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SHORT COMMUNICATION

The Effect of Long-term Ingestion of Glucocorticoids on Liver and Serum Plasma in Rats

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Summary

The effect of chronic hydrocortisone administration (0.5 mg/kg) on the liver and plasma lipid content was assessed in Wistar rats. It was found after that the liver cholesterol content was significantly increased 6 months of hydrocortisone treatment. At the same time, the distribution of liver phospholipid fractions was altered. The fatty acid composition of liver lipids showed a significant increase of 22:6 n-3. Decreased levels of cholesterol and LDL-cholesterol were found in the plasma of the hydrocortisone-treated rats.

Key words

Hydrocortisone – Liver lipids – Plasma Lipids

Glucocorticoid hormones play an important role both as therapeutic agents and as metabolic regulators in almost all tissues. They are used pharmacologically in the treatment of wide-spread and diverse conditions such as arthritis, leukaemia, severe allergies, autoimmune disorders, etc. Ingestion of glucocorticoids can cause hyperlipidaemia in man (Stern *et al.* 1973) and in other animal species including rats (Reaven *et al.* 1974, Cole *et al.* 1982, Mitamura 1987). The pattern and extent of hyperlipidaemia vary depending on the dosage of glucocorticoids and on the animal species. It is well known that cell membrane function may be influenced by phospholipid composition, the fatty acid composition of phospholipids and phospholipid-cholesterol ratios (Murray *et al.* 1979).

The aim of the present study was to obtain additional information on the treatment with a low dose of hydrocortisone acetate. Male Wistar rats, aged 60 days at the beginning of the experiment, were divided into two groups containing 12 rats in each. Animals of both groups were fed daily with 15 g of pelleted balanced nonpurified diet prepared according to the formula recommended in the Report of American Institute of Nutrition (1977), containing the following ingredients (w/w): protein 21 %, carbohydrates 62 %, fat 5 %. All animals were weighed biweekly. The experimental group drank a hydrocortisone solution in a dose of 0.5 mg/kg and the control group the same volume of water (30 ml). After 6 months, the animals fasted for 24 h and were killed under Nembutal anaesthesia. Samples of blood were withdrawn by heart puncture. The plasma lipids (total lipids, triacylglycerol, phospholipids, cholesterol) were estimated as described previously (Ristic *et al.* 1985) using commercial kits purchased from Sigma Diagnostic Reagents (St. Louis, USA). The liver total lipid extracts were prepared by the method of Folch *et al.* (1957). Liver lipids were determined as lipids. Liver phospholipids were separated by two-dimensional thin-layer chromatography (TLC) into seven fractions, i.e. phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), diphosphatidylglycerols (DPG), sphingophospholipids (SPH) and phosphatidic acid (PA). The spots of individual phospholipids were scraped off for determination of lipid phosphorus, as described by Kostic *et al.* (1972). The fatty acid composition of total liver lipids was analyzed by gas liquid chromatography (Varian GC 3400). All results were expressed as means

± S.D. The significance of differences in liver and plasma lipids after hydrocortisone treatment was analysed by Student's t-test.

The average daily body weight gain during the experiment was reduced in the hydrocortisone-treated rats (0.61±0.03 g/day), compared with the control group (0.81±0.12 g/day). Significant differences were observed in plasma and liver lipids after hydrocortisone treatment. The plasma cholesterol and LDL-cholesterol levels were significantly lower in the hydrocortisone-treated (1.67±0.04 and 0.05±0.003 mmol/l) than in the control rats (1.99±0.09 and 0.36±0.09 mmol/l). However, plasma triacylglycerol, HDL-cholesterol and phospholipid concentrations were unaffected by the hydrocortisone treatment. Total liver lipids and cholesterol were significantly higher in the hydrocortisone-treated (64.74±10.90 and 2.87±0.53 mg/g liver) than in the control rats (53.43±7.67 and 1.95±0.40 mg/g liver). The results show that hydrocortisone produced hypocholesterolaemia associated with liver cholesterol accumulation.

Table 1
Distribution of liver phospholipids in control and hydrocortisone-treated rats

Phospholipids[1]	Control	Hydrocortisone
PC	43.21±3.49	45.90±3.19
PE	29.09±4.00	23.04±1.03**
PI	16.56±1.51	15.78±1.87
PA	2.14±0.38	1.92±0.44
SPH[2]	4.55±1.10	7.50±1.96**
PS	2.77±0.71	3.71±0.79*
DPH	1.67±0.25	2.14±0.64

[1]Values are expressed as the weight percentage of total phospholipids (means ±S.D.) [2]Sphingomyelin. Significantly different values from the controls: * $p<0.05$, ** $p<0.01$

The effects of hydrocortisone treatment on liver phospholipid content are presented in Table 1.

The results show that long-term intake of hydrocortisone significantly affected liver phospholipid distribution. The percentage of PE was significantly lower in the hydrocortisone group, while the percentage of SPH and PS were significantly higher. A decrease in PE and an increase in SPH, PS and cholesterol content may occur due to *de novo* synthesis, remodelling of existing phospholipids, altered metabolism or transfer from a pool to the site in question (Murray *et al.* 1979).

Table 2
Fatty acid composition of liver lipids in hydrocortisone treated and control rats

Fatty acids[1]	Controls	Hydrocortisone
16:0	16.62±1.30	16.27±2.22
16:1	0.74±0.08	0.37±0.01**
18:0	28.25±2.43	27.00±6.55
18:1 n-9	7.36±1.02	7.60±1.19
18:2 n-6	18.14±1.17	20.69±3.54**
18:3 n-3	1.17±0.38	0.82±0.19
20:4 n-6	20.56±2.22	19.69±3.59
20:3 n-6	0.88±0.27	0.58±0.11*
20:5 n-3	2.19±0.74	1.18±0.40*
22:6 n-3	4.32±0.75	6.46±1.14**

[1]Values are expressed as the percentage of total fatty acid methyl esters (means±S.D.) *Significantly different values from the controls at $p<0,05$ **$p<0.01$

The fatty acid composition of liver lipids show a significant increase of 22:6 n-3 and a decrease of 16:0, 20:3 n-6 and 20:5 n-3 after hydrocortisone treatment (Table 2).

Hydrocortisone-stimulated changes in PE, SPH, PS, cholesterol and polyunsaturated fatty acids could affect hepatocyte membranes by altering membrane stability and fluidity, facilitating or inhibiting the coupling of receptors and enzymes. It may be supposed that the hypocholesterolaemia results from these changes occurring in hepatocyte membranes.

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