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Effects of phycocyanin administration on some hormones and thermogenic factors related to metabolism in old mice

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Maintaining metabolic homeostasis in elderly individuals is crucial for preventing metabolic diseases, as age-related metabolic irregularities, including insulin resistance, are common. Phycocyanin (PC), a phycobiliprotein derived from Spirulina, is known for its various bioactive properties and its applications in medicine, pharmacy, aquaculture, and the cosmetic industry. Given its potential as a nutritional supplement, this study aimed to investigate the effects of PC on key metabolic-regulating hormones; ghrelin, leptin, adiponectin, and resistin and thermogenic factors; SIRT1 and UCP1 in old mice. A total of 15 male C57BL/6 mice, aged 12-15 months, were randomly assigned to two groups: a control group (n=7) and a phycocyanin-administered group (n=8). PC was administered through drinking water at a dose of 150 mg/kg for a period of two weeks. Blood serum and adipose tissue samples were collected for subsequent analysis of hormone and thermogenic factor levels. According to our results, PC a significant increased adiponectin levels (1.98 ng/L) and a significant decreased ghrelin levels (1.48 ng/L). Considering the metabolic changes mediated by adiponectin in muscle and liver tissue, the fact that PC increases adiponectin levels in old mice is promising that it can compensate for some metabolic irregularities, especially insulin resistance, that develop with old age. Although the clinical effect of PC in reducing ghrelin levels in aged mice is not fully known, it may be useful in preventing weight gain that occurs with aging.

Keywords: Spirulina, Aging, Leptin, SIRT1

Microalgae are used in different areas due to their rich nutritional content and effects on health and have been consumed by people as food and food additives for centuries. The nutritional composition of microalgae consists of different chemical and biological compounds such as carbohydrates, proteins, vitamins (A, B1, B2, B3, B6, B12, C, E, biotin, folic acid and pantothenic acid), minerals (Na, K, Ca, Mg, Fe, Zn), lipids, antioxidants and other trace elements[1,2].

Spirulina, which has an important place among microalgae, is a cyanobacteria that contains various bioactive substances. It is used as a nutritional supplement in the form of tablets, capsules or dried powder[3]. Phycocyanin (PC), a phycobiliprotein produced from *Spirulina sp*., is a blue-colored, odorless, non-toxic, water-soluble, natural color substance with strong fluorescent properties. This water-soluble protein pigment is used as a nutritional supplement, colorant, and fluorescent probe in many countries. It also has uses in medicine, pharmacy, aquaculture and the cosmetic industry[4,5]. Additionally, PC has hepatoprotective, neuroprotective, nephroprotective, cardioprotective, immunomodulatory, anti-inflammatory, antioxidant, antibacterial, anti-angiogenic, antidiabetic, anti-tumor and anti-proliferative properties[5, 6].

In this study, the effects of PC on ghrelin, leptin, adiponectin and resistin, which are important hormones in terms of metabolic regulation, were investigated. Ghrelin is a peptide hormone containing 28 amino acids that is produced by the gastrointestinal system and regulates appetite and body weight depending on eating behavior through the effect of the central nervous system[7]. Ghrelin is produced mostly by X/A cells with endocrine functions located in the oxyntic mucosa of the stomach and in small amounts by the intestine, kidney, pituitary gland, placenta and hypothalamus[8,9]. Ghrelin level in humans increases before eating and decreases to its lowest 90 min after eating. Ghrelin stimulates hyperglycemia and reduces insulin levels, while hyperglycemia and insulin reduce ghrelin levels[10].

Leptin is a peptide hormone containing 167 amino acids. It is secreted from fat tissue and transported to the brain by blood and has its effect on reducing appetite by stimulating receptors in the hypothalamus[11]. Studies have shown that leptin is a very important hormone in regulating body weight and food intake in both animals and humans. In addition, it has been stated that a decrease in leptin may cause an increase in the amount of body fat in humans[12]. The half-life of leptin, which is considered to be the hormone affecting the feeling of satiety, is approximately 25 min in humans, 3 to 10 min in rats,

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and 1-3 h in mice. In addition to being produced in white adipose tissue and a small amount in brown adipose tissue[13], it has been reported that leptin is also released from the liver, stomach, breast tissue, bone marrow, intestine, ovary, testes, skeletal muscle, gastric fundus and placenta[14-16].

Adiponectin, produced mainly from white adipose tissue, is a protein that increases insulin sensitivity. There is an inverse relationship between circulating leptin concentration and insulin sensitivity and adiponectin level. In general, while the amount of leptin increases, there is a decrease in the level of adiponectin[17]. It has been shown that adiponectin, an adipocyte-derived hormone, works on energy balance in muscle and liver tissue by increasing insulin sensitivity[18]. It suppresses gluconeogenetic enzymes and glucose production rate in the liver, but has no effect on glucose uptake rate and glycolysis or glycogen synthesis[19].

Resistin was identified as a fat tissue-specific hormone in 2001 while investigating the effect of antidiabetic drugs on gene expression. It was named resistin because it stimulated insulin resistance in the first studies conducted with mice. It is a cysteine-rich protein containing 108 amino acids and weighing 12.5 kDA[20]. However, unlike mice, the primary source of circulating resistin in humans is cells other than adipocytes, including peripheral blood mononuclear cells, macrophages, and bone marrow cells. Additionally, inflammatory conditions affect circulating resistin levels. As new studies are performed, the debate regarding the role of resistin in the development of IR and obesity in humans continues to rage. It is thought that factors such as the fact that the studies were conducted in healthy, obese or people with metabolic diseases, gender differences, etc. may have played a role in the seemingly contradictory results obtained in various published studies. However, it is thought possible that resistin is a biomarker for IR or contributes to IR only in certain situations[21].

In this study, the changes of SIRT1 and UCP1 proteins in brown adipose tissue as a result of PC application were also investigated. Sirtuin1 (SIRT1), a member of the sirtuin family of NAD+-dependent protein deacetylases, has played important roles in the regulation of gene expression, programmed cell death, DNA repair, cancer, metabolism, reproduction and aging mechanisms. SIRT1 is a key cellular energy sensor and a mediator of the beneficial effects of caloric restriction in some animal models[22]. SIRT1 is also involved in the regulation of glucose and fat metabolism[23,24]. Sirtuins are believed to have important roles in aging and even life extension. SIRT1 is a conserved histone deacetylase with widespread effects on cellular metabolism and aging. It protects cells from exposure to reactive oxygen species (ROS), which directly affects aging[25]. Many studies have been conducted to examine the effects of SIRT1 in mammalian tissues and organs. As a result of overexpression of SIRT1 in the hearts of transgenic mice; The aging of the heart is delayed and oxidative stress and apoptosis are prevented. In light of recent data, it is thought that SIRT1 reduces aging in the organism and increases stress resistance[26].

Mammals have brown and beige thermogenic adipocytes that are both rich in mitochondria and express uncoupling protein 1 (UCP1). UCP1 is localized to the mitochondrial inner membrane. There is significant evidence that UCP1 is crucial in brown adipose tissue thermogenesis and systemic energy homeostasis. Mitochondrial uncoupling protein UCP1 (Uncoupling Protein), called thermogenin, is responsible for the release of energy as heat by the oxidation of fatty acids in adipocytes. Many studies have investigated whether UCP1 is required for thermogenesis in thermogenic adipocytes. UCP1 knockout (KO) mice are unable to maintain body temperature and develop hypothermia upon acute cold challenge[27].

This study aimed to determine the levels of ghrelin, leptin, adiponectin and resistin hormones in serum and SIRT1 and UCP1 levels in adipose tissue in old mice fed a normal diet and in old mice given PC (150mg/kg) as a nutritional supplement. There are no studies in the literature regarding the effects of PC on the parameters in this study in old mice.

Materials and Methods

Supply and care of experimental animals

This study was carried out in accordance with the decision numbered 883 dated 20.04.2022 taken from Burdur Mehmet Akif Ersoy University (MAKU) Experimental Animals Local Ethics Committee. C57BL/6 male mice were obtained from Burdur Mehmet Akif Ersoy University (MAKU) Experimental Animal Production and Experimental Research Laboratory, with an age range of 12-15 months and a similar weight. Following the acclimatization process, experimental applications

lasted for 2 weeks, and throughout the entire study, the housing and care of the mice were carried out within the Burdur Mehmet Akif Ersoy University Experimental Animal Production and Experimental Research Center. The ambient temperature in the care rooms was maintained at 24±1°C, and the humidity ranged between 50-55%. All procedures were conducted in accordance with national and international guidelines. The mice were randomly selected and divided into two groups: control (n = 7) and phycocyanin (n = 8) group. The body weights of the mice were measured and recorded at the beginning and end of the experiment. Feed and liquid intake were allowed *ad-libitum* for both groups throughout the study.

Mice in the control group were fed with standard mouse chow and drinking water until the end of the study. The initial and final weights of the mice were measured and recorded. Phycocyanin was mixed into the drinking water of the phycocyanin group at a dose of 150 mg/kg. The average weight of the mice was 20 grams, and their daily water consumption was observed to be approximately 4 mL. A phycocyanin dose of 150 mg/kg/day was targeted, and a solution containing 3 mg of phycocyanin in 4 mL of water was prepared. For this purpose, 187.5 mg of phycocyanin was added to 250 mL of water. The amount of phycocyanin (P2201, TCI America™) to be used in the study was determined based on previous studies[28-31].

Termination of the study, collection of blood and fat tissue

At the end of 2 weeks of application, the mice were fasted for 10 h before euthanasia. After the mice were anesthetized with intraperitoneal injection of thiopental sodium (50 mg/kg), blood was taken from the hearts of the mice. Following blood collection, euthanasia was performed by cervical dislocation, and then fat tissues were taken from the interscapular regions of the mice. Blood obtained intracardiac from mice was centrifuged at 3000 rpm for 10 min to obtain blood serum. Serum was stored at -20 °C until analysis, and fat tissues were stored at -80 °C before homogenization.

Tissue homogenization

For homogenization, 0.01 M PBS buffer at *p*H 7.4 was prepared. Tissue and PBS buffer were added to the tube at a ratio of 1:9 (w/v). After ultrasonic digestion, refrigerated centrifugation was performed at 3000 rpm for 20 min and the supernatant was stored at -20 °C before analysis.

Measurement of hormones and thermogenic factors levels

Levels of ghrelin, leptin, adiponectin and resistin hormones and thermogenic factors SIRT1 and UCP1 were determined by ELISA analysis. Blood serum was used to analyse hormones, and homogenized adipose tissue supernatants were used to analyse thermogenic factors. Ghrelin (E1133Mo, Bt-Lab), leptin (E0625Mo, Bt-Lab), adiponectin (E0246Mo, Bt-Lab), resistin (E0263Mo, Bt-Lab) ELISA kits were used for analyses. For thermogenic factors, uncoupling protein 1 (UCP1) (E1465Mo, Bt-Lab) mouse NAD-dependent protein deacetylase Sirtuin-1 (SIRT1) (E1563Mo, Bt-Lab) ELISA kit was used. Analyzes were performed by following the instructions for the kits. The data were analyzed using the Mann-Whitney U test with the IBM SPSS (version 26.0) statistical program.

Results and Discussion

In the control group, no significant change was observed between the initial (20.70±1.30 g) and final (20.70±0.76 g) body weights ($P > 0.05$). In contrast, a statistically significant increase was detected in the phycocyanin-administered group between the initial (22.29±1.70 g) and final (22.36±1.22 g) body weights ($P = 0.023$, $P < 0.05$) (Table 1). Leptin, ghrelin, adiponectin, resistin, SIRT1 and UCP1 values measured by ELISA method to investigate the metabolic effects of PC administration in C57BL/6 mice aged between 12-15 months are shown in Table 2 & Table 3. As a result of the statistical analysis performed with the Mann-Whitney test, statistical differences were observed in the levels of ghrelin and adiponectin hormones in the blood serum of the control and phycocyanin-administered groups (P <0.05). There was no statistically significant difference in leptin, resistin, SIRT1 and UCP1 levels between the control and phycocyanin groups (P >0.05).

Phycocyanin, one of the main pigment components of *Spirulina sp*. used as a natural food source and also a natural edible pigment, used as a food ingredient

Table 1 — Effects of phycocyanin administration on body weight in aged mice

ΔWeight (g)	Control	Phycocyanin
Initial Body Weight	20.70±1.30[a]	22.29±1.70[a]
Final Body Weight	20.70±0.76[a]	22.36±1.22[b]

[Data are presented as mean value ± standard deviation. P< 0.05 was considered statistically significant. [a],[b]Different superscripts in the same column indicate differences between groups (independent samples t test)]

Table 2 — Hormone values in phycocyanin and control groups

Groups	Leptin (ng/L)	Ghrelin (ng/L)	Adiponectin (ng/L)	Resistin (ng/L)
Control	2.47±0.31[a]	1.6±0.07[a]	1.79±0.08[a]	2.83±0.04[a]
Phycocyanin	2.51±0.39[a]	1.48±0.08[b]	1.98±0.07[b]	2.8±0.03[a]
P value	.655	.032	.002	.141

[Data are presented as mean value ± standard error. $P< 0.05$ was considered statistically significant. [a,b]Different superscripts in the same column indicate differences between groups (Mann-Whitney Test)]

Table 3 — Thermogenic factor levels in Phycocyanin and Control groups

Groups	SIRT1 (ng/L)	UCP1 (ng/L)
Control	2.42±0.27[a]	2.39±0.07[a]
Phycocyanin	2.43±0.19[a]	2.37±0.2[a]
P value	.949	.949

[Data are presented as mean value ± standard error. $P< 0.05$ was considered statistically significant. [a,b]Different superscripts in the same column indicate differences between groups (Mann-Whitney Test)]

and health product, is known to exhibit a number of physiological and pharmacological activities without causing toxicity and harm[32].

There are either no articles in the literature about the effects of PC on the levels of some molecules whose levels were investigated in this study, and a very limited number of articles were found for some of them. There are a few articles in the literature regarding the effects of Spirulina on these parameters, but none of them are related to elderly individuals. Generally, there are several publications on the effects of Spirulina on obese, diabetic individuals or its effects in combination with various exercises, etc.

Phycocyanin is known for its various metabolic effects, including its influence on body weight regulation[33]. While previous studies have predominantly reported that phycocyanin reduces body weight in young and adult rodents[34-36], our findings suggest that its effects in aged mice differ. This variation may be due to age-related metabolic changes that alter the physiological response to phycocyanin. Factors such as slowed energy metabolism, changes in nutrient absorption, and differences in inflammatory processes in older individuals could contribute to the age-dependent modulation of phycocyanin's impact on body weight.

In this study, PC was shown to increase adiponectin hormone levels in mice. Adiponectin, an adipocyte-derived hormone, increases fatty acid oxidation in skeletal muscle[37]. It is also known as a hormone that reduces hepatic glucose production and increases insulin sensitivity[38,39]. It has been shown that adiponectin works on energy balance in muscle and liver tissue by increasing insulin sensitivity. It suppresses gluconeogenetic enzymes and glucose production rate in the liver, but has no effect on glucose uptake rate and glycolysis or glycogen synthesis[19]. Therefore, increasing adiponectin levels is expected to lead to metabolically beneficial consequences.

Few studies in the literature show the effects of Spirulina and PC on adiponectin. In the study conducted by Ichimura *et al*., a statistically non-significant increase in serum adiponectin level was observed in rats fed a PC-rich diet[40]. Another study conducted in 2008 found that Spirulina increased adiponectin levels[41]. Oriquat and his colleagues also observed in their study published in 2019 that Spirulina caused an increase in adiponectin levels[42].

Although there are publications showing that Spirulina increases the level of the leptin hormone[43], there are more studies showing that it decreases it[44-46]. Studies on PC are very few. In the study of Omar *et al*. published in 2022, it was determined that PC application in chickens did not change plasma leptin levels. In our study, it did not create a statistically significant change[47].

The effect of Spirulina on ghrelin has generally been tested in combination with resistance exercises or other diets. There is no article in the literature where Spirulina or PC is applied in a similar way to ours and its effect on serum ghrelin levels. It has been observed that Spirulina applied with exercise increases ghrelin levels[48]. A study conducted in fish found that Spirulina increased ghrelin expression when taken as a supplement[49]. In our current study, PC reduced ghrelin levels in old mice. The clinical impact of decreasing ghrelin levels is unclear[50].

There are also a few articles on the effect of Spirulina on resistin levels. In their study, Akbarpour & Samari found that serum resistin levels decreased in type 2 diabetic patients given Spirulina[51]. In our study, no significant change was observed in resistin levels.

It was shown in a study conducted in 2018 that Spirulina causes SIRT1 activation[52]. It was also shown in another study that Spirulina peptides increase the amount of SIRT1[53]. There is little information in the literature about the *in-vivo* effects of Spirulina. The little information about PC is based on cell culture studies. PC increased the levels of SIRT1, which is reduced by hydrogen peroxide, in mesenchymal stem cells treated with hydrogen peroxide in cell culture[54].

It was observed that the ethanol extract of Spirulina increased the UCP1 level in obese mice[55]. In the study of de Mattos *et al*., it was determined that Spirulina reduced UCP1 expression in fish[56]. No publications have been found regarding the direct effects of PC. In our study, no effect of PC was detected on UCP1 and SIRT1 levels.

Considering the metabolic changes mediated by adiponectin in muscle and liver tissue, the fact that PC increases adiponectin levels in aged mice gives hope that it may compensate for some metabolic disorders that develop with old age. Although the clinical effect of PC in reducing ghrelin levels in aged mice is not fully known, it may be useful in preventing weight gain that occurs with old age.

Conclusion

This study investigated the effects of phycocyanin (PC) supplementation on metabolic regulatory hormones and thermogenic factors in aged mice. Our findings indicate that PC supplementation increases adiponectin levels while decreasing ghrelin levels. Although previous studies have generally reported that PC reduces body weight in young and adult rodents, the results obtained in this study suggest that the metabolic effects of PC may differ in aged individuals. This implies that age-related metabolic changes may alter the physiological response to PC.

The aging process is associated with a slowdown in energy metabolism, changes in nutrient absorption, and differences in inflammatory response mechanisms, all of which may influence the effects of PC on metabolic parameters. Therefore, PC is considered to have potential as a dietary supplement to support metabolic homeostasis in elderly individuals. However, long-term studies involving different age groups are necessary to better understand the mechanisms underlying these effects. In particular, comprehensive research is needed to evaluate the long-term effects of PC on age-related metabolic changes.

Conflict of interest

The authors declare no competing interests.

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