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Effect of Dietary Protein on Locomotor Activity During Chronic Lead Exposure in Male and Female Rats

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Lead intoxication has been recognized as a public health problem for approximately two decades. Acute lead exposure produces a well defined toxic syndrome (lead encephalopathy) with resultant frank neurological deficits that are easily recognized (BAROCAS & WEISS 1974; LIN-FU 1980). In contrast, the effects of chronic exposure to lead are much less clearly defined. It is suggested that neurological deficits, hypoactivity, or hyperactivity may result from chronic exposure to low levels of lead. The immature or developing animal appears to be under a more substantial health risk than the adult following chronic exposure to lead. Studies have demonstrated behavioral disorders indicative of minimal brain dysfunction in children exposed to low lead levels (DAVID et al. 1972, 1977). Low level lead exposure is also believed to induce changes in cognitive ability in children (HAMMOND & BELILES 1980). A study of 210 institutionalized children with nonclassified mental retardation revealed that the incidence of elevated blood-lead levels was 45%, while in the 80 normal controls, the incidence was 2.5% (MONCRIEFF et al. 1964).

The questions surrounding the toxic effects of chronic low level exposure to lead in humans are further compounded by the probability that specific toxic responses to lead exposure are modified by external variables. These include but may not be limited to socioeconomic status, health status, nutrition, emotional status and environmental stress, (VERLANGIERI 1978). Nutritional components may play a significant role in the mediation of response to chronic lead intoxication in children. Rats exposed to lead while receiving a diet deficient in protein, gained less weight, showed decreased hemoglobin levels and had a higher mortality rate than controls (BAERNSTEIN & GRAND 1942). A more recent study (ANDERS 1982) suggests that the dietary protein source may modify lead toxicity.

The purpose of the study described here was to examine the interaction between chronic lead intake and running wheel activity in weanling rats while under various dietary protein regimes. The objective of this study was to ultimately provide insight into the effects of chronic lead exposure on locomotor activity in animals under different levels of nutritional stress.

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MATERIALS AND METHODS

1. Experimental Animals:

Twenty-five, in-house, female Wistar rats, 200-225g, were mated with proven male breeders. They were housed in individual stainless steel cages and maintained on standard rat chow (Ralston-Purina) and tap water ad libitum. The animal room was maintained at 18±1°C and set on a 12h light:dark photoperiod.

Day-zero of pregnancy was determined by vaginal lavage with saline and subsequent microscopic examination for the presence of sperm. Once impregnation by this criteria was established, the females were placed in individual lactation cages throughout gestation, parturition and nursing. Tap water and rat chow was available ad libitum.

Weaning began after the pups were 21 days of age. All pups were identified as to dam, sorted by sex and pooled. From the pool 18 males and 18 females were chosen at random and assigned to the experimental groups. (See Table I.) Each pup was housed singly and the animal room set on a 19:5h light:dark photoperiod to maximize running activity (VERLANGIERI 1978).

2. Lead Exposure:

All treated animals received lead via the drinking water. The animals received 0.136mg lead acetate ($Pb(C_2H_3O_2)_2.3H_2O$) per ml of deionized water. This concentration is equivalent to 74.5ppm lead. This level is at least 5 times lower than that considered a safe exposure level for children (on a body-weight basis) (GOLDFRANK & OSBORN 1977). Control (no lead) animals received deionized water. Water was available ad libitum to all groups.

3. Diet:

Diets were obtained from Teklad Test Diets (Madison, WI). The various experimental groups were fed diets containing the following percentages of protein (as vitamin free casein): Group I (low protein)-3.2%, Group II (normal protein)-23.7% and Group III (high protein)-50.0%. The description of each experimental group is shown in Table I.

TABLE I
EXPERIMENTAL GROUPS

GROUP	DIET	TREATMENT	NUMBER OF ANIMALS	
	% PROTEIN		MALE	FEMALE
I(L)[a]	3.2	Lead	3	3
		No Lead	3	3
II(N)	23.7	Lead	3	3
		No Lead	3	3
III(H)	50.0	Lead	3	3
		No Lead	3	3

[a]L = low protein; N = normal protein; H = high protein

4. Experimental Design:

Running wheel activity was measured in Wahman LC33 wheel cages for five-hour intervals, during the dark period, once per week. Experimental animals were rotated to different wheels on each running day to compensate for difference in wheel torque, revolution counters or wheel placement within the running room. Only water was available during the activity period.

Body weights, water and food consumption were measured throughout the 52 week study. Random urine collections were made to monitor urinary Δ-aminolevulinic acid (ALA), as an indicator of lead intoxication, and analyzed according to KORNFELD et al. (1972). Blood samples were drawn in the last week of the study and hematocrits determined.

All data was statistically analyzed by either the Student's "t-test" or one-way ANOVA followed by Duncan's Multiple Range Test.

RESULTS

1. Body Weight:

The low protein diet regime (Group I) retarded growth significantly in both sexes compared to Group II, normal protein (see Table II). In addition, lead treatment further reduced body weights in the low protein group significantly. However, lead treatment did not adversely affect body weight gains in either the normal (Group II) or high protein (Group III) diet groups. Body weights of rats (male or female) fed the high protein diet for 52 weeks were not significantly different from those receiving normal protein diets; however, in both groups body weights by 52 weeks were significantly different from baseline values.

TABLE II
MEAN BODY WEIGHTS OF MALE AND FEMALE RATS
BEFORE AND AFTER INITIATION OF DIET/TREATMENT REGIMES

GROUP	TREATMENT	MALES		FEMALES	
		Baseline*	52 Weeks	Baseline	52 Weeks
I (L)	Lead	119 (4.2)[a]	122 (7.1)[b]	102 (9.7)	98.3 (2.7)[b]
	No Lead	118 (7.3)	166 (11.4)[b,c]	119 (5.4)	185 (3.1)[b,c]
II (N)	Lead	117 (6.6)	606 (108.7)[c]	114 (2.9)	385 (23.8)[c]
	No Lead	128 (10.2)	552 (55.9)[c]	116 (6.5)	396 (28.2)[c]
III (H)	Lead	154 (12.1)	619 (43.8)[c]	98.0 (3.7)	361 (12.8)[c]
	No Lead	124 (9.7)	606 (59.0)[c]	124 (8.8)	393 (18.6)[c]

L = low protein; N = normal protein; H = high protein

*Baseline levels prior to initiation of diet/treatment regime

[a]Standard error

[b]Significantly different from Group II treatment-matched control ($p \leq 0.05$) by ANOVA followed by Duncan's Multiple Range Test.

[c]Significantly different from baseline value ($p \leq 0.05$) by Student's "t-test."

2. Water (Lead) and Food Consumption:

Water consumption (ml/kg/d) did not vary significantly between any diet or lead-treated groups. (See Table III.) Accordingly, the total lead intake per day was equivalent for all treatment groups.

TABLE III
DAILY MEAN WATER AND LEAD INTAKE
OVER 52 WEEKS IN MALE AND FEMALE RATS

DIET	TREATMENT	MALES		FEMALES	
		ml/kg/d	µgPb/kg/d	ml/kg/d	µgPb/kg/d
I(L)	Lead	130(16)[a]	9.8(1.2)	113(8)	8.3(0.7)
	No Lead	121(12)	--	107(11)	--
II(N)	Lead	81(9)	6.8(0.5)	80(14)	7.1(0.1)
	No Lead	72(13)	--	86(21)	--
III(H)	Lead	97(6)	7.8(0.2)	112(9)	9.1(0.8)
	No Lead	89(14)	--	106(17)	--

L = low protein; N = normal protein; H = high protein
[a]Standard error

Food consumption (g/kg) was significantly elevated in both non-treated male and female rats on the low protein regime (Group I) compared to animals on normal protein diets. Lead treatment in this group (low protein) further increased the parameter. There were no other significant differences in food consumption between any other diet/treatment groups.

3. Urinary Δ-Aminolevulinic Acid (ALA):

Urinary ALA (mg%) was significantly elevated in all lead-treated groups (independent of diet) by the last week of the study (week 52) compared to baseline levels. Values ranged from 0.34-0.69 and 0.31-0.57 mg% for untreated males and females respectively compared to 0.68-0.91 and 0.67-0.86 mg% for lead-treated males and females respectively.

4. Hematocrits:

There were no significant differences in mean terminal hematocrits between any of the diet/treatment groups. Female animals tended to have slightly lower values (0.44±0.04) than males (0.51 ±0.07); this was not significant however.

5. Running Wheel Activity:

The low protein diet regime alone produced a significant increase in running wheel activity (compared to Group II, normal protein) in males as shown in Table IV. Additionally, lead treatment in the low protein group, further elevated this parameter (hyperactivity) in both males and females (with respect to diet-matched controls.)

Lead treatment in animals receiving the normal protein dietary regime resulted in a significant decrease in activity in males

(hypoactivity) and a significant increase in this parameter in females (hyperactivity) when compared to diet-matched controls.

A significant increase in running wheel activity was observed in lead-treated males receiving the high protein diet (Group III) compared to diet-matched controls. This hyperactivity was not found in lead-treated females of this group however.

TABLE IV
MEAN RUNNING WHEEL ACTIVITY (REVS/5HR) BETWEEN WEEKS 46-52 IN MALE AND FEMALE RATS WITH RESPECT TO LEAD TREATMENT AND DIETARY PROTEIN CONTENT

GROUP	TREATMENT	MALE	FEMALE
I (L)	Lead	4352 (75)[a,c]	3437 (50[c]
	No Lead	2579 (524)[b]	1447 (719)
II (N)	Lead	167 (53)[c]	1520 (199)[c]
	No Lead	551 (104)	854 (208)
III (H)	Lead	432 (11)[c]	1424 (354)
	No Lead	136 (91)	1263 (94)

L = low protein; N = normal protein; H = high protein
[a]Standard error
[b]Significantly different from Group II treatment-matched control ($p \leq 0.05$) by ANOVA followed by Duncan's Multiple Range Test.
[c]Significantly different from diet-matched control ($p \leq 0.05$) by Student's "t-test."

DISCUSSION

The body weight data presented herein appear to indicate that the low protein diet (Group I) induced a substantial "dietary stress" in animals in this group. Despite increased food intake (relative to the normal protein group), minimal body weight gain occurred. Lead treatment interacted in the low protein group to further retard growth.

Urinary ALA levels were significantly elevated in all lead-treatment groups, however there was no effect on hematocrit values. This phenomenon demonstrates the chronic nature of this mode of exposure, since certain hematological effects are only expected in acute high-level lead intoxications (BARLTROP 1977).

The hyperactivity of the non-lead-treated animals in Group I is apparently due to a protein deficiency or carbohydrate excess. The diets were formulated by substituting cornstarch for casein when altering the protein content. The low protein diet then, is also a high carbohydrate diet. COLLIER et al. (1972) has suggested that rats increase their running wheel activity on high carbohydrate diets in order to maintain the body weights. Lead treatment in this group potentiated this hyperactivity.

This may be due at least in part to the action of lead on the central nervous system, the severity of which may be dictated by the dietary characteristics of this group. Running wheel activity in this group of lead-treated animals was an order of magnitude higher than in other groups. It is possible that the animals had a greater absorption of lead from the gastro-intestinal tract than did animals in other groups. Lead is known to be chelated by sulfur-containing amino acids (SHIH & HANIN 1978) which may limit its absorption. It would therefore be expected that protein-deficient diets would result in increased absorption of ingested lead.

Lead-treated males in Group II (normal protein) exhibited a significant hypoactivity. This type of response has been observed previously by VERLANGIERI (1978) who reported hypoactivity in males under normal non-stressful conditions, and hyperactivity under conditions of "auditory-stress." Lead-treated females in Group II became hyperactive however. This may be the result of a sex-dependent behavioral response to lead intoxication which is mediated by hormonal factors.

Group III lead-treated males exhibited hyperactivity while no effect of lead treatment was observed in females of this group. It is interesting to speculate that the high protein diet of animals in Group III may be producing a form of "metabolic-stress" via amino acid metabolism. Flooding the system with excess amino acids, as would be expected in protein-rich diets may result in increased levels of circulatory and tissue ammonia. It has been demonstrated that exposure to sub-lethal levels of ammonia may result in hepatotoxic changes related to declinations in the nutritional status of the animal (KAPEGHIAN et al. 1982). Under these conditions, dietary-induced stress may interact with lead-induced changes to cause a hyperactive animal. Why hyper-activity was only seen in males of this group is unexplained; there may be some important hormonal factors which play a role in these interactions as earlier speculated.

The results of this study suggest that a component in the expression of lead toxicity (as measured by changes in running activity) is the interaction of the effects of chronic lead ingestion with nutritional factors. These factors may either be in the form of nutritional deficiencies or excesses which serve to alter normal growth and metabolic processes. The identification of populations at risk from chronic lead exposure may be better accomplished by parallel assessments of both the nutritional profile and metabolic stresses present.

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