Original Research Article

Age-Dependent Effect of Cyclic Feeding Regimen on The Level of Serum Apolipoproteins in Young and Old Rats

ABSTRACT

Aims: the aim of this study was to investigate the influence of intermittent fasting (IF) on the content of blood serum apolipoproteins in young and old animals and to ascertain whether there exist adaptation mechanisms to this dietary regimen.

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): (AL) - fed *ad libitum*; (IF1) - 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; (R1) - refeeded *ad libitum* for 20 days after IF1; (IF2) - provided the same regimen as for IF1, but after successive IF1 and R1; (R2) - refeeded *ad libitum* for 10 days after IF2.

Methodology: plasma proteins were separated by one dimensional SDS-PAGE using a 7.5-15% gradient separating gel.

Results: IF1 with 30% weight loss resulted in ~28% and ~24% decrease and ~121% increase in serum apolipoprotein A-I (apoA-I), apolipoprotein B-100 (apoB-100), and apolipoprotein E (apoE) levels of young rats, respectively, and after R1 the level of these proteins was characterized by ~37%, ~66%, ~22% increase in comparison with control. IF1 and R1 in old animals were followed by ~53% increase in apoE content and ~13% decrease in the amount of apoB. IF2 was coupled with ~32% decrease in apoA-I level and ~133% increase in apoE concentration in young animals and ~39% and ~38% decrease in apoA-I and apoB-100 level in old animals, respectively. R2 produced ~48% and ~49% increase in apoA-I and apoE levels of young rats, respectively, and to ~31% increase in apoA-I content of old rats.

Conclusion: the main outcome reached is the identification of differences in the effects of the dietary regimen during reapplication on apoE and apoB-100 serum levels in old animals and on apoA-I level in the case of young animals.

Keywords: Cyclic Feeding Regimen, Intermittent Fasting, Apolipoprotein A-I, Apolipoprotein B-100, Apolipoprotein E

1. INTRODUCTION

The experimental proofs that restriction in dietary intake (DR) had beneficial effects on longevity, age-related diseases, cancer and adaptation to stress in rodents were obtained in the early 1900s by Rous, and Osborne *et al.* After the work of McCay *et al.* It has been postulated for more than a half of a century that the main factor in DR is only the limitation of energy consumed by animals [1-2]. This idea was in a very good accord with the free radical

theory of aging (FRTA). This theory states that every organism ages because of the accumulation of free radical damage which is mainly the result of reactive oxygen species (ROS) production during cellular respiration in mitochondria [3]. Hence, the restriction on calorie consumption should reduce ROS production and, as a consequence, delay aging and retard age-associated diseases. Nowadays, caloric restriction (CR), reduction of daily energy intake by 15-40%, is the only universally recognized non-genetic way of increasing the average and maximum life-span and health-span of almost every eukaryotic organism tested [4] as well as a very powerful approach to prevention and therapy of a wide variety of age-related and chronic diseases and cancer [5]. Another striking feature of CR is the ability to increase resistance to stress [2], immunological [6] and brain function [7]. However, over the past decades, a growing body of evidence suggests that the beneficial effects of CR are not simply the passive consequence of lower energy intake, but is the active response to food scarcity that is resulting in metabolic reprogramming with beneficial effects [4]. Nowadays, accumulating evidence indicates that another dietary restriction paradigm, intermittent fasting (IF), in which 24 h period of fasting alternates with 24 h period of ad libitum feeding results in the same beneficial effects as classical daily CR [8]. It is believed that the mechanisms underlying the beneficial effects of both dietary regiments (CR and IF) are common, but, intriguingly, there are numerous findings pointing to the differences between responses to CR and IF [2]. Accordingly, the comparison of the metabolic changes caused by CR and IF would be a unique way to elucidate the factors involved in health and life-span increase. Blood serum proteome is one of the best sources for the biomarkers of such changes because it reflects the myriad processes running in cells, tissues and organs [9]. Among a variety of blood proteins as potential candidates for such biomarkers, apolipoproteins take pride of place because their level undergoes substantial alterations under a variety of pathological conditions [10-11]. A plethora of CR studies provides indisputable evidence for the strong influence of this diet on apolipoproteins through alterations in gene expression and protein metabolism, but little is known about the effects of IF [12-14]. In view of this, the purpose of our study was to investigate the influence of IF on the content of blood serum apolipoproteins, in particular, apolipoprotein A-I (apoA-I), apolipoprotein B-100 (apoB-100) and apolipoprotein E (apoE). To do this, both young (3 month) and old (20 month) Wistar rats were subjected to intermittent dietary restriction where the fasting days alternate the days with the provision of a limited amount of food [15]. The second part of the study was aimed at answering the question of whether IF could have a long-term effect on the parameters estimated. To this end, the 10 days period of diet was followed by the 20 days period of *ad libitum* feeding. 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. MATERIAL AND METHODS

2.1 Materials

Ammonium persulfate, Trizma base, and glycerol were purchased from Sigma (St. Louis, MO). Acrylamide, BIS (N,N-Methylene-bis-acrylamide), and TEMED were from BIO-RAD (Richmond, CA). Glycine and bromophenol blue were from Merck (Darmstadt, Germany). Coomassie Brilliant Blue R-250 and G-250 were provided by DIA-M (Moscow, Russia). All other chemicals were of analytical grade and used without further purification.

2.2 Animals and Treatment

The 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℃ ± 2℃), 50% ± 10% relative humid ity and free access to water.

The animals were fed *ad libitum* with a pelleted feed developed in our laboratory (modified NIH-07 formula), 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). This dietary regimen continued until reaching 3 and 20 months of age for young and old age groups, respectively. The weight of animals from control groups were 240g ± 5g and 450g ± 5g for 3- and 19-months rats, respectively.

At that time, the rats of both age groups were individually housed and randomly assigned to one of the five groups (with 10 animals *per* group): (AL) fed *ad libitum*; (IF1) 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; (R1) refeeded *ad libitum* for 20 days after IF1; (IF2) provided the same regimen as for IF1, but after the application of IF1 and R1; (R2) refeeded *ad libitum* for 10 days after IF2. Feeding, body weight measurement and cage cleaning were performed daily at the same time.

2.3 Blood Sample Preparation and SDS-PAGE

After a 12 h period of fasting, rats of each group were sacrificed by decapitation between 7:00 and 9:00 am. The blood was collected and allowed to clot at 15℃ for 20 min. The serum was separated by centrifugation and the concentration of serum proteins was determined by the Bradford method [16]. All samples were handled at 4℃. To adjust the different protein concentrations in the samples, the serum was diluted with reducing Laemmli sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% (w/v) glycerol, 5% β-mercaptoethanol, 2% SDS,0.01% bromophenol blue) to the concentration of 20 mg/ml. The serum samples were heated at 95℃ for 2 minutes before being subjected to electrophoresis.

Plasma proteins were separated by one dimensional SDS-PAGE using a 4% stacking gel and a 7.5-15% gradient separating gel. SDS-PAGE analysis was carried out with the Tris/Glycine buffer system according to Laemmli [17] in a Gel electrophoresis apparatus "Model 91" (REANAL) at 4℃. The gels were stained with Coomassie Brilliant Blue R-250 (CBB R-250) as described by Wang et al. [18].

Images were acquired with an EOS 300D digital camera (Canon) and analyzed using GelAnalyzer (http://www.gelanalyzer.com/) and OriginPro 7.5 (OriginLab Corporation, Northampton, MA, USA). In order to correct for the differences in gel staining, relative optical density and relative volume were calculated and the proteins expressed in control animals were used as standard references for comparison (taken as 100%). After this normalization, the resulting band intensity volume percentage (relative content of protein) was used for comparison of different groups.

Identification of protein bands was based on their relative migration on SDS-PAGE and known molecular weights (28 kDa, 34 kDa, 512 kDa for apoA-I, apoE and apoB-100, respectively). All proteins studied migrate as a single band without any visible contamination by other polypeptides and with mobility similar to those reported elsewhere for these proteins [19-21].

2.4 Statistics

For proteins 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 OriginPro software (OriginLab Corporation, Northampton, MA, USA). The differences

between groups were considered to be statistically significant when P<0.05. The data in all figures are presented as means ± S.E.M.

3. RESULTS AND DISCUSSION

3.1. Rat weight

Ten days of intermittent fasting (IF1) resulted in ~ 30% weight loss in both young and old rats. We found that under conditions used in the present study such decrease is critical for young rats because if the weight falls below this level the mortality will be too high (unpublished data). The same weight loss during the period of IF1 was achieved by providing old rats with only half amount of calories on feeding day compared to young ones. This approach, in our opinion, made it possible to elude the potential artifacts associated with a higher amount of fat energy reserve and lower energy consumption of old animals. In other words, the diet hardness can be regarded as identical for old and young rats only when relative weight losses are similar for both ages. As illustrated in Fig.1., after 20 days of *ad libitum* refeeding (R1) the weight of the old animals returned back to prefasting control level (AL), but the weight of the young ones was ~ 1.4-fold greater than that of the control animals. In the case of old rats the second application of intermittent fasting (IF2) produced the weight variations similar to those observed for IF1. At the same time, IF2 caused the weight of young rats to return to its initial level (AL). Subsequent refeeding (R2) was followed by the weight increase in the group of old animals (up to ~ 90% relative to control level), while the weight of young rats reached the value close to that of R1 (1.4-fold larger relative to AL group).

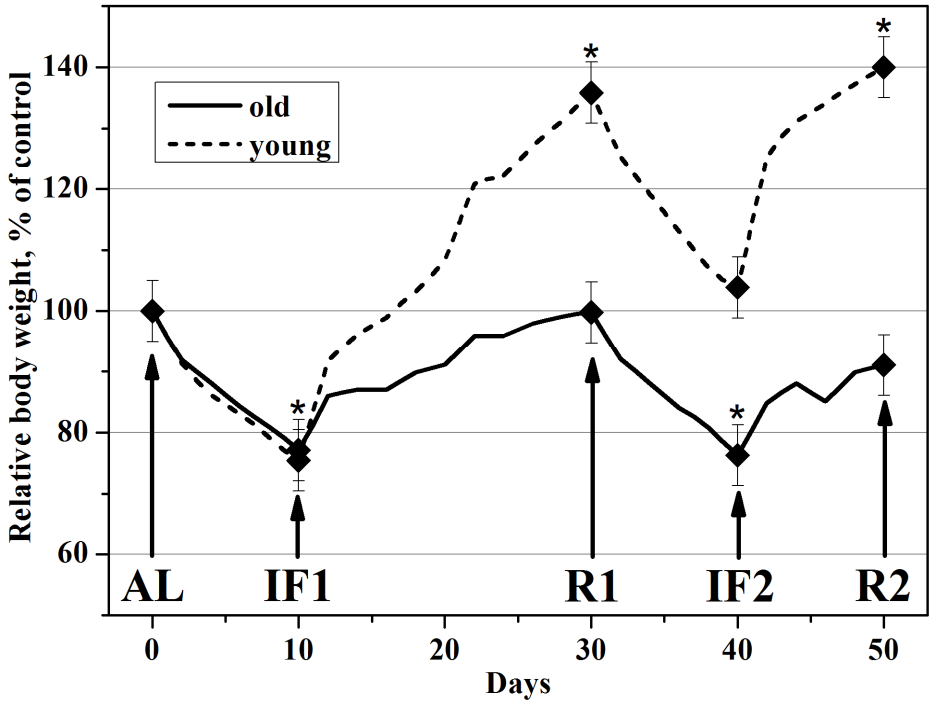

Fig.1. Body weight of rats during the course of two cycles of intermittent fasting/refeeding. Data are means ± SEM for 10 rats *per* group. AL: control rats fed *ad libitum*; IF1: rats after intermittent fasting for 10 days; R1: refeeded *ad libitum* for 20

days after IF1; IF2: the same as IF1 but after R1; R2: refeeded *ad libitum* for 10 days but after IF2. *Marked by asterisks are statistically significant differences relative to the control group, * P < .05*

3.2. Apolipoprotein A-I

As demonstrated in Fig. 2, the first intermittent fasting (IF1) brought about the decrease in apoA-I relative content (~28%) in the group of young rats. On the contrary, the first refeeding (R1) was accompanied by a sharp increase in this parameter (~37%) compared to control. Remarkably, neither IF1 nor R1 induced any significant changes in apoA-I level of old animals. In contrast, the apoA-I levels observed for the second cycle (IF2/R2) are featured by apoA-I changes typical for young rats (~32/39% decrease and ~48/31% increase for old and young groups, respectively) and the absence of any statistically significant difference between the age groups. The former observation suggests that the age-related difference in metabolic responses of young and old individuals to this dietary regimen is not stable and would be different upon repeated exposure.

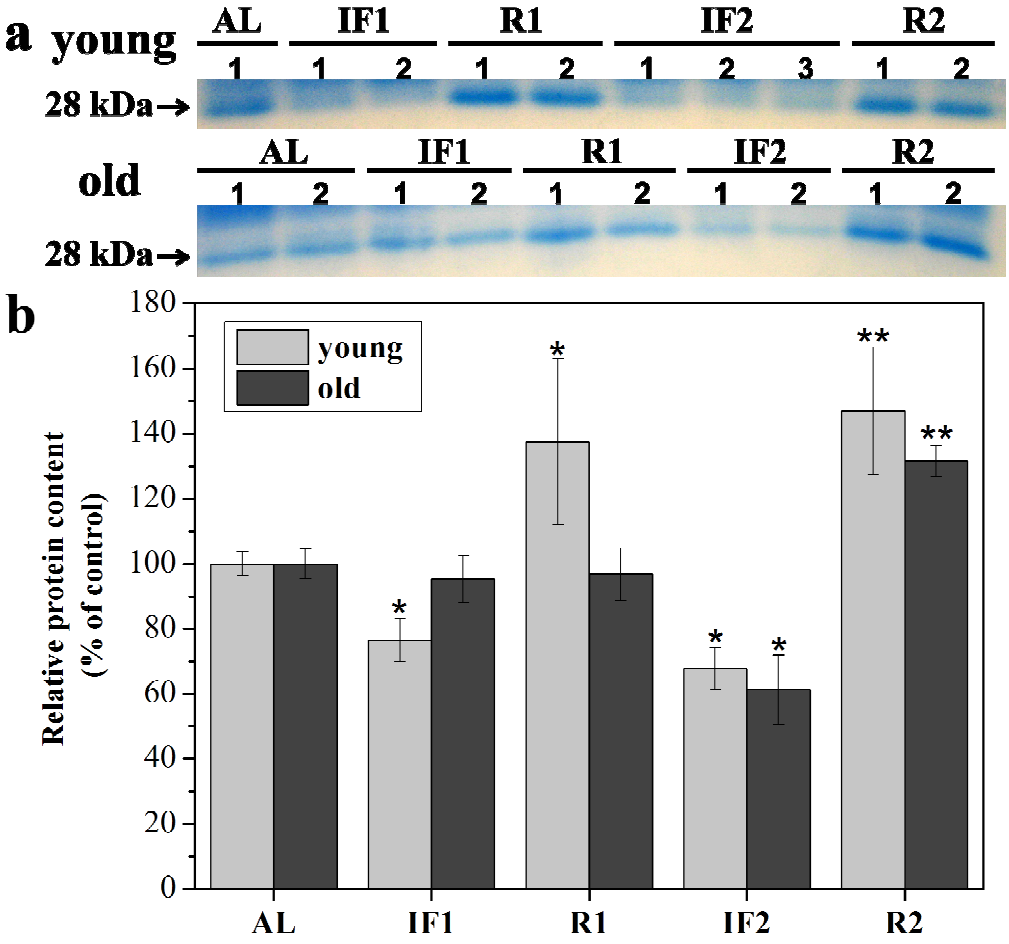

Fig.2. Effect of two cycles of intermittent fasting/refeeding on apoA-I serum content in young and old rats. (a) Representative CBB G-250 stained gel images of serum proteins of rats after different dietary regimens. Arrows indicate the position of apoA-I bands with molecular weight ~ 28 kDa. (b) Plasma levels of apoA-I identified on the

gels were assessed by image analysis. The amount of protein in bands was determined by calculating the average intensity using the GelAnalyzer software. Values presented are means ± SEM of 10 rats from each group. The mean of control group serves as 100%. AL: control rats fed *ad libitum*; IF1: rats after intermittent fasting for 10 days; R1: refeeded *ad libitum* for 20 days after IF1; IF2: the same as IF1 but after R1; R2: refeeded *ad libitum* for 10 but after IF2. *Marked by asterisks are statistically significant differences relative to the control group, * P < .05; ** P < .001*

ApoA-I is the major constituent of high density lipoproteins in plasma taking part in a variety of processes including cholesterol efflux, liver and neuron regeneration, lipid metabolism, etc [22]. This protein readily reacts on nutritional state and, as hypothesized, may be involved in the beneficial outcome of IF. It has been demonstrated for young (6 month) Wistar rats that classical CR (every day 40% calorie restriction) does not significantly change the serum level of apoA-I [23], although it causes 1.5 decrease of the protein content in the liver [14]. As for IF diet paradigm, to the best of our knowledge, there is only one work concerning this protein where the expression of apoA-I gene in liver of 6 month old rats showed no detectable change or 1.3-fold increase after feeding and fasting day, respectively [24]. In this work, however, only the first cycle of IF/refeeding in old rats fits the above observations: there were no significant changes in this parameter after IF1 and R1 relative to control. On the contrary, after all periods of IF in young animals and the second IF in old ones we observed pronounced decreases in apoA-I amount which reached nearly the same level relative to control. These results are corroborated by the studies of starvation where fasting for more than 48h was followed by a sharp decrease in apoA-I blood level by 50% after 3 or 4 days of starvation and by 75% after 6 or 7 days of starvation [25]. It has been shown that obesity-related decrease in apoA-I blood level is the result of down-regulation and increased clearance of this protein [10]. However, while considering the fact that even 24h starvation resulted in the increase in apoA-I mRNA level both in liver and intestine, the organs in which this protein is solely expressed, and that the ApoA-I gene is characterized by the most pronounced up-regulation among all genes in the liver during this process [26] we should dismiss the role of down-regulation. These facts strongly suggest the existence of some post-translational mechanisms that may be responsible for decreased apoA-I plasma levels in young rats as well as the activation of such mechanisms after the first IF1/R1 cycle in old ones. One may consider the process of protein degradation before secretion and/or the accelerated clearance/autophagy. Interestingly, when such fasting was followed by the refeeding, 1.5-fold rise in serum apoA-I was observed [27], which is similar to that obtained for R1/R2 of young and R2 of old animals. This observation allowed us to put forward an idea that the changes in apoA-I serum concentration in the case of starvation/refeeding and our model of IF/refeeding reflect similar mechanisms of adaptation to energy depletion and that the classical CR somehow falls beyond this paradigm. Obviously, IF has more auspicious effects than classical CR [2], as a consequence of the existence of such mechanism. Gaining further insights into these biological processes seems to be of great value for deeper understanding of IF beneficial effects.

3.3. Apolipoprotein B-100

As illustrated in Fig.3, apoB-100 level of young rats tends to decrease gradually with time after IF1, R1 and IF2 (for 13%, 18% and 38%, respectively), but during the period of R2 it was fully recovered to the control level. However, in old animals, while IF1 induced the decrease (24%) of serum protein level, R1 was coupled with a significant increase (22%). We did not observe any significant changes relative to control in apoB-100 upon the second cycle of IF/refeeding in old rats.

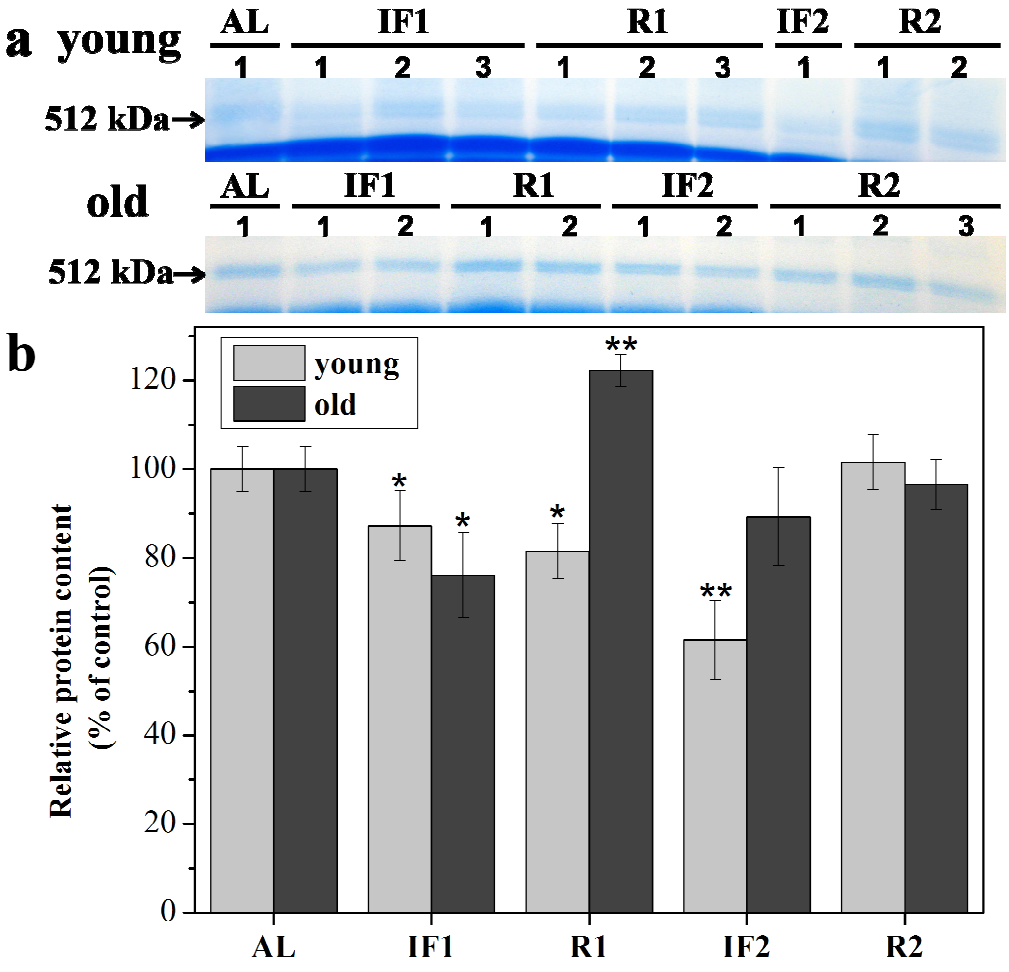

Fig.3. Effect of two cycles of intermittent fasting/refeeding on apoB-100 serum content in young and old rats. (a) Representative CBB G-250 stained gel images of serum proteins of rats after different dietary regimens. Arrows indicate the position of apoB-100 bands with molecular weight ~ 512 kDa. (b) Plasma levels of apoB-100 identified on gels were assessed by image analysis. The amount of protein in bands was determined by calculating the average intensity using the GelAnalyzer software. Presented values are mean ± SEM of 10 rats from each group. The mean of control group serves as 100%. AL: control rats fed *ad libitum*; IF1: rats after intermittent fasting for 10 days; R1: refeeded *ad libitum* for 20 days after IF1; IF2: the same as IF1 but after R1; R2: refeeded *ad libitum* for 10 days after IF2. *Marked by asterisks are statistically significant differences relative to the control group, * P < .05; ** P < .001*

ApoB-100 is the primary constituent of low-density lipoproteins and chylomicrons and the ligand for LDL receptors. Notably, there is one apoB-100 molecule per such particle, hence, the concentration of this protein exactly reflects the level of corresponding lipoproteins [22]. It has been demonstrated that both 48h-fasting and CR in rodents leads to more than 2-fold upregulation of apoB-100 expression in hepatocytes, while, paradoxically, several lines of evidence indicate that during such dietary regimens the blood level of this protein did not increase, but showed a noticeable decrease [28-29]. A vast majority of studies showed that the concentration of apoB-100 in blood, in contrast to the case of most secreted proteins, is regulated via degradation. Specifically, apoB-100 degradation is activated in response to the

lack of triglycerides (TG) and goes through the processes of ubiquitination, release from the endoplasmic reticulum and proteasomal degradation before the formation of VLDL [30]. Interestingly, if there is sufficient supply of TG, but the level of polyunsaturated fatty acids (PUFAs) is very high, one could observe another type of degradation – through the process of autophagy. This phenomenon is believed to be the result of misfolding with subsequent aggregation of apoB-100 during the assembly of VLDL particles where PUFAs plays role of misfolding agents [31]. Accordingly, the diets containing high amount of PUFAs may result in the decrease of apoB-100 content and, as a consequence, could be used to affect metabolism of TG and cholesterol. All these considerations led us to assume that the decrease in apoB-100 serum content after IF periods (IF1 and IF2) in both young and old rats observed in this work come from proteosomic degradation as a result of severe restriction in fat consumption. In turn, it seems highly probable that the rise in apoB-100 expression has been conserved during evolution as the mechanism of rapid replenishment of fat reserves. We believe that the long-term decrease of apoB-100 level in young rats arises from accelerated clearance of chylomicrons and LDL, whereas the sharp increase of the protein level in old animals originates from the suppression of such process. This suggestion is in line with the results of Field et al. [32], who observed more than 2-fold decrease in expression of LDL receptors (i.e. apoB-100 receptors) in peripheral tissues with age, leading to competition between the particles at apoB-100 receptor and concomitant increase of blood particle concentration and time of circulation. The situation with insufficient quantities of LDL receptors and high content of apoB-100 is one of the main cause of atherosclerosis and heart disease. Therefore, it could be assumed that the first cycle of IF in the case of old animals may be accompanied by high risk of the development of such pathologies. During the second cycle of IF/refeeding, after IF2 in young rats we observed further decrease of apoB-100 level (38% relative to control and 20% relative to R1) and after R2 the level of this protein was comparable to the control level. In contrast, IF2 in old animals leads to decrease of apoB-100 to the level of control animals (33%), but after the refeeding (R2) it remained unaffected. What is the reason for the difference in responses to the first and the second cycles? The fact that eventually apoB-100 level returns to normal level allows us to suggest that the observed difference is not a consequence of accidental coincidence. Most likely it is due to the process of adaptation to periods of energy/nutrient deficiency. The validity of this viewpoint is corroborated by the fact that alterations in some parameters in the liver could be suppressed during several consecutive fastings [33]. Knowing the mechanism of this process is not only of academic interest, but, as we can see from the beneficial effects on old animals, is a prerequisite for development of more effective treatment strategies for apoB-100 related diseases. Particularly, the refeeding of the second cycle is no longer conducive to the sharp rise in apoB-100, probably because of restoration of LDL receptor level typical for young animal with the decrease in risk of cardiovascular diseases. In addition, although decrease in apoB-100 blood content is thought to be beneficial, it can be followed by attenuation of antibacterial immunity [34]. In view of this possibility, the highly infectious disease incidence in mammals on calorie restriction [35] can be explained in terms of the above assumption.

3.4. Apolipoprotein E

As seen in Fig.4, dramatically different behavior was observed for apoE where IF1 and IF2 resulted in more than twofold increase in the serum apoE content of young animals (~121% and ~133%, respectively). Moreover, although the protein level after R1 and R2 was decreased relative to IF1 and IF2, it remained significantly larger relative to control (~66% and ~49%, respectively). The old animals demonstrated similar, but much less pronounced changes in apoE level and only in the case of the first cycle (~53% increase and ~24% decrease). Intriguingly, during the second cycle, we failed to detect any significant changes

relative to control in old rats, so that the repeat in response to such feeding model affected apoE in reverse manner with regard to the apoA-I.

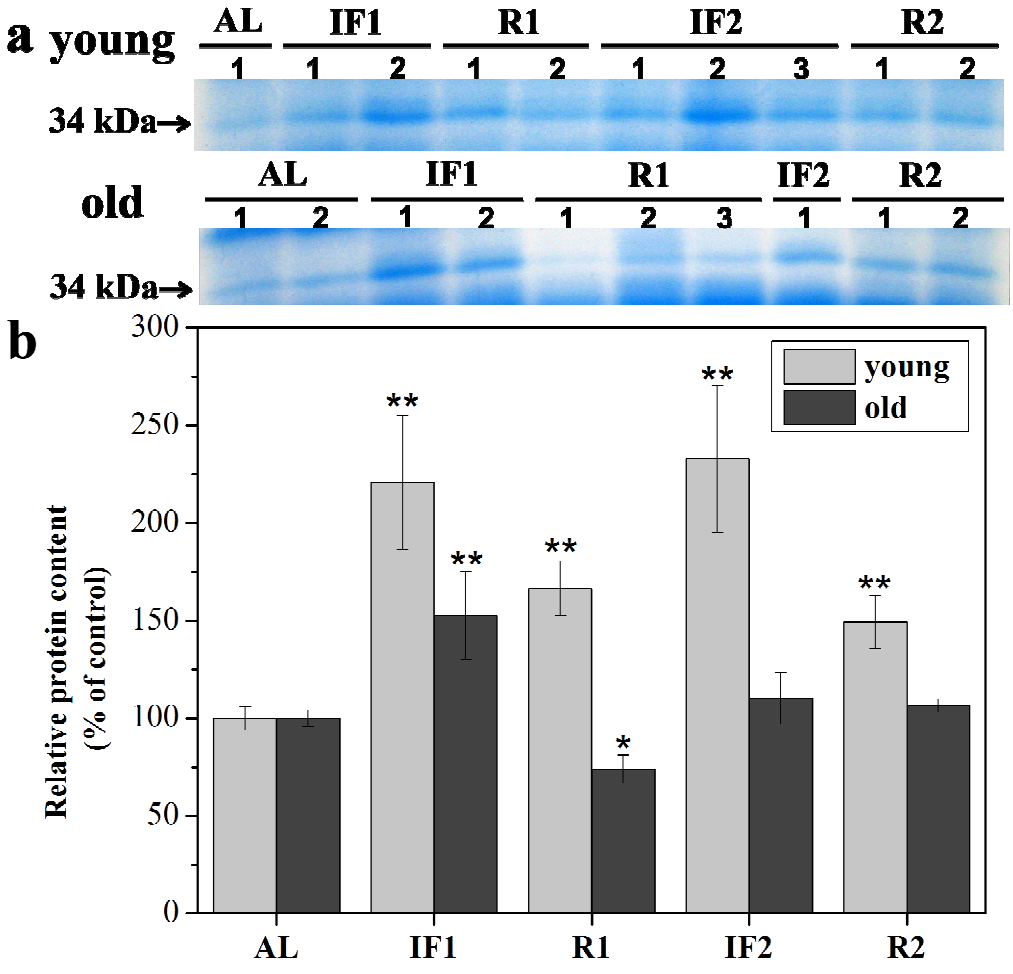

Fig.4. Effect of two cycles of intermittent fasting/refeeding on apoE serum content in young and old rats. (a) Representative CBB G-250 stained gel images of serum proteins of rats after different dietary regimens. Arrows indicate the position of apoE bands with molecular weight ~ 34 kDa. (b) Plasma levels of apoE identified on gels were assessed by image analysis. The amount of protein in bands was determined by calculating the average intensity using the GelAnalyzer software. Presented values are mean ± SEM of 10 rats from each group. The mean of control group serves as 100%. AL: control rats fed *ad libitum*; IF1: rats after intermittent fasting for 10 days; R1: refeeded *ad libitum* for 20 days after IF1; IF2: the same as IF1 but after R1; R2: refeeded ad libitum for 10 days after IF2. *Marked by asterisks are statistically significant differences relative to the control group, * P < .05; ** P < .001*

ApoE is vital for the normal metabolism of lipoproteins and chylomicrons and it serves as a transporter of cholesterol in central nervous system. ApoE-deficiency is the most widely used model of atherosclerosis and also it is conducive to age-dependent blood-brain barrier leakage [29]. Under normal physiological conditions blood pool of apoE is maintained by the liver and macrophages [36]. However, starvation and CR activate expression of this protein in adipocytes and intestine while in the liver it is constant even in the case of sublethal

fasting [25]. It is known that when expression of apoE in adipocytes is suppressed, they are not capable of producing TG and uptaking of VLDL, the property resulting in the inability of these cells to increase their fat reserve [37]. On the contrary, activation of apoE expression triggers TG storing, i.e. one could see the effect similar to apoB-100 [38]. But, in contrast to apoB-100, all periods of IF were followed by a pronounced rise of apoE level in all age groups (121%/53% after IF1 and 66%/37% after IF2 for young and old rats, respectively). It is established that increase in apoE expression in adipocytes is the mechanism of preservation of TG reserves from rapid depletion [39]. We hypothesize that this process, like in the situation with apoB-100, plays role in the mechanism of preparation to maximizing the uptake of lipids by adipocytes and enterocytes. This tentative mechanism is in good agreement with the data obtained by Poullain *et al.* where 72h fasting rats displayed 1.5-fold increase in apoE level [28]. It should be noted that in the experiments where the animals were subjected to CR for a long time (more than 4 weeks) 2.5-fold decrease of blood apoE was observed, emphasizing the reversibility of expression activation of this protein by energy restriction [13]. The less pronounced activation of apoE expression in the case of old animals may be the result of a trivial decrease of its expression with age [12]. In young animals the refeeding was coupled with the decrease in both cycles and the level of this protein remained heightened (~1.5-fold). The long-term effect of IF in young rats with such elevated level of apoE is in keeping with the lower risks of atherosclerosis and CVD [29]. Indeed, as evidenced from our results, the young rats tend to put on weight rapidly in contrast to the old ones. The level of apoE in old rats after R1 was lower than in control, but after R2 it was close to the level of the control and IF2 groups. ApoE non-responsiveness to the second cycle of IF/refeeding in old animals, similar to apoB-100, provides some grounds for believing that the mechanism underlying the regulation of the content of these proteins is common. Additional proof for this idea comes from the observation that both these proteins are critical for normal metabolism of chylomicrons and VLDLs [40].

4. CONCLUSION

Cumulatively, principal findings of the present study can be outlined as follows:

(i) IF for ten days with 30% weight loss results in the 1.3-fold decrease in serum apoA-I content in young, rather than in old rats and after the refeeding of at least 20 days the level of this protein in young rats undergoes 1.8-fold increase. The repeat of IF/refeeding leads to similar effect in the case of young rats, but old ones show the 1.4-fold increase in apoA-I suggesting that the first IF/refeeding cycle could somehow induce the metabolic response similar to that of young rats.

(ii) apoB-100 serum level in young rats is characterized by a gradual decrease with periods of IF1, R2 and IF2, but it is fully recovered to the control level after R2. On the contrary, IF1 and R1 in old animals are followed by 1.2-fold decrease and 1.4-increase in the content of this protein, respectively. However, after IF of the second cycle its amount reaches the control level and remains unaffected after second refeeding.

(iii) All periods of IF are followed by pronounced rise of apoE level in all age groups. The refeeding in young animals is coupled with the decrease in protein content in both cycles but the level remains invariant (~1.5-fold). The level of apoE in old rats after R1 is lower than that in control and after R2 it is about the level of the control and IF2 groups.

The main outcome of this work is the identification of differences in the effects of dietary regimens during reapplication on apoE and apoB-100 serum levels in old animals and on apoA-I level in the case of young animals. Gaining further insight into the mechanisms underlying such differences seems to shed light on age-related changes in the regulation of

processes in which the examined proteins take part. Furthermore, the existence of such changes should be taken into account during the development of diet therapies. For example, recent studies revealed that the low apoB100/apoA-I ratio is the best indicator of heart attack risk and in this work we observed very low value of such ratio in old animals only after repeated the cycle of IF/refeeding. Although the present results are acquired using the classical SDS-PAGE, they can serve as a starting point for further refinement of blood serum protein levels using more sophisticated approaches.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

ETHICAL APPROVAL

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

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