Journal of Advances in Biology & Biotechnology
2(1): 38-50, 2015; Article no.JABB.2015.006

SCIENCEDOMAIN *international*
www.sciencedomain.org

Cyclic Intermittent Fasting Influences the Structure of Hepatocyte Nuclear Membrane in Young and Old Rats

M. S. Girych[1*], N. I. Kurguzova[1] and A. I. Bozhkov[1]

[1]Department of Molecular Biology of Ontogenesis, Research Institute of Biology, V.N. KarazinKharkiv National University, Svobody Sq. 4, 61022, Kharkiv, Ukraine.

Authors' contributions

This work was carried out in collaboration between all authors. Author MSG performed the experiments, wrote the protocol, prepared graphics and wrote the first draft of the manuscript. Author NIK worked with animals. Author AIB planned the study, contributed the experiments and participated in the discussion and interpretation of the obtained results. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/JABB/2015/13771
Editor(s):
(1) Rafael A. Cañas, Department of Molecular Biology and Biochemistry, Málaga University, Spain.
Reviewers:
(1) Atef Mahmoud Mahmoud Attia, Biochemistry department, Biophysical laboratory, National Research Centre, Egypt.
(2) Ahmed E. Abdel Moneim, Zoology, Helwan University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=681&id=39&aid=6601

Original Research Article

Received 2nd September 2014
Accepted 20th September 2014
Published 23rd October 2014

ABSTRACT

Aims: The present study was undertaken to monitor the changes in structural and dynamic state of hepatocyte nuclear membrane of young (3-months) and old (20-months) rats subjected to intermittent fasting (IF).
Study Design: young (3-month) and old (20-month) rats were individually housed and randomly assigned to one of five groups (with 10 rats per group): (Control)-fed *Ad libitum*; (1IF)-provided access to a limited amount of food (4g/100g and 2g/100g of food/body weight for young and old rats, respectively) every other day for 10 days; (1R)-refeeded *Ad libitum* for 20 days after 1IF; (2IF)-provided the same regimen as for 1IF but after successive 1IF and 1R; (2R)-refeeded *Ad libitum* for 10 days after 2IF.
Methodology: The magnitude of fluidity changes was evaluated through measuring the excimer-to-monomer intensity ratio (E/M) in the pyrene emission spectra. The changes in membrane hydration were assessed using Laurdan generalized polarization (GP).
Results: During two cycles of intermittent fasting/refeeding statistically significant differences in E/M

**Corresponding author: E-mail: girichms@gmail.com*;

for young animals are observed only after the first refeeding (increase by 11% compared to control). 1IF and 1R in old animals were followed by ~26% and ~18% decrease in E/M value. The second cycle of dietary regimen brought about ~20% and ~36% decrease in E/M of old rats after 2IF and 1R, respectively. The first intermittent fasting resulted in ~74% and ~101% increase in GP value of young and old rats, respectively, and the refeeding period GP parameter underwent ~221% and ~89% increase in comparison with control for young and old rats, respectively. After the second IF following the first refeeding the young and old rats were characterized by ~200% and ~44% increase in GP, respectively. On the contrary, second refeeding leads to ~182% and ~27% increase in GP for young and old rats, respectively.

Conclusion: The main outcome reached is the identification of differences in the effects of re-applicated IF on nuclear membrane fluidity and hydration in animals of different age, suggesting that membrane responses to IF are governed by age-dependent mechanisms.

Keywords: Aging; nuclei membrane; membrane fluidity; membrane hydration; intermittent fasting.

1. INTRODUCTION

Caloric restriction (CR), reduction of daily energy intake by 15-40%, is currently regarded as one of the most powerful approaches to prevention and therapy of a variety of age-related and chronic diseases as well as represents the only universally recognized non-genetic way of increasing the average and maximum life-span of almost every eukaryotic organism tested [1]. However, the use of CR in mammals is associated with two serious problems. First, this dietary regimen is highly effective only when it starts from weaning [2]. Second, the first several weeks of CR are usually accompanied by death of significant part of experimental animals, thereby creating prerequisites for selection of individuals for medium and maximum life span [3]. Nowadays, accumulating evidence indicates that another dietary restriction paradigm, intermittent fasting (IF), in which 24h period of fasting alternates with 24h period of *ad libitum* feeding results in similar beneficial effects as classical daily CR [4]. IF approach is easier to use, its effective application can be performed during short time periods and the effects of this dietary regimen appear even in the case of old animals. Intriguingly, in comparison with CR, some protective effects of IF are even more pronounced [5]. It is believed that the mechanisms underlying the beneficial effects of both dietary regiments (CR and IF) are common, but information concerning IF is still scarce [6].

A number of studies support the idea that aging and development of age-related diseases is accompanied by an impairment of biological membrane functions, originating predominantly from the change in structural and dynamic state of lipid bilayer [7]. In turn, functional state of cell membranes is known to be largely controlled by physicochemical parameters (fluidity, hydrationdegree, acyl chain order etc.) of lipid bilayer, affecting a broad spectrum of biochemical processes implicating membrane-bound transporters, receptors, enzyme systems, etc. [8]. A plethora of studies provides indisputable evidence for strong influence of CR and IF on the structure and dynamics of membranes from different cell types, showing that these diets are capable of producing some age-retarding and even rejuvenating effects [9-10]. However, to the best of our knowledge, nothing is known about the effects of such dietary restrictions on hepatocyte nuclear membrane. It is becoming increasingly appreciated that the key metabolic and regulatory systems localized in hepatocyte nuclear membrane could be modulated by dynamic state of lipid bilayer. In particular, such kind of modulation is reported for nucleotide-triphosphatases which reside close to nuclear pores and regulate RNA efflux [11-12] and for the process of phosphorylation of nuclear components responsible for regulation of posttranscriptional modification and cell cycle [13]. Moreover, physical state of hepatocyte nuclear membrane controls the activity of steroid and thyroid hormone receptors [14-15]. In addition, it has been shown that membrane environment of hepatocyte nuclear envelope directly modulates insulin receptor kinetics, insulin binding affinity and capacity, and insulin receptor kinase activity [16]. In light of these facts and taking into account the enormous functional role of aforementioned hormones in metabolic regulation during dietary restrictions, it is very likely that at least some beneficial effects of IF may arise from the change in physical state of hepatocyte nuclear membrane. Therefore, the present study was undertaken to

monitor the changes in structural and dynamic state of hepatocyte nuclear membrane of young (3-months) and old (20-months) rats subjected to intermittent fasting. To this end, two nuclear membrane parameters, fluidity and hydration, were assessed using fluorescence spectroscopy technique with pyreneand Laurdan fluorescent probes. We were particularly interested in answering the question of whether IF with 10 days period of diet, followed by the 20 days period of *Ad libitum* feeding, could have a long-term effect on the estimated membrane. The final step of the study was dedicated to looking for the adaptation pathways to this dietary restriction, viz. the search for the differences between the effects of first and second cycles of IF/refeeding.

2. MATERIALS AND METHODS

2.1 Materials

HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), PMSF (phenylmethylsulfonyl fluoride), pyrene, and glycerol were purchased from Sigma-Aldrich (St. Louis, MO). Laurdan (6-Lauroyl-2-dimethylaminonaphthalene) was from Invitrogen Molecular Probes (Eugene, OR, USA). All other chemicals were of analytical grade and used without further purification.

2.2 Animals and Treatment

3-and 19-months male rodents were obtained from Wistar rat colony maintained at the Research Institute of Biology vivarium under light- and temperature-controlled conditions (12-h light/dark cycle (7:00-19:00), 22°C±2°C), 50%±10% relative humidity and free access to water. The rats of both age groups were individually housed and randomly assigned to one of the five groups (with 10 animals per group): (Control) animals on standard diet; (1IF) animals which were provided with a limited amount of food (4g/100g and 2g/100g of food/body weight for young and old rats, respectively) every other day for 10 days; (1R) animals which were refeeded *Ad libitum* for 20 days after 1IF; (2IF) animals which were provided the same regimen as for 1IF, but after the application of 1IF and 1R; (2R) animals which were refeeded *Ad libitum* for 10 days after 2IF.Feeding, body weight measurement and cage cleaning were performed daily at the same time. Under *Ad libitum* diet the animals were fed with a pelleted feed developed in our laboratory, its composition was as follows: 33.5% wheat, 28% barley, 11% corn, 7% dried milk, 5.5% sunflower seeds, 5.5% dried fish, 5% dried brewer's yeast, 2% alfalfa meal, 1% chalk, 0.5% egg powder, 0.5% dietary salt mix, 0.5% gelatin (3450 kcal/kg). The weight of animals from control groups were 240g±5g and 450g±5g for 3- and 19-months rats, respectively.

2.3 Nuclei Isolation

Nuclei from rat liver were isolated according to the procedure described by Graham [17]. Buffer A: Sucrose (250mM), KCl (25mM), MgCl2 (5mM), HEPES-NaOH (10mM), PMSF (1mM), pH=7.4. Buffer B: Sucrose (2.2mM, refractive index is 1.276±0.0005), KCl (25mM), MgCl2(10mM), HEPES-NaOH (10mM), PMSF (1mM), pH=7.4. All solutions and tubes were kept in ice and all operations were carried out at 0–4°C. After overnight starvation, the experimental animals have been sacrificed by cervical dislocation. The abdominal cavity was quickly opened and the liver was weighed and perfused with physiological solution. The liver was transported to the chilled handy press and extracted to the homogenizer containing 25ml of buffer B. The samples were homogenized by 7-8 tractions at 500-700rpm of the pestle of Potter-Elvenjem homogenizer (clearance approx. 0.09mm). After the filtration through a single layer of nylon gauze (pore size 75μm), the homogenate was transferred into a 30-ml centrifuge tube and twice centrifuged at 25,000g for 30min. After decantation of the supernatant, the nuclei-enriched pellet was separated from the nuclear debris by washing two times in buffer A with 1,500g centrifugation for 10min. The homogenization efficiency and purification quality of nuclei were checked by phase-contrast microscopy. The obtained nuclei were diluted with buffer A supplemented with 10% (v/v) glycerol and frozen in liquid nitrogen until use.

2.4 Fluorescence Measurments

The changes in hepatocyte nuclear membrane fluidity were assessed using the classical fluorescent probe pyrene [18]. The method is based on the correlation between membrane fluidity and the rate of pyrene excimeri sation under the conditions of constant temperature and invariable probe concentration. The value of excimer to monomer fluorescence intensities ratio (E/M) is directly proportional to the fluidity of the membrane environment of the probe and is

widely used to monitor physical state of different biological membranes [19].

The rate of membrane hydration were assessed using fluorescent probe Laurdan. In a lipid bilayer Laurdan is localized at lipid-water interface with the lauric acid tail anchored in the acyl chain region. Laurdan is sensitive to polarity, displaying a large red shift of the fluorescence emission when the polarity of the solvent is increased. It has been proposed that this process is related to the number and motional freedom of water molecules around the Laurdan fluorescent moiety [20]. This spectrum gives an immediate qualitative view of the probe environment characteristics.

The spectral changes of Laurdan fluorescence can be quantitatively characterized by the steady-state fluorescence parameter known as the generalized polarization (GP) [21]. This parameter rises with the decrease of membrane hydration. The generalized polarization (GP) of Laurdan fluorescence was determined as

$$GP = \frac{I_B - I_R}{I_B + I_R}$$

Where IB and IR are the maximum fluorescence intensities of the blue (440nm) and red (490nm) spectral components, respectively.

Hepatocyte nuclei were suspended in the media containing 0.25M sucrose, 10mM HEPES, 2mM MgCl2, pH 7.4 to give a protein content of ~1mg/mL. Pyrene and Laurdan were dissolved in ethanol and added to the membrane samples to a final probe concentration of 4μM. The ethanol concentration was less than 0.5%. This mixture was equilibrated for 30min at 37°C. Fluorescence measurements were performed at 37ºC with CM 2203 spectrometer (SOLAR, Belarus). Pyrene and Laurdan emission spectra were excited at 340nm and 360nm, respectively. Excitation and emission slit widths were set at 5nm. The excimer-to-monomer fluorescence intensity ratio (IE/IM) was determined by measuring fluorescence intensity at the monomer (389nm) and excimer (480nm) peaks.

2.5 Statistics

For data of each group, the mean and standard deviation of the mean were calculated and pairwised by multiple comparisons of the mean values performed using Scheffé's test in Origin Pro software (Origin Lab Corporation, Northampton, MA, USA). The differences between groups were considered to be statistically significant for $P<0.05$. The data in all fig. are presented as means±S.E.M.

3. RESULTS AND DISCUSSION

3.1 Rat Body and Liver Weight

As illustrated in Fig. 1, ten days of intermittent fasting (1IF) resulted in about 20% weight loss in young rats, while in old ones the changes of this parameter were not statistically significant. We found that under conditions employed in the present study such decrease is critical for young rats because if the weight falls below this level the mortality was too high (data not shown). After 20 days of *Ad libitum* refeeding (1R) the weight of animals in young age group returned back to prefasting control level and nearly the same changes were observed after 2IF and 2R periods. The first *Ad libitum* refeeding in old rats brought about 23% rise in body weight. The second cycle of intermittent fasting/refeeding was not characterized by any statistically significant difference as compared to control. The age-related discrepancies in body weight dynamics during the application of this dietary regimen, in our opinion, can be associated with a higher amount of fat energy reserve of old animals and differences in energy metabolism of young and old rats.

The liver is a central organ for carbohydrate and lipid metabolism and is responsible for the regulation of metabolic rate during dietary restrictions. Therefore, it was of interest to determine the dynamics of liver weight change during cyclic intermittent fasting-refeeding. As shown in Fig. 2, after 10 days of intermittent fasting the liver weight of young and old animals was markedly decreased (by 55% and 25% as compared to control, respectively). 20 days of *Ad libitum* refeeding in young rats lead to the restoration of this parameter to the level of control group, but the liver weight of the old animals was ~1.22-fold greater than control. In the case of young animals, the second application of intermittent fasting/refeeding (2IF/2R) produced the weight variations similar to those observed for 1IF/1R. At the same time, 2IF caused the weight of old rats to return to its initial level. Subsequent refeeding (2R) was followed by the statistically insignificant weight increase

(in comparison to control level) in the group of old rats.

3.2 Membrane Fluidity

Presented in Fig. 3 (A) are the typical fluorescence spectra of pyrene in hepatocyte nuclear membranes of young and old rats subjected to two cycles of intermittent fasting and refeeding.

Statistically significant differences in E/M for young animals are observed only after the first refeeding (increase by 11% compared to control), pointing to marked increase of hepatocyte nuclear membrane fluidity in this experimental group (Fig. 3 (B).

Several lines of evidence indicate that the dietary model of intermittent fasting/refeeding proposed by Strittmatted et al. [22] results in several fold rise in the activity of microsomal desaturases in rat liver [23].These enzymes play key role in biosynthesis of unsaturated fatty acids and the changes in their activity are directly correlated with the double bond amount in cell membranes [24]. The increased activity of desaturases may provoke the alterations in membrane physical state, viz. the elevated double bond level in phospholipid acyl chains would disturb lipid bilayer order producing the rise in membrane fluidity. Indeed, Stoch et al. showed that Strittmatter's diet leads to significant increase in the content of monoenoic (mainly 18:1) and polyenoic (mainly 20:4) acids coupled with the growth of membrane double bond index (the sum of the fraction of each fatty acid times the number of double bonds in that acid) and considerable lowering in the content of saturated fatty acids in plasma membranes of rat hepatocytes [10]. In turn, this is followed by the drastic increase in the plasma membrane fluidity and resulting changes in the activity of membrane enzymes but physiological role of these changes still remains poorly understood. At the same time in hepatocyte microsomal membranes, which are distinguished by the presence of desaturases, a different situation takes place: increase in the amount ofmonoenoic acids (16:1 and 18:1) is accompanied by the sharp drop in the level of polyenoic acids, thereby ensuring the invariance of the double bond index [25-26]. Likewise, no alterations in the dynamic state of hepatocyte microsomal membranes were observed for the rats subjected to intermittent fasting/refeeding [23]. On the other hand, significant decrease in cholesterol was reported for both plasma and microsomal membranes while employing such kind of dietary restriction [10,24]. This effect is thought to arise from consumption of acetyl coenzyme A in fatty acid synthesis and its lack in cholesterol biosynthesis, along with inhibiting effect of starvation on hydroxymethyl coenzyme A reductase, key enzyme of cholesterol production [27]. The fact that reduction of cholesterol level in microsomal membrane is not followed by the increase in membrane fluidity is explained by compensatory rise in the content of 16:0 fatty acids that does not occur in plasma membranes [10,23]. Based on the above rationales, it can be hypothesized that fluidity increase in hepatocyte nuclear membranes after the first cycle of intermittent fasting/refeeding (1IF/1R), stems from higher level of phospholipid acyl chain unsaturation and decrease cholesterol-to-phospholipid ratio. Interestingly, no statistically significant fluidity changes relative to control were found after the second cycle of intermittent fasting/refeeding (2IF/2R). This led us to suppose that the absence of fluidity shifts after repeated application of the above dietary restriction can be the considered as manifestation of adaptive processes. This notion is in harmony with the idea that the change in membrane fluidity and concomitant impairment of membrane functions brought about by various dietary restrictions is not functionally reasonable and is likely to be side effect of the drastic change in lipid metabolism [10]. It is also reported that increase of membrane fluidity is associated with a variety of pathological states [28] and underlies side effects of many pharmaceuticals [29]. In these regard, the absence of perturbation in the hepatocyte nuclear membrane after repeated application of dietary restriction can be considered as one of the beneficial effects of cyclic intermittent fasting/refeeding. On the one hand, this may be a consequence of "homeoviscous adaptation", i.e. the restoration of membrane fluidity following perturbation and, on the other hand, more rational functioning of lipid metabolism [8]. Notably, the reduction in the magnitude of changes with every subsequent application of dietary restrictions is characteristic of a range of biochemical parameters [30].

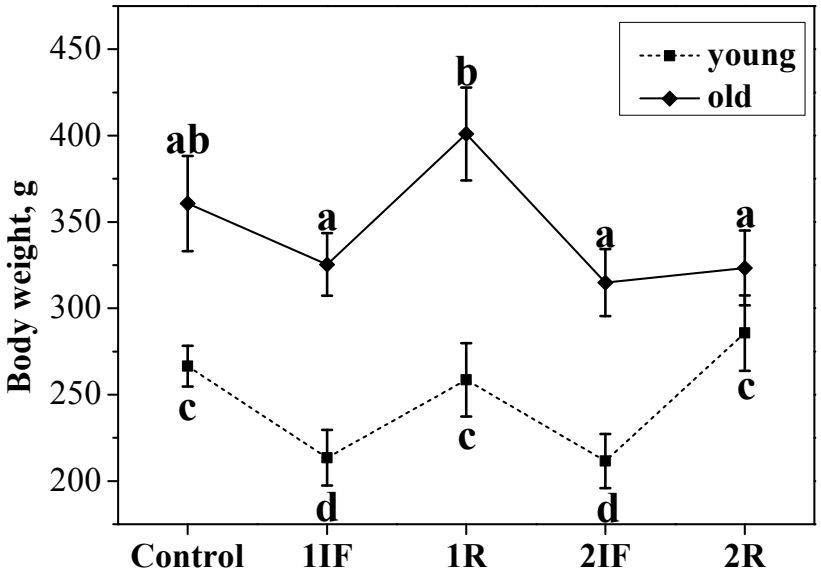

Fig. 1. Body weight of 3-and 19-months (young and old) rats during the course of two cycles of intermittent fasting/refeeding. Data are means±SEM for 10 rats *per* group. Control: rats fed with standard chow; 1IF: rats after intermittent fasting for 10 days; 1R: refeeded *Ad libitum* for 20 days after 1IF; 2IF: the same as 1IF but after 1R; 2R: refeeded *Ad libitum* for 10 days but after 2IF

Values that do not share the same superscript letter are significantly different from each other (P<0.05)

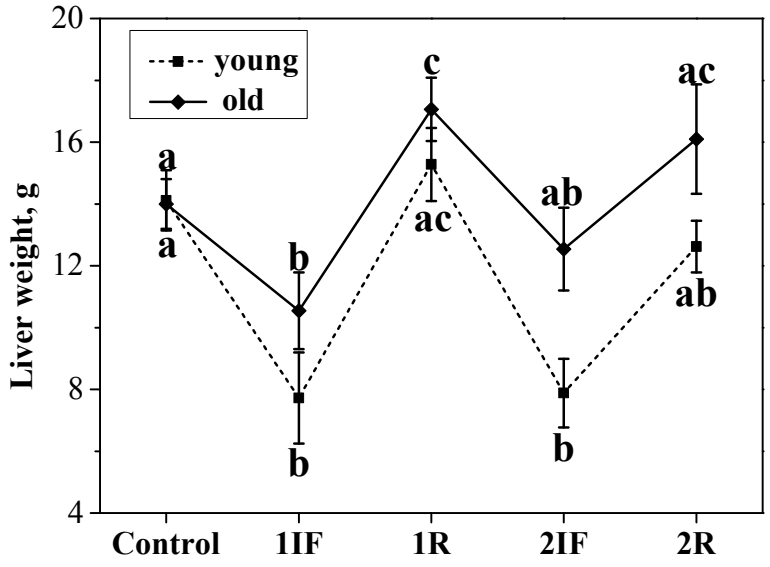

Fig. 2. Liver weight of 3- and 19-months (young and old) rats during the course of two cycles of intermittent fasting/refeeding. Data are means±SEM for 10 rats *per* group. Control: rats fed with standard chow; 1IF: rats after intermittent fasting for 10 days; 1R: refeeded *Ad libitum* for 20 days after 1IF; 2IF: the same as 1IF but after 1R; 2R: refeeded *Ad libitum* for 10 days but after 2IF

Values that do not share the same superscript letter are significantly different from each other (P<0.05)

In the group of old animals both periodic starvations evoked decrease of E/M value relative to control (by 26% and 20%, respectively) (Fig. 4). After the first refeeding *Ad libitum* (1R) E/M value of old animals displayed statistically significant increase (~11%) as compared to the group subjected to first intermittent fasting suggesting the rise of nuclear membrane fluidity upon refeeding (Fig. 4).

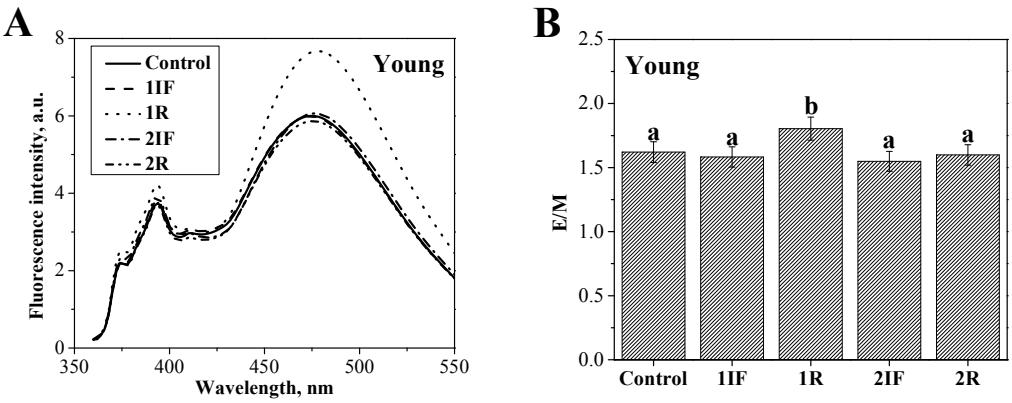

Fig. 3(A). Representative pyrene fluorescence spectra in hepatocyte nuclear membranes of 3-months (young) rats during the course of two cycles of intermittent fasting/refeeding. (B) Effect of cyclic intermittent fasting/refeedingon pyrene excimer ization degree (E/M) in hepatocyte nuclear membrane of 3-months (young) rats. Data are means±SEM for 10 rats per group. Control: rats fed with standard chow; 1IF: rats after intermittent fasting for 10 days; 1R: refeeded *Ad libitum* for 20 days after 1IF; 2IF: the same as 1IF but after 1R; 2R: refeeded *Ad libitum* for 10 days but after 2IF

Bars that do not share the same superscript letter are significantly different from each other (P<0.05)

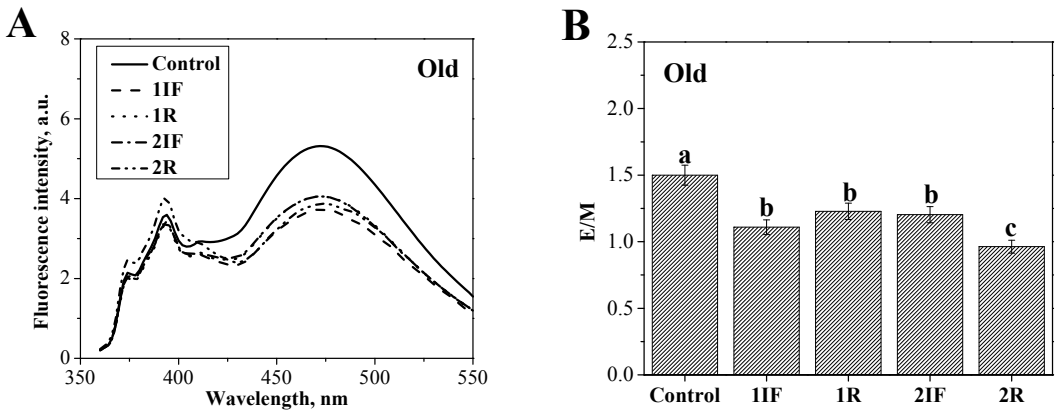

Fig. 4(A). Representative pyrene fluorescence spectra in hepatocyte nuclear membranes of 19-months (old) rats during the course of two cycles of intermittent fasting/refeeding. (B) Effect of cyclic intermittent fasting/refeeding on pyrene excimer ization degree (E/M) in hepatocyte nuclear membrane of 19-months (old) rats. Data are means±SEM for 10 rats per group. Control: rats fed with standard chow; 1IF: rats after intermittent fasting for 10 days; 1R: refeeded *Ad libitum* for 20 days after 1IF; 2IF: the same as 1IF but after 1R; 2R: refeeded *Ad libitum* for 10 days but after 2IF

Bars that do not share the same superscript letter are significantly different from each other (P<0.05)

It is known that peroxide ability index of plasma, mitochondrial and microsomal membranes of rat liver hepatocytes increases with age, along with reduction in the efficiency of cellular enzymatic antioxidant processes [31-32]. This hinders hepatocyte ability to maintain prooxidant-antioxidant balance. [33]. Furthermore, dietary restrictions have been reported to cause enhanced generation of reactive oxygen species, mainly hydrogen peroxide in rat liver [34]. The origin of this effect is supposed to lie in the increased activity of H2O2-producing oxidases in peroxisomes due to enhanced influx of fatty acids [35]. Membrane rigidification resulting from oxidative modification of lipids was attributed to both (i) Structural derangement of polyunsaturated phospholipid acyl chains and (ii) Cross-link formation induced by lipid peroxidation products, mainly by 4-hydroxynanonal [36]. In addition, an enzyme such as phospholipase A2 implicated in utilization of oxidized lipids through hydrolysis and substitution of oxidatively damaged unsaturated phospholipid acyl chains (a.k.a. retailoring) can also contribute to the rise in membrane rigidity [37]. Nuclear phospholipase A2 is distinguished by elevated activity and similar specificity for a broad spectrum of phospholipids [38]. Moreover, the activity of this enzyme was reported to increase upon reduction of membrane fluidity [39]. Intensification of phospholipase A2 activity under malnutrition conditions leading to deficiency in essential fatty acids would result in a sharp decrease of membrane double bound index and, as a consequence, to significant reduction of lipid bilayer fluidity [8]. It seems logical to suppose that this effect would be much more pronounced in old animals whose desaturase activity is significantly lowered [24]. It is worth noting that since starvation gives rise to decreased cholesterol/phospholipid ratio in rat hepatocyte membrane the involvement of this factor in membrane rigidification upon periodic starvation is hardly probable [10,23]. Both young and old animals showed increase of membrane fluidity after the first cycle of intermittent fasting/refeeding. However, in old rats after the first cycle of this dietary regimen membrane fluidity was lower relative to control. This finding can be explained by superposition of the membrane effects of lipid peroxidation and decrease of desaturase activity with age [24,32]. Additional support to this idea comes from the observation that the content of TBA-active products, the main indicator of oxidative membrane damage, was the highest in the group of old rats after the first refeeding (data not shown). Importantly, the lowest E/M value, i.e. the strongest decrease of nuclear membrane fluidity, was observed for old animals after the second refeeding. Such response to repeated application of intermittent fasting/refeeding may originate from the absence of desaturase reaction (similar to the case of young animals) against a background of pronounced membrane oxidative damage. However, molecular determinants of the differences in responses to repeated applications of intermittent fasting/refeeding are so far obscure and require further clarification. If these differences are determined by the change in desaturase activity that is regulated mainly at the level of gene expression, cyclic intermittent fasting/refeeding would appear suitable model for investigation of epigenetic regulation of lipid metabolism. Likewise, of great interest is tentative role of such changes in determining the beneficial effects of this dietary regime.

3.3 Membrane Hydration

To gain more detailed information about membrane structural perturbation upon cyclic intermittent fasting/refeeding pyrene fluorescence measurement were complemented by the analysis of Laurdan fluorescence spectra.

In both age group cyclic intermittent fasting/refeeding brought about significant modification of Laurdan spectral behavior, reflecting the changes in nuclear membrane hydration degree at the polar-nonpolar boundary (Fig. 5 (A), Fig. 6 (A).

In young animals GP parameter showed significant increase after the first periodic starvation (by 75%), reached its maximum after the first refeeding (by 224% relative to control), and displayed slight decrease during the second cycle of this dietary regimen (Fig. 5 (B).

The change in membrane hydration at the level of phospholipid glycerol backbone sensed by Laur dancan be induced by the rearrangement of lipid molecules that, in turn, may cause conformational alterations of membrane proteins [40]. To exemplify, it was found that activity of erythrocyte Na+, K+-ATP ase is directly correlated with GP value, being inversely proportional to membrane hydration [41]. It is generally recognized that hydration degree of biological membranes is controlled mainly by cholesterol/phospholipid ratio and the extent of

lipid oxidative damage [42-43]. The studies of Laurdan spectral behavior in the model and biological membranes showed that GP parameter is directly correlated with the content of cholesterol that is able to decrease membrane hydration at the level of glycerol backbone and increase acyl chain order [20,44]. Likewise, it is reported that oxidative damage of biological membranes results in GP increase [45-46]. For instance, Zhu et al. showed that increase in exposition time and H2O2 concentration induced drastic GP rise in the astrocyte plasma membrane [47]. Taking into account that the first refeeding resulted in the increase of membrane fluidity, while cholesterol/phospholipid ratio is expected to decrease during periodic starvation, we are prone to rule out the involvement of cholesterol in the observed GP increase. Hence, lipid peroxidation seem to play the dominating role in GP change. Notably, we found strict correlation between GP value and MDA content in hepatocyte nuclear membrane (to be published elsevere).

In the case of old animals the cyclic intermittent fasting/refeeding also leads to abrupt increase in GP parameter after the first cycle (by 100% and 84% relative to control for 1IF and 1R, respectively) (Fig. 6 (B)). During the second cycle of this dietary regimen GP value gradually approached the control level.

Numerous studies support the hypothesis that life-span-prolonging effects of the dietary restrictions are coupled with considerable increase in oxidative stress resistance [48]. Moreover, an analogous growth in stress resistance is seen in animals with life-span prolonging mutations [49]. The most commonly accepted theory explaining such stress resistance effect is that dietary restrictions cause activation of specific signal transduction pathways responsible for the induction of cellular defense mechanisms (so called “hormesis”) and, as a consequence, direct or indirect influence on life-span and health [50]. The observed restoration of membrane hydration to the level typical of control animals after the second cycle of intermittent fasting/refeeding is in keeping with the above-stated view. Thus, our findings provide additional arguments for relation between the beneficial effects of cyclic intermittent fasting/refeeding and the increase of resistance against disturbances in structural and dynamic state of hepatocyte nuclear membrane.

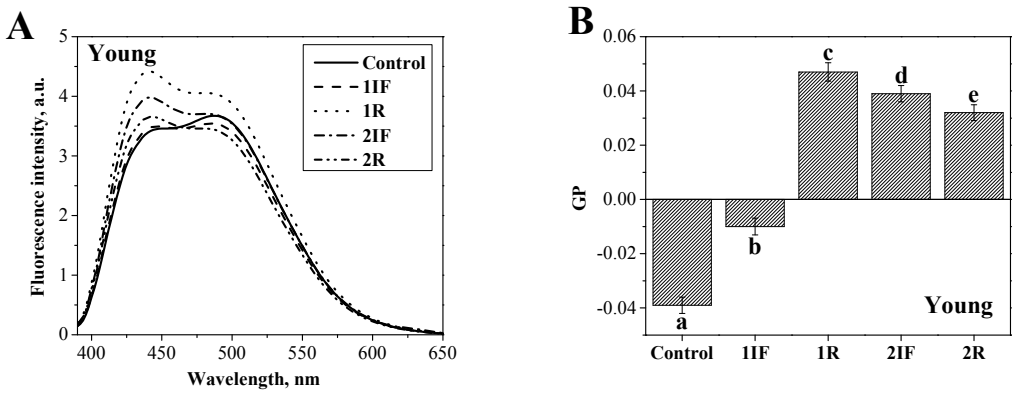

Fig. 5(A). Representative Laurdan fluorescence spectra in hepatocyte nuclear membranes of 3-months (young) rats during the course of two cycles of intermittent fasting/refeeding. (B) Effect of cyclic intermittent fasting/refeeding on generalized polarization of Laurdan in hepatocyte nuclear membrane of 3-months (young) rats. Data are means±SEM for 10 rats per group. Control: rats fed with standard chow; 1IF: rats after intermittent fasting for 10 days; 1R: refeeded *Ad libitum* for 20 days after 1IF; 2IF: the same as 1IF but after 1R; 2R: refeeded *Ad libitum* for 10 days but after 2IF

Bars that do not share the same superscript letter are significantly different from each other (P<0.05)

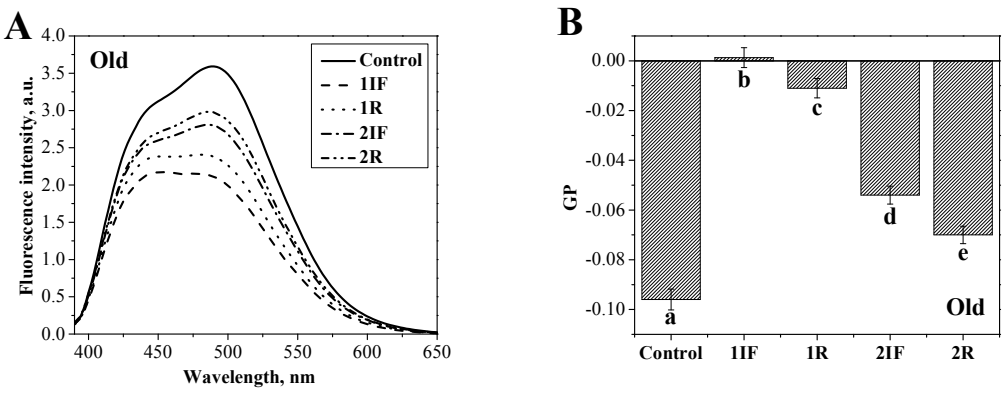

Fig. 6. (A) Representative Laurdan fluorescence spectra in hepatocyte nuclear membranes of 19-months (old) rats during the course of two cycles of intermittent fasting/refeeding. (B) Effect of cyclic intermittent fasting/refeeding on generalized polarization of Laurdan in hepatocyte nuclear membrane of 19-months (old) rats. Data are means±SEM for 10 rats per group. Control: rats fed with standard chow; 1IF: rats after intermittent fasting for 10 days; 1R: refeeded *Ad libitum* for 20 days after 1IF; 2IF: the same as 1IF but after 1R; 2R: refeeded *Ad libitum* for 10 days but after 2IF

Bars that do not share the same superscript letter are significantly different from each other (P<0.05)

4. CONCLUSION

To sum, the present study revealed that:

(i) In young animals both cycles of intermittent fasting/refeeding cause about 20% body weight loss and about 55% decrease of liver weight, whereas after periods of *Ad libitum* refeeding these parameters returned back to prefasting control levels. On the contrary, in the case of old animals the variations of body and liver weight in the course of the dietary regimen were much less pronounced and statistically significant changes were observed only for liver weight at the first cycle of intermittent fasting/refeeding.

(ii) Statistically significant differences in the fluidity of hepatocyte nuclear membrane for young animals are observed only after the first refeeding (E/M increase by 11% compared to control). In the group of old animals both periodic starvations evoked decrease of the fluidity relative to control (by 26% and 20%, respectively). After the first refeeding *Ad libitum* the nuclear membrane fluidity of old animals displayed statistically significant increase (E/M increase by ~11%) as compared to the group subjected to the first intermittent fasting. The strongest decrease of nuclear membrane fluidity was observed for old animals after the second refeeding.

(iii) In young animals membrane hydration showed significant decrease after the first intermittent fasting (GP increase by 75%), reached its minimum after the first refeeding (GP increase by 224% relative to control), and displayed slight increase during the second cycle of this dietary regimen. The cyclic intermittent fasting/refeeding in case of old animals also lead to abrupt decrease in membrane hydration after the first cycle (GP increase by 100% and 84% relative to control for 1IF and 1R, respectively), but during the second cycle this parameter gradually approached the control level.

These findings may prove of importance for gaining deeper insights into membrane-related processes contributing to beneficial effects of cyclic intermittent fasting/refeeding as well as for development of more effective calorie restriction strategies aimed at prevention and therapy of age-related diseases.

ETHICAL APPROVAL

All procedures followed the Ukrainian Cabinet Committee on Bioethics and Ukrainian Academy of Science guidelines for the use of animals in research.

ACKNOWLEDGEMENTS

The authors greatly indebted to Prof. Galyna Gorbenko for invaluable help and useful discussion.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Masoro EJ. Overview of caloric restriction and ageing. Mech Ageing Dev. 2005;9:913-922.
2. Weindruch R, Walford RL, Fligiel S, Guthrie D. The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake. J Nutr. 1986;116(4):641-54.
3. Sonneborn JS. The myth and reality of reversal of aging by hormesis. Ann N Y Acad Sci. 2005;1057:165-76.
4. Varady KA, Hellerstein MK. Alternate-day fasting and chronic disease prevention: a review of human and animal trials. Am J ClinNutr. 2007;86(1):7-13.
5. Anson RM, Jones B, de Cabod R. The diet restriction paradigm: a brief review of the effects of every-other-day feeding. Age. 2005;27(1):17-25. DOI: 10.1007/s11357-005-3286-2.
6. Everitt A, Rattan S, Couteur D. Calorie restriction, aging and longevity. Springer. 2010;16:500p.
7. Yu BP, Suescun EA, Yang SY. Effect of age-related lipid peroxidation on membrane fluidity and phospholipase A2: Modulation by dietary restriction. Mech Ageing Dev. 1992;65(1):17-33.
8. McMurchie EJ. Dietary lipids and the regulation of membrane fluidity and function, in Physiological Regulation of Membrane Fluidity. Aloia RC, Curtain CC, Gordon LM, Eds, Alan R. Liss, New York. 1988;189-237.
9. Laganiere S, Yu BP. Modulation of membrane phospholipid fatty acid composition by age and food restriction. Gerontology. 1993;39(1):7-18.
10. Storch J, Schachter D. Dietary induction of acyl chain desaturases alters the lipid composition and fluidity of rat hepatocyte plasma membranes. Biochemistry. 1984;23(6):1165-70.
11. Herlan G, Giese G, Wunderlich F. Influence of nuclear membrane lipid fluidity on nuclear RNA release. Exp Cell Res. 1979;118(2):305-9.
12. Agutter P. In Nuclear Envelope Structure and RNA Maturation. Alan R. Liss, New Yourk. 1982;561-578.
13. Smith CD, Wells WW. Phosphorylation of rat liver nuclear envelopes. II. Characterization of in vitro lipid phosphorylation. J Biol Chem. 1983;258(15):9368-73.
14. Venkatraman JT, Lefebvre YA. Multiple thyroid hormone binding sites on rat liver nuclear envelopes. BiochemBiophys Res Commun. 1985;132(1):35-41.
15. Venkatraman JT, Lefebvre YA, Clandinin MT. Diet fat alters the structure and function of the nuclear envelope: Modulation of membrane fatty acid composition, NTPase activity and binding of triiodothyronine. BiochemBiophys Res Commun. 1986;135(2):655-61.
16. Gletsu NA, Clandinin MT. Impact of dietary fatty acid composition on insulin action at the nucleus. Ann NY Acad Sci. 1997;827:188-99.
17. Graham JM. Preparation of Nuclei from Rat Liver using Sucrose // eLS–Published online; 2004.
18. Galla HJ, Sackmann E. Lateral diffusion in the hydrophobic region of membranes: use of pyrene excimers as optical probes. Biochim Biophys Acta. 1974;339(1):103-15.
19. Hashimoto M, Hossain S, Shimada T, Shido O. Docosahexaenoic acid-induced protective effect against impaired learning in amyloid beta-infused rats is associated with increased synaptosomal membrane fluidity. Clin Exp Pharmacol Physiol. 2006;33(10):934-9.
20. Parasassi T, Giusti AM, Raimondi M, Gratton E. Abrupt modifications of phospholipid bilayer properties at critical cholesterol concentrations. Biophys J. 1995;68(5):1895-902.
21. Parasassi T, Stasio GD, Ravagnan G, Rusch RM, Gratton E. Quantitation of lipid phases in phospholipid vesicles by the generalized polarization of Laurdan fluorescence. Biophys J. 1991;60(1):179–189.
22. Strittmatter P, Spatz L, Corcoran D, Rogers MJ, Setlow B, Redline R. Purification and properties of rat liver microsomal stearyl coenzyme A

desaturase. Proc Natl Acad Sci U S A. 1974;71(11):4565-9.
23. Pugh EL, Kates M, Szabo AG. Fluorescence polarization studies of rat liver microsomes with altered phospholipid desaturase activities. Can J Biochem. 1980;58(10):952-8.
24. Brenner RR. Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog Lipid Res. 1981;20:41-7.
25. Allmann DW, Hubbard DD, Gibson DM. Fatty acid synthesis during fat-free refeeding of starved rats. The Journal of Lipid Research. 1965;6:63-74.
26. Kates M, Pugh EL, Ferrante G. Regulation of Membrane Fluidity by Lipid Desaturases Biomembranes. 1984;19:379-95.
27. Bucher NL, Overath P, Lynen F. β-hydroxy-β-methylglutaryl coenzyme a reductase, cleavage and condensing enzymes in relation to cholesterol formation in rat liver. Biochim Biophys Acta. 1960;40:491–501.
28. Cooper RA. Abnormalities of cell-membrane fluidity in the pathogenesis of disease. N Engl J Med. 1977;297(7):371-7.
29. Goldstein DB. The effects of drugs on membrane fluidity. Annu Rev Pharmacol Toxicol. 1984;24:43-64.
30. Nishio H, Kuwabara H, Mori H, Suzuki K. Repeated fasting stress causes activation of mitogen-activated protein kinases (ERK/JNK) in rat liver. Hepatology. 2002;36(1):72-80.
31. Laganiere S, Yu BP. Anti-lipoperoxidation action of food restriction. Biochem Biophys Res Commun. 1987;145(3):1185-91.
32. Yu BP, Laganiere S, Kim JW. Influence of life-prolonging food restriction on membrane lipo peroxidation and antioxidant status. Basic Life Sci. 1988;49:1067-73.
33. Rikans LE, Hornbrook KR. Lipid peroxidation, antioxidant protection and aging. Biochim Biophys Acta. 1997;1362(2-3):116-27.
34. Marczuk-Krynicka D, Hryniewiecki T, Piatek J, Paluszak J. The effect of brief food withdrawal on the level of free radicals and other parameters of oxidative status in the liver. Med Sci Monit. 2003;9(3):BR131-5.
35. Orellana M, Fuentes O, Rosenbluth H, Lara M, Valdés E. Modulation of rat liver peroxisomal and microsomal fatty acid oxidation by starvation. FEBS Lett. 1992;310(2):193-6.
36. Chen JJ, Yu BP. Alterations in mitochondrial membrane fluidity by lipid peroxidation products. Free Radic Biol Med. 1994;17(5):411-8.
37. Stubbs CD, Smith AD. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. Biochim Biophys Acta. 1984;779(1):89-137.
38. Neitcheva T, Peeva D. Phospholipid composition, phospholipase A2 and sphingomyelinase activities in rat liver nuclear membrane and matrix. Int. J. Biochem. Cell Biol. 1995;27:995–1001.
39. Yu BP, Suescun EA, Yang SY. Effect of age-related lipid peroxidation on membrane fluidity and phospholipase A2: Modulation by dietary restriction. Mech Ageing Dev. 1992;65(1):17-33.
40. Jo E, Darabie AA, Han K, Tandon A, Fraser PE, McLaurin J. Alpha-Synuclein-synaptosomal membrane interactions: Implications for fibrillogenesis. Eur J Biochem. 2004;271(15):3180-9.
41. Faloia E, Garrapa GG, Martarelli D, Camilloni MA, Lucarelli G, Staffolani R, et al. Physicochemical and functional modifications induced by obesity on human erythrocyte membranes. Eur J Clin Invest. 1999;29(5):432-7.
42. Harris FM, Best KB, Bell JD. Use of laurdan fluorescence intensity and polarization to distinguish between changes in membrane fluidity and phospholipid order. Biochim Biophys Acta. 2002;1565(1):123-8.
43. Soto-Arriaza MA, Sotomayor CP, Lissi EA. Relationship between lipid peroxidation and rigidity in L-alpha-phosphatidylcholine-DPPC vesicles. J Colloid Interface Sci. 2008;323(1):70-4. DOI: 10.1016/j.jcis.2008.04.034.
44. Ambrosini A, Zolese G, Balercia G, Bertoli E, Arnaldi G, Mantero F. Laurdan fluorescence: A simple method to evaluate sperm plasma membrane alterations. FertilSteril. 2001;76(3):501-5.
45. Verstraeten SV, Oteiza PI. Effects of Al3+ and Related Metals on Membrane Phase State and Hydration: Correlation with Lipid Oxidation. Archives of Biochemistry and Biophysics. 2000;375:340-346.
46. Dousset N, Ferretti G, Galeazzi T, Taus M, Gouaze V, Berthon G, Curatola G. Effect of aluminium ions on liposomal

membranes as detected by Laurdan fluorescence. Free Radic Res. 1997;27(3):291-9.
47. Zhu D, Tan KS, Zhang X, Sun AY, Sun GY, Lee JC. Hydrogen peroxide alters membrane and cytoskeleton properties and increases intercellular connections in astrocytes. J Cell Sci. 2005;118(16):3695-703.
48. Qiu X, Brown K, Hirschey MD, Verdin E, Chen D. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. Cell Metab. 2010;12(6):662-7. DOI:10.1016/j.cmet.2010.11.015.
49. Holzenberger M, Dupont J, Ducos B, Leneuve P, Géloën A, Even PC. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. Nature. 2003;421(6919):182-7.
50. Ristow M, Schmeisser S. Extending life span by increasing oxidative stress. Free Radic Biol Med. 2011;51(2):327-36. DOI: 10.1016/j.freeradbiomed.2011.05.010.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=681&id=39&aid=6601