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Astaxanthin lowers blood pressure and lessens the activity of the renin-angiotensin system in Zucker Fatty Rats

Harry G. Preuss[a,*], *Bobby Echard*[a], *Debasis Bagchi*[b], *Nicholas V. Perricone*[c], *Eiji Yamashita*[d]

[a]*Departments of Physiology, Medicine and Pathology, Georgetown University Medical Center, Basic Science Building, Room 231B, 3900 Reservoir Road, Washington, DC 20057, USA*
[b]*Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX 77204, USA*
[c]*Michigan State University College of Human Medicine, East Lansing, MI 48824, USA*
[d]*Life Science Division, Fuji Chemical Industry Co., Ltd., Toyama 930-0397, Japan*

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ABSTRACT

The ability of astaxanthin to favorably influence the renin-angiotensin system (RAS), blood pressure (BP), and metabolic parameters in Zucker Fatty Rats (ZFR) was examined. In separate experiments, 96 ZFR were equally divided into four groups: control, captopril (30 mg/kg), low astaxanthin (5 mg/kg) and high astaxanthin (25 mg/kg). RAS and insulin systems were examined following recovery from heat stress. RAS was lower in test groups; however, there was no evidence of enhanced insulin sensitivity. Test groups decreased SBP (systolic blood pressure) significantly compared to the control. The tests carried out suggested that RAS was involved in the ability of astaxanthin to lower BP. Astaxanthin at high dosage influenced circulating TNF-α and MCP-1 and lessened fat oxidation in liver and kidneys. Thus, astaxanthin may be considered as a good stress reducer with regards to heat stress. Astaxanthin's effects on RAS indicate it might overcome perturbations associated with increased activity, especially those related to the cardiovascular system.

1. Introduction

Astaxanthin, a natural carotenoid antioxidant with an impressive safety record, occurs in a wide variety of living organisms (Torrissen et al., 2000; Naguib, 2001; Goto et al., 2001). Previous studies have indicated that *per os* consumption of astaxanthin can lower blood pressure, delay the incidence of stroke, and favorably affect many of the perturbations of the metabolic syndrome in spontaneously hypertensive rats (SHR) (Hussein et al., 2005a,b, 2006; Karagiannis et al., 2007). In contrast, blood pressure (BP) was not significantly lowered in normotensive Wistar rats (Hussein et al., 2005a,b). Because the mechanisms behind the decrease in elevated BP are uncertain, the primary purpose of the present investigation was to examine the ability of oral astaxanthin to favorably influence the renin-angiotensin system (RAS). Other constituents of the metabolic syndrome in Zucker Fatty Rats (ZFR) were also studied.

2. Materials and methods

The Animal Welfare Board at Georgetown University Medical Center approved the protocol for this investigation. For two experiments, a total of 96 Zucker Fatty Rats (ZFR) (Charles Rivers, Wilmington, MA) were used: 48 in each experiment were divided into four groups of 12 rats. Initial body weights ranged

* *Corresponding author:*
E-mail address: preusshg@georgetown.edu (H.G. Preuss).
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between 434 and 624 g in the first experiment and between 388 and 520 g in the second. All rats consumed a regular rat diet, Purina rat chow, in crushed form (Purina, Richmond, IN). Sucrose was added to the pulverized rat chow (30%, w/w). The four groups consisted of a control eating the described diet and three test groups eating the same diet, but with the addition of captopril or astaxanthin (two different doses). Captopril was added to the feed at 30 mg/kg, astaxanthin at 5 mg/kg to the feed of the group designated as Lo Asta, and astaxanthin at 25 mg/kg to the feed of the group designated as Hi Asta. The amount of astaxanthin added was based on 2% (w/w) content of astaxanthin to powder. The first study continued for 57 days, while the second continued for 75 days.

2.1. Body weight (BW)

BW was estimated by routine scale measurements. Two readings taken at least 10 min apart on a given day had to be within 2 g of each other, or the procedure was repeated until the weight measurements were within this range. Water and food intakes were estimated by subtracting the volume or weight of the remaining fluid and food from the amounts premeasured 24 h earlier.

2.2. Systolic blood pressure (SBP)

SBP was measured by tail plethysmography (Bunag, 1973) using two different instruments. As in many previous studies, an instrument from Narco Biosciences (Houston, TX) was used (Preuss & Preuss, 1980; Preuss et al., 1988). This allowed us to rapidly measure SBP with a beeper sound system. The second reading was performed on an instrument obtained from Kent Scientific Corporation (Torrington, CT). The latter is a computerized, non-invasive tail cuff acquisition system that utilizes a specially designed differential pressure transducer to noninvasively measure the blood volume in the tail. This instrument not only records SBP, but also provides measurements of mean blood pressure (MBP), diastolic blood pressure (DBP), and cardiac rate. The two instruments were used interchangeably. Previous experience has shown that the SBP readings were virtually the same by either instrument (Perricone et al., 2008). Rats were allowed free access to their diet and water until SBP readings were obtained between 13.00 and 17.00 h after a slight warming. Multiple readings on individual rats were taken. To be accepted, SBP measurements on a given rat had to be stable (consistently within 5 mm Hg).

2.3. Blood chemistries

Blood for chemical analysis was obtained at the end of the study following removal of food 4 h earlier. Blood chemistry values were obtained via dry chemistry procedures using a Johnson and Johnson Vitros 250 instrument (Johnson and Johnson, Langhorne, PA).

2.4. Intraperitoneal glucose tolerance test (ipGTT)

During the ipGTT, glucose (2.5 g/kg BW) was injected intraperitoneally (i.p.) to challenge the tolerance to glucose. Drops of blood were obtained from the tail at 0, 30, 60, 120, 180 and 240 min post injection. Glucose was estimated using commercial glucose strips (Lifescan, One Touch Ultra, Melitas, CA).

2.5. Insulin challenge testing (ICT)

Testing was commenced after 17–19 h of food deprivation. For ICT, 0.6 unit of regular insulin/kg BW (Eli Lilly Co., Indianapolis, IN) was administered, and blood for glucose determination was obtained from the tail vein at 7.5 min after injection (Chang et al., 2006; Preuss et al., 2007; Williams et al., 1999, 2000). Glucose was estimated using commercial glucose strips (Lifescan, One Touch Ultra, Melitas, CA).

2.6. Losartan challenge

After performing baseline SBP readings, SHR from all dietary groups were given 40 mg/kg losartan orally via gastric lavage (Wong et al., 1991). Three and six hours after lavage, SBP was remeasured. The decreased SBP after losartan was used to estimate activity of the RAS (Mohamadi et al., 2000).

2.7. Serum angiotensin-2 levels

Serum angiotensin-2 was trapped by octylminicolumns (Amprep-C8, 500 mg, Amersham, Buckingshire, UK). Each minicolumn was prewashed with 5 mL of 100% methanol and then with 10 mL of 0.1% trifluoroacetic acid (TFA) in distilled water. After the serum was passed through the minicolumn, it was washed with 0.1% TFA again. The trapped peptides were eluted with 3 mL of methanol/water/TFA (80%:19.9%:0.1%, v/v/v). Samples were collected and dried in a vacuum centrifuge and dissolved in 0.1 M Tris–acetate buffer (pH 7.4) containing 2.6 mM disodium salt of ethylenediaminetetra-acetic acid (EDTA), 1 mM phenylmethylsulphonyl fluoride, and 0.1% bovine serum albumin (Mohamadi et al., 2000).

Angiotensin-2 was measured by radioimmunoassay using an angiotensin-2 antibody (Peninsula Laboratories Inc., Belmont, CA). The sensitivity of the assay is 1.0 pg/tube. The 500-fold diluted angiotensin-2 antibody (100 μL) was added to the sample (400 μL) and standard tubes that were incubated 24 h at 4 °C. The ([3-^{125}I] iodotyrosyl) angiotensin-2 (Peninsula) 100 μL was reconstituted in 0.1 M Tris–acetate buffer, and was added to each tube at a concentration of approximately 15,000 cpm with further incubation 24 h at 4 °C. On the third day, 300 μL of 1.25% bovine-albumin was added to each tube and mixed, and 800 μL of 25% polyethylene glycol 8,000 was added and mixed once again. After centrifugation at 1500*g* for 30 min at 4 °C, the supernatant was aspirated and the pellet was counted with a gamma-counter (Titertek, Huntsville, AL).

2.8. Serum angiotensin converting enzyme (ACE) activity

Serum ACE activity was measured by a commercial kit (Sigma Co. Ltd., St. Louis, MO) (Aviram & Dornfeld, 2001). This spectrophotometric method utilizes the synthetic tripeptide substrate N-[3-(2-furyl)acryloyl]-phenylalanylglycylglcine (FAPGG). FAPGG is hydrolyzed by ACE to furylacryloylphenylalanine

(FAP) and glycylglycine. Hydrolysis of FAPGG results in a decreased absorbency at 340 nm. Serum ACE activity was determined by comparing the sample reaction rate to that obtained with an appropriate ACE calibrator.

2.9. Cytokine assay

Various cytokines were measured by ELISA methodology using kits from the following sources: rat MCP-1 – Assay Designs Inc., Ann Arbor, MI; rat adiponectin – ALPCO Diagnostics, Salem, NH; TNF-a, ALPCO Diagnostics, Salem, NH; RAT IL-1B – Assay Designs Inc., Ann Arbor; RAT IL-6 – ALPCO Diagnostics, Salem, NH.

2.10. Organ weights

Organ weights were measured immediately after sacrifice of the rats.

2.11. Lipid peroxidation and DNA fragmentation

2.11.1. Assessment of lipid peroxidation

Thiobarbituric acid-reactive substances (TBARS) as an index of lipid peroxidation were determined on hepatic and kidney homogenates from control and treated animals according to the method of Buege and Aust (1978), and as previously reported (Bagchi et al., 1993), malondialdehyde (MDA) was used as the standard. Absorbance values were measured at 535 nm, and an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ was used (Buege & Aust, 1978; Bagchi et al., 1998).

2.11.2. Assessment of DNA fragmentation

Frozen liver and kidney samples were homogenized in lysis buffer (5 mM Tris–HCl, 20 mM EDTA (ethyleneoliaminetetraacetic acid), 0.5% Triton X-100, pH 8.0). Homogenates were centrifuged at 27,000*g* for 20 min to separate intact chromatin in the pellet from fragmented DNA in the supernatant fraction. Pellets were re-suspended in 0.5 M perchloric acid and 5.5 M perchloric acid was added to supernatant samples to reach a concentration of 0.5 M. Samples were heated at 90 °C for 15 min and centrifuged at 1500*g* for 10 min to remove protein. Resulting supernatants were reacted with diphenylamine for 16–20 h at room temperature. Absorbance was measured at 600 nm. DNA fragmentation in control samples was expressed as percentage of total DNA appearing in the supernatant fraction. Treatment effects are reported as percent of control fragmentation (Bagchi et al., 1998).

2.12. Statistical analyses

Results are presented as mean ± SEM of 4–6 experiments. SBP and BW were examined by repeated measures, two-way analyses of variance (one factor being group and the second factor being time of examination). Where a significant effect of regimen was detected by ANOVA ($p < 0.05$), the Dunnett *t* test was used to establish which differences between means reached statistical significance (Dunnett, 1955). Other measurements were assessed by one-way analyses of variance. Statistical significance was set at a $p < 0.05$.

3. Results

3.1. Experiment 1

3.1.1. Body weight (BW)

Over the initial 33 days of the first study, BW increased slightly over control in the test groups, i.e., ZFR receiving captopril, and low (Lo) and high (Hi) levels of astaxanthin, although these differences did not reach statistical significance at any timed measurements (Fig. 1).

3.1.2. Food and water intake

After three weeks on the various regimens, average food and water intake were similar in all groups. The possible exception was in the captopril group that showed a trend toward increased water intake (data not shown).

3.1.3. Systolic blood pressure (SBP)

During the first month, the SBP of the control group rose steadily from the initial reading of 126.7 mm Hg ± 1.7 (SEM)–145.8 mm Hg ± 2.1 (SEM) and remained around this level until the end of study (Fig. 2). On the 33rd day, all BPs of the test groups were significantly lower than those of control. The captopril and Hi Astaxanthin groups showed a significantly lower SBP over the last month; while there was a tendency for the SBP to be lower in the Lo Asta group.

3.1.4. Heat stress

On the 42nd day, the temperature of rodent quarters rose from 22 °F to the range of 30–32 °F. It was estimated that the rats were exposed to this elevated temperature for 5–6 h. By the time the rats were removed from the heat, approximately one third each of the control (4/12), captopril (4/11) and Lo

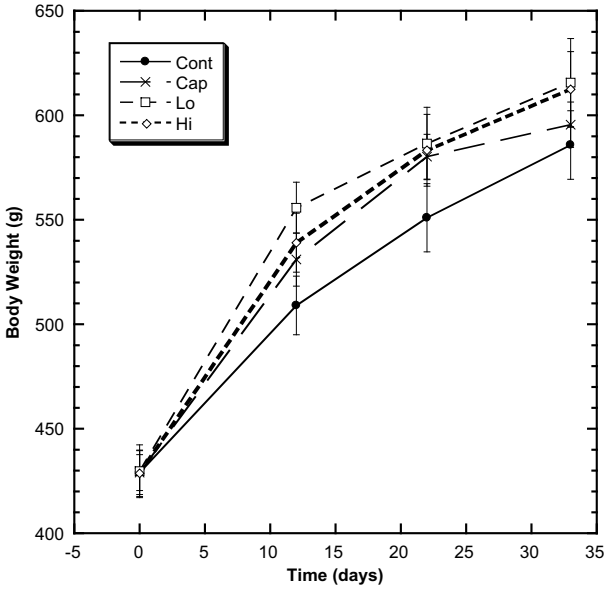

Fig. 1 – Body weight (BW) of rats in control (Cont), captopril (Cap), and low (Lo) and high (Hi) astaxanthin groups over a period of 33 days (EXPT 1). Data are expressed as mean ± SEM.

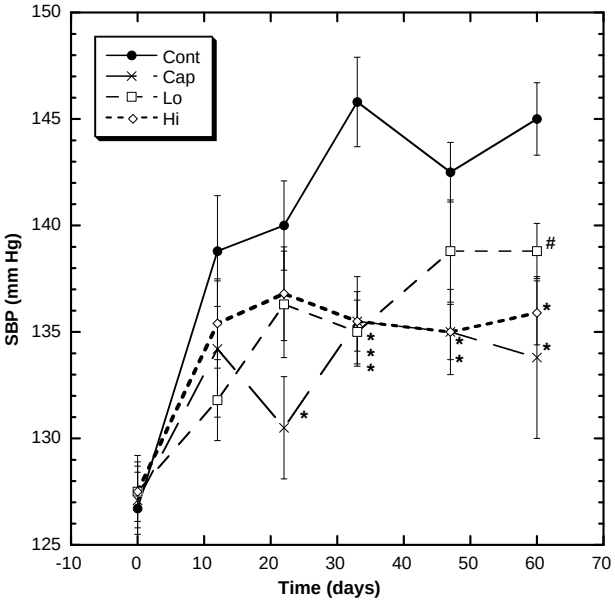

Fig. 2 – Systolic blood pressure (SBP) of rats in control (Cont), captopril (Cap), and low (Lo) and high (Hi) astaxanthin groups over a period of 60 days (EXPT 1). Data are expressed as mean ± SEM. After day 33, SBP represents remaining rats.

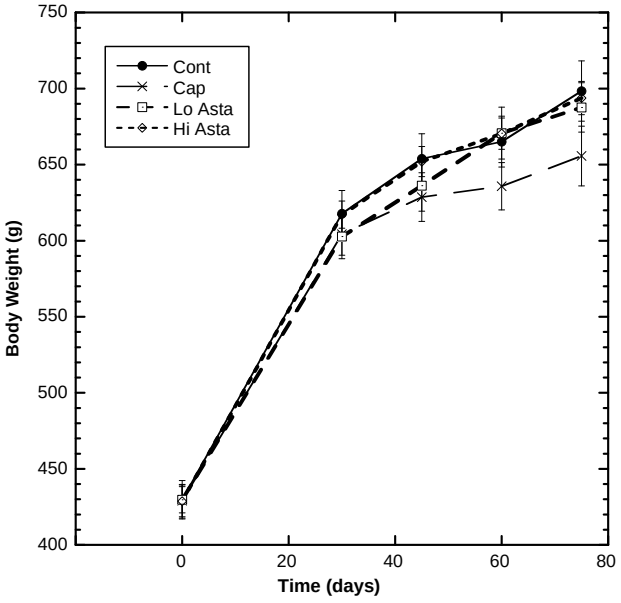

Fig. 3 – Body weight (BW) of rats in control (Cont), captopril (Cap), and low (Lo) and high (Hi) astaxanthin groups over a period of 77 days (EXPT 2). Data are expressed as mean ± SEM.

Asta (4/12) groups survived. In contrast all ZFR in the Hi Asta group survived (11/11).

3.1.5. *Tests after heat stress*

Twelve days after the heat stress, the average increase in BW among the surviving rats was not statistically different (see Table 1). The average SBP of the captopril and Hi Asta groups remained statistically lower than the control. Although the average SBP was lower than control in the Lo Asta group, this value was not statistically significant from the control. In the following week, two additional tests were run on the survivors. Compared to the control, SBP decreased significantly less after losartan challenge in the Hi Asta group showed a trend toward a lesser decrease in the captopril group, but was not significantly different in the Lo Asta group. Following an insulin challenge (ICT), the decreases in circulating glucose levels were not statistically different among the groups.

3.2. *Experiment 2*

3.2.1. *Body weight (BW)*

Over the 11 weeks of study, the average increase in BW was not statistically different among the four groups (Fig. 3). The control group did not show a tendency to have a lower body weight as in the first experiment.

3.2.2. *Food and water intake*

When food and water intake were measured at one month and two months, no statistically significant differences were seen among the groups (data not shown).

3.2.3. *Glucose tolerance test*

ZFR had high circulating glucose levels that showed marked differences among groups at baseline (Fig. 4a). Although not statistically different due to the wide range of readings, rats from the Hi Asta group had a higher average baseline glucose level (406 mg/dL ± 40.8 [SEM]) compared to the control (300 mg/dL ± 29.8 [SEM]), the captopril (302 mg/dL ± 46.1 [SEM]), and the Lo Asta (346 mg/dL ± 36.5 [SEM]) groups. Examining the area under the curve after glucose challenge for the four groups of six rats, the animals in the Hi Asta group had a statistically significant lower average reading than control. Baseline insulin concentrations were: control (0.92 ng/mL ± 0.09 [SEM]), captopril (0.82 ng/mL ± 0.06 [SEM]), Lo Asta (0.80 ng/mL ± 0.08 [SEM]), and Hi Asta (0.90 ng/mL ± 0.07 [SEM]). One hour after the glucose challenge, the

Table 1 – Changes in systolic blood pressure (SBP) and body weight (BW) after heat stress (12 days)

Group	#	Body weight (g)	SBP (mm Hg)	ICT (mg/dL glucose)	Losartan
Control	(4)	36.5 ± 7.8	146.3 ± 1.3	−8.8 ± 3.0	39.0 ± 2.4
Captopril	(4)	37.0 ± 18.4	133.8 ± 2.4*	−9.8 ± 3.5	29.0 ± 2.4#
Lo Asta	(4)	14.5 ± 13.2	140.0 ± 2.0	−8.5 ± 4.1	38.0 ± 3.2
Hi Asta	(11)	24.3 ± 6.8	133.5 ± 1.5*	−7.6 ± 2.5	28.0 ± 2.5*
ANOVA (p)		0.51	0.0005	0.97	0.020

#Trend toward significance; *statistically significant.

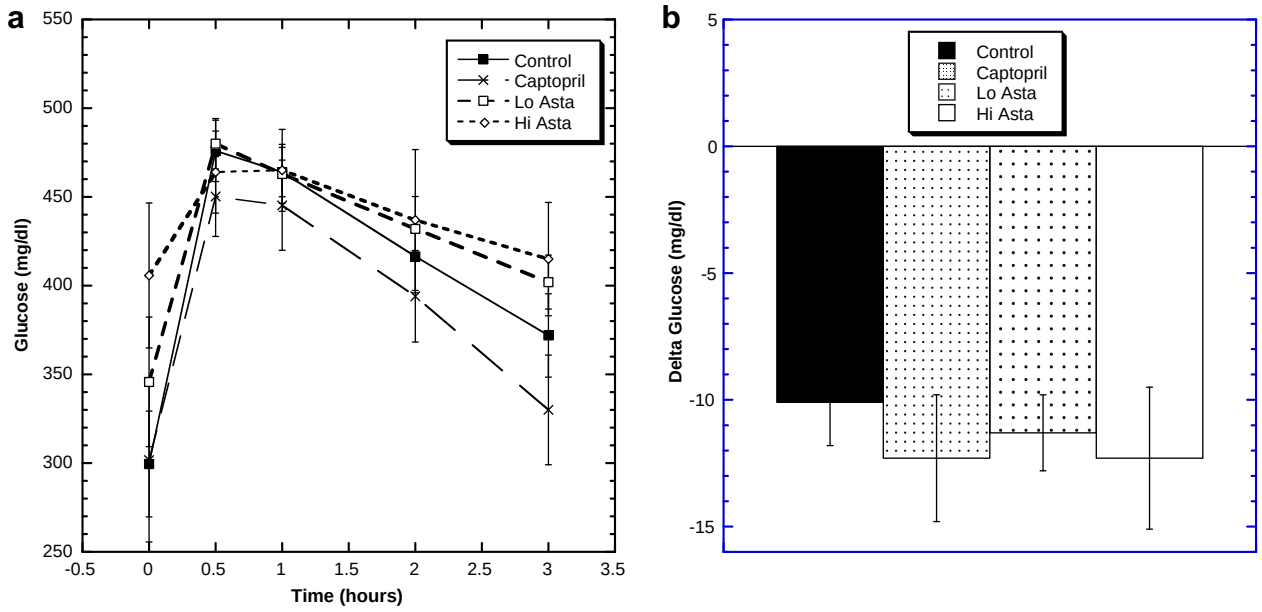

Fig. 4 – (a) Glucose tolerance test in control (Cont), captopril (Cap), and low (Lo) and high (Hi) astaxanthin groups after 70 days (EXPT 2). Data are expressed as mean ± SEM. (b) Insulin challenge test at end of study. Average ± SEM shown.

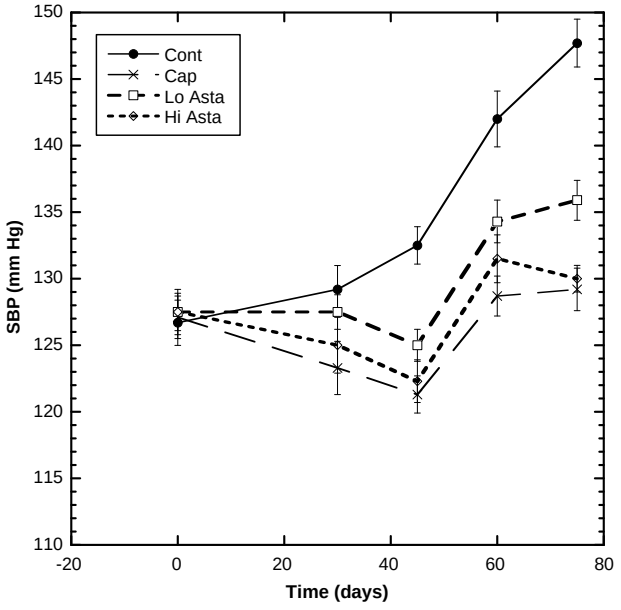

Fig. 5 – Systolic blood pressure (SBP) of rats in control (Cont), captopril (Cap), and low (Lo) and high (Hi) astaxanthin groups over a period of 75 days (EXPT 2). Data are expressed as mean ± SEM.

insulin concentrations were: control (2.50 ng/mL ± 0.22 [SEM]), captopril (2.37 ng/mL ± 0.14 [SEM]), Lo Asta (2.15 ng/mL ± 0.14 [SEM]), and Hi Asta (2.23 ng/mL ± 0.14 [SEM]).

3.2.4. *Insulin challenge test (ICT)*

Seven and one half minutes after i.p. challenge with 4 units of regular insulin, the average decrease in circulating glucose showed no significant difference among all groups (see Fig. 4b).

3.2.5. *Systolic blood pressure (SBP)*

By 40 days, the average SBP of the three test groups (captopril, Lo Asta and Hi Asta) was statistically significantly different, remaining significantly lower than control throughout the rest of the study (Fig. 5). At the end of study (75 days), the SBP of the captopril and Hi Asta groups was significantly lower than in the Lo Asta group. At 60 days, a more complete evaluation was performed as depicted in Table 2. While the SBP was lower in all the test groups, the diastolic blood pressure (DBP) was significantly lower only in the captopril and Hi Asta groups. No significant differences in cardiac rates were seen among the four groups.

3.2.6. *Evaluation of RAS system*

When losartan was given to each group of rats, the decrease in SBP of the three test groups was significantly less than the control. Examination of serum angiotensin converting enzyme (ACE) activity showed lesser activity in the captopril and Hi Asta groups, not in the Low Asta rats compared to control. Circulating angiotensin-2 concentrations were statistically lower in the captopril group, showed a trend toward a lower circulating level in the Hi Asta group and were not significantly different in the Lo Asta group compared to the control. These results are summarized in Table 3.

3.2.7. *Blood chemistries*

Among the values depicted, the only statistical differences and trends occurred in the blood urea nitrogen (BUN), potassium and creatinine levels in the Lo Asta group compared to control. All other chemistries (see Table 4) seemed essentially the same in all groups.

Table 2 – Cardiovascular readings at 60 days

Parameter	Control	Captopril	Lo Asta	Hi Asta
SBP (mm Hg)	142.0 ± 2.1	129.0 ± 1.5*	134.0 ± 1.6*	132.0 ± 1.8*
DBP (mm Hg)	111.0 ± 2.1	103.0 ± 1.8*	108.0 ± 2.1	102.0 ± 2.0*
Cardiac rate	413.0 ± 10.0	393.0 ± 9.3	387.0 ± 8.6	401.0 ± 10.6

Average ± SEM of 12 rats is shown; *statistically significant compared to control. SBP – systolic blood pressure, DBP – diastolic blood pressure.

Table 3 – Evaluation of renin-angiotensin system (RAS)

Parameter	Control	Captopril	Lo Asta	Hi Asta
Losartan (mm Hg)	42.5 ± 2.8	30.6 ± 1.8*	33.8 ± 2.8*	27.5 ± 1.3*
ACE activity (units)	29.9 ± 1.7	21.5 ± 1.2*	27.1 ± 1.4	24.2 ± 1.3*
Angio 2 (pg/mL)	204.0 ± 12.7	162.0 ± 6.4*	197.0 ± 7.2	177.0 ± 5.2#

Average ± SEM of 10 rats; *statistical significance compared to control ($p < 0.05$); #trend toward statistical significance (>0.05 < 0.10). ACE – angiotensin converting enzyme.

Table 4 – Blood chemistries

Parameter	Control	Captopril	Lo Asta	Hi Asta
Glucose	320.0 ± 14.6	327.0 ± 23.0	317.0 ± 30.6	327.0 ± 14.2
BUN	13.8 ± 0.3	14.0 ± 0.5	25.3 ± 5.4*	15.0 ± 0.5
Creatinine	0.5 ± 0.02	0.4 ± 0.02	0.6 ± 0.90#	0.5 ± 0.01
Sodium	140.0 ± 0.5	140.0 ± 0.5	141.0 ± 0.7	142.0 ± 0.8
Potassium	5.9 ± 0.06	6.0 ± 0.04	6.5 ± 0.04	6.0 ± 0.13
Chloride	98.9 ± 1.3	98.6 ± 1.0	98.4 ± 0.7	98.9 ± 0.8
CO_2	24.8 ± 1.0	24.1 ± 0.9	22.5 ± 1.0	23.5 ± 0.5
Calcium	11.0 ± 0.2	11.3 ± 0.6	11.3 ± 0.2	11.4 ± 0.1
Cholesterol	295.0 ± 20.4	281.0 ± 15.6	308.0 ± 27.8	314.0 ± 13.6
Triglycerides	1226.0 ± 70.5	1029.0 ± 73.6	1167.0 ± 71.6	1172.0 ± 51.2
AST	95.3 ± 16.9	93.0 ± 12.8	105.8 ± 20.9	106.1 ± 21.0
ALT	68.3 ± 8.0	89.4 ± 13.9	70.9 ± 12.4	86.2 ± 13.7
ALP	337.0 ± 35.6	333.0 ± 22.1	364.0 ± 33.4	331.0 ± 27.3

Average ± SEM is depicted for 10–12 rats per group; #significantly different from control. Values are mg/dL with exception of AST (aspartate aminotransferase) and ALT (alanine aminotransferase) that are in units. BUN – blood urea nitrogen, ALP – alkaline phosphatase.

Table 5 – Cytokines

Parameter	Control	Captopril	Lo Asta	Hi Asta
TNF-α	383.0 ± 10.2	242.0 ± 13.6*	349.0 ± 11.9	294.0 ± 13.0*
IL-6	483.0 ± 30	391.0 ± 12*	470.0 ± 21	433.0 ± 9.3
Adiponectin	3.92 ± 0.35	4.27 ± 0.39	4.11 ± 0.27	4.03 ± 0.40
IL-1B-α	488.0 ± 24.7	416.0 ± 14.8#	490.0 ± 21.7	440.0 ± 25.5
MCP-1	112.0 ± 6.7	81.0 ± 4.1*	110.0 ± 5.2	96.0 ± 4.1#

Average ± SEM of 11–12 rats; *statistical significance compared to control ($p < 0.05$); #trend toward statistical significance (>0.05 < 0.10).

3.2.8. *Cytokines*

Tumor necrosis factor-alpha (TNF-α) levels were statistically lower in the captopril and Hi Asta groups, and IL-6 was significantly lower in the captopril group compared to control (Table 5). No significant differences were noted in circulating adiponectin levels and a trend for a lowering was noted in IL-1b-a concentrations in the captopril group compared to control. Concerning MCP-1 concentrations, captopril showed a significant lowering while the Hi Asta group showed a trend toward a lowering compared to the control.

3.2.9. *Organ weights*

After sacrifice, the average weights of the liver, right and left kidneys and epididymal fat pads were not significantly different among groups (see Table 6).

Table 6 – Organ weights

Group	Control	Captopril	Lo Asta	Hi Asta
Liver	41.2 ± 2.1	43.1 ± 2.0	40.4 ± 2.4	43.3 ± 2.3
Kidney (right)	2.4 ± 0.10	2.3 ± 0.09	2.5 ± 0.34	2.5 ± 0.10
Kidney (left)	2.3 ± 0.11	2.3 ± 0.09	2.5 ± 0.16	2.4 ± 0.08
Epididymal fat pad	22.1 ± 1.5	19.1 ± 1.2	19.3 ± 0.9	21.7 ± 1.2

Average ± SEM of 10–12 rats are shown.

Table 7 – Lipid peroxidation and DNA fragmentation data in liver and kidney tissues of rats

	Liver	Kidney
Lipid peroxidation (nmols MDA eq/mg protein)		
Control	5.4 ± 0.45	6.2 ± 0.39
Captopril	4.0 ± 0.29*	3.4 ± 0.44*
Lo Asta	4.7 ± 0.53#	3.9 ± 0.48*
Hi Asta	3.9 ± 0.46*	3.5 ± 0.30*
DNA fragmentation (% control)		
Control	4.0 ± 0.26 (100%)	4.3 ± 0.62 (100%)
Captopril	4.0 ± 0.17 (100%)	4.5 ± 0.51 (105%)
Lo Asta	4.3 ± 0.38 (108%)	4.4 ± 0.37 (102%)
Hi Asta	4.1 ± 0.33 (103%)	4.3 ± 0.48 (100%)

Average ± SEM for each group of eight rats are depicted; *statistically different from control ($p < 0.05$); #significantly different from Picolinate. MDA – malondialdehyde.

3.2.10. *MDA and DNA fragmentation*

Lipid peroxidation in the liver and kidneys was significantly less than control in the captopril and Hi Asta groups (Table 7). In the Lo Asta group, the decrease was significant in the kidneys but showed only a trend toward a lowering in the liver. DNA fragmentation was not different among the groups.

4. Discussion

Astaxanthin is an orange pigment that provides colour to many living organisms – giving salmon, lobsters, and shrimp their reddish colour upon cooking (Torrissen et al., 2000; Naguib, 2001; Shahidi et al., 1998). It exhibits strong free radical scavenging activity by protecting against lipid peroxidation and oxidative damage of LDL-cholesterol, cell membranes, cells, and tissues (Torrissen et al., 2000; Naguib, 2001). The efficient antioxidant activity of this naturally occurring carotenoid pigment is believed to be due to the unique structure of the terminal ring moiety (Goto et al., 2001).

The primary purpose in the present study was to examine the potential of astaxanthin to favorably affect the renin-angiotensin system (RAS) and certain aspects of the metabolic syndrome. In particular, we wished to associate the effects of the RAS with BP lowering. Many studies have linked, at least in some manner, the RAS with insulin resistance, obesity, hormonal changes, and the aging phenomenon leading to a hypothesis that activity of the RAS might be as important to these chronic conditions as insulin resistance (Sower et al., 1982; Basso et al., 2005; deCavanagh et al., 2007; Ernsberger & Koletsky, 2007). We selected Zucker Fatty Rats (ZFR) for our animal model as a first approximation, because this rat manifests many of the characteristics making up the metabolic syndrome (Zucker & Zucker, 1961; Bray, 1977; Bach et al., 1981; Talpur et al., 2002). These genetically obese rats have an increased amount of adipose tissue and manifest hyperlipidemia, insulin resistance, and hypertension.

The doses of astaxanthin added to the food were initially based on a ratio of body weights: comparing a 500 g rat to a 70 kg human (delta = 140). However, many believe it is more appropriate to consider surface area rather than body weight in calculating dosing (Freireich et al., 1966; Reagan-Shaw et al., 2008). The conversion factor for surface area for a rat compared to a human is seven, which makes the delta 20 instead of 140. We estimated that rats eat approximately 25 g of food per day. Throughout the study, the body weight increased and food and water intake among the groups appeared similar suggesting that the ZFR were consuming the desired doses. Thus, at 5 mg/kg in the food, rats would eat 0.125 mg and multiplied by 20, this would approximate a human dose of 2.5 mg. At 25 mg/kg, the rats would eat five times more astaxanthin, roughly the equivalent to a human daily dose of 12.5 mg.

The initial experiment was cut short by an accident in the animal facility, necessitating the second study. However, the data emanating from this misfortune are intriguing and bring up many interesting possibilities. On a hot summer day, the air conditioning system in the animal quarters ceased functioning. The internal temperature of the animal quarters rose to a Fahrenheit range in the high 80s. Due to this heat stress, several ZFR that had already undergone almost six weeks of study expired. Interestingly, the ZFR in this study were the only rats in the entire animal quarters that died from the heat. We can only attribute this to poor stress handling of ZFR (Zucker & Zucker, 1961; Bray, 1977; Bach et al., 1981; Talpur et al., 2002). Although the study might have ended

there, the outcome of this disaster was too fascinating to dismiss. We are referring to the pattern of survival among the four groups. The following survival percentages were found among the four groups: control 4/12 (33%), captopril 4/11 (36%), Lo Asta 4/12 (33%) and Hi Asta 11/11 (100%).

Prior to the accident, the average food and water intake and weight gain were essentially the same among the four groups; and the average SBP was lower in all test groups compared to the control, although the Lo Asta group appeared to have a return of SBP toward control values as the study progressed. Following the acute stress, the surviving rats seemed to recover fully. The consistent weight gain and SBP levels continued in the same fashion as previous to the accident, i.e. SBP in the captopril and Hi Asta groups still remained significantly lower, whereas average SBP in the Lo Asta group approached that of control and was not significantly different. In the survivors, the test for insulin sensitivity showed no differences among the four groups. In contrast, the losartan challenge showed significant differences among groups even with the lesser number of ZFR to study. Compared to the control, the lower values in the Hi Asta group showed a significant difference, a trend was seen in the captopril group (positive control). Differently the Lo Asta group was similar to the control. All these suggested that astaxanthin and captopril were decreasing the activity of the RAS – astaxanthin in a dose-dependent manner.

The inadvertent finding of the ability of astaxanthin to ameliorate the effects of heat stress at the higher dose obligated us to examine the literature. Bollengier-Lee et al. (1999) had previously reported that a dietary supplement of another antioxidant, vitamin E, provided before, during and after heat stress alleviated the effects of the heat stress on egg production in laying hens. Nishikawa et al. (2005) examined the ability of astaxanthin to overcome stress-induced gastric ulcers in rats. Ulcer indices were lower with the rat group fed astaxanthin extracted compared to control. Two United States patents (Ito et al., 1997; Asami et al., 2001) discussed the ability of astaxanthin to be an anti-stress agent. Our findings corroborate the anti-stress potential of astaxanthin.

In a second repeat study necessitated by the mishap, many of the original findings were confirmed. Over the course of study, body weights were not statistically different among the groups. However, this time the captopril group seemed to lag somewhat instead of the control group. The pattern of SBP was virtually similar to the first experiment with all three groups showing a lowering – the Lo Asta group showed less lowering with time than the other two test groups. A new finding was that the captopril and Hi Asta groups showed a significantly lower diastolic BP as well. Cardiac rates were similar among all groups. The second study allowed us to examine our primary aim: could astaxanthin affect the RAS similar to captopril (Ruiz-Ortega et al., 2001)? Captopril, a well-established angiotensin converting enzyme (ACE) inhibitor, was used as a positive control for comparison.

Activity of the RAS was estimated by examining the decrease in SBP after a challenge with the angiotensin receptor blocker, losartan (Wong et al., 1991). All three test groups showed a lesser decrease in SBP compared to the control, suggesting a lesser activity (Mohamadi et al., 2000). When examining circulating ACE activity directly (Aviram & Dornfeld, 2001), the captopril and Hi Asta groups showed lower serum activity compared to the control. Although the activity of the Lo Asta group was lesser than the control, the differences were not significant. Captopril is a well-recognized ACE inhibitor: our results suggest that astaxanthin possesses this ability as well. Concerning the levels of circulating angiotensin-2, captopril showed significantly lower circulating concentrations, while Hi Asta showed a trend toward a lowering compared to the control. Because circulating angiotensin has been associated with vascular damage (Sower et al., 1982; Basso et al., 2005; deCavanagh et al., 2007; Ernsberger & Koletsky, 2007), lowering these levels suggests that astaxanthin could be important in the battle to ameliorate many cardiovascular disorders (Basso et al., 2005; deCavanagh et al., 2007; Ruiz-Ortega et al., 2001).

In addition to BP changes, previous published work reports that astaxanthin can overcome insulin resistance (Asami et al., 2001), similar to other antioxidants (Preuss, 1998). However, our results, in this regard, were inconclusive. We were unable to substantiate this using an insulin challenge test, neither in the first nor in the second experiment. The results with the ipGTT were difficult to interpret due to the wide range of the baseline blood sugars among the ZFR. Other reasons behind these differences may relate to the different species used (SHR vs. ZFR) or the dosages of astaxanthin used. Hussein et al. (Asami et al., 2001) reported that astaxanthin gavaged at 50 mg/kg/day for 22 weeks significantly reduced arterial blood pressure, fasting blood sugar, homeostasis model assessment (HOMA). The latter two parameters suggest improved insulin sensitivity. Based on BW and the conversion factor for surface area of rats as discussed above, this roughly calculates to a dose of 500 mg for a 70 kg human compared to our daily doses which calculated to 2.5 and 12 mg per day.

In addition, Hussein et al. (2007) also showed increased HDL cholesterol, and a decreased triglycerides and improved adiponectin levels. In the present study, we did not see this. Again the rat species and dosing of astaxanthin may have played a role in the differences. Interestingly, the Hussein study showed elevated adiponectin in the 6th, but not in the 18th week.

In general, the blood chemistries were similar among the groups with a single exception, evidence of renal damage occurred in three ZFR of the Lo Asta group based on the increased circulating concentrations of urea, creatinine and potassium. The explanation of this is not clear, but does not seem to be related to the astaxanthin intake as none of the ZFR in the Hi Asta group showed any evidence of elevations in the BUN and creatinine concentrations. At even higher doses of astaxanthin (Asami et al., 2001), other studies saw no renal damage. Examining liver and kidneys at the end of the study corroborated previous experience that astaxanthin is a powerful antioxidant, although no effects on DNA damage were observed, unlike previous reports (Santocono et al., 2006).

Captopril was able to significantly lower TNF-α, IL-6, and MCP-1. There was a trend toward a lowering of the proinflammatory IL-1B-α. For the Hi Asta group, the TNF-α was significantly lower while the MCP-1 showed a trend to be lower. TNF-α is a cytokine that causes tumor necrosis. Production of TNF-α is increased in persons who are obese and may be

related to induction of insulin resistance. IL-α is another cytokine produced by adipose tissue that has been positively correlated with obesity, impaired glucose tolerance and insulin resistance. Levels of adiponectin were not statistically significantly different: results from other studies showed much variation and were difficult to interpret (Hussein et al., 2007). In any case, astaxanthin has the ability to affect some cytokines and appears to possess anti-inflammatory properties.

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