

SHORT REPORT

The effect of hydrocortisone on the concentration of triacylglycerols and cholesterol in the liver and kidney of mice maintained on two diets differing in protein level

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Used were mice aged 6 weeks and maintained on diets containing 16 or 10% crude protein. Over 4 or 6 consecutive days the mice were given intraperitoneally 0.0075 mg hydrocortisone per kg body weight. Control mice were intraperitoneally injected with 0.9% NaCl. In the liver of mice kept on low-protein diet the concentration of triacylglycerols was found significantly lower after 6 days injections while in the kidney of those kept on standard diet (16% protein) – increased after 4 days injections.

KEY WORDS: cholesterol / dietary protein / hydrocortisone / mice / triacylglycerol

The principal activity of cortisone is related to the stimulation of glucose production by liver cells, principally in the process of gluconeogenesis [Ardeshev and Kiseleva 2000, Greenstein *et al.* 2002, Gulevich *et al.* 2000, Hibberd *et al.* [2000], LeBlanc and Ducharme 2005]. Cortisone is also known to have a lipolytic effect, stimulating the

mobilization of fatty acids from the fat tissue, intensifying their oxidation resulting in the production of ketone bodies and leading to an increased level of free fatty acids (FFA) fraction in blood plasma [Gazdarov *et al.* 1984, Laderach and Straub 2001]. Simultaneously, cortisone increases the transport of fatty acids to the cells and their use as a source of energy. An increased use of fatty acids in place of glucose for the production of energy is a characteristic feature of the activity of the hormone [Gardner and Zhang 1999, Ridges *et al.* 2001].

Considering its physiological importance, the effect of cortisone was examined on the possible changes in the concentration of triacylglycerols and cholesterol in the liver and kidney of mice (model animals) maintained on two diets differing in protein level.

Material and methods

The study was conducted on 60 Swiss line, sexually mature male mice, bred at the Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, 6-8 weeks old, weighing 20 ± 2 g, the parents of which were mated randomly. The animals were maintained under standard conditions, at a temperature of 22°C and regulated lighting (12 hours light/12 hours dark), with free access to water and remained under constant veterinary control.

The mice were weaned at the age of 6 weeks, randomly divided into two main groups of 30 and then fed pelleted diets similar in energy level and containing 10% or 16% crude protein (Tab. 1 and 2).

Table1. Per cent composition of diets

Ingredient	Diet 1 (10% protein)	Diet 2 (16% protein)
Corn meal	89.32	64.73
Solvent-extracted soybean oil meal	1.94	10.14
Cereal germs	0.29	15.12
Milk powder	4.33	5.80
Yeasts	0.30	0.30
Premix LSM	1.02	1.01
Chalk	1.65	1.64
Phosphates	0.97	0.97
Salt	0.29	0.29
Dry matter	88.94	88.49
Crude fibre	3.27	4.06
Crude ash	4.37	4.08
Ether extract	3.07	3.17
Lysine	3.9	4.7
Leucine	11.6	8.9

Pellets constituting each diet were produced by the Łomna-Las Farm belonging to the Witold Stefański Institute of Parasitology, Polish Academy of Sciences, while

Table 2. Gross energy and crude protein content of diets¹

Diet	Gross energy content (MJ/kg)*	Crude protein content (%)*
1. Containing 10% protein	13.47	10.38
2. Containing 16% protein	14.04	15.51

¹According to precise determinations made at the Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences, Jabłonna.

their chemical composition was determined at the Jan Kielanowski Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences, Jabłonna.

At the age of 8 weeks each of two main groups (10% and 16% crude protein content of diet) was randomly divided into three sub-groups (n=10), as shown below.

- control – 250 µl of 0.9% NaCl given intraperitoneally, twice a day (8:00 and 20:00);
- 250 µl (0.0075 mg/g body weight) of Hydrocortisonum hemisuccinatum (JELFA) given intraperitoneally, twice a day (8:00 and 20:00) over a period of four consecutive days;
- as above but injections repeated over a period of six consecutive days.

Two hours after the last cortisone injection the mice were decapitated and their liver and kidneys prepared. The liver was subjected to perfusion in a solution of physiological salt (4°C) in order to remove the blood, while the kidneys were suspended in a 0.1M phosphate buffer, pH 7.4, containing 10 mM EDTA. Tissues thus prepared were homogenized at 200 rev./min. in a glass homogenizer (POTTER-ELVEHJEM) with a teflon piston, placed in a container with crushed ice.

The final solutions for analyses were obtained by centrifuging liver and kidney homogenates for 10 min at 12,000 rev./min., at a temperature of about 4°C in a JANETZKI K-24 centrifuge.

The concentration of triacylglycerols in liver and kidney supernatants obtained from all animals tested, was determined using ALPHA DIAGNOSTIC TESTS (Poland). The determination is based on the method of Wako, as described by Searcy [1974]. The levels of cholesterol and total lipids were determined using BIOCHEM TESTS [Gliwice, Poland) based on the method of Chromy *et al.* [1975].

Technical, analytical and statistical part of experiment were conducted at the Department of Animal Physiology, Institute of Biology, Świętokrzyska Academy, Kielce.

Results and discussion

As shown in Table 3 the concentration of triacylglycerols in the liver and kidney of control mice (0.9% NaCl) kept on a low protein diet (10% crude protein),

was significantly lower than that recorded for animals kept on a standard diet (i.e. containing 16% crude protein) – 15.0 vs 18.3 $\mu\text{mol/g}$ tissue in liver and 9.6 vs 17.9 $\mu\text{mol/g}$ tissue in kidney.

Protein dietary deficiency significantly intensifies the catabolism of endogenous proteins in order to maintain the necessary level of metabolic substrates [Chromy *et al.* 1975] and simultaneously decreases the synthesis rate of triacylglycerols [Hecker 2001]. This study seems to corroborate those results.

After four days of cortisone injections a significant drop ($P \leq 0.05$) was observed in the level of triacylglycerols in the kidneys of mice maintained on a diet containing 16% protein – from 17.9 to 14.2 $\mu\text{mol/g}$. In turn, cortisone injections continued over a period of six days resulted in a decrease ($P \leq 0.01$) of the level of triacylglycerols only in the liver of mice fed a low protein diet – from 15.0 to 10.6 $\mu\text{mol/g}$ tissue.

The caused by cortisone decreased level of triacylglycerols, very clearly visible in the liver of animals fed the diet containing 10% protein, was probably the effect of its activity leading to the intensification of lipolysis, as cortisone is a hormone of a long-lasting stress response. The present work confirms the results obtained in studies on mice by Sukhova and Ukhina [2004] and Berg *et al.* [2006] as well as by LeBlanc and Ducharme [2005] on humans.

The results presented in Table 4 indicate that in control animals the diet containing 10% protein significantly increased the level of cholesterol in the liver (from 3.6 to 4.5 $\mu\text{mol/g}$) while only slightly in the kidney (from 3.6 to 3.9 $\mu\text{mol/g}$), what may be an indication of an enhanced functional readiness of the organism under the protein deficiency stress – the organism prepares more adrenal cortical steroid hormones. The level of cholesterol in the blood also depends on the protein content in the diet as this significantly affects its changes. Gazdarov *et al.* [1984]

Table 3. Means and their standard deviations (SD) for the concentration of triacylglycerols ($\mu\text{mol/g}$ tissue) in the liver and kidney of unselected mice kept on diets differing in crude protein level

Subgroup	Diet containing 16% crude protein				Diet containing 10% protein			
	liver (n=10)		kidney (n=10)		liver (n=10)		kidney (n=10)	
	mean	SD	mean	%	mean	SD	mean	%
Control	18.3	3.3	17.9	100	15.0	2.0	9.6	100
Cortisone for 4 days	15.7	2.5	14.2*	80	12.7	1.6	11.4	85
Cortisone for 6 days	15.7	2.0	15.8	88	10.6**	1.0	9.2	71

* $P \leq 0.05$; ** $P \leq 0.01$ – differences as related to control confirmed statistically.

observed that the level of fat and protein in the diet and cholesterol in the blood both affect the “generation” of enzymes mobilizing fatty acids in tissues. Hecker [2001] and Ridges *et al.* [2001] suggest that the level of cholesterol in the blood of animals as well as in the fat of their tissues, considerably depends upon the type of diet and in particular

on the presence in the diet of soybean protein, casein and protein of animal muscles.

Four days of cortisone injections resulted in a significant increase in the cholesterol concentration (from 3.6 to 4.2 $\mu\text{mol/g}$) only in the kidney of mice kept on a diet containing 16% protein.

The results obtained and presented here did not show any significant effect of cortisone (with the exception of the one case, mentioned above) on cholesterol concentration in liver and kidney of mice. Cholesterol is utilized as a precursor of hormones of the adrenal cortex during adaptation responses. Thus, the excess of cortisone as a steroid hormone did not create the necessity of its increased usage and thus also did not lead to significant changes of its concentration in the organs of both basal groups examined. However, the increased genesis of cholesterol in kidney might also be caused by a lower degradation of cholesterol to bile acids.

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Table 4. Means and their standard deviations (SD) for the concentration of cholesterol ($\mu\text{mol/g}$ tissue) in the liver and kidney of unselected mice kept on diets differing in crude protein level

Subgroup	Diet containing 16% crude protein				Diet containing 10% crude protein			
	liver (n=10)		kidney (n=10)		liver (n=10)		kidney (n=10)	
	mean	SD	mean	SD	mean	SD	mean	SD
Control	3.6	0.4	3.6	0.2	4.5	0.1	3.9	0.4
Cortisone for 4 days	4.0	0.5	4.2*	0.3	4.4	0.2	3.6	0.5
Cortisone for 6 days	3.1	0.3	3.1	0.3	4.7	0.3	4.4	0.2

* $p \leq 0.05$ – differences as related to control confirmed statistically.

- fed at various levels of energy. *Bulleten Vsiesojuznogo Nauczno- Issledovatielskogo Instituta Fizjologii, Biochimii i Pitanja Sielskocozastviennych Životnyh* 1, 43-46.
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Wpływ hydrokortyzonu na koncentrację triacylogliceroli i cholesterolu w wątrobie i nerce myszy utrzymywanych na dietach o różnym poziomie białka

Streszczenie

Myszy doświadczalne w wieku 6 tygodni i o masie ciała $20 \pm 2,0$ g pozostające na kontrolnej diecie zawierającej 16 bądź 10% białka otrzymywały przez 4 i 6 kolejnych dni dootrzewnowe iniekcje 0,0075 mg hydrokortyzonu/kg masy ciała. Osobnikom kontrolnym wstrzykiwano 0,9% roztwór NaCl. Koncentracja triacylogliceroli w wątrobie myszy utrzymywanych na diecie ubogiej w białko (10%) obniżyła się istotnie po 6 dniach, a w nerce myszy żywionych paszą o normalnej zawartości białka (16%) wzrosła po 4 dniach stosowania hydrokortyzonu.

