In case of obesity, longevity-related mechanisms lead to anti-inflammation

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Received: 11 June 2013 / Accepted: 4 November 2013 / Published online: 4 December 2013
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Abstract The exact mechanisms which contribute to longevity have not been figured out yet. Our aim was to find out a common way for prompting longevity by bringing together the well-known applications such as food restriction, exercise, and probiotic supplementing in an experimental obesity model. Experimental obesity was promoted in a total of 32 young (2 months old) and 32 aged (16 months old) male Wistar albino rats through 8-week cafeteria diet (salami, chocolate, chips, and biscuits). Old and young animals were divided into groups each consisting of eight animals and also divided into four subgroups as obese control, obese food restriction, obese probiotic-fed and obese exercise groups. Probiotic group diet contained 0.05 %w/total diet inactive and lyophilized *Lactobacillus casei* str. *Shirota*. The exercise group was subjected to treadmill running 1 h/day, at 21 m/min and at an uphill incline of 15 % for 5 days a week. Food restriction group was formed by giving 40 % less food than the others. The control group was fed regular pellet feed ad libitum. This program was continued for 16 weeks. Blood samples from all the

groups were analyzed for fasting glucose, insulin, IGF-1, insulin-like growth factor binding protein 3 (IGFBP-3), interleukin (IL)-6, IL-12, malondialdehyde (MDA), fT3, TT3, fT4, TT4, and liver tissue MDA levels were measured. All applications showed anti-inflammatory effects through the observed changes in the levels of IGFBP-3, IL-6, and IL-12 in the young and old obese rats. While the interventions normally contribute to longevity by recruiting different action mechanisms, anti-inflammatory effect is the only mode of action for all the applications in the obesity model.

Keywords Obesity · Food restriction · Exercise · Probiotic bacteria · Longevity · Young and aged rats

Introduction

Obesity could increase the appearance of some pathological conditions such as insulin resistant diabetes, cardiovascular diseases, non-alcoholic fatty liver disease, endocrine problems, and various different types of cancers. Therefore, obesity is regarded as a negative indicator of longevity (Gurevich-Panigrahi et al. 2009).

Longevity of mice can be increased by spontaneous or experimentally induced mutations that interfere with the biosynthesis or actions of growth hormone (GH), insulin-like growth factor 1 (IGF-1), or insulin in the adipose tissue (Bartke 2011). In a study, very low IGF-1 serum levels were observed under obesity conditions. Although serum levels of insulin-like growth factor binding protein 3 (IGFBP-3) were informed to increase

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slightly under obesity conditions (Voskuil et al. 2001; Frystyk et al. 1999), some other studies did not confirm these findings (Chang et al. 2002; Schoen et al. 2002). Interleukin (IL)-6 secretion was increased in adipocytes of obese individuals and this increase could be explained either by its acting like a hormone in circulation or a local regulator on insulin levels (Mohamed-Ali et al. 1997). Some studies suggest that IL-6 levels have stronger association with obesity-induced insulin resistance compared to the levels of tumor necrosis factor alpha (TNF- α) and leptin (Bastard et al. 2000). Increased inflammation levels and augmented levels of IL-6 and C-reactive protein are related with the increased risk of morbidity and mortality in the elderly (Michaud et al. 2013). To maintain a healthy and long life, it is important to keep the basal immune activity in controlled low levels. Even though a robust immune system is one of the very important components of health in early periods of life, its over-activity in older ages could be detrimental for the living host. Therefore, the less inflammation the living beings get exposed to, the longer life they would lead (DeVeale et al. 2005; Caruso et al. 2005).

Probiotics could have significant impacts on cellular, humoral, and nonspecific immunity. These beneficial bacteria have been proved to increase natural killer cell activity and nonspecific host protective mechanisms, especially in the elderly. Prebiotics and probiotics may modulate the gut microbiota to maintain health in the elderly (Duncan and Flint 2013).

Reports suggest that commensal bacteria and inflammation process were among the main reasons accounting for diet-induced obesity and insulin resistance (Ma et al. 2008). In diabetic rats, probiotic applications were reported to cause decreased blood glucose levels with the help of non-insulin dependent mechanisms; therefore, it might be useful in diabetes therapy (Al-Salami et al. 2008).

As well as increasing insulin sensitivity, calorie restriction also decreases insulin secretion without significant alteration of the glucose amount in blood. Some studies suggest that the selective mutations in the genes of the components which make up the signaling pathway of the insulin/insulin-like growth factor (IIS) have induced the extended life spans in many living species. Based on these findings, the reduced IIS seems to be a very appropriate candidate for increasing the longevity. Genetic manipulations such as GH-deficient Ames dwarf, GH gene knock-out, IGF-1 receptor gene knock-out heterozygote, adipose tissue-specific insulin

receptor gene deleted, and IRS-2 substrate gene knock-out mouse have been reported to have significant extended life spans (Mair and Dillin 2008). When the rats were allowed to consume 40 % less food than ad libitum, their maximum life spans were increased by 25–40 % (Weindruch and Walford 1988). In addition, food restriction has been reported to cause life span extensions in the range of from yeasts (*Saccharomyces cerevisiae*) to nematodes (*Caenorhabditis elegans*), from fruit flies (*Drosophila melanogaster*) to rodents (Mair and Dillin 2008).

While exercise reestablishes the distorted energy/fat balance, it also ameliorates the pathological conditions of the obesity/excess weight (Hansen et al. 2005).

The exercise, probiotic, and food restriction interventions are regarded as important longevity inducers in almost all species. While the longevity works have been trying to elucidate the exact mechanisms responsible for the effect of various interventions, up to now, there is no clear definition accounting for this effect. We proposed that different aforementioned interventions could be joined in one common experimental obesity model to unfold complex mechanism. We hypothesize that since the organism in obesity conditions already have been fighting chronic inflammation which takes a toll on life expectancy, the other longevity interventions should share the common concern instead of exerting their effects using different mechanisms. As a result, they are likely to show a common action mode for exerting their effects in that special occasion.

Materials and methods

In this study, 50 young (2 months old) and 60 old (16 months old) male Wistar albino rats were used. After the obesity was formed, the young and old rats were subdivided into four groups, each consisting of eight animals such as control, food restriction, probiotic supplement, and exercise. Following the 16-week treatments, blood and tissue samples taken from the animals were analyzed.

Experimental obesity induction The animals obtained with the normal body weight were fed with high-fat diet (60 % fat, 10 % protein, and 30 % carbohydrate) by using cafeteria diet consisting of biscuits, chocolate, chips, and salami (1:1:1:2.5). After 8-weeks application of obesity-induced diet, the animals showing at least

20 % body weight increase compared to control groups were regarded as obese (Widdowson et al. 1997). The animals whose weight gains were not significant compared to the control were kept out of the study. After obtaining obesity, the diet was shifted to the normal rat feeding (12 % fat, 16 % protein, 72 % carbohydrate).

Feed restriction For this purpose, daily food consumptions of the animals were monitored during 15 days and on the basis of mean daily consumption of the each animal, 60 % of these amounts were given to the animals to apply 40 % restriction. For preparing the feeds of the restriction group, the vitamin and mineral contents were adjusted accordingly.

Exercise The young and old animals in this group were allowed to exercise just for 5 min and at the lowest speed (stand by position) on the zero incline at the first day for the adaptation purpose. The animals in the exercise group were allowed to exercise 21 m/min speed at 15 % incline for 5 days a week on the tread mill device for 16 weeks (Steinberg et al. 2004).

Probiotic bacteria supplementation The feeds for the group were prepared with probiotic bacteria additive. For this purpose, the bacteria (*Lactobacillus casei* str. *Shirota*) obtained from the Dutch National Institute for Public Health and the Environment, Netherlands, were inoculated with the MRS mediums and incubated at 30 °C for 72 h. Following this incubation period, the grown bacteria were collected by centrifuging and rinsed by sterile distilled water three times. Then, the bacteria were kept at 100 °C for 15 min in order to inactivate the bacteria and followed by the lyophilization process. While preparing the diets of this group, the lyophilized bacteria were added to their feeds by 0.05 % weight. These diets were freshly prepared every week and kept on 4 °C before being given to the animals ad libitum (Hori et al. 2002).

Blood collection and analyses After the treatment period, the blood samples of all the animals were drawn through intracardiac route and sera were separated. The blood samples taken from the tail ven under the anesthesia were used to determine fasting blood glucose levels by using the strips of eBsensor. Insulin (SPI-Bio A05105), IGF-1 (DSL-10-29200), IGFBP-3 (Mediagnost E031), fT3 (Monobind), TT3 (Monobind), fT4 (Monobind),

TT4 (Monobind), IL-6 (Bender MedSystems rat interleukin-6 ELISA Kit) and IL-12 (BioSource rat IL-12+p40) measures were done by using specific ELISA kits. Lipid peroxidation measurement in the liver tissue was done by using the method which Sushil et al. described (Sushil et al. 1989).

Statistical analyses The results are expressed as mean $\pm$ standard deviation. Statistical evaluations of the data obtained from the study were performed by using Duncan and ANOVA tests. Differences were considered significant at $p < 0.05$.

Results

Table 1 shows the analyzed fasting blood glucose, insulin, IGF-1, IGFBP-3, IL-6, IL-12, fT3, TT3, fT4, TT4, and liver tissue malondialdehyde (MDA) values of young and old obese groups in statistical comparison to one another and obese controls.

Old and young exercise groups' insulin levels have decreased significantly compared to the control groups (in order $p < 0.01$, $p < 0.001$). The hypoinsulinemia effects of exercise have been observed in both groups (Fig. 1).

Old obese probiotic, exercise, and food restriction applications have increased IGF-1 levels compared to the controls ($p < 0.001$); this shows that these applications show a GH-like effect. However, in the young groups, this effect was observed in the exercise groups ($p < 0.05$) (Fig. 2).

In the young probiotic, exercise, and old exercise group, significant increases of IGFBP-3 levels were observed ($p < 0.001$). Statistically, there was a tendency to increase in the old probiotic and restriction group, but it was not significant (Fig. 3).

Significant decreases were observed in the levels of plasma IL-6 levels both in young and old probiotic, exercise, and food restriction groups ($p < 0.05$) (Fig. 4).

In young and old obese probiotic and restriction groups, there were significant increases in IL-12 levels compared to their controls ($p < 0.01$). While exercise made a significant increase of IL-12 in the young group ($p < 0.001$), no significant increase was observed in the old group (Fig. 5).

Table 1 Comparison of all the groups

		Mean±Standard Error (X±SE)					
		N	Glucose (mg/dl)	Insulin (ng/ml)	IGF-1 (ng/ml)	IGFBP-3 (ng/ml)	IL-6 (pg/ml)
Aged obese	Control	8	99.12±2.71b	0.9±0.19a	428.96±14.77b	179.85±8.68b	212.47±5.34a
	Probiotic	7	102.71±2.56b	0.91±0.07a	671.7±28.32a	203.25±6.31b	114.13±3.27d
	Exercise	8	80.50±2.26c	0.5±0.05b	737.07±27.92a	246.18±8.42a	183.28±5.57b
	Restriction	5	125±5.77a	0.54±0.05b	684.34±24.79a	195.65±7.91b	164.27±6.95c
Young obese	Control	8	112.25±3.29c	0.92±0.06a	1093.66±57.23b	247.4±21.33b	153.06±1.20a
	Probiotic	8	115.50±4c	0.39±0.09b	632.29±28.52c	321.83±12.36a	124.05±1.88b
	Exercise	7	130.14±2.02b	0.33±0.03b	1225.43±97.69a	308.67±8.07a	111.95±3.40c
	Restriction	8	145.87±5.05a	0.86±0.05a	685.38±23.70c	199.82±5.57c	93.15±1.40d
		Mean±Standard Error (X±SE)					
		IL-12 (pg/ml)	MDA (nmol/g)	FT3 (pg/ml)	TT (ng/ml)	3FT4 (ng/dl)	TT4 (ug/dl)
Aged obese	Control	417.97±38.60b	471.28±14.24	4.53±0.31c	0.99±0.09	1.38±0.07b	5.81±0.13a
	Probiotic	679.47±45.16a	467.85±12.34	7.73±1b	1.16±0.09	1.36±0.15b	6.14±0.46a
	Exercise	462.33±19.84b	479.72±17.41	10.41±0.33a	1.49±0.13	2.1±0.10a	4.56±0.26b
	Restriction	647.47±17.02a	459.67±9.02	3.28±0.33c	1.1±0.12	1.5±0.18b	6.2±0.66a
Young obese	Control	466.42±13.23c	465.31±10.28	3.85±0.45a	0.67±0.08	1.02±0.11c	1.69±0.31c
	Probiotic	591.18±22.22b	474.36±16.66	3.73±0.32a	1.24±0.06	1.95±0.16a	5.25±0.39b
	Exercise	712.92±22.16a	473.73±7.59	3.44±0.32a	1.16±0.09	1.44±0.09b	6.43±0.40a
	Restriction	705.24±33.82a	419.13±10.56	3.17±0.15a	0.67±0.08	1.9±0.22a	1.75±0.13c

Comparison of application groups (control, probiotic, exercise and restriction) within the age groups (young and old). Different letters within the same age group show significance between the treatment groups ($p<0.05$)

Fig. 1 Comparison of insulin values of all application groups (ng/ml). The letters on the standard error lines of each bar shows the comparison of application and control groups (control, probiotic, exercise, and restriction) in young and old animals. Different letters within the same age group show significance between the treatment groups ($p < 0.05$)

Discussion

It is known that the certain applications such as diet and exercise could delay insulin resistance and helps the regulation of body composition changes during the aging process. It has been reported that reductions in body weight could have increased the insulin sensitivity and ameliorated the glucose tolerance. Furthermore, insulin resistance caused by advanced aging could be improved to some extent by using certain exercise applications (Ryan 2000). In the present study, the hypoinsulinemic effects of exercise intervention in either group have been observed clearly. So, these results prove that the exercise is the most effective hypoinsulinemic application for young and old subjects.

It is suggested that regular physical activity could improve life quality by increasing the levels of IGF-1 in circulation (Tissandier et al. 2001). Low IGF-1 levels suggest decreased GH amounts. As a result of exogenous treatments of GH or GHRH in both young and old

male subjects, IGF-1 levels in circulation were reported to increase in the same way (Corpas et al. 1993). As the applications of probiotic, exercise, and partly food restriction in old group caused to increase IGF-1 levels in circulation in our study, these applications have been shown to exert GH-like effects. However, this effect in the young group appeared in exercise group only.

In 10 adult patients with inflammatory bowel disease, IGFBP-3 levels have been reported to decrease compared to healthy control groups. Anti-inflammatory steroid therapy was reported to improve IGFBP-3 levels (Katsanos et al. 2001). In the present work, we found increased IGFBP-3 levels in young and old groups. But in the old probiotic group, there was not any statistical significance even though there was a tendency to elevate. This specific finding of the study shows the potential anti-inflammatory effect of the probiotic application. However, it is suggested that supporting enteric microflora with probiotics could be a novel approach for therapy in the inflammatory bowel disease (Linskens

Fig. 2 Comparison of insulin-like growth factor-1 (IGF-1) values of all application groups (ng/ml). The letters on the standard error lines of each bar shows the comparison of application and control groups (control, probiotic, exercise and restriction) in young and old animals. Different letters within the same age group show significance between the treatment groups ($p < 0.05$)

Fig. 3 Comparison of IGF binding protein-3 (IGFBP-3) values of all application groups (ng/ml). The letters on the standard error lines of each bar shows the comparison of application and control groups (control, probiotic, exercise, and restriction) in young and old animals. Different letters within the same age group show significance between the treatment groups ($p < 0.05$)

et al. 2001). A study suggests that short-chain fatty acids like butyric acid, which comes up as a result of fermentation of fiber by probiotic bacteria in intestine could exert anti-inflammatory effect by preventing the transcriptions of pro-inflammatory cytokines (Segain et al. 2000).

It has been recognized that although mostly regarded as a pro-inflammatory cytokine, IL-6 also has many regenerative or anti-inflammatory activities (Scheller et al. 2011). Obesity is a chronic low-grade inflammatory disorder, producing a state where responses to subsequent inflammatory challenges are potentiated (Iannitti et al. 2012). The most acceptable explanation for the relevance between cytokine production and obesity-associated insulin resistance is the increased TNF- α and IL-6 secretion by the adipose tissue. Plasma IL-6 level and insulin sensitivity relationship is very remarkable and there is a very strong controversial connection between them (Kern et al. 2001). It is suggested that the shift of the secretory profile of adipose tissue from pro- to anti-inflammatory importantly

contributes to improved insulin sensitivity-reduced rate of aging and extended longevity in mice lacking GH signals (Masternak and Bartke 2012). It is said that the exercise program applied along with low-calorie diet could decrease the inflammation rate by reducing the plasma levels of some cytokines like IL-6, CRP, and TNF- α (Brunn et al. 2006).

In contrast to adipose-derived IL-6, which is commonly thought of as pro-inflammatory, episodic elevations in skeletal muscle derived IL-6 trigger an anti-inflammatory cascade by inhibiting the release of pro-inflammatory cytokines (TNF- α and IL-1 β) via the stimulation of their antagonistic receptors (Petersen and Pedersen 2005). Regular exercise induces anti-inflammatory actions. During exercise, IL-6 is produced by muscle fibers. IL-6 enhances lipid turnover, stimulating lipolysis as well as fat oxidation. IL-6 may be involved in mediating the beneficial health effects against chronic diseases associated with low-grade inflammation (Pedersen 2006). Physical activity induces an increase in the systemic levels of a number of

Fig. 4 Comparison of interleukin-6 (IL-6) values of all application groups (pg/ml). The letters on the standard error lines of each bar shows the comparison of application and control groups (control, probiotic, exercise, and restriction) in young and old animals. Different letters within the same age group show significance between the treatment groups ($p < 0.05$)

Fig. 5 Comparison of Interleukin-12 (IL-12) values of all application groups (pg/ml)*. The letters on the standard error lines of each bar shows the comparison of application and control groups (control, probiotic, exercise and restriction) in young and old animals. Different letters within the same age group show significance between the treatment groups ($P<0.05$)

cytokines with anti-inflammatory properties (Petersen and Pedersen 2005). The level of circulating IL-6 increases in an exponential fashion (up to 100-fold) in response to exercise, and declines in the post-exercise period (Febbraio and Pedersen 2002). In the present study, due to the fact that all measurements were completed after the exercise program, IL-6 levels were found lower in the groups of old and young compared to control groups. Thus, during the exercise, IL-6 plays an antiinflammatar action by elevating, but after the exercise program, the same effect is likely to be obtained by decreased levels.

Probiotics are able to develop the mucosal barrier by increasing the production of innate immune molecules and some strains stimulate the adaptive immune responses (Iannitti and Palmieri 2010). Probiotic bacteria of *Lactococcus lactis* subsp. *lactis* G50 was stated to increase cytokines related with Th1 and showed anti-inflammatory effect by suppressing the hypersensitivity reactions (Kimoto et al. 2004). We found that IL-6 levels were significantly decreased in the probiotic, exercise, and restriction subgroups of both young and old groups. It seems that these three applications exert their effects on the body through a common anti-inflammatory action.

IL-12 is a 70-kDa heterodimeric cytokine comprising two disulfide-linked subunits p40 and p35 (Trinchieri 2003). The genes encoding p35 and p40 are located on separate chromosomes (Wolf et al. 1991; Sieburth et al. 1992; Schoenhaut et al. 1992) and therefore their expression is independently regulated. Co-expression of both subunits is required to form the biologically active, secreted p70 heterodimer (Gubler et al. 1991). IL-12 p40 is produced in much greater quantities than is required to form heterodimers (Wysocka et al. 1995).

Excess p40 is secreted as free monomers and homodimers (Dasgupta et al. 2008). Homodimeric p40 has been ascribed immunomodulatory roles. It can act as an antagonist by competing for the IL-12 receptor and dampen the immune response (Gillesen et al. 1995). In other cases, it has been reported to be immunostimulatory (Piccotti et al. 1998). However, homodimeric p40 has not been detected in human cells and is not considered a physiological IL-12 antagonist in humans (Carra et al. 2000). Caloric restriction was suggested to eliminate the impotence of the immune system with advanced aging (Nikolich-Zugich and Messaoudi 2005). Also, caloric restriction would significantly help reduce the serum levels of interferon- γ and IL-12 which was considered as pro-inflammatory cytokines (Shibolet et al. 2002). In the present study, IL-12 levels were found to be increased in the groups of old and young probiotic and restriction groups compared to control groups. Though our findings seemed to be contradictory with the above mentioned work's findings, the measures of IL-12 along with p40 subunit levels might have caused such discrepancy by exerting anti-inflammatory effects of p40 subunit. Moreover, IL-12 is an important mediator of cell-mediated immunity and is expected to augment the natural immune defense against infections and cancers (Trinchieri 2003). It stimulates T cells to secrete interferon-gamma (IFN-g), promotes Th1 cell development, and, directly or indirectly, augments the cytotoxic activity of natural killer (NK) cells and macrophages. It also suppresses redundant Th2 cell responses for the control of allergy (Gavett et al. 1995). Two important immunoregulatory cytokines produced by cells of the innate defense system in response to bacteria are IL-12 and IL-10. IL-12 stimulates IFN-g production from T cells and NK cells and increases their

cytotoxicity (Trinchieri 1994). Since certain lactobacilli can adhere to human intestinal epithelial cells (Adlerberth et al. 1996) and are able to translocate over the gut barrier (Herias et al. 1995), they occupy a position favorable for interaction with cells in the gut-associated immune system. One of the reasons why they scarcely cause disease might be their potent ability to induce the production of IL-12 and thereby activate macrophages to a more effective clearance of the bacteria, decreasing their infectivity (Hessle et al. 1999).

There are many reports that probiotic bacteria prevent murine inflammatory bowel disease (IBD) (Madsen et al. 1999; O'Mahony et al. 2001). In one of them, lactobacillus- and bifidobacterium-fermented milk prevented ileal inflammation in SAMP1/Yit, a murine model of CD, by down-regulating Th1-cytokine responses (Matsumoto et al. 2001). Some other reporters stated that this probiotics induced improvement in murine chronic IBD is associated with the down-regulation of pro-inflammatory cytokines such as IL-6 in LPMC. The safety of this probiotic strain has been demonstrated in a clinical trial (Aso et al. 1995). In the same way, it was postulated that *Lactobacillus casei* str. *Shirota* may be a useful probiotic for the treatment of IBD (Matsumoto et al. 2005). In one report, it was again pointed that endurance exercise and CR exert powerful anti-inflammatory function through suppression of pro-inflammatory cytokine expression in white adipose tissues (Huang et al. 2010).

Successful aging seems to be related to an optimal functioning of the immune system, pointing out that polymorphisms for the immune system genes, which are involved in the regulation of immune-inflammatory responses, may play a key role in the genetics of aging (Iannitti and Palmieri 2011).

Importantly, human studies have shown that exercise intervention without weight loss decreases circulating inflammatory biomarkers in lean and obese men with and without type II diabetes (Dekker et al. 2007). Reduction of the inflammation levels in plasma and adipose tissue through the body weight control is one of the main protective mechanisms of body (Petersen and Pedersen 2005). The increased levels of anti-inflammatory cytokines during exercise not only restricts pro-inflammatory cytokines against muscle destruction (Toft et al. 2002), but also decrease the production of the numerous cytokines which account for many diseases (Petersen and Pedersen 2005).

Accumulation of adipose tissue in obesity and increased body weight may increase the risk of developing age-related diseases. This imbalance in body weight and body composition is now defined as a situation in increased oxidative stress and inflammation for the organism (Tzanetakou et al. 2012). It was reported that optimum exercise could lead to increases in certain anti-inflammatory mediators (Petersen and Pedersen 2005) and boost the cellular immunity (Davis et al. 2004). In a study, *Tai Chi Chuan* exercise program for 12 weeks was found to increase IL-12 levels significantly in blood and also reverse the situation in the diabetic patients with propensity to obesity and sensitive for proinflammatory response (Yeh et al. 2008). In the present work, daily regular exercise caused a significant increase in the blood IL-12 levels in the young group, although this increase was not found important in the old group. In our opinion, that difference could be related with the suppression of the anti-inflammatory effect of the exercise as it takes longer to restore the organ and tissue injuries in the old group and as well the weaker antioxidation mechanisms in older ages.

As a conclusion, the hypoinsulinemia effects of exercise both in the young and old groups have been observed clearly. This finding shows that exercise has been the most potent hypoinsulinemia application in either group. The exercise application has been found to exert GH-like effect by using the same mechanism in either groups, whereas probiotic and restriction application groups were found to use different mechanisms on IGF-1 system in both young and old groups. Again, all applications in the young and old obese groups on IGFBP-3, IL-6, and IL-12 levels were found to have anti-inflammatory actions. In our work, the levels of IL-6 in all applications of both young and old groups were found to have decreased significantly. Since IL-6 is a very strong indicator of inflammation, these applications have been proved to have an anti-inflammatory impact. In the light of these findings, all three applications were found to have exerted their effects using a common way by inhibiting the inflammation, when the obesity was a common base for all the applications. There was not any change in MDA levels of all the groups and applications. The lack of lipid peroxidation increase in liver tissues of all groups could be explained by the possible presence of high levels of vitamin E, as an antioxidant, in liver adipose tissue. Meanwhile, some thyroid hormone changes in the findings were suggested

to restore obesity-induced energy imbalance in both young and old groups.

The exercise, probiotic, and food restriction interventions contribute to longevity in non-obese animals by recruiting different and some not yet identified mechanisms (Froy and Miskin 2010; Piazza et al. 2009). In our knowledge, our work firstly demonstrated that each of every application, in first hand, exerted their effects through using one shared mechanism; anti-inflammatory impact, in the case of obesity. Obesity is known to cause chronic inflammation, has anti-longevity influence, and it is also a serious threat for the living beings' health. This also shows that the applications improving the parameters related with longevity could recruit surprisingly different mechanisms depending on the animal's physiologically altered state, such as obesity. As a final word, it could be said that the most influential intervention regarding longevity in obese subjects is to decrease the systemic inflammation in body.

Acknowledgments This study was supported by both the Scientific and Technological Research Council of Turkey (TUBITAK)—Agriculture, Forestry and Veterinary Research Support Group research grants (TOVAG-106O344) and Yuzuncu Yil University Grant Commission of research numbered with YYU-BAP-2006-SBE-105.

Conflict of interest The authors declare that they have no conflict of interest.

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