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Effects of six-week consumption of lard or palm oil on blood pressure and blood vessel H_2S in middle-aged male rats

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ABSTRACT

Background: Cardiovascular disease is the leading cause of death. The etiology of this disease is multifactorial, with unhealthy nutrition being one of the main risk factors. Diets high in animal fats and saturated fatty acid have been associated with an increased risk for cardiovascular diseases. However, the results of investigations on the effects of lard (LO)- and palm oil (PO) on cardiovascular risk are still controversial due to the dosages used and the age of the animals investigated.

Objective: We investigated whether LO or PO consumption led to different effects on blood pressure, vascular functions, and lipid profiles within middle-aged rats.

Methods: The study was performed in middle-aged male rats, $n = 6$ for each group. LO, PO, or distilled water (control) 1 or 3 ml/kg were orally gavaged once a day for 6 weeks. Basal blood pressure and heart rate were measured in anesthetized rats. Fasting serum lipids were measured by enzymatic methods. The vascular functions of isolated thoracic aorta were studied using pharmacological techniques in the absence or presence of N-nitro-L-arginine, a nitric oxide synthase (eNOS) inhibitor and or DL-propargylglycine (PAG), a cystothionine-γ-lyase

(CSE) inhibitor. Additionally, the aortic wall eNOS and CSE protein expression were measured by Western blotting.

Results: In comparison to the control group (distilled water, DW), no differences were observed in any of the parameters studied after the rats took 1 ml/kg of LO or PO. However, PO caused an increase in neutrophil/lymphocyte ratio and body fat. At 3 ml/kg dosage, LO caused increased basal blood pressure (LO, 153.4 ± 3.2; DW, 131.4 ± 3.2 mm Hg for systolic blood pressure and LO, 130.9 ± 2.5; DW, 107.9 ± 5.8 mmHg for diastolic blood pressure) body and liver cell lipid accumulation, while PO led to increased body fat and fasting serum triglyceride (PO, 131.5 ± 13.2; DW, 91.8 ± 4.8 mg %). Neither LO nor PO treatment had any effect on vascular contraction to phenylephrine, except in the presence of PAG which led to an increased contractile response to phenylephrine. PO but not LO treatment caused increased vascular wall CSE protein expression.

Conclusion: The results document how both LO and PO at a dose of 3 ml/kg (corresponding to three servings of Thai fast food) cause increased cardiovascular risk factors. However, the blood vessel H_2S production increased while the lower dose had a minimal effect.

Keywords: Lard oil, Palm oil, blood vessel, liver lipid, NO, H_2S

Animal Ethic Number: Ref. 065/7

BACKGROUND

Cardiovascular disease is the leading cause of death globally. Its etiology is multifactorial and unhealthy nutrition is one of the main risk factors. Diets high in animal fats and saturated fatty acid have been associated with an increased risk of cardiovascular diseases [1-3]. However, fat is an essential macronutrient. Accordingly, it is important that types and amount of fat/oil consumption do not reach harmful levels [3].

In Thai and other Asian cuisines, lard oil (LO) is used as the major cooking oil for most deep-fried food and fried dishes. For example, fried rice, vegetable stirred fried, omelet, etc. Palm oil (PO) was introduced to Thailand about 30 years ago with the belief that plant oil is healthier than LO [4-7]. As a result, PO became more popular than LO. However, metabolic syndrome and cardiovascular diseases have also increased in the Thai people with the same trends seen in the rest of the global population [8-13]. Although other plant oils have been introduced, PO is still popular as it is inexpensive and affordable for the majority of the population within developing and third world countries [14-15].

Refined LO and PO contain a high proportion of palmitic acid, having saturated fatty acid and oleic acid (ω-9-unsaturated fatty acid, and ω-6- linoleic acid) [16-17]. They are considered atherogenic. However, the results of investigations of the effects of LO and PO on cardiovascular risk are still controversial.

Regarding LO, Hartog et al. [17] discovered that 9.1 % lard fat supplemented diet caused increased plasma levels of total cholesterol and HDL-C after 6 weeks in young pigs. Consistent with these results, a high fat diet using 50-60% kcal lard caused increased body weight, plasma triglycerides, plasma cholesterol and/or liver lipid accumulation after 12-weeks consumption

in mice [18-20]. However, the excess amount of lard as a fat supplement in the diet of these studies may not reflect the normal metabolism of lard consumption.

Concerning PO, in studies using weaned rats, Manorama and Rukmini [21] discovered that 10% of refined palm-olein oil consumption for 90 days did not affect serum triglyceride and cholesterol levels. In contrast, Boon et al. [22] discovered no change in systolic blood pressure or lipid profile with a reduction in LDL-C level after taking 15% palm olein for 15 weeks. In young rats, Go et al. [23] discovered no changes in plasma lipid level after taking PO 2.5 ml/rat/day for 22 days. In contrast, Onyeali et al. [24] discovered that 20% PO supplemented diet caused an increase in serum LDL-C and triglyceride levels after 4 weeks. However, sustained intake up to 12 weeks reduced serum triglyceride, total cholesterol, and LDL. A meta-analysis of PO consumption within humans revealed that PO caused higher LDL-C than vegetable oils low in saturated fat, in addition to higher HDL-C compared to trans-fat-containing oils. Cardiovascular disease risk markers occurred when PO was substituted for the primary dietary fats [25-26], where food patterns were not related to the confounding nature of the dietary saturated fats on cardiovascular risk [27].

Controversies are partially due to the dosages of LO and PO used and the age of individuals investigated. Furthermore, there are no reports on the effects of dosages of LO and PO consumption on lipid profile in relation to blood pressure and vascular functions, especially in older individuals where the development of cardiovascular risk is more relevant. Therefore, the present study was performed on middle-aged rats. Additionally, we not only studied classical risk factors such as blood pressure but also vascular function and lipid profile. Two doses (1 and 3 ml/kg) of LO and PO were used, the higher dose (3 ml/kg) corresponding to the amount of oil used for preparing three servings of Thai fast food. The vascular parameters that we studied were especially related to endothelial function, where nitric oxide (NO) and H_2S (gasotransmitters produced from blood vessels) are critical regulators [28-36]. These parameters were chosen as aging is associated with endothelial dysfunction and consequently decreased NO/H_2S production [37-38], which is the early stage of pathophysiological changes in the development of cardiovascular disease [39-41].

The results were expected to provide new information about the potential risks of LO and PO consumption.

MEDTHODS

Lard oil (LO) preparation

Pig lumbar lard sheet was obtained from the Faculty of Natural Resource-pork butcher, Prince of Songkla University, Hat-Yai, Thailand. The lard was chopped into small pieces and fried to obtain lard oil (LO) and was kept in a refrigerator until used.

Palm oil (PO) preparation

Refined palm oil was obtained from Chompol Palm oil industry, Chompol province, Thailand.

Analysis of lard- and palm oil fatty acid composition and cholesterol concentration

The LO and PO were analyzed for fatty acid composition by LC-MS (using the service of the Central Equipment Center, Prince of Songkla University), and analyzed for cholesterol

concentration by In-house method TE-CH-143 base on AOAC (2016) 976.26 (using the service of the Central Laboratory (Thailand) Co., Ltd.

Pharmacological studies

Middle-aged (12-14 month old) Wistar male rats were bought from the Southern Laboratory Animal Facility, Faculty of Science, Prince of Songkla University. The animals were housed in controlled environmental conditions at 25°C on a 12 h dark and 12 h light cycle and allowed access to standard food (Perfect Companion Group Co. Ltd, Thailand) and tap water *ad libitum*. The animal methods employed in this study were approved by the Prince of Songkla University Animal Ethics Committee (Ethic Number: Ref. 06/57). The investigation conformed to the Guide for the Care and Use of Laboratory Animals (CIOMS Guidelines). The rats were randomly selected into five groups, 6 animals for each group. The experimental group was treated by oral administration of 1 or 3 ml/kg LO, PO (corresponding to the amount of oil used to prepare one or three servings of Thai fast food respectively), or distilled water once a day for 6 weeks. The body weight and 24 h food intake (one day before receiving oral gavage of each oils or distilled water) were recorded at day 0 every consecutive 7th day within the 6-week period.

Effects of the LO or PO treatment on basal blood pressure and on the haematology and clinical biochemical analysis

The same methods as previously described [42] were used. At the end of the 6 weeks of either the LO, PO, or distilled treatment, each rat (13-15 h fasting) was anaesthetized with pentobarbital (60 mg/kg). Blood pressure and heart rate were recorded via the right common carotid artery by a polyethylene catheter which connected to a Polygraph. The data were collected after a 40-min equilibration period.

After measuring the basal blood pressure and heart rate, the rat was killed by decapitation with a guillotine and two tubes of blood sample was collected. One sample was used for glucose and lipid levels analysis by enzymatic methods using an automatic chemistry analyzer which was routinely operated at the Prince of Songkla University Hospital. The other sample was sent to the hematology laboratory for a total blood count procedure measured by an automated hematology analyzer.

Effects of LO, PO or distilled water treatment on internal organs and lipid accumulation

The decapitated rat was dissected as previously described [42]. Heart, lung, liver, adrenal gland, kidney, testes, visceral fats from the epididymis, testis, retroperitoneal, and subcutaneous fats were removed and weighed.

Two pieces of liver (middle lobe) were cut, embedded into a cryostat gel, the sections (20 μm thick) were stained with oil red O (0.5% in absolute propylene glycol), and mounted with glycerine jelly for observation by light microscopy. The oil red O of each slide was extracted with 1 ml of 100% dimethyl sulfoxide (DMSO) and its absorbance was measured at 520 nm. The concentration of the oil red O was obtained from the standard curve of known concentrations of the oil red O in 100% DMSO (μg/ml). The area of a whole liver thin section

was measured using the Auto CAD 2005 program. The amount of the accumulated liver lipid was expressed in terms of μg/ml/cm^2 of the liver tissue thin section area.

Preparation of the thoracic aortic rings

The thoracic aorta was removed from the decapitated rat and placed in oxygenated 37 °C Krebs-Henseleit solution. Then adhering connective tissue was removed. Six adjacent rings of 4-5 mm in length were cut. For one ring, the endothelium layer was removed by a small cotton bud. Each aortic ring was mounted with two stainless steel hooks in a 20-ml organ bath containing Krebs-Henseleit solution of the following composition (mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 1.9, $MgSO_4 \cdot 7H_2O$ 0.45, KH_2PO_4 1.18, $NaHCO_3$ 25.0, glucose 11.66, Na_2EDTA 0.024, and ascorbic acid 0.09. The solution was maintained at 37°C and bubbled with carbogen (95% O_2 and 5% CO_2 gas mixture). One of the hooks was fixed at the bottom and the other was connected to a transducer for recording the isometric tension by a Polygraph. The tissues were equilibrated for 60 min under a resting tension of 1 g and the bath solution was replaced with pre-warmed oxygenated Krebs-Henseleit solution every 15 min.

At the end of the equilibration period, each aortic ring was tested for the viability of the endothelium by precontraction with phenylephrine (3 μM) until the response reached a plateau (5-8 min), which was followed by the addition of acetylcholine (30 μM). Endothelial viability was judged by a > 65% vasorelaxation back to the tension generated by the ring before adding the phenylephrine. Denudation was confirmed by the absence of vasorelaxation following the response to the addition of acetylcholine. The preparations were then washed several times with Krebs-Henseleit solution and allowed to fully relax for 45 min before the experimental protocol began.

Effects of the LO, PO or distilled water treatment on the pharmacological vascular functions

Role of nitric oxide

At the end of the 45-min re-equilibration after the functional endothelium testing, the basal tension of the thoracic aortic rings with intact endothelium and the rings without endothelium was adjusted to the optimal tension of 2 g and equilibrated for another 10 min. Then the contractile response to a cumulative concentration-response (*C-R*) curve of phenylephrine was obtained. After the response was obtained there were several washings and the aortic ring was allowed to fully relax for 50 min. Next, the endothelium-intact aortic rings were preincubated with L-NA for 40 min and then the second *C-R* curve to phenylephrine was obtained.

Using another set of endothelium-intact thoracic aortic rings, each ring was equilibrated under a basal tension of 2 g for 10 min and then was precontracted with phenylephrine (3 μM) for 10-15 min followed by determination of the cumulative dilator *C-R* curves to acetylcholine.

Role of H_2S

After equilibration, the endothelium-denuded thoracic aortic rings were incubated with L-NA for 40 min under a basal tension of 3 g. Then a cumulative *C-R* curve to phenylephrine was obtained in the presence of L-NA, followed by several washings and re-equilibration for 60 min in the presence of L-NA to allow full relaxation of the blood vessels to their original baseline of 3 g. Afterwards, PAG was added to the incubation and left for 10-15 min until the

aortic contraction reached a plateau and the cumulative *C-R* curve to phenylephrine was obtained in the presence of L-NA and PAG.

eNOS and CSE Western blot analysis

Thoracic aortae of the LO- and PO- treated groups and the distilled water control groups (*n* = 4) were obtained in order to measure the expression level of enzyme eNOS and CSE. After removal of the adhering connective tissue, the blood vessel was then cut in to small rings. The endothelium was removed by a small cotton bud and kept at -70 °C until used. Protein extraction from the tissues and Western blot analysis were carried out as previously described [42]. Total proteins extracted from homogenized tissue of each animal was briefly placed in lysis RIPA buffer [25 mM Tris-HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 0.5 mM EDTA containing the protease inhibitor cocktail (GE Healthcare)]. The protein lysate of each animal was centrifuged and supernatant was used to quantitate the protein content by Bradford assay. Protein at 50 μg was run on 12% SDS-polyacrylamide gel electrophoresis. Then the protein bands were transferred onto nitrocellulose membrane. The membranes were blocked with 5% low fat dry milk in TBS-T (Tris buffer saline- 0.1% Tween 20) for 1 hour, followed by primary antibodies incubation against eNOS (1:250), CSE (1:1,000), and β-actin (1:1,000) dissolved in 1% low fat dry milk in TBS-T overnight at 4°C [rabbit eNOS and rabbit β-actin antibodies were from Cell Signaling (USA); mouse CSE was from Abnova (U.S.A)]. Membranes were then incubated with HRP-conjugated rabbit IgG (1: 5,000) for eNOS and β-actin and mouse IgG antibody (1: 5,000) for CSE. The membrane was incubated with chemiluminesescence detection kit (Pierce, Rockford, USA) and the protein signal was detected by Fusion FX5XT spectra/Superbright (Vilber Lourmat).

Drugs

The following drugs were used. Acetylcholine chloride, N^G-nitro-L-arginine (L-NA), norepinephrine, phenylephrine hydrochloride, DL-propagylglycine (PAG), pentobarbital, and oil red O obtained from Sigma, U.S.A. Glyceryl trinitrate was obtained from Mycomed, Denmark. Acetylcholine chloride and phenylephrine were dissolved in a solution containing NaCl 9 g/l, NaH_2P0_4 0.19 g/l, and ascorbic acid 0.03 g/l.

Statistical analysis

The results were expressed as the mean ± standard error of the mean (SEM) (*n* = 6 for vascular function study and *n* = 4 for Western blotting). "*n*" is the number of animals. Statistical differences were determined by the Student's unpaired *t*-test or by one-way analysis of variance (ANOVA), followed by Tukey's range test using GraphPad Prism 5.00. A p value < 0.05 was considered to identify a significant difference between values.

RESULTS

Fatty acid composition of the oils

The main component of fatty acid composition of the LO and PO is oleic acid (40%); followed by palmitic acid (LO, 21%; PO, 36%), linoleic acid (ω-6-unsaturated fatty acid, LO, 19%; PO, 7%), stearic acid (LO, 9%; PO, 4%). The other components only contained about 0.5-1.5%; myristeric acid, alpha linoleic acid (ω-3-unsaturated fatty acid), eicosenoic acid and cis 11, 14

eicosadienoic acid (in LO but not in PO). The cholesterol content (mg/100 g) is 55.40 and 2.92 for lard- and palm oil respectively.

Effects of 6 weeks LO or PO treatment on the body weight, food intake, animal blood pressure, internal organs and adipose tissue and blood chemistry

In comparison to the distilled water control group, there was no difference in animal body weights or food intake after treatment with 1 or 3 ml/kg LO or PO (Table 1). At the dosage of 1 ml/kg, neither the LO or PO treatments caused changes in basal blood pressure and heart rate. However, when the dosage increased to 3 ml/kg LO but not PO caused increased basal systolic and diastolic blood pressure with no changes in basal heart rate (Table 1). None of the internal organ weights changed after treatment with LO or PO either 1 or 3 ml/kg (Supplementary Table 1). However, the relative adipose tissue weight per 100 gm body weight around the prostate glands, retroperitoneal, and subcutaneous increased after treatment with LO or PO except the 1 ml/kg LO (Table 2). Liver cell lipid accumulation increased after treatment with 3 ml/kg LO but not with the 1 ml/kg LO or with the PO (Figure 1A-D). Only 3 ml/kg PO treatment caused increased fasting plasma triglycerides (Table 2). There were no changes in plasma glucose in any of the groups studies (Supplementary Table 2). With the complete blood cell counts, only neutrophils were discovered to increase. Lymphocytes decreased after LO or PO treatment, while the other parameters were similar to the distilled water control group (Supplementary Table 3). The neutrophil-to-lymphocyte ratio was also discovered to increase for the 3 ml/kg LO and the (1 and 3 ml/kg) PO treatments (Table 3).

Effects of LO and PO treatment on vascular functions

Effect on contraction and relaxation of the thoracic aorta

There were no changes in contractile responses to phenylephrine of the endothelium-intact thoracic aortic rings obtained from LO- or PO-treated rats in comparison with the distilled water control group.

Table 1. Effects of lard oil (LO), palm oil (PO), or distilled water (DW, control) consumption on body weight, food intake, basal blood pressure, and heart rate in anesthetized rats.

Treatments	Body weight (g)		Food intake	Basal Systolic BP	Basal Diastolic BP	Basal heart rate
	Initial	Final	(g/day)	(mmHg)	(mmHg)	(bpm)
DW	461.8 ± 8.2	477.2 ± 12.9	14.86 ± 0.91	131.7 ± 4.9	107.9 ± 5.8	421.7 ± 13.9
LO 1 ml/kg	455.2 ± 7.7	487.8 ± 8.8	14.30 ± 1.07	144.1 ± 5.3	119.6 ± 5.8	467.5 ± 12.6
LO 3 ml/kg	453.5 ± 8.8	475.0 ± 7.0	12.77 ± 0.83	153.4 ± 3.2[a]	130.9 ± 2.5[a]	448.3 ± 9.9
PO 1 ml/kg	454.5 ± 6.9	483.0 ± 8.2	14.18 ± 0.65	127.2 ± 6.7	107.0 ± 5.8	421.0 ± 0.5
PO 3 ml/kg	455.5 ± 9.9	471.3 ± 3.4	12.62 ±1.38	139.6 ± 3.1	115.8 ± 1.5	446.7 ± 7.6

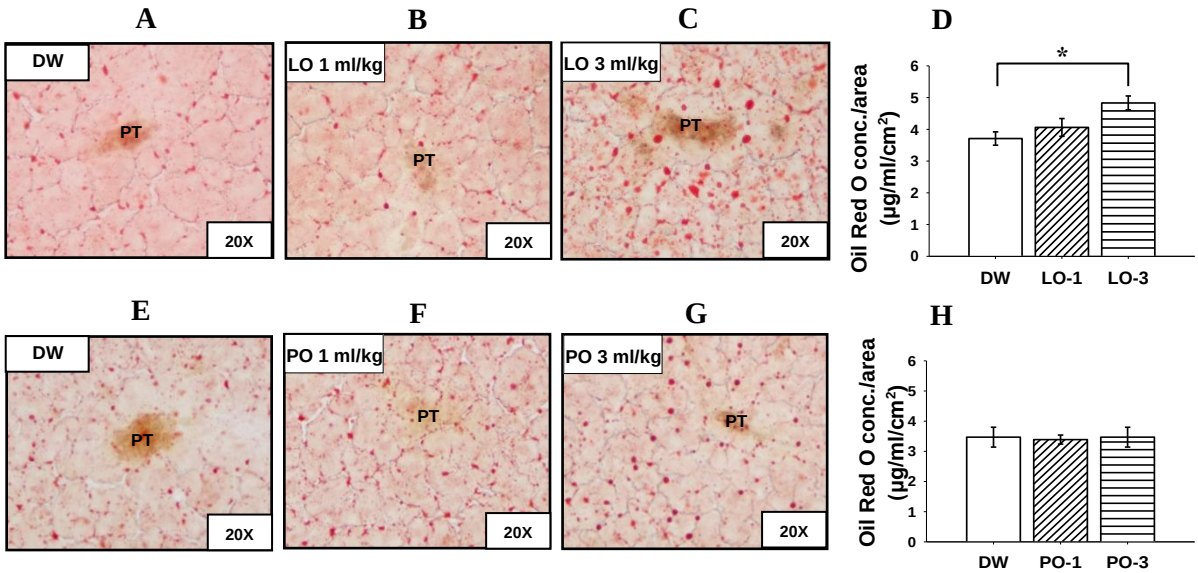
Values were expressed as mean ± SEM, $n = 6$. Significantly higher than the control group was expressed as $p < 0.05$.

Table 2. Effects of lard oil (LO), palm oil (PO) or distilled water (DW) consumption on body adipose tissue accumulation and fasting serum triglycerides level.

Treatments	Adipose tissue weight/100 g body weight (% g)			Triglycerides (mg %)
	Prostate	Retroperitoneal	Subcutaneous	
DW	0.10 ± 0.01	2.3 ± 0.1	6.5 ± 0.5	91.8 ± 4.8
LO 1 ml/kg	0.10 ± 0.01	2.4 ± 0.3	6.8 ± 0.4	94.7 ± 11.3
LO 3 ml/kg	0.17 ± 0.02[a]	3.0 ± 0.2[a]	8.0 ± 0.3[a]	86.2 ± 7.7
PO 1 ml/kg	0.13 ± 0.01[a]	2.7 ± 0.2[a]	7.9 ± 0.4[a]	87.8 ± 6.1
PO 3 ml/kg	0.12 ± 0.01[a]	2.8 ± 0.1[a]	8.1 ± 0.4[a]	131.5 ± 13.2[a]

Values were expressed as mean ± SEM, n = 6. [a]significantly higher than control group, $p < 0.05$.

Pretreatment of the intact-aortic rings with N^G-nitro-L-arginine (L-NA) or removal of the vascular endothelium caused an increase in sensitivity and the maximal contractile responses of the aortic rings to phenylephrine to the same extent compared to the distilled water control group (Figure 2A-C; LO, E-G; PO). The relative relaxation to acetylcholine of endothelium-intact aortic rings precontracted with phenylephrine obtained from the LO- or PO-treated rats were similar to that of the distilled water control group (Figure 3A; LO and B; PO).



(A) distilled water (DW), (B) LO 1 ml/kg, (C) LO 3 ml/kg, and (D) their oil red O concentrations. (E) distilled water (DW), (F) PO 1 ml/kg, (G) PO 3 ml/kg, and (H) their oil red O concentrations. Values represent mean ± SEM of 6 experiments. *Significantly higher compared to the control group, $p < 0.05$. (PT = Portal triad; oil red O staining of liver tissue frozen section, 20 mm thick, 20X magnification).

Figure 1. Effects of lard oil (LO), palm oil (PO), or distilled water (DW) consumption on liver cell lipid accumulation.

Table 3. Effects of lard oil (LO), palm oil (PO) or distilled water (DW, control) consumption on white blood cell count.

Treatment	Neutrophil (%)	LYMPH (%)	N/L ratio
DW	79.0 ± 2.1	20.8 ± 1.5	3.9 ± 0.4
LO 1 ml/kg	90.2 ± 0.4[a]	19.7 ± 0.8	4.6 ± 0.2
LO 3 ml/kg	84.6 ± 1.7[a]	15.3 ± 1.7[b]	6.0 ± 0.9[a]
PO 1 ml/kg	84.7 ± 2.0[a]	15.3 ± 1.7[b]	6.0 ± 1.0[a]
PO 3 ml/kg	86.3 ± 2.0[a]	13.5 ± 1.8[b]	7.1 ± 1.1[a]

Values were expressed as mean ± SEM, $n = 6$. [a]significantly higher and [b]significantly lower than the control group, $p < 0.05$.

Role of H_2S: Addition of DL-propargylglycine (PAG) into the incubation medium caused a spontaneous contraction of the endothelium-denuded thoracic aortic rings in the presence of L-NA for all groups. However, responses obtained from LO [1 ml/kg (2.52 ± 0.7 g), 3 ml/kg (2.36 ± 0.7 g)] and PO [1 ml/kg (1.9 ± 0.63 g), 3 ml/kg (4.29 ± 0.39)] were higher than those from the distilled control group (0.63 ± 0.11 g). These effects subsequently resulted in a greater contraction for low concentrations of the phenylephrine *C-R* curves of the thoracic aortic rings obtained from LO- or PO-treated rats than that of the vehicle control group (Figure 2D and H).

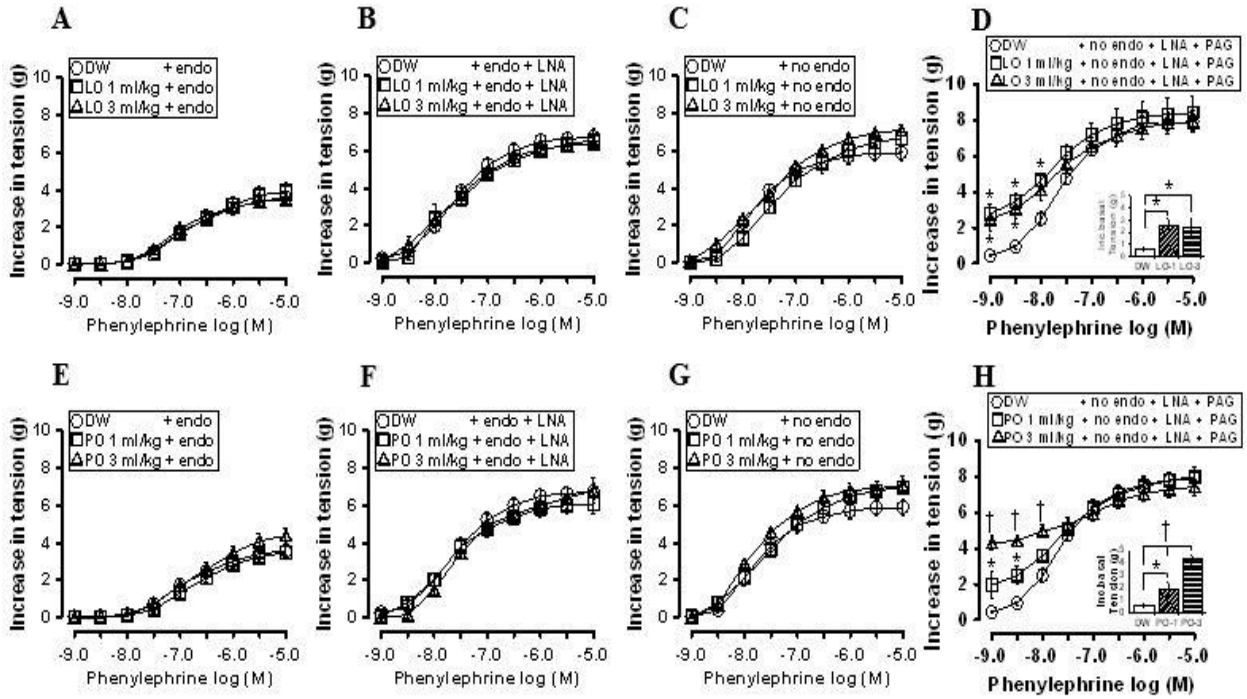


Figure 2. Effects of lard oil (LO, A-D), palm oil (PO, E-H) or distilled water (DW, control) consumption on contractile response to phenylephrine of thoracic aorta.

(A and E) endothelium-intact, (B and F) endothelium-intact with L-NA, (C and G) without endothelium, and (D and H) without endothelium plus N^G-nitro-L-arginine (L-NA) and DL-propargylglycine (PAG). Values represent mean ± SEM; $n = 6$. *Significantly higher than the control groups and †significantly higher than the other groups, $p < 0.05$.

Note: Miniature bar graphs in D and H showed the increased basal tension after addition of PAG.

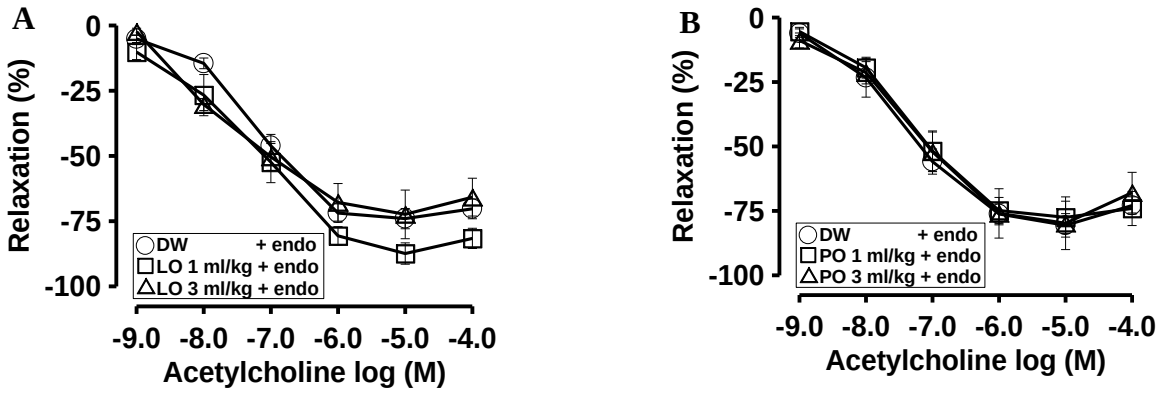


Figure 3. Effect of lard oil (LO, A), palm oil (PO, B) or distilled water (DW, control) consumption on relaxation of the endothelium-intact thoracic aortic ring precontracted with phenylephrine to acetylcholine. Values represent as mean ± SEM; $n = 6$.

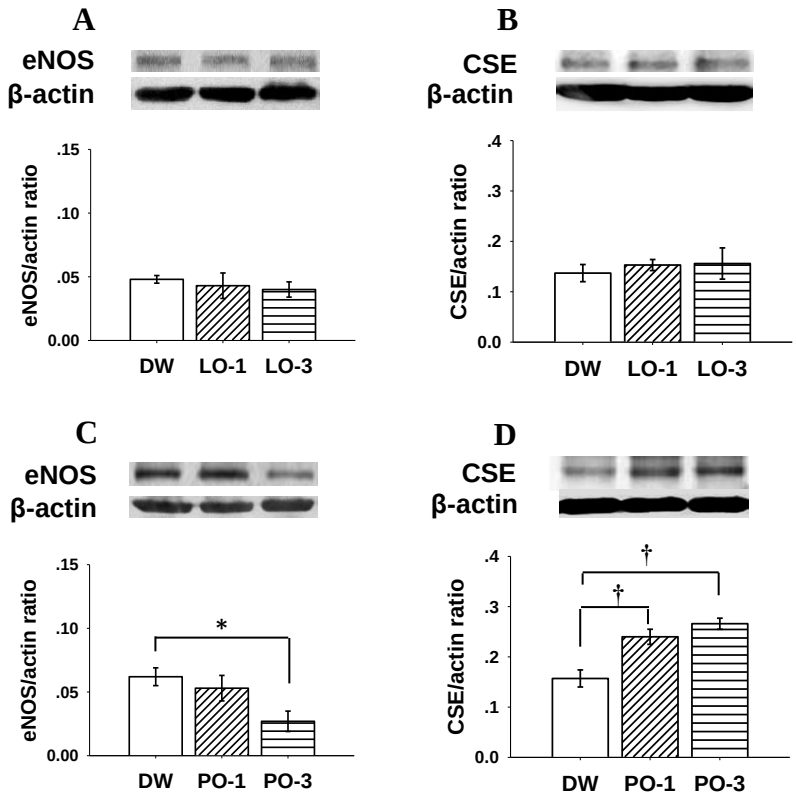


Figure 4. Effect of lard oil (LO, A), palm oil (PO, B) or distilled water (DW, control) consumption on eNOS (A, C) or CSE (B, D) protein expression of the thoracic aorta.

For each blot, β-actin expression is shown as a loading control. Values represent mean ± SEM; $n = 4$. *Significantly lower or †significantly higher than the control group (DW), $p < 0.05$.

eNOS and CSE Western blot analysis

The quantitative expression of the vascular eNOS protein expression of the LO-treated rats was not different from that of the distilled water control group, whereas that of the 3 ml/kg but not of the 1 ml/kg PO-treated rats was significantly lower than that of the distilled water control group (Figures 4A and C).

The quantitative expression of the vascular wall CSE protein expression of the PO but not the LO-treated rats were significantly higher than the distilled water control rats (Figures 4B and D).

DISCUSSION

The present study demonstrated that high dosage (3 ml/kg, corresponding to the amount of oil used to prepare three servings of Thai fast food) consumption of LO had several harmful effects on the cardiovascular system in the middle-aged male rats. Firstly, it caused increased basal systolic and diastolic blood pressure, which is an early symptom of cardiovascular disease. Secondly, it caused increased body fat accumulation in the internal organs and subcutaneously. Thirdly, it caused liver cell lipid accumulation and liver cell inflammation, consistent with increased amount of oil red O staining of the liver tissue. Finally, the finding of increased neutrophil-to-lymphocyte ratio suggests that the LO-fed animals are in lipid-driven inflammatory state [43-45].

High dosage consumption of PO 3 ml/kg also induced several cardiovascular risk factors. While it did not induce hypertension, the increase in body fat accumulation and fasting serum of triglycerides were also indicative of high-risk conditions for cardiovascular disease development in PO-treated rats [46-48]. Furthermore, the finding of an increase in neutrophil-to-lymphocyte ratio indicated that a lipid-driven inflammation occurred after taking PO in the middle-aged rats [43-45]. Nevertheless, as suggested by the unchanged oil red O staining, PO consumption at the dosage used did not induce fatty liver. These results suggest that PO consumption is probably less harmful than LO with respect to the cardiovascular risk factors found.

Although high dosage of the LO caused hypertension in the middle-aged rats, there was no alteration in the vascular responsiveness to phenylephrine in either intact or denuded thoracic aortic ring either in the absence or presence of L-NA, a nitric oxide synthase inhibitor. These results indicate that the increase in blood pressure after 3 ml/kg LO treatment might not be responsible for alteration of the vascular wall function. Additionally, dilatation to acetylcholine of the aortic ring pre-contracted with phenylephrine was not different from the control group. These results also suggest that LO consumption did not alter the nitric oxide production from the blood vessel, which was confirmed in the results as there was no difference in vascular eNOS protein expression of the LO-treated group compared to that of the control group.

With 3 ml/kg PO consumption, a similar result was found for the responsiveness of the blood vessel to phenylephrine and acetylcholine, but the amount of vascular eNOS protein expression of the PO treatment group was significantly lower compared to the distilled water control group. These results suggested that the active component of the PO might stimulate the release of another vasodilator substance to compensate for the reduction of the NO production in order to re-store the responsiveness of the blood vessel to both vasoconstrictor and dilator agonists.

Hwang et al. [49] demonstrated that mice fed high-fat diet for 5 weeks developed fatty liver with increased CBS and CSE enzyme expression and caused a significant elevation in H_2S production in the liver. Additionally, Artuna et al. [50] recently reported that NO and H_2S has a crosstalk with the regulation of vascular tone: endogenously produced H_2S can compensate for impaired vasodilatory responses in the absence of NO of the eNOS deficiency blood vessels. As a result, it is possible that middle-aged rats fed high dosage of LO and PO might have an increase in vascular CSE and/or H_2S production to play an adaptive role against the reduction of NO to maintain normal vascular functions. Our studies on blood vessel

function discovered that addition of the PAG into the incubation medium caused an increase in basal vascular tone of the LO and PO treated blood vessels higher than that of the control group. This effect caused increased contraction of the vessels from PO and LO treated animals to small concentration of the phenylephrine when compared to the results obtained from the control group. This was consistent with the results of how the blood vessel wall CSE enzyme protein expression of the PO-treated rats significantly increased compared to the control group. In contrast, the CSE protein expression of the LO-treated blood vessel did not change when compared to the control group. Consequently, our results suggested that increase in H_2S production of the blood vessels in LO-treated animals may occur via different mechanisms. One possibility may be that LO-treatment led to increased activity of the blood vessel CSE. Evidence from our indirect assay measuring the effect of basal blood vessel H_2S production on the magnitude of basal vascular tone developed after the adding of PAG, a CSE inhibitor, had no difference in the magnitude of the basal tone developed between those obtained from 1 or 3 ml/kg LO-treated rats, although both of them were higher than that of the control group. However, further studies measuring the H_2S concentration/production rate directly from the blood vessel would be required to clarify this possibility. Nevertheless, these results indicated that LO or PO consumption caused increased blood vessel H_2S production to regulate vascular tone in middle-aged male rats.

The increased vascular H_2S and/or CSE protein expression after the feeding of PO in the present study is contrary to those reported by Peh et al. [51] and Jenkins et al. [52]. The reason for these might be due to differences in fat diet composition. In the present study, we used LO or PO, while Peh et al. [51] and Jenkins et al. [52] used 16% fat and 12.5% cholesterol and high-fat western diet (SF05-031; 21% fat, 0.15% cholesterol) respectively. Consequently, the difference may be due to cholesterol content which was discovered to only be 0.05% in LO and 0.003% in PO within the present study. However, further study in cholesterol-fed middle-aged rats would be needed to clarify the possibility.

At lower dosage, 1 ml/kg (corresponding to the amount of oil used to prepare one serving of Thai fast food), LO or PO consumption did not show any harmful cardiovascular risk factor except the increase in subcutaneous fat and neutrophil to lymphocyte ratio by the PO but not the LO consumption. The finding of increased H_2S production with an increased vascular wall CSE enzyme expression of the PO-treated rats suggests that the lipid-driven body inflammation might be protected by an increased endogenous H_2S from the vessel wall.

To date, this is the first report using appropriate methods to provide good evidence that LO and PO increase cardiovascular risk factors in a dose-dependent manner in middle-aged animals. At the higher dosage of 3 ml/kg/day for 6 weeks, both LO and PO generated a series of harmful cardiovascular risk factors: lipid-driven body inflammation, increased body and liver lipid accumulation, increased fasting serum triglyceride, and finally hypertension with LO. Although both LO and PO seemed to increase H_2S production, the amount generated by the LO was not sufficient to normalize the basal blood pressure of the middle-aged rats.

CONCLUSION

These animal experiments suggest that in order to maintain longer cardiovascular health, one should avoid LO or PO consumption or consume as little as possible. Intake should be no grater than 1 ml/kg/day (9.5 % of total cal/day or 3.2% of total food intake/day), which is in the same range as the agreement of the international guidelines, that saturated fatty acids intake should be kept to <10% of total energy, in a balanced diet; within these limits, no effect of palm oil consumption on human health can be foreseen [53]. Our results suggest that PO is slightly less detrimental than LO.

Limitations of the Study: The present study was limited to middle-aged male rats, and did not measure directly H_2S concentration/production rate in the vascular wall. Therefore, it could not be confirmed whether the augmented role of H_2S in the LO fed group is due to an increase in CSE activity. Accordingly, our next study should include female rats with additional experiments for a direct assay of H_2S production.

List of Abbreviations: LO, lard oil; PO, palm oil; NO, nitric oxide; L-NA, N^G-nitro-L-arginine; CSE, cystathionine-γ-lyase; PAG, DL-propargylglycine.

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Authors' Contributions: CJ designed and conducted the research. JN and LSC conducted the research and performed statistical analysis. KK assisted for Western blot technique. NR assisted for oil red O staining technique. CJ, JN and LSC wrote the manuscript. All authors read and approved the final version of the manuscript.

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