Effects of astaxanthin supplementation in healthy and obese dogs

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Background: Since astaxanthin (ASX) has potent antioxidative effects with inhibitory action of lipid peroxidation and singlet oxygen quenching activity, it is widely used as a functional food for keeping good health in human. Obesity is a risk factor for various metabolic disorders. It is characterized by low-grade chronic inflammation based on oxidative stress by excessively produced ROS. From the point of preventive medicine, natural compounds have been proposed as potential therapeutic agents in the prevention of metabolic disorder in companion animals. The purpose of this study is to evaluate the effects of ASX supplementation in healthy and obese dogs.

Materials and methods: Ten healthy beagle dogs and 5 clinically obese dogs were used in this study. The healthy beagle dogs were randomly divided into 2 groups as follows: control and test groups. The test group dogs received ASX supplementation mixed with the food for 6 weeks. Five clinically obese dogs received ASX supplementation for 8 weeks. Metabolites, hormones and enzymes were measured before and after ASX supplementation.

Results: In the healthy dog groups, after 6 weeks, plasma triglyceride (TG) and malondialdehyde concentrations and lactate dehydrogenase (LDH) values significantly decreased in the test group. There was no significant difference in the control group. In clinically obese dogs, plasma TG concentration decreased after 8 weeks of ASX supplementation. Plasma alanine aminotransferase and LDH values clearly decreased in all 5 dogs and 4 dogs out of 5 dogs, respectively.

Conclusion: ASX supplementation (0.3 mg/kg body weight/day) for 6 weeks in healthy dogs and 8 weeks in obese dogs induced the elevation of antioxidant function and of liver function by ameliorating lipid metabolism.

Keywords: astaxanthin, obese dogs, lipid metabolism, anti-oxidative activity, liver function

Introduction
The incidence of obesity and its associated diseases has been increased in dogs and cats as well as in human.1,2 Since obesity causes physical inactivity and oxidative stress related diseases that are induced by obesity-based metabolic syndrome, body weight (BW) reduction is required for obese animals. However, the satisfactory result of weight reduction is limited in the animals with pathological obesity induced by accumulated visceral fat, causing slight systemic inflammation.3 Fat accumulation and oxidative stress impair the function of mitochondria via morphological alteration, increased membrane peroxidation, decreased ATP level, increased ROS production, defective mitochondrial β-oxidation and increased mitochondrial permeabilization.4 Increase in circulating nonesterified fatty acids (NEFAs) due to excessive accumulated...
visceral fat is confirmed in obese animals. Fat accumulation triggers free radical production and insults additional inflammation. Excess amount of ROS is also produced via accelerated β-oxidation of fatty acids. Such overproduced ROS is attributed to one of the pathogens for obesity and its associated diseases. Consequently, some antioxidants appear to be effective to ameliorate obesity conditions in animals. Experimental studies of mice administered obesity-inducing diet combined with ASX showed anti-diabetic and anti-obesity effects by improved insulin (INS) sensitivities and liver function. The study showed the suppression of fat tissue weight gain by ASX in a dose-dependent manner.

*Haematococcus pluvialis*, known as an important source of natural astaxanthin (ASX), is a freshwater microalga belonging to the family *Chlamydomonadaceae*. When the alga experiences environmental stress conditions, ASX is created and acts like a force field that protects the nuclear DNA and lipids against UV-induced oxidation. ASX (3,3′-dihydroxy-β,β′-carotene-4,4′-dione) is a nontoxic and organic fat-soluble xanthophyll carotenoid. In comparison to other phytochemicals, ASX has previously been reported to possess a significantly greater antioxidant function, with its antioxidant activities quantified like a force field that protects the nuclear DNA and lipids against UV-induced oxidation. ASX accumulates in the liver, especially in the microsomal and mitochondrial fractions of the liver tissue. This substance has been shown to prevent oxidative damage to the liver, improve metabolic profiles, and reduce hepatic inflammation. From the above, excessive fat accumulation and oxidative stress and liver function are closely related.

In this study, we measured plasma metabolites and hormone concentrations and enzyme activities involved in energy metabolism in healthy and obese dogs with ASX supplementation for several weeks. The purpose of this study was to evaluate the effect of ASX supplementation in obese and healthy dogs.

### Materials and methods

#### Animals

Ten healthy beagle dogs and 5 clinically obese dogs were used in this study. Their body condition score (BCS) was evaluated by the 5-point scale system (1, very thin; 2, underweight; 3, ideal; 4, overweight and 5, obese). BCS of 10 healthy dogs was 3. The average age of them was 2 years (1–3 years), and the average BW was 10.4 kg (9.7–11.1 kg). They were randomly divided into 2 groups: control group (dog no 1–5) and test group (dog no 6–10). As preparation of the study, they were given commercial diet (Nippon Pet Food Co., Ltd., Tokyo, Japan) for 2 months. The nutrient composition of the food is crude protein 21.7%, crude fat 10.1%, crude ash 6.3%, crude fiber 2.7%, linoleic acid 1.72%, moisture 8.7% and nitrogen-free extract 50.5%. All the dogs were kept under controlled conditions and professionally supervised at Narita Animal Science Laboratory Co., Ltd. (Narita, Japan) prior to and during the study period. Ethical approval for this study was obtained from Narita Animal Science Laboratory co., Ltd. Research Animal Ethical Committee (17-C042).

Five clinically obese dogs were recruited from local primary veterinary practice as shown in Table 1. The selected 5 dogs met the following 3 conditions: 1) BW must be more than 20% of its ideal weight; 2) symptoms of chronic disease are properly managed and 3) owner is reliable, and good compliance can be received with the complete consent. Case numbers 1, 2 and 3 have been treated for their chronic diseases by certain therapeutic agents for years, and the clinical signs have been well controlled for long time. Case numbers 4 and 5 have taken no medications.

#### ASX supplementation

ASX that is *H. pluvialis* biomass (AstaReal® AW1011; Astar Real Inc., Moses Lake, WA, USA) was used in this study. In the healthy beagle dogs, 5 test group dogs were given 1 dose of

### Table 1 Profiles of clinically overweight and obese dogs

<table>
<thead>
<tr>
<th>Case Number</th>
<th>0W</th>
<th>8W</th>
<th>0W</th>
<th>8W</th>
<th>0W</th>
<th>8W</th>
<th>0W</th>
<th>8W</th>
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<tbody>
<tr>
<td>Breed</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Clinical complication</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW (Kg)</td>
<td>10.0</td>
<td>11.3</td>
<td>15.4</td>
<td>15.6</td>
<td>7.3</td>
<td>7.2</td>
<td>25.5</td>
<td>25.5</td>
<td>59.5</td>
<td>59.6</td>
</tr>
<tr>
<td>Ideal weight (Kg)</td>
<td>7</td>
<td>11</td>
<td>6</td>
<td>4</td>
<td>15</td>
<td>35</td>
<td></td>
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<td>BCS</td>
<td>5</td>
<td>5</td>
<td>4</td>
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<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

**Abbreviations:** BCS, body condition score; BW, body weight; NF, neutered female; NM, neutered male; W, weeks.
0.3 mg/kg ASX with a meal per day. BW and BCS were measured every week, and the ASX supplement dose was adjusted. For clinical cases, 0.3 mg /kg /day of ASX was given in a single dose or in divided doses with the food on a daily basis.

Blood sampling
Fasting blood samples were collected before initiation of the study, after 6 weeks in healthy dogs, and after 8 weeks in clinically obese dogs, respectively. Collected blood was dispensed in a heparinized tube and centrifuged at 400 x g for 10 minutes at 4°C to collect plasma. Plasma was stored at –80°C until use.

Metabolite, hormone and enzyme analyses
Glucose (GLU), total cholesterol (TC), triglyceride (TG), total protein (TP), blood urea nitrogen (BUN), creatinine (CRE) concentrations and alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were measured using an auto-analyzer (JCA-BM2250; JEOL, Tokyo, Japan) and lactate dehydrogenase (LDH) activities were measured using the NEFA-C test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma malondialdehyde (MDA) concentration was measured using the NWLSS™ Malondialdehyde assay kit (Northwest Life Science Specialties, LLC, Vancouver, Canada). Plasma INS, adiponectin (ADN) and TNFα were measured by the Rat Insulin ELISA kit (AKRIN-010T; Shibayagi Co., Gunma, Japan), mouse/rat adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and TNFα ELISA kit (LS-F1347-1; Life Span Bioscience, Inc, Seattle, WA, USA), respectively.

Statistical analysis
All values were calculated using Microsoft Excel. The data were expressed as mean±standard error (SE). Statistical analysis was performed using the 2-tailed, paired t-test. Statistical significance was designated as *P<0.05, and a high level of significance was designated as **P<0.01.

**Results**
Comparisons of biomarker levels in healthy dogs in the control group and the test group are shown in Table 2. The values of plasma TG and MDA concentrations and LDH significantly decreased in the test group dogs (*P<0.05, **P<0.01), and there was no significant difference in the control group. After 6 weeks, TGs significantly decreased (*P<0.05) in the test group, MDA and LDH also significantly decreased in

### Table 2 Comparison of biomarkers level of healthy dogs with and without astaxanthin supplementation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>without ASX (n=5)</th>
<th>6 weeks</th>
<th>with ASX (n=5)</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>10.5 ± 0.2</td>
<td>11.3 ± 0.3</td>
<td>10.3 ± 0.2</td>
<td>11.1 ± 0.3</td>
</tr>
<tr>
<td>Body condition score</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>87.4 ± 1.8</td>
<td>79.8 ± 2.1</td>
<td>90.6 ± 2.5</td>
<td>87.4 ± 2.3</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>30.6 ± 1.3</td>
<td>38.0 ± 6.6</td>
<td>37.2 ± 1.3</td>
<td>23.6 ± 4.1</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>144.8 ± 20.0</td>
<td>131.6 ± 8.9</td>
<td>113.8 ± 6.5</td>
<td>133.6 ± 9.1</td>
</tr>
<tr>
<td>NEFA (mEq/L)</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.1 ± 0.1</td>
<td>7.0 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>6.6 ± 0.1</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>13.4 ± 0.7</td>
<td>12.0 ± 0.7</td>
<td>12.4 ± 0.7</td>
<td>10.2 ± 0.6</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Malondialdehyde (µmol/L)</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>0.9 ± 0.0***</td>
</tr>
<tr>
<td>Insulin (ng/mL)</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>18.4 ± 4.0</td>
<td>27.5 ± 3.3</td>
<td>27.0 ± 6.3</td>
<td>30.5 ± 4.3</td>
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<tr>
<td>TNF-α (pg/mL)</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>32.2 ± 1.8</td>
<td>35.2 ± 2.4</td>
<td>37.2 ± 1.3</td>
<td>33.6 ± 1.5</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>28.5 ± 4.1</td>
<td>30.2 ± 5.2</td>
<td>31.6 ± 1.2</td>
<td>36.4 ± 2.9</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>184.0 ± 46.3</td>
<td>166.6 ± 36.0</td>
<td>152.4 ± 8.7</td>
<td>152.6 ± 8.7</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>83.4 ± 5.7</td>
<td>113.6 ± 12.6</td>
<td>89.4 ± 6.3</td>
<td>57.4 ± 2.4***</td>
</tr>
</tbody>
</table>

**Notes:** Data are presented as the mean ± SE. Statistical significance is indicated by asterisks. *Significantly different (p<0.05) from the value at 0 week in the test group with ASX (paired t-test). **Significantly different (p<0.01) from the value at 0 week in the test group with ASX (paired t-test). ***Significantly different (p<0.01) from the value at 6 weeks of the control group without ASX (paired t-test).

**Abbreviations:** ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ASX, astaxanthin; LDH, lactate dehydrogenase; NEFA, non-esterified fatty acid; SE, standard error; SE, standard error.
the test group ($P<0.01$) (Figure 1). Moreover, upon comparing control and test groups in 6 weeks, the values of MDA and LDH significantly decreased ($P<0.01$) in the test group ($P<0.01$) (Figure 1). In healthy dogs, with or without ASX supplementation, plasma GLU, TC, NEFA, TP, BUN, CRE, INS, ADN, AST and ALP values showed no significant change before and after 6 weeks of trail (Table 2).

The results of obese dogs are shown in Table 3. TG values clearly decreased after 8 weeks of ASX supplementation, and ALT and LDH values also remarkably decreased in all 5 dogs and in 4 out of the 5 dogs, respectively. Plasma GLU, TC, NEFA, TP, BUN, CRE, INS, ADN, AST and ALP values showed no major change in clinically obese dogs on ASX supplementation after 8 weeks.

Although statistical comparison is impossible, simple comparison of the TG, ALT, MDA and LDH values of the healthy dog groups with individual obese dogs is done (Figure 2). The change before and after ASX supplementation was more pronounced in obese dogs. In each obese dog, ALT values were always higher than those of healthy dogs regardless of ASX supplementation.

BW and BCS showed no changes after ASX supplementation.

**Discussion**

Obesity is characterized by low-grade chronic inflammation. This continuous inflammation due to obesity induces severe metabolic disorders such as hypertension, vascular disorders, diabetes mellitus and others. Increased circulating NEFAs from accumulated visceral fat cause inflammation and INS resistance by directly activating plasma membrane receptors, such as toll-like receptor 4, followed by elevation of inflammatory reaction via NF-κβ. On the other hand, excessive amount of NEFA enhances overproduction of ROS in the process of β-oxidation of fatty acids in the mitochondria of various tissues. Overproduction of ROS induces oxidative stress. Consequently, systemic inflammatory components were confirmed in obesity. Adipose tissue, a population of adipocytes, not only acts as an energy reservoir but also has physiological activities such as angiogenesis and wound healing, and adipocytes produce and secrete adipokines involved in energy metabolism. Accumulated visceral fat in obese animals induces high concentrations of plasma NEFA, circulating C-reactive protein (CRP) and MDA. Excessive amount of visceral fat is suspected as a contributing factor to various metabolic disorders in obese animals. From the above mentioned findings, antioxidant substances are considered to decrease oxidative stress and to ameliorate metabolic disorders caused due to obesity.

Obese animals encountered in veterinary practice are individually unique in their background. In general, dietary supplements and functional foods are often recommended for animals that require the health care intervention from veterinary professionals, such as aging, chronic diseases and...
critical obesity as well. Considering these circumstances, obese dogs participated in this experiment were various age, sex, and species.

In this study, ASX supplementation showed clear antioxidative effects in both healthy and obese dogs. In the healthy dogs, after ASX supplementation for 6 weeks, plasma TG and oxidative stress biomarker MDA concentrations decreased significantly. ASX revealed antioxidant activity in healthy dogs. In the same way, plasma TG, MDA, ALT and LDH values decreased, especially, TG and ALT values remarkably decreased. Antioxidant effects of ASX supplementation in obese dogs are more apparent than those in the healthy dogs. Since those positive effects were observed in all obese dogs with different underlying diseases, it is considered that ASX supplementation could be effective on the antioxidant activity and also improve the hepatic function.

ASX prevents diseases in heart and kidneys and liver from oxidative stress, in addition ASX can decrease plasma MDA concentrations and improve the pathological signs of animal diabetic nephropathy. In this study, ASX supplementation indicated to be effective on elevation of antioxidant function and on amelioration of metabolic functions in liver. Long-term intake of ASX inhibits the elevations in BW and adipose tissue weight caused by a high-fat diet in mice tested for 60 days. Chronic ASX administration significantly improve increased body weight, hyperglycemia, hyperinsulinemia and increased plasma levels of TNF-α and IL-6 observed in the study of obese model mice. However, ASX supplementation does not influence BW reduction in obese dogs in this study. In the mice experiments, ASX was given in relatively high dose such as 6, 12 and 30 mg/kg for 60 days. The dose of ASX used in this experiment was set up based on the standard recommended dose to human. Considering the lifespan of the mouse, the duration of 60 days for the mouse corresponds to 1 year or more in the dog. We speculate that longer administration period in obese dogs of more than 2 months could bring the farther positive effect. ASX improved oxidative stress biomarkers by suppressing lipid peroxidation and stimulating the liver function. However, weight reduction effect could not be achieved only by supplementation of ASX to obese dogs.

Moreover, safety of ASX supplementation is advocated by EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) in previous report. Considering that ASX has wide safety range, continuous supplementation of ASX seems to be effective to prevent the prevalence

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Changes in biomarkers level of clinically overweight and obese dogs with astaxanthin supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case Number</td>
<td>1</td>
</tr>
<tr>
<td>Breed</td>
<td>Miniature Dachshund</td>
</tr>
<tr>
<td>Age (years)</td>
<td>10</td>
</tr>
<tr>
<td>Sex</td>
<td>NM</td>
</tr>
<tr>
<td>Clinical complication</td>
<td>Hypothyroidism, Cushing syndrome</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>10</td>
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<tr>
<td>Body condition score</td>
<td>5</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>87</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>89</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>162</td>
</tr>
<tr>
<td>NEFA (mEq/L)</td>
<td>0.54</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>8.2</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dL)</td>
<td>23</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.6</td>
</tr>
<tr>
<td>Malondialdehyde (μmol/L)</td>
<td>1.63</td>
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<tr>
<td>Insulin (ng/ml)</td>
<td>3.6</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>10.8</td>
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<tr>
<td>AST (IU/L)</td>
<td>31</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>250</td>
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<tr>
<td>ALP (IU/L)</td>
<td>1460</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>68</td>
</tr>
</tbody>
</table>

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ASX, astaxanthin; LDH, lactate dehydrogenase; NEFA, non-esterified fatty acid; NF, neutered female; NM, neutered male; NT, not tested; W, weeks.
of visceral fat-type obesity. Further studies with different doses of ASX supplementation are necessary to clarify the usefulness of ASX as an antioxidant supplement in a large number of dogs with different severities of obesity.

**Conclusion**

ASX supplementation (0.3 mg/kg BW/day) in food for 6 weeks in healthy dogs and for 8 weeks in obese dogs effectively activated antioxidant function and liver function followed by improved lipid metabolism.

**Acknowledgments**

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**Disclosure**

Kumi Tominaga is a researcher working in AstaReal Co.Ltd. The authors report no other conflicts of interest in this work.

**References**

   restricts weight gain, promotes insulin sensitivity and curtails fatty
   liver disease in mice fed a obesity-promoting diet. Process Biochem.
   2010;45(8):1406–1414.
11. Ikeuchi M, Koyama T, Takahashi J, Yazzawa K. Effects of astaxanthin
   production in Haematococcus pluvialis grown under different culture
13. Kurashige M, Okimasa E, Inoue M, Utsumi K. Inhibition of oxidative
   injury of biological membranes by astaxanthin. Physiol Chem Phys
14. Shimidzu N, Goto M, Miki W. Carotenoids as singlet oxygen quenchers
15. Miki W. Biological functions and activities of animal carotenoids. Pure
   of astaxanthin in several tissues and plasma lipoproteins in male broiler
   chickens fed a yeast (Phaffia rhodozyma) with a high concentration of
17. Showalter LA, Weimann SA, Østerle M, Lockwood SF. Plasma appearance
   and tissue accumulation of non-esterified, free astaxanthin in C57BL/6
   mice after oral dosing of a disodium disuccinate diester of astaxanthin
   tocellular injury following ischemia/reperfusion. Toxicology.
19. Ferramosca, A, Di Giacomo M, Zara V. Antioxidant dietary approach in
   treatment of fatty liver: new insights and updates. World J Gastroenterol.
   2017;23(23):4146–4157.
20. Islam MA, Al Mamun MA, Faruk M. Astaxanthin ameliorates hepatic
   damage and oxidative stress in carbon tetrachloride-administered rats.
21. DeFronzo RA. Insulin resistance, lipotoxicity, type 2 diabetes and
22. Prado NJ, Ferder L, Manucha W, Diez ER, Raul Diez E. Anti-inflammatory
   effects of melatonin in obesity and hypertension. Curr Hypertens
   expression and signaling in muscle from insulin-resistant subjects.
   kappaB and stimulation of inhibitor kappaB by troglitazone: evidence
   for an anti-inflammatory effect and a potential antithrombotic effect
25. Evans JL, Goldfine ID, Maddux BA, Gродsky GM. Oxidative stress and
26. Dorresteijn JA, Visseren FL, Spiering W. Mechanisms linking obesity to
27. Renna NF, Diez ER, Lembo C, Miattolo RM. Role of Cox-2 in vascular
   inflammation: an experimental model of metabolic syndrome. Mediators
   hormone leptin is a direct regulator of aldosterone secretion, which
   promotes endothelial dysfunction and cardiac fibrosis. Circulation.
   visceral obesity and postoperative inflammatory response following
   3651–3657.
   acid on insulin responsiveness and insulinemia in visceral adipose
   tissue from obese individuals: possible role for PTP1B. Int J Obes.
   oxidative stress in heart and kidneys of isoproterenol-administered aged
32. Zhu X, Chen Y, Chen Q, Yang H, Xie X. Astaxanthin promotes Nrf2/
   ARE signaling to alleviate renal fibronectin and collagen IV accumulation
33. Arunkumar E, Bhuvaneswari S, Anuradha CV. An intervention study
   in obese mice with astaxanthin, a marine carotenoid: effects on insulin
   signaling and pro-inflammatory cytokines. Food Funct. 2012;3(2):
   120–126.
34. European Food Safety Authority. Scientific opinion on the safety and
   other fish, ornamental fish, crusrtaceans and ornamental birds. EFSAJ
35. Brown DR, Gough LA, Deb SK, Sparks SA, McNaughton LR. Astaxan-
   thin in exercise metabolism, performance and recovery: a review.
   Front Nutr. 2018;4:76.