

Effect of Sex Hormones on Plasma Phospholipid Fatty Acid Composition in Intact Rats and Rats With Bilaterally Occluded Carotid Arteries

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Summary

The effects of 8-days treatment with 17 α -estradiol (33.3 μ g/kg) and progesterone (1.7 mg/kg) on plasma lipids and fatty acid composition of plasma phospholipids were examined in intact (INT) and bilaterally common carotid arteries occluded (BCO) male Wistar rats. Significant decrease of triglyceride level was found in BCO rats after the estradiol treatment. Both hormones elevated proportion of 18:1n-7 fatty acid in INT, but they failed to have such an effect in BCO. Estradiol increased 22:5n-3 and total n-3 polyunsaturated fatty acids (PUFA) in intact, and decreased 18:2n-6 in BCO rats. Significantly lower level of total n-3 was found in progesterone-treated than in estradiol-treated BCO rats. Given that n-3 PUFA have many beneficial effects on cell and tissue function, while n-6 PUFA have mostly the opposite effects, estradiol, rather than progesterone, was seen to improve plasma lipids and phospholipids FA profiles in INT and BCO animals. Estradiol significantly elevated the estimated activity of Δ 9-desaturases and progesterone of Δ 5-desaturase in BCO group, with no effects in INT rats.

Key words

Estradiol • Progesterone • Male rats • Plasma lipids • Fatty acids

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Introduction

Sex hormones are well known to influence lipid metabolism and fatty acid (FA) content in different mammal's tissue such as liver, muscle, pancreas, arterial wall, adipose tissue and brain (D'Eon and Braun 2002, Oztekin *et al.* 2007, Irwin *et al.* 2008). Estrogen increases the activity of hormone sensitive lipase, leading to the rise in circulating FA (D'Eon and Braun 2002). On the other hand, progesterone counteracts this stimulatory effect of estrogen and reduces plasma free FA, mostly acting *via* phosphodiesterase IV and cAMP (Saleh *et al.* 1999, Campbell and Febbraio 2001). In rats, estradiol decreases circulating total cholesterol and low density lipoproteins (LDL) level, and prevents LDL binding to the artery wall, while no cholesterol-lowering effect of progesterone was found (Lundeen *et al.* 1997, Walsh *et al.* 2000). Plasma triglycerides (TG) are also affected by estradiol. Brooks-Asplund *et al.* (2002) detected an estradiol-induced increase of plasma TG level in male lean and obese Zucker rats, the same effect was detected in ovariectomized female rats (Liu *et al.* 2004). In humans, estrogen therapy is associated with improved plasma lipoprotein profiles (McCrohon *et al.* 1999).

There is plenty of evidence about beneficial role of female sex hormones in maintaining the structure and function of the nervous system when compromised by various brain damages and injuries (Simpkins *et al.* 2005, Gibson *et al.* 2006, Stein 2008). Due to poor blood

supply, bilateral occlusion of the common carotid arteries (BCO) in rats has been established as a model for chronic cerebral hypoperfusion and related neurodegenerative diseases (Farkas *et al.* 2007). BCO is closely associated with ischemic derangement of the brain metabolism and neuronal energy failure, depletions of oxygen and ATP, generation of reactive oxygen species (ROS) and decrease of antioxidant enzymes activity, as well as with lipid peroxidation and damage of neuronal cell membrane (Farkas *et al.* 2007, Anil Kumar *et al.* 2011). Like in other pathophysiological conditions, sex steroid hormones, stimulate enzyme activity of antioxidant defense system, reduce intracellular ROS and alleviate lipid peroxidation, having a stabilizing effect on physical and functional properties of biomembranes following hypoxic-ischemic brain injury in BCO (Pajovic *et al.* 2003, Irwin *et al.* 2008, Ozacmak and Sayan 2009, Ishrat *et al.* 2009).

Phospholipids (PL) are the major lipid components of cellular membranes in the brain as in the other tissues. Their FA compositions, together with membrane cholesterol content, significantly determine overall properties of biomembranes. Moreover, membrane fluidity depends on chain length, degree and type of unsaturation of PL fatty acids, as well as on the ratio between saturated and unsaturated fatty acids in PL (Tepsic *et al.* 2009). In pathological conditions, such as brain ischemia and metabolic stress caused by BCO, membrane PL precursors can be cleaved and released, possibly triggering peripheral lipid dysregulation (Quartu *et al.* 2012). Markers of lipid peroxidation have been found to be elevated in brain tissues and body fluids in neurodegenerative diseases (Anil Kumar *et al.* 2011).

This study was performed to assess the potential biochemical alternations in plasma lipids parameters and fatty acid composition of plasma phospholipids in intact and male rats with bilaterally occluded carotid arteries, subjected to short-term, 8-days, treatment with estrogen or progesterone.

Material and Methods

Animals and treatments

The experiments were carried out on 2 months old male Wistar rats. The animals were kept under controlled conditions, 12 h light-dark cycle, 22±2 °C, and had free access to water and food. Commercial normo-protein pelted cereal-based diet (Veterinary Institute, Subotica) was applied, containing (w/w) 17.2 % protein,

60.9 % carbohydrate, 3.7 % fat with polyunsaturated/saturated fatty acid ratio (PUFA/SFA) of 1.3 and n-3/n-6 PUFA ratio of 0.05, 5.6 % fiber and adequate amount of vitamins and minerals (ash 7.6 %). The animals were fasted overnight before decapitation. All experimental procedures were done according to our Institutional guidelines for animal research and principals of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other (Official Daily N.L 358/1-358/6, 18, December 1986).

For this study, rats were randomly divided into two main groups. The first group of twenty-one animals remained intact (INT). The second group, composed of 26 animals was subjected to bilateral occlusion of the common carotid arteries (BCO). Rats were anesthetized with intraperitoneal administration of 400 mg/kg chloral hydrate. Midline cervical incision and blunt dissection of muscles were performed, exposing right and left common carotid arteries. Both arteries were permanently ligated with surgical thread, while leaving the vagus nerve intact. The survival rate was 80.8 %.

Both groups were divided into three subgroups (n=7) based on the treatment as follows. The first subgroup of animals of both INT and BCO groups received 33.3 µg/kg of 17α-estradiol (Sigma Chemical Co, St Louis, MO, USA) given in linseed oil (INT-E, BCO-E respectively). The second set of animals was treated with 1.7 mg/kg progesterone (Sigma Chemical Co, St Louis, MO, USA) in linseed oil (INT-P, BCO-P). The third set of animals received only vehicle, 0.2 ml of linseed oil (INT-V, BCO-V). Fatty acid composition of linseed oil was as follows (mol %): C16:0 6.74±0.10, C18:0 6.07±0.05, C18:1*cis* 18.69±0.08, C18:1*trans* 1.29±0.03, C18:2n-6 13.59±0.13, C18:3n-3 53.63±0.31, SFA 12.81±0.08, MUFA 19.98±0.11, PUFA 67.21±0.19 (Vucic *et al.* 2012). The first injection was applied 30 min after surgery and the treatment was continued for 8 consecutive days. After the treatment, animals were decapitated with Harvard Guillotine and blood samples (6-8 ml) from all rats were collected in tubes containing sodium citrate (3.8 % w/v) as anticoagulant. Samples were centrifuged at 1500 g for 10 min, and obtained plasma was immediately used for biochemical analysis. Remaining plasma was frozen at -80 °C until analysis of FA profiles.

Biochemical analysis

Cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol in plasma were analyzed using the

automated enzymatic methods with cholesterol oxidase, glycerol oxidase, PEG modified cholesterol esterase and cholesterol esterase, respectively (Roche Diagnostics,

Mannheim, Germany), on Cobas c111 analyzer (Roche, Basel, Switzerland).

Table 1. Weight gain of experimental animals.

Subgroup	Initial body weight (g)	Final body weight (g)	Body weight gain (g)
INT-V	281 ± 26	305 ± 21	24 ± 2.3
INT-E	319 ± 17	339 ± 24	20 ± 3.3
INT-P	298 ± 19	319 ± 23	22 ± 2.1
BCO-V	291 ± 20	309 ± 37	18 ± 2.4**
BCO-E	300 ± 25	317 ± 44	17 ± 3.2
BCO-P	310 ± 28	329 ± 45	19 ± 3.5*

Values are means ± SD (g). * $p < 0.05$ when compared INT-P and BCO-P. ** $p < 0.01$ when compared INT-V and BCO-V.

Analysis of plasma phospholipids fatty acid composition

Plasma lipids were extracted by the method of Folch *et al.* (1957) using a chloroform-methanol mixture (2:1 v/v). During the extraction procedure, lipids were protected against oxidation by addition of 10 mg / 100 ml 2,6-di-tert-butyl-4-methylphenol (BHT) to the solvents. One-dimensional thin-layer chromatography (TLC) on Silica Gel GF plates (Merck, Darmstadt, Germany) using neutral lipid solvent system of hexane: diethyl ether: acetic acid (87:12:1) was applied to isolate PL fraction. Methyl esters of PL fatty acids were prepared by transmethylation, as described previously (Popovic *et al.* 2012).

Fatty acids methyl esters were separated by Gas Chromatography (GC) using Shimadzu GC 2014 (Shimadzu Co, Tokyo, Japan) equipped with a flame ionization detector and Rtx 2330 fused silica gel capillary column, (60 m x 0.25 mm x 0.2 µm) (Restek Co, Bellefonte, PA, USA). The flame ionization detector was set at 260 °C and the injection port at 220 °C, the oven temperature was programmed at 140 °C for 5 minutes and then from 140 °C to 220 °C at the heating rate 3 °C/min. Individual FA methyl esters in the samples were identified by comparing sample peak retention times with authentic standards (Sigma Chemical Co, St Louis, MO, USA) and/or (PUFA)-2 standard mixture (Restek Co, Bellefonte, PA, USA). The results are expressed as percentages of total identified FA.

The activities of certain enzymes involved in FA biosynthesis, desaturases and elongases, were estimated as the product-to-precursor ratios of the percentages of individual FA. The ratio of 20:4n-6/20:3n-6 was used as a

measure of estimated $\Delta 5$ -desaturase. The 20:3n-6/18:2n-6 ratio was used as a measure of $\Delta 6$ -desaturase and elongase activities, while 16:1n-7/16:0 and 18:1n-9/18:0 ratios represented estimated activities of $\Delta 9$ -desaturase 1 and 2, respectively. The index of elongase activity was calculated as the ratio 18:0/16:0.

Statistical analysis

The results are expressed as means ± SD. The normal distribution of variables was tested using the Shapiro-Wilk test before statistical analysis. When variables showed normal distribution, statistical analysis was performed using one-way ANOVA, followed by Tukey *post hoc* test to identify differences between groups. For non-normally distributed variables, Kruskal-Wallis and Mann-Whitney tests were applied (18:3n-3, 22:4n-6 and MUFA). The differences were considered significant at $p < 0.05$.

Results

As presented in Table 1, there were no statistically significant differences in body weight gains among -V, -E, -P subgroups in the same group (INT or BCO) but BCO-V and BCO-P rats had lower weight gain than the INT-V, *e.g.* INT-P groups.

Changes of biochemical parameters in plasma of estradiol-treated (INT-E) and progesterone-treated (INT-P) intact rats are shown in Figure 1. In comparison with the control animals (INT-V), there were no significant effects of hormones on lipid profile. As in the case of intact animals, in rats with bilateral occluded carotid

arteries treatment with progesterone (BCO-P), as well as estradiol (BCO-E) slightly reduced total, HDL- and LDL-cholesterol, when compared to the control subgroup (BCO-V) (Fig. 2), but no statistical significance of

differences between subgroups was found. Contrary to the intact rats, statistically significant decrease of triglyceride level after the estradiol treatment was found, while progesterone showed no effect.

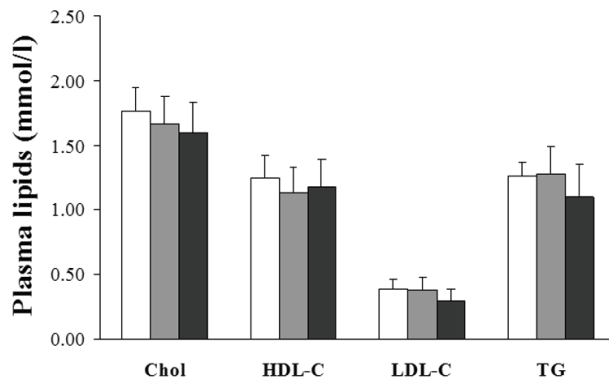


Fig. 1. The effects of estrogen and progesterone on plasma total, HDL- and LDL- cholesterol and triglyceride levels (mmol/l) in intact rats (INT-V, white; INT-E, gray; INT-P, black). Data are the mean \pm SD. * $p < 0.05$ when compared to the control rats (INT-V).

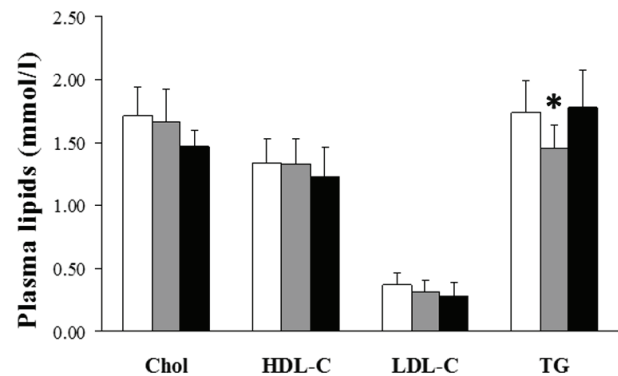


Fig. 2. The effects of estrogen and progesterone on plasma total, HDL- and LDL- cholesterol and triglyceride levels (mmol/l) in rats with bilaterally occluded carotid arteries (BCO-V, white; BCO-E, gray; BCO-P, black). Data are the mean \pm SD. * $p < 0.05$ when compared to the control rats (BCO-V).

Table 2. The effects of estradiol and progesterone on plasma phospholipids fatty acid profile in intact (INT) rats and in rats with bilaterally occluded carotid arteries (BCO).

Fatty acids	INT-V	INT-E	INT-P	BCO-V	BCO-E	BCO-P
16:0	22.89 \pm 1.94	22.43 \pm 1.89	24.37 \pm 2.54	22.74 \pm 1.71	21.18 \pm 2.07	24.19 \pm 2.42
16:1n-7	0.31 \pm 0.10	0.30 \pm 0.17	0.33 \pm 0.12	0.23 \pm 0.08	0.34 \pm 0.20	0.21 \pm 0.05
18:0	23.71 \pm 0.65	22.63 \pm 3.09	22.84 \pm 2.55	22.21 \pm 1.63	21.74 \pm 1.30	21.97 \pm 1.14
18:1n-9	5.00 \pm 0.43	4.73 \pm 0.17	5.06 \pm 0.68	4.30 \pm 0.30 ^a	4.68 \pm 0.43	4.30 \pm 0.35
18:1n-7	1.81 \pm 0.07	2.39 \pm 0.10***	2.07 \pm 0.29#	1.59 \pm 0.11 ^a	1.64 \pm 0.12	1.69 \pm 0.11
18:2n-6	24.83 \pm 1.82	23.93 \pm 3.18	24.59 \pm 1.05	24.52 \pm 1.90	21.55 \pm 0.86*	22.53 \pm 0.66
18:3n-3	0.18 \pm 0.06	0.15 \pm 0.03	0.16 \pm 0.04	0.34 \pm 0.26	0.68 \pm 0.53	0.22 \pm 0.06
20:3n-6	1.66 \pm 0.16	1.47 \pm 0.48	1.44 \pm 0.31	1.68 \pm 0.29	1.44 \pm 0.26	1.21 \pm 0.22*
20:4n-6	13.35 \pm 3.17	13.40 \pm 2.75	12.14 \pm 2.30	15.39 \pm 2.82	19.14 \pm 2.24	17.32 \pm 1.01
20:5n-3	0.82 \pm 0.15	0.96 \pm 0.21	0.80 \pm 0.20	1.25 \pm 0.41	0.92 \pm 0.24	0.72 \pm 0.06
22:4n-6	0.34 \pm 0.11	0.55 \pm 0.30	0.30 \pm 0.09	0.27 \pm 0.04	0.26 \pm 0.09	0.19 \pm 0.08
22:5n-3	1.40 \pm 0.33	1.92 \pm 0.49*	1.37 \pm 0.29#	1.06 \pm 0.32	1.06 \pm 0.24	0.78 \pm 0.10
22:6n-3	4.18 \pm 0.32	5.11 \pm 0.74	4.53 \pm 0.48	4.56 \pm 1.08	5.37 \pm 0.69	4.71 \pm 0.36
SFA	46.60 \pm 2.37	45.06 \pm 2.61	47.21 \pm 1.73	44.95 \pm 1.82	42.92 \pm 2.88	46.16 \pm 2.39
MUFA	7.13 \pm 0.43	7.41 \pm 0.25	7.45 \pm 1.03	6.12 \pm 0.42 ^a	6.65 \pm 0.44	6.20 \pm 0.46
PUFA	46.77 \pm 2.03	47.48 \pm 2.64	45.33 \pm 1.78	49.07 \pm 2.28	50.42 \pm 2.81	47.68 \pm 2.12
n-6	40.18 \pm 2.02	39.35 \pm 3.30	38.47 \pm 1.63	41.86 \pm 1.80	42.39 \pm 2.23	41.24 \pm 1.67
n-3	6.58 \pm 0.54	8.14 \pm 1.08*	6.86 \pm 0.81	7.21 \pm 1.46	8.03 \pm 1.13	6.44 \pm 0.49#
n-6/n-3	6.14 \pm 0.62	4.95 \pm 1.09	5.67 \pm 0.67	6.05 \pm 1.45	5.36 \pm 0.68	6.42 \pm 0.28

Values are means \pm SD (mol %). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. * $p < 0.05$, *** $p < 0.001$ – when compared to the proper hormone-untreated subgroup (INT-V or BCO-V). ^a $p < 0.05$ when compared the INT-V subgroup. # $p < 0.05$ – when compared to the proper estradiol-treated subgroup (INT-E or BCO-E), as determined by ANOVA followed by the Tukey *post hoc* test.

Table 3. The estimated plasma desaturase and elongase activities in intact rats and rats with bilateral occlusion of carotid arteries.

Desaturases and elongases	INT-V	INT-E	INT-P
20:4n-6/20:3n-6 ($\Delta 5$)	8.13 \pm 2.20	10.34 \pm 4.75	8.88 \pm 2.86
20:3n-6/18:2n-6 ($\Delta 6$)	0.07 \pm 0.01	0.06 \pm 0.02	0.06 \pm 0.01
16:1n-7/16:0 ($\Delta 9-1$)	0.015 \pm 0.003	0.014 \pm 0.007	0.015 \pm 0.002
18:1n-9/18:0 ($\Delta 9-2$)	0.29 \pm 0.02	0.33 \pm 0.02	0.32 \pm 0.07
18:0/16:0	1.04 \pm 0.08	1.02 \pm 0.20	0.95 \pm 0.19
Desaturases and elongases	BCO-V	BCO-E	BCO-P
20:4n-6/20:3n-6 ($\Delta 5$)	9.35 \pm 2.21	13.81 \pm 3.52	14.47 \pm 2.60*
20:3n-6/18:2n-6 ($\Delta 6$)	0.07 \pm 0.01	0.07 \pm 0.01	0.05 \pm 0.01
16:1n-7/16:0 ($\Delta 9-1$)	0.009 \pm 0.002 ^a	0.020 \pm 0.007*	0.009 \pm 0.002
18:1n-9/18:0 ($\Delta 9-2$)	0.26 \pm 0.02 ^a	0.29 \pm 0.02*	0.28 \pm 0.02
18:0/16:0	0.98 \pm 0.12	1.03 \pm 0.09	0.92 \pm 0.10

^a p<0.05 – when compared to intact hormone-untreated animals (INT-V), * p<0.05 – when compared to control group (BCO-V), as determined by ANOVA followed by the Tukey *post hoc* test.

Alternations in percentage of individual fatty acids observed in plasma phospholipids of control and hormone-treated intact and BCO rats are presented in Table 2. In comparison with INT-V, statistically significant increase in a monounsaturated fatty acid (MUFA), 18:1n-7 (vaccenic acid) was found in both INT-E and INT-P subgroups. There was also a significant difference in vaccenic acid between INT-E and INT-P, showing a stronger raising effect of estradiol on this FA. The percentage of a polyunsaturated fatty acid (PUFA) 22:5n-3 (eicosapentanoic acid, EPA) elevated significantly in estradiol-treated rats, but not in the INT-P subgroup, making a significant difference between EPA in INT-E and INT-P. These changes induced significant increase in total n-3 PUFA in INT-E. The relative fatty acid composition of plasma PL in rats with bilateral occlusion of carotid arteries was very slightly affected by estradiol or progesterone. Statistically significant decrease in linoleic acid (18:2n-6, LA) in estradiol-treated subgroup (BCO-E) was found when compared to the control subgroup (BCO-V). In progesterone-treated subgroup (BCO-P), a significant decrease in dihomo-gamma-linoleic acid (20:3n-6, DGLA) was detected in comparison to BCO-V, and also in total n-3 PUFA, when compared to BCO-E. All other FA remained unchanged in both treated groups.

Treatment with estradiol and progesterone did not significantly affect the estimated activity of desaturase and elongase systems in plasma phospholipids

of intact rats (Table 3). In BCO rats, progesterone treatment increased the activity of $\Delta 5$ -desaturase, while estradiol positively affected the estimated activity of $\Delta 9$ -desaturases 1 and 2. Moreover, hormone-untreated BCO rats (BCO-V) had significantly lower estimated activities of $\Delta 9$ -desaturases 1 and 2, when compared to intact hormone-untreated animals (INT-V). There is also an upward trend of $\Delta 5$ -desaturase activity in INT-E and BCO-E subgroups, compared to INT-V and BCO-V, respectively (p<0.1).

Discussion

Short term hormone treatments applied in this study had no effect on body weight gains in both INT and BCO rats. A lower weight gains in all BCO subgroups, when compared to the corresponding INT subgroups, could be explained by discomfort which BCO animals felt during movement of the head, mastication, and swallowing in the first days after surgery (Farkas *et al.* 2007).

Our results have shown that physiological concentration of steroid hormones 17 α - estradiol and progesterone had no effect on plasma cholesterol level in intact 2 months old male rats and BCO rats. A significant modulatory effect of estradiol was found only on triglyceride level in BCO rats. In all (hormone-treated and untreated) BCO subgroups an elevated level of plasma triglycerides (TG) was found, when compared to

the appropriate INT subgroups. It suggests that bilateral occlusion of carotid arteries induced a sharp increase in plasma TG (1.26 mmol/l in INT-V vs. 1.74 mmol/l in BCO-V). Cerebral ischemia has already been reported to increase circulating TG in spontaneously hypertensive male and female rats subjected to BCO (Wexler 1980), that is in line with our results. However, in this study, short-term, 8 days, treatment with physiological doses of progesterone and 17 α -estradiol did not affect plasma TG level in male intact rats, while estradiol decreased TG in rats with bilateral occlusion. Previous studies showed increasing effect of estradiol. It is possible that physiologic doses of estradiol mitigated the increase in TG after the operation in BCO rats. Namely, subcutaneously administered ovarian hormones for 7 days alleviate stroke injury in reproductively senescent female rats (Alkayed *et al.* 2000). After the treatment of same duration physiologic doses of progesterone reduces mitochondrial dysfunction and hippocampal cell loss after traumatic brain injury in female rats (Robertson *et al.* 2006), while estradiol reduces cortical and caudate infarction volume in castrated male rats (Toung *et al.* 1998). Therefore, physiologic doses of ovarian hormones induced significant changes in brain and it is possible that the same doses and duration of treatment affect lipid profile as well.

Even one-time to one-week hormone treatment decreases brain oxidative stress markers, increases endogenous antioxidant levels, reduces the extent of brain injury and promotes the neurogenesis after carotid occlusion, stroke and traumatic brain injury (Ozacmak and Sayan 2009, Barha *et al.* 2011, Zhang *et al.* 2013). At least some of these effects may be related to hormonal capability to attenuate increase of disintegration and decrease of resynthesize of membrane phospholipids following hypoxic-ischemic brain injury. Busto *et al.* (1989) assumed that ischemia induced fluctuations of palmitic, stearic, oleic and arachidonic acids in local cerebral blood flow, following increase in their concentrations detected in cortex and striatum of experimental rats. In addition, hormonal treatment could induce systemic changes in FA metabolism, which could be seen in plasma phospholipids, as examined in this paper. Although 8 days may be rather short time for the analyses of brain lipid metabolism, short-term estradiol and progesterone administration is very commonly used in treatment of different brain injuries, thus it is important to examine its possible effects on plasma lipids and phospholipids.

Bilateral occlusion of carotid arteries in rats led to a significant decline in plasma PL 18:1n-7 (vaccenic acid) and 18:1n-9 (oleic acid) content, and consequently in the concentration of total MUFA in BCO-V, when compared with intact control. It could be assumed that Δ 9-desaturase activity was reduced in BCO rats. Although both estradiol and progesterone elevated proportion of vaccenic acid in intact rats, they failed to have such an effect in BCO groups. Estradiol also induced increases of 22:5n-3 (eicosapentanoic acid, EPA) and total n-3 PUFA in intact rats. Elevated concentrations of n-3 PUFA in rat plasma are positively associated with circulating concentrations of estradiol and progesterone (Childs *et al.* 2008). However, in our study progesterone had no effect on n-3 PUFA proportion in intact rats. Moreover, in BCO rats, significantly lower level of total n-3 was found in BCO-P than in the BCO-E subgroup. Given that n-3 PUFA have many beneficial effects on cell and tissue function, including anti-inflammatory, anticancer, cardioprotective and neuroprotective effects (Ristic-Medic *et al.* 2013), our results confirmed favorable effects of estradiol in intact animals. In BCO rats, estradiol significantly reduced linolenic acid (18:2n-6), with no effect on MUFA or n-3 PUFA. Regarding that n-6 PUFA have mostly the opposite effects of n-3 FA (pro-inflammatory and pro-cancerogenic), the total effect of estradiol is beneficial in BCO animals as well.

As primarily observed in erythrocytes, there is a positive correlation between the alternations in plasma phospholipid composition and cell membranes (Peet *et al.* 1998). The alternations in plasma n-3 and n-6 long-chain PUFA detected in this study, are expected to reflect on the phospholipid composition of the neuronal membrane, in a way that improves physicochemical environment to promote protein-lipid and protein-protein interactions essential for neuronal function, synaptic transmission and neurotransmitter signaling (Fabelo *et al.* 2011).

The sex hormones act to modify plasma and tissue PUFA content, possibly by altering the expression and activity of desaturase and elongase enzymes (Childs *et al.* 2008). In BCO animals, estimated activity of Δ 9-desaturases 1 and 2 was significantly lower than in the intact rats, as we previously assumed based on lower levels of MUFA. Furthermore, estradiol treatment significantly increased the activity of this enzyme in BCO rats (but not in intact ones), while progesterone elevated the estimated activity of Δ 5-desaturase. In line with our results, Marks *et al.* (2013) have recently reported increased Δ 9-desaturase 1 expression in HepG2 cells,

after 17 β -estradiol treatment, although they also found decreased expression of this enzyme after treatment with progesterone. However, these alterations were not found when compared male and female rats. They also found elevated expression of hepatic elongase in females relative to male rats, which appears to be mediated by sex hormones based on observations of hormonal treatments of HepG2 cells (Marks *et al.* 2013). Nevertheless, both hormones had no significant effects on desaturase/elongase in intact rats in our study, although we detected some alterations in FA profiles in these animals as well. In addition, we should not neglect other possible reasons for the observed changes in phospholipid fatty acid composition, including PUFA mobilization from the adipose tissue, phospholipids remodeling of brain tissue or effects of oxidative stress.

In summary, applied hormones induced different effects on FA in intact and BCO rats. Estradiol showed beneficial effect on FA composition in intact rats,

increasing EPA and n-3 PUFA proportion, which are crucial for cardiovascular health and synthesis of anti-inflammatory eicosanoids. In BCO rats, estradiol reduced level of LA, which is a precursor of n-6 arachidonic acid and pro-inflammatory eicosanoids. In contrast, progesterone showed no positive effects on plasma fatty acid profile. The possible reasons for overall weak effects of hormones include short term treatment and influence of endogenous testosterone. Thus, further investigations of longer duration on more animals should be required.

Conflict of Interest

There is no conflict of interest.

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References

- ALKAYED NJ, MURPHY SJ, TRAYSTMAN RJ, HURN PD: Neuroprotective effects of female gonadal steroids in reproductively senescent female rats. *Stroke* **31**: 161-168, 2000.
- ANIL KUMAR S, SAIF SA, OOTHUMAN P, MUSTAFA MIA: Lipid peroxidation in chronic cerebral hypoperfusion-induced neurodegeneration in rats. *Int Med J Malaysia* **10**: 3-6, 2011.
- BARHA CK, ISHRAT T, EPP JR, GALEA LA, STEIN DG: Progesterone treatment normalizes the levels of cell proliferation and cell death in the dentate gyrus of the hippocampus after traumatic brain injury. *Exp Neurol* **231**: 72-81, 2011.
- BROOKS-ASPLUND EM, SHOUKAS AA, KIM SY, BURKE SA, BERKOWITZ DE: Estrogen has opposing effects on vascular reactivity in obese, insulin-resistant male Zucker rats. *J Appl Physiol* **92**: 2035-2044, 2002.
- BUSTO R, GLOBUS MY, DIETRICH WD, MARTINEZ E, VALDES I, GINSBERG MD: Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. *Stroke* **20**: 904-910, 1989.
- CAMPBELL SE, FEBBRAIO MA: Effects of ovarian hormones on exercise metabolism. *Curr Opin Clin Nutr Metab Care* **4**: 515-520, 2001.
- CHILDS CE, ROMEU-NADAL M, BURDGE GC, CALDER PC: Gender differences in the n-3 fatty acid content of tissues. *Proc Nutr Soc* **67**: 19-27, 2008.
- D'EON T, BRAUN B: The roles of estrogen and progesterone in regulating carbohydrate and fat utilization at rest and during exercise. *J Womens Health Gend Based Med* **11**: 225-237, 2002.
- FABELO N, MARTIN V, SANTPERE G, MARIN R, TORRENT L, FERRER I, DIAZ M: Severe alterations in lipid composition of frontal cortex lipid rafts from Parkinson's disease and incidental Parkinson's disease. *Mol Med* **17**: 1107-1118, 2011.
- FARKAS E, LUITEN PG, BARI F: Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev* **54**: 162-180, 2007.
- FOLCH J, LEES M, SLOANE STANLEY GH: A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**: 497-509, 1957.
- GIBSON CL, GRAY LJ, MURPHY SP, BATH PM: Estrogens and experimental ischemic stroke: a systematic review. *J Cereb Blood Flow Metab* **26**: 1103-1113, 2006.

- IRWIN RW, YAO J, HAMILTON RT, CADENAS E, BRINTON RD, NILSEN J: Progesterone and estrogen regulate oxidative metabolism in brain mitochondria. *Endocrinology* **149**: 3167-3175, 2008.
- ISHRAT T, SAYEED I, ATIF F, STEIN DG: Effects of progesterone administration on infarct volume and functional deficits following permanent focal cerebral ischemia in rats. *Brain Res* **1257**: 94-101, 2009.
- LIU ML, XU X, RANG WQ, LI YJ, SONG HP: Influence of ovariectomy and 17beta-estradiol treatment on insulin sensitivity, lipid metabolism and post-ischemic cardiac function. *Int J Cardiol* **97**: 485-493, 2004.
- LUNDEEN SG, CARVER JM, MCKEAN ML, WINNEKER RC: Characterization of the ovariectomized rat model for the evaluation of estrogen effects on plasma cholesterol levels. *Endocrinology* **138**: 1552-1558, 1997.
- MARKS KA, KITSON AP, STARK KD: Hepatic and plasma sex differences in saturated and monounsaturated fatty acids are associated with differences in expression of elongase 6, but not stearoyl-CoA desaturase in Sprague-Dawley rats. *Genes Nutr* **8**: 317-327, 2013.
- MCCROHON JA, NAKHLA S, JESSUP W, STANLEY KK, CELERMAJER DS: Estrogen and progesterone reduce lipid accumulation in human monocyte-derived macrophages: a sex-specific effect. *Circulation* **100**: 2319-2325, 1999.
- OZACMAK VH, SAYAN H: The effects of 17beta estradiol, 17alpha estradiol and progesterone on oxidative stress biomarkers in ovariectomized female rat brain subjected to global cerebral ischemia. *Physiol Res* **58**: 909-912, 2009.
- OZTEKIN E, TIFTIK AM, BALTAÇI AK, MOGULKOC R: Lipid peroxidation in liver tissue of ovariectomized and pinealectomized rats: effect of estradiol and progesterone supplementation. *Cell Biochem Funct* **25**: 401-405, 2007.
- PAJOVIC SB, SAICIC ZS, SPASIC MB, PETROVIC VM: The effect of ovarian hormones on antioxidant enzyme activities in the brain of male rats. *Physiol Res* **52**: 189-194, 2003.
- PEET M, MURPHY B, SHAY J, HORROBIN D: Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biol Psychiatry* **43**: 315-319, 1998.
- POPOVIC T, BOROZAN S, ARSIC A, MARTACIC JD, VUCIC V, TRBOVIC A, MANDIC L, GLIBETIC M: Fish oil supplementation improved liver phospholipids fatty acid composition and parameters of oxidative stress in male Wistar rats. *J Anim Physiol Anim Nutr* **96**: 1020-1029, 2012.
- QUARTU M, SERRA MP, BOI M, PILLLOLA G, MELIS T, PODDIGHE L, DEL FIACCO M, FALCONIERI D, CARTA G, MURRU E, CORDEDDU L, PIRAS A, COLLU M, BANNI S: Effect of acute administration of Pistacia lentiscus L. essential oil on rat cerebral cortex following transient bilateral common carotid artery occlusion. *Lipids Health Dis* **11**: 8, 2012.
- RISTIC-MEDIC D, VUCIC V, TAKIC M, KARADZIC I, GLIBETIC M: Polyunsaturated fatty acids in health and disease. *J Serb Chem Soc* **78**: 1269-1289, 2013.
- ROBERTSON CL, PUSKAR A, HOFFMAN GE, MURPHY AZ, SARASWATI M, FISKUM G: Physiologic progesterone reduces mitochondrial dysfunction and hippocampal cell loss after traumatic brain injury in female rats. *Exp Neurol* **197**: 235-243, 2006.
- SALEH J, SNIDERMAN AD, CIANFLONE K: Regulation of Plasma fatty acid metabolism. *Clin Chim Acta* **286**: 163-180, 1999.
- SIMPKINS JW, YANG SH, WEN Y, SINGH M: Estrogens, progestins, menopause and neurodegeneration: basic and clinical studies. *Cell Mol Life Sci* **62**: 271-280, 2005.
- STEIN DG: Progesterone exerts neuroprotective effects after brain injury. *Brain Res Rev* **57**: 386-397, 2008.
- TEPSIC J, VUCIC V, ARSIC A, BLAZENCIC-MLADENOVIC V, MAZIC S, GLIBETIC M: Plasma and erythrocyte phospholipid fatty acid profile in professional basketball and football players. *Eur J Appl Physiol* **107**: 359-365, 2009.
- TOUNG TJK, TRAYSTMAN RJ, HURN PD: Estrogen-mediated neuroprotection after experimental stroke in male rats. *Stroke* **29**: 1666-1670, 1998.
- VUCIC V, TEPSIC J, ARSIC A, POPOVIC T, DEBELJAK-MARTACIC J, GLIBETIC M: Fatty acid content of vegetable oils and assessment of their consumption in Serbia. *Acta Alimentaria* **41**: 343-350, 2012.
- WALSH BA, MULLICK AE, BANKA CE, RUTLEDGE JC: 17beta-estradiol acts separately on the LDL particle and artery wall to reduce LDL accumulation. *J Lipid Res* **41**: 134-141, 2000.

WEXLER BC: Comparative effects of unilateral and bilateral carotid artery ligation in the spontaneously hypertensive rat. *Stroke* **11**: 72-78, 1980.

ZHANG H, LIU Y, WANG L, LI Z, WU J, RAHMAN N, GUO Y, LI D, LI N, HUHTANIEMI I, TSANG SY, GAO GF, LI X: Differential effects of estrogen/androgen on the prevention of nonalcoholic fatty liver disease in the male rat. *J Lipid Res* **54**: 345-357, 2013.
