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Research Article

Recycled Deep-frying Oil Causes Blood Pressure Elevation and Vascular Hypertrophy in Sprague-Dawley Rats

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ABSTRACT

Background: Recycling cooking oil for deep frying makes the oil more vulnerable to lipid peroxidation that generates oxidative compounds, linking to an increased risk of cardiovascular diseases. The study aimed to investigate the long-term effect of recycled deep-frying oil on blood pressure and aortic structure in rats. **Methods:** Adult male Sprague-Dawley rats were divided into four groups, namely (a) control; (b) fresh oil (FO); (c) deep-frying oil (DO); and (d) recycled deep-frying oil (RDO). Feeding duration was six months. Blood pressure was measured at baseline and the end of the study using tail-cuff method. After six months the rats were sacrificed and aortic arches were obtained to quantitate intimal and media thickness as well as to examine pathological changes. **Results:** Both FO and DO groups did not show any significant blood pressure and aortic microscopic structure. There was a significant increase in blood pressure in rats fed RDO compared to other groups. Aortic media thickness was significantly increased in RDO group, while intimal thickness did not differ among the groups. Microscopic examination showed an enlarged space between elastic lamellae in aorta of RDO group. **Conclusions:** Long-term intake of food containing recycled deep-frying oil causes blood pressure elevation and vascular hypertrophy in rats.

Keywords: aorta, hypertension, heating, lipid peroxidation, vegetable oil

1. Introduction

Due to its convenient preparation and the desirable properties of colour, texture, smell of the food, deep frying is now gaining more popularity [1]. During deep frying, the cooking oil is heated at high temperature with exposure to air and moisture, resulting in lipid peroxidation [2]. This thermal deterioration generates harmful oxygen reactive species which might be deleterious to the cardiovascular system. Repeated heating the cooking oil makes it even more susceptible to lipid peroxidation [3]. However, reusing the same cooking oil for deep frying before discarding it is common in the society, not only by roadside vendors but in household as well [4]. It reduces the cost of food preparation but they may not be aware that such practice may cause exposure to harmful oxidative compounds.

Several studies have also demonstrated the detrimental effects of oxidised oil, including alterations in platelet function [5], liver dysfunction [6], and endothelial impairment [7]. Although a previous study reported that ingestion of deep frying oil did not seem to influence blood pressure and its related parameters in rats [8], the risk of hypertension was found to be positively associated with the intake of cooking oil oxidative compounds in human subjects [9].

Considering the hazardous potential of consuming repeatedly heated deep frying oil, the present study was undertaken to determine the adverse effects of the oil on blood pressure and blood vessel histology in experimental rat models. Palm oil was chose in this study because it is widely used in Malaysia [10]. The oil which is popular in food industry and family kitchen due to its oxidative stability, contains saturated and unsaturated fatty acids at almost equal levels [11].

Since cooking oil constitutes a major part in daily food intake, the effect of the oil on cardiovascular health is a long-term issue. Therefore, the feeding duration for the experimental rats was set at six months

2. Design and Methods

Animals

Twenty-four (n = 24) adult male Sprague-Dawley rats with weight averaging 200-260 g were obtained from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia. The animals were housed in stainless steel cages and kept at room temperature of 27˚C ± 2˚C and a 12-hour light cycle in the department's animal house. All rats had free access to standard rat chow and tap water *ad libitum* throughout the experiment. The experimental procedures and animal handling were monitored and approved by the Universiti Kebangsaan Malaysia Animal Ethnics Committee (UKMAEC).

Deep frying and diet preparation

Palm oil (Lam Soon Edible oil, Kuala Lumpur, Malaysia) used in this study was purchased from a local market. The oils given to rats were either freshly, deep-fried once or repeatedly. The deep-frying oil (DO) was prepared by frying 1 kg sliced sweet potatoes in 2.5 L of oil in a stainless-steel wok at about 180°C for 10 min. The heated oil was then allowed to cool for at least five hours before continuing the deep frying process for four cycles to get recycled deep-frying oil (RDO). This step was done to simulate the practice of reusing the same oil for deep frying in the community. Standard rat chow (Gold Coin, Kepong, Malaysia) was ground and formulated by mixing 15% weight per weight (w/w) of respective oils. The mixture was reformed into pellets and dried in an oven at 80°C overnight.

Study design

After one week of adaptation, the rats were randomly and equally assigned to four groups. The rats were fed with the following diets for six months:

a) Rat chow only (control);

b) Rat chow mixed with 15% w/w fresh oil (FO);

c) Rat chow mixed with 15% w/w deep-frying oil (DO);

d) Rat chow mixed with 15% w/w recycled deep-frying oil (RDO).

Systolic blood pressure was measured at the beginning and the end of the study. After six months of feeding, all of the rats were humanely sacrificed. Arches of aorta were obtained and processed for histological examination.

Blood pressure measurement

A non-invasive blood pressure measurement by tail cuff method was employed. The rats were pre-warmed for 10 minutes to enhance the blood flow to the tails. Systolic blood pressure was then determined using the PowerLab Data Acquisition Systems (ADI Instruments, Castle Hill, NSW, Australia). Five readings were obtained from each rat and averaged.

Histological examination of aorta

The specimens were vertically embedded with paraffin. Five consecutive cross sections of aorta at thickness of 5 µm from each rat were accomplished. Verhoeff van Gieson (VVG) staining method was applied to stain the sections and then visualised under a Nikon Eclipse 80i microscope (Nikon Corporation, Tokyo, Japan). Intimal and media thickness of aorta were analysed using the software Image-Pro Plus (Media Cybernetics, Silver Spring, MD, USA). The histological slides were then examined for any pathological changes by two blinded investigators at magnification of 100X, 200X and 400X.

Statistical analysis

One-way analysis of variances (ANOVA) followed by Tukey's Honestly Significant Differences (HSD) post-hoc test was used to evaluate the differences of mean among the groups. Differences were considered statistically significant at $p < 0.05$. Results were presented as the mean ± SEM. Analyses were carried out using the Statistical Product and Service Solutions (SPSS) version 16.0 (SPSS, Inc., Chicago, IL).

3. Results

Blood pressure

There was a significant increase in blood pressure in rats following the six months dietary intervention with RDO. The percentage of blood pressure increment was significantly higher ($p < 0.05$) than all other groups. The rats fed FO or DO did not demonstrate any significant changes in blood pressure before and after feeding (Figure 1).

Intimal thickness of aorta

We did not observe significant changes in the thickness of tunica intima of aorta in all groups (Figure 2).

Media thickness of aorta

There was significant thickening of tunica media in rats fed RDO compared to the other groups ($p < 0.05$). The rats fed FO and DO did not show any changes in aortic media thickness (Figure 3).

Intima-to-media ratio

There was no significant difference in the ratio between intimal and media thickness among the groups (Figure 4).

Microscopic changes of aorta

Compared to control, the tunica media of aorta in the rats fed RDO was noticeably increased. The spaces between elastic lamellae were enlarged. Moreover, the alignment of lamellae was not organised in RDO group. However, we did not observe any remarkable pathological alterations in the aortic sections of the FO and DO groups (Figure 5).

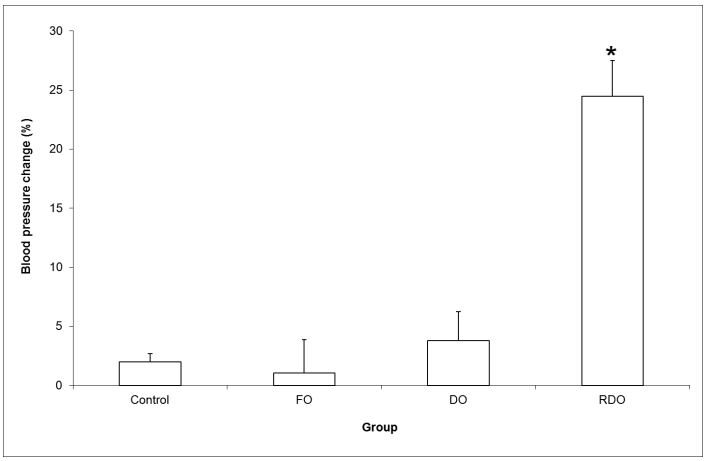

Figure 1 Changes in blood pressure in rats following six months of feeding with either rat chow (control) or rat chow mixed with fresh oil (FO), deep-frying oil (DO) or recycled deep-frying oil (RDO). Data were presented as percentage compared to respective pre-treatment blood pressure. Results were given as mean ± SEM.

* $p < 0.05$ vs. control, FO and DO

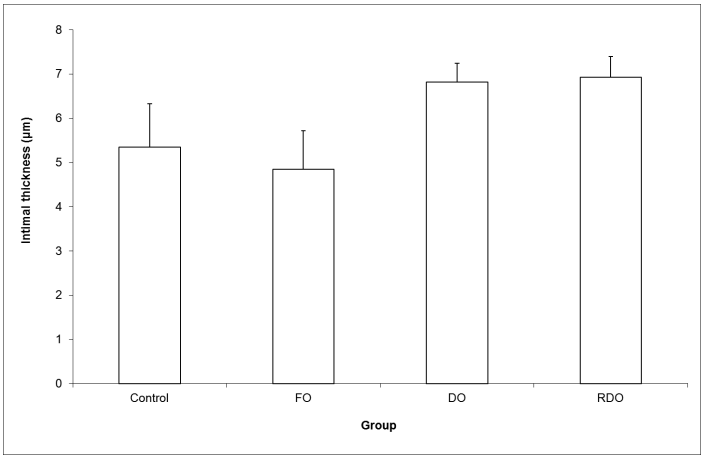

Figure 2 Intimal thickness of aorta in rats following intake of either rat chow (control) or rat chow mixed with fresh oil (FO), deep-frying oil (DO) or recycled deep-frying oil (RDO) for six months. Results were given as mean ± SEM.

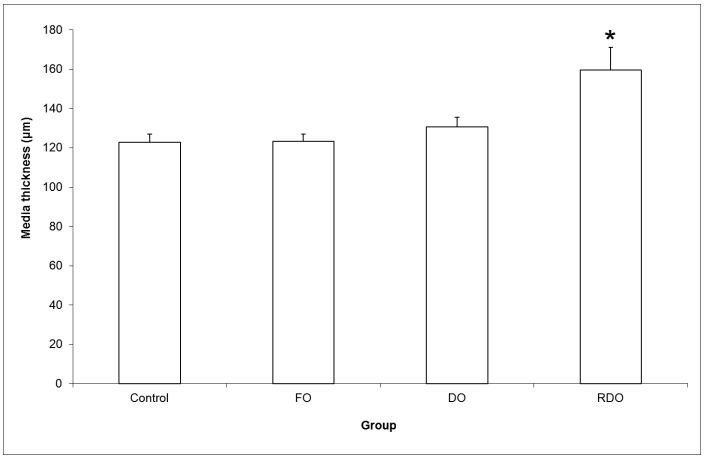

Figure 3 Media thickness of aorta in rats following intake of either rat chow (control) or rat chow mixed with fresh oil (FO), deep-frying oil (DO) or recycled deep-frying oil (RDO) for six months. Results were given as mean ± SEM.

* $p < 0.05$ vs. control, FO and DO

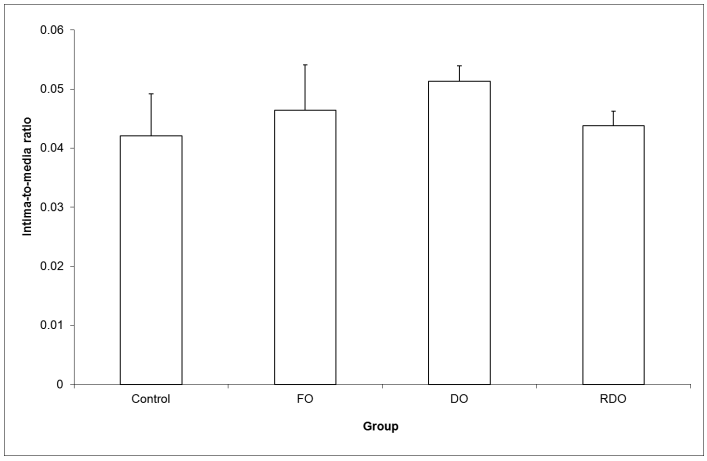

Figure 4 Intima-to-media ratio of aorta in rats following intake of either rat chow (control) or rat chow supplemented with fresh oil (FO), deep-frying oil (DO) or recycled deep-frying oil (RDO) for six months. Results were given as mean ± SEM.

4. Discussion

Based on the present findings, prolonged intake of food containing cooking oil that has been used for deep frying once did not cause much adverse effects in rats. The situation however became detrimental following consumption of repeatedly used deep-frying oil, as it caused increases in blood pressure after six months. A previous study has also reported that repeatedly heated palm oil resulted in high blood pressure with necrosis of cardiac tissue in experimental rats [12]. This may be due to over-production of oxidative compounds in the cooking oil by the process of repeated deep frying. Frequent heating of cooking oil is capable of making it more vulnerable to lipid peroxidation, thus producing extreme level of invasive by-products [13]. In addition, deep frying also reduces the antioxidative vitamin E constituents in palm oil [14]. Therefore, recycled deep-frying oil may demolish the antioxidant capacity and elevate oxidative stress in rats [8]. Disturbed oxidative status alters blood pressure and endothelial function, probably via the imbalance between vasodilators and vasoconstrictors [15].

Vascular thickening observed in the rats fed RDO may indicate remodelling process in response to vascular haemodynamic alterations. Elevated blood pressure causes an increase in the stresses acting on the vascular wall. Vascular smooth muscle cells respond to the pathophysiological stimuli and remodel their architecture to maintain the blood pressure [16]. In fact, this vascular remodelling may be adaptive initially, but it could be maladaptive eventually after some prolonged period, leading to unfavourable compromises. This can be seen by the lamellar disorganisation in the RDO group. As the result, these maladaptive structural alterations may impair the normal physio-physical compliance of blood vessel in the regulation of blood pressure [17].

Aside from high blood pressure, vascular remodelling may also be affected by the biochemical environment. Hypertension can be accompanied by an altered biochemical environment such as alteration of the renin-angiotensin-aldosterone system [17]. Our earlier

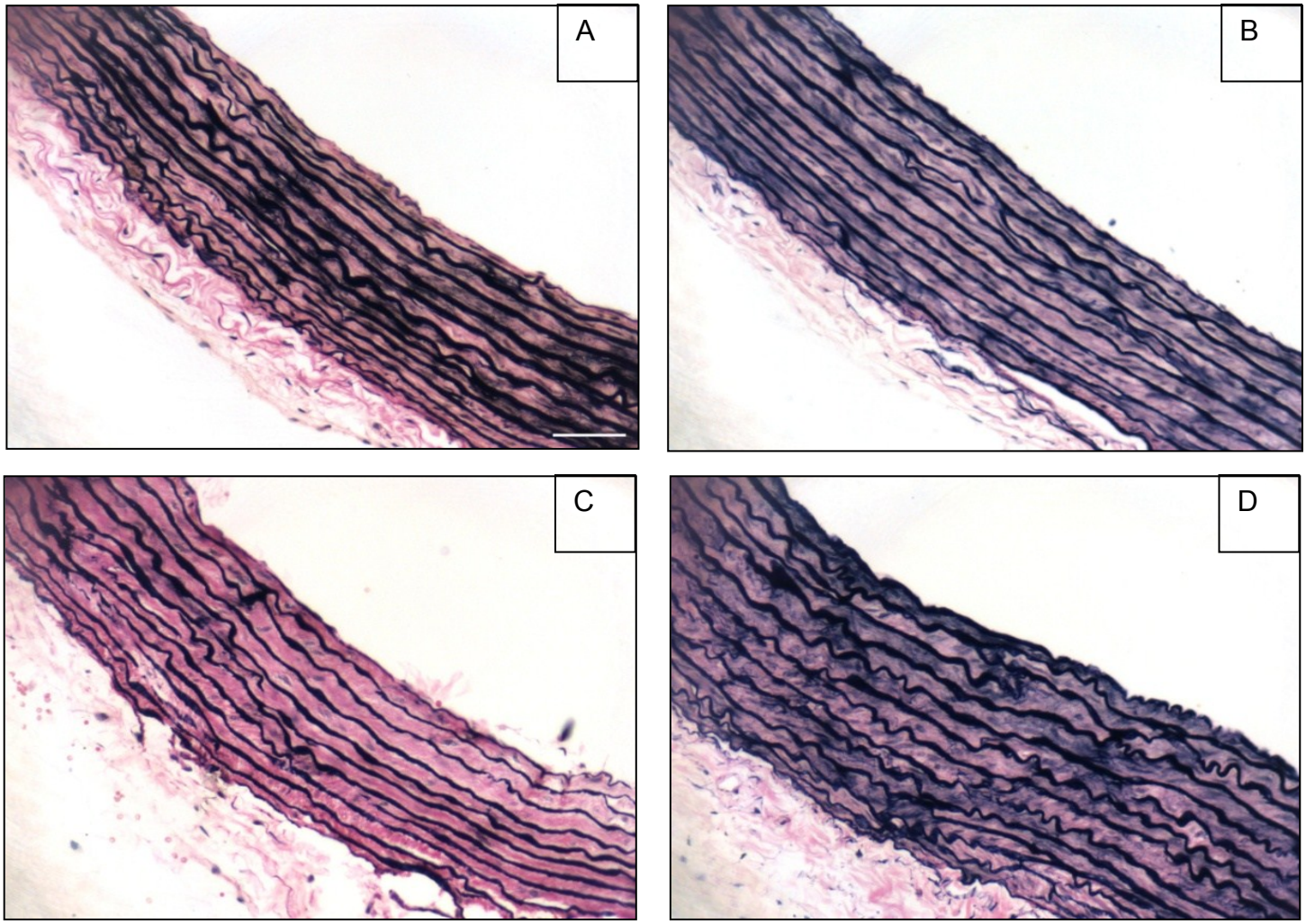

Figure 5 Representative photomicrographs of aorta stained by VVG (magnification X200; calibration bar 50µm) in rats following intake of (A.) rat chow (control) or rat chow mixed with (B.) fresh oil (FO), (C.) deep-frying oil (DO) or (D.) recycled deep-frying oil (RDO) for six months. Note the hypertrophied media in the RDO groups with an enlarged space between disorganised elastic lamellae compared to the other groups. The DO group showed no remarkable structural difference than the control and FO group. Intimal thickness was similar between all the groups.

experiment found that long-term consumption of repeatedly heated oil elevated the levels of angiotensin-converting enzyme [18], but reduced the production of vasodilator nitric oxide [19]. Angiotensin-converting enzyme converts inactive angiotensin I into potent vasoconstrictor angiotensin II that also acts as a cell growth promoter. Nitric oxide, on the contrary, inhibits cellular proliferation. We believe that an imbalance between the factors controlling cell growth and death may, at least in part, be responsible for vascular hypertrophy in the rats.

Intimal thickening may indicate formation of lipid plague and therefore the initiation of atherosclerosis. In the present study, the long-term intake of RDO did not significantly induce thickening of tunica intima of aorta, even though its thickness in the group seemed to be higher than control. These results were contradictory to an earlier study which had reported that the consumption of repeatedly heated oil increased the serum parameters related to atherosclerosis in rats [20]. The differences could be attributed to the animal models used in the study. Male adult Sprague-Dawley rats were fed heated cooking oil in the current study instead of oestrogen-deficient ovariectomised rats.

5. Conclusion

Consumption of cooking oil that has been heated once does not affect blood pressure in rats. However, consumption of recycled deep-frying oil increased blood pressure and caused vascular hypertrophy in rats. Due to its harmful implications, cooking oil should not be reused several times for deep frying of daily food.

6. Acknowledgement

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