https://doi.org/10.46389/rjd-2022-1180
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2022; volume 29, issue 4, pages 395-400

Original Article

Astaxanthin administration reduces body weight and abdominal fat weight, but does not lower the neutrophil-lymphocyte ratio in obese male rats (*rattus norvegicus*)

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Received: 31 May 2022 / Accepted: 4 August 2022

Abstract

Obesity has been known as global health. Astaxanthin is an antioxidant which also has an anti-obesity effect. This research aimed to identify the effect of astaxanthin on body weight, abdominal fat weight, and neutrophil-to-lymphocyte ratio (NLR) in obese male *Wistar* rats. A group of male *Wistar* rats was divided into three and fed either a standard or high-fat diet. Twenty-eight days later, rats in the high-fat diet group were divided into two and treated with astaxanthin 6 mg/kg body weight in glycerin or glycerin alone. On the 74th day, body weight and abdominal fat weight were measured. 1cc blood sample was obtained from medical canthus sinus orbitalis to perform a routine blood count (NLR). The results showed that body weight in the treatment group was lower than in the placebo group ($p<0.001$ 95%CI 41.16–82.84). Consistently, the abdominal fat weight in the treatment group was also lower in the placebo group ($p<0.009$ 95%CI 0.34–2.13). Furthermore, NLR's mean was not statistically significant ($p<0.118$). Astaxanthin reduces body weight and abdominal fat weight but does not lower NLR in obese male *Wistar* rats.

Keywords: astaxanthin, obesity, abdominal fat, neutrophil, lymphocyte.

Introduction

Obesity has been an epidemic that has been challenging to global health. Obesity can reduce life expectancy and cause chronic diseases [1]. Obesity is also a condition that may cause a low degree of chronic inflammation [2]. Obesity is a pathologic condition that has been considered one of the biggest risk factors not only for various non-communicable diseases, such as type 2 diabetes mellitus, cholelithiasis, cardiovascular disease, nonalcoholic steatohepatitis, dyslipidemia, depression, obstructive sleep apnea, and malignancy but also for communicable diseases, such as severe degree of coronavirus disease 2019 (COVID-19) [3, 4]. Fat accumulation, especially in the abdomen area, plays an important role in the occurrence of metabolic disease in obesity [5].

The prevalence of obesity globally has almost tripled since 1975. In 2016, more than 1.9 billion adults were overweight and more than 650 million adults were obese [6]. The high prevalence of obesity has become the most worrying health and socioeconomic problem globally. In addition, obesity has a multifactorial etiology [3, 5]. Because of the dramatic rise in obesity, multiple approaches are applied to treat obesity. Lifestyle modifications, such as healthy diet and

physical activity, pharmacology, and bariatric surgery, are the treatment options for obesity. However, these approaches have some limitations [7].

Nowadays, several potential anti-obesity treatments have been investigated to treat obesity [7]. Astaxanthin has been evaluated as it has various pharmacologic effects on human health. Astaxanthin is a carotenoid pigment found in aquatic animals and some algae. Astaxanthin is widely found in the human diet, mainly in lobster, salmon, crustaceans, and other fish [8]. Furthermore, several studies exhibited that astaxanthin administration could reduce body weight, abdominal fat weight, and some inflammatory markers (TNF-α and IL-6) in high-fat high carbohydrate diet mice [9, 10].

Neutrophils and lymphocytes play a key role in inflammation and contribute as the first defense mechanism against infection. The neutrophil-to-lymphocyte ratio (NLR) is the ratio of absolute neutrophils to lymphocytes, which is also a prognostic factor of various diseases [11]. Unlike other inflammatory markers, NLR is inexpensive and can be routinely checked [12]. A previous study showed that NLR was positively correlated with visceral fat. Moreover, NLR correlated better with adipose tissue than other pro-inflammatory markers (TNF-α, IL-1β, leptin, and CRP) [13]. However, study about astaxanthin and NLR in obesity is still limited. Therefore, this study aims to determine the effect of astaxanthin on body weight, abdominal fat weight, and NLR in obese rats.

Material and methods

This was an experimental study using a randomized post-test-only control group design. This study was conducted in the Integrated Biomedical Laboratory of the Faculty of Medicine Universitas Udayana, Bali. This research was carried out from February 2022 to March 2022.

Thirty male *Wistar* rats, at 6–8-week age, were purchased from the Faculty of Medicine Universitas Udayana Bali animal unit. Rats were housed individually in a standard laboratory rat cage under a 12-hour light-dark cycle and hygienic conditions (250C and 70% humidity). The animals obtained a standard diet and water *ad libitum*. After one week of acclimatization, the rats were assigned randomly into 3 groups (n=10). The first group (P0) was treated with a standard diet (days 1 to 28). The placebo (P1) dan treatment group (P2) were treated with a high-fat diet (days 1 to 28). At the end of 28 days, the Lee index was obtained. P0 (Lee index<0.3) was treated with a standard diet. P1 (Lee index>0.3) received a standard diet and 1 cc glycerin as a placebo. P2 (Lee index>0.3) obtained standard diet and oral astaxanthin 6 mg/kg body weight. After 45 days of treatment, body weights, abdominal fat weights, and NLR were measured. Body weights were measured by using the Sartorius scale.

Examination of NLR

The rats were anesthetized before taking the blood sample using Ketamine 10% (50 mg/kg body weight) and xylazine 2% (10 mg/kg body weight) intramuscularly. The blood samples were obtained from medial canthus sinus orbitalis. The routine blood check was conducted in a standard animal laboratory (Balai Besar Veteriner Denpasar).

Examination of abdominal fat weight

The rats were euthanatized intracardially using Ketamine 10% (50 mg/kg body weight) and xylazine 2% (10 mg/kg body weight). A midline incision on the abdominal wall was done from the sternum to the symphysis pubis. Then, both subcutaneous (inguinal fat) and visceral (perirenal, retroperitoneal, mesenteric, and epididymal) abdominal fat were found. Abdominal fat weights were measured by using the Sartorius scale.

Data analysis

Statistical analysis was conducted using SPSS (Statistical Package for Social Science) software. Data were expressed as mean+SD (standard deviation), median, minimum, and maximum. All data were tested normality using Shapiro Wilk and homogeneity using Levene's Test. One-way ANOVA was performed to identify significant differences between groups. The LSD (Least Significant Different) Post Hoc Test would be conducted if the tests were statistically significant. P values less than 0.5 were considered significant statistically.

Results

Three rats were dropped out, while 27 rats remained in this study. The descriptive analysis of the data is shown in Table 1. The mean body weight in the treatment group was 207.33+16.87 grams, while the mean body

https://doi.org/10.46389/rjd-2022-1180

Table 1: Descriptive analysis.

Groups	Mean	Standard Deviation	Median	Minimum	Maximum
Body weight (gram)					
Control (P0)	181.44	18.15	182	155	215
Placebo (P1)	269.33	16.46	268	245	291
Treatment (P2)	207.33	16.87	205	183	236
Abdominal fat weight (gram)					
Control (P0)	0.09	0.05	0.09	0.00	0.17
Placebo (P1)	1.27	0.89	1.22	0.06	3.17
Treatment (P2)	0.04	0.02	0.04	0.00	0.07
Neutrophil-to-Lymphocyte Ratio (NLR)					
Control (P0)	0.45	0.19	0.43	0.22	0.85
Placebo (P1)	0.56	0.21	0.58	0.26	0.84
Treatment (P2)	0.35	0.22	0.30	0.11	0.83

weight in the placebo group was 269.33+16.46 grams. The mean abdominal fat weight in the treatment group was 0.04+0.02 grams, whereas the mean abdominal fat weight in the placebo group was 1.27+0.89 grams. The mean of NLR in the treatment and placebo groups were 0.35+0.22 and 0.56+0.21, respectively.

One-way ANOVA results showed that the mean of both body weight and abdominal fat weight groups were statistically different (p=0.001). The Post Hoc LSD test showed that the body weight in the treatment group was significantly lower than in the placebo group (p=0.001 95%CI 45.29–78.71) (Figure 1). Furthermore, the Post Hoc LSD test also revealed that the abdominal fat weight in the treatment group was significantly lower than in the placebo group (p=0.001 95%CI 2.3–3.88) (Figure 2). However, One-way ANOVA exhibited that the mean of NLR between groups was not statistically different with p=0.118 (Figure 3).

Discussion

Obesity is a condition of excess adipose tissue which links to morbidity and mortality [14]. Obesity has a multifactorial etiology, which is an interaction among genetics, social, behavioral, and environmental factors [3]. High energy consumption and a sedentary lifestyle have increased obesity prevalence [15, 16].

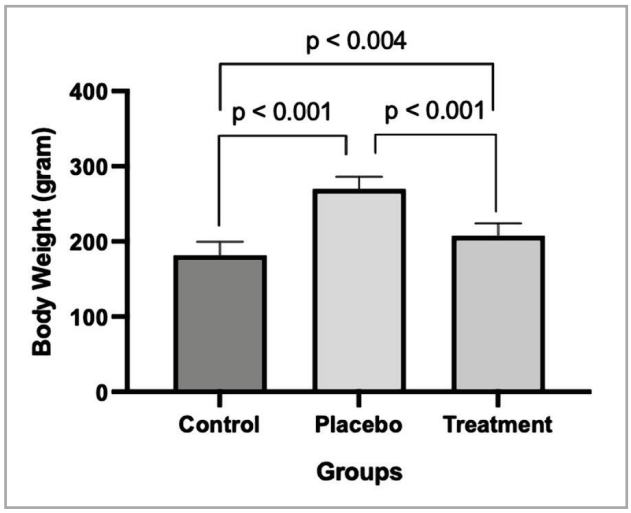

Figure 1: One-way ANOVA and post-hoc body weight data.

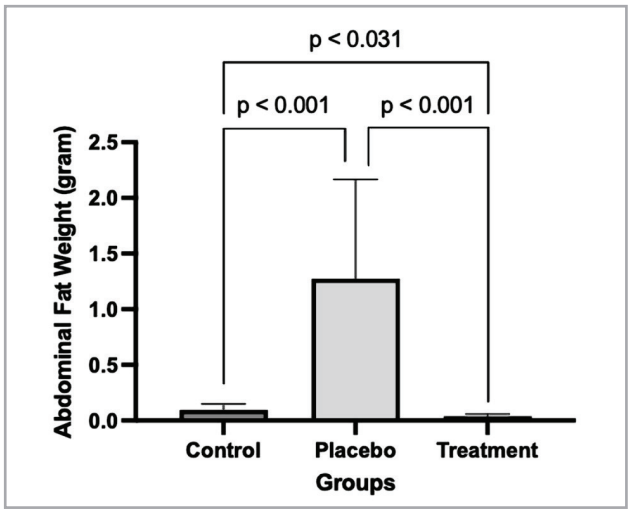

Figure 2: One-way ANOVA and post-hoc abdominal fat weight data.

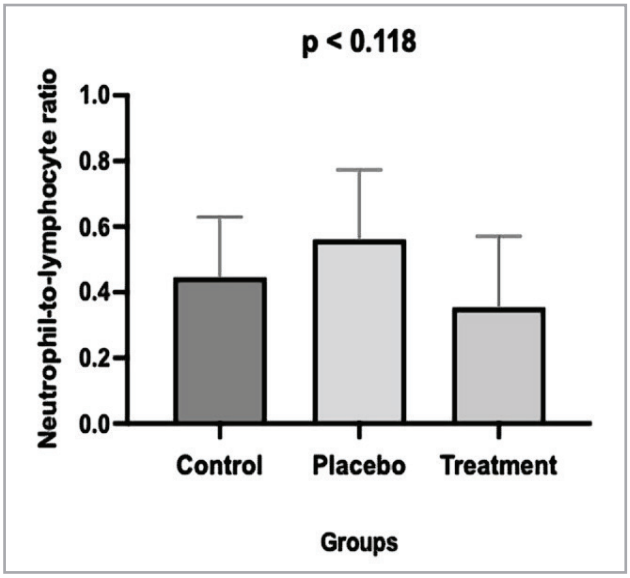

Figure 3: One-way ANOVA and post-hoc NLR data.

Obesogenic environments, such as lack of recreational activity and high consumption of high carbohydrate, high fat diet and sugary drinks, can cause calory imbalance [17]. Nevertheless, some medical conditions can also cause obesity, including Cushing's syndrome, hypothyroid, insulinoma, craniopharyngioma, and the list continues [14].

Energy consumption and energy expenditure are the basic mechanisms in calory balance. Obesity is a condition of imbalanced energy consumption that is higher than energy expenditure [18, 19]. Obesity is usually related to macronutrient metabolism impairment, mainly fat and carbohydrate [3]. The excess energy is converted to triglyceride in adipose tissue. Hydrolysis of triglyceride produces free fatty acid. Plasma-free fatty acid commonly increases obesity and causes adipose tissue mass enlargement, also resulting in increased body weight and abdominal fat weight [18, 19].

Excess adipose tissue develops over time relates to excess energy intake. In addition, excess adipose tissue will be transported to various body compartments as weight gains [19]. There are two kinds of adipose tissue in humans: white adipose tissue and brown adipose tissue. Anatomically, white adipose tissue consists of subcutaneous and visceral adipose tissue [20]. Subcutaneous adipose tissue commonly stores more fat in various anatomic sites. Visceral adipose tissue generally links with metabolic disturbances and complications related to obesity [19].

Obesity is usually followed by increasing macrophages and other immune cells in adipose tissue [19]. The number of accumulated macrophages in adipose tissue in obese individuals links with body weight, body mass index, and total body fat [21]. The immune cells produce pro-inflammatory cytokines, which result in insulin resistance associated with obesity. Moreover, adipocytes and macrophages produce pro-inflammatory adipokine in adipose tissue, which leads to a low degree of systemic inflammation in obesity [19].

Therefore, foods containing antioxidants and anti-inflammation are important to consider as they can prevent complications associated with obesity [22]. Astaxanthin is a red-orange oxycarotenoid pigment and lipid soluble [23]. Astaxanthin is synthesized by phytoplankton or algae, accumulated in zooplankton, crustaceans, and substantial in fish [24]. Astaxanthin belongs to a group of carotenoids called xanthophylls. The absorption of carotenoids is similar to the absorption of fat [25]. The carotenoid absorption from diet occurs by passive diffusion into the intestinal epithelium and is facilitated by lysophosphatidylcholine, produced by pancreatic phospholipase A2. Carotenoids are merged with chylomicrons following absorption and transported to the liver by a blood vessel or lymph [26]. Xanthophylls, including astaxanthin, are secreted with high-density lipoprotein (HDL) and in a small amount with very low-density lipoprotein (VLDL) [25].

Peroxisome proliferated-activated receptors (PPARs) are nuclear receptors belonging to the superfamily of steroid/thyroid receptor hormone and activated by ligands to induce gene transcription, which regulates carbohydrate, lipid, and protein metabolism. There are three isoforms of PPAR: PPAR-α, PPAR-γ, and PPAR-δ/β [27]. Astaxanthin may increase the level of adiponectin and decrease the level of leptin. PPAR-γ regulates adiponectin. The dysregulation of the PPAR receptor may relate to obesity. Astaxanthin can bind to PPAR-γ by inhibiting PPAR-γ ligand (rosiglitazone) and PPAR-γ transcriptional activity. Furthermore, activation of PPAR-δ/β by astaxanthin can reduce fat accumulation in adipocytes [28].

Our study exhibits that administration of astaxanthin reduces body weight ($p<0.001$ 95%CI 45.29–78.71) and abdominal fat weight ($p<0.001$ 95%CI 2.30–3.88) in diet-induced obese rats. Consistently, previous studies also found that astaxanthin could reduce body weight ($p<0.05$) and epididymal fat weight ($p<0.05$) in high fat-high fructose diet mice significantly compared to control [9]. Furthermore, astaxanthin was also reported that it could significantly reduce the weight gain ($p<0.05$) and body fat index ($p<0.01$) in high-fat diet mice significantly compared to control [10].

Adipose tissue contributes to initiating and maintaining systemic inflammation. Adipose tissue enhanced

https://doi.org/10.46389/rjd-2022-1180

various immune responses, including neutrophils in early stage and followed by macrophage and mast cell involvement. The increment of neutrophils in adipose tissue suggests that obesity is related to systemic inflammation [29]. However, NLR is not statistically different from our findings ($p<0.118$). In our study, NLR is the highest in the placebo group, while NLR is the lowest in the treatment group. This may be caused by the absence of complications associated with obesity in this study.

The previous study reported that astaxanthin administration reduced pro-inflammatory cytokines, such as TNF-α and IL-6. The study was conducted in insulin resistance-associated obese mice. However, the present study does not compare astaxanthin's effect in complications associated with obese mice. Therefore, further study should be done to compare the effect of astaxanthin on NLR in the obese group with and without complications.

Conclusion

Obesity has been the most alarming health problem and is associated with a low degree of systemic inflammation. Our study proves that astaxanthin administration reduces body weight and abdominal fat weight but does not reduce NLR in diet-induced obese rats.

Acknowledgments

The authors are greatly thankful to Mr. I Gede Wiranatha, S.Si, who helped the authors take care of the rats and to the Balai Besar Veteriner Denpasar staff, who contributed to helping the authors conduct the routine blood check.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This research has been ethically reviewed by the committee ethics of Faculty of Medicine of Universitas Udayana, Denpasar, Bali, to obtain ethical clearance (B/248/UN14.2.9/PT.01.04/2021).

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