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Effects of lead deficiency on lipid metabolism

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Summary: The criteria of lipid metabolism in lead deficiency were determined. For this purpose an experiment was performed with Sprague-Dawley rats distributed in depletion and control groups. The depletion rats were fed an extremely low lead content diet (20 ± 5 ppb), whereas the diet for the control animals was supplemented with 800 ppb Pb^{++}. Dependent on generation, depletion influenced triglyceride content in serum and liver, phospholipid and cholesterol content in liver as well as the whole body fat of the offspring. The results indicate that in mothers the absorption of lipids is impaired. Consequently it is possible that the mothers do not excrete enough fat with milk leading to an energetical deficiency and therefore to reduced growth.

Zusammenfassung: Nachdem sich in Modellstudien zum Nachweis der Essentialität von Blei bei unzureichender Pb-Versorgung von Muttertieren Veränderungen im Lipid-Stoffwechsel bei ihren Nachkommen zeigten, sollten im vorliegenden Versuch Parameter des Lipidstoffwechsels sowohl bei Muttertieren als auch bei deren Nachkommen untersucht werden. Der Versuch wurde mit weiblichen Sprague-Dawley-Ratten über mehrere Generationen durchgeführt. Eine teilweise gereinigte halbsynthetische Diät sowie Trinkwasser wurden ad libitum angeboten. Der Pb-Gehalt der Basisdiät, die als Depletionsdiät eingesetzt wurde, betrug 20 ± 5 ppb. Für die Kontrolltiere wurde die Basisdiät mit 800 ppb Pb^{++} supplementiert. Durch die Depletion an Blei wurde in Abhängigkeit von der Generation der Triglyceridgehalt in Serum und Leber, der Phospholipid- und Cholesterolgehalt der Leber sowie der Gesamtlipidgehalt des Körpers beeinflußt. Im Vergleich zu Kontrolltieren war bei den depletierten Tieren der P_0-Generation der Triglyceridgehalt des Serums reduziert und der Gesamtfettgehalt des Körpers unverändert, bei den depletierten Tieren der F_1-Generation der Triglyceridgehalt in Serum und Leber reduziert und der Phospholipid- und Cholesterolgehalt der Leber erhöht, bei den depletierten Tieren der P_1-Generation der Triglyceridgehalt der Leber erhöht, der Triglyceridgehalt des Serums erheblich vermindert und der Phospholipid- und Cholesterolgehalt der Leber sowie der Gesamtfettgehalt des Körpers unverändert und bei den depletierten Tieren der F_2-Generation der Triglycerid-, Phospholipid- und Cholesterolgehalt der Leber unverändert, der Triglyceridgehalt im Serum erhöht sowie der Gesamtfettgehalt des Körpers vermindert.

Key words: lead depletion, lipid metabolism, serum triglycerides, liver triglycerides, liver phospholipids, total body lipids

Introduction

In previous animal studies in which rats were experimentally depleted of lead, clinical symptoms of lead deficiency could be reproducibly induced by providing rats with insufficient alimentary lead. Thus, lead

was shown to be an essential element for the organism. Symptoms of lead deficiency, which are growth depression (4, 5) and anemia (6), were observed in the offspring of lead-depleted female rats. These symptoms were shown to be related to biochemical changes in the organism, and in particular to changes in iron and lipid metabolism (1, 2, 7).

In this study, we investigated criteria of lipid metabolism in adult female rats and their offspring after the rats were provided with either a deficient, or sufficient, lead supply.

Materials and Methods

Twenty female rats with initial weights of 30 ± 1 g were divided into a depletion group and a control group. At sexual maturity (ca. 200 g) the rats were mated, after gravida and 21 days' lactation, dams were decapitated under ether narcosis, bled, and then the liver and pancreas of each animal were removed (p_0 generation). Offspring of the p_0 generation dams, with the exception of one representative female per litter, were also decapitated at the end of the suckling period, liver and pancreas were removed (f_1 generation). The representative female from each litter was raised to sexual maturity, mated, allowed to lactate for 21 days and then was also decapitated and dissected (p_1 generation). The offspring of the p_1 generation dams were likewise sacrificed and treated at the end of lactation (f_2 generation).

The rats were supplied, ad libitum, a half-synthetic diet consisting of the following: 20 % casein, 34.8 % starch, 28 % sucrose, 8 % coconut fat, 4 % mineral "mix", 3 % cellulose, 2 % vitamin "mix", and 0.2 % DL-methionine. The mineral "mix" consisted of 8.28 g $Na_2HPO_4 \cdot H_2O$, 8.2 g KH_2PO_4, 6.0 g KCL, 3.4 g $MgCl_2 \cdot 6\ H_2O$ and 13.6 g $CaCO_3$ per 10 kg diet. The following trace elements were added: Fe (50 ppm), Zn (50 ppm), Cu (12 ppm), Mn (40 ppm), I (7 ppm), Ni (1 ppm), F (540 ppb), Mo (200 ppb), Sn (300 ppb), Se (200 ppb), Cr (100 ppb), V (100 ppb) and Si (200 ppb). Casein was isolated from skimmed curds and purified according to the method of Schnegg and Kirchgeßner (9). Cellulose was also purified by the a method of Schnegg and Kirchgeßner (9). Minerals were purified using a modification of the method of Williams and Mills (10). The depletion diet contained 20 ± 5 ppb lead. 800 ppb lead, in the form of supra-pure lead acetate, was added to the control diet. The rats were provided with pure drinking water with 0.014 % supra-pure NaCl ad libitum.

Rats were housed in fully air-conditioned rooms (temperature 23 ± 1 °C, relative humidity 60 ± 10 %) in macrolon® cages. The rats were kept on a 12 h night-day cycle.

Rat liver, pancreas, carcass and serum were deep-frozen until they could be analyzed. Homogenization of liver and pancreas was performed with a Potter Elevehjem homogenisator. Triglyceride, phospholipids and cholesterol were determined with kits from Boehringer, Mannheim. Total body fat from the rat carcass (minus liver and pancreas) was determined after disintegration of each carcass in HCL and subsequent ether extraction (3). Livers from dams of the p_0 generation as well as carcasses from the f_1 generation rats could not be used for these determinations, as these livers and carcasses were used for trace element determinations.

Data were evaluated using the analysis of variance. The multiple t-test was used to test for significant variation between group mean values. The results show group mean values together with standard errors of the mean (± values). Group mean values which are significantly different from one another are indicated by raised characters.

Results

Table 1 summarizes the liver triglyceride concentrations from 21 day old rats of the f_1 and f_2 generation and from dams of the p_1 generation. The triglyceride concentration in livers of f_1 generation lead-depleted rats was 25 % lower than the concentration in livers of the f_1 control group rats, although no difference in liver triglyceride levels was observed between depletion and control animals of the f_2 generation. In contrast, the liver triglyceride levels in lead depleted dams (p_1) was 88 % higher than levels in the p_1 control group dams.

Table 2 demonstrates triglyceride levels in serum of p_0 and f_1 generation dams and their 21 day old f_1 and f_2 generation offspring. Serum triglyceride concentrations for the depletion dams of the p_0 and p_1 generation and for the young depletion rats of the f_1 generation were reduced in comparison to concentrations for the respective control animals. On the other hand, depletion animals of the f_2 generation had higher serum triglyceride levels than did the f_2 control animals.

In Table 3, liver phospholipid concentrations from 21 day old f_1 and f_2 generation rats and p_1 generation dams are depicted. The phospholipid

Table 1. Triglyceride content in liver of p_1 generation mothers and offspring from f_1 and f_2 generations dependent on deficient and sufficient Pb supply (FM = fresh matter).

Generation	Depletion mg/g FM	Control mg/g FM	Depletion mg/g Protein	Control mg/g Protein
f_1	24.3^a ± 1.8	35.6^a ± 3.9	381^a ± 41	512^b ± 56
p_1	9.3^b ± 0.6	5.4^a ± 0.8	86.3^b ± 13.3	45.7^a ± 6.4
f_2	33.6^a ± 4.3	27.3^a ± 3.6	582^a ± 87	496^a ± 65

Table 2. Triglyceride content in serum of mothers from p_0 and p_1 generations and their offspring (f_1 and f_2 generations) resulting from deficient and sufficient Pb supply.

Generation	Depletion	Control
p_0	3.0^a ± 0.5	5.7^b ± 0.9
f_1	3.6^a ± 0.2	4.3^b ± 0.3
p_1	4.2^a ± 0.9	20.1^b ± 1.4
f_2	4.3^b ± 0.4	3.2^a ± 0.3

Table 3. Phospholipid content in liver of mothers from the g_1 generation and the offspring of f_1 and f_2 generations dependent on deficient and sufficient Pb supply (FM = fresh matter).

Generation	Depletion mg/g FM	Control mg/g FM	Depletion mg/g Protein	Control mg/g Protein
f_1	13.2[b] ± 0.7	10.5[a] ± 0.4	196[b] ± 11	151[a] ± 6
p_1	9.8[a] ± 1.1	9.3[a] ± 0.4	91[a] ± 10	81[a] ± 4
f_2	6.5[a] ± 0.6	6.5[a] ± 0.5	112[a] ± 12	117[a] ± 11

Table 4. Cholesterol content in liver of mothers from g_1 generations and offspring from f_1 and f_2 generations resulting from deficient and sufficient Pb supply (FM = fresh matter).

Generation	Depletion mg/g FM	Control mg/g FM	Depletion mg/g Protein	Control mg/g Protein
f_1	2.7[a] ± 0.2	4.0[b] ± 0.1	39.7[a] ± 2.2	53.1[b] ± 2.2
p_1	9.2[a] ± 0.8	9.3[a] ± 0.9	79.7[a] ± 5.6	85.8[a] ± 7.3
f_2	4.0[a] ± 0.2	3.9[a] ± 0.1	65.6[a] ± 2.8	69.5[a] ± 2.2

Table 5. Whole body fat (% of dry matter) of mothers from p_0 and p_1 generations and offspring from the f_2 generation resulting from deficient and sufficient Pb supply.

Generation	Depletion	Control
p_0	23.0[a] ± 2.9	27.4[a] ± 3.2
p_1	33.7[a] ± 1.5	33.5[a] ± 3.1
f_2	26.1[a] ± 0.6	30.0[b] ± 0.8

concentration in the livers of f_1 depletion animals was 26 % higher than the value for the f_1 control rats. In contrast, there was no observable difference between phospholipid concentrations of depletion and control group animals of the f_2 and the p_1 generations.

The cholesterol concentration in livers of p_1 generation dams and 21 day old f_1 and f_2 generations animals are shown in Table 4. Only in the f_1 generation rats did liver cholesterol concentrations differ between deple-

tion and control group animals, f_1 offspring of lead depleted dams had lower liver cholesterol levels than did the f_1 control group animals.

Table 5 demonstrates total body fat in the carcasses (sacrificed, bled rats minus liver and pancreas) of the p_0 and p_1 generation and f_2 offspring animals. Between depletion and control groups of p_0 and p_1 dams, there was no observable difference, however, depletion rats of the f_2 generation had lower total body fat concentrations than did the f_2 generation control rats.

Discussion

In general, experiments presented here show that insufficient lead supply affects lipid metabolism in both rat dams and their offspring. Hereby, triglyceride levels are affected to a greater, and phospholipid and cholesterol levels to a lesser, extent. The metabolism changes in the rat dams were obviously less intensive than in their offspring, as the total body fat content had not yet been reduced. In dams, the triglyceride concentration was most affected in serum. The extremely low triglyceride level in serum of p_1 generation dams is most likely the cause of raised triglyceride levels in the liver, which in turn possibly represent a strong compensatory triglyceride synthesis in the liver. The elevated lipase activity in the dam pancreas can be interpreted as a compensatory measure in order to cover the especially high energy requirement present during lactation (8).

The reduced triglyceride concentration in the serum and liver of f_1 rats at the end of their suckling period confirms results from earlier experiments on lead depletion. The reduced triglyceride levels are most likely related to the consistent negative changes caused by insufficient lead supply, once again confirming the essentiality of lead.

In contrast, f_2 generation depletion rats had, when compared to control rats, unchanged triglyceride levels in the liver and elevated triglyceride concentrations in the serum. These most likely result from compensatory mechanisms, as the total body fat content was lower in carcasses of the depleted f_2 animals than it was in the f_2 control animals. Assumably, the reduced total body fat of f_2 depletion animals is due to either reduced lipid absorption or lowered fat intake over the mothers' milk. It is possible that the triglyceride level in milk of p_1 dams was reduced, as disturbances in the lipid metabolism of these dams had already been observed. This hypothesis must be tested in further experiments, and the question must be asked as to why the triglyceride content in mothers' milk is reduced, eventually as a result of reduced lead supply. Consistent with their reduced fat intake and hampered fat absorption is the reduced lipase activity in the pancreas of these rats (8).

In terms of weight, the reduced total body fat concentration in carcasses of the lead depleted animals corresponds to the growth depression observed (5). Thus, disturbances in lipid metabolism are probably the primary cause of the reduced growth in offspring of lead depleted female rats which had been observed during the suckling period. This hypothesis is supported by the fact that the raw protein content in carcasses of

depletion animals was unchanged in comparison to the value for control animals (unpublished data).

As growth depression in offspring always appeared post-partum (4, 5), the actual disturbances in lipid metabolism must be searched for in lactating animals.

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