Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 01, pp.25421-25428, January, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

INVESTIGATION OF AGE-RELATED CHANGES IN LIPID LEVELS IN THE BRAIN AND LIVER TISSUES EXTRACTS OF MALE WISTAR RATS: EXTRACTION, SEPARATION, QUALITATIVE AND QUANTITATIVE DETERMINATION

[1,2,*]Loay Khaled Mohammad Hassouneh, [1]Amjad Abuirmeileh, [1]Zead Helmi Mahmoud Abudayeh, [3]Khaldun Mohammad Al Azzam, [1]Qais Abualassal and [4]Uliana Vladimirovna Karpiuk

[1]Faculty of Pharmacy, Isra University, P.O. Box 22, Amman, 11622, Jordan
[2]Department of Physiology of Ontogenesis, Institute of Biology, Kharkov Karazin National University, 4 Svobody pl., Kharkov, 61077, Ukraine
[3]Pharmacy Program, Batterjee Medical College for Sciences and Technology (BMC), 21442 Jeddah, Kingdom of Saudi Arabia
[4]O.O. Bogomolets National Medical University, T. Shevchenko Boulevard, 13, Kiev, Ukraine

ARTICLE INFO

Article History:
Received 14th October, 2015
Received in revised form
20th November, 2015
Accepted 25th December, 2015
Published online 31st January, 2016

Key words:

Ceramide,
Diacylglycerol,
Glucosylceramide,
Phosphatidylcholine,
Sphingomyelin.

ABSTRACT

Physiological aging encompasses progressive changes in an organism which results in a decrease in organ function and an increase in the risk of mortality over time. This innate phenomenon engages several cellular components including lipids. Determining the influence of different ages on the amounts of these lipids is a mean to better understanding of their role in physiological aging. Male wistar rats were utilized to investigate the effect of age on these lipids. The results demonstrated an increase in free fatty acids, diaceylglicerol, ceramide and sphingomyline and a decrease in phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine in the liver and/or brain of 24 months old rats when compared to 3 months old. The changes in lipids were possibly due to the consequence of alteration in metabolic pathways where diaceylglicerol increased as a result of this precursor's accumulation and lack of its utilization in the *de novo* synthesis of phosphatidylcholine and phosphatidylethanolamine. Also, the increase in ceramide and sphingomyline levels was plausibly due to an increase in degradation of sphingolipids such as glucosylceramide. The results revealed possible metabolic pathways implicated in diaceylglicerol and ceramide accumulation during different ages, thus the potential involvement of these lipids in some age related pathologies.

Citation: **Loay Khaled Mohammad Hassouneh, Amjad Abuirmeileh, Zead Helmi Mahmoud Abudayeh, Khaldun Mohammad Al Azzam, Qais Abualassal and Uliana Vladimirovna Karpiuk, 2016.** "Investigation of age-related changes in lipid levels in the brain and liver tissues extracts of male wistar rats: extraction, separation, qualitative and quantitative determination", *International Journal of Current Research,* 8, (01), 25421-25428.

INTRODUCTION

Lipids are important components of biological membranes and they participate in the regulation of physiological functions of cells. These functions include proliferation, differentiation, apoptosis, secretion, and transportation of particulates. Numerous metabolites of glycerol- and sphingolipids possess high biological activities and are involved in the processes of signal transduction and formation of an adequate stimulus (Abdel-Latif, 1986; Nishizuka, 1992; Wim *et al*., 2003; Eyster, 2007). A chronic accumulation of diaceylglicerol (DAG) in cells is a characteristic of pathological processes such as adiposity, insulin resistance, diabetes, and oncogenesis (Unger, 2002, 2003; Stratford *et al*., 2004). It has been established that phospholipase-C and phospholipase-D activation under the influence of hormones and age factors leads to the generation of DAG (Hannun *et al*., 2001). Sphingomyelin (SM) cycle is an intracellular process which involves the formation of ceramide (CER) and phosphorylcholine (PC). These metabolites are formed as a result of SM cleavage by various sphingomyelinases. Subsequently, CER can be further consumed by sphingomyelin synthase (SMS) (David and Hannun, 1988). SMS is one of the most intriguing enzymes that regulate CER levels. This enzyme transfers the PC group from PC to CER and thus generates SM and DAG (Ullman and

Corresponding author: Loay Khaled Mohammad Hassouneh,
Faculty of Pharmacy, Isra University, P.O. Box 22, Amman, 11622, Jordan.

Radin, 1974; Hatch and Vance, 1992). SMS can also potentially be involved in the retrograde regulation of CER and DAG (Luberto and Hannun, 1998). The sphingolipid CER is involved in the regulation of many important physiological processes such as differentiation, proliferation and apoptosis (Wim *et al*., 2003; Hannun *et al*., 2001; David and Hannun, 1988). The increase of the basal level of CER in skeletal muscle cells inhibits phosphoinositides-3-kinase (PISK)-mediated insulin signaling. This inhibition has been proven to be a major contributor to insulin resistance (Unger, 2002, 2003; Stratford *et al*., 2004). Furthermore, an increase in the basal level of CER in hippocampal neurons and other cerebral structures is a characteristic of age related neurodegenerative diseases such as Alzheimer's disease (Cutler and Mattson, 2001). Here, the induction of the *de novo* synthesis of CER in cortical neurons where macroglia has been linked to the intensification of amyloidogenesis and hyperphosphorylation of tau protein (Patil *et al*., 2007).

Another peptide involved in the process of aging is phosphatidylserine (PS); It is involved in signal transduction as a co-factor protein kinase C (Bell and Burns, 1991), benzodiazepine receptor modulator synaptosomal membrane (Levi *et al*., 1989), and activator protein-1 sRaf (Ghosh *et al*., 1994). PS is the major acidic phospholipid of nerve cell membranes and plays an exclusively important role in supporting neural functions (Mozzi *et al*., 2003). In the current study, considering the role of signaling lipids in the regulation of biologically active glycerol, sphingolipids and the development of human age-dependent pathologies, thus it is important to further investigate their age related adaptation. Moreover, age-related changes in lipid levels in the brain and liver tissues of male wistar rats have also been studied. Moreover, lipids were fractionated followed by thin-layer chromatography (TLC). The identifiaction of compounds has been done by comparing the R_f values with standards. Additionally, quantitative determination of the lipid and protein contents in liver and brain tissues were evaluated. The metabolism in the liver homogenate SM was also investigated.

Experimental

Reagents and chemicals

Triton X-100, HEPES ≥99.5%, 0.4% trypan blue solution were purchased from Serva (Heidelberg, Germany). Dithiothreitol and Sorbfil TLC plates were purchased from Sorbpolymer, (Krasnodar, Russia). Palmitic acid (HVA 2.07 /mmol) was purchased from GE Healthcare (Amersham, UK). Chloroform ($CHCl_3$), methanol HPLC grade (CH_3OH), *n*-hexane (C_6H_{14}), diethyl ether ($(C_2H_5)_2O$), acetic acid (CH_3COOH), sodium hydroxide (NaOH), formic acid (CH_2O_2), potassium chloride (KCl), sodium chloride (NaCl), ninhydrin ($C_9H_6O_4$), *n*-butanol (C_4H_9OH), ethyl acetate ($C_4H_8O_2$), isopropyl alcohol (C_3H_8O), iodine (I), ammonium hydroxide solution (NH_4OH), sulfuric acid 99.9% (H_2SO_4), hydrochloric acid 37% (HCl), triaminomethane (Tris) ($C_4H_{11}NO_3$), ethylenediaminetetraacetic acid (EDTA) ($C_{10}H_{16}N_2O_8$), magnesium chloride ($MgCl_2$), magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), monopotassium phosphate (KH_2PO_4), sucrose 0.2% bovine serum albumin, penicillin, streptomycin, fetal bovine serum, Eagle's medium, Krebs-Henseleit buffer, 2 μCi / mL of [^{14}C] palmitic acid, palmitic acid (1-14C) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Wistar rats

Male wistar rats of 3 and 24 months age (n=20) were used in the current experiments. They were kept under standard vivarium conditions in Kharkiv National University, Ukraine (Ruth, 1935; Andreollo *et al*., 2012; Pallav, 2013). All animal studies were carried out in compliance with the international principles of the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Kehr and Weidner, 1987). Experimental procedures were approved by the Institutional Animal Care and Use Committees at the Kharkov Karazin National University. The rats had a free access to a standard chow diet and drinking water ad libitum. Animals were divided into groups depending on the purpose of the experiment where intact animals 3 and 24 months age were sacrificed and their liver, hippocampus and cerebral cortex were removed and then homogenized and used to study age-related features and lipid metabolism.

Preparation of tissue homogenates

After anesthesia with diethyl ether, animals were sacrificed via decapitation. Liver, hippocampus, and cerebral cortex were homogenized and performed in situ chilled to 4°C in isotonic solution of NaCl. It was removed from the abdominal cavity and then pressed through a perforated plate having a pore diameter of 0.3 mm at 4°C. Hippocampal and cortical tissues were crushed and washed with chilled to 4°C in isotonic solution of NaCl. These pieces of tissues homogenized were used to further study the lipid contents and the inclusion of [^{14}C]-labeled precursors into lipids. Synthesis of lipids and lipid extraction of the studied tissues produced as described in extraction of lipids section.

Preparation of isolated hepatocytes

Native cell membrane of hepatocytes was isolated non-enzymatically as previously reported by the work of Kanaeva *et al*., (Kanaeva *et al*., 1975). It was evaluated using 0.4% trypan blue solution. The yield of viable cells was 90 ± 5.0%. Hepatocytes were resuspended (at a concentration of $6 \cdot 10^6$ cells in 1 mL) in Eagle's medium, (pH 7.4) containing 25 mM HERES, penicillin (61 mg / L), streptomycin (100 mg / L), 10% fetal bovine serum and 2 μCi / mL of [^{14}C] palmitic acid, and incubated at 37˚C for 90 min. After incubation, it was washed twice with an excess of a buffer containing: 118 mM NaCl, 5 mM KCl, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 2 mM $CaCl_2$, 0.2% $NaHCO_3$, 0.1% BSA, 61 mg /L penicillin, 100 mg /L streptomycin solutions, pH 7.5 (medium A), chilled at 4˚C. Subsequently, hepatocytes were diluted to a final concentration of 10^6 cells per 1 mL; then the reaction was stopped by chilling to 4˚C. Chloroform and methanol as a mixture (1:2, v/v) was used as a medium for lipids extraction as described in section extraction of lipids section.

Extraction of lipids

Lipids from the homogenated tissue and isolated hepatocytes were extracted using mixture of chloroform: methanol (1:2, v/v) with the aid of shaking as prescribed in the method of Bligh and Dyer (Bligh and Dyer, 1959). The resulting extract was centrifuged at 3000 rpm for 10 min. The upper phase was removed, and the fabric was further extracted with a mixture of chloroform: methanol: water (1:2:0.8, v/v) and then centrifuged at 3000 rpm for 10 min. The collected supernatants were combined and extracted again using the above mentioned mixture. Samples were centrifuged at 3000 rpm for 10 min. The upper phase was discarded, and the bottom layer was evaporated under vacuum at 37˚C till dryness. Dried precipitate was dissolved in chloroform: methanol mixture (2:1, v/v) and was used in thin layer chromatography (TLC).

Separation of lipids by thin layer chromatography (TLC)

Lipids were fractionated by thin-layer chromatography with the aid of silica gel plates on a commercial *Sorbfil* plates. DAG and FFA were partitioned in the developing solvent system consisting of *n*-hexane: diethyl ether: acetic acid (73:25:2, v/v). To separate SM, PC, PEA, and CER, two solvent systems were used namely; 1 - diethyl ether, 2 - chloroform: methanol: water (40:10:1, v/v) (Kates, 1975). For separation of glucosylceramide (GLC), the evaporated lipid extract was incubated for 60 min at 37˚C in medium consisting of chloroform: methanol (1:1, v/v) and containing NaOH (0.1 M) (hydrolysis acetyl glycerol). For separation of PS, chloroform: methanol: acetic acid: formic acid: water (35:15:6:2:1, v/v) was used as developing solvent. Lipids were extracted again according to the work reported by Bligh and Dyer (Bligh and Dyer, 1959), and used for the division into classes in a solvent system containing chloroform: ethyl acetate: isopropyl alcohol: methanol: 0.25% KCl. Spots of DAG, phospholipids and GLC were developed in iodine vapor, and the spots of SM were detected using 3% (w/v) solution of ninhydrin in *n*-butanol. The identifiaction of compounds has been achieved by comparing the R_f values with standards.

Elution of lipid fractions

Lipid spots were scraped from the chromatographic plates and transferred to centrifugation test tubes with the aid of 2 mL of chloroform: methanol: 14 N ammonium hydroxide solution (56:42:2, v/v). After that, tubes were incubated in a water bath at 55˚C for 15 min followed by centrifugation at 3000 rpm for 10 min. After that, the eluates were collected in tubes. This step was repeated twice.

Quantification of lipid fractions

Quantitative determination of the lipid contents was performed based on the method of March and Weinstein (March and Weinstein, 1966). 2 mL of concentrated H_2SO_4 was added to each test tube containing the eluate of interest and then evaporated and placed into blocks for combustion for 15 min at 200˚C. After cooling, 2 mL of water was added to each tube, and the content was stirred and cooled again. The absorbance of samples was measured at a wavelength of 375 nm on a spectrophotometer SF-26 (LOMO, Russia). For the quantitative determination of the content of CER, tissue stains lipids were transferred into tubes and then eluted with the aid of chloroform: methanol (1:1, v/v) followed by elution with methanol (Sathishkumar *et al*., 2005). After that, the eluates were evaporated in vacuum and then subjected to hydrolysis using 0.5 M HCl in methanol at 65˚C for 15 h. The mass of CER was determined by the release of long chain base in the hydrolysis of lipids by the method Trams and Lauter (Trams and Lauter, 1962).

Determination of protein content in liver tissue

Protein content in the samples was determined by the method of (Lowry *et al*., 1951).

Preparation of labeled [^{14}C] palmitic acid endogenous sphingolipids

Freshly isolated hepatocytes were resuspended (at a concentration of 10^8cells /mL) in Eagle's medium containing [^{14}C] palmitic acid (370 kBq /mL), 25 mM HERES, penicillin (61 mg /L), and streptomycin (100 mg /L) 10% fetal bovine serum and then incubated for 24 h in plastic petri dishes at 37˚C, pH 7.5. After incubation , it was washed twice with an excess of Krebs-Henseleit buffer containing 118 mM NaCl; 5 mM KCl; 1 mM KH_2PO_4; 1 mM $MgSO_4$; 2 mM $CaCl_2$; 0.2% $NaHCO_3$; 0.2% bovine serum albumin; 61 mg /L penicillin, and 100 mg /L streptomycin (pH 7.5; 4˚C), and then lysed. The extraction of lipids was carried out as described in extraction of lipids section. The chloroform extracts phase were evaporated and sphingolipids were separated by preparative chromatography. Elution of SM was performed as described in section elution of lipid fractions. Determination of radioactivity of SM was carried out with a BETA-1 counter radioactivity (Medpribor, Kiev). The extract of [^{14}C] SM was evaporated under vacuum before use. The obtained residue was dissolved in a mixture consisting of 1.5 mL of water and 1.5 mL of a solution containing 100 mM acetate buffer (pH 5.0), 2 mM EDTA and 1.75% Triton X-100 ; or 1.5 mL of a solution containing 100 mM Tris HCl (pH 7.35), 2 mM EDTA, 20 mM $MgCl_2$ and 1.75% Triton X-100.

Determination of metabolic sphingomyline (SM) liver homogenates

To determine the metabolism in the liver homogenate SM, labeled [^{14}C] palmitic acid SM was resuspended in a mixture of 50 mM acetate buffer (pH 5.0), 1 mM EDTA and 0.9% Triton X-100 or in buffer containing 50 mM Tris-HCl (pH 7.35), 1 mM EDTA, 10 mM $MgCl_2$, and 0.9% Triton X-100. Samples were incubated at 37˚C for 60 min. The reaction was stopped by chilling to 4˚C. Lipids were extracted using chloroform: methanol mixture (1:2, v/v) (Bligh and Dyer, 1959). The extracts were evaporated under vacuum and then acylglycerol hydrolyzed (Trams and Lauter, 1962). Sphingolipids were fractionated as described earlier in extraction of lipids section. The activity of the acid and neutral sphingomyelinase was judged by the formation of [^{14}C] CER content, not the destroyed [^{14}C]SM. Radioactivity in the samples containing the labeled [^{14}C] lipids was determined by the radioactivity meter.

Statistical data processing

In the study of age-related nature of the content and lipid metabolism in rat tissues, nonparametric methods (Mann-Whitney and Kruskal-Wallis) was used to compare the two groups using the parametric Student's *t*-test. For multiple comparisons, the analysis of variance (ANOVA, Turkey criterion and Fisher LSD - test) was conducted. Differences between groups were considered statistically significant at $p \leq 0.05$.

RESULTS

It is known that there are numerous ways that DAG and CER are generated in cell (Giusto *et al*., 2002; Kavok *et al*., 2003). The purpose of this experiment was to detect the different types of lipid metabolism of age rats DAG, CER, and PS synthesized in liver hippocampal, and cortical tissue of young and old rats. From the results it can be clearly seen that DAG levels significantly increased in 24 months old mice in comparison to 3 months old (Fig. 1). This was observed in liver, hippocampal, and cortical tissue.

Table 1. The contents of phosphatidylserine (PS) in the tissues of rats of different ages (M ± SD; n = 10)

Tissue type	Age of male wistar rats			
	3 months		24 months	
	(nmol /mg protein)	(mmol /g tissue)	(nmol /mg protein)	(mmol /g tissue)
Liver	11.7 ± 1.85	2.28 ± 0.40	16.2 ± 2.05	2.45 ± 0.42
Hippocampus	102.7 ± 6.13	5.42 ± 0.32	56.9 ± 2.26*	4.06 ± 0.71

*P<0.05 relative to 3-months-old animals

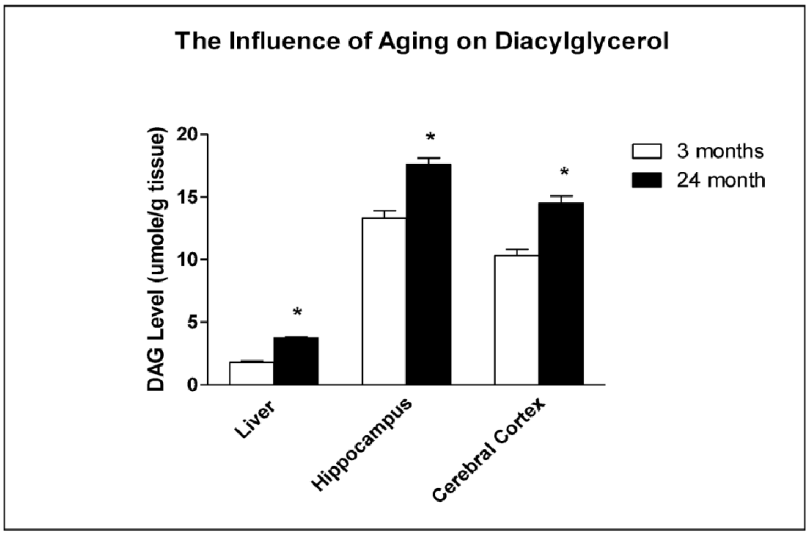

Figure 1. The difference in DAG levels between 3 and 24 months old micro DAG levels were estimated in liver, hippocampus and cerebral cortex. * Indicates significant difference where p<0.05

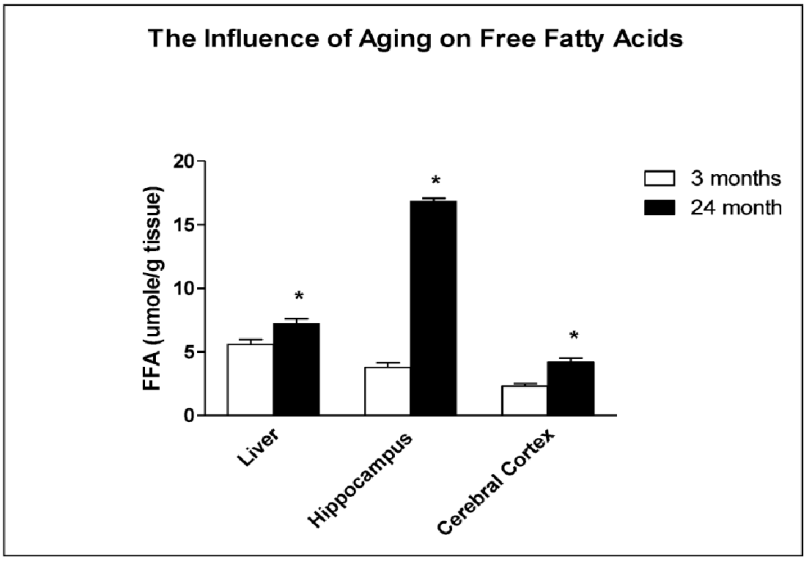

Figure 2. The difference in FFA levels between 3 and 24 months old mice. *Indicates significant difference where p<0.05

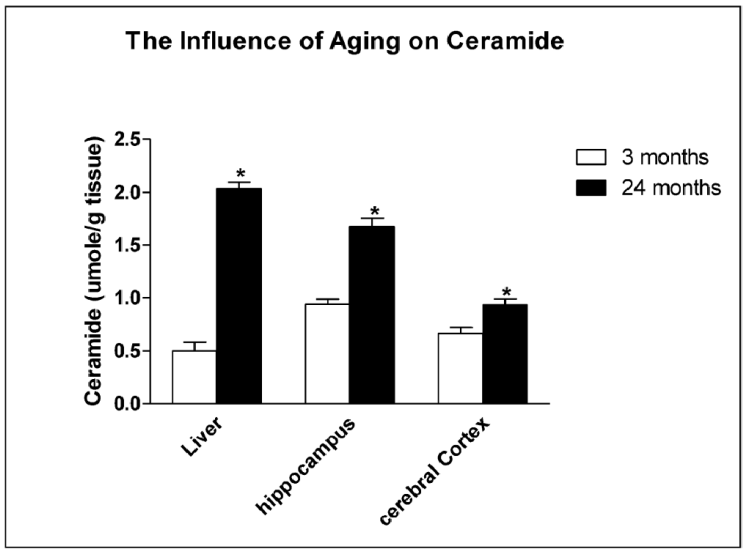

Figure 3. The difference in CER levels between 3 and 24 months old mice. CER levels were estimated in liver, hippocampus and cerebral cortex. *Indicates significant difference where p<0.05

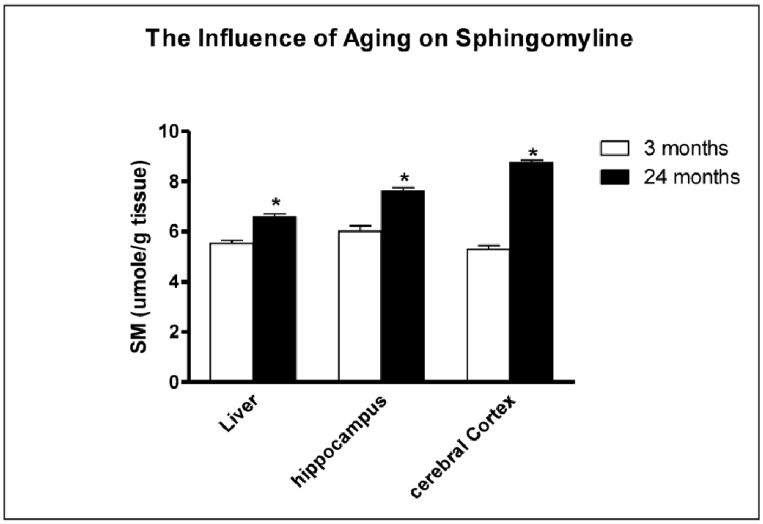

Figure 4. The difference in SM levels between 3 and 24 months old mice. SM levels were estimated in liver, hippocampus and cerebral cortex. *Indicates significant difference where p<0.05

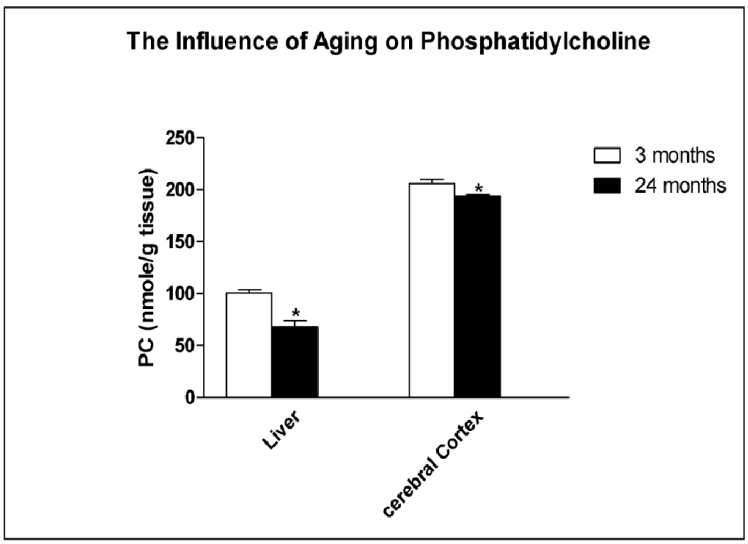

Figure 5. The difference in PC levels between 3 and 24 months old mice. PC levels were estimated in liver and cerebral cortex. *Indicates significant difference where p<0.05

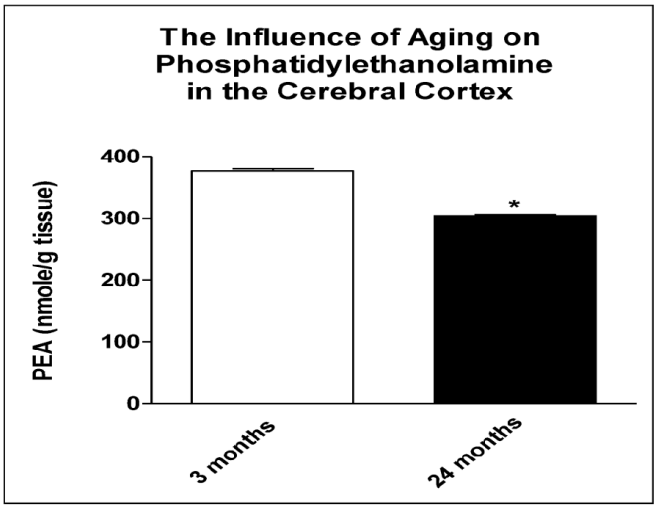

Figure 6. The difference in cerebral cortex PEA levels between 3 and 24 months old mice. *Indicates significant difference where p<0.05

This significant increase was also seen when measuring FFA, CER and SM levels (Fig. 2, 3, and 4), where the liver, hippocampus and cerebral cortex of 24 months old mice demonstrated a significant increase in these parameters when compared to 3 months old mice. Additionally, when studying the effect of age on tissue levels containing PC and PEA, it was revealed that PC levels underwent a significant reduction in 24 months old mice when compared to 3 months old (Fig. 5). Here, both liver and cerebral cortex showed a significant reduction in these levels. This age related decline was also seen with cortical PEA levels where 24 months old mice exhibited reduced levels of cerebral cortex PEA levels when compared to 3 months old mice (Fig. 6). The ratio of GLC: CER; decreased from 0.60 ± 0.09 in the liver of three-months-old mice to 0.38 ± 0.07 (p<0.05) in the liver of 24-months-old animals. At the same time, the level of PS is dramatically reduced in the hippocampus of old animals compared with adult rats, and the content of the PS changed little with age in liver (Table 1).

DISCUSSION

Increased fatty acids in liver, skeletal muscle and adipose tissue, correlates with the increased synthesis of DAG and increase in their mass (Schmitz-Pfeiffer, 2002). It is possible that increased content of fatty acids in the tissues of old rats compared with adult animals leads to an increase in the basal level of other signaling lipid – DAG. The content of the newly synthesized PC in the presence of [^{14}C] palmitic acid in the liver and brain of 24-months-old rats was 1.5 - 2 times lower than in three-months-old rats. We can consider that the increase of the DAG synthesis due to the increase *de novo* synthesis from FFA. Also, is due to the inhibition of its use in the synthesis of phospholipids (PC and PEA) it is an important reason of the increase of its content in the liver and brain of old rats. The reduction of PC can be attributed to the inhibition of SM synthase catalyzes which transfer of the phosphorylcholine from one molecule of PC to one molecule of CER. This leads to the formation of one molecule of SM and DAG (Satoi, *et al*., 2005). However, a chronic increase in fatty acids in cells causes not only DAG accumulation, but also a CER (Schmitz-Pfeiffer, 2002). Given, this may suggest that the increase in mass of fatty acids is important reason for the increase of the content of CER in the liver and studied parts of the brain in older rats, and also, for their change of the sphingolipids *de novo* synthesis begins with the condensation reaction palmityl-CoA and serine (Perry *et al*., 2000). Also, it might be due to the increase of degradation of complex sphingolipids, such as GLC. Moreover, GLC is converting to the CER during the reaction which is catalyzed by galactosylceramidase (Galceramidase) (Mielke *et al*., 2013). In the cerebral cortex, it was found that a simultaneous increase in the content of fatty acids, CER and SM (Fig. 2, 3 and 4). These data may indicate increased sphingolipids *de novo* synthesis in the cerebral cortex during aging. For instance, it was found that the intensity increases with age including [14C] serine to SM brain cells, which correlates with the increasing content of SM in neurons of the cerebral cortex (Giusto *et al*., 2002).

The results of this study in accordance with the results obtained in previous studies that showed the increase of the neutral membrane connected sphingomyelinase in the hippocampus and cerebral cortex of 22 months-old rats in comparison with 6 month-old animals serve support the hypothesis (Crivello *et al*., 2005; Murray *et al*., 2007). PS has the normal functioning of neurons in the brain. Thus, the induced amyloid beta peptide (Aβ1-40) death of cortical neurons and hippocampal preceded not only the accumulation of SM, but significant changes in metabolism of endogenous PS (Crivello *et al*., 2005). It is established that the content of the PS changes slightly with age in liver (Table 1). At the same time, the level of PS is dramatically reduced in the hippocampus of old animals compared with adult rats. Given this data, we can assume that the decline in PS in the hippocampus of aged animals is one of the reasons for increasing the sensitivity of its cells to oxidative stress and other adverse factors in old age. These data suggest that the accumulation occurred in tissues in the old aged animal is attributed to the FFA and SM. Otherwise, in the liver, the increase in the basal level of DAG, and in the brain structures of old animals – SM increased and PS reduced significantly. Accumulation of this neutral lipids and sphingolipids in liver

cells and various parts of the brain can lead to a violation of the sensitivity of these cells to regulatory incentives and hormones.

Conclusion

In summary, peculiarities of the terminated regulation of the metabolism of glycerol- and sphingolipids in target cells under conditions of the physiological aging have been studied. When comparing older animals to young ones, an increase of the level of FFA, DAG, CER, and SM takes place in the liver, hippocampus, and cerebral cortex. The increase of the content of DAG in certain tissues of old age animal takes place due to an increase in their *de novo* synthesis from FFA and to the decrease of their use in the synthesis of PC and PEA. In liver, hippocampus, and brain of 24-months-old rats in comparison with three-month-old animals, the increase level of CER in old rats is due to an increase in their sphingolipids *de novo* synthesis, which also accompanied by the increase of the product degradation with the participation of GLC. The data show the possible metabolic pathways or processes by which the DAG and CER accumulated in the target cells during aging. Knowing the mechanisms by which DAG and CER accumulated in aging tissues, we can use by the impact or effect of some physiological factors that regulate these two categories to avoid their accumulation in critical tissues, which is considered one of the most reasons or precursors for pathological diseases (Unger, 2002, 2003; Stratford *et al*., 2004; Cutler and Mattson, 2001; Satoi *et al*., 2005). In our future studies, consideration of the gene expression of enzymes involved in the metabolism and synthesis of DAG, CER (CER Synthase), SM, and PC, as the mice age, will be investigated. Global gene expression is considered a very powerful strategy for interpreting new and important issues in biology and other fields as well. Even several methods are available nowadays, but investigators must be cautious when using them, as many technical problems and experimental biases can influence the obtained results. As this field gets matures, it will lead to new horizons in understanding its role in regulating network that correlate gene expression with changing in lipid levels. Furthermore, these discoveries will certainly guide future human disease treatment.

Acknowledgements

This experimental work was supported by the research program "Approaches to overcome cell resistance to physiological stimuli in animals of different age" (State Registration No. 0106U001577, Kharkov Karazin National University, 4, Svobody pl., 61077 - Ukraine). At the same time, it was supported by the faculty of pharmacy at Isra University of Jordan.

Disclosures

None relevant to this study

Conflict of interest

There is no conflict of interest

REFERENCES

Abdel-Latif, A.A. 1986. Calcium-mobilizing receptors, polyphosphoinositides, and the generation of second messengers. *Pharmacolo.,* 38: 227 – 272.

Andreollo, N.A., Santos, E.F., Araújo, M.R., Lopes, L.R. 2012. Rat's age versus human's age: what is the relationship? *Arq. Bras.s Cir. Dig*., 25: 49 – 51.

Bell, R.M., Burns, D.J. 1991. Lipids activation of protein kinase C. J. Biol. Chem., 266: 4661 – 4664.

Bligh, E.G., Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.,* 37: 911 – 917.

Crivello, N.A., Rosenberg, I.H., Dallal, G.E., Bielinski, D., Joseph, J.A. 2005. Age-related changes in neutral sphingomyelin specific phospholipase C activity in striatum,hippocampus, and frontal cortex: implication for sensivity to stress and inflammation. *Neurochem. Int*., 47: 573 – 579.

Cutler, R., Mattson, M.P. 2001. Sphingomyelin and ceramide as regulators of development and lifespan. *Mech.Ageing Dev*., 122: 895 – 908.

David, K.P., Hannun, Y.A. 1988. The role of ceramide in cell signaling. Biochim. et Biophys. Acta., 1436: 233 – 243.

Eyster, K.M. 2007. The membrane and lipids as integral participants in signal transduction: lipid signal transduction for the non-lipid biochemist. *Adv. Physiol. Ed*., 31: 5 – 15.

Ghosh, S., Xie, W.Q., Quest, A.F., Mabrouk, G.M., Strum, J.C., Bell, R.M. 1994. The cysteine-rich region of Raf-1 contains zinc, translocates to liposomes and is adjacent to a segment that binds GTP-ras. *J. Biolo. Chem.,* 269: 10000 – 10007.

Giusto, N.M., Salvador, G.A., Castagnet, P.I., Pasquaré, S.J., Ilincheta, de Boschero MG. 2002. Age-associated changes in central nervous system glycerolipid composition and metabolism. *Neurochem. Res*., 27: 1513 – 1523.

Hannun, Y.A., Luberto, C., Argraves, K.M. 2001. Enzymes of sphingolipid metabolism: from modular to integrative signaling. *J. Biochem.,* 40: 4893 – 4903.

Hatch, G.M., Vance, D.E. 1992. Stimulation of sphingomyelin biosynthesis by brefeldin A and sphingomyelin breakdown by okadaic acid treatment of rat hepatocytes. *J. Biolo. Chem*., 267: 12443 – 12451.

Kanaeva, I.P., Kariakin, A.V., Alenicheva, T.V., Burmantova, N.P., Alimov, G.A. 1975. Respiration and oxidative-phosphorelation in isolated rat-liver cells. *Tsitologiya,* 17: 545 – 550.

Kates, M. 1975. Techniques of lipidology, North-Holland Pub. Co.

Kavok, N.S., Krasilnikova, O.A., Babenko, N.A. 2003. Increase in diacylglycerol production by liver and liver cell nuclei at old age. *Exp. Geront*., 38: 441 – 447.

Kehr, P., Weidner, A. 1987. Startbourg. Springer_Verlag/Wien, France.

Levi, D.M., Medina, J.N., DeRobertis, E.D. 1989. In vivo and in vitro modulation of central type of benzodiazepine receptors by phosphatidylserine. *Mol. Brain Res*., 5: 9 – 15.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem*., 193: 365 – 375.

Luberto, C., Hannun, Y.A. 1998. Sphingomyelin synthase, a potential regulator of intracellular levels of ceramide and diacylglycerol during SV40 transformation. Does sphingomyelin synthase account for the putative phosphatidylcholine-specific phospholipase C. *J. Biolo. Chem.,* 273: 14550 – 14559.

March, J.B., Weinstein, D.B. 1966. Simple charring method for determination of lipids. *J. Lipid Res*., 7: 574 – 580.

Mielke, M.M., Maetzler, W., Haughey, N.J., Bandaru, V.V., Savica, R., Deuschle, C., Gasser, T., Hauser, A.K., Gräber-Sultan, S., Schleicher, E., Berg, D., Liepelt-Scarfone, I. 2013. Plasma Ceramide and Glucosylceramide Metabolism Is Altered in Sporadic Parkinson's Disease and Associated with Cognitive Impairment: A Pilot Study. PLoS One 8: 1 – 6.

Mozzi, R., Buratta, S., Goracci, G. 2003. Metabolism and functions of phosphatidylserine in mammalian brain. *Neurochem*. *Res*., 28: 195 – 214.

Murray, I., Fried, K.E.B., Shermal, J. 2007. Determination of the phospholipid and sphingolipid content in the feces of uninfected balb/c mice and those infected with echinostoma caproni by high performance silica gel thin layer chromatography with densitometry. *Acta Chromatogr*., 18: 190 – 198.

Nishizuka, Y. 1992. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science*, 258: 607 – 614.

Pallav, S. 2013. The Laboratory Rat: Relating Its Age with Human's. *Int. J. Prev.Med*., 4: 624 – 630.

Patil, S., Melrose, J., Chan, C. 2007. Involvement of astroglial ceramide in palmitic acid-induced Alzheimer-like changes in primary neurons. *Eur. J. Neurosci*., 26: 2131 – 2141.

Perry, D.K.,, Carton, J., Shah, A.K., Meredith, F., Uhlinger, D.J., Hannun, Y.A. 2000. Serine Palmitoyltransferase Regulates de Novo Ceramide Generation during Etoposide-induced Apoptosis. *J. Biol. Chem*., 275: 9078 – 9084.

Ruth, E.B. 1935. Metamorphosis of the pubic symphysis. I. The white rat (Mus norvegicus albinus). *Anat. Rec*., 64: 1– 7.

Sathishkumar, S., Boyanovsky, B., Karakashian, A.A., Rozenova, K., Giltiay, N.V., Kudrimoti, M., Mohiuddin, M., Ahmed, M.M., Nikolova-Karakashian, M. 2005. Elevated sphingomyelinase activity and ceramide concentration in serum of patients undergoing high dose spatially fractionated radiation treatment: implications for endothelial apoptosis. *Cancer Biol. Ther*., 4: 979 – 986.

Satoi, H., Tomimoto, H., Ohtani, R., Kitano, T., Kondo, T., Watanabe, M., Oka, N., Akiguchi, I., Furuya, S., Hirabayashi, Y., Okazaki, T. 2005. Astroglial expression of ceramide in Alzheimers disease brains: role during neuronal apoptosis. *Neuroscience,* 130: 657 – 666.

Schmitz-Pfeiffer, C. 2002. Protein kinase C and lipid-induced insulin resistance in skeletal muscle. *Ann. NY. Acad. Sci*., 967: 146 – 157.

Stratford, S., Hoehn, K.L., Liu, F., Summers, S.A. 2004. Regulation of insulin action by ceramide: dual mechanisms linking ceramide accumulation to the inhibition of Akt/protein kinase B. *J. Biol. Chem*., 279: 36608 – 36615.

Trams, E.G., Lauter, C.J. 1962. On the isolation and characterization of gangliosides. Biochem. *et Biophys. Acta*, 3: 350 – 380.

Ullman, M.D., Radin, N.S. 1974The Enzymatic Formation of Sphingomyelin from Ceramide and Lecithin in Mouse Liver. *J. Biol. Chem*., 1974; 249: 1506 – 1512.

Unger, R.H. 2002. Lipotoxic diseases. *Annu. Rev. Med*., 53: 319 – 336.

Unger, R.H. 2003. Lipid overload and overflow: metabolic trauma and the metabolic syndrome. *Trends Endocrinol. Metab.,* 14: 398 – 403.

Wim, J., van Blitterswijk, W.J., Van der Luit, A.H., Veldman, R.J., Verheij, M., Borst, J. 2003. Ceramide: second messenger or modulator of membrane structure and dynamics? *J. Biochem*., 369: 199 – 211.

****