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IDENTIFICATION OF FACTORS CONNECTED WITH AUTOPHAGY ACTIVITY UNDER CONDITIONS OF PARTIAL NUTRITIONAL DEPRIVATION IN MEN

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Autophagy activity was studied on men of different ages and weights under conditions of partial food deprivation (800-120 kcal per day). Beclin- 1 marker, which was determined by enzyme immunoassay, was chosen as an indicator of autophagy activity. Autophagy activity was determined by the difference in the concentration of beclin-1 before the onset of food deprivation and 12 days later, expressed as a percentage of the initial one. The study showed that the leading factor influencing the activity of autophagy in men is age and certain components of body composition. The authors assess the degree of activation of the autophagy process in men in relation to various factors, such as weight, body composition, age, biochemical parameters of lipid metabolism under conditions of partial food deprivation (PFD). Autophagy activity was determined by the difference in the concentration of beclin-1 before the start of food deprivation and after 12 days. The authors found that the weight category of the patient does not play a significant role in the degree of autophagy activation during calorie restriction in men. The most significant factor influencing the activity of autophagy is age and is a factor influencing the blood lipid spectrum. The authors found a negative correlation between delta-beclin-1 and high-density lipoproteins at the young age, and negative correlations with LDL, total cholesterol and positive correlations with HDL in the elderly.

Keywords: food deprivation, autophagy, age, weight, Beclin-1, men

The works devoted to autophagy show the important physiological role of this process in maintaining cellular and systemic homeostasis in mammals [1]. However, most studies have been carried out either on experimental animals or on individual cell cultures [2, 3]. It is generally accepted that calorie restriction stimulates the process of autophagy, which was the argument for the widespread introduction of various restrictive diets into the masses of the population [4, 5, 6]. The autophagy marker Beclin-1 protein is used to characterise the autophagy process. In 2001, Tamotsu Yoshimori and colleagues [7] showed that Beclin-1 forms a complex with phosphatidylinositol-3-kinase (PI3K) and in this composition initiates the nucleation and formation of the autophagosome membrane, and plays a key role in other stages of autophagy [8,9]. At the same time, the analysis of literature data shows the paucity of studies of this issue specifically on humans. In this regard, the study of the process of autophagy and factors affecting the activity of this process is an urgent problem.

Purpose of the study: to determine the degree of activation of the autophagy process in men in connection with various factors: weight, body composition, age, biochemical parameters of lipid metabolism under conditions of partial food deprivation (PFD).

Materials and methods of research

This study involved 20 men. The concentration of Beclin-1 was highly variable, and therefore the interpercentile interval of 5%-95% was chosen. This interval included 17 men aged 30 to 69 years and weighing 68.6 to 198 kg. The subjects were examined and received partial food deprivation on the basis of the clinic LLC "Health Center" in the city of Maykop (Russia, Republic of Adygea). This diet included caloric restriction to 1100 kcal/day. Clinical and biochemical blood tests included complete blood count, total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides (TG). Indicators of body composition (muscle mass – M.M., lean mass – L.M., fat mass – F.M., total body water – TBW, extracellular water – Ext. W., intracellular water – Int. W.) were determined by the impedance method on the device Medi Ld (France) using EIS – ESTECK (USA) software. Body mass index (BMI) was calculated by the Quetelet coefficient: the ratio of body weight (kg) to height (m^2). Obesity was diagnosed with BMI ≥30 kg/m^2. The activity of autophagy processes was determined using the Beclin-1 protein. To characterize the activity of autophagy, the indicator "delta-beclin-1" was introduced – the difference between the initial concentration and the repeated determination, expressed as a percentage. The concentration of beclin-1 was determined by enzyme immunoassay using the apparatus " CLARIOstarplus " BMG LABTECH (Germany) with Cloud – Clone test kits Corp " (USA). Blood sampling was carried out in the morning on an empty stomach upon admission to the health center and on the 12th day of arrival. The marker concentration was expressed in pg /mL.

The subjects were divided into 3 age groups, according to WHO recommendations: young age from 18-44 years old, average age from 45 to 60 years old and old age from 61 to 75 years old.

IBM software SPSSStatistics (26.0). To characterise the statistical series, descriptive statistics (percentiles 5%-95%) were used with the calculation of the median, mean, error of the mean, minimum and maximum values. To compare the mean values, the nonparametric Mann-Whitney U-test and the parametric Student's t-test were used. Pearson's correlation analysis was used to identify relationships between the studied parameters. The association was considered significant at $p < 0.05$.

Results of the research and discussions

First of all, the obtained data were compared depending on the age and weight of the patients (Tables 1 and 2).

When comparing the parameters of delta-becklin between groups depending on age (Table 1) and weight (Table 2), no significant differences could be found. However, when analysing the lipid spectrum and autophagy activity, interesting data were obtained (Table 3).

Correlation analysis (Table 3) revealed a positive correlation at a young age between LDL and beclin-1 deltas and a negative correlation between HDL and beclin-1 delta. In old age, a high direct correlation was found between HDL, TC and Beclin-1 delta and a negative one between LDL and Beclin-1 delta. This trend was not observed in the middle age group. When analysing the indicators of autophagy activity depending on weight, no differences were found (Table 4).

Analysis of the body composition delta parameters (Table 5) revealed direct correlations at a young age between the levels of muscle mass, lean mass, total body water, and Beclin-1 delta. In the elderly, an inverse correlation was found between the amount of fat mass and Beclin-1 delta.

When examining the parameters of patients with different weight categories depending on body composition, no significant relationships were found (Table 6).

Table 1

Comparison of the beclin concentration delta (in % of baseline) before and after FPD depending on age

Study groups/age	M cf.	σ	m0	Median	Min	Max	P
1. Young (n= 4)	164.7	234.9	117.5	123.5	-40	452	$P_{1-2} = 0.78$
2.Medium (n=8)	222.1	358.9	126.9	66.5	-35	987	$P_{1-3} = 0.81$
3. Elderly (n= 5)	129.2	191.1	85.4	66.0	-17	462	$P_{2-3} = 0.61$

Table 2

Comparison of beclin concentration delta (in % of baseline) before and after FPD depending on weight

Study groups/age	M cf.	σ	m0	Median	Min	Max	P
1.Normal weight (n= 5)	89.6	104.9	46.9	66.0	-14	261	$P_{1-2} = 0.4$
2. Obesity (n= 12)	219.5	321.7	92.9	66.5	-40	987	

Table 3

Correlation analysis between beclin-1 concentration deltas and lipid spectrum indices depending on age

Age groups/age	Correlation coefficient			
	LDL	HDL	Total cholesterol	Triglycerides
1. Whole group (n=17)	0.18	0.02	0.21	0.19
2. Young (n= 4)	0.94*	-0.91*	0.59	-0.32
3.Medium (n= 8)	0.44	-0.41	0.5	0.46
4. Elderly (n=5)	-0.99*	0.94**	-0.98**	-0.33

Note: *significance – $p < 0.05$; **significance – $p < 0.01$

Table 4

Correlation analysis between beclin-1 concentration deltas and lipid spectrum indices depending on body weight

Study Groups	Correlation coefficient			
	LDL	HDL	Total cholesterol	Triglycerides
1.Normal weight (n= 5)	0.29	-0.6	0.55	0.3
2.Obesity (n= 12)	0.2	0.006	0.25	0.32

Table 5

Correlation analysis between deltas of beclin-1 concentration and indicators of body composition depending on age

Study groups/age	Correlation coefficient							
	Weight	BMI	MM.	F. M.	L. M.	TBW	Ext. W.	Int. W.
1. The whole group (n= 17)	-0.20	-0.33	-0.18	-0.18	-0.24	0.09	-0.01	-0.08
2. Young (n= 4)	0.27	0.27	0.96*	0.96*	0.07	0.96*	0.86	-0.66
3.Medium (n= 8)	-0.28	-0.58	-0.57	-0.57	-0.23	0.13	-0.22	-0.02
4. Elderly (n= 5)	-0.50	-0.50	-0.79	-0.79	-0.87*	-0.79	-0.05	-0.23

Note: *significance – $p < 0.05$.

Table 6

Correlation analysis between beclin-1 concentration deltas and body composition indices depending on body weight

Study Groups	Correlation coefficient							
	Weight	BMI	MM.	T. M.	L. M.	TBW	Ext. W.	Int. W.
1.Normal weight (n= 5)	-0.12	-0.14	0.1	0.1	0.1	0.1	0.29	-0.4
2.Obesity (n= 12)	-0.26	-0.45	-0.27	-0.27	-0.31	0.13	0.02	-0.15

Conclusion

The data obtained indicate that the weight category of the patient does not play a significant role in the degree of autophagy activation during caloric restriction in men. The most significant factor affecting the activity of autophagy is age. In particular, this is confirmed by the presence of direct correlations in the young examined with muscle, lean mass and total water content, in the elderly – the presence of a negative relationship with the total fat content. Age also turned out to be a factor influencing changes in the blood lipid spectrum: at a young age, a negative correlation was found between delta-beclin-1 and high-density lipoproteins, and in old age, negative correlations with LDL, total cholesterol and positive correlations with HDL.

In this study, the fact of a decrease in the activity of autophagy as a person grows older was confirmed. Previously, the data obtained concerned only laboratory animals or individual cell cultures [10]. Restriction of caloric intake is the starting point for the activation of internal signaling pathways for changing metabolism. One such regulator is the sirtuin-1 enzyme protein (SIRT-1). Experimental studies have shown that calorie restriction contributes to longevity and cell protection from hypoxia through the sirtuin-1-dependent autophagy process [11, 12].

From these positions, SIRT1 acts as an energy sensor of cells. Sirtuin-1 is a representative of NAD-dependent deacetylases, which, when over-expressed, can increase the lifespan of individual mammalian representatives [13]. Through the process of acetylation, sirtuins are able to regulate apoptosis, gluconeogenesis, fatty acid oxidation, insulin sensitivity, and differentiation of adipose tissue cells [14].

Deficiency of energy substrates can act as a trigger for the expression of sirtuin-1 and 3. In their review, Rusakova E. et al. [15] showed that the SIRT1 gene promotes the process of gluco-

neogenesis in the liver, fatty acid oxidation in muscles, and fat mobilization in adipose tissue, which gives the right to consider this gene an important regulator of lipid metabolism.

Indeed, a number of studies have shown that SIRT1 activates gluconeogenesis in the liver, fatty acid oxidation in muscles, and fat mobilization in adipose tissue.

In an experiment under conditions of hyperglycemia, SIRT1 prevents the accumulation of lipids in the liver through the activation reaction of AMPK (AMP-activated protein kinase). SIRT1 can regulate lipid metabolism in the liver through deacetylation of SREBPs (Sterol Regulatory Element-Binding Proteins) proteins that regulate lipogenesis and cholesterol synthesis. Activation of SIRT1 inhibits SREBPs gene expression and reduces signs of steatosis in mice fed a restricted diet and mice with genetic obesity. In terms of the problem studied by the authors, it is important to note that the impaired function of SIRT1 in adipose tissue in genetically fatty mice (ADIPO-H363Y) was restored with calorie restriction [16].

Deacetylation of beclin-1 under the influence of SIRT1, as follows from the literature data [17, 18], is accompanied by an increase in the number of autophagosomes. They require components to build, including cholesterol, LDL, HDL. It is possible that this regulation of lipid metabolism is carried out through sirtuin-1. The consensual response of SIRT1 and beclin-1 in elderly men seemed to manifest itself most physiologically, namely, in a trend towards a decrease in LDL and an increase in HDL. However, the matter requires a further study.

References

1. Galluzzi L., Pietrocola F., Levine B., Kroemer G. Metabolic Control of Autophagy. Cell. 2014. Vol. 159. No. 6. P. 1263-1276. DOI: 10.1016/j.cell.2014.11.006.

2. Chen X., Kondo K., Motoki K., Homma H., Okazawa H. Fasting activates macroautophagy in neurons of Alzheimer's disease mouse model but is insufficient to degrade amyloid-beta. Scientific reports. 2015. Vol. 5. P. 12115. DOI: 10.1038/srep12115.

3. Mizushima N., Levine B. Autophagy in mammalian development and differentiation. Nature cell biology. 2010. Vol. 12. P. 823-830. DOI: 10.1038/ncb0910-823.

4. Aris J.P., Alvers A.L., Ferraiuolo R.A., Fishwick L.K., Hanvivatpong A., Hu D., Kirlew C., Leonard M.T., Losin K.J., Marraffini M., Seo A.Y., Swanberg V., Westcott J.L., Wood M.S., Leeuwenburgh C., Dunn Jr. WA Autophagy and leucine promote chronological longevity and respiration proficiency during calorie restriction in yeast. Experimental gerontology. 2013. Vol. 48. No. 10. P. 1107-1119. DOI: 10.1016/j.exger.2013.01.006.

5. Libert S., Guarente L. Metabolic and neuropsychiatric effects of calorie restriction and sirtuins. Annual review of physiology. 2013. Vol. 75. P. 669-684. DOI: 10.1146/annurev-physiol-030212-183800.

6. Rickenbacher A., Jang J.H., Limani P., Graf R., Humar B., Clavien P.A. Fasting protects liver from ischemic injury through Sirt1-mediated downregulation of circulating HMGB1 in mice. Journal of hepatology. 2014. Vol. 61. No. 2. P. 301-308. DOI: 10.1016/j.jhep.2014.04.010.

7. Kihara A., Kabeya Y., Ohsumi Y., Yoshimori T. Beclin-phosphatidylinositol 3-kinase complex functions at the trans-Golgi network. EMBO. 2001. Vol. 2. P. 330–335. DOI: 10.1093/embo-reports/kve061.

8. Margariti A., Li H., Chen T., Martin D., Vizcay-Barrena G., Alam S., Karamariti E., Xiao Q., Zampetaki A., Zhang Z., Wang W., Jiang Z., Gao C., Ma B., Chen Y.G., Cockerill G., Hu Y., Xu Q., Zeng L. XBP1 mRNA splicing triggers an autophagic response in endothelial cells through BECLIN-1 transcriptional activation. The journal of biological chemistry. 2013. Vol. 288. No. 2. P. 859-872. DOI: 10.1074/jbc.M112.412783.

9. Ashkenazi A., Bento C.F., Ricketts T., Vicinanza M., Siddiqi F., Pavel M., Squitieri F., Hardenberg M.C., Imarisio S., Menzies F.M., Rubinsztein D.C. Polyglutamine tracts regulate beclin 1-dependent autophagy. Nature. 2017. Vol. 545. No. 7652. P. 108-111. DOI: 10.1038/nature22078. DOI:

10. Luévano-Martínez L.A., Forni M.F., Peloggia J., Watanabe L.S., Kowaltowski A.J. Calorie restriction promotes cardiolipin biosynthesis and distribution between mitochondrial membranes. Mechanism of ageing and development. 2017. Vol. 169. P. 9-17. DOI: 10.1016/j.mad.2017.02.004.

11. Morselli E., Maiuri M. C., Markaki M., Megalou E., Pasparaki A., Palikaras K., Criollo A., Galluzzi L., Malik S. A., Vitale I., Michaud M., Madeo F., Tavernarakis N., Kroemer G. Caloric restriction and resveratrol promote longevity through the Sirtuin-1-dependent induction of autophagy. Cell death and disease. 2010. Vol. 1. No. 1. DOI: 10.1038/cddis.2009.8.

12. Kume S., Uzu T., Horiike K., Chin-Kanasaki M., Isshiki K., Araki S. I., Sugimoto T., Haneda M., Kashiwagi A., Koya D. Calorie restriction enhances cell adaptation to hypoxia through Sirt1-dependent mitochondrial autophagy in mouse aged kidney. The journal of clinical investigation. 2010. Vol. 120. No. 4. P. 1043-1055. DOI: 10.1172/JCI41376.

13. Kanfi Y., Naiman S., Amir G., Peshti V., Zinman G., Nahum L., Bar-Joseph Z., Cohen H.Y. The sirtuin SIRT6 regulates lifespan in male mice. Nature. 2012. Vol. 483. No. 7388. P. 218-221. DOI: 10.1038/nature10815.

14. Lee S.H., Lee J.H., Lee H.Y., Min K.J. Sirtuin signaling in cellular senescence and aging. BMB reports. 2019. Vol. 52. No. 1. P. 24-34. DOI: 10.5483/BMBRep.2019.52.1.290.

15. Rusakova E.A., Kosyan D.B., Myroshnikov S.A. SIRT3, SIRT1 as potential candidate genes for the regulation of lipid metabolism in cattle. Animal husbandry and fodder production. 2018. Vol. 101. No. 4. P. 35-42.

16. Xu C., Cai Y., Fan P., et al. Calorie restriction prevents metabolic aging caused by abnormal SIRT1 function in adipose tissues. Diabetes. 2015. Vol. 64. P. 1576–1590. DOI: 10.2337/db14-1180.

17. Sun T., Li X., Zhang P., Chen W.D., Zhang H.L., Li D.D., Deng R., Qian X.J., Jiao L.J., Ji J., Li Y.T., Wu R.Y., Yu Y., Feng G.K., Zhu X.F. Acetylation of Beclin 1 inhibits autophagosome maturation and promotes tumour growth. Nature communications. 2015. Vol. 6. P. 7215. DOI: 10.1038/ncomms8215.

18. Qiu G., Li X., Wei C., Che X., He S., Lu J., Wang S., Pang K., Fan L. The Prognostic Role of SIRT1-Autophagy Axis in Gastric Cancer. Disease markers. 2016. Vol. 2016. DOI: 10.1155/2016/6869415.