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HOMEOSTASIS OF THE MATERNO-FETAL COMPLEX OF EXPERIMENTAL ANIMALS AFTER ETHANOL ADMINISTRATION. NOTE I. LIPID HOMEOSTASIS

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ABSTRACT. Blood samples collected from pregnant Wistar strain rats randomized in three groups (C – control, E_1 – consumed 20% ethanol ad libitum in the drinking water during pregnancy for 20 days, E_2 – received 20 % ethanol ad libitum in the drinking water 20 days before pregnancy and for 20 days during pregnancy) reveal differences in serum total cholesterol, HDL-cholesterol, LDL-cholesterol and triacyglycerols, in the order E_2>E_1>C. These results differ from those obtained in our previous experiments where ethanol was directly injected into the animals.

Keywords: *ethanol consumption - Wistar rats; pregnancy - lipid biomarkers*

INTRODUCTION

Chronic ethanol consumption has complex influences on metabolism. Experimental animal models have offered valuable insight in this respect [1 - 5]. In animals ethanol is first oxidized to acetaldehyde via three different pathways, and then further oxidized to acetic acid by aldehyde dehydrogenase – AlDH [3]. At low ethanol intake, acetaldehyde is produced by alcohol dehydrogenase (ADH) and nicotinamide adenine dinucleotide, NAD^+, in the cytosol and mithocondria of hepatic cells. With larger amounts of ethanol, acetaldehyde production is taken over by NADPH and H_2O_2 or O_2. Here, we follow up on previous studies on the effects of alcohol upon the organism causing the alcohol embryo- and fetopathy [6 - 10].Our investigations pursue the evaluation of the biochemical homeostasis status of lipid metabolites after chronic ad libitum alcohol administration in pregnant Wistar strain rats. This first note presents results on biomarkers of lipid homeostasis.

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RESULTS AND DISCUSSION

Table 1 shows a synopsis of the animal model used in this research (cf. Experimental section). Serum concentrations were determined for total-cholesterol (T-C), high density lipoprotein-cholesterol (HDL-C), and triacylglycerols (TAG); the values of serum low density lipoprotein-cholesterol (LDL-C) were obtained using the Friedewald equation, cf. [11]. These are considered as biochemical markers of lipid metabolism [12 - 14].

Table 1. Synopsis of the animal experimental model

Grp	No. anim	Admin. subst.	Conc (%)	Adm. way	Quan-tity	Period of admin (days)	
						before pregnancy	pregnancy
C	8	tap water	-	per os	ad libitum	-	-
E_1	8	ethanol	20			-	20
E_2	8	ethanol	20			20	20

Table 2 shows that, in good agreement with previous literature data [15, 16], serum T-C, LDL-C and HDL-C were increased in both experimental groups (E_1 and E_2). For E_2 the individual values show a more remarkable variation, perceptible by the SD values. While HDL-C is considered a beneficial lipoprotein, as its large particles can remove cholesterol from atheroma and have a negative effect on the development of fatty liver., elevated LDL-C is known to promote atheroma formation on the arteries walls, a principal cause of cardiovascular diseases. [14, 17, 18]

Table 2. Concentrations of lipid biomarkers from the class of sterols

Group	No. anim	T-C (mg/dL) X ± SD	HDL-C (mg/dL) X ± SD	LDL-C (mg/dL) X ± SD
C	8	92.71± 8.27	36.14 ± 2.94	44.99 ± 4.17
E_1	8	103.83± 3.45**	39.61±5.88***	48.13±6.82***
ΔX_1		+ 11.12	+ 3.47	+ 3.14
E_2	8	114.86±11.34**	43.52 ± 4.19**	52.88 ± 5.09**
ΔX_2		+ 22.15	+ 7.38	+ 7.89

* statistically significant : ** $P < 0.01$; *** $P < 0.05$

Table 3. Concentrations of triacylglycerols

Group	No. anim	TAG (mg/dL) X ± SD
C	8	57.86 ± 6.12
E_1	8	80.41 ± 9.42**
ΔX_1		+ 22.55
E_2	8	92.28 ± 8.06*
ΔX_2		+ 34.42

* Significancy : * P < 0.001; ** P< 0.01

Values for triacylglycerols (TAG), as specific biomarkers for lipid metabolism, are presented in Table 3. E_1 showed an elevation by 38% and E_2 by 59%, in qualitative agreement with previous literature data [5, 19-21]. While serum TAG result mostly from dietary glycerides, ethanol increases the endogenous production of TAG, as liver cells eliminate the excess hydrogen formed from the breakdown of alcohol by utilizing it to form alpha-glycerophosphates and fatty acids.

CONCLUSIONS

Chronic ethanol consumption by pregnant female rats induces dyshomeostatic effects characterized by increases in total cholesterol, its lipoprotein derivatives HDL-C and LDL-C, and triacylglycerols (TAG). The effects are more notable in group E_2 (i.e., a longer period of consumption). Notably, these data differ from our own previous reports where alcohol was administered by injection,[3.6,7] as opposed to the ad libitum oral route followed here - an important methodological observation, that affects the meaningfulness of ethanol-related studies in animals.

EXPERIMENTAL SECTION

*A*nimals were randomly divided in one control (C) and two experimental groups (E_1 and E_2). Each group included 8 animals with an average body weight of 200 ± 20 g. Ethanol with a concentration of 20% in drinking water was provided ad libitum to animals of group E_1 during pregnancy. The same concentration of ethanol was provided 20 days before pregnancy and 20 days during pregnancy to the animals of group E_2 On the 20^{th} day of pregnancy all the animals were euthanized after ketanest anaesthesia. After laparotomy blood samples were obtained by puncture of vena cava caudalis and collected in clean non-heparinized centrifuge tubes. Blood was allowed to coagulate and the tubes were centrifuged for serum separation. Analytical determinations were made by using a LABSYSTEM-901 analyzer and specific Clinilab reagents. The method of Fossatti and Prencipe [22] was used for triacyglycerols and the method of Allain et al. [23] for total cholesterol determination. HDL-cholesterol were determined by the enzymatic method elaborated by Burnstein at al. [24] and perfected by Lopez-Virella et al. [25]. Spectrophotometric measurements were made at 505 nm. Values of LDL-C were calculated [29] according to the equation: LDL-C = T-C – [(HDL-C) – (TAG/5)]. Results are expressed as means (X) and standard deviation (SD) and the statistical significance as the *t* test was used as appropriate.

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