Edible oils have different effects on lipid profiles and on the propensity for producing lipid peroxidation products. These two properties of edible oils can affect arterial structure, ultimately leading to atherosclerosis. Hypertension is said to be a predisposing factor for atherosclerosis and can accelerate its process. This paper investigates the effects of three edible oils, namely soya bean oil, palm oil and ghee, on the ultrastructure of the aortas of spontaneously hypertensive rats at the end of a 4 month feeding period. It was found that ghee produced significant structural changes to the aortic wall when compared with palm oil or soya bean oil, and that no noticeable structural differences were seen to occur on the aortas of the palm oil-fed and soya bean oil-fed groups of rats. This study suggests that the consumption of ghee, rather than palm or soya bean oil, is more likely to lead to the development of atherosclerosis.

Key words: aorta, palm oil, soya bean oil, ghee, spontaneously hypertensive rats.

Introduction

Edible oils vary in their content of saturated and unsaturated fatty acids. It is well documented that saturated fatty acids increase serum total cholesterol while unsaturated fatty acids lower it. An increase in serum total cholesterol is known to cause changes in arterial structure ultimately leading to atherosclerosis. A better correlate with clinical events is measurement of high and low density lipoproteins and a series of apolipoproteins. Ischemic heart disease is inversely correlated with high density lipoproteins and directly correlated with low density lipoproteins. Further, a high level of apolipoprotein B and a low level of apolipoprotein A are associated with increased incidence of coronary heart disease. Recent evidence suggests that besides cholesterol level, lipid peroxidation may also play a role in the process of atherosclerosis.

Edible oils which are polyunsaturated are prone to lipid peroxidation, can damage cellular structures and may influence the process of atherosclerosis. Different mechanisms by which lipid peroxidation might propagate atherogenesis and cause thrombotic events leading to myocardial ischemia or stroke have been postulated. Besides the fatty acid composition of an oil, other components of an oil may be protective or detrimental. Tocopherols found in palm oil and soya bean oil as well as tocotrienols found in palm oil are antioxidants; tocotrienols can also lower cholesterol levels while cholesterol oxides found in ghee have been reported to be atherogenic.

A predisposing factor for atherosclerosis is hypertension. It has been reported that with hypertension there is an increase in lipid peroxidation products of erythrocytic membranes of humans and of aortas of spontaneously hypertensive rats (SHR). Thus, lipid peroxidation may be partly responsible for the detrimental effects of changes in arterial structure caused by hypertension.

Palm oil, soya bean oil and ghee show varying degrees of unsaturation in their fatty acid composition and antioxidant properties. Thus, their consumption may result in different effects on lipid profile and lipid peroxidation parameters which can cause changes in arterial structure, finally leading to atherosclerosis. Because hypertension is a predisposing factor of atherosclerosis, it may exacerbate any contribution edible oils have on the process of atherosclerosis and unmask their potentially atherogenic properties. This study examines the effects of consumption of edible oils for a period of 4 months on the ultrastructure of the SHR aorta.

Materials and methods

Twenty-four two-month-old SHR with initial body weights varying from 140 to 160 g were randomly divided into four groups of six rats each. The first group (control) was fed only rat chow, which was ground to a coarse powder. The composition of the rat chow is given in Table 1. The other three groups were fed one type of dietary oil, either soya bean oil, palm oil or ghee, respectively. It was found that ghee produced significant structural changes to the aortic wall when compared with palm oil or soya bean oil, and that no noticeable structural differences were seen to occur on the aortas of the palm oil-fed and soya bean oil-fed groups of rats. This study suggests that the consumption of ghee, rather than palm or soya bean oil, is more likely to lead to the development of atherosclerosis.

Correspondence address: Prof. Zainudin Merican, Department of Pharmacy, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.
Tel: 60 3 440 5284; Fax: 60 3 291 2659.
Email: zainudin@medic.ukm.my
Accepted 9 October 1998.
Effects of edible oils on rat aorta

palm oil, or ghee, 20% weight/weight (w/w) mixed with rat chow, which was also ground to a coarse powder. The fatty acid composition of the dietary oils is given in Table 2. The duration of feeding was 4 months. The initial and final body weights of the rats are given in Table 3.

At the end of 4 months, the SHR were anaesthetized by intraperitoneal injections of nembutal. The aorta of each SHR was dissected free from surrounding tissues. Several specimens from different parts of the aortic arch each measuring about 4 mm long were obtained and dipped in a pool of cold 3% glutaraldehyde. The same tissues were further cut to approximately 1 mm in size and fixed in the glutaraldehyde at pH 7.2 for 3 h at 4°C. The aorta was sectioned at right angles to the longitudinal axis. Postfixation of the specimens was done in 1% solution of osmium tetroxide for 1 h at 4°C. The specimens were then dehydrated in increasing concentrations of alcohol before being embedding in epoxy resin (epon or agar resin 100, Agar Scientific Ltd, UK) and polymerised in an oven at 60°C for 48 h. Nine sections of 1 µm thickness from different parts of the aortic arch were obtained using an LKB 1 V ultramicrotome (LKPProdukter, Sweden), stained with toluidine blue and viewed under a light microscope.

The ultrathin sections obtained were collected on copper grids and stained with 3% uranyl acetate and Reynold’s lead citrate before they were viewed under an electron microscope (Philips EM 400; NV Philips Gloeilampenfabrieken, Eindhoven, Netherlands) and micrographs taken.

### Measurement of thickened intima area

Areas with the greatest focal thickenings were located and measured, the rest of the intima being relatively thin. The area of thickened intima was measured by projecting the image of the transverse section of the aorta on to a paper placed against the wall at a fixed distance throughout the measurement in order to keep the magnification constant for all measurements. A projection microscope was used with an objective lens magnification of ×40 and an eyepiece magnification of ×10. The tracings were then retracted onto a Summasketch graphic tablet (Summagraphics Corporation, USA) using a stylus pen. The graphic tablet was attached to a compatible IBM computer using the Autocad programme (version 2.6; Autodraft Incorporation, USA) in order to measure the projected area of thickened intima. The thickened areas of nine sections of the aortas of each rat were measured, averaged and taken as the value for one aorta. The data was then converted to its actual area by using a stage micrometer. The average area of intimal thickening for each group of rats is shown in Table 4.

### Statistical analysis

Data are presented as means ± SEM. Statistical significance of the thickened areas were determined by unpaired t-test after a one way analysis of variance using PCSTAT.

---

**Table 1. Composition of rat chow**

<table>
<thead>
<tr>
<th>Material Composition (%)</th>
</tr>
</thead>
</table>
| Crude protein (maximum) | 20  
| Crude fibre (maximum)   | 5   
| Crude fat (minimum)     | 2.5  
| Moisture (maximum)      | 13  
| Ash (maximum)           | 7.0  
| Calcium                 | 0.7-1.4  
| Total phosphorus        | 0.6-1.2  
| Nitrogen free extract   | 51  

Additives consisted of vitamin A, D3, E, C, K, B12, thiamin, riboflavin, panthothenic acid, niacin, pyridoxine, folic acid, choline, antioxidant, trace minerals. *Rat chow was obtained from Gold Coin (Malaysia) Ltd. (Port Klang, Selangor, Malaysia.

**Table 2. Fatty acid composition of edible oils**

<table>
<thead>
<tr>
<th>Fatty acid*</th>
<th>Soya bean oil</th>
<th>Ghee</th>
<th>Palm olein</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4:0 Butyric</td>
<td>—</td>
<td>3.1</td>
<td>—</td>
</tr>
<tr>
<td>C6:0 Caproic</td>
<td>—</td>
<td>1.3</td>
<td>—</td>
</tr>
<tr>
<td>C8:0 Caprylic</td>
<td>—</td>
<td>1.5</td>
<td>—</td>
</tr>
<tr>
<td>C10:0 Capric</td>
<td>—</td>
<td>2.7</td>
<td>—</td>
</tr>
<tr>
<td>C12:0 Lauric</td>
<td>—</td>
<td>2.6</td>
<td>0.2</td>
</tr>
<tr>
<td>C14:0 Myristic</td>
<td>0.1</td>
<td>11.0</td>
<td>0.8</td>
</tr>
<tr>
<td>C16:0 Palmitic</td>
<td>11.0</td>
<td>27.1</td>
<td>37.2</td>
</tr>
<tr>
<td>C16:1 Palmitoleic</td>
<td>—</td>
<td>2.1</td>
<td>0.4</td>
</tr>
<tr>
<td>C18:0 Stearic</td>
<td>4.0</td>
<td>13.3</td>
<td>4.2</td>
</tr>
<tr>
<td>C18:1 Oleic</td>
<td>23.4</td>
<td>27.0</td>
<td>43.6</td>
</tr>
<tr>
<td>C18:2 Linoleic</td>
<td>53.2</td>
<td>2.5</td>
<td>11.7</td>
</tr>
<tr>
<td>C18:3 Linolenic</td>
<td>7.8</td>
<td>1.9</td>
<td>0.3</td>
</tr>
<tr>
<td>C20:0 Arachidic</td>
<td>—</td>
<td>—</td>
<td>0.3</td>
</tr>
<tr>
<td>C20:1 Eicosenoic</td>
<td>—</td>
<td>—</td>
<td>0.2</td>
</tr>
</tbody>
</table>

*S ratios represent the number of carbon chains of fatty acids in the edible oils.

**Table 3. Initial and final body weights of spontaneously hypertensive rats (SHR)**

<table>
<thead>
<tr>
<th>Diet</th>
<th>n</th>
<th>Initial (g)</th>
<th>Final (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC</td>
<td>6</td>
<td>147 ± 4</td>
<td>337 ± 11</td>
</tr>
<tr>
<td>RC + SB</td>
<td>6</td>
<td>148 ± 3</td>
<td>343 ± 10</td>
</tr>
<tr>
<td>RC + PO</td>
<td>6</td>
<td>146 ± 3</td>
<td>321 ± 9</td>
</tr>
<tr>
<td>RC + Ghee</td>
<td>6</td>
<td>147 ± 6</td>
<td>322 ± 17</td>
</tr>
</tbody>
</table>

Initial and final body weights of SHR after 4 months of being fed rat chow (control) and rat chow mixed with 20% w/w edible oils. The initial weights of the four groups of SHR were not significantly different from each other (P > 0.05). The final weights were also not significantly different from each other (P > 0.05). Values are mean ± SEM; RC, rat chow; SB, soya bean oil; PO, palm oil; n, number of SHR.

**Table 4. Areas of thickened intima of aortas of spontaneously hypertensive rats (SHR)**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Area of thickened intima (µm²)</th>
<th>No. of SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC</td>
<td>4947 ± 779</td>
<td>6</td>
</tr>
<tr>
<td>RC + SB</td>
<td>4568 ± 402</td>
<td>6</td>
</tr>
<tr>
<td>RC + PO</td>
<td>5408 ± 747</td>
<td>6</td>
</tr>
<tr>
<td>RC + Ghee</td>
<td>7617 ± 871*</td>
<td>6</td>
</tr>
</tbody>
</table>

Areas of thickened intima of aortas of SHR fed rat chow (control) and rat chow mixed with 20% w/w edible oils for 4 months. Values are mean ± SEM; RC, rat chow; SB, soya bean oil; PO, palm oil. * indicates significant difference from other means (P < 0.05).
Results
This experiment was carried out by a non-blinded observer and naked eye examination of the aortic arch was not done. All nine areas of the aortic sections were taken at the same site of the aortic arch of each rat, avoiding the branching sites.

Light microscopy revealed no significant structural changes in the tunica intima and tunica media of the aortas of the control, soya bean oil- and palm oil-fed groups of SHR (Fig. 1a–c). The endothelial cells were seen located close to the internal elastic laminae, leaving a relatively narrow subendothelial space with the least amount of connective tissue fibres. In the tunica media, several lamellae of elastic laminae were seen to be lying somewhat parallel to each other with smooth muscle cells interposed between them. The intimal thickness of the aortas of the above three groups of SHR showed no significant differences from each other (Table 4) but were significantly different from the ghee-fed group of SHR (Table 4). Cellular elements and extracellular material were more noticeable in the subendothelial space of the thickened regions of the aortas of ghee-fed groups of rats (Fig. 1d). The tunica media of the ghee-fed SHR, however, did not show any difference from the other three groups of SHR.

At the ultrastructural level, the endothelial cells of the control, soya bean oil- and palm oil-fed groups of SHR (Fig. 2a–c) were found to be irregular in shape. Except for pinocytotic vesicles, cytoplasmic vacuoles were rarely seen. When present they were of various sizes, mostly clear, and some were filled with granular material. In a number of instances, the endothelial cells were seen to have cytoplasmic processes projecting into the lumen, most of these being short and stubby. Endothelial cells with the above features were very rarely seen but when they were seen, they were in approximately equal numbers in the control, soya bean oil- and palm oil-fed SHR.

The ghee-fed SHR showed an increase in the density and frequency of occurrences of cytoplasmic vacuoles and cytoplasmic processes of the endothelial cells. The cytoplasmic processes of the ghee-fed groups appeared to be longer and more slender, sometimes appearing to be hook-like. Certain areas of the subendothelial layer were seen to be relatively thicker in the ghee-fed groups of SHR when compared with the control, soya bean oil- or palm oil-fed groups of SHR. The thickened areas showed an apparent increase in the density of connective tissue elements, namely the fibrils of collagen and those of some connective tissue cells (Fig. 3). Cells such as fibroblasts (Fig. 4a); macrophages (Fig. 3), identified by the presence of lysosomes; and smooth muscle cells (Fig. 4b), identified by the presence of myofilaments as well as abundant endoplasmic reticula and well-defined basement membranes at higher magnifications, were more frequently

Figure 1. (a–d) Light micrographs of the aortas of spontaneously hypertensive rats fed with various edible oils for a period of 4 months. a, Control; b, palm oil-fed; c, soya bean oil-fed; d, ghee-fed. Note the thickened intima in Fig. 1d. L., Lumen; EC, endothelial cell; I, intimal layer; M, medial layer. Epon-embedded sections with toluidine blue stain (light microscope ×1000).
found in the intimal layer of the ghee-fed SHR but rarely in the other three groups of SHR. There was discontinuity of the internal elastic laminae in the ghee-fed SHR indicating possible migration of smooth muscle cells into the intima, although this was not seen in the other three groups of SHR (Figs 3 and 5).

Morphological changes in the tunica media were only noticeable in the aortas of the ghee-fed SHR. In this layer some of the smooth muscle cells were seen to contain vacuoles of various sizes with various degrees of osmiophilia (Fig. 6). However, no changes were apparent in the adventitia of any of the four groups of SHR.

Discussion
Hypertension is one of the predisposing factors for atherosclerosis and is known to accelerate its process. Spontaneously hypertensive rats were used in this study in an attempt to unmask any potential atherogenic effect edible oils may have on the process of atherosclerosis. Hypertensive rats, rabbits and monkeys have been shown to develop atherosclerotic lesions when their plasma lipoprotein levels are increased by dietary cholesterol feeding. Although the rabbit is said to be more susceptible to atherosclerosis compared with the rat, the latter was chosen for this study.
because, like humans, they are more resistant to the development of atherosclerosis and in this respect present a more realistic human model compared with the rabbit.21,22

In this study, after 4 months of feeding the rats with various edible oils, intimal thickening occurred in the ghee-fed SHR (Fig. 1d) but not in the control, soya bean oil- or palm oil-fed groups of SHR (Fig. 1a–c and Table 4). Although the fat content of feed in the palm oil- and soya bean oil-fed groups was 20% w/w, more than that of the control group, the effect on the arteries was found to be similar to the control group in that no intimal thickening occurred. This shows that although palm oil is 50% saturated, which is midway between ghee and soya bean oil, its effect on the intimal lining does not produce the same result as that of ghee, a saturated oil. It is believed that the intimal thickening is probably a reactive response to the oil and it is possible that fatty acid

Figure 4 (a,b) Electronmicrographs of the aortic intima of a ghee-fed spontaneously hypertensive rat showing (a) two fibroblasts (fb) and (b) a single myointimal cell (smc) that has invaded into the aortic intima. Elsewhere, in the intima the thickened area is filled with collagen and elastic bundles. Internal elastic lamina (iel) is intact; L, lumen; EC, endothelial cell (×3700).

Figure 5. Electronmicrograph showing an apparent migration of a smooth muscle cell (smc) into the intima of a ghee-fed spontaneously hypertensive rat. Internal elastic lamina, iel; I, intima (×6000).

Figure 6. Electronmicrograph showing smooth muscle cells (smc) in the tunica media of a ghee-fed spontaneously hypertensive rat containing vacuoles (v) with various degrees of osmiophilia (×10 500).
composition, lipid peroxidation potential and/or other properties of an oil are responsible for this effect.

The other changes observed in the intimal layer of the aorta such as vacuoles and cytoplasmic processes in the endothelial cells and increases in the density of connective tissue matrix are normal occurrences in hypertensive animals. Proliferation of smooth muscle cells and their migration from the medial into the intimal layer is also part of the pathology of hypertension. However, the extent of the lesions were more obvious in the ghee-fed SHR indicating an atherogenic effect of ghee, while the effects of palm oil and soya bean oil on the arteries are similar to the control in that less lesions occurred. Together with the presence of macrophages, this type of early lesion has been classified as a type I lesion.

The changes caused by ghee are not likely to be due to differences in the blood pressure of the groups of rats, as a study with normotensive rats fed soya bean oil, palm oil or ghee for a period of 4 and 9 months did not show any significant differences in systolic blood pressure among the three groups of rats. However, ghee is known to increase serum cholesterol levels, increase formation of lipid peroxidation products, and accelerate the process of atherosclerosis. High concentrations of serum cholesterol, principally LDL, and their metabolic products have adverse effects on endothelial cells, enhance monocyte adhesion to endothelial cells, promote foam cell accumulation and vascular smooth muscle cell proliferation.

Palm oil is generally regarded as a saturated oil because it contains approximately 50% saturated fatty acids. Recent controlled studies in humans have shown that serum cholesterol does not increase when palm oil is used. This is in contrast to substitution with the more saturated coconut oil. Recent observations may explain why palm oil does not raise plasma cholesterol in the way that is usually expected of an oil containing 50% saturated fatty acids. Besides not raising serum cholesterol, an effect qualitatively similar to soya bean oil, it has an advantage over soya bean oil in producing less lipid peroxidation products. Thus, although palm oil is 50% saturated its properties with respect to atherogenicity are quite different from ghee but appear to be more like those of soya bean oil.

This study suggests that even in hypertension, a pathologic state conducive to the formation of atherosclerosis, neither palm oil nor soya bean oil, unlike ghee, cause changes in the aorta indicative of the early development of atherosclerosis.

Acknowledgements. This work was supported by a Universiti Kebangsaan Malaysia research grant. The authors gratefully acknowledge the technical assistance of Baharom Suboh.

References