BASIC RESEARCH

The effects of heated vegetable oils on blood pressure in rats

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OBJECTIVES: The goal of this study was to determine the possible mechanism that is involved in the blood pressure-raising effect of heated vegetable oils.

METHODS: Adult male Sprague-Dawley rats were divided into 11 groups; the control group was fed with rat chow, and the other groups were fed with chow that was mixed with 15% weight/weight palm or soy oils, which were either in a fresh form or heated once, twice, five, or ten times. Blood pressures were measured at the baseline and throughout the 24-week study. Plasma nitric oxide levels were assessed prior to treatment and at the end of the study. Following 24 weeks, the rats were sacrificed to investigate their vascular reactivity using the thoracic aorta.

RESULTS: Palm and soy oils had no detrimental effects on blood pressure, and they significantly elevated the nitric oxide contents and reduced the contractile responses to phenylephrine. However, trials using palm and soy oils that were repeatedly heated showed an increase in blood pressure, enhanced phenylephrine-induced contractions, reduced acetylcholine- and sodium nitroprusside-induced relaxations relative to the control and rats that were fed fresh vegetable oils.

CONCLUSIONS: The blood pressure-raising effect of the heated vegetable cooking oils is associated with increased vascular reactivity and a reduction in nitric oxide levels. The chronic consumption of heated vegetable oils leads to disturbances in endogenous vascular regulatory substances, such as nitric oxide. The thermal oxidation of the cooking oils promotes the generation of free radicals and may play an important contributory role in the pathogenesis of hypertension in rats.

KEYWORDS: Palm oil; Soy oil; Heating; Blood Pressure; Aorta.

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INTRODUCTION

Various studies have been performed in humans and animals to determine the role of saturated and unsaturated fatty acids in hypertension. The consumption of a diet that is rich in monounsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs), such as olive oil and fish oil, has been found to decrease blood pressure (BP).[1,2] Conversely, diets that are rich in saturated fatty acids (SFAs) have been found to increase BP.[3,4]

The pathogenesis of hypertension is often associated with endothelial dysfunction and oxidative stress. Palm oil has been found to decrease the mean arterial BP in rats by reducing thromboxane levels and vascular resistance in the aorta and renal arteries.[5] Furthermore, palm oil can reduce oxidative stress and BP via changes in endothelium-derived factors.[6] A previous study has reported that rats fed oxidized oil experienced a significant increase in BP relative to a control group and rats that were fed fresh oil.[7]

Cooking oil is sometimes reused due to its stability at high temperatures.[8] During the frying process, various chemical reactions occur, such as thermal oxidation, hydrolysis, and polymerization, due to the exposure of the oil to high temperatures in the presence of air and moisture. As a result, cooking oil decomposes and forms volatile compounds and various monomers, and polymers.[9] Several factors can affect the quality of cooking oil during heating, including ventilation, temperature, heating duration, the type of oil, the saturation ratio of the oil, and the presence of a catalyst/antioxidant.[8]

The repeated heating of oil at high temperatures (≥180°C) results in the thermal oxidation of the oil, which causes the configuration of the fatty acid to change from the *cis* isomer to the *trans* isomer. This configuration change causes the PUFAs to acquire undesirable properties associated with SFAs, such as their correlation with increased serum cholesterol levels and higher low-density lipoprotein (LDL) cholesterol in

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particular.[10] The fats that are found in processed foods are exposed to high heat primarily during preparation and may be affected by these configuration changes.

The practice of reusing oil during food preparation processes is widespread. This practice is not only confined to roadside food stalls; established food outlets in large cities throughout Malaysia also use this method to reduce costs. The repeated heating of cooking oil will result in oil that is more prone to lipid peroxidation.[11] However, the potential adverse effects that are associated with this practice are quite insidious. The purpose of this study was to elucidate the possible mechanism of the BP-raising effect of heated palm and soy oils in rats.

MATERIALS AND METHODS

Experimental animals and study design

A total of 110 three-month-old adult male Sprague-Dawley rats were randomly and equally divided into eleven groups. The control group was fed a basal diet (rat chow), and the diets of the remaining groups were fortified with 15% weight/weight (w/w) of one of the following oils: fresh palm oil (FPO); palm oil that was heated once (1HPO), twice (2HPO), five times (5HPO), or ten times (10HPO); fresh soy oil (FSO); or soy oil that was heated once (1HSO), twice (2HSO), five times (5HSO), or ten times (10HSO). The rats were kept in stainless-steel cages and maintained with a 12:12-hour light-dark schedule at room temperature (27℃ ± 2℃). All of the rats were provided with food and water *ad libitum* for 24 weeks. The animals were handled according to the guidelines set down by Commonwealth and Malaysian legislation and the recommendations of the University Federation of Animal Welfare. The studies were approved by the University Animal Ethics Committee (UKMAEC: FP/FAR/2008/KAMSIAH/9-APR/220-APR-2008-FEB-2011). Blood pressure measurements were recorded at the baseline and once every four weeks for 24 weeks. Non-fasting blood samples were collected over ethylenediaminetetraacetic acid (EDTA) through the orbital sinus prior to treatment and at the end of the study. The blood samples were then centrifuged to separate the plasma. The plasma aliquots were stored at -70℃ until the analysis. The rats were then sacrificed, after 24 weeks of study, and their thoracic aortas were dissected to measure the vascular reactivity. All of the animals were anesthetized by placing them into a jar that contained cotton soaked in diethyl ether, and they were allowed to go into an unconscious state during the blood collection and after the end of the feeding protocol when the tissue was harvested.

Preparation of heated-oil diets

Commercially purchased palm and soy oils were either used fresh or were heated according to the modified method of Owu *et al*.[12] In the heating process, the oils were used to fry sweet potatoes in a stainless-steel wok for ten minutes. The temperature of the heated oils reached approximately 180℃. The hot oils were cooled to room temperature, and the entire frying process was repeated to fry a fresh batch of sweet potatoes without the addition of oils. The process was repeated up to ten times. Standard rat chow (Gold Coin, Port Klang, Selangor, Malaysia) was ground and formulated by mixing 15% w/w of the respective prepared oils; the chow was then dried in an oven at 70℃ overnight. The oil diets were stored in the dark and prepared weekly.

Measurement of blood pressure (BP)

A non-invasive BP monitoring method was employed. A monitoring cuff was placed proximally on the tail to detect changes in blood flow that occurred during the occlusion or release of the cuff. The rats were placed in an appropriately body-sized plastic container prior to obtaining the BP measurement. This step ensured that the animals were acclimated and provided a faster BP measurement. The animals were pre-warmed for 15 min to enhance blood flow to the tail. The measurements were taken using a PowerLab data acquisition system (ADInstruments, Castle Hill, NSW, Australia) at room temperature (27℃ ± 2℃) to avoid a reduction in the tail blood flow in a cool environment. The BP was obtained as an average of five readings.

Measurement of nitric oxide (NO)

Plasma NO levels were assessed by the presence of nitrite metabolites according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). Samples of 50 μL each were transferred to a microtiter plate, and 50 μL of modified Griess reagent was added (Sigma-Aldrich, St. Louis, MO, USA). After 15 min of incubation at room temperature, the nitrite concentrations were measured spectrophotometrically at 540 nm using an Emax ELISA microplate reader with the SoftMax Pro software (Molecular Devices, Sunnyvale, CA, USA). The procedures were performed in a dark environment. The nitrite concentrations were quantified with a standard curve generated using known concentrations of sodium nitrite (Sigma-Aldrich, St. Louis, MO, USA).

Aortic rings preparation and vascular reactivity

The descending thoracic aorta of each rat was excised using a midline incision, and the aorta was cleaned of fat and connective tissues. The aorta was cut into 3- to 5-mm ring segments and were suspended in individual 5-mL organ baths that were filled with Krebs solution containing the following components (mM): NaCl (118.0), KCl (4.7), $CaCl_2 \cdot 2H_2O$ (2.5), KH_2PO_4 (1.2), $MgSO_4$ (1.2), glucose (11.7), $NaHCO_3$ (25.0), and EDTA (0.026). The bathing solution was warmed to 37℃ and was continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Care was taken to not injure the endothelium during the preparation of the rings. Changes in the tension of the tissues (g) were measured isometrically and recorded using a force-displacement transducer (F-D-Transducer FT03E, Grass Instruments, West Warwick, RI, USA) that was connected to a MacLab computerized system (model 8S, ADInstruments, Castle Hill, NSW, Australia). The aortic rings were then progressively stretched to a basal tension of 1 g. Each ring preparation was allowed to equilibrate for 30 min prior to the initiation of the experimental protocol. During this period, the incubation media was changed every 15 min, and the resting tension was readjusted to a basal tension of 1 g.

After the equilibration period, the vascular reactivity experiments were initiated by obtaining a reference contractile response to an isotonic KCl solution (high K^+, 80 mM). To confirm the stability of the tissue, three consecutive equal contractions were performed. Following the washout of the responses to high K^+, the relaxation responses (vasodilatations) to cumulatively increasing concentrations of acetylcholine (ACh, 10^{-10} M to 10^{-5} M) and sodium nitroprusside (SNP, 10^{-11} M to 10^{-6} M) were recorded in phenylephrine (PE, 10^{-6} M) pre-contracted aortic rings. In addition, the contractile responses (vasoconstrictions) to cumulatively increasing concentrations of PE (10^{-10} M to 10^{-5} M) were

recorded in the rings. The rings were constricted with PE (10^{-7} M) to test the endothelial integrity with the addition of ACh (10^{-5} M) after the washout of the responses to high K^+. Only the endothelium-intact rings (i.e., rings showing greater than 50% relaxation in response to ACh) were used.[13] Different aortic rings with an intact endothelium were used in each experiment.

Drugs used in the vascular reactivity studies

Acetylcholine chloride, phenylephrine-HCl (Sigma Chemical Co., St. Louis, MO, USA), sodium nitroprusside, and Krebs salts (BDH Limited and BDH Laboratory Supplies, Poole, England) were used.

Statistical analyses

The results were expressed as the means ± SEM. The level of statistical significance was fixed at 0.05. The distribution of the data was determined using the Kolmogorov-Smirnov test. The data were analyzed using an unpaired Student's *t* test or a one-way ANOVA followed by a post-hoc Tukey's HSD test using the SPSS software package version 13.0 (SPSS Inc., Chicago, IL, USA). The data that were not normally distributed were analyzed using non-parametric tests, such as the Kruskal-Wallis *H* and Mann-Whitney *U* tests.

RESULTS

Effect of heated vegetable oils on blood pressure (BP)

The rats in the control, FPO and FSO groups did not show any significant changes in BP. The 2HPO, 5HPO, and 10HPO groups displayed a significant increase (p end of the study with increases of 22.4%, 23.9%, and 25.4%, respectively, when compared to the control group (4.3%). The BP increases in the 1HSO, 2HSO, 5HSO, and 10HSO groups (16.0%, 23.0%, 25.9%, and 34.4%, respectively) were higher than those of the heated palm oil groups. In addition, we observed that the BP increase was significantly higher in the 10HSO group than in the 10HPO group (Figure 1).

Effect of heated vegetable oils on nitric oxide (NO) contents

The rats that were fed FPO- and FSO-amended diets displayed NO metabolite (nitrite) levels that increased by 22.5% and 23.1%, respectively, when compared to their baseline values. However, the groups that were fed heated palm and soy oils displayed reduced levels of nitrites when compared to the fresh oil groups (Figure 2).

Effect of heated vegetable oils on vascular reactivity in aortic rings

i. Relaxation in response to acetylcholine (ACh) and sodium nitroprusside (SNP)

ACh and SNP relaxed the phenylephrine pre-contracted aortic rings from various rat groups. The relaxant effect of ACh at the maximum tested concentration (10^{-5} M) was significantly reduced ($p<0.05$) in the aortic rings that were obtained from the 5HSO, 5HPO, 10HSO, and 10HPO groups when compared to the control and other test groups (Figure 3).

In addition, vasodilatation in response to the highest tested SNP concentration (10^{-6} M) was significantly attenuated

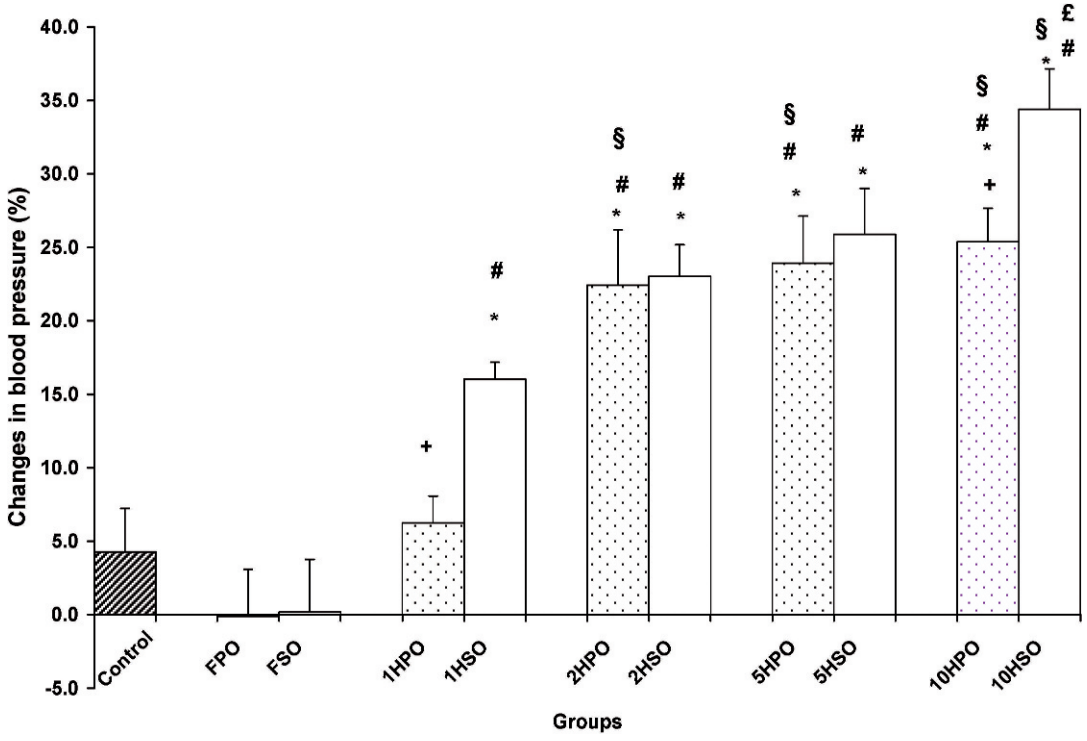

Figure 1 - Changes in blood pressure after 24 weeks of feeding with a basal diet (control) or chow amended with one of the following oils: fresh palm oil (FPO); palm oil that was heated once (1HPO), twice (2HPO), five times (5HPO), or ten times (10HPO); fresh soy oil (FSO); or soy oil that was heated once (1HSO), twice (2HSO), five times (5HSO), or ten times (10HSO). The results are the means ± SEM **(n = 10)**. Significant difference ($p<0.05$) versus the *control, #fresh oil group, §once-heated-oil group, or £twice-heated-oil group. +Significant difference ($p<0.05$) between the palm and soy oil groups. The data are expressed as percentage based on baseline values.

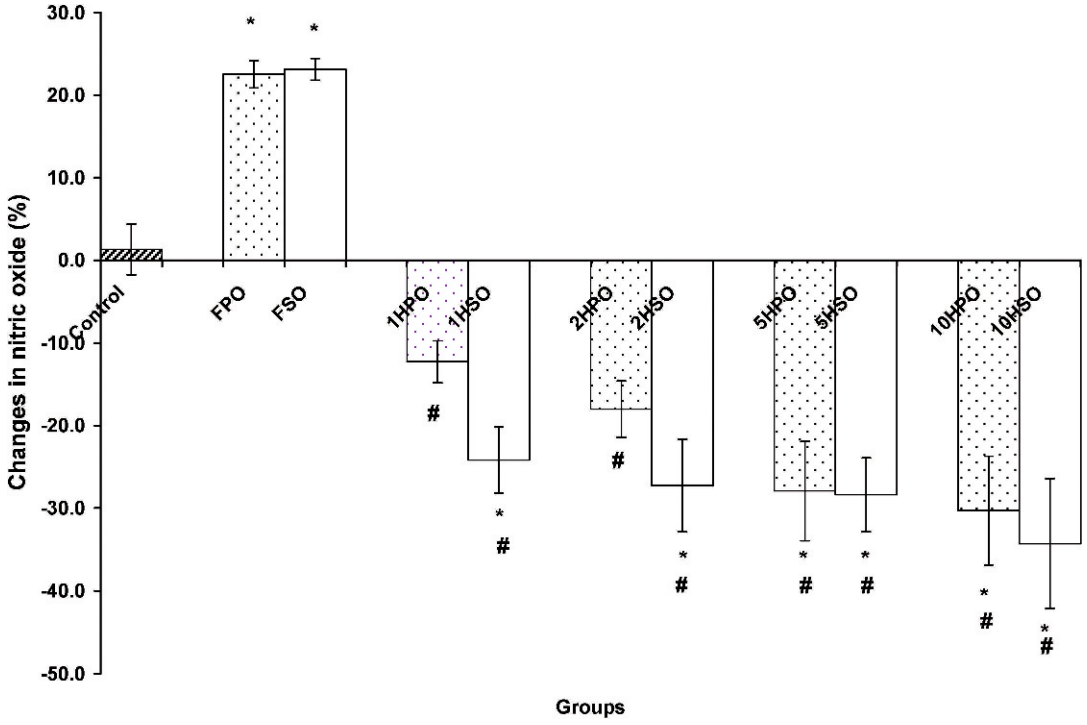

Figure 2 - Changes in plasma nitric oxide levels after 24 weeks of feeding with a basal diet (control) or chow amended with one of the following oils: fresh palm oil (FPO); palm oil that was heated once (1HPO), twice (2HPO), five times (5HPO), or ten times (10HPO); fresh soy oil (FSO); or soy oil that was heated once (1HSO), twice (2HSO), five times (5HSO), or ten times (10HSO). The results are the means ± SEM **(n = 10)**. Significant difference ($p<0.05$) versus the *control or #fresh oil group. The data are expressed as percentage based on baseline values.

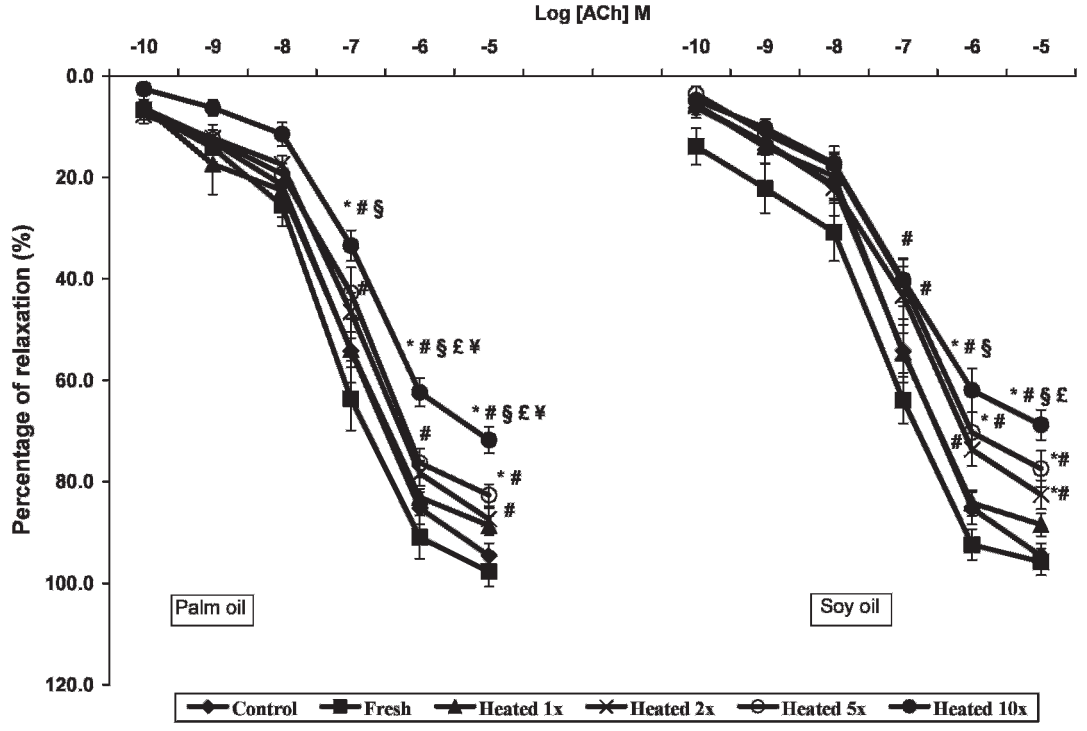

Figure 3 - Endothelium-dependent relaxation induced by acetylcholine (ACh) in aortic rings that were isolated from rats that were fed for 24 weeks with a basal diet (control), fresh palm or soy oil, or palm or soy oil that was heated once (1x), twice (2x), five times (5x) or ten times (10x). The results are the means±SEM **(n = 10)**. Significant difference ($p<0.05$) versus the *control, #fresh-oil group, §1x heated-oil group, £2x heated-oil group, or ¥5x heated-oil group. Dose response curves are plotted as percentage of relaxation against the maximal phenylephrine (PE, 10^{-6} M).

($p<0.05$) in the aortic rings obtained from the 5HSO, 5HPO, 10HSO, and 10HPO groups. The relaxation effects remained unaltered in the aortic rings of the rats fed fresh oils relative to the control rats (Figure 4).

ii. Contractile response to phenylephrine (PE)

The vasoconstriction in response to the highest tested concentration of the alpha-$_1$ adrenergic agonist PE (10^{-5} M) was significantly augmented ($p<0.05$) in the aortic rings of the 5HSO, 5HPO, 10HSO, and 10HPO groups when compared to the control and other dietary groups (Figure 5).

DISCUSSION

The chronic ingestion of heated palm and soy oils for 24 weeks caused a significant increase in BP. The increased BP was affected by the number of heating repetitions as evidenced by a greater increase in BP when the oils were heated ten times compared to the BP increase when the oils were heated two or five times. The BP increase was significantly higher for the 10HSO group than the 10HPO group, and it is possible that the higher degree of PUFA lipid peroxidation may contribute to this result. The results of this study were in agreement with earlier studies that reported that repeatedly heated oils increased BP.[14,15] However, FPO and FSO had a tendency to reduce BP, although the effect was not significant. In this study, the effect of fresh oils on BP was not in agreement with previous studies.[5,6,16] The results could be due to differences in duration of the study, the types of oils and the animals used.

NO plays a vital contributory role in BP regulation.[17] The impairment of NO bioavailability may be an important aspect to the pathogenesis of diet-induced hypertension that follows the prolonged intake of heated vegetable oils. In this study, we found that feeding with heated palm and soy oils led to reduced plasma NO contents. However, feeding with FPO or FSO increased the plasma nitrite levels. These findings suggest that fresh oils, which have higher antioxidant contents, led to increased NO levels.

We postulate that thermally oxidized oils generate free radicals, such as the superoxide anion, which react with NO to form peroxynitrite.[18] The reduction in the plasma nitrite levels could be explained by the enhanced NO sequestration by free radicals and its inactivation due to the imbalance between the antioxidant/oxidant status. Therefore, it appears that the BP increase occurs due to the loss of NO-dependent relaxation. It is possible that increased peroxynitrite levels are responsible for endothelial dysfunction, which leads to increased BP.[19] The generation of free radicals from thermally oxidized oils may be due to a significant reduction in antioxidant levels. Previous findings from our laboratory have shown that vitamin E contents progressively diminish as palm and soy oils are repeatedly heated.[20]

In this present study, the rats that were fed with FPO or FSO displayed increased plasma nitrite levels. It has been suggested that natural antioxidants found in fresh oils may provide protective effects by decreasing oxidative stress or stimulating NO formation, which subsequently preserves the bioavailability of NO.[21] Soy intake was also inversely associated with BP.[22,23] Dietary soy has been shown to enhance nitric oxide synthase expression, which further promotes NO production.[24] The identification of increased plasma NO metabolite levels after 24 weeks in both of the

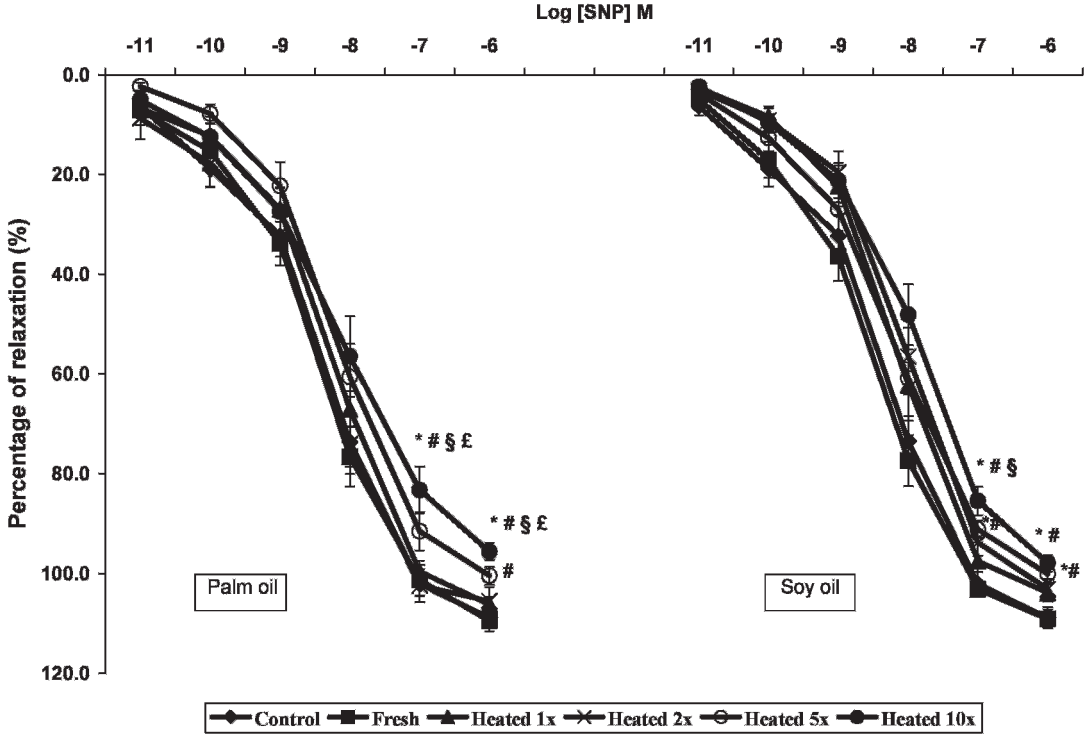

Figure 4 - Endothelium-independent relaxation induced by sodium nitroprusside (SNP) in aortic rings isolated from rats that were fed for 24 weeks with a basal diet (control), fresh palm or soy oil, or palm or soy oil that was heated once (1x), twice (2x), five times (5x) or ten times (10x). The results are the means ± SEM **(n = 10)**. Significant difference ($p<0.05$) versus the *control, #fresh-oil group, §1x heated-oil group, or £2x heated-oil group. Dose response curves are plotted as percentage of relaxation against the maximal phenylephrine (PE, 10^{-6} M).

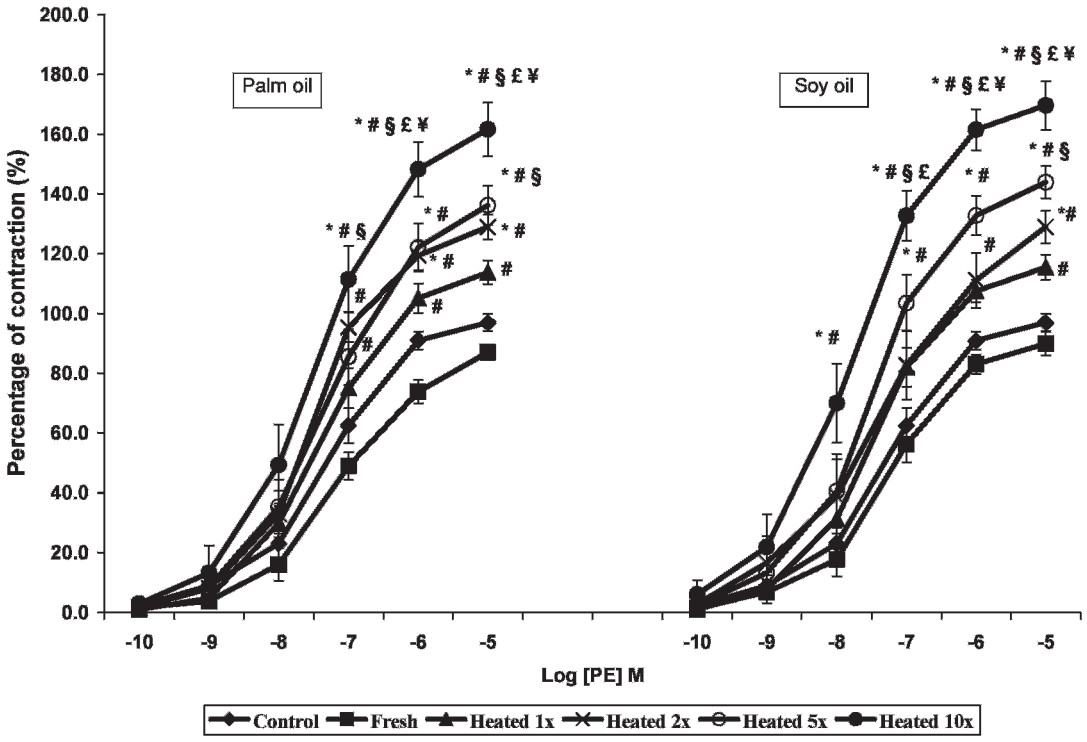

Figure 5 - Contractile response induced by phenylephrine (PE) in aortic rings isolated from rats that were fed for 24 weeks with a basal diet (control), fresh palm or soy oil, or palm or soy oil that was heated once (1x), twice (2x), five times (5x), or ten times (10x). The results are the means ± SEM **(n = 10)**. Significant difference ($p<0.05$) versus the *control, #fresh oil group, §1x heated-oil group, £2x heated-oil group, or ¥5x heated-oil group. Dose response curves are plotted as percentage of maximum contraction obtained with high K^+.

fresh oil groups reinforces the importance of the influence of NO levels on BP in rats.

Previous studies have documented that NO has a vasodilatory effect, and its release may be triggered by several pharmacologically vasoactive substances, such as ACh.[25,26] NO is synthesized predominantly in the vascular endothelium and diffuses into the adjacent smooth muscle cells to activate guanylate cyclase (GC); this increases the formation of cyclic guanosine monophosphate (cGMP) and causes vascular smooth muscle relaxation.[27] In the aorta, endothelium-dependent relaxation induced by ACh increases NO bioavailability via the release of NO from the endothelium. SNP breakdown spontaneously generates exogenous NO and causes endothelium-independent relaxation.

The data presented here suggest that the treatment with heated vegetable oils may decrease the endothelium-derived NO bioavailability and enhance the contraction that is induced by PE when compared to the control and fresh oil groups. The increased vascular contractile reactivity contributes to increased vascular tone.[28] Heated vegetable oils have reduced radical-scavenging properties, which may result in reduced NO bioavailability in the aorta. A previous study has also reported impaired vasorelaxation following the ingestion of heated palm oil.[29] In addition, the consumption of heated oils has been reported to cause abnormal ultrastructural changes in the aorta.[30,31]

As observed in this current study, the effect of repeatedly heated palm oil had a more stable effect on BP than repeatedly heated soy oil. This effect may be due to the unique composition of fatty acids and vitamin E in palm oil. Palm oil is widely used as cooking oil in both household and food outlets due to its low cost. FPO contains an approximately 1:1 ratio of saturated and unsaturated fatty acids[32] and contains lower levels of PUFAs, but higher MUFA levels, relative to soy oil. Attack by free radicals typically targets the unsaturated bonds in fatty acid chains. Therefore, a frying oil with a greater number of unsaturated bonds is less stable and more readily broken down by heat. The repeated heating of edible oils that are rich in PUFAs increases the formation of toxic compounds, which are associated with an increased risk of hypertension. Soy oil has a higher percentage of PUFAs than palm oil and is therefore more prone to oxidation.

Both palm and soy oils contain vitamin E, which is an antioxidant that can scavenge free radicals. During the frying process, the oil is aged and becomes more oxidized when the natural antioxidants are depleted; thus, after repeated heating, the antioxidants can no longer prevent the oxidation of the fatty acids in the oil. Previous studies have reported that vitamin E is destroyed when frying oil is repeatedly heated.[9,10] The reduction in the vitamin E content of frying oils may contribute to the increased production of reactive oxygen species (ROS) and may cause oxidative damage. Palm oil contains both tocopherol and tocotrienol compounds, whereas tocotrienols are not present in soy oil. Tocotrienols have a stronger antioxidant activity than tocopherols,[33,34] the presence of tocotrienols in palm oil may contribute to the greater resistance to oxidation with repeated heating relative to soy oil observed in this study.

This study had several limitations. The number of animals that were investigated was relatively large. A smaller study using eight rats from each group instead of ten may have

yielded similar results. We used ten rats to avoid a true difference between tested groups from not being detected. Although indirect BP measurements may yield results with a lower accuracy, we chose a non-invasive technique (the tail-cuff method) because it was possible to take repeated BP measurements without injuring the rats. Regardless of the lower accuracy, this method enabled us to detect substantial differences in BP between the groups and changes in BP over time for a large numbers of rats.

Our earlier laboratory findings reported that heated vegetable cooking oils caused increased lipid peroxidation as indicated by elevated serum acid thiobarbituric acid (TBARS) levels.[35,36] We suggest that repeated heating may increase the number of free radicals. In addition, our laboratory also documented that heating causes a reduction in vitamin E levels in cooking oils.[20] Therefore, the reduction in the antioxidant levels of palm and soy oil was inversely proportional to the increase in lipid peroxidation.

We conclude that fresh palm and soy oils do not detrimentally affect blood pressure, and they significantly elevate nitric oxide levels and reduce contractile responses. When these oils are heated repeatedly during the cooking process, they generate free radicals and reduce the levels of antioxidants and vitamins, which lead to oxidative stress. Increased ROS and the altered balance between NO and ROS lead to impaired NO bioavailability, which results in decreased endothelium-dependent vasorelaxations leading to hypertension.

Project location: this project was conducted at the Universiti Kebangsaan Malaysia and the University of Malaya.

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AUTHOR CONTRIBUTIONS

Jaarin K designed and organized the study, interpreted the results and drafted the manuscript. Mustafa MR designed and organized the study, interpreted the results and revised the manuscript. Leong XF performed the studies, collected the data, performed data analyses, interpreted the results and revised the manuscript. All of the authors have read the final manuscript.

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