Original Article

Effect of palm oil on blood pressure, endothelial function and oxidative stress

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The pathogenesis of hypertension has been associated with endothelial dysfunction and oxidative stress. We have previously shown that palm oil (PO), with an unsaturated-to-saturated fatty acid ratio close to one and rich in antioxidants vitamins, reduces oxidative stress-induced hypertension in normal rats. Here, we investigated the cardiovascular effects of natural vitamin-rich PO using the Dahl Salt-sensitive hypertension model. Male rats were fed either a high salt (8% NaCl, HS) or low salt (0.3% NaCl, LS) diet with or without PO (Carotino, 5 g/kg daily) for four weeks. Mean arterial pressure (MAP), heart rate, blood flow and vascular resistance, vascular reactivity *in vitro* as well as remodelling of second-order mesenteric arteries were measured. Plasma levels of nitric oxide (NO), prostacyclin, thromboxane A_2 (TXA_2) and isoprostane (ISO), were determined by enzyme immunoassay. Plasma, heart and kidney GSH and GSSG levels were analyzed by HPLC and aortic superoxide ($^{\cdot}O_2$-) production by fluorescence spectrometry. High salt induced an elevation in MAP that was associated with decreased NO, prostacyclin and GSH: GSSG ratio. Plasma ISO and TXA_2, aortic and renal vascular resistance as well as aortic $^{\cdot}O_2$- were increased. Palm oil reduced MAP, plasma TXA_2 and vascular resistance of the renal and aortic arteries, and increased the GSH: GSSG ratio and NO in the LS group. The HS-induced elevation in ISO and $^{\cdot}O_2$- production and the reductions in kidney GSH: GSSG ratio, were attenuated by PO. The effect of PO was also associated with a reduced vessel wall-thickness: lumen diameter ratio and a greater relaxant effect of mesenteric arteries to acetylcholine, in the LS group. The mortality associated with HS was reduced by PO. Thus, palm oil attenuates the progression of salt-induced hypertension and mortality, via mechanisms involving modulation of endothelial function and reduction in oxidative stress.

Key Words: vitamin-rich palm oil, oxidative stress, endothelial function, hypertension

Introduction

Palm oil, obtained from the fruit of the tropical plant *Elaeis guineensis*, is the second major edible oil used worldwide, contributing approximately 23% of the consumption rate.[1] Previous studies have demonstrated beneficial effects of PO on arterial thrombosis[2,3] and blood pressure.[4] In a recent study, using Sprague-Dawley rats, treatment with natural vitamin-rich PO for five weeks was associated with a reduction in oxidative stress and increases in plasma prostacyclin and NO and reductions in TXA_2 suggesting a protective effect on the endothelium.[5] Natural vitamin rich PO, produced using a Palm Oil Research Institute of Malaysia (PORIM)-patented refining short-path distillation, retains most of the qualities of fresh PO, which would otherwise be reduced in the refined, bleached or deodorized brands.[6] Palm oil is unique from other forms of vegetable and animal oils in that it has a high amount of tocopherols and tocotrienols as well as beta-carotene, which act as potent antioxidants that make it relatively stable to oxidation[7] and does not contain the lipid-raising fatty acid (myristic acid) as its saturated fatty acid component.[8] It has been suggested that the high monounsaturation, with oleic acid, at sn2-position of the oil's triacylglycerol, accounts for the beneficial effects of palm oil described in several nutritional studies.[9] Unsaturated fatty acids reduce the formation of endothelial contracting substance[10] and increase the production of vasodilator prostaglandins, PGE_2 and PGI_2.[11]

In Dahl Salt-sensitive rats fed a high salt diet, the development of hypertension involves impairment of endothelium-dependent vasodilation[12] and suppressed nitric oxide (NO) synthesis.[13] Endogenous NO plays an important role in renal hemodynamics and sodium homeostasis, inducing renal vasodilation and natriuresis.[14] The inhibition of NO synthesis, in these rats, has been associated with increased total peripheral resistance and decreased blood flow to many vascular beds.[15] Also, the progression of hypertension in the Dahl SS rat has been associated with impairment of vascular prostacyclin (PGI_2) production and enhanced thromboxane A_2 (TXA_2) release resulting in

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Accepted 30th June 2005

further impairment of endothelium-dependent vasodilation.[16] In the Dahl SS hypertensive rat, there is also evidence of increased oxidative stress characterized by an elevated number of circulating leukocytes that produce superoxide compared with its normotensive control, the Dahl salt-resistant rat.[17] When Dahl SS rats were fed a high sodium chloride diet they displayed decreased antioxidant capacity of the hypertrophied heart[18] and had increased mortality compared to those on a low salt diet.[19] Human studies have also shown that levels of some free radical scavengers such as vitamin E and superoxide dismutase are depressed in hypertensive patients.[20] A range of antioxidant defenses however, has evolved to detoxify reactive oxygen species (ROS), a major one of which is the glutathione redox cycle.[21] Glutathione is the most abundant non-protein intracellular thiol, with multiple roles as an antioxidant agent.[22] Reduced glutathione (GSH) acts to scavenge ROS as well as to regenerate other antioxidants from their oxidized forms.[23] In this process, glutathione is converted to its oxidized form (GSSG) which must be reduced by the combination of glutathione reductase and NADPH. Thus, an index of cellular oxidative events is the ratio of the levels of the reduced and oxidized forms of glutathione. Reactive oxygen species, such as superoxide radicals, form vasoconstrictor isoprostanes from non-enzymatic peroxidation of arachidonic acid[24] and inactivate nitric oxide.[25] Thus, oxygen free radicals may contribute to arteriolar vasoconstriction and vascular resistance, by inactivating nitric oxide; decreasing formation and release of prostacyclin; or increasing formation of vasoconstrictor prostanoids .[21]

In this study, the possible mechanisms underlying the protective effect of palm oil on the vascular endothelium were investigated using the Dahl SS hypertension rat model.

Materials and Methods

Experimental design

Male Dahl Salt-sensitive rats (Harlan Sprague-Dawley, Indianapolis, IN), four to five weeks old, were grouped five per cage in our animal facility that has 12- hour light/dark cycles, with the temperature controlled at 21-23ºC. Rodent Lab Chow™ (Purina Mills Inc., Richmond, IN) and water were made available ad lib for one week. Prior to being placed on the test diets and weekly thereafter, the indirect mean arterial pressure (MAP), heart rate (HR) and body weight were measured. Following acclimatization, six animals were sacrificed and used to provide basal data. The rest were individually housed and separated into two dietary groups (low and high salt groups and fed 0.3% and 8.0% NaCl, respectively) and, as required, were further split into control and palm oil (5g/kg/d by gavage) treated groups for two and four-week duration. The PO used in these experiments, Carotino™, was a kind gift from PORIM. The composition of this oil as specified by the manufacturer is: saturated fat, 50%; monounsaturated fat, 39%; polyunsaturated fat, 11%; carotene (provitamin A), 500 ppm; and vitamin E, 800 ppm.

Animal surgery

After the final MAP and HR measurements were taken, each animal was anesthetized (using 70 mg/kg ketamine and 10 mg/kg xylazine, i.m.) and its carotid and jugular vein were cannulated using PE-50 tubing containing heparin (20 iu/ml) in 0.9% NaCl. The cannulae were externalized in the posterior cervical region and occluded with a metal plug. Patency of cannulae was maintained by flushing with heparinized saline every twelve hours.

Indirect blood pressure measurement in conscious rats

Tail cuff plethysmography (Rat Tail Blood Pressure Monitor and Universal Oscillograph, Harvard Apparatus Inc., Holliston, MA) was used to measure indirect MAP. Heart rate was calculated from the arterial pulse wave at the same time.

Collection and storage of blood samples

Twenty-four hours after surgery, blood samples (2.0ml) for measurement of NO and prostanoids, were collected by free flow via the polyethylene cannula in the right carotid artery into chilled heparinized and indomethacin (100 mM) rinsed (for prostaglandin samples) tubes and replaced with an equal volume of saline. For glutathione and total isoprostane, blood samples (5ml) were withdrawn via cardiac puncture from all animals under anesthesia (ketamine/xylazine mixture) prior to sacrifice. For determination of reduced (GSH) and oxidized (GSSG) glutathione, 0.5ml of ice-cold 0.2M boric acid/10% perchloric acid/10μM γ-glutamylglutamate solution (BA/PCA/γGG) was mixed with 0.5 ml of whole blood and processed for plasma. All blood samples were centrifuged at 3,000x g for 25 min at 4ºC. For isoprostane, butylated hydroxytoluene was added to 1.0ml of plasma to give a final concentration of 0.005% (v/v). All plasma samples were frozen in aliquots and stored at -80ºC until assayed.

Blood flow and vascular resistance studies

The rats were anesthetized using ketamine/xylazine mixture, a midline laparotomy was performed and the lower abdominal aorta (above bifurcation), and renal arteries were carefully isolated. A reasonable length (7-10 mm) of each artery was freely isolated from surrounding tissues while avoiding injury to the vessel and adjacent nerves and veins. Subsequently, miniaturized flow probes (1R and 2SD) were placed around the blood vessels. Ultrasonic coupling gel was placed in the probe vessel contact site to prevent air bubbles. The probe wires were then connected to a small animal ultrasonic flow meter (T206 Dual Channel, Transonic Systems, Inc., Ithaca, NY) which was coupled to an IBM compatible computer. Basal blood flow, blood pressure and heart rate were monitored until stable readings were obtained. Vascular resistance was calculated by dividing MAP by blood flow.

Tissue harvesting for in vitro studies

Immediately following cardiac puncture the heart and both kidneys were harvested from all the animals and some were frozen in liquid nitrogen and stored at -80ºC. Tissue weights were measured and expressed as a ratio of tissue weight to body weight. Selected tissues (heart and kidney) and blood vessels (aorta, carotid, renal or mesenteric arteries) were put into cold oxygenated (95%O_2: 5% CO_2 mixture) HEPES-buffered Ringer solution

(composition in mM: 115 NaCl; 5 KCl; 1.8 $CaCl_2$; 0.8 $MgSO_4$; 0.9 NaH_2PO_4; 26 $NaHCO_3$; 10 glucose; 10 HEPES, pH 7.4).

Measurements of plasma NO, prostanoids, psoprostane and glutathione

Enzyme immunoassay kits (Cayman Chem. Corp., Ann Arbor, MI) were used to measure plasma levels of thromboxane A_2 (as TXB_2), prostacyclin (as 6-keto-PG $F_{1\alpha}$), and total 8-isoprostanes (free and esterified) according to the manufacturer's instructions. Plasma nitric oxide (as nitrates + nitrites) was measured by microplate assay using the Greiss reagent (Cayman Chemical Corp.). Total, oxidized and reduced glutathione were simultaneously measured by HPLC fluorescence detection as described previously,[26] with modifications according to protocols of Abhukhalaf and colleagues.[27]

Measurement of tissue levels of glutathione

Prior to homogenization, tissues (heart and kidney) were mixed 1:1 (w:v) with ice-cold 10% PCA/BA/γGG solution. The mixture was then centrifuged at 3,000 xg for 25 min at 4°C. The supernatant was collected and frozen at -80°C until assayed. An aliquot was taken for protein determination by the BioRad method.[28] Total, reduced, and oxidized glutathione were measured as described above for plasma.

Measurement of superoxide production

Superoxide production was measured by the dihydroethidium/DNA fluorescence assay as previously described.[29] Briefly, aortas were thawed, cleaned of connective tissue and homogenized (glass/glass) in ice cold HEPES buffer (containing 25 mM HEPES, 1 mM EDTA and 0.1 mM phenylmethylsuflonyl fluoride) (1:10, w/v). After centrifugation at 6,000x g for 5 min at 4°C, the supernatant was collected, frozen and stored at -80°C. Protein was determined in an aliquot of each homogenate by the Bio-Rad method.[28]

Superoxide production was measured in the following reaction mixture (0.2 ml total): 10μM dihydroethidium, 0.5mg/ml salmon testes DNA, 10 μg of homogenate protein and the appropriate substrate for either NADH or NADPH oxidase (0.1mM NADH or NADPH). This mixture was incubated on a 96 well microplate for 30 min at 37°C. Ethidium/DNA fluorescence was measured at an excitation of 475nm and an emission of 610nm on a Cytofluor II fluorescence plate reader (Biosearch Products, Bedford, MA). The data was expressed as fluorescence units/min/mg protein.

Microvessel reactivity

In these studies, the vasodilator responses to acetylcholine (Ach) were assessed after preconstriction with 10μM norepinephrine (NE), using the Living Systems Instrumentation (Burlington, Vermont, USA) as previously described.[30] The measurements were performed at intravascular pressures determined from blood pressure readings from each group of animals. Vascular responses were assessed by measuring the internal and external diameters of each vessel. In addition to wall to lumen ratios, dose-response curves were expressed as the percent of NE-induced constriction.

Chemicals

Chemicals were purchased from Sigma Chemical Col. (St. Louis, MO). The 0.3% and 8% NaCl diets were obtained from Harlan Teklad (Madison, WI). Enzyme immunoassay kits were obtained from Cayman Chemical Co. (Ann Arbor, MI).

Data analysis

Values were reported as mean ± standard error (SEM), where *n* refers to the number of rats used. Statistical significance ($P<0.05$) was evaluated using either Student's "t"-test, or for multiple groups, analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. For microvessel reactivity, each dose-response curve was expressed as the percent of the NE-induced constriction by each vessel by the formula: Vascular Response (%) = [(diameter before NE-current diameter)/ (diameter before NE-diameter after NE)] x100. Dose-response curves were compared using a two-way ANOVA for repeated measurements followed by the Student-Newman-Keuls method for pairwise multiple comparisons.

Results

Changes in mean arterial blood pressure and heart rate

Salt-sensitive (SS) rats, on the HS diet, showed a progressively greater MAP elevation at compared to those on LS and LS + PO, $P<0.05$. Rats treated with PO in both the HS and LS groups had lower pressures compared to those given salt diets alone, $P<0.05$. At 4 weeks, the BP change was 56 ± 2 mmHg in the HS, 39 ± 1 mmHg in the HS + PO, 22 ± 1 mmHg in the LS group, and 6 ± 2 mm Hg in the LS + PO, (Fig. 1A). There were no significant changes in heart rate regardless of the level of dietary salt or PO intake (Fig. 1B).

Plasma, kidney and heart GSH: GSSH ratio

The plasma GSH:GSSG ratio progressively decreased from a basal value of 8.2 ± 1 to 4.9 ± 0.2 and 3.9 ± 0.4 in the HS, and 3.7 ± 0.3 and 4.2 ± 0.2 in the HS + PO, at 2 and 4 weeks respectively, $P<0.05$. It did not significantly change in the LS group, (7.1 ± 0.3 and 6.0 ± 0.5), but increased in the LS + PO, to 8.9 ± 1 and 12.8 ± 1, at 2 and 4 weeks, respectively (Fig. 2A). The kidney GSH: GSSG ratio decreased from a basal value of 7.2 ± 0.3, to 5.8 ± 0.4 and 5.5 ± 0.6 at 2 and 4 weeks respectively, in the HS; but was not much different from the basal in the HS + PO (6.3 ± 0.6 and 7.8 ± 0.4) and the LS group (6.1 ± 0.3 and 7.0 ± 0.6). It was higher in the LS + PO (10.6 ± 0.8) at 4 weeks, $P<0.05$ (Fig. 2B). The heart GSH: GSSG decreased from a basal value of 5.4 ± 0.6, to 3.9 ± 0.3 and 3.6 ± 0.3 at 2 and 4 weeks, respectively, in the HS, and 3.9 ± 0.4 at 4 weeks in the HS + PO. It didn't significantly change in the LS group (4.6 ± 0.3 and 4.5 ± 0.1), but increased in the LS + PO at 4 weeks to 8.1 ± 1.3, $P<0.05$ (Fig. 2C).

Aortic superoxide production

Superoxide production, via NADH and NADPH oxidases at 2 and 4 weeks was higher in the aortas from rats on HS diet compared to the basal and LS values, $P<0.05$. The effect of HS was attenuated and normalized in the

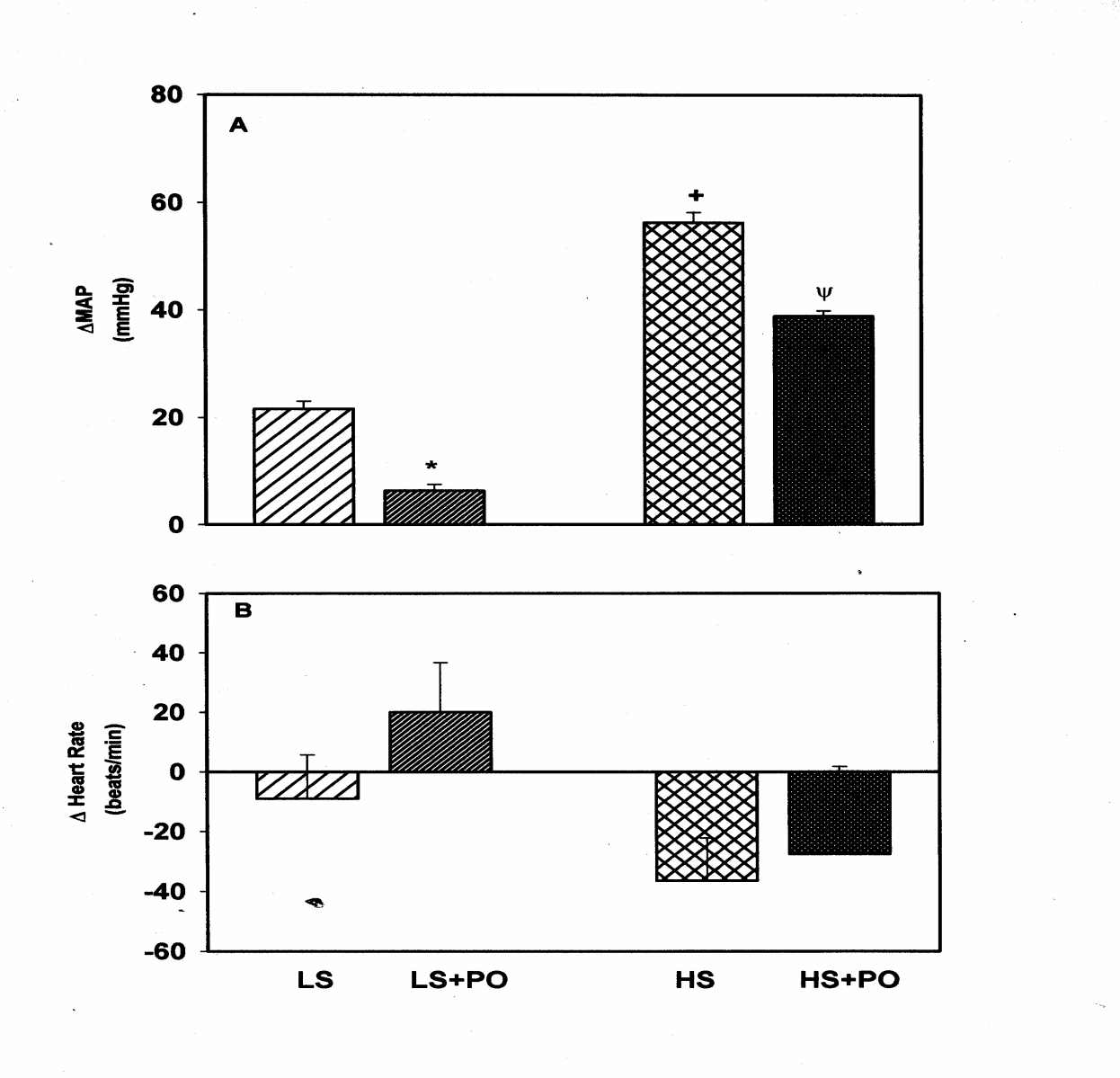

Figure 1. Effect of Palm Oil and Dietary Salt on the change in (A) mean arterial pressure and (B) heart rate, from the basal values, in Dahl Salt-sensitive rats. Data are represented as mean ± SEM for N = 12 animals per group at 2 weeks, and N = 6 at 4 weeks. Significant difference (P <0.05) is denoted as asterisk (*) for effect of LS+PO, (+) for effect of HS and (ψ) for effect of HS+PO.

presence of PO. In the LS + PO group, superoxide production via these enzymes, at both 2 and 4 weeks was lower compared to the HS group, P<0.05 (Fig. 3A and 3B).

Plasma levels of Isoprostane and Thromboxane A_2

Plasma levels of total isoprostane (pg/ml) were elevated at 2 and 4 weeks in the HS group (236 ± 41 and 269 ± 161) compared to the basal (65 ± 10 pg/ml), LS (88 ± 14 and 129 ± 5), and LS + PO (71 ± 8 and 99 ± 12), respectively; P<0.05. The effect of HS was attenuated in the PO + HS group at both 2 and 4 weeks, (140 ± 8 and 167 ± 16, respectively), P<0.05; but not completely normalized, (Fig. 4A). Plasma TXA_2 (pg/ml) progressively increased in the HS group and was higher at 4 weeks (426 ± 49) compared to basal (185 ± 25) and LS (238 ± 15), P<0.05. The level in the HS + PO (391 ± 14) was not much different from the HS group. In the LS + PO group TXA_2 was decreased (131 ± 5), compared to basal and LS, P<0.05, (Fig. 4B).

Plasma levels of nitric oxide, prostacyclin

Plasma prostacyclin (pg/ml) progressively decreased in the HS group, and was lower at 4 weeks (115 ± 7) compared to the basal (303 ± 9) and LS at both 2 and 4 weeks (261 ± 42 and 29 ± 35), P<0.01. In the HS + PO group (330 ± 9), the level was normalized to the basal value, P <0.05. It did not change significantly in the LS and LS + PO groups, (Fig. 5A). Plasma NO (μM) was decreased at 2 and 4 weeks in the HS group (32 ± 5 and 25 ± 2), compared to basal (38 ± 6) and LS (52 ± 4). The level in the HS + PO was not different from the HS. At 4 weeks,

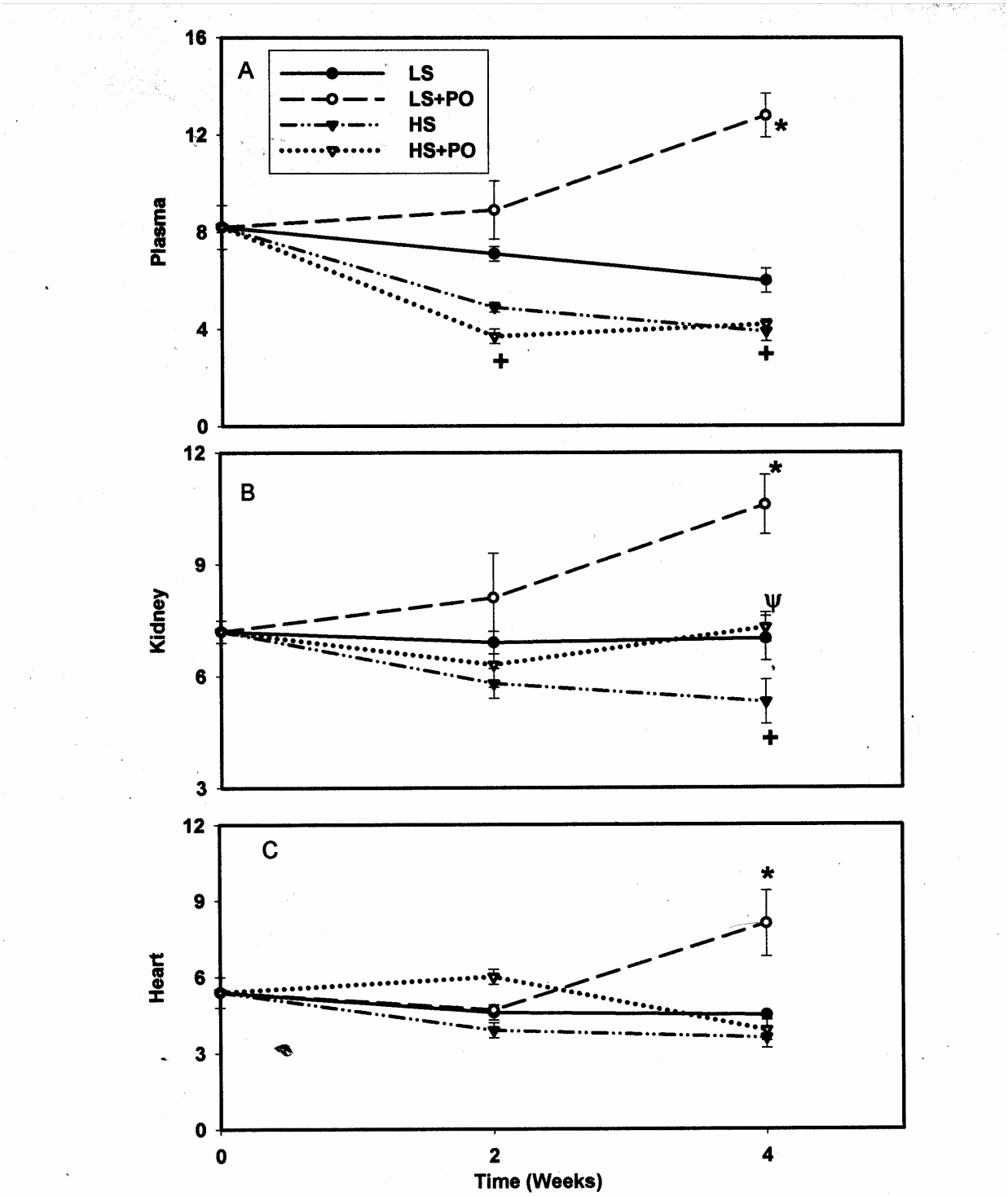

Figure 2. Effect of Palm Oil and Dietary Salt on (A) plasma, (B) heart and (C) kidney GSH:GSSG ratio in Dahl Salt-sensitive rats. Data are represented as mean ± SEM for six animals per group. Significant difference (P<0.05) is denoted as asterisk (*) for effect of LS+PO, (+) for effect of HS and (±) for effect of HS+PO.

plasma NO was significantly elevated in the LS + PO group (78 ± 5), compared to basal and LS, P <0.01 (Fig. 5B).

Effect of palm oil and dietary salt on aortic and renal blood flow and vascular resistance in dahl salt-sensitive rats

As shown in Figure 6, rats fed a HS diet had significantly lower rates of blood flow in both the aortic (11.6 ± 0.3) and renal (2.8 ± 0.1 ml/min) arteries compared to the LS (17.8 ± 0.6 ml/min) and (4.3 ± 0.2 ml/min), respectively. Administration of PO resulted in higher rates of blood flow in the LS group (23.2 ± 0.8 vs 17.8 ± 0.6 for the aortic artery and 5.6 ± 0.4 vs 4.3 ± 0.3 for the renal artery), but not the HS (15.2 ± 0.8 vs 11.6 ± 0.3 for the aortic artery and 3.5 ± 0.2 vs 2.8 ± 0.1 for the renal artery). Vascular resistance in the HS group was higher compared to the LS in both the aortic (16 ± 0.6 mmHg/ml/min vs 8.1 ± 0.5) and renal (65.6 ± 3.1 vs 34.6 ± 3.2) blood vessels, respectively, P<0.05. In both groups, concurrent consumption of PO resulted in reduced vascular resistance; in the aorta (11.7 ± 1.1 vs 16 ± 0.6 for the HS

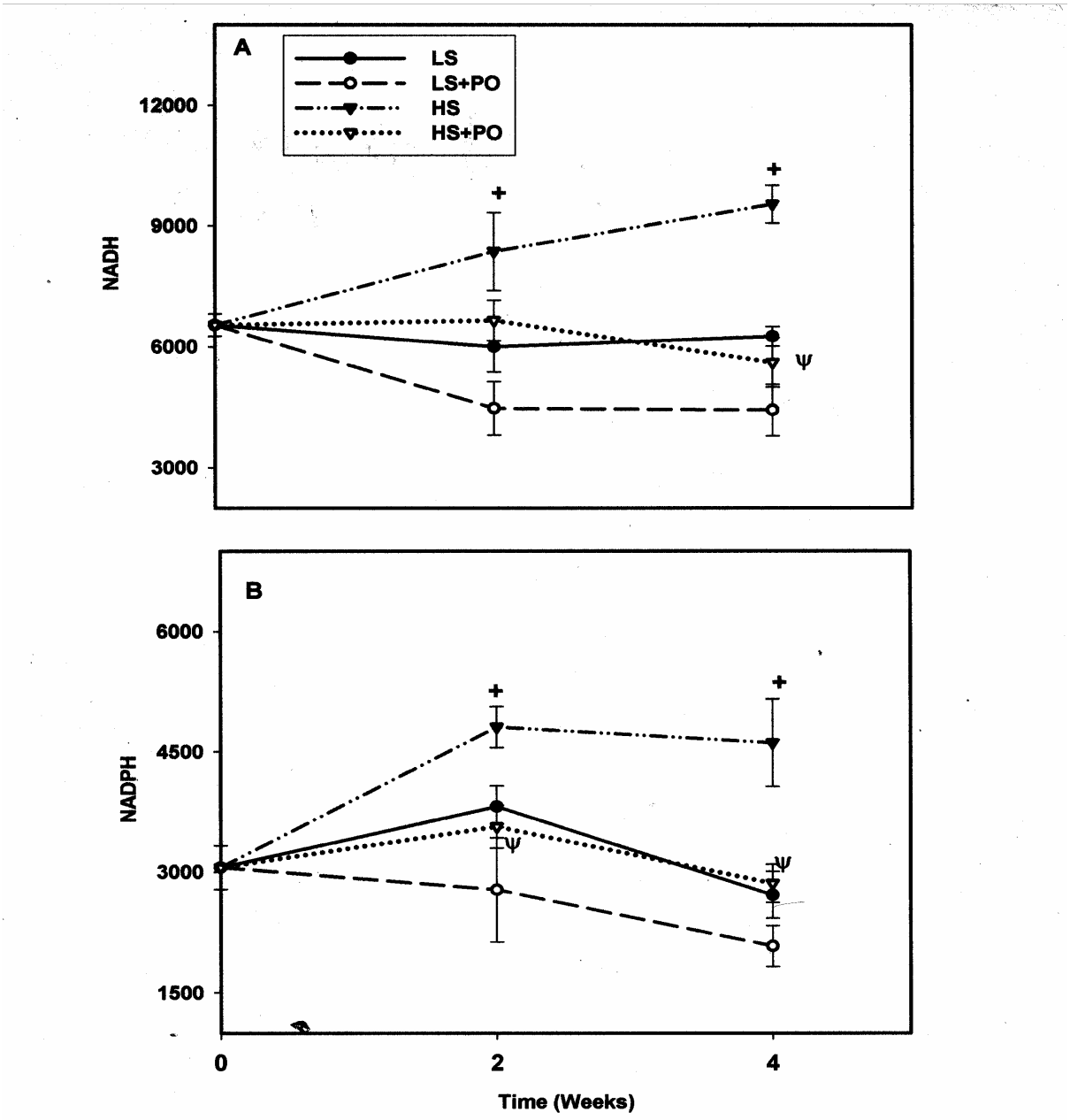

Figure 3. Effect of Palm Oil and Dietary Salt on aortic superoxide production via (A) NADH and (B) NADPH in Dahl Salt-sensitive rats. Data are represented as mean ± SEM for six animals per group. Significant difference (P<0.05) is denoted as asterisk (*) for effect of LS+PO, (+) for effect of HS and (ψ) for effect of HS+PO.

and 5.3 ± 0.2 vs 8.1 ± 0.5 for the LS), P<0.05, and in the renal artery, (51.9 ± 5.8 vs 65.6 ± 3.1 and 222.0 ± 1.2 vs 34.6 ± 3.2 mmHg/ml/min) (Fig. 7).

Effect of palm oil and dietary salt on in-vitro micro-vascular reactivity of the mesenteric arteries in dahl salt-sensitive rats

The vasodilator response to Acetylcholine (Ach) was significantly lower in the HS group compared to the LS. At the maximum dose of Ach, (10^{-4} M), vessels in the HS group maintained response of about 61% of NE-induced constriction compared to 15% in the LS group, P<0.01. Concurrent administration of PO did not significantly alter the response induced by HS, but in the LS group, the vascular response to Ach was further increased to a level of 2% of NE-induced constriction, P <0.05 (Fig 8).

Effect of palm oil and dietary salt on wall thickness and lumen diameter of the mesenteric arteries in dahl salt-sensitive rats

The wall thickness to lumen diameter ratio of the Dahl SS rats given HS and HS + PO was higher compared to the LS and LS+PO, P<0.05. Concurrent consumption of PO resulted in a lower ratio in the HS group, P<0.05, (Fig. 9).

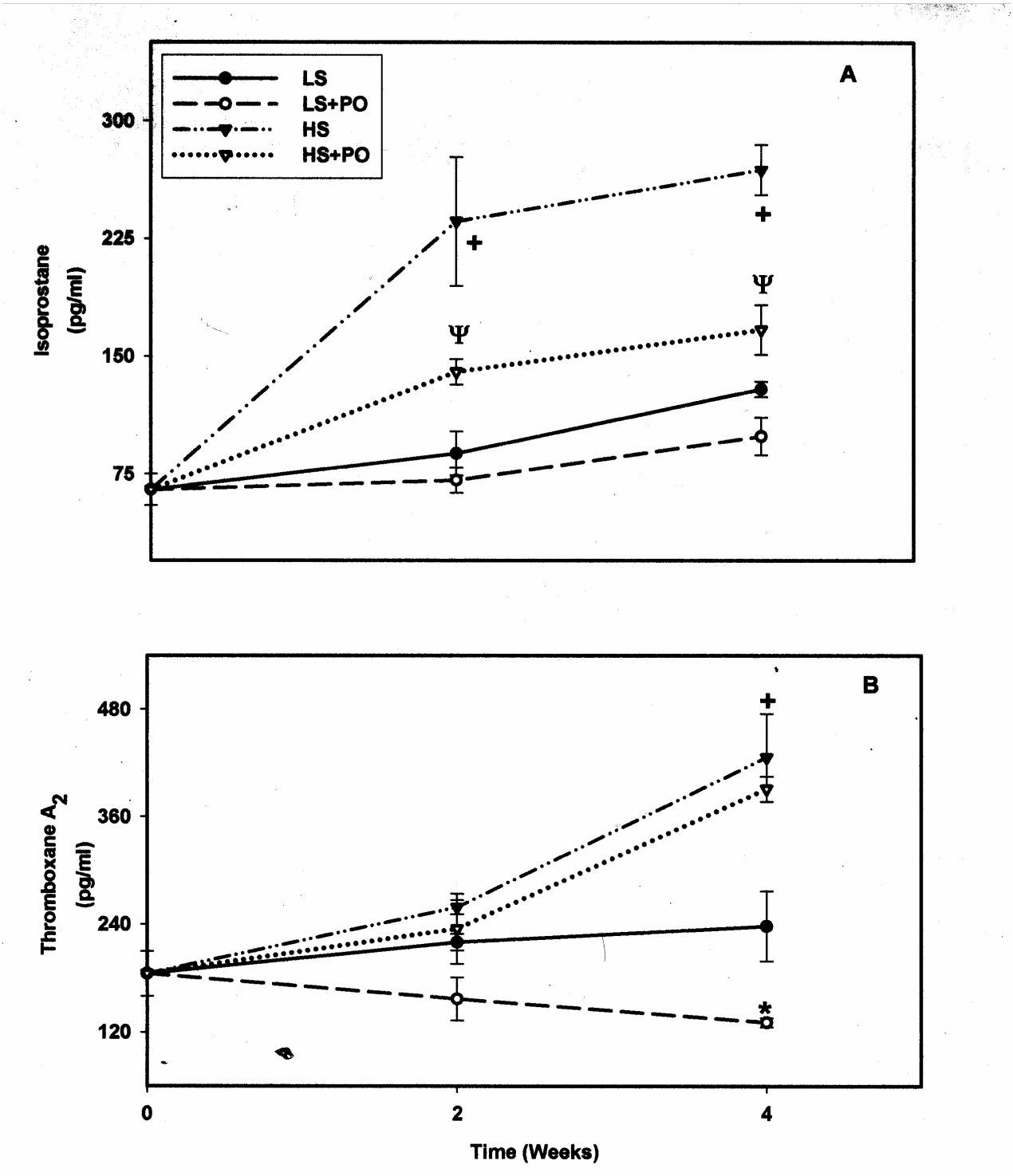

Figure 4. Effect of Palm Oil and Dietary Salt on plasma (A) isoprostane and (B) thromboxane levels in Dahl Salt-sensitive rats. Data are represented as mean ± SEM for six animals per group. Significant difference (P <0.05) is denoted as asterisk (*) for effect of LS+PO, (+) for effect of HS and (ψ) for effect of HS+PO.

Mortality

All the rats in the low salt groups, with or without palm oil, survived the diets. However only 58% of the rats on the high salt survived during the observation period. In contrast, all of the rats on high salt supplemented with palm oil survived (Table 1).

Discussion

Data from this study show that dietary supplementation with natural vitamin-rich palm oil (PO) attenuates endothelial dysfunction, oxidative stress and the mortality associated with salt-induced hypertension in Dahl salt-sensitive (SS) rats. The pressor response in SS rats fed a high salt was associated with increased production of superoxide anion in the aorta, isoprostanes in plasma and reductions in plasma, kidney and heart GSH: GSSG ratio. Aortic superoxide production was increased, via both NADH/NADPH oxidases. The above effects of HS were

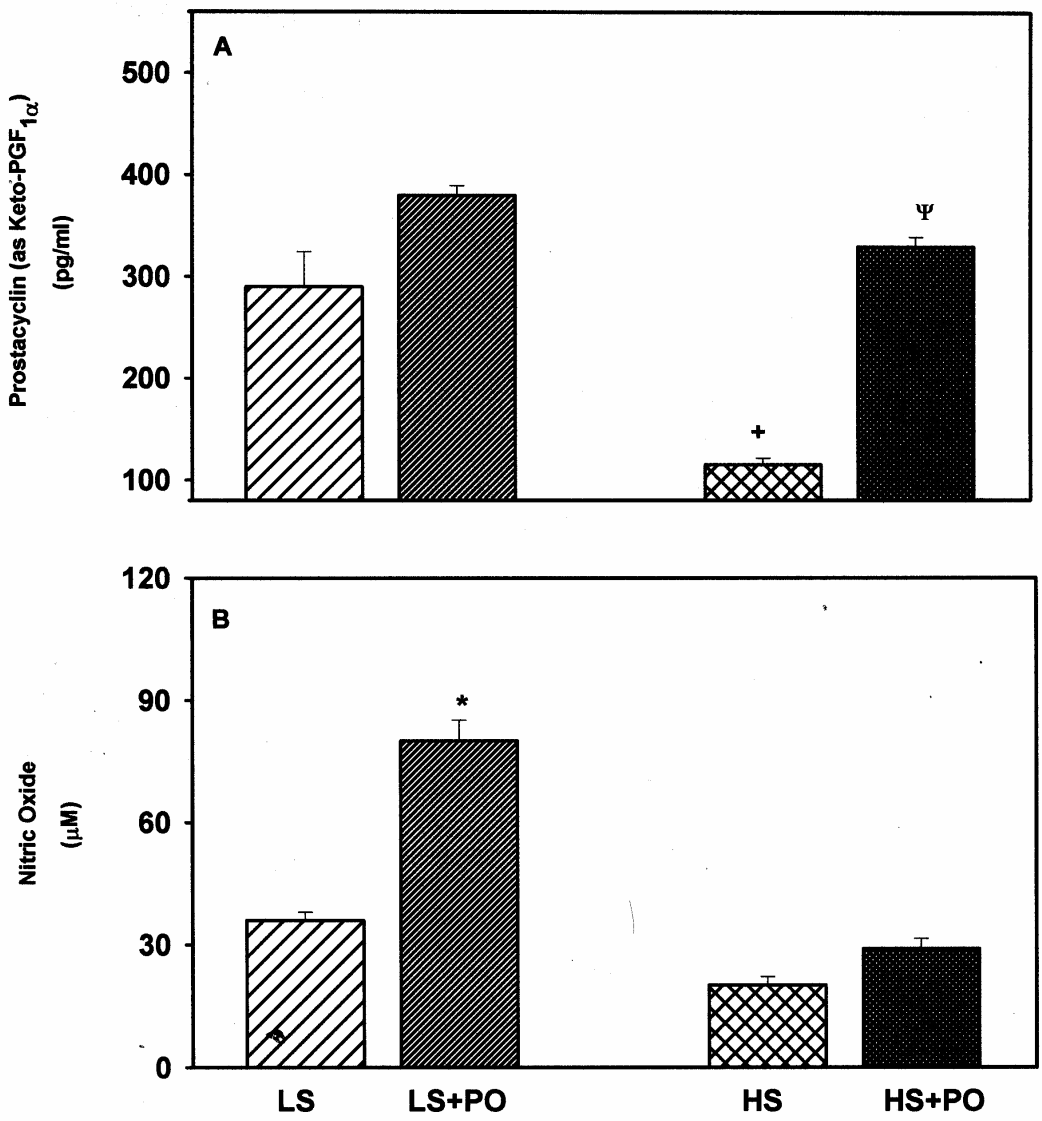

Figure 5. Effect of Palm Oil and Dietary Salt on plasma (A) prostacyclin and (B) nitric oxide in Dahl Salt-sensitive rats. Data are represented as mean ± SEM for six animals per group. Significant difference (P<0.05) is denoted as asterisk (*) for effect of LS+PO, (+) for effect of HS and (ψ) for effect of HS+PO.

Table 1. Percent survival of dahl salt-sensitive rats fed PO and Low or High Salt for four weeks

Diet	Percent Survival
Low Salt	100
Low Salt +Palm Oil	100
High Salt	58[a]
High Salt + Palm Oil	100

P <0.05, effect of HS versus LS, LS+PO and HS+PO.

attenuated and normalized in the presence of PO. Also, in the rats fed a normal salt diet, consumption of PO resulted in lower levels of superoxide production. PO is a rich source of tocopherol and tocotrienol fractions of vitamin E, as well as beta-carotene, essential components of the diet that function as antioxidants.[9]

Consistent with the superoxide data, there was a progressive reduction in the GSH: GSSG ratio in rats fed HS in plasma, kidney and the heart. The development of hypertension in Dahl SS has been associated with a significantly decreased glutathione and GSH-peroxidase acti vity, which is exaggerated by NaCl loading.[18] Administration of PO normalized the GSH: GSSG ratio in the

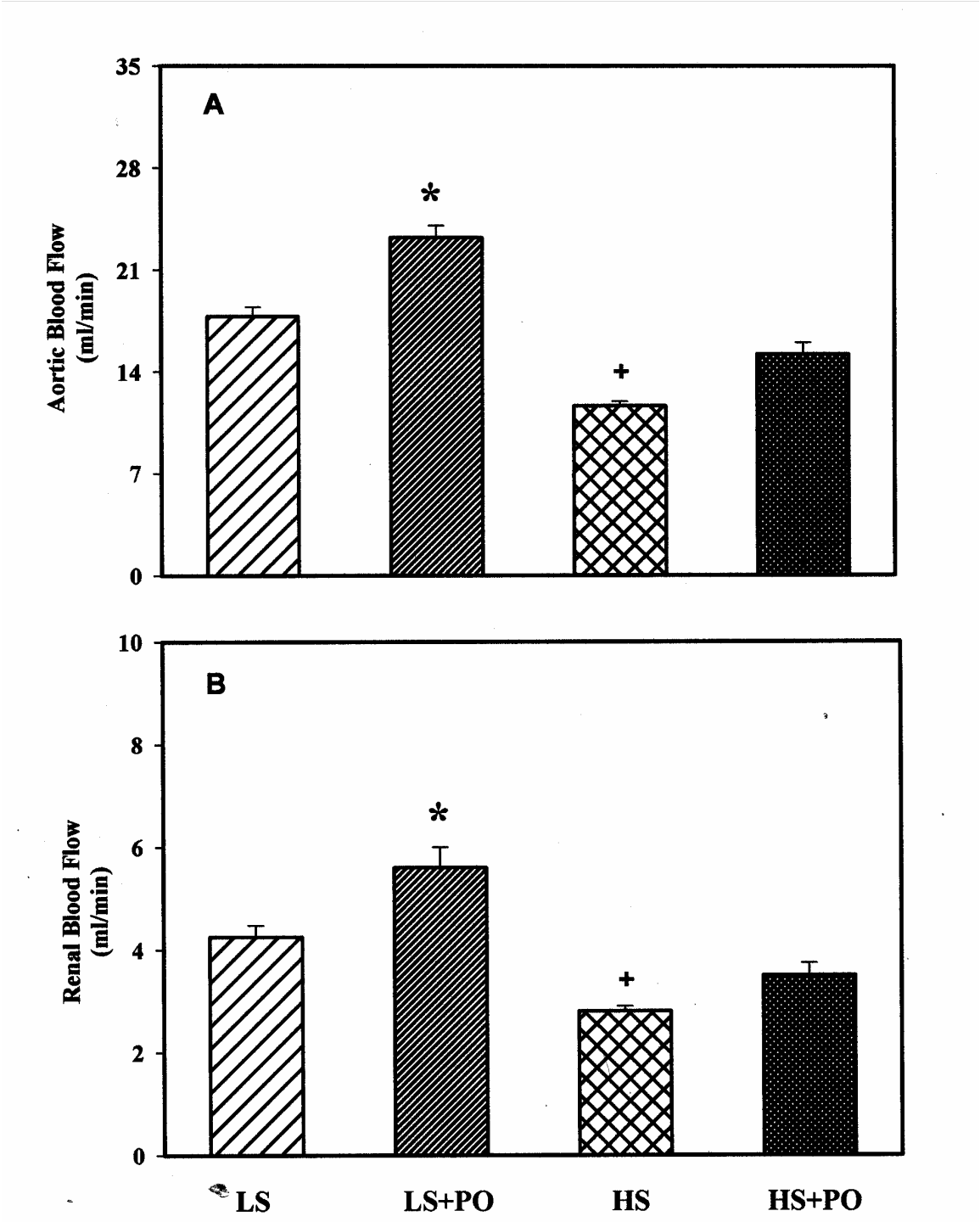

Figure 6. Effect of palm oil and dietary salt on (A) aortic and (B) renal blood flow in dahl salt-sensitive rats. Data are represented as mean ±SEM for six animals per group. Significant difference (P<0.05) is denoted as asterisk (*) for effect of LS+PO, (+) for effect of HS.

kidney but not the heart or plasma. This pattern of change is consistent with the inter-organ translocation, turnover, and metabolism of GSH; there is a relatively rapid turnover of GSH in the kidney compared to the other tissues, where GSH is resistant to transpeptidation.[18] In the low salt group fed PO, the GSH: GSSG ratio was increased over time, which implies that in the presence of PO, GSH is preserved and a high antioxidant potential is maintained. Long term feeding of PO, in experimental animals, has been associated with increased activity of superoxide dismutase (SOD), GSH- peroxidase and GSH content.[32]

Further supporting evidence for the antioxidant potential of PO was shown by its ability to reduce the salt-induced isoprostane elevation. This effect may be attributed to its vitamin E fraction, which has been demonstrated to suppress isoprostane generation *in vivo* and reduce atherosclerosis in ApoE-deficient mice.[33] Plasma levels of isoprostane are considered to be a sensitive and reliable measure of in-vivo oxidative stress.[34] It should be noted, however, that the isoprostane levels were not normalized, implying that the high salt-induced oxidative stress and vascular remodelling, which was evident as early as two weeks, may not be completely reversed by

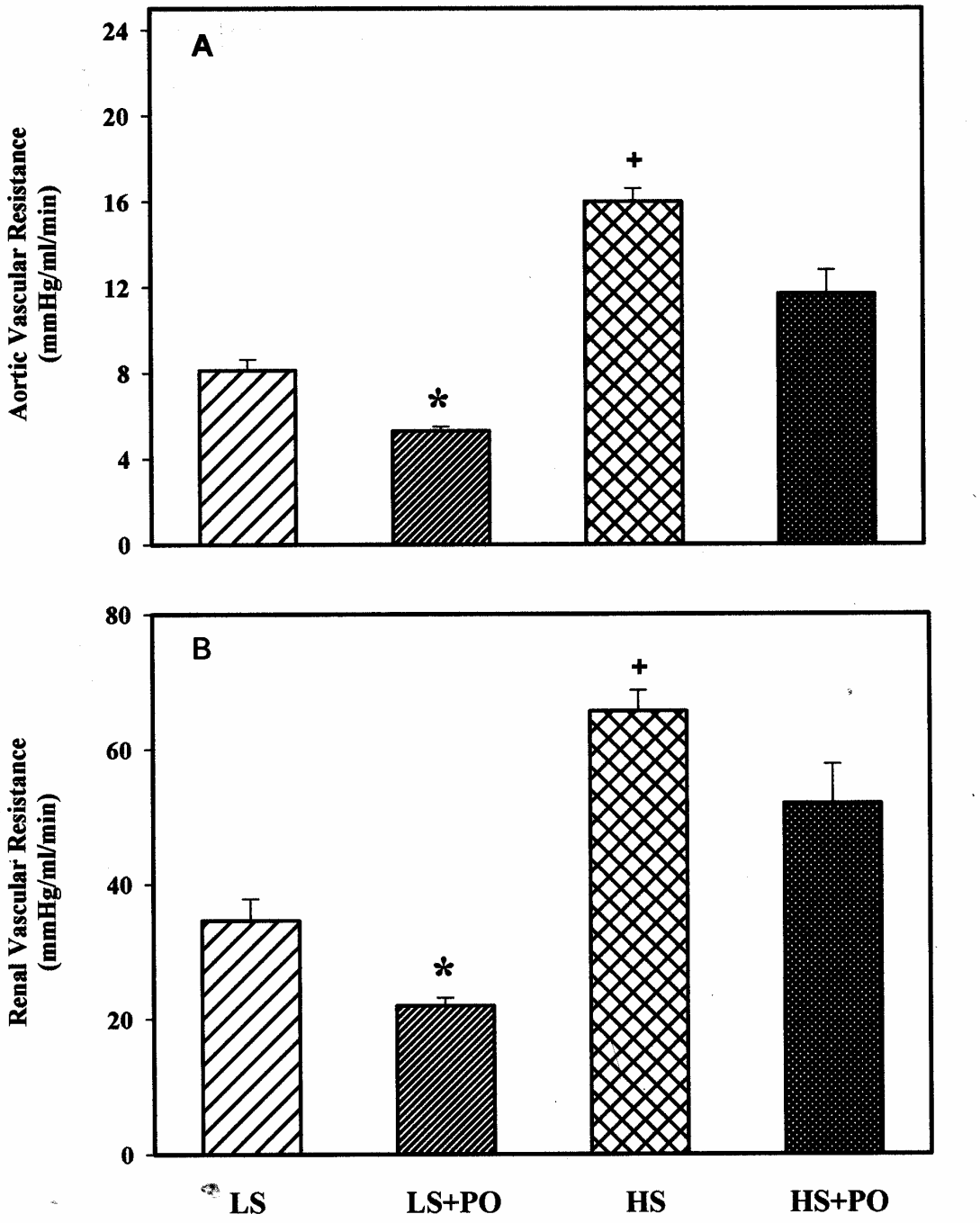

Figure 7. Effect of Palm Oil and Dietary Salt on (A) aortic and (B) renal vascular resistance in Dahl Salt-sensitive rats. Data are represented as mean ± SEM for six animals per group. Significant difference (P<0.05) is denoted as asterisk (*) for effect of LS+PO, (+) for effect of HS.

PO. Endothelial dysfunction, associated with oxidative stress induced by HS, was manifested by alterations in the plasma levels of TXA_2, prostacyclin and NO. The role of TXA_2 in the development of salt-induced hypertension has previously been demonstrated. For instance, Gomi *et al*, (1995) showed that administration of the thromboxane synthetase inhibitor, OKY-046, had a protective effect on hypertensive renal damage in Dahl SS rats.[35] Likewise, our findings show that administration of PO was associated with reduction in the plasma levels of TXA_2 in the low salt group. Palmvitee™, a PO-fraction rich in tocotrienols, has been shown to decrease thromboxane synthesis in humans.[36] In the high salt group administered PO, however, the levels of TXA_2 were not significantly altered, further suggesting that high salt-induced oxidative stress may partially impair endothelial protective effect of PO. As regards prostacyclin, plasma levels in the high salt group decreased over time. It is possible that the alterations in GSH and increases in superoxide anion associated with a high salt diet have an inhibitory effect on prostacyclin formation. Prostacyclin levels, however, were normalized to the basal values in the high salt group following the administration of PO. Earlier studies in rats showed that treatment with PO reduced TXA_2 and facilitated the utilization of arachidonate for prostacyclin synthesis, hence decreasing the thromboxane/prostacyclin ratio.[9]

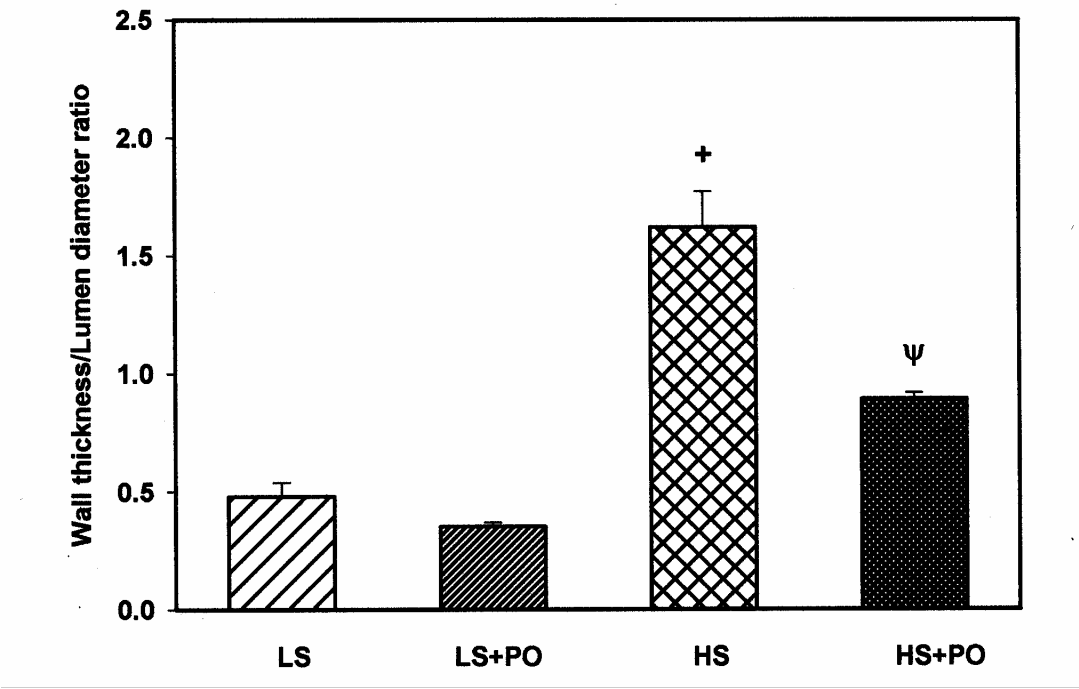

Figure 8. Effect of Palm Oil and Dietary Salt on the response of mesenteric arteries, preconstricted with Norepinephrine, to incremental doses of Acetylcholine in Dahl Salt-sensitive rats. Data are represented as mean ± SEM for six animals per group. Significant difference (P<0.05) is denoted as asterisk (*) for effect of LS+PO, (+) for effect of HS.

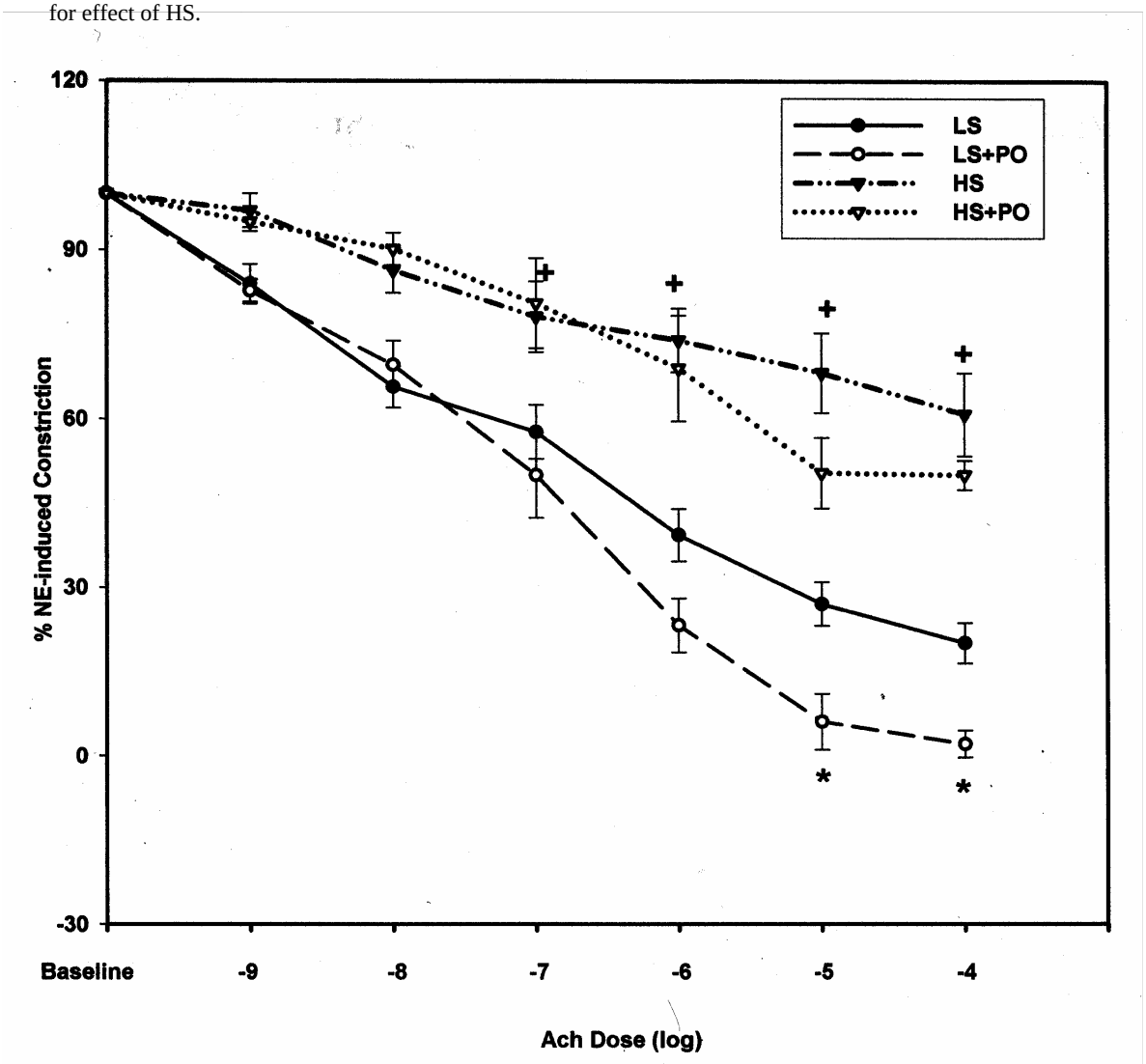

Figure 9. Effect of Palm Oil and Dietary Salt on the Wall-Thickness to Lumen Diameter ratio of second-order mesenteric arteries in Dahl Salt-sensitive rats. Data are represented as mean ± SEM for six animals per group. Significant difference (P <0.05) is denoted as (+) for effect of HS and (+) for effect of HS+PO.

The unsaturated fatty acid fraction of PO may be responsible for the increased production of PGI_2.[11] Also, alterations in the GSH-GSSG system are capable of activating and/or inactivating many enzymes that may affect prostanoid synthesis. For instance, changes in the enzyme activities of glutathione peroxidase and glutathione reductase following antioxidant therapy with vitamin E has been linked to an increase in the GSH: GSSG ratio and prostacyclin levels.[37]

Nitric oxide levels were reduced in the SS rats on high salt compared to those on low salt, consistent with previous observations in our laboratory.[38] During oxidative stress, superoxide anion reacts with NO at a higher rate than it does with SOD to form peroxynitrite, a potent cytotoxic oxidant, hence reducing the available NO.[39,40] This is probably one of the major mechanisms by which oxygen free radicals affect vascular resistance, and contribute to arteriolar vasoconstriction and elevation of peripheral resistance. Palm oil, on the other hand, increased plasma levels of NO in the LS group, but did not attenuate the suppressive effect of high salt. It has been observed that some natural antioxidants may preserve the biological activity of endothelium-derived nitric oxide, by either decreasing oxidative stress or directly stimulating NO synthesis.[41]

Consistent with the observed alterations in endothelial factors, there was a reduction in blood flow and an increase in vascular resistance of both the renal and aortic arteries in Dahl SS rats fed high salt. Furthermore, there was an increase in the wall-thickness to lumen diameter ratio, as well as reduced response to acetylcholine in the mesenteric arteries. In the HS group, PO did not significantly alter these parameters, whereas in the LS group the MAP and vascular resistance were reduced, and blood flow was increased. Administration of PO in the LS group caused a much greater response to Ach in the mesenteric arteries *in vitro* and reduced wall-thickness to lumen diameter ratios.

These observations on the effect of HS are consistent with earlier studies, which suggested that the development of high blood pressure in Dahl salt-sensitive rats is related to an inability of the renal vasculature to dilate following high-salt diet supplementation.[42] This is thought to be due to inhibition of NO synthesis, that leads to increased total peripheral resistance and decreased blood flow to many vascular beds.[15] The increase in blood flow and reduction in vascular resistance in the LS group administered PO may be related to increased availability of NO. We previously showed that administration of PO in normal rats was associated with elevation of NO and increased cGMP levels.[5] NO diffuses out of endothelial cells, where it is synthesized, and stimulates guanylate cyclase in vascular smooth muscle cells, causing vascular relaxation.[41] Palm oil did not completely modify the HS effect, probably because of suppressed NO synthesis or the elevation in superoxide anion, induced by HS. Increased superoxide anion, per se, has been associated with impaired endothelium-dependent vascular relaxation and increased vascular contractile reactivity.[43] These effects may be mediated directly by increasing $\{Ca^{2+}\}_i$ or indirectly by reducing the concentrations of NO. It is also possible that the increase in prostacyclin levels and reduction in thromboxane levels induced by PO contribute to the vasodilator effect in the LS group. Consistent with the changes in blood flow and resistance in the aorta and renal arteries, administration of HS diet in the Dahl SS rats impaired endothelium-dependent vascular relaxation to acetylcholine in mesenteric arteries, suggesting altered responsiveness of vascular smooth muscle cells to NO. With administration of PO, the HS effect was not significantly modified, but in the LS group endothelium-dependent relaxation to acetylcholine was much greater. It is possible that HS diet induces significant vascular remodelling that may not be completely reversed by PO. Consumption of HS in the Dahl SS rat, also increased the wall-thickness to lumen diameter ratio of the mesenteric arteries. An increase in the optical density of the media, probably due to thickening of the walls of the artery, and narrowing of the lumen was observed in blood vessels in the HS group. This is suggestive of hypertrophic remodelling to allow for the adaptation to the increase in wall stress during hypertension.[44] Arterial remodelling is mediated, in part, by synthesis and release of locally produced growth and vasoactive factors, and is an adaptive process occurring in response to chronic changes in arterial pressure or flow. In hypertension, oxidative stress promotes vascular smooth muscle cell proliferation and hypertrophy, collagen deposition, and alterations in activity of matrix metalloproteinases, which lead to thickening of the vascular media and arterial remodelling.[45] Administration of PO in the HS group was associated with a significant reduction in the wall-thickness to lumen diameter ratio, suggesting that PO has a protective effect on vascular remodelling induced by HS.

Our findings also show that dietary supplementation with PO reduced the mortality associated with high salt. Although renal failure[46] and cerebral vascular disease[47] have been shown to be a prominent factor in the mortality induced by high salt, it could be speculated that the protective effect of PO may be related to the decrease in oxidative stress and preservation of endothelial function. Taken together, these findings suggest that palm oil atenuates the progression of salt-induced hypertension and mortality, via mechanisms involving reduction in oxidative stress and modulation of endothelial function. The effect of PO involves improvement in endothelium-dependent relaxation and a reduction in vascular resistance and remodelling induced by HS.

Acknowledgements

This work was supported, in part, by NIH grants S06GM08248-12 (M.A.B) and P20RR11104-07 (I.K.A), NASA and PORIM.

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Effect of palm oil on blood pressure, endothelial function and oxidative stress
棕榈油在血压、内皮功能和氧化应激中的功效

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高血压的发病机理已经被证明与内皮功能紊乱和氧化应激有关。以前已经显示棕榈油中，不饱和与饱和脂肪酸比例接近 1，富含抗氧化维他命，对普通老鼠可以减少氧化应激产生的高血压。本文采用达拉克群岛盐敏感高血压样本研究富含天然维他命棕榈油的心血管作用。喂给雄性老鼠 8% NaCl 的高盐，0.3% NaCl 的低盐饮食饮食有或无棕榈油（胡萝卜素 5 g/kg daily）四个星期。测量平均动脉压，心率，血流和血管阻力，体外血流动力和肠系膜动脉的二次塑造。血浆的一氧化氮，环前列腺素和凝血噁烷(TXA_2)和 ISO 水平用酶免疫测定。血浆，心脏和肾脏的 GSH 和 GSSG 水平用高效液相色谱，动脉超氧化物的产生用荧光光谱测定。高盐使平均动脉压增高，降低一氧化氮，环前列腺素和 GSH 与 GSSG 的比例。血浆 ISO 和 TXA_2,大动脉和肾血管阻力，大动脉$\cdot O_2$-都增加。在低盐组棕榈油减少平均动脉压，血浆 TXA_2,，肾血管和大动脉阻力，增加 GSH 与 GSSG 比例，一氧化氮。高盐组棕榈油升高 ISO，$\cdot O_2$- ，减少肾 GSH 与 GSSG 的比例。在低盐组棕榈油的功效还能减少血管壁的厚度即内腔内外径比例，对肠系膜动脉乙酰胆碱的弛缓释放增加作用。棕榈油可以减少高盐组的死亡率。因此，棕榈油通过包含调制内皮功能的机制合降低氧化应激减少食盐性高血压的发展和死亡率。

关键词：富含维他命棕榈油，氧化应激，内皮功能，高血压