Modulation of vascular endothelial cell function by palm oil antioxidants

MY Abeywardena[1] PhD, RJ Head[1] PhD, A Gapor[2] MSc

[1]CSIRO, Division of Human Nutrition, Kintore Avenue, Adelaide, SA 5000, Australia
[2]PORIM, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

Several cardiovascular risk factors including, hypercholesterolaemia and hypertension, lead to diseased blood vessels due to endothelial cell dysfunction. Recent studies also indicate that such alterations in blood vessel function may involve free radical related mechanism(s). Therefore, in the present study, two different preparations of palm oils with variable antioxidant profiles, as well as a purified antioxidant fraction extracted from unprocessed palm oil (tocotrienol-rich-factor; TRF), were tested for their ability to influence blood vessel dysfunction in the spontaneously hypertensive rat (SHR). Adult SHRs were fed a synthetic diet supplemented (5% w/w) with either physically refined palm oil (PO), golden palm cooking oil (Nutrolein; GPO) or olive oil (OO; control diet). Antioxidant rich diet (TRF diet) was prepared by supplementing the OO diet with 0.2% (w/w) TRF. After 12 weeks of pre-feeding, segments of thoracic aorta were used to evaluate vascular function. Compared to the normotensive Wistar-Kyoto (WKY) control rats, aortic rings from the SHR showed impaired endothelium dependent relaxation to acetylcholine (ACh) which was restored by dietary TRF ($p<0.05$, ANOVA and Tukey's test). In addition, the paradoxical increase in tension in control hypertensive vessels observed at higher doses of ACh was prevented by TRF and also by the PO and GPO diets. Although the development of thromboxane-like constrictor response, after the inhibition of nitric oxide in hypertensive vessels, was unaffected by test diets, both TRF and GPO feeding prevented the amplification of this unwanted constriction by a threshold dose (7.2×10^{-10} M) of noradrenaline. Results suggest a modulatory role for minor constituents of edible oils and are in agreement with the recently reported benefits of natural antioxidants against cardiovascular diseases.

Key words: endothelium, dietary antioxidants, acetylcholine, spontaneously hypertensive rat

Introduction

The luminal surface of blood vessel is covered by a monolayer of cells commonly referred to as the vascular endothelium. The endothelium not only acts as a selective barrier against the infiltration of various molecules into the underlying tissue, but also modulates vascular tone, maintains cardiovascular homeostasis and cell growth as well as inflammatory and immune responses in blood vessels through the production of an array of both relaxant and constrictor compounds[1-3]. The major endothelial cell derived relaxant factors include; nitric oxide, prostacyclin, endothelium-derived hyperpolarising factor and adenosine whilst thromboxane, free radicals, lipid hydroperoxides and the vasoactive peptide endothelin are the main constrictor factors[2-4]. Abnormalities in endothelial cell function therefore may promote vasospasm, myocardial ischaemia, thrombosis, atherosclerosis or restenosis[1-3].

Recent findings also suggest that endothelial function is influenced by sustained hypertension, hypercholesterolaemia and also by ageing[1-5]. Furthermore, data implies that an imbalanced production of both the relaxant and constrictor factors may account for the abnormal vasoconstriction observed in these disease conditions. There is also an increasing body of evidence which indicates that oxidative stress - oxygen derived free radicals and related products - to be an important determinant in endothelial cell dysfunction[6] and thus in the onset of several cardiovascular diseases.

Such findings implicate dietary antioxidants as well as edible oils rich in endogenous antioxidants as potential candidates to extend vascular protective actions. Indeed, several recent studies, both in animal models and human subjects, have reported improvement of blood vessel function by dietary antioxidant vitamin supplementation[7-10]. Furthermore, a recent investigation in this laboratory also found specific dietary n-3 polyunsaturated fatty acids[11] and several flavonoid compounds[12] to offer vasoprotective actions against the development of vascular dysfunction in the spontaneously hypertensive rat.

Whilst it seems likely that cardiovascular benefits of dietary n-3 polyunsaturates mediate through favourable changes in the eicosanoid profile due to alterations in precursor/substrate fatty acid availability[11,13,14], it has also been reported that eicosanoid production, hence cardiovascular function, may be influenced by the non-fatty acid constituents in edible oils[14,15]. In this context it can be speculated that palm oil, due to its fatty acid composition (low polyunsaturated fatty acids) and high endogenous antioxidant (tocotrienol and tocopherol) content[14], may be effective in influencing the peroxidation of membrane phospholipids by oxygen radicals and may limit the consequent functional changes of oxidative stress. In the newer preparations of palm oil (eg, golden palm oil; Nutrolein), the carotenoids have also been preserved thus improving the overall composition and content of endogenous antioxidants.

Therefore, in the present study two different palm oil preparations with variable endogenous antioxidant profiles and a purified fraction of palm oil antioxidants extracted from unprocessed palm oil (tocotrienol-rich-factor; TRF), were evaluated for their ability to influence vascular function in the spontaneously hypertensive rat.

Materials and methods

Animals and diets

Four month old adult spontaneously hypertensive rats (SHR; N=8 per group) and normotensive Wistar-Kyoto (WKY) control rats were obtained from the colony established at this division. After an equilibration period of two weeks, animals were fed *ad libitum* a synthetic diet[16], based on the American Institution of Nutrition rodent diet (AIN-86). The total lipid content of the test diets was 5% (w/w). Physically refined palm oil (PO), golden palm cooking

Corresponding author: Mahinda Y Abeywardena, CSIRO, Division of Human Nutrition, P.O. Box 10041, Gouger Street, Adelaide, SA 5000, Australia
Tel. +61 8 8303 8889; Fax. +61 8 8303 8899;
Email: mahinda@dhn.csiro.au

oil (Nutrolein; GPO) or olive oil (OO) served as the dietary lipid source. The tocotrienol enriched diet was prepared by supplementing the base diet with 0.2% (w/w) tocotrienol-rich-factor (TRF). The α-tocopherol level of the base diet was 0.04% (w/w). Olive oil was the source of dietary lipid for the TRF supplemented diet and the unsupplemented control diet. TRF and PO were supplied by the Palm Oil Research Institute of Malaysia whilst GPO was kindly provided by the Hai Loo Enterprise Sdn Bhd, (Johore Bahru, Malaysia). OO was purchased locally.

Aortic ring preparation

Upon completion of the feeding period, rats were stunned, killed by decapitation and aorta from the thoracic region was carefully excised and cleared of adhering tissue. Aorta was then cut into eight rings, approximately 3 mm in length, and mounted under isometric conditions at a resting tension of 4g in an organ bath chamber containing oxygenated (95%/5% mixture of O_2/CO_2) Krebs-Henselit buffer at 37°C. The composition of the buffer solution was as follows: (mM) 113 NaCl, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 2.5 $CaCl_2$, 11.2 glucose and ascorbic acid (0.57mM) in deionised water. The aortic rings were allowed to equilibrate for 60 minutes before contracting with KCl (20 mM) to test tissue viability. The increase in tension was detected by Grass FT03 force transducers and recorded on a Graphtech Linearecorder (FW33701) via an amplifier[17].

Pharmacological protocol

After establishing the tissue viability with KCl, concentration response curves to noradrenaline (NA; 10^{-10} 10^{-5} M) were constructed by cumulative additions to the bath. Vascular relaxation to acetylcholine (ACh) was studied in tissues pre-contracted with NA. In brief, after repeated washing and re-equilibration for an hour, the rings were pre-contracted with NA (10^{-7} M) before concentration response curves to ACh (10^{-9} -10^{-5} M) were constructed.

To study the spontaneous release of constrictor factor(s) in hypertensive blood vessels[12], several rings were incubated with the inhibitor of nitric oxide (NO), N_ω-Nitro-L-Arginine (NOLA; 10^{-4} M), for a period up to 60 minutes. Rings used for these studies have not been exposed to noradrenaline prior to the addition of NOLA. In some experiments, rings were pre-incubated with a threshold concentration of NA ($7.2x10^{-10}$ M) for 15 minutes before the addition of NOLA.

Statistics

Contractile responses are expressed as % contraction to KCl (20 mM) for each ring. Relaxation to acetylcholine are presented as % contraction to 10^{-7} M to noradrenaline which normally elicited a half-maximal response. Where appropriate, the results are presented as Mean ± SEM. The means were compared with a one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons[18]. A p value of <0.05 was considered as statistically significant.

Results

Table 1 shows the major fatty acids of dietary oil supplements used in the present study. It is clear that the two palm oil preparations differed in the proportions of palmitic (16:0), oleic (18:1,n-9) and linoleic (18:2,n-6) acids. For example, physically refined palm oil (PO) supplement contained a higher 16:0 (about 12% higher) than golden palm cooking oil (GPO; Nutrolein) but a lower proportion of monounsaturated 18:1, (n-9) and polyunsaturated linoleic acid. In the GPO, this reduction in palmitic acid was offset by an increase in oleic (7%) and also in linoleic acid. Olive oil was rich in oleic acid (75%), and compared to the two palm oil supplements, contained a lower proportion of 16:0.

The oil preparations also differed in their antioxidant profiles. For instance, whilst both PO and OO supplements contained no carotenoids, the GPO was rich in these antioxidants (approx. 450 ppm). However, the total vitamin E levels (tocopherol and tocotrienol contents) were similar between the two palm oil samples and ranged between 630-700 ppm. In contrast OO contained no tocotrienol, and compared to palm oil, a lower tocopherol content (130 ppm).

Table 1. Major fatty acids of dietary oil supplements.

	Oil Supplement		
Fatty acid[#]	OO	PO	GPO
14:0	ND	1.0	1.0
16:0	8.8	46.1	34.4
18:0	3.1	4.3	3.4
18:1 n-9	75.1	38.7	45.7
18:2 n-6	11.2	9.0	14.2
18:3 n-3	0.9	0.1	0.4

[#]Relative proportions of fatty acids are expressed as % (w/w) of total fatty acids. Oil supplements are; OO (olive oil); PO (palm oil) and GPO (golden palm cooking oil). Fatty acids profiles were determined as reported previously[14]. ND: not detected.

Figure 1. Restoration of impaired endothelium dependant vascular relaxation in the SHR by dietary antioxidants. Aortic rings were pre-contracted with noradrenaline (10^{-7}M) and exposed to increasing concentrations of acetylcholine. Data are the mean ± SEM in preparations from 7-8 rats per group. Asterisk indicates significant difference compared to hypertensive rats fed the control diet (SHR-OO; $p<0.05$, ANOVA and Tukey's test for multiple comparisons). WKY(OO) - normotensive and SHR(OO) hypertensive - rats fed the control (olive oil) diet. PO (palm oil); GPO (golden palm oil) and TRF (tocotrienol-rich-factor) supplemented diets fed groups.

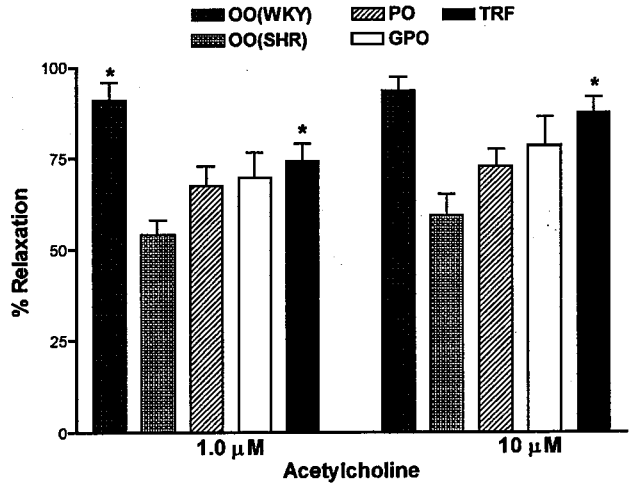


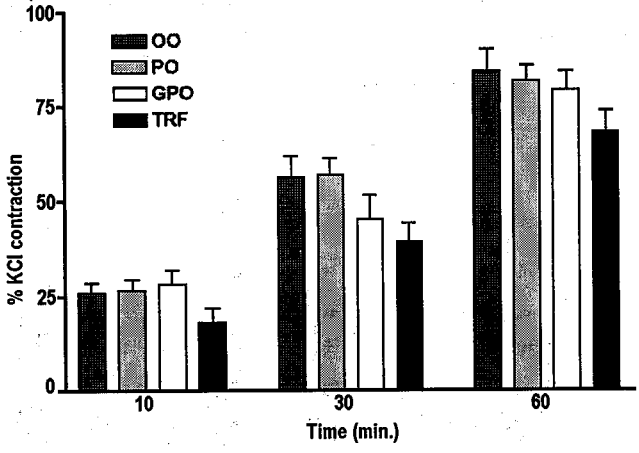
Figure 1 demonstrates the impaired vascular relaxation to acetylcholine (ACh) in the spontaneously hypertensive rat. It is clear that compared to the normotensive WKY control rats, the hypertensive vessels relax only partially in OO rats. For example, the dose-dependant increase in relaxation evident in the control vessels was not observed with the diseased vessels and the maximal relaxation achieved at the highest ACh dose amounted to only 59%. Incorporation of PO into the diet tended to increase the relaxation response, to 67.7% and 72.9% at 1 and 10 mM ACh respectively, but these changes failed to achieve significance at the 5% level (Figure 1). A similar effect was observed with the GPO diet fed animals. In contrast, TRF supplementation found to be effective in restoring the impaired relaxation in hypertensive vessels ($p<0.05$ vs SHR fed OO); over 87% relaxation was observed at 10 µM ACh.

It was also found that ACh at higher doses (1-10 µM) resulted in a paradoxical increase in contraction (30-40% of KCl response) in vessels from SHR fed the control (OO) diet. In contrast, this

response was considerably reduced in animals fed different experimental diets. For example, at the highest dose of ACh (10 mM) the contractions elicited (expressed as % KCl contraction) were; PO 9.8±2.1*; GPO 6.4±1.7*; TRF 2.1±0.4* (* indicates significant difference; $p<0.05$ vs OO).

Figure 2 shows the time dependant release of constrictor factor(s) from the blood vessels after the inhibition of nitric oxide (NO) with N_ω-Nitro-L-Arginine (NOLA; 10^{-4} M). It is clear that the release of the constrictor factor(s) is a slow process as there appears to be a gradual rise in tension with time. This increase in contraction normally tends to plateau 45 minutes after the inhibition of NO (data not shown). None of the treatments was found to significantly modify this constrictor response in hypertensive vessels ($P>0.05$, ANOVA and Tukey's test for multiple comparisons). TRF dietary rats however, displayed the lowest mean levels for all time points studied. Compared to hypertensive rats, the constrictor response was virtually absent in the normotensive control animals. For example, at 60 minutes the NOLA induced contraction was only 2.2% of the KCl contraction (data not shown).

Figure 2. Abnormal thromboxane-like constrictor response in the SHR. After contraction with KCl (20 mM), aortic rings were incubated with an inhibitor of endothelial cell nitric oxide, N_ω-Nitro-L-Arginine (NOLA; 10^{-4}M), for a period up to 60 minutes, to unmask the release of constrictor factor(s). The tension developed is expressed as a percentage of maximal contractile response to KCl. Results are the mean ± SEM; $p>0.05$.

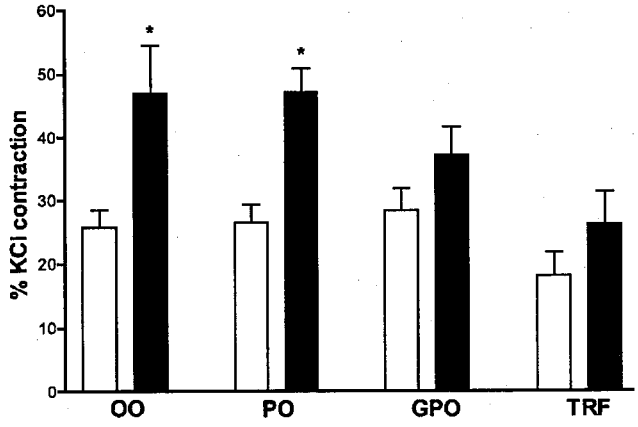


It was also found (Figure 3) that the release of this constrictor factor(s) after inhibition of nitric oxide with NOLA was considerably potentiated by the presence of a threshold dose ($7.2x10^{-10}$ M) of noradrenaline (NA). For example, in the control SHR fed the olive oil diet, presence of this low dose of NA resulted in over 80% increase in the constriction at the 10 minute (Figure 3) and over 30% at the 30 minute time point following the inhibition of nitric oxide. This amplification of the NOLA induced unwanted constriction by NA was prevented by both TRF and GPO supplementation of the diet.

Discussion

The present study supports several recent investigations which have demonstrated that blood vessel function can be influenced by dietary lipids and antioxidants[7,8,11]. For instance, natural antioxidants vitamin E and β-carotene have recently been shown to restore the impaired vascular relaxation observed in hypercholesterolaemia and atherosclerosis[7-10]. Similarly, vitamin E and several plant flavonoid compounds have also been found to exert vasoprotective actions in hypertensive vessels[7,12]. In the present investigation, it was found that tocotrienol-rich-factor (TRF), extracted from palm oil, mimicked the previously reported[14] effects of vitamin E (α-tocopherol) and restored the impaired vascular relaxation in the spontaneously hypertensive rat (SHR). Both PO and GPO feeding also displayed increased relaxation to ACh compared to the control group, although this failed to reach statistical significance. Taken together, these findings tend to imply that the improvement of vascular relaxation in this model is likely to be mediated through the antioxidant components rather than the fatty acid constituents of edible oils. Both PO and GPO were rich in natural antioxidants whilst OO was found to contain a lower vitamin E content. Data also indicate that endogenous antioxidant content of edible oils alone may not be sufficient to fully restore the impaired vascular function in diseased vessels.

Figure 3. Effect of dietary antioxidants on noradrenaline potentiation of abnormal constrictor response. Blood vessels were pre-incubated with a threshold dose of noradrenaline ($7.2x10^{-10}$M) before unmasking of the spontaneous constriction with NOLA (10^{-4}M). Asterisk indicates significance at the 5% level (N=7 per group). Both TRF and GPO supplementation prevented the amplification of tension development by noradrenaline. (□) basal release; (■) in the presence of noradrenaline.



As in the case with the TRF supplemented diet, both PO and GPO groups prevented the paradoxical increase in contractions to ACh which occurred mainly at the upper end of the dose-response curve. Such ACh induced contractions have previously been observed in blood vessels from the SHR and are thought to be due to the release of endothelium derived contracting factors (EDCFs; 3,4). The candidate mediators include prostaglandin H_2 (PGH_2) and oxygen derived free radical superoxide anion[4,19]. The ability of antioxidant rich diets to prevent the formation of EDCFs tends to imply a role for superoxide anion in mediating the ACh induced constriction. Furthermore, the effectiveness of antioxidant supplementation in restoring the endothelium dependent vascular relaxation to ACh, reported prev-iously[7-9] and also observed in the present investigation, implies that a free-radical related mechanism is involved in causing vascular dysfunction in the SHR. This speculation is further supported by recent reports which suggest that in this model of hypertension (SHR) the main endothelium derived relaxing factor pathway (L-arginine/nitric oxide) to be functioning normally[2,4,20].

In the present study, the thromboxane-like constrictor response which is evident only after the inhibition of endothelial cell NO, was not influenced by antioxidant or edible oil supplementation of the diet. However, GPO diet as well TRF feeding prevented the amplification by a low dose of NA of this NOLA induced constriction. The mechanism responsible for this action is not clear, but parallel studies with several pharmacological tools (MY Abeywardena, unpublished observations) suggest an involvement of eicosanoid metabolites in this process. In this regard it is also worth noting that minor constituents in dietary palm oil have previously been reported to modulate eicosanoid biosynthesis in cardiac muscle[14,21]. In addition, our recent findings suggested that the nature of this unwanted constriction to be a prostaglandin intermediate such as PGH_2[12] further strengthening the above speculation of a possible

modulation of eicosanoid metabolism in blood vessels by dietary antioxidants.

In conclusion, the findings of the present study are in agreement with recent reports which assign a beneficial role for natural antioxidants against cardiovascular diseases[22-24].

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棕櫚油抗氧化劑對血管内皮細胞功能的調節

摘要

若干心血管病危險因素包括高膽固醇血症和高血壓，會使血管内皮細胞功能障礙，引起疾病．最近研究亦指出這些血管功能的改變也許與自由基有關．作者在該研究中，選用自發性高血壓大鼠〔SHR〕爲對象，研究含不同抗氧化劑的棕櫚油制劑〔包括含豐富生育三烯酚因子TRF的制劑〕對血管功能障礙的影響．將合成膳食喂成年SHR，一組喂5%物理純化棕櫚油〔PO〕，一組喂金棕櫚烹調油〔Nutrolein,GPO〕，一組喂橄欖油〔OO，對照膳食〕，加入0．2%〔W/W〕TRF于OO中制成抗氧化劑豐富的膳食．經喂養12周后，取胸主動脈段評價血管功能，與正常血壓的Wistar-Kyoto〔WKY〕對照鼠比較，SHR的主動脈環顯示内皮細胞依賴乙酰膽鹼松弛的作用減弱，這種減弱可進食TRF膳而得到恢復〔$P<0.05$，ANOVA和Tokey's試驗〕．雖然由氧化氮對高血壓血管抑制后，建立的血栓烷樣收縮物反應，不受上述試驗膳食的影響．但是用TRF及GPO膳食喂養大鼠，可預防臨界劑量正腎上腺素〔7.2×10^{-10}M〕所引起血管的强烈收縮作用．該研究結果指出食用油抗氧化劑的調節作用，與目前報告的天然抗氧化劑有益于心血管疾病相符合。

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