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FREE FATTY ACID MOBILIZATION IN THE DEVELOPMENT OF CERIUM-INDUCED FATTY LIVER

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Summary: Following cerium injection to female rats: (1) Plasma free fatty acids (FFA) concentration increases during the first 24 hours, then remains constant up to 48 hours. (2) Adipose tissue lipolytic activity increases tremendously during the first 12 hours (+380%), maintaining high values throughout the study (48hrs). These modifications are followed by a time-dependent increase of total liver lipids consisting mainly of triglycerides and to a less extent of cholesterol. (3) Adrenalectomy prevented the development of cerium--induced fatty liver : plasma FFA and lipolysis failed to increase in adrenalectomized cerium-treated animals. Thus, our study demonstrates the involvement of adrenergic stimulation of adipose tissue lipase as an obligatory step in the development of cerium-induced fatty liver.

Introduction: The development of fatty liver after intravenous injection of rare earth salts has been fairly well studied (1-4). Although the increase of plasma free fatty acids (FFA) resulting from rare earth administration is thought to be responsible for the accumulation of liver triglycerides (5), the determination of glyceride fatty acids composition in liver and adipose tissue does not define the origin of fatty acids which accumulate in the rare earth fatty liver (4). It has been previously established that hormones play a major role in the toxicity of rare earth salts : while intact female and castrated male rats develop a fatty liver, hypophysectomy protects the liver of female rats from fatty infiltration (2). Nevertheless, the complexity of hormonal interactions precludes any definite interpretation of previously published data concerning the particular role of each endocrine gland.

ABBREVIATIONS: FFA : free fatty acids; b.w. : body weight.

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In this study, we demonstrate the existence of a direct relationship between plasma FFA, adipose tissue lipolytic activity and the development of fatty liver. In addition, we make evident an essential role of adipose tissue lipase activation through adrenal hormones in the cerium-induced fatty liver.

MATERIALS and METHODS:

Animals: Female Wistar rats weighing 200-250 g received under light ether anesthesia 0.5 ml of 155 mM sodium chloride (control rats) or cerium chloride (3 mg of cerium, as element, per kg b.w.) by intravenous injection in the femoral vein. As cerium administration induces a reduced food intake, control- and cerium-treated rats were pair-fed. All animals were fasted overnight before the sacrifice with free access to tap water. Where mentionned, bilateral adrenalectomy was performed under pentobarbital anesthesia (30 mg/kg b.w.) 72 hours before saline or cerium injection.

Analytical methods:At the time indicated after saline or cerium injection, blood was sampled under light ether anesthesia by aortic puncture using heparinized syringe. After perfusion of isotonic saline through the portal vein and complete blanching, the liver was removed, blotted on filter paper and weighed. The lipids of about 1 g of liver were extracted in duplicate samples (6). Triglycerides (7), cholesterol (8) and phospholipids (9) determinations were carried out on aliquots of the lipid extract. Total liver lipids were determined gravimetrically. After centrifugation of the blood at +4°C, plasma FFA concentration was determined (10).

Determination of lipolytic activity: Fat pads were sampled bilaterally, minced with scissors, washed with isotonic saline to remove blood and blotted on filter paper. Aliquots of adipose tissue weighing about 200 mg were incubated at 37°C during 60 minutes in 3 ml of Krebs-Ringer buffer, pH=7.4, containing 10 mM glucose and 0.3 mM delipidated albumin (11). Lipolytic activity was calculated after determination of FFA medium concentration (10) at the beginning and at the end of the incubation period. In order to test the direct effect of cerium ions on the lipolytic activity, adipose tissue lipase was extracted and its activity determined according to the method of Rizack (12).

Chemicals: All the chemicals for routine use were obtained from Prolabo (Paris, France). Pentobarbital sodium was obtained from Lathévet (Paris, France) and cerium chloride from Koch-Light (Colnbrook, England).

Statistical methods: The significance of differences between control and cerium-treated rats was determined with the Mann-Whitney U-test.

RESULTS and DISCUSSION: Following cerium injection, plasma FFA concentration increases and reaches maximum values 24 hours later (Figure 1). This increase is statistically significant as early as 12 hours and is more pronounced between 12 and 24 hours after cerium. High values of plasma FFA are then maintained with a tendency to decrease between 24 and 48 hours (Figure 1). Our results are in agreement with those published by Snyder and Stephens (5). These authors suggested that the

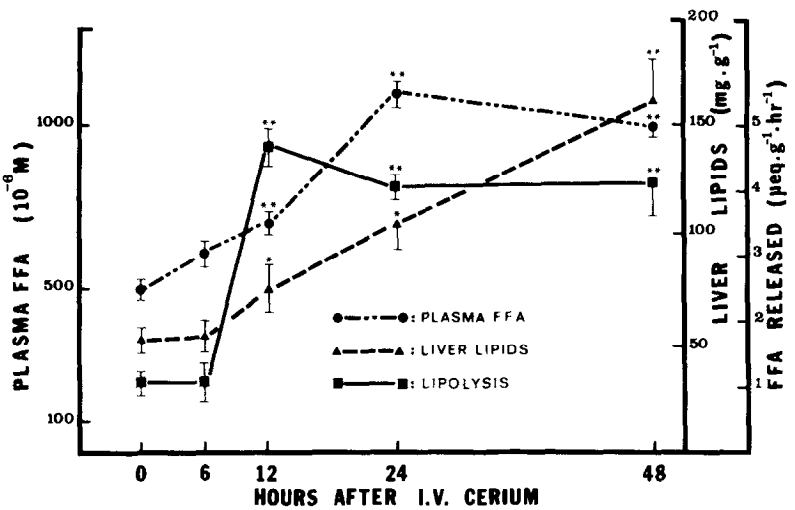

FIGURE 1 : Evolution of plasma FFA, adipose tissue lipolytic activity and liver lipids after cerium injection. Each point represents the mean ± SEM of 6 determinations. All values were determined and compared as described in MATERIALS and METHODS.

observed increase in plasma FFA concentration resulted from extrahepatic mobilization (5).

In order to verify this hypothesis, we determined the lipolytic activity of adipose tissue at different times after cerium injection. Between 6 and 12 hours we observed a 4-fold increase of adipose tissue lipase activity which precedes the increase of plasma FFA (Figure 1). This kinetic study constitutes the first direct evidence that FFA accumulation in the plasma observed after cerium injection results from increased adipose tissue lipolytic activity.

In order to clear up the mechanism of adipose tissue lipase activation, we tested the possibility of a direct action of cerium ions on this enzyme. After extraction of lipase from adipose tissue obtained from intact rats, we measured the effect of cerium chloride addition *in vitro* on the lipase activity. Our results rule out a direct effect of cerium on the lipase : without cerium: 551.2 ± 41.9 nmol fatty acids released.hr^{-1} (n=4); cerium added (2.8 mM) : 570.3 ± 28.4 nmol fatty acids released.hr^{-1} (n=3). This lack of effect

contrasts with the lipase inhibition observed after addition of tetracyclin *in vitro*, another potent steatogenic agent (13).

As FFA uptake rate by the liver is directly related to their plasma concentration (14,15), the amount of fatty acids taken up by the liver of cerium-treated animals is greatly increased compared to control rats. It has been previously shown that in response to an increased influx of fatty acids into the liver, the amount of low density lipoproteins secreted by the liver is highly enhanced (16,17). On the contrary, despite a 2-fold increase of plasma FFA induced by cerium injection, plasma lipid concentration remains steady (4,18) or even decreases(19). This disproportion between FFA influx and lipid secretion leads to a progressive accumulation of liver lipids (Fig. 1). Previous investigations have shown that the bulk accumulation of liver lipids is achieved within 48 hours (2) and then remains constant (4) or slightly increases (3) 72 hours after cerium injection. Later on, a decrease of lipid content of the liver is observed (3), showing much variation. This recovery period is difficult to study as only a few rats survive longer than 72 hours following cerium injection (2).

This progressive accumulation of lipids contrasts with the tetracyclin-induced fatty liver where a 2-fold increase in liver lipids is observed as early as 3 hours after injection of the antibiotic (13), and suggests a progressive perturbation of lipid metabolism in the liver of cerium-intoxicated animals. The lipids which accumulate consist mainly of triglycerides : we observe a 3-fold increase 12 hours and a 6-fold increase 24 hours after Ce, and to a less extent of cholesterol : +18% at 12 hours and +180% 24 hours after Ce. On the contrary, the liver phospholipid content remains unchanged (Table 1).

If high plasma FFA levels resulting from increased adipose tissue lipase activity are responsible for the fatty infiltration observed after

TABLE 1. EFFECT OF ADRENALECTOMY ON THE CERIUM-INDUCED FATTY LIVER.

Cerium administered ($mg.kg^{-1}$ b.w.)	TRIGLYCERIDES ($nmol.mg^{-1}prot.$)		CHOLESTEROL ($nmol.mg^{-1}prot.$)		PHOSPHOLIPIDS ($nmol.mg^{-1}prot.$)	
	A	B	A	B	A	B
0 (Control rats)	65 ± 3	55 ± 2	34 ± 2	27 ± 2	266 ± 27	209 ± 13
3.0 [a]	193 ± 30**	52 ± 5(NS)	41 ± 10*	29 ± 1(NS)	260 ± 31	192 ± 13(NS)
3.0 [b]	366 ± 14**	67 ±10(NS)	60 ± 3**	30 ± 2(NS)	254 ± 20	192 ± 2(NS)

All data are given as the mean ± SEM from 6 rats per group, determined and compared as described in MATERIALS and METHODS. Animals were sacrificed a: 12 or b: 24 hours after intravenous injection of $CeCl_3$. *: $p < 0.05$; **: $p < 0.01$; NS: not significant. A: intact rats; B: adrenalectomized rats.

TABLE 2. EFFECT OF ADRENALECTOMY ON THE CERIUM-INDUCED FREE FATTY ACID MOBILIZATION.

Cerium administered ($mg.kg^{-1}$ b.w.)	PLASMA FFA (μM)		LIPOLYTIC ACTIVITY FFA released ($\mu mol.g^{-1}.hr^{-1}$)	
	A	B	A	B
0 (Control rats)	368 ± 76	337 ± 28	2.4 ± 0.1	2.0 ± 0.2
3.0 [a]	700 ± 215**	278 ± 32 (NS)	3.6 ± 0.2*	2.5 ± 0.1 (NS)
3.0 [b]	1100 ± 196**	379 ± 26 (NS)	4.1 ± 0.4**	1.9 ± 0.1 (NS)

All data are given as the mean ± SEM from 6 rats per group, determined and compared as described in MATERIALS and METHODS. Animals were sacrificed a : 12 or b : 24 hours after intravenous injection of $CeCl_3$. * : $p < 0.05$; ** : $p < 0.01$; NS: not significant. A: intact rats; B: adrenalectomized rats.

cerium injection, a suppression of lipase activation should protect the liver from steatosis. As epinephrine controls the rate of adipose tissue lipase (12), we tested the possibility that adrenalectomy could suppress the lipolytic response observed after cerium injection into intact animals.

In fact, adrenalectomy performed 72 hours before cerium injection abolishes completely the development of fatty liver : triglycerides and cholesterol contents of the cerium- and control- group are similar (Table 1). Contrasting with our results, Snyder et al. found no or incomplete prevention of cerium-induced fatty liver after adrenalectomy depending on the strain of rats used (2). It is likely that differences in the response of adipose tissue lipase to epinephrine stimulation are responsible for these variations.

The complete prevention of liver triglycerides and cholesterol accumulation after injection of cerium into adrenalectomized rats indicates that adrenal hormones play a role of first importance in the etiology of the cerium-induced fatty liver.

Indeed, injection of cerium into adrenalectomized animals does not produce the important increase of plasma FFA observed 12 or 24 hrs after cerium injection into intact animals (Table 2). This observation prompted us to measure the adipose tissue lipolytic activity in vitro 12 or 24 hours after injection of cerium into adrenalectomized rats. Again, adrenalectomy prevents completely the considerable increase of lipolytic activity induced by cerium in intact rats (Table 2).

Thus, in the cerium-induced fatty liver, we have established a direct cause-effect relationship between : 1-The lipolytic activity of adipose tissue and the plasma FFA concentration. 2-The excess of plasma FFA and the accumulation of triglycerides in the liver. It is noteworthy to compare the mechanism of cerium-fatty liver with the tetracyclin-fatty liver : in the latter case, the steatosis results mainly

from impaired lipoprotein secretion and not from increased FFA influx since tetracyclin inhibits directly the adipose tissue lipase, resulting in a very low plasma FFA concentration (13). It is not known however if cerium chloride impairs the secretion of lipoproteins by the liver, a phenomenon enhancing the accumulation of hepatic triglycerides. This study is under current investigation in our laboratory.

REFERENCES:

1. Kyker, G.C., Cress, E.A., Sivaramakrishnan, V.M., Steffee, C.H. and Stewart, M. (1957) Fed. Proc. 16, 207-208.
2. Snyder, F., Cress, E.A. and Kyker, G.C. (1959) J. Lipid Res. 1, 125-131.
3. Snyder, F., Cress, E.A. and Kyker, G.C. (1960) Nature 185, 480-481.
4. Snyder, F. and Kyker, G.C. (1964) Proc. Soc. Exp. Biol. Med. 116, 890-893.
5. Snyder, F. and Stephens, N. (1961) Proc. Soc. Exp. Biol. Med. 106, 202-204.
6. Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497-509.
7. Kessler, G. and Lederer, H. (1965) Automation in Analytical Chemistry, pp. 341-344, L.T. Skeggs, New-York.
8. Sperry, W.M. and Webb, M. (1950) J. Biol. Chem. 187, 97-106.
9. Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375-400.
10. Trout, D.L., Estes, E.H. and Friedberg, S.J. (1960) J. Lipid Res. 1, 199-202.
11. Chen, R.F. (1967) J. Biol. Chem. 242, 173-181.
12. Rizack, M.A. (1961) J. Biol. Chem. 236, 657-662.
13. Infante, R., Koumanov, K. and Caroli, J. (1971) Gut 12, p. 765.
14. Soler-Argilaga, C., Infante, R., Renaud, G. and Polonovski, J. (1974) Biochimie 56, 757-761.
15. Heimberg, M., Weinstein, I. and Kohout, M. (1969) J. Biol. Chem. 244, 5131-5139.
16. Alcindor, L.G., Infante, R., Soler-Argilaga, C., Raisonnier, A., Polonovski, J. and Caroli, J. (1970) Biochim. Biophys. Acta 210, 483-486.
17. Nestel, P.J. and Steinberg, D. (1963) J. Lipid Res. 4, 461-469.
18. Arvela, P. (1974) Experientia 30, 1061-1062.
19. Lehman, B.V., Grajewski, O., Arntz, H.R. and Oberdisse, E. (1976) Acta Hepatogastroenterol. 24, 328-333.