0.628 ± 0.010	0.632 ± 0.012	0.636 ± 0.016
	5	0.648 ± 0.008	0.581 ± 0.020	0.624 ± 0.008	0.603 ± 0.016
Males	0	0.633 ± 0.017	0.636 ± 0.006	0.619 ± 0.012	0.648 ± 0.015
	0.5	0.638 ± 0.008	0.644 ± 0.008	0.628 ± 0.011	0.626 ± 0.009
	1	0.641 ± 0.014	0.631 ± 0.013	0.619 ± 0.014	0.638 ± 0.011
	5	0.564 ± 0.017	0.608 ± 0.034	0.529 ± 0.015	0.607 ± 0.020

TABLE 4

Cortical index CA/TA of rat femur measured after eight months of exposure to coal ash and/or toxic elements. Results are presented as arithmetic means of seven animals in each group ± S.E.M.

Rats	Ash in diet (%)	Toxic element in drinking water			
		None	Cadmium (100 ppm)	Manganese (2000 ppm)	Mercury (50 ppm)
Females	0	0.630 ± 0.013	0.599 ± 0.017	0.639 ± 0.013	0.632 ± 0.013
	0.5	0.655 ± 0.016	0.645 ± 0.011	0.637 ± 0.018	0.652 ± 0.012
	1	0.647 ± 0.012	0.628 ± 0.016	0.645 ± 0.011	0.638 ± 0.015
	5	0.588 ± 0.010	0.573 ± 0.018	0.578 ± 0.011	0.550 ± 0.018
Males	0	0.588 ± 0.015	0.599 ± 0.011	0.570 ± 0.009	0.589 ± 0.013
	0.5	0.592 ± 0.019	0.578 ± 0.012	0.587 ± 0.008	0.560 ± 0.021
	1	0.592 ± 0.012	0.578 ± 0.019	0.589 ± 0.014	0.604 ± 0.013
	5	0.513 ± 0.007	0.498 ± 0.013	0.514 ± 0.013	0.524 ± 0.027

not significant after the second interval of exposure. A possible explanation for such results is adaptation of the animals to applied doses of cadmium after a longer exposure.

Very little or no influence of cadmium and other toxic metals on bone could be explained by the relatively high calcium and iron content of our control diet (1.2% Ca and 0.06% Fe). The diet with ash additive had an even higher content of these elements[15] and increased calcium and iron in the diet might cause decreased absorption of toxic elements in the gut[14].

The fact that the addition of toxic metals did not potentiate the effect of ash on bone is in agreement with the results of our pharmacokinetic studies[12] where we found that ash additive to diet did not influence the absorption, retention and distribution of cadmium, manganese and mercury in rats.

TABLE 5
Analysis of variance of cortical index CA/TA obtained after four months of treatment.

Source of variance	DF	Females		Males	
		F ratio	P value	F ratio	P value
Coal ash	3	5.29	<0.01	4.68	<0.01
Cadmium	1	6.15	<0.05	0.81	N.S.
Coal ash × cadmium	3	3.69	N.S.	0.94	N.S.
Coal ash	3	4.49	<0.01	18.51	<0.01
Manganese	1	2.34	N.S.	4.45	N.S.
Coal ash × manganese	3	0.26	N.S.	0.31	N.S.
Coal ash	3	2.08	N.S.	6.68	<0.01
Mercury	1	4.23	<0.05	1.18	N.S.
Coal ash × mercury	3	2.42	N.S.	1.41	N.S.

TABLE 6
Analysis of variance of cortical index CA/TA obtained after eight months of treatment.

Source of variance	DF	Females		Males	
		F ratio	P value	F ratio	P value
Coal ash	3	10.16	<0.01	18.55	<0.05
Cadmium	1	3.84	N.S.	0.77	N.S.
Coal ash × cadmium	3	0.24	N.S.	0.31	N.S.
Coal ash	3	12.42	<0.01	17.64	<0.01
Manganese	1	0.44	N.S.	0.64	N.S.
Coal ash × manganese	3	0.45	N.S.	0.34	N.S.
Coal ash	3	16.58	<0.01	10.49	<0.01
Mercury	1	1.65	N.S.	0.02	N.S.
Coal ash × mercury	3	0.92	N.S.	0.92	N.S.

The results of this study indicate that the high ash content in the diet significantly reduces bone density parameter – the cortical index (CA/TA). Whether the diet with a high ash content affects calcium metabolism in the rat through its toxic components or the reduction of bone mass and body weight is a consequence of malnutrition, remains to be explained. No synergistic effect of toxic elements in our experimental conditions was observed.

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