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Concentration of triacylglycerols and cholesterol in liver, kidneys and muscles of mice following exposure to ethyl alcohol

Introduction and aim

The effect of ethyl alcohol on biochemical transformations of triacylglycerols is a widely discussed research problem. Indeed, the recognition of its effect on biochemical transformations of triacylglycerols and cholesterol may have priceless practical importance for medicine and veterinary practice and procedures (Jóźwik et al, 2012). Triacylglycerols in mammals constitute approximately 98% of the total fatty tissue pool, and their level in blood depends on hormonal regulation, age and muscle work (Klusek et al, 1998, Levy et al, 2004, Sacchetti et al, 2004). There are also many reports concerning cholesterol, due to its biochemical functions and physiological importance (Chien et al, 2008, Fremont et al., 2000, Klusek et al, 2001). Considering the above-mentioned data, the aim of the study was to assess the effect of specified doses of ethyl alcohol on the concentration of triacylglycerols and cholesterol in the liver, kidneys, and thigh muscles tissue in experimental mice selectively bred for high and low analgesia.

Material and methods

The experiment was conducted on 80 male Swiss mice bred at the Institute of Genetics and Animal Breeding, Polish Academy of Sciences. When the animals reached sexual maturity, at the age of 6 weeks, they were randomly selected for high and low analgesia. In order to investigate sensitivity to pain, the mice were placed on a copper plate with a temperature of 56 °C. The animals which did not react to this high temperature for 10 seconds were marked as HA (High Analgetic Group) and designated as parents of subsequent generations of mice with high analgesia. A male and a female reacting as early as after 3 seconds, marked as LA (Low Analgetic Group) were the precursors of the generations of animals with low analgesia. In this way, two genetic lines of animals differing with respect to sensitivity to pain were mated.

The experimental animals were kept in standard conditions of an experimental farm, in a 12-hour light cycle (L:D), in a room with a controlled temperature of 21–22 °C, air exchange, and relative humidity 60–75%. They were placed in typical plastic cages, 30 × 15 × 10 cm, on a bedding of soft wood shavings. The cages were covered with a stainless steel mesh. On its mesh, there was given constantly granulated

standard feed, produced specially for feeding experimental mice, containing 16% of proteins and 14.04 MJ/kg of energy. All the mice had access to water *ad libitum*. For the entire period of the experiment the mice were provided with very good veterinary care.

Experimental animals were divided into 8 study groups.

1 – Experimental Group I – mice showing high analgesia (HA), administered 8% ethyl alcohol every day for a period of 21 days.

2 – Experimental Group II – mice showing low analgesia (LA), administered 8% ethyl alcohol every day for a period of 21 days.

3 – Control Group I – mice showing high analgesia, administered water every day for a period of 21 days.

4 – Control Group II – mice displaying low analgesia, administered water every day for a period of 21 days.

Administration to animals *per os* of ethyl alcohol at a dose of 500 µl/mouse, or the same amount of water was performed using an automatic micropipette, twice daily, at 10:00 and 20:00.

5 – Experimental Group III – mice showing high analgesia, given an intraperitoneal injection of 8% ethyl alcohol at a dose of 500 µl/mouse;

6 – Experimental Group IV – mice displaying low analgesia, given an intraperitoneal injection of 8% ethyl alcohol at a dose of 500 µl/mouse;

7 – Control Group III – mice showing high analgesia, given an intraperitoneal injection of 0.9% NaCl a dose of 500 µl/mouse;

8 – Control Group IV – mice showing low analgesia, given an intraperitoneal injection of 0.9% NaCl a dose of 500 µl/mouse.

Intraperitoneal injections of ethyl alcohol and saline solution were performed twice daily only at 10:00 and 20:00.

At the cessation of the experiment, the mice were anaesthetized by izofluorane narcose (a dose of 3030 µl/kg) and subsequently decapitated. Immediately after decapitation, the liver, kidneys and the left thigh muscles were isolated.

The liver was subjected to perfusion with saline solution cooled to +4 °C, and subsequently, a mass of 600 mg of tissue/6 ml 0.1 M phosphate buffer pH 7.4 was homogenized in Potter's homogenizer with a teflon piston (name of manufacturer, city, country), at 200 rpm/minute, performing four 'up-down' cycles for the liver and kidney, and twenty cycles for the muscle. The homogenates obtained were centrifuged in a Janetzki K-24 centrifuge for 10 minutes at 12 000 rpn/minute. Both homogenization and centrifugation were performed in a cold room at the temperature of +4 °C.

In order to determine the level of triacylglycerols in the liver, kidneys and muscle supernatant, the 'Alpha Diagnostic' test was used (Poland). The determination was based on the method of Wako, as described by Searcy (1974).

The cholesterol level was determined by means of 'Biochemtest' (Gliwice, Poland) based on the method of Allain et al. (1974).

Extinction levels were determined using the Perkins-Elmer Lambda Bio 20 spectrometer (USA). The concentration of triacylglycerols and cholesterol were expressed in µmol/g fresh tissue.

Analysis of variance was performed according to the following statistical model:

1. Analysis for each organ separately:

$$y_{ijk} = P_i + C_j + P \times C_{ij} + e_{ijk}$$

y_{ijk} – level of triacylglycerols and cholesterol
P_i – constant effect of alcohol
C_j – constant effect of selection
$P \times C_{ij}$ – constant effect of alcohol-selection interaction
e_{ijk} – error

2. Analysis for each alcohol group separately:

$$y_{ijk} = N_i + C_j + N \times C_{ij} + e_{ijk}$$

y_{ijk} – level of triacylglycerols and cholesterol
N_i – constant effect of the organ examined
C_j – constant effect of selection
$N \times C_{ij}$ – constant effect of organ-selection interaction
e_{ijk} – error

Experiments on mice were carried out with the consent of the Bioethical Commission operating at the Świetokrzyska Medical Chamber, ul. Wojska Polskiego 52 25-399 Kielce, Poland [No. 46/2016 dated 06.2016]

Results

Table 1 presents the results of changes in the concentration of triacylglycerols and cholesterol in the examined organs of mice displaying high and low analgesia, administered ethyl alcohol for 21 days and intraperitoneally.

The data presented in Table 1 show that alcohol caused a significant ($p<0.05$) increase in the concentration of triacylglycerols in the liver in both experimental groups of mice (HA and LA) – up to 120.6% and 144.2% of control values. However, it did not reveal any changes in the kidneys, and significantly decreased their level in the muscle of LA group (to 71%). Concentration of triacylglycerols in all examined organs in control animals (LA/HA) was higher (132.3% in the liver, 139.2% in the kidneys and 133.03% in the muscle).

The table reveals that alcohol doses administered intraperitoneally significantly increased the level of triacylglycerols in the liver of animals HA group (to 136.9%) and in LA group (to 133.9%).

The animals in HA group had significantly ($p<0.05$) higher concentration of cholesterol than LA group (8.2 and 5.7 μmol/g). The administration of ethyl alcohol to mice *per os* significantly decreased the level of cholesterol only in the liver of the HA group (down to 74.2% of the control value).

Intraperitoneal injection of alcohol decreased the level of cholesterol only in the HA group of animals in the liver and muscle (down to 78% and 73% of control values, respectively).

The data in Table 2 show that the alcohol significantly changed the level of triacylglycerols in the liver and muscle. No analogous changes were noted in the

kidneys, similarly as no significant changes were observed in cholesterol level in all examined organs.

Table.1. Concentration ($\overline{X}$ ±SD) of triacylglycerols (μmol/g tissue) and cholesterol (μmol/g tissue) in the liver, kidneys and muscle of mice administered ethyl alcohol for 21 day and 2 × intraperitoneally;

Group of mice administrated alcohol Control		Liver		Kidney		Muscle	
		Alcohol	Control	Alcohol	Control	Alcohol	
Triacylglycerols							
21 days administrated	HA	13.0±4.81,a	15.7±6.61	12.5±5.1b	11.6±5.9	11.2±2.5c	11.2±4.8
	%	100	120.6	100	93.2	100	99.4
	LA	17.2±3.32,a	24.8±4.52	17.4±8,.6b	15.1±7.1	14.9±6.13,c	10.6±5.53
	%	100	144.2	100	86.9	100	71.0
2 × injection	HA	14.1±4.71	19.3±8.61	14.8±5.3	13.8±5.1	12.8±1.5	12.5±1.8
	%	100	136.88	100	93.24	100	97.6
	LA	16.5±7.92	22.1±8.02	16.7±2.7	15.4±1.8	13.6±1.7	13.0±2.8
	%	100	133.94	100	92.3	100	95.6
Cholesterol							
21 days administreated	HA	8.2±6.11,a	6.1±3.31	8.5±4.3	9.0±4.4	3.4±1.4	3.5±1.3
	%	100	74.2	100	106.8	100	103.26
	LA	5.7±1.7a	6.2±2.4	8.4±3.4	8.1±4.2	3.3±0.8	3.5±1.0
	%	100	108.6	100	95.9	100	108.9
2 × injection	HA	8.9±2.471	6.99±1.681	7.89±2.53	6.58±1.38	3.25±0.982	2.37±0.952
	%	100	78.65	100	83.4	100	72.92
	LA	7.0±2.6	6.09±2.16	7.89±2.56	6.79±3.14	3.03±0.99	3.30±0.88
	%	100	87.20	100	86.06	100	108.91

1-1, 2-2, 3-3, a-a, b-b,c-c – statistically significant changes, $p<0.05$;

Table 2. Analysis of variance (F values) for the content of triacylglycerols and cholesterol in the liver, kidneys and muscle of mice administered alcohol during 21 days.

Independent variable	Mean square value	F value
Liver		
Triacylglycerols	185.546	7.5*
Cholesterol	33.805	2.42
Kidney		
Triacylglycerols	88.850	2.45
Cholesterol	33.702	3.09
Muscle		
Triacylglycerols	46.092	3.45*
Cholesterol	1.389	1.14

* - statistical significance, $p<0.05$

Discussion

The problems concerning triacylglycerols and cholesterol was analyzed during our previous studies, also with relation to response to the effect of alcohol (Klusek et al, 2002). In the liver and blood plasma of the experimental quail, administration of alcohol resulted in a decrease in the level of cholesterol and elevation of the concentration of triacylglycerols.

Many authors discuss the relations between the response to the alcohol taken and the changes of lipid metabolism rate according to the administration of its various doses (Oczkowski et al., 2013, Tan et al., 2012, Zhong et al., 2012).

Triacylglycerols

The results of our study indicate that 21-day administration of ethyl alcohol to experimental animals revealed a significant ($p<0.05$) increase in the level of triacylglycerols in their liver, both in the groups of high and low analgesia, and a decrease of this level in the muscle of LA animals. In the kidney ethanol did not cause any statistically significant differences.

After intraperitoneal injection of ethyl alcohol, a significant ($p<0.05$) increase in the investigated indicator was observed only in the liver of mice displaying high and low analgesia. The observed changes may be due to a different rate of the lipogenesis process, generated after taking considerably higher doses of alcohol during long-lasting 21-day exposure, than in the form of two intraperitoneal injections only. According to Aruna et al. (2005), the consequence of alcohol abuse may also be the impairment of the lipolysis process, which leads to an increase the concentration of triacylglycerols.

The results demonstrated that among control animals, administered water for 21 days, the concentration of triacylglycerols was significantly ($p<0.05$) higher in groups showing low analgesia (LA), compared to HA group both in the liver, kidney, and muscle. It may be presumed that animals displaying high analgesia may more quickly and intensively utilize energy proceed from triacylglycerols than the mice showing low analgesia. Thus, the level of triacylglycerols in these organs of LA mice maybe higher because that substance is not utilized as quickly as in the tissues of animals displaying high analgesia. In the opinion of Kołątaj (1993), pain exerts an effect on energy expenditure. It is a stressful phenomenon, and the stress response requires energy outlay.

Song et al. (2004) and You and Crabb (2004) stated that chronic consumption of alcohol leads to hypertriglyceridemia and accumulation of triacylglycerols in hepatocytes. Conclusions from studies by Klop et al. (2013) show that the consumption of excessive amounts of alcohol is associated with excessive secretion of low density lipoproteins (LDL), a decrease of lipolysis rate, and an increase of the transport of free fatty acids from the fatty tissue to the liver. In effect, it leads to the elevation of the level of alcohol-induced triacylglycerols. Yoon et al. (2004) also expressed an opinion that the consumption of ethanol by humans in doses higher than 30 g/daily leads to an increase in the concentration of triacylglycerols; however, the consumption of a half of this dose (15 g/daily) causes a significant ($p<0.05$) decrease in

their concentration. Aruna et al. (2005), noted a significantly higher concentration of triacylglycerols in the plasma of rats after 45 days of administration of 20% alcohol solution. Similar results were obtained by Umamaheswari et al. (2012), also in studies on rats which were administered 20% alcohol for one month.

Cholesterol

Analysis of the results obtained indicates a relative stability of the level of cholesterol under the effect of alcohol in selected organs of mice. Its concentration decreased significantly in the liver of mice displaying high analgesia, only which were administered alcohol per os, and also in the liver and muscle of HD mice after intraperitoneal injection.

These results are consistent with the studies conducted by Klusek et al. (1998) which showed that administration of ethanol to mice may cause a reduction in the concentration of cholesterol in the liver, with a simultaneous increase in the level of triacylglycerols.

A decrease in the concentration of cholesterol in the liver of mice after administration of ethyl alcohol may result from disorders in the synthesis and degradation of lipoproteins under the effect of alcohol, which is related to the defective protein glycosylation in the liver (Song et al, 2004).

Furthermore, the reduction of the cholesterol level may be the result of reduced activity of the enzyme responsible for estrification of cholesterol – *Acyl-CoA cholesterol acyltransferase (*ACAT) induced by alcohol.

However, it should be emphasized that the effect of alcohol on the concentration of cholesterol is equivocal, which is confirmed by studies on animals. Umamaheswari et al. (2012) observed a significant ($p<0.05$) increase of total cholesterol concentration in blood plasma of mice receiving 20% alcohol for 4 weeks, similar to Lee (2004), who administered 25% alcohol to rats for 5 weeks. However, Ehrlich et al. (2012) did not find any significant differences in the cholesterol concentration in the plasma of rats which consumed a 20% alcohol solution for a period of 12 months, compared to animals from the control group.

These results revealed some differences in the concentration of cholesterol in the liver and partly in muscle between animals of control groups and after received alcohol. It seems possible that the lack of an essential participation of cholesterol in the intensification of response is generated by a high or low ethyl alcohol threshold of sensitiveness of animals.

In conclusion, it may be presumed that the differences observed mainly in the liver were induced by the effect of alcohol and a varied level of analgesia. These changes may be the result of adaptation response in order to maintain the systemic physiological homeostasis.

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Abstract

Introduction and aim. The effect of ethyl alcohol on biochemical transformations of triacylglycerols is a widely discussed research problem. It may have practical importance for medicine and veterinary practice and procedures. The aim of the study was to assess the effect of specified doses of ethyl alcohol on the concentration of triacylglycerols and cholesterol in the liver, kidneys, and thigh muscles tissue in experimental mice selectively bred for high and low analgesia.

Material and methods. The experiment was conducted on mice selected for high (HA) and low (LA) analgesia. They were given specified doses of ethyl alcohol to assess the effect of alcohol on triacylglycerols and cholesterol concentration in the liver, kidneys, and thigh muscle tissues. It was measured by spectrofotomeric method.

Results. A significant ($p<0.05$) increase of triacylglycerols was observed in the liver in both the HA and LA groups. However, in the muscle of LA the level of triacylglycerols after exposure to alcohol *per os* was significantly ($p<0.05$) lower *vs.* control values. Ethyl alcohol did not affect renal function. The level of cholesterol in the liver of control HA animals was significantly ($p<0.05$) exacerbated, with respect to LA animals.

Conclusions. Observed changes, induced by the effect of alcohol and a varied level of analgesia, may be the result of adaptation response in order to maintain the systemic physiological homeostasis.

Keywords: triacylglycerols, cholesterol, mice, ethyl alcohol, analgesia

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