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The effect of adrenaline on the concentration of triacylglycerols and cholesterol in the liver and kidneys of mice selected for high body weight and kept on two levels of dietary protein

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Used were mice selected for high body weight gain and control mice being the progeny of randomly mated parents. Within each group two subgroups were considered – kept on diet containing 10 and 16% crude protein. Intraperitoneal injections of 0.01 μg adrenaline per g body weight led to significant increase of triacylglycerols in both liver and kidney of selected mice kept on low-protein diet and in the liver of mice unselected kept on the same dietary protein level.
After adrenaline injections the level of cholesterol increased significantly in the kidney of selected, and in the liver of unselected mice kept on diet with 16 and 10% protein as well as in the kidney of unselected mice kept on low-protein diet.

KEY WORDS: adrenaline / cholesterol / protein / triacylglycerol

The role of adrenaline is, among much else, to mobilize the energy reserves by stimulating glycogenolysis in the liver, increasing the level of sugar in the blood, as well as accelerating the rate of lipolysis and the release of free fatty acids to the blood [Malpica *et al*. 2002, Errasti *et al* 2007, Silva *et al*. 2007, Silverberg and Manoach 2007, Yamaguchi-Shima *et al*. 2007]. However, no reports were found on the response to adrenaline of animals selected for increased body weight and offered low protein diet.

The present work aimed at determining the effect of adrenaline on the concentration of triacylglycerols and cholesterol in the liver and kidneys of mice maintained on diets with different protein levels and selected for high body weight gain.

Material and methods

The study was conducted on 40 Swiss sexually mature male mice, bred at the Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, 6-8 weeks old, weighing 35±2.5 g and belonging to the line of animals selected over 25 generations for high body weight (selected mice). The other group (unselected mice) comprised 40 sexually mature unselected males (with a mean live body weight of 20±1.5 g), the parents of which were mated randomly. The selection of selected mice was based on mating the parental animals, females and males, selected from the original, initial population and showing the highest body weight. The animals were maintained on a mouse farm under standard conditions, at a temperature of 21-22°C and natural lighting, with constant access to water and under veterinary control.

At the age of six weeks the mice were weaned and for a period of two weeks offered two complete pelleted diets containing 10 or 16% crude protein. Both diets were prepared at the Łomna-Las Farm belonging to the Witold Stefański Institute of Parasitology, Polish Academy of Sciences. Their nutritive value was determined at the Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences, Jabłonna. Per cent composition and nutritive value of both diets are presented in another report published in this issue [Klusek *et al*. 2007].

The experimental design is presented in Table 1.

Table 1. Experimental design

Diet	Group	N	Intramuscular injection
16% protein			selected mice
	I control	10	0.9% NaCl
	II adrenaline	10	*Adrenalinum hydrotartaricum* 0.1% (POLFA, Poland), 0.01µg/g body weight
			unselected mice
	III control	10	0.9% NaCl
	IV adrenaline	10	*Adrenalinum hydrotartaricum* 0.1% (POLFA, Poland), 0.01µg/g body weight
10% protein			selected mice
	V control	10	0.9% NaCl
	VI adrenaline	10	*Adrenalinum hydrotartaricum* 0.1% (POLFA, Poland), 0.01µg/g body weight
			unselected mice
	VII control	10	0.9% NaCl
	VIII adrenaline	10	*Adrenalinum hydrotartaricum* 0.1% (POLFA, Poland), 0.01µg/g body weight

Animals from control groups (I, III, V, VII) received three times daily (at 8:00, 10:00 and 12:00) intramuscular injections of 250 µl of 0.9% NaCl solution, while those from experimental groups (II, IV, VI, VIII), three times daily (8:00, 10:00 and 12:00), intramuscular injections of 250 µl adrenaline (0.01µg/g body weight).

Two hours after the last NaCl or adrenaline injection the animals were decapitated and the liver and kidneys were collected. The liver was perfused 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 liver and kidney homogenates obtained were centrifuged for 10 minutes with 12 000 rev./min, at about 4°C in a JANETZKI K-24 centrifuge.

Triacylglycerols content of supernatants from liver and kidney of all animals was determined according to Searcy [1974] using ALPHA Diagnostic tests (Poland) The level of cholesterol was determined using BIOCHEMTESTS test kits (Gliwice, Poland].

The significance of the effect of experimental factors on the concentration of triacylglycerols and cholesterol in the liver and kidneys was assessed based on a multi-factor analysis of variance using the SAS/STAT (1999-2001) User's Guide, and Origin Software version 5.0, Microcal Software Inc. Northampton, USA.

The methods used have been accepted by the respective Ethics Commission for Experimentation with Animals of the Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec.

Results and discussion

As shown in Table 2, the concentration of triacylglycerols in the liver and kidneys of selected mice receiving diets with a 16% or 10% protein content was very similar (15.1 *vs* 16.2 µm/g for liver and 17.0 *vs* 14.6 µm/g for kidneys) for all control groups (0.9% NaCl). The content of triacylglycerols in the liver and kidneys of unselected mice differed significantly between the two groups of different protein content of diet (18.3 *vs* 15.0 and 17.9 *vs* 9.6, respectively).

Adrenaline injecting did not lead to significant changes either in the liver (16.5 *vs* 15.1µm) or kidneys (15.3 *vs* 17.0) of selected mice receiving a diet containing 16% protein, but did significantly increase the level of triacylglycerols in the liver of selected mice maintained on a low-protein diet (10%) – from 16.2 to 18.9 µm/g tissue ($P \leq 0.05$) as well as in the kidneys of those animals – from 14.6 to 17.5 µm/g tissue ($P \leq 0.05$). The liver of un- selected mice fed a diet containing 10% protein responded similarly (increase from 15.0 to 19.5 µm/g, $P \leq 0.05$). However, the liver of the former but maintained on a high protein diet did not respond significantly to an adrenaline injection. A similar situation was recorded for the kidneys of animals kept on both types of diet.

The results presented are concordant with the accepted opinion that adrenaline accelerates the rate of lipolysis in the liver and increases its intake of free fatty acids as an energy source [McCann *et al*. 1995, Arimoto *et al*. 1998]. Palmer *et al*. [1981] working with rats observed that high doses of adrenaline (exceeding 1 µmol) activated lipase and thus decreased the level of triacylglycerols in the heart. On the other hand, small adrenaline doses (0.005 µmol) lead to a considerable increase of the concentration of triacylglycerols by blocking the activity of lipase.

The increased concentration of triacylglycerols observed in the present work, may indicate an intensified metabolism on non-oxidative routs, *i.e*. estrification of fatty acids, and thus the activation of the adrenergic system. In animals receiving a low-protein diet all the values obtained for triacylglycerol concentration, both in the liver and in kidneys, appeared higher after the adrenaline injections compared to the control mice injected with 0.9% NaCl (in 3 out of 4 cases the difference was confirmed statistically). However, the selected control animals (NaCl), receiving a high-protein diet (16%) showed a lower concentration of triacylglycerols in the liver than unselected mice (15.1 *vs* 18.3 µmol/g). In the case of kidneys the difference proved not significant.

Table 2. Means and their standard deviations (SD) for concentration of triacylglycerols (µmol/g tissue) in liver and kidney of mice from line selected *vs*. unselected for increased body weight. Mice were kept on two diets differing in protein content (n=10)

Experimental factor	Mice selected over 24 generations for increased body weight						Unselected mice					
	liver			kidney			liver			kidney		
	mean	SD	%	mean	SD	%	mean	SD	%	mean	SD	%
Diet with 16% protein												
control	15.1[a]	2.0	100	17.0	2.3	100	18.3[a1]	3.3	100	17.9[2]	1.0	100
adrenaline	16.5	3.2	110	15.3	1.9	90	18.3	3.4	103	16.7	1.6	93
Diet with 10% protein												
control	16.2	2.1	100	14.6	1.9	100	15.0[1]	2.0	100	9.6[2]	1.4	100
adrenaline	18.9*	3.3	117	17.5*	3.4	119	19.5**	1.8	130	10.7	1.6	111

*P≤0.05; **P≤0.01; 1-1, 2-2; a-a – differences confirmed statistically.

The results presented in Table 3 indicate that the concentration of cholesterol in the liver of selected and unselected mice of the control groups (0.9% NaCl), kept on a diet containing 16% protein, was similar and even identical (3.6 and 3.6 µmol/g), while in animals receiving a diet containing 10% protein it was significantly higher in the liver of selected mice (5.8 *vs* 4.5 µmol/g). The cholesterol content of kidneys did not differ significantly (3.1 *vs* 3.6 and 3.8 *vs* 3.9 µmol/g).

In the kidney of selected mice the lowest content of cholesterol was observed in animals fed the high-protein diet (3.1 µmol/g), while mice fed the low-protein diet showed a slightly, though not significantly higher level (3.8 µmol/g). A similar situation was observed in unselected mice (3.6 *vs* 3.9 µmol/g).

The adrenaline injection did not lead to significant changes in the level of cholesterol in the liver of selected animals maintained on both types of diet (3.1 *vs* 3.6 and 5.5 *vs* 5.8 µmol/g). However, this effect did prove significant in the case of kidneys of selected mice receiving high-protein (increase from 3.1 to 3.6 µmol/g; P≤0.05) or low-protein diet (from 3.8 to 4.6 µmol/g; P≤0.05). Moreover, adrenaline injections resulted also in an increase in the cholesterol level in the kidney of unselected mice kept on a low-protein diet (from 3.9 to 4.8 µmol/g; P≤0.05). This increase may constitute a natural adaptation reaction, because during a stress response caused by excess of adrenaline, cholesterol may be used more intensively as a precursor of suprarenal cortex hormones.

The significant increase observed in the level of cholesterol in kidneys of selected mice maintained on a diet containing 16% protein, as well as the highly significant increase in the liver of unselected mice, also fed a diet with protein level considered to be standard (*i.e.*16 %), seems to corroborate the results reported by Nitecka and Tymolewska-Niebuda [1993] as well as Gawęcki and Czarnocińska [1991], indicating that the level of cholesterol in the blood depends on the amount and source of dietary protein.

Table 3. Means and their standard deviations (SD) for concentration of cholesterol (µmol/g) in liver and kidney of mice from line selected *vs* unselected for increased body weight. Mice were kept on two diets differing in protein content (n=10)

Experimental factor	Mice selected over 24 generations for increased body weight						Unselected mice					
	liver			kidney			liver			kidney		
	mean	SD	%	mean	SD	%	mean	SD	%	mean	SD	%
Diet with 16% protein												
control	3.6[1]	0.6	100	3.1	0.4	100	3.6	0.4	100	3.6	0.2	100
adrenaline	3.1	0.6	84	3.6*	0.3	117	4.6**	0.2	127	4.1	0.3	112
Diet with 10% protein												
control	5.8[a1]	0.5	100	3.8	0.6	100	4.5[a]	0.1	100	3.9	0.4	100
adrenaline	5.5	0.5	94	4.6*	0.6	123	4.0	0.4	89	4.8*	0.3	122

*P≤0.05; **P≤0.01; 1-1; a-a – differences confirmed statistically.

Beside nutrition, the artificial selection is the most powerful factor deciding about progress in the improvement of farm animals [e.g. Essen 1989]. The results presented in Table 4 show that selection conducted for high body weight led to a significant decrease (compared to unselected mice) of the level of triacylglycerols only in the liver of mice fed a diet containing 16% protein (15.1 *vs* 18.3 μmol/g). In turn, the concentration of cholesterol (Tab. 5) was found significantly higher in the liver of selected mice fed a diet containing 10% protein compared to unselected animals (5.8 *vs* 4.5 μmol/g). These results indicate that the organisms of mice of the two genetic groups (selected *vs* unselected) responded differently to the hormone and diets tested. The unselected mice proved somewhat more resistant to the adrenaline administered as the changes in the level of triacylglycerols were lower than those observed in selected animals.

The experimental factors applied, *i.e*. exogenous adrenaline, diets differing in the level of protein as well as selection proved to modify the concentration of the lipid indicators in the liver and kidneys. One may assume that the changes in concentration revealed were related to adaptation in the process of creating a new homeostasis within cells, resulting from the protein deficit and adrenaline injection.

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Wpływ adrenaliny na koncentrację triacylogliceroli i cholesterolu w wątrobie i nerce myszy selekcjonowanych na wysoką masę ciała i utrzymywanych na dietach różniących się poziomem białka

S t r e s z c z e n i e

Myszy selekcjonowane w kierunku szybkiego tempa wzrostu ciała i myszy kontrolne, których rodziców kojarzono losowo utrzymywano na dietach zawierających 10 i 16% białka. Po trzykrotnych domięśniowych iniekcjach 0,01 μg adrenaliny/g masy ciała stwierdzono istotny wzrost koncentracji triacylogliceroli w wątrobie i nerce myszy selekcjonowanych pozostających na diecie ubogiej w białko oraz w wątrobie myszy nieselekcjonowanych, żywionych tą samą dietą. Po iniekcjach adrenaliny poziom cholesterolu wzrósł istotnie w nerce zwierząt selekcjonowanych i w wątrobie zwierząt nieselekcjonowanych pozostających na diecie zawierającej 16% białka i 10% białka oraz w nerce myszy nieselekcjonowanych utrzymywanych na paszy z 10% zawartością białka.