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Cold Swimming Stress: Effects on Serum Lipids, Lipoproteins and LCAT Activity in Male and Female Rats

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TSOPANAKIS, C. AND C. TESSEROMMATIS. *Cold swimming stress: Effects on serum lipids, lipoproteins and LCAT activity in male and female rats.* PHARMACOL BIOCHEM BEHAV **38**(4) 813–816, 1991.—Effects of consistent cold swimming stress on lipid and lipoprotein metabolism parameters were studied using male and female rats over a period of 60 and 20 days respectively. At the end of treatment serum total cholesterol, high density lipoproteins (HDL-C) and lecithin:cholesterol acetyltransferase (LCAT) activity declined in both male and female rats. TC/HDL-C ratio declined in 20 days in females, while in males it did not change. Free fatty acids increased, while triglycerides remained unchanged in both sexes. %Lipoprotein distribution in male animals did not show any phenotype alteration except in the group of 40 days where %VLDL declined and %LDL-C increased. Body weights did not change, except in males in 60 days. Consistent cold swimming stress by lowering HDL-C and LCAT activity seems to influence lipoprotein metabolism.

Cold stress HDL-C Lecithin:cholesterol Acetyltransferase

MANY of the physical and psychological demands of everyday life are intense stress factors (16, 19, 24, 26, 27); equally intense is the "nonspecific response of the body" the well known stress.

It has been reported that stress affects cholesterol levels in animals (22,32), while exposure to cold and the ensuing stress have been found to have the same effects as other acute stress producing conditions (8,23).

Changes in lipid and lipoprotein levels are considered to be closely connected with atherosclerosis and CHD (6). Studies concerning the distribution of cholesterol between different lipoprotein fractions have shown that high density lipoprotein (HDL-C) cholesterol has a negative correlation with CHD and atherosclerosis (14). Also various ways by which these levels could be increased have been thoroughly investigated and are even now so (10). However, not many studies have been conducted on the direct effects of stress on lipoprotein metabolism.

Recently it has been shown that consistent cold stress caused in male rabbits a marked reduction in the levels of serum cholesterol, high density lipoproteins (HDL-C) and the activity of lecithin:cholesterol acetyltransferase (LCAT), a key enzyme in their metabolism (30).

The purpose of the present study was to investigate the effects of consistent cold swimming stress on parameters of lipid metabolism in male and female animals. In this work we used rats instead of rabbits because their low density lipoprotein metabolism seems to vary slightly from human and rabbit: the rat liver produced LDL-C has $ApoB_{48}$ as its main apoprotein: human and rabbit liver produced LDL-C seem similar having $ApoB_{100}$ as main apoprotein (4). We also used different stress conditions in an attempt to find the most suitable stress model. Investigators have used forced cold swimming stress and comparing it with other stress conditions found similar effects (5).

The results of subjecting normal rats of both sexes to consistent stress and following with time its effects mediated on HDL-C, total cholesterol, triglycerides, free fatty acids, lipoprotein phenotypes and LCAT, are reported and discussed in this work.

METHOD

Animals and Stress Conditions

Subjects were 40 female and 54 male Wistar rats (age 40 ± 5 days) separated in the following groups:

1. Female: A_1 n:10, A_2 n:10 and B n:20. Groups $A_{1,2}$ were subjected to everyday cold swimming stress 5°C 10 min, that is until exhaustion, for 10 and 20 days respectively: distribution of four rats per barrel ensured continuous swimming activity. Group B were kept under normal sedentary conditions and were used as controls.

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TABLE 1

SERUM HDL-C, LCAT, TOTAL CHOLESTEROL, TRIGLYCERIDES, FREE FATTY ACIDS, AND BODY WEIGHTS OF FEMALE RATS

Groups	HDL-C $mmol \cdot l^{-1}$	LCAT $\mu mol \cdot l^{-1} \cdot h^{-1}$	TC $mmol \cdot l^{-1}$	TG $mmol \cdot l^{-1}$	FFA $mEq \cdot l^{-1}$	b.wt. g	TC/HDL-C
A_1 n: 10 (10 days)	0.515 ±0.112†	43.01 ±13.42‡	1.534 ±0.218†	0.828 ±0.163	0.98 ±0.13‡	169 ±17	3.09 ±0.80
A_2 n: 10 (20 days)	0.591 ±0.085	54.66 ±27.07*	1.301 ±0.488†	0.810 ±0.138	0.69 ±0.07‡	177 ±30	2.18 ±0.75*
B n: 10 controls	0.697 ±0.157	74.58 ±23.99	1.851 ±0.215	0.788 ±0.350	0.35 ±0.04	151 ±29	2.78 ±0.73

Values are mean ± SD (p values: <0.05,* <0.01,† <0.001‡). TC: total cholesterol, TG: triglycerides, FFA: free fatty acids.

2. Male: C_1 n:10, C_2 n:10, C_3 n:10 and D n:24. Groups $C_{1,2,3}$ received the same as above treatment for 20, 40, 60 days respectively, while D were used as controls. All animals were fed ad lib a regular diet containing: proteins 2%, celluloses 6%, Ca, P 1.8%, fat 2%. Rats were allowed free access to water. Body weights were obtained before sacrifice.

Biochemical Methods

Animals were sacrificed by decapitation after 24 h of food withdrawal at the end of treatment. They were killed 20–24 h after their final exercise period. Blood was collected, serum was separated at 1500 × g and 4°C within 1 h and analyses were performed within 48 h. Serum total cholesterol (TC) was determined enzymatically (2) (Boehringer Mannheim, W. Germany, Cat. No. 237564), triglycerides (TG) enzymatically (33) (Human, W. Germany, Cat. No. H5008), HDL-C by $MgCl_2$-Na phosphotungstate precipitation (17) (Boehringer Mannheim, Cat. No. 543004) and free fatty acids (FFA) colorimetrically (Wacko Ch., W. Germany, Cat. No. 991-22309). Serum lecithin:cholesterol acetyltransferase (LCAT EC 2.3.1.43.) activity was determined as a function of the quantity of free cholesterol which is transformed to esterified cholesterol during incubation at 37°C as follows (12): A pool of normal rat serum was heated for 30 min at 56°C to inactivate LCAT. The serum was further incubated at 4°C for 15 min with 0.2% (w/v) dextran sulphate (M_r 2.10^6). One part dextran sulphate was used for 20 parts of inactivated serum. This treatment produced an elimination of LDL and VLDL. This step was followed by centrifugation for 10 min at 1750 × g; the supernatant rich in HDL was used as the LCAT substrate. For the determination of LCAT activity, for each sample were prepared two tubes containing 100 μl LDL- and VLDL-free serum and 100 μl substrate: in tube 1 free cholesterol was determined after incubation for 80 min at 37°C, in tube 2 after incubation for 160 min at 37°C. The transformation of free cholesterol to esterified cholesterol was measured during incubation at 37°C every 30 min from 0 to 4 h. The linearity of the reaction was better after incubation for 80 min. Therefore, we measured the disappearance of free cholesterol after incubation between 80 and 160 min. Free cholesterol was measured enzymatically (2) (Boehringer Mannheim, W. Germany, Cat. No. 310328). Results are expressed in $\mu mol \cdot l^{-1} \cdot h^{-1}$ of serum. Serum lipoproteins were separated by electrophoresis on cellulose acetate using a Helena Laboratories system: the relative % values of each lipoprotein were computed by using a densitometer (Helena Laboratories, Beaumont, TX) for qualitative and quantitative screening of phenotypes.

Statistical analysis of the results was performed by Student's t-test and was carried out by using a Hewlett-Packard HP-85 computer. Values are presented as mean ± SD.

RESULTS

Cold swimming stress in female rats was associated with a significant decline in HDL-C levels and LCAT activity in 10 days (group A_1) ($p<0.01,<0.001$), which in 20 days (group A_2) increased slightly but still remained significantly lower than the controls ($p<0.05,<0.05$) (Table 1). Total cholesterol levels declined continuously until 20 days ($p<0.01$). TC/HDL ratio declined in 20 days ($p<0.05$). Free fatty acid (FFA) concentration increased significantly in both 10 and 20 days ($p<0.001$), showing lower values in the second group (A_2). Triglyceride concentration and body weights did not show any change from the controls.

The groups of male animals demonstrated a more dramatic response (Table 2). HDL-C levels did not change in group C_1 (20 days), but in 40 days they declined and remained low until 60 days ($p<0.05,<0.001$). Serum cholesterol followed the same pattern ($p<0.01,<0.001$). TC/HDL ratio did not change. LCAT activity was reduced from 20 days ($p<0.001$) and remained very low until the end of treatment ($p<0.01$). Free fatty acids increased continuously from the first 20 days of treatment ($p<0.05,<0.001,<0.001$). Triglyceride levels did not change from controls, while body weight increased only at 60 days ($p<0.05$). Serum lipoprotein electrophoresis showed no alteration in phenotypes in 20 and 60 days. In 40 days it showed a decrease in %VLDL ($p<0.01$) and an increase in %LDL-C ($p<0.05$).

Between the two sexes there appear to be some differences: the females have lower control FFA levels and the FFA response to stress in them follows a different pattern rising rapidly to very high values in 10 days and falling slightly in 20 days, while in male rats it increases steadily. Differences appear in the control values of HDL-C, which are lower in the male animals. LCAT values are also different, the control male ones being higher than the female, while after stress the enzyme activity seems to decline to lower levels in males than in females.

Females showed a different response to stress; they seemed to cope with much difficulty each time after stress and for this reason they had to be sacrificed earlier than planned.

DISCUSSION

It has been reported that exposure of rats to cold stress increases plasma ACTH-like material (8). Stress liberates catecholamines, which together with ACTH and the ensuing corticosteroid production, cause lipid mobilization. This includes increased TG hydrolysis and concomitantly increased FFA cir-

TABLE 2

SERUM HDL-C, LCAT, TOTAL CHOLESTEROL, TRIGLYCERIDES, FREE FATTY ACIDS, BODY WEIGHTS, AND % LIPOPROTEIN DISTRIBUTION OF MALE RATS

Groups	HDL-C $mmol \cdot l^{-1}$	LCAT $\mu mol \cdot l^{-1} \cdot h^{-1}$	TC $mmol \cdot l^{-1}$	TG $mmol \cdot l^{-1}$	FFA $mEq \cdot l^{-1}$
C_1 n: 10	0.575	15.97	2.122	0.668	0.62
(20 days)	±0.120	±4.56‡	±0.489	±0.171	±0.14*
C_2 n: 10	0.353	31.96	1.760	0.716	0.79
(40 days)	±0.147*	±15.81†	±0.342†	±0.094	±0.04‡
C_3 n: 10	0.310	20.87	1.827	0.854	0.81
(60 days)	±0.100‡	±9.04†	±0.254‡	±0.096	±0.21‡
D n: 24	0.486	90.02	2.086	0.764	0.54
controls	±0.093	±29.63	±0.333	±0.174	±0.07

Groups	b.wt. g	% HDL-C	% VLDL	% LDL-C	TC/ HDL-C
C_1 n: 10	176	23.66	30.69	41.04	3.81
(20 days)	±14	±4.09	±5.60	±10.60	±1.08
C_2 n: 10	198	21.59	23.99	54.37	5.10
(40 days)	±32	±8.08	±4.2†	±10.37	±1.50
C_3 n: 10	203	17.75	37.18	43.61	5.50
(60 days)	±13*	±5.43	±9.54	±13.40	±1.53
D n: 24	179	21.11	33.64	45.38	4.61
controls	±26	±4.12	±6.07	±8.28	±0.88

Values are mean ± SD (p values: <0.05,* <0.01,† <0.001‡). TC: total cholesterol, TG: triglycerides, FFA: free fatty acids.

culation. The slow body weight rise observed in our animals may probably be explained by the above.

Studies that span a remarkable breadth of stress conditions in humans have found that serum lipids are noticeably influenced by them. Increased FFA levels in stressed humans of both sexes have been reported (28,29). Increased FFA levels have been found in swimming rats (20). The significant concentration increase observed in both our male and female rats is in agreement with the above. However, there appears to exist a difference between sexes in their FFA level response to stress; it is possible that females developed a quicker adaptation to stress.

Furthermore, it is generally accepted that sexual dimorphism characterizes the adrenal size of the rat, the females having larger adrenals than the males (31). This dimorphism may be responsible for higher levels of corticosteroids produced during stress thus mobilizing higher amounts of FFA in the circulation.

Reports on stress elicited effects on TG values are contradictory: changes varying from a 33% decrease to a 111% increase have been found in humans (28,29), while a significant decrease has been found in rats after strenuous swimming (20,34) and in male rabbits after cold stress (30). In both our sex groups TG levels did not show any response to stress. Rats exposed to stress have shown decreased levels of serum total lipids after 3 months (32), while cold stress in rabbits has decreased their levels after 9 weeks (3).

The significant lowering of serum total cholesterol concentration in both our sex groups agrees with other reports (20, 30, 32, 34) who found reduced serum TC levels in male rabbits stressed for similar time periods.

Also increased cholesterol catabolism was found in stressed male rats (18). This serum cholesterol level reduction was accompanied in the present study by a significant HDL-C one and also by a lowering of LCAT activity that followed the same pattern in both sexes. This is in agreement with our previous report of concomitant TC and HDL-C decline and of LCAT activity lowering after cold stress in male rabbits (30).

The mechanism by which stress may affect cholesterol metabolism is not known. However, neurogenic pathways are thought to be involved (25). Stress caused ACTH production, increases also the ensuing cortisol secretion (7,15). Established actions of ACTH include activation of cholesterol side-chain cleavage (1,13) and increased uptake of cholesterol rich lipoproteins (11), while cortisol synthesis needs cholesterol as precursor. It may be possible that the combination of the above stress-provoked functions has as a result the depletion of both cholesterol and cholesterol carrying lipoproteins observed in our stress groups. This may also explain the decline in LCAT activity, since the enzyme has free HDL cholesterol as its main substrate and ApoAI (of HDL) as its main cofactor. However, there may also exist other mechanisms concerning the reduction of HDL-C. It has been reported that LCAT acts as a complex with other proteins (3); to what extent stress can influence this complex and (or) affect the availability of the substrate to its enzyme is not known: the marked HDL-C reduction could be explained by such an effect.

Lipoprotein electrophoresis did not show any significant effects of stress on lipoprotein phenotypes, except in the male group of C_2 (40 days) where a decreased loading of VLDL may signify an increased lipoprotein uptake (11).

Forced exercise is regarded as a form of stress to the animal (21); this combined with cold may enhance stress effects on the organism even further. Therefore, the beneficial effects of voluntary human or animal exercise as far as lipid and lipoprotein metabolism are concerned (i.e., HDL-C increase) cannot be compared to the effects of the above conditions on the same factors.

It can be concluded that cold swimming stress exerts profound effects on lipid and lipoprotein metabolic parameters, namely HDL-C and LCAT decrease which may reflect adaptations of HDL-C metabolism in rats of both sexes.

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