EFFECTS OF SHORT-TERM AND LONG-TERM ACTH ON PLASMA LIPID, LIPOPROTEIN AND GLUCOSE LEVELS IN MICE

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SUMMARY : This study was designed to evaluate the changes of lipoproteins, triglycerides and plasma glucose levels of mice under treatment of ACTH. In these experiments sixty mice between 6-8 weeks of age weighing about 30 g were used. They were divided into three groups [control, Group I (short-term) and Group II (long-term)], each containing 20 animals. Control mice were treated with 0.9%NaCl solution twice a day for 3.5 days during experiment. Mice in Group I were treated with ACTH (500 µg/kg) once a day, 4 hours before the sacrification, and mice in Group II treated with ACTH twice a day for 3.5 days at dose of 500 µg/kg per day. Blood samples were obtained under deep anesthesia before they were sacrificed. The results revealed increased levels of plasma cholesterol, LDL, HDL, non-HDL cholesterols and VLDL in the experimental group treated with ACTH. However, ACTH injection did not produce any alterations in plasma glucose, and triglyceride levels in the same group. Our data show that prolonged ACTH treatment increased plasma cholesterol, LDL, HDL, non-HDL cholesterol and VLDL levels.

Key Words: ACTH, plasma lipoprotein, cholesterol, glucose levels.

INTRODUCTION

Stress is caused by strong physical or environmental changes threatening internal homeostasis, while psychosocial or psychological stress is evoked by subjective perceptions (1, 2). Stress activates the hypothalamic-pituitary-adrenal axis by increasing secretion corticotropin releasing hormone (CRH), leading to production of glucocorticoids which down-regulate immune responses (3). Stress, however, aggravates a number of neuroinflammatory disorders (4). CRH also has proinflammatory effects (5).

Cholesterol constitutes a component of extra and intracellular membranes and is needed to maintain normal cell growth and multiplication (6-8). It is the precursor of steroid hormones and bile acids and is a structural component of lipoprotein particles involved in the transport of lipid energy to the tissues (6, 8). Cell may acquire cholesterol by *de novo* synthesis or from circulating lipoproteins (9).

Certain hormones have a powerful influence on lipoprotein receptor expression (10, 11). Adrenal steroid hormones are largely derived from lipoprotein cholesterol (12). A major physiological effect of ACTH is to increase adrenal glucocorticoid secretion (6). In rats and mice, chronic administration of ACTH results in suppression of hepatic lipoprotein receptors and increases in plasma lipoprotein cholesterol. The chronic exposure of rodents to ACTH leads to elevated plasma LDL and HDL cholesterol (6).

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Table 1 : Plasma cholesterol, LDL, HDL, Non-HDL, VLDL, triglycerides, glucose and Cholesterol/HDL concentrations.

	Control n=20(mg/dl)	Group I n= 20(mg/dl)	Group II n= 18(mg/dl)
Plasma cholesterol	71.5 ± 12.4	93.1 ± 25.7	144.5 ± 35.1*
LDL	10.1 ± 3.0	12.7 ± 5.4	15.8 ± 12.3
HDL cholesterol	36.7 ± 16.0	48.9 ± 21.2	92.2 ± 19.8*
Non-HDL cholesterol	34.8 ± 11.9	44.2 ± 15.6	52.3 ± 26.4
VLDL	38.6 ± 15.4	44.3 ± 32.1	50.5 ± 18.7
Triglycerides	299.5 ± 49.8	258.6 ± 65.1	289.1 ± 54.3
Plasma glucose	157.6 ± 35.4	167.4 ± 46.8	159.7 ± 26.4
Cholesterol/HDL ratio	1.95 ± 0.22	1.90 ± 0.92	1.57 ± 0.51#

Values are mean ± Standart deviation (SE)

* Significantly higher in Group II compared with Group I and Control ($p<0.05$).

Significanly lower in Group II compared with Group I and Control ($p<0.05$).

Hypercholesterolemia has been recognized as a risk factor for cardiovascular disease. The report of the Coronary Primary Prevention Trial has suggested an association between clinical complications of atherosclerosis and hypercholesterolemia (13). Some drugs, such as diazepam, inducing anxiety that is supposedly an emotional response to psychosocial stress, is also a cardiovascular risk factor (14).

The aim of the present study was to describe the effect of a short lasting and long-time ACTH that on plasma cholesterol, LDL, HDL, non-HDL, VLDL, triglyceride and glucose levels.

MATERIALS AND METHODS

Animals

Sixty mice with the age of 6-8 weeks weighing about 30 g were obtained from the Laboratory of Animal Science, Cerrahpasa Medical School, Istanbul University, Istanbul, Turkey. The animals were given standart mouse pellets (Antalya Food Factory, Antalya, Turkey) and tap water *ad libitum*. All animals were housed in stainless cages under standard laboratory conditions (light 0700 to 2000 h, 21 ± 2°C, relative humidity 55%), and received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health. They were divided into three groups: control, short-term (Group I) and 3.5 days (Group II) injected Tetracosactide (ACTH) (Synacthen depot, Ciba-Geigy, Horsham, Sussex, UK), each containing 20 animals. Mice of the first experimental group were treated with ACTH once (500 µg/kg), 4 hours before the sacrification (Group I), and Group II were treated with ACTH twice a day for 3.5 days at dose of 500 µg/kg per day and 0.9%NaCl was injected to control group intramuscular at 0900h and 1600h for 3.5 d. There were no deaths in Group I, whereas 2 animals died after second injection in Group II.

Biochemical analyses

Blood samples were obtained after the injection of 4 hours and 3.5 days for the measurement of plasma lipoproteins, plasma triglycerides, and plasma glucose. Plasma total cholesterol and triglycerides were determined enzymatically with commercial kits adapted to a BM / HITACHI 911 autoanalyzer (Boehering GmbH, Mannheim, Germany). Triglycerides determinations were corrected for the free glycerol present in plasma (Sigma, St. Louis, MO). High density lipoprotein (HDL) cholesterol was measured after precipitation with phosphotungstic acid and magnesium ions (Boehering GmbH). Non-HDL cholesterol was calculated as the difference between total and HDL cholesterols (15). Plasma glucose levels were analyzed using an enzymatic kit (# 315-100, Sigma) and measured using a SpectraMax 250 Spectrophotometer.

Statistical Analysis

All values are given as means ± SEM. Student's t-test was used for comparing groups. A value of $P < 0.05$ was considered significant.

RESULTS

In this experiment, we wanted to determine the effects of ACTH on the levels of plasma total cholesterol, LDL, HDL, Non-HDL, triglycerides and glucose. Mice were injected with ACTH, and groups of animals were sacrificed after 4 h (one injection) and 3.5 days (twice a day, ACTH /

500 μg/kg per day). ACTH treatment increased plasma cholesterol in mice injected once and for 3.5 days ($P< 0.05$). It is important to note that this was to an increase mainly of HDL cholesterol. Because non-HDL cholesterol decreased in groups I and II. Levels of plasma glucose were measured in postheparin plasma. No significant differences in the plasma glucose levels were observed when the different groups of mice were compared ($P>0.05$) (Table 1). Triglyceride levels revealed an unimportant decline in Group I (258.6±65.9) and Group II (299.1±54.3) compared to control (229.5±49.8). We have no explanation for this decrease in triglycerides. Also, cholesterol /HDL ratio of experimental groups decreased compared to control group

DISCUSSION

We studied the plasma levels of the total cholesterol, and LDL, HDL, non-HDL, VLDL and triglycerides, serum glucose in normal control and ACTH treated mice.

After exposure to ACTH the animals did not reveal any change in plasma glucose. Triglycerids in the same group of animals demonstrated a minor reduction (Table 1) without any statistical significance.

In a previous study conducted on wild mice ACTH treatment caused significant increase in HDL-c and LDL-c and as a result produced an increase in the level of total cholesterol (6). However, in LDLR deficient mice treated with ACTH total plasma cholesterol did not increase (6). In previous long-term exercise training studies, the most common lipid change observed has been increased HDL cholesterol (16). Furthermore, in another long-term exercise study, it has been observed that plasma total cholesterol, LDL cholesterol and total cholesterol/HDL cholesterol ratio has been effected positively (17). Although previous results on the chronic effects of regular exercise are inconsistent, increases in HDL cholesterol and decreases in total and LDL cholesterol and triglycerides have been often reported (16, 18). In another study, regular exercise resulted in decreases in total and LDL cholesterol levels, but mean triglyceride level did not change (17).

Several studies have shown that diazepam lowers blood lipid concentration(s) in hyperlipidemic experimental animals (19, 20). Consistent with early studies, ACTH stress induced hypercholesterolemia. However, our present results, similar to those of Galman *et al.* (6), successfully demonstrated significant hyperlipidemic effects due to physiological stress injection of ACTH. In some studies, Berg and Nillson -Ehle (21) and Arnadottir *et al.* (22) have reported that ACTH administration to volunteers for 4 days resulted in a lowering of LDL and an increase in HDL cholesterol in plasma (21, 22). However, in a previous study, a moderate increase in triglycerides and non-HDL cholesterol was found in the mice (15). One experimental issue in the use of STZ-induced diabetic mouse, plasma HDL and LDL and VLDL cholesterols were increased compared to controls. After STZ-treatments there were no significant changes in triglyceride (23). In some models, diabetes leads to increased plasma cholesterol. It is likely that this dyslipidemia is responsible for greater disease (24).

During the present study, in Group II, plasma cholesterol and HDL cholesterol levels increased, when compared to Group I and control group. Statistically significant increase was found compared to control group ($p<0.05$). In groups I and II a reduction of triglyceride levels was observed compared to control group ($p>0.05$). Besides, there has been no statistically significant change at non-HDL, VLDL, plasma glucose levels between all groups ($p>0.05$). According to these results, ACTH stress has no effect on plasma glucose levels, but has negative effects on plasma cholesterol, HDL and cholesterol/HDL ratio.

These results are similar to the observations of a number of other investigators. Galman *et al.* (6) observed that ACTH treatment increased total plasma cholesterol in rats receiving cholesterol and normal rat chow, and this was owing to an increase mainly in HDL cholesterol in both situations.

As a conclusion, the results of ACTH treatment, an increment was observed in plasma cholesterol and HDL cholesterol, and a decline was observed in Cholesterol/ HDL ratio. Thus, stress that is formed by ACTH, has changed the value of blood plasma lipoproteins and however did not influence the level of plasma glucose.

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