

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: (i) fed ad libitum (AL); (ii) 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 14 days (IF1); (iii) refeeded ad libitum for 14 days after F1 (R1); (iv) provided the same regimen as for F1, but after successive F1 and R1 (IF2); (v) refeeded ad libitum after F2.

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

Results: 1IF 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) has beneficial effects on longevity, age-related diseases, cancer and adaptation to stress in rodents were obtained in the early 1900s by Rous [1], and Osborne et al.[2]. One reasonable hypothesis to account for such effects was concerned with individual components of food. Numerous laboratory

experiments have been conducted in an attempt to substantiate this hypothesis, but after the work of McCay et al. it has been postulated for more than a half of century that the main factor in DR is only the limitation of energy consumed by animals [3,4]. This idea was in a very good accord with 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 [5]. Hence, the restriction in 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 median and maximum life-span and health-span of almost every eukaryotic organism tested [6] as well as a very powerful approach in prevention and therapy of a wide variety of age-related and chronic diseases (such as Alzheimer's or Parkinson's diseases, hypertension, diabetes, atherosclerosis, cardiomyopathy, autoimmune, cardiovascular, respiratory and kidney deceases, etc.) and cancer [7]. Another striking feature of CR is the ability to increase resistance to stress [4], immunological [8] and brain function [9]. However, over the past decades, a growing body of evidence suggests that the beneficial effects of CR is 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]. First, since the 2000s, with the creation of specific gene knockouts, numerous studies have shown no good correlation between the level of ROS production/scavenging and life span [10]. Second, progress in life science research methods provided vast information about CR influence on a number of metabolic and signaling pathways including sirtuins [11], the AMP-dependent kinase [12], the endocrine insulin/IGF-1 [13], the nutrient-responsive kinases target of rapamycin (TOR) [14], cyclic AMP-dependent kinase (PKA) [15], Sch9/AKT [16] pathways et al. Third, it is established that enhanced consumption of calories due to increased energy expenditure does not shorten life span [17]. Finally, 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 [18]. The main feature of such paradigm is that animals could gorge restricted calories so that the overall energy intake is not decreased. 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 that pointing the differences in response to them [4]. Accordingly, 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 [19]. Among a variety of blood proteins as potential candidates for such biomarkers, apolipoproteins take pride of place because of their property to reflect physiological state of an organism and to serve as markers of many diseases. A plethora of results from studies of CR provided indisputable evidence for existence of strong influence of this diet on apolipoproteins through alterations in gene expression and protein metabolism, but little is known concerning the effects of IF. Hence, 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 subjected to intermittent dietary restriction where the fasting days alternate the days with the provision of a limited amount of food [20]. The second part of the study was aimed at answering the question whether IF could have a long-term effects on the parameters estimated. In an attempt to approach this question, the 14 days period of diet was followed by the same 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 effect of the first cycle of IF/refeeding and the second one.

76 2. MATERIAL AND METHODS

77

78 2.1 Animals and Treatment

79 The male rodents were obtained from Wistar rat colony maintained at the Research institute
80 of Biology vivarium under light- and temperature-controlled conditions (12-h light/dark cycle
81 (7:00-19:00), 22°C ± 2°C), 50% ± 10% relative humidity and free access to water.

82 The animals were fed ad libitum with a pelleted feed developed in our laboratory (modified
83 NIH-07 formula), its composition as follows: 33.5% wheat, 28% barley, 11% corn, 7% dried
84 milk, 5.5% sunflower seeds, 5.5% dried fish, 5% dried brewer's yeast, 2% alfalfa meal, 1%
85 chalk, 0.5% egg powder, 0.5% dietary salt mix, 0.5% gelatin (3450 kcal/kg). This dietary
86 regimen was continued until reaching 3 and 20 month of age for young and old groups,
87 respectively.

88 At that time, both young and old rats were individually housed and randomly assigned to one
89 of the five groups: (i) fed ad libitum (control); (ii) provided access to a limited amount of food
90 (4g/100g and 2g/100g of food/body weight for young and old rats, respectively) every other
91 day for 14 days (F1); (iii) refeeded ad libitum for 14 days after F1 (R1); (iv) provided the
92 same regimen as for F1, but after successive F1 and R1 (F2); (v) refeeded ad libitum after
93 F2. Feeding, body weight measurement and cage cleaning were performed daily at the
94 same time.

95 2.2 Blood Sample Preparation

96 After a 12 h period of fasting, rats of every group were sacrificed by decapitation between
97 7:00 and 9:00 am. The blood was collected in a covered polypropylene tubes and allowed to
98 clot at 15°C for 20 min before centrifugation at 3000g for 10 min. The separated serum was
99 removed and the concentration of serum proteins was determined by the Bradford method
100 [21]. All samples were handled at 4°C. To adjust the different protein concentrations in the
101 samples, the serum was diluted with reducing Laemmli sample buffer (62.5 mM Tris-HCl, pH
102 6.8, 10% (w/v) glycerol, 5% β-mercaptoethanol, 2% SDS, 0.01% bromophenol blue) to the
103 concentration of 20 mg/ml and incubated for 30 min at 37°C for reduction of disulfide bonds
104 between peptide chains in the proteins. The serum samples were heated at 95°C for 2
105 minutes before being subjected to electrophoresis.

106 2.3. SDS-PAGE

107 Plasma proteins were separated by one dimensional SDS-PAGE using a 4% stacking gel
108 and a 7.5-15% gradient separating gel. Linear gradient gels were prepared by mixing 7.5%
109 and 15% acrylamide (10 ml of each) in a two-chambered gradient mixer according to the
110 recipe described in Table 1. The amount of protein loaded was 100 µg per well. SDS-PAGE
111 analysis was carried out with the Tris/Glycine buffer system according to Laemmli [22] in a
112 Gel electrophoresis apparatus "Model 91" (REANAL) at 4°C at the following conditions: 5
113 mA/gel for 2.5 h; 20 mA/gel until the end of the run.

114 After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250 (CBB R-250) as
115 described by Wang et al. [23]. Briefly, gels were fixed with 10% acetic acid, 10% methanol
116 and 40% ethanol for 1 h followed by sensitisation in 1% acetic acid and 10% ammonium
117 sulfate solution for 2 h. After that, they were stained with 5% acetic acid, 45% ethanol and
118 0.125% CBB R-250 for 4 h and washed overnight with 3% acetic acid and 30% ethanol.

Images were acquired with 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 volumes percentage (relative content of protein) was used for comparison of different groups.

Identification of protein bands were 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 [24-26]. Moreover, these bands are well separated from each other on 7.5-15% gradient SDS-PAGE under reducing conditions allowing to measure the relative protein content as described above.

Table 1. Components of separating gels (7.5-15% gradient SDS-PAGE, 10 ml of each)

Component	SDS-PAGE (15%)	SDS-PAGE (7.5%)
Urea (g)	2	-
60%:0.4% acrylamide:bisacrylamide (ml)	2.5	1.5
Tris (mg)	730	730
1N HCl (ml)	1	1
ddH ₂ O (ml)	6	7
N,N,N',N'-tetramethylethylenediamine (μl)	30	30
SDS (mg)	20	20
10% APS (μl)	150	300

2.4 Statistics

For proteins of each group, the mean and standard deviation of the mean were calculated and pairwise multiple comparisons of the mean values were 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 $\pm$ S.E.M.

142 3. RESULTS AND DISCUSSION

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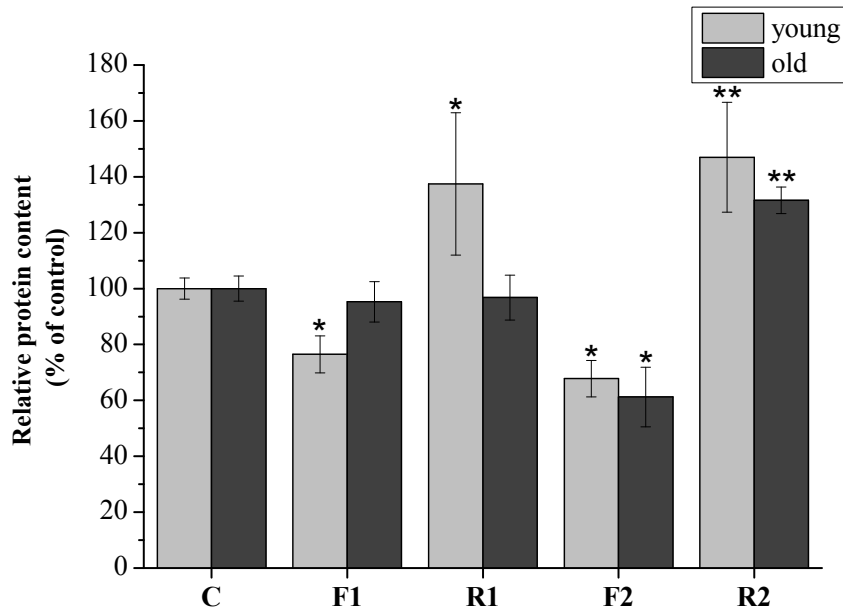
144 3.1. Rat weight

145 Ten days of intermittent fasting (F1) resulted in about 30% weight loss in both young and old
146 rats. We found that under conditions used in the present study such decrease is critical for
147 young rats because if the weight falls below this level the mortality will be too high
148 (unpublished data). The same weight loss during the period of F1 was achieved by the fact
149 that the old rats received only half amount of calories on feeding day in comparison with old
150 ones. This approach, in our opinion, made it possible to elude the potential artifacts
151 associated with a higher amount of fat energy reserve and lower energy consumption of old
152 animals. After 20 days of ad libitum refeeding (R1) the weights of all animals in all age
153 groups returned back to prefasting control levels (F0). The same changes were observed for
154 F2 and R2 periods. This fact gave us the opportunity to compare metabolic responses to the
155 first and the second cycles of intermittent fasting/refeeding considering that both cycles have
156 been subjected to similar impact.

157 3.2. Apolipoprotein A-I

158 As demonstrated in Fig. 1, the first intermittent fasting (F1) in young rats brought about
159 decrease in apoA-I relative content (~28%). On the contrary, the first refeeding (R1) was
160 accompanied by sharp increase in this parameter (~37%) compared to control. Remarkably,
161 neither F1 nor R1 induced any significant changes in apoA-I level of old animals, suggesting
162 that the protein expression/clearance remained virtually unchanged. In contrast, the apoA-I
163 levels observed for the second cycle (F2/R2) are featured by apoA-I changes typical for
164 young rats (~32/39% decrease and ~48/31% increase for old and young groups,
165 respectively) and the absence of any statistically significant difference between the age
166 groups. The former observation suggests that the age-related difference in metabolic
167 responses of young and old individuals to this dietary regimen is not stable and would be
168 different upon repeated exposure.

169



170

171 **Fig.1. Effect of two cycles of intermittent fasting/refeeding on apoA-I serum content in**
 172 **young and old rats. Presented values are Mean values $\pm$ Standard error of means of**
 173 **10 rats from each group. The mean of control group serves as 100%. C: control rats**
 174 **fed ad libitum; F1: after intermittent fasting for 14 days; R1: refeeded ad libitum for 14**
 175 **days after F1; F2: the same as F1 but after R1; R2: the same as R1 but after F2.**
 176 *Marked by asterisks are statistically significant differences relative to control group, * $P < .05$; ** $P <$*
 177 *.001*

178

179 apoA-I is the major constituent of high density lipoproteins in plasma and it takes part in a
 180 variety of processes including cholesterol efflux, liver and neuron regeneration, lipid
 181 metabolism, etc [34]. This protein readily reacts on nutritional state and, as hypothesized,
 182 may be involved in the beneficial outcome of IF. It has been demonstrated for young (6
 183 month) Wistar rats that classical CR (every day 40% calorie restriction) does not significantly
 184 change the serum level of apoA-I [27], although it causes 1.5 decrease of the protein content
 185 in the liver [28]. As for IF diet paradigm, to the best of our knowledge, there is only one work
 186 concerning this protein where the expression of apoA-I gene in liver of 6 month old rats
 187 showed no detectable change or 1.3-fold increase after feeding and fasting day, respectively
 188 [29]. In this work, however, only the first cycle of IF/refeeding in old rats fit the above
 189 observations: there were no significant changes in this parameter after F1 and R1 relative to
 190 control. On the contrary, after all periods of IF in young animals and the second IF in old
 191 ones we observed pronounced decreases which were about at the same level relative to
 192 control. These results are corroborated by the studies of starvation where fasting for more
 193 than 48h was followed by sharp decrease in apoA-I blood level by 50% after 3 or 4 days of
 194 starvation and by 75% after 6 or 7 days of starvation [30]. It has been shown [31] that
 195 obesity-related decrease in apoA-I blood level is the result of down-regulation and increased
 196 clearance. However, while considering the fact that even 24h starvation resulted in the
 197 increase in apoA-I mRNA level both in liver and intestine, the organs in which this protein is
 198 solely expressed, and that the ApoA-I gene is characterized by the most pronounced up-
 199 regulation among all genes in the liver during this process [32] we should dismiss the role of
 200 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 mechanism after the first F1/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 [33], 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. It is possible that IF has more beneficial effects than classical CR [4], 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.2, apoB-100 level of young rats tends to decrease gradually with time after F1, R1 and F2 (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 F1 induced the decrease (24%) of serum protein level, R1 was coupled with 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, indicating the age-related differences in metabolic answer of young and old individuals to this dietary restriction.

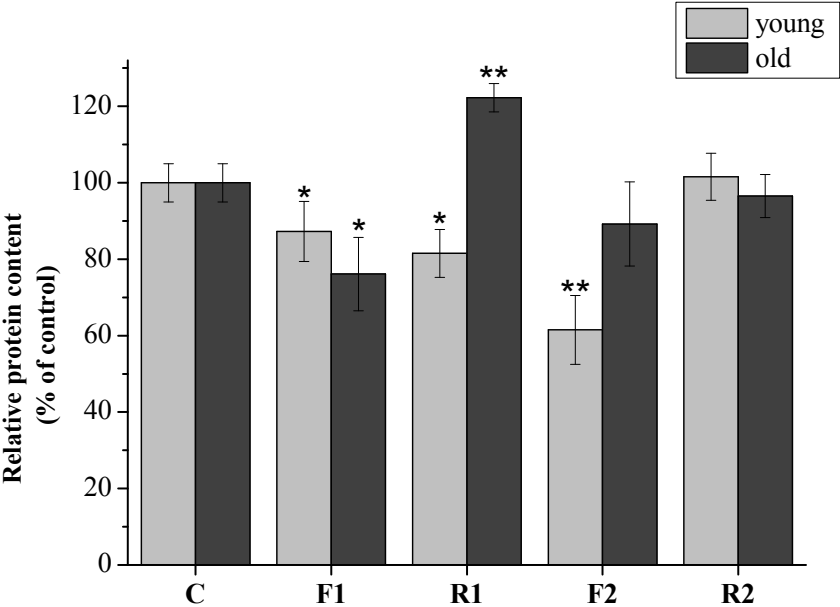


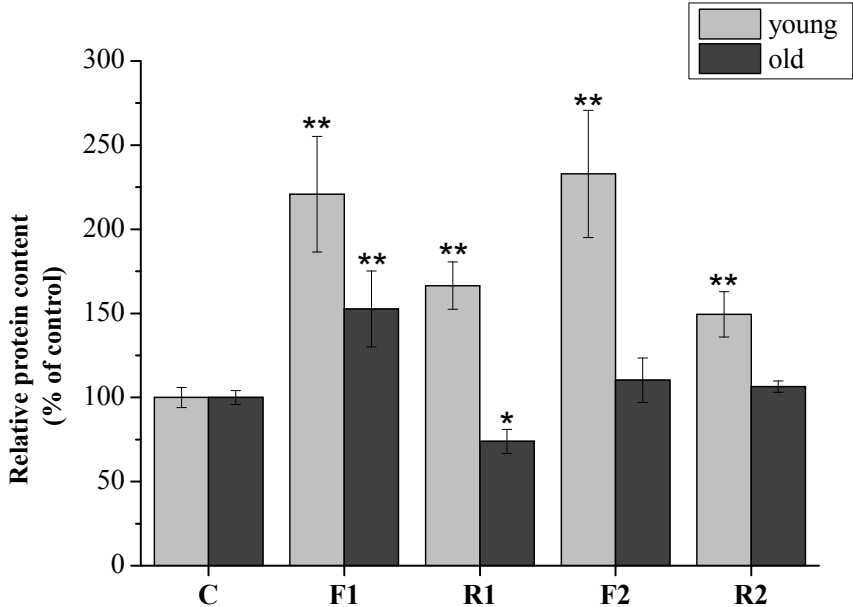
Fig.2. Effect of two cycles of intermittent fasting/refeeding on apoB-100 serum content in young and old rats. Values are mean $\pm$ SE of 10 rats from each group. The mean of control group serves as 100%. C: control rats fed ad libitum; F1: after intermittent fasting for 14 days; R1: refeeded ad libitum for 14 days after F1; F2: the same as F1 but after R1; R2: the same as R1 but after F2.

228 *Marked by asterisks are statistically significant differences relative to control group, * $P < .05$; ** $P <$*
229 *.001*

230 apoB-100 is the primary constituent of low-density lipoproteins and chylomicrons and the
231 ligand for LDL receptors. Notably, there is one apoB-100 molecule per such particle, hence,
232 the concentration of this protein exactly reflects the level of corresponding lipoproteins [34]. It
233 has been demonstrated that both fasting of 48h and CR in rodents lead to more than 2-fold
234 upregulation of apoB-100 expression in hepatocytes, while, paradoxically, several lines of
235 evidence indicated that during such dietary regiments the blood level of this protein did not
236 increase, but showed noticeable decrease [35,36]. A vast majority of studies indicate that the
237 concentration of apoB-100 in blood, in contrast to the case of most secreted proteins, is
238 regulated via degradation. Specifically, apoB-100 degradation is activated in response to the
239 lack of triglycerides (TG) and goes through the processes of ubiquitination, release from
240 endoplasmic reticulum and proteasomal degradation before the formation of VLDL [37].
241 Interestingly, if there is sufficient supply of TG, but the level of polyunsaturated fatty acids
242 (PUFAs) is very high, then one could observe another type of degradation – through the
243 process of autophagy. This phenomenon is believed to be the result of misfolding with
244 following aggregation of apoB-100 during the assembly of VLDL particles where PUFAs play
245 role of misfolding agents [38]. Accordingly, diets containing high amount of PUFAs may
246 result in the decrease of apoB-100 content and, as a consequence, could be used to affect
247 metabolism of TG and cholesterol. All these considerations led us to conclude that the
248 decrease in apoB-100 serum content after IF periods (F1 and F2) in both young and old rats
249 observed in this work come from proteasomal degradation as a result of severe restriction in
250 fat consumption. In turn, it seems highly probable that the rise in apoB-100 expression has
251 been conserved during evolution as the mechanism of rapid replenishment of fat reserves.
252 We believe that the long-term decrease of apoB-100 level in young rats arises from
253 accelerated clearance of chylomicrons and LDL, whereas the sharp increase of the protein
254 level in old animals comes from the suppression of such process. This suggestion is in line
255 with the results of Field et al. [39], who showed that one could observe more than 2-fold
256 decrease in expression of LDL receptors (i.e. apoB-100 receptors) in peripheral tissues with
257 age which leads to competition between the particles at apoB-100 receptor resulting in the
258 increase of blood particle concentration and time of circulation. The situation with insufficient
259 quantity of LDL receptors and high content of apoB-100 is one of the main cause of
260 atherosclerosis and heart disease. Therefore, it could be assumed that the first cycle of IF in
261 the case of old animals may be accompanied by high risk of the development of such
262 pathologies. During the second cycle of IF/refeeding, after F2 in young rats we observed
263 further decrease of apoB-100 level (38% relative to control and 20% relative to R1) and after
264 R2 the level of this protein was comparable with the control level. In contrast, F2 in old
265 animals leads to decrease of apoB-100 to the level of control animals (33%), but after the
266 refeeding (R2) it remained unaffected. What is the reason for the difference in response to
267 the first and the second cycles? The fact that eventually apoB-100 level returns to normal
268 level allows us to suggest that the observed difference is not a consequence of accidental
269 coincidence. Most likely it is due to the process of adaptation to periods of energy/nutrient
270 deficiency. Knowing the mechanism of this process is not only of academic interest, but, as
271 we can see from beneficial effects on old animals, is a prerequisite for development of more
272 effective treatment strategies for apoB-100 related diseases. Particularly, the refeeding of
273 the second cycle is no longer conducive to the sharp rise in apoB-100, probably because of
274 restoration of LDL receptor level typical for young organism with the decrease in risk of
275 cardiovascular diseases. In addition, although decrease in apoB-100 blood content is
276 thought to be beneficial, it can be followed by attenuation of antibacterial immunity [40]. In
277 view of this possibility, the high infectious disease incidence in mammals on calorie
278 restriction [41] can be explained in terms of the above assumption.

279 3.4. Apolipoprotein E

280 As seen in Fig.3, dramatically different behavior was observed for apoE where F1 and F2
 281 resulted in more than twofold increase in serum apoE content of young animals (~121% and
 282 ~133%, respectively). Moreover, although the protein level after R1 and R2 was decreased
 283 relative to F1 and F2, it remained significantly larger relative to control (~66% and ~49%,
 284 respectively). The old animals demonstrated similar, but much less pronounced changes in
 285 apoE level and only in the case of the first cycle (~53% increase and ~24% decrease).
 286 Intriguingly, during the second cycle we failed to detect any significant change relative to
 287 control in old rats, so that the repeat in response to such feeding model affected apoE in
 288 reverse manner with regard to the apoA-I.



289
 290 **Fig.3. Effect of two cycles of intermittent fasting/refeeding on apoE serum content in**
 291 **young and old rats. Values are mean $\pm$ SE of 10 rats from each group. The mean of**
 292 **control group serves as 100%. C: control rats fed ad libitum; F1: after intermittent**
 293 **fasting for 14 days; R1: refeeded ad libitum for 14 days after F1; F2: the same as F1**
 294 **but after R1; R2: the same as R1 but after F2.**

295 *Marked by asterisks are statistically significant differences relative to control group, * $P <$*
 296 *.05; ** $P <$.001*

297 ApoE is vital for the normal metabolism of lipoproteins and chylomicrons and it serves as a
 298 transporter of cholesterol in central nervous system. ApoE-deficiency is the most widely
 299 used model of atherosclerosis and also it is conducive to age-dependent blood-brain barrier
 300 leakage [34]. Under normal physiological conditions blood pool of apoE is maintained by
 301 liver and macrophages [42]. However, starvation and CR activate expression of this protein
 302 in adipocytes and intestine while in liver it is constant even in the case of sublethal fasting
 303 [30]. It is known that when expression of apoE in adipocytes is suppressed they are not
 304 capable of producing TG and uptake of VLDL, which result in inability of these cells to

increase their fat reserve [43]. On the contrary, activation of apoE expression triggers TG storing, i.e. one could see the effect similar to apoB-100 [44]. But, in contrast to apoB-100, all periods of IF were followed by pronounced rise of apoE level in all age groups (121%/53% after F1 and 66%/37% after F2 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 [45]. We hypothesize that this process, like in the situation with apoB-100, plays role in the mechanism of preparation to maximize 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 were characterized by 1.5-fold increase in apoE level [33]. 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 [46]. The less pronounced activation of apoE expression in the case of old animals may be associated with the known considerable decrease of its expression with age [47]. 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 [34], but such condition is supposed to be associated with the tendency to obesity. 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 F2 groups. apoE non-responsiveness to second cycle of IF/refeeding in old animals, as in the case of apoB-100, provides some grounds for believing that the mechanism underlying the regulation of protein content for 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 [48].

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 but not in old rats and after the refeeding of at least 20 days the level of this protein in young rats is characterized by 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 young rats.

(ii) apoB-100 serum level in young rats is characterized by gradual decrease with periods of F1, R2 and F2, but it is fully recovered to the control level after R2. On the contrary, F1 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 in control and after R2 it is about the level of the control and F2 groups.

The main outcome of this work is the identification of differences in the effects of dietary regiments 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 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 cycle of IF/refeeding.]

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359 **COMPETING INTERESTS**

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361 Authors have declared that no competing interests exist.

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364 **ETHICAL APPROVAL**

365

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

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