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Serum lipids, lipid peroxidation and glutathione peroxidase activity in rats on long-term feeding with coconut oil or butterfat (ghee)

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We determined the relative atherogenicity of two saturated fats by studying their effects on lipid peroxidation (LP), by way of malonaldehyde (MDA) and conjugated dienes (CD) and glutathione peroxidase (GSHPx) activity in serum, liver and heart; and on serum lipid profile after 4 months and 9 months of feeding. Male Rattus norwegicus rats were fed a basal diet (control) or basal diet fortified with 20% weight/weight butterfat (ghee) (BF) or coconut oil (CO). Serum high-density-lipoprotein-cholesterol (HDL-chol) and HDL-chol:LDL-chol ratio was lower in the BF group compared to CO after both feeding periods. Conjugated dienes (CDs) were higher in the serum and liver after 4 months, and heart after 9 months, of the rats fed BF compared to CO. Serum low-density-lipoprotein-cholesterol (LDL-chol) was higher, but CD were lower at 9 months than at 4 months feeding for all three groups. Liver and heart MDA and CD were higher in both groups after 9 months compared to 4 months. Liver GSHPx activity was higher after 9 months compared to 4 months in the BF group. Heart GSHPx activity was lower after 9 months compared to 4 months for both BF and CO groups. In conclusion, BF is potentially more atherogenic than CO in terms of serum lipids and LP. The unfavourable responses in serum lipids, with the exception of triglycerides, and LP were exaggerated with the longer duration of feeding with both oils.

Key words: coconut oil, butterfat, ghee, serum lipids, lipid peroxidation, glutathione peroxidase, rats

Introduction

Low-density lipoprotein (LDL) is an integral component of the atheromatous plaque. Peroxidised LDL do not interact with receptors in cholesterol-requiring cells, causing it to accumulate and form atherosclerotic plaques[1,2]. Human monocyte-macrophage incubated with malonaldehyde (MDA) treated-LDL was found to accumulate more cholesteryl esters compared to native LDL[3]. Previous studies demonstrated a positive correlation between high levels of LP products and an atherogenic lipid profile[4,5], and a negative correlation between the activity of the antioxidant enzyme, glutathione peroxidase (GSHPx) with serum LDL-cholesterol (LDL-chol)[6].

Polyunsaturated fatty acids are more prone to lipid peroxidation (LP) as compared to saturated fatty acids[1]. However, previous studies have shown that this may not necessarily be true in the case of dietary oils. D'Aquino et al[6] found less LP, but higher GSHPx activity in the liver of rats fed CO compared to fish oil, but Dhanakoti and Draper[7] found more urinary MDA in rats fed hydrogenated CO compared to fish oil and corn oil. Butterfat (BF), also known locally as ghee, is a saturated fat of bovine origin. Rats fed BF produced higher levels of serum MDA compared to rats fed palm oil[8].

Saturated fats are reported to affect serum lipids unfavourably. CO was found to increase total cholesterol (T-chol), but decrease LDL-chol:HDL-chol ratio in the rabbit as compared to corn oil[9]. BF was found to raise plasma triglycerides (TG) and reduce plasma LDL and HDL compared to the more monounsaturated palm oil[10]. However Foxall and Schwaery[11] observed that a BF-enriched diet was less hypercholesterolaemic and less atherogenic compared with a fish oil-enriched diet.

In this study, we determined the atherogenic potential of two saturated fats of different origins. namely CO of plant origin, and BF of animal origin, by comparing their effects on serum lipid profile, LP and GSHPx activity. We also compared LP and GSHPx activity in liver and heart homogenates. We also studied the effects of long-term feeding of these two fats on the above parameters.

Materials and Methods

Animals and diets

Male *Rattus norwegicus* rats weighing between 145-165g (age approximately 2 months) were divided into 3 groups of 8 rats each and fed basal diet (Gold Coin, Malaysia) (control) or basal diet fortified with 20% w/w (weight/weight) either coconut oil (CO) (Star Enterprise, Kuala Lumpur, Malaysia) or butterfat (BF) (QBB, Butter Producers, Brisbane, Australia). The composition of the basal diet was given earlier[12]. The fatty acid composition of the oils used were given by other authors[13,14] (Table 1). A group of 8 rats were sacrificed at the beginning of the experiment for baseline values.

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Table 1. Fatty acid composition of the oils used in this study.

Fatty acid	Coconut oil	Butterfat
4:0	-	3.1
6:0	0.5	1.3
8:0	8.0	1.5
10:0	6.4	2.7
12:0	48.5	2.6
14:0	17.6	11.0
16:0	8.4	27.1
18:0	2.5	13.3
18:1	6.5	27.0
18:2	1.5	2.5
18:3	-	1.9
Saturates	91.9	62.6
Monounsaturates	6.5	27.0
Polyunsaturates	1.5	4.4

(adapted from Chong and Ng[13], Karanja et al[14])

The rats were housed 4 per cage with a 12-hour light/dark cycle. The respective diets and tap water were given ad libitum for 4 or 9 months. At the end of each feeding period the rats were with fasted overnight and sacrificed under Pentobarbitone Sodium 35 mg/kg. Samples of serum, liver and heart were taken for analyses of MDA and conjugated dienes (CD) concentrations, GSHPx activity and serum lipid profile.

Sample preparation

Blood was taken from the common carotid artery and allowed to clot at room temperature for 30 minutes. The clotted blood was centrifuged at 3,000 rpm for 25 minutes. Liver and heart homogenates were prepared as described by Stocks et alts . The samples were divided into aliquots and stored at -70°C until analysed. All work was done at 0°C - 4°C to minimise peroxidation in vitro.

Biochemical analyses

Measurement of MDA was performed according to Ledwozyw et al[4] and Yagi[16]. Distilled water 0.1 ml was added to 0.4 ml of the serum or homogenate samples and mixed. 2.5 ml 1.22M trichloroacetic acid in 0.6M HCl was added, mixed and left to stand at room temperature for 15 minutes. 1.5 ml 0.67% thiobarbituric acid in 0.05M NaOH was then added to the mixture, mixed and incubated in boiling water for 30 minutes.

The mixture was cooled to room temperature. 4 ml n-buthanol was added to the mixture and mixed. The top layer consisting of n-buthanol was taken and the fluorescence measured using spectrofluorometer (Shimadzu RF-5000) at excitation and emission wavelengths 515 nm and 553 nm respectively.

Serum and tissue CD levels was determined according to Buege and Aust[17]. Measurement of GSHPx activity was described by Beutler et al[18].

The above measurements were expressed per g protein. Total protein content of the samples were determined using the computerised autoanalyser, Hitachi 717, based on the Biuret method.

Serum lipid parameters measured were T-chol, TG and HDL-chol. The assays was done using kits (Boehringer Mannheim, Germany). All measurements were made using Hitachi 717 computerised autoanalyser. LDL-chol concentration was obtained by calculation.

Analysis of data

Data obtained were expressed as means ± standard deviation and analysed via analysis of variance and Student's t test. $p<0.05$ was considered significant.

Ethical approval

This study was approved by the Research and Ethical Committee, Medical Faculty, Universiti Kebangsaan Malaysia, and confirmed by the University's Central Research Committee.

Results

Serum lipid profile

HDL-chol declined, and LDL-chol increased after 4 months feeding. LDL-chol for the CO and BF groups continued to increase until 9 months of feeding. The group fed BF had lower HDL-chol concentrations than CO, and higher LDL-chol than control group after both feeding periods. T-chol did not increase for the control group, but increased at 9 months feeding for both the CO and BF groups. T-chol was higher in the CO and BF groups compared to control after 4 months and continued to be higher in the BF group after 9 months. TG concentrations increased at 4 months feeding for the CO and BF groups, but declined to control values after 9 months (Table 2).

Serum lipid peroxidation products and glutathione peroxidase activity

MDA concentrations increased after 4 months feeding in all three diet groups, but there was no difference between groups. CD concentrations declined at 9 months for all three

Table 2. Serum lipid profile of rats fed coconut oil or butterfat after 4 and 9 months of feeding.

Diet	Duration of feeding (months)	Serum lipids (mmol/l) HDL-chol	LDL-chol	HDL-chol / LDL-chol	T-chol	TG
Control	0	0.77±0.08	0.58±0.14	1.41±0.46	1.5±0.1	0.52±0.07
	4	0.51±0.06*	^{c}0.67±0.12*	^{e}0.76±0.13*	^{gh}1.3±0.2	^{j}0.70±0.22
	9	0.52±0.07*	^{d}0.89±0.17*	^{f}0.60±0.13*	^{i}1.5±0.2	0.62±0.17
CO	4	^{a}0.54±0.06*	0.83±0.18*	0.68±0.13*	^{g}1.6±0.2	^{j}0.96±0.26*
	9	^{b}0.55±0.05*	1.05±0.17*°	0.54±0.11*	1.7±0.2*	0.67±0.13°
BF	4	^{a}0.47±0.05*	^{c}0.95±0.18*	^{e}0.52±0.12*	^{h}1.6±0.2	0.83±0.24*
	9	^{b}0.48±0.06*	^{d}1.18±0.18*°	^{f}0.41±0.08*	^{i}1.8±0.2*	0.60±0.13°

Values with marker ° are different from values at 4 months feeding within the same diet group at $p<0.05$. Values with marker * are different from values at beginning of feeding (0 months) at $p<0.05$. Values bearing the same alphabetical superscript are different at $p<0.05$ Values are given as mean± S.D. (n = 8).

groups, but only BF showed an initial rise at 4 months feeding. CD concentration was higher in the CO and BF groups compared to control after 4 months feeding, but declined to control levels at 9 months. GSHPx activity increased in the control group after 4 months feeding, but for the group fed BF, the increase was seen after 9 months. GSHPx activity was lower in the CO group than the control group after 4 months feeding (Table 3).

Table 3. Serum malonaldehyde and conjugated diene concentrations, and glutathione peroxidase activity in rats fed coconut oil or butterfat after 4 and 9 months of feeding.

Diet	Duration of feeding (months)	MDA (umol/g protein)	CD (OD/g protein)	GSHPx (OD/g protein)
Control	0	0.65±0.05	9.5±1.7	17.8±4.8
	4	0.79±0.08*	^{ab}1.0±1.8	^{d}30.9±9.7*
	9	0.88±0.15*	4.7±1.4*°	29.4±7.9*
CO	4	0.81±0.12*	^{ac}8.5±2.5	^{d}17.7±5.5
	9	0.88±0.09*	5.7±1.3*°	22.7±3.8
BF	4	0.77±0.13*	^{bc}14.7±2.3*	21.7±7.8
	9	0.86±0.10*	5.2±1.6*°	28.9±7.5*

Values with marker ° are different from values at 4 months feeding within the same group at $p<0.05$. Values with marker * are different from values at beginning of feeding (0 months) at $p<0.05$. Values bearing the same alphabetical superscript are different at $p<0.05$ Values are given as mean ± S.D. (n = 8).

Liver and heart lipid peroxidation products and glutathione peroxidase activity

Liver and heart MDA and CD concentrations increased in all three groups after 4 months and continued until 9 months. Liver MDA was higher in the CO group compared to control at 4 months feeding. Heart MDA was higher in both the CO and BF groups than in the control group after both feeding periods. Liver CD was higher in the BF group compared to control and CO after 4 months feeding. Heart CD was lower at 4 months, but higher at 9 months in the BF group as compared to control (Table 4).

Liver GSHPx activity increased after 4 months in the BF and CO groups, and the increased continued until 9 months for the BF group. Liver GSHPx in the control group activity increased only at 9 months feeding. Heart GSHPx activity initially increased at 4 months feeding, then declined until 9 months for all three groups. Liver GSHPx activity was higher in the CO group than control at 4 months, while heart GSHPx was higher in both the CO and BF groups at 9 months (Table 4).

Discussion

In the control group, we found that the decline in HDL-chol and the rise in LDL-chol had already reached maximum levels at 4 months of study, while no rise was seen in T-chol and TG levels, suggesting that by 2 months of age (beginning of study), these lipids had already reached their peak values. But other workers observed an increase in T-chol and LDL-chol between rats age 10 months and 24 months[19]. In this study, both BF and CO increased plasma T-chol compared to control, and this was also seen in human studies[20,21]. However no differences in T-chol and TG were observed between the two saturated fats, a finding similar to other studies[22,23]. But the other studies[22,23] differed from ours in that cholesterol was added to the CO oil diet to provide a similar amount of dietary cholesterol as the BF diet.

Table 4. Malonaldehyde and conjugated diene concentrations, and glutathione peroxidase activity in liver and heart of rats fed coconut oil or butterfat after 4 and 9 months of feeding.

Diet	Duration of feeding (months)	MDA (umol/g protein)	CD (OD/g protein)	GSHPx (OD/g protein)
Liver				
Control	0	0.5±0.1	25.7±4.0	626.2±58.1
	4	^{a}1.4±0.3*	^{b}69.0±8.3*	^{d}592.2±167.9
	9	11.4±3.0*°	189.0±26.4*°	1084.3±133.3*°
CO	4	^{a}2.5±0.4*	^{c}59.1±10.1*	^{d}1123.7±321.9*
	9	13.1±2.1*°	157.8±31.0*°	1364.0±388.2*
BF	4	2.1±0.6*	^{bc}138.1±42.8*	962.9±325.8*
	9	12.6±2.8*°	179.9±31.4*°	1391.5±199.4*°
Heart				
Control	0	2.9±0.5	66.7±8.5	633.7±68.6
	4	^{ab}3.6±1.1	^{e}133.8±36.8*	2200.4±531.8*
	9	^{cd}22.8±4.7*°	^{f}336.6±44.9*°	^{gh}723.6±88.8*°
CO	4	^{a}5.0±1.1*	106.2±26.6*	2295.2±660.8*
	9	^{c}34.9±4.6*°	323.6±34.0*°	^{g}1037.6±236.3*°
BF	4	^{d}5.4±1.4*	^{e}87.5±21.5*	2169.0±527.0*
	9	^{d}36.4±8.9*°	^{f}390.1±67.4*°	^{h}1168.1±185.2*°

Values with marker ° are different from values at 4 months feeding within the same group at least $p<0.05$. Values with marker * are different from values at beginning of feeding (0 months) at $p<0.05$. Values bearing the same alphabetical superscript are different at $p<0.05$ Values are given as mean ± S.D. (n = 8).

Our study showed that addition of BF, but not CO increased LDL-chol concentration compared to control. An important observation to note is that an upward trend is seen in LDL-chol with both saturated fats. This indicate that long-term ingestion of 20% saturated fats adversely affects serum lipids compared to a basal diet which contains only 2.5% fats[12].

Lindsey et al[24] found that palmitic acid (16:0) enhanced HDL production. However, we found that the diet enriched with CO had higher serum HDL-chol and HDL-chol:LDL-chol ratio compared to BF, despite the fact that BF contained more palmitic acid, and less overall saturation compared to CO (Table 1). A reason for our results could be that BF, being an animal fat, contains cholesterol, while CO which is devoid of cholesterol. Anhydrous BF contains 0.25 g cholesterol/100 g butterfat[22,23].

Palmitic acid (16:0) raised plasma TG in hamsters after a 4-week feeding period[24]. BF contained a larger proportion of palmitic acid compared to CO (Table 1). However, in our study both fats increased TG transiently, and CO demonstrated a significant increase compared to control at 4 months. Lai et al[22] showed that different dietary saturated fats have unique effects on TG metabolism after feeding for 5 weeks. An important point in our study is the long feeding period (9 months). We found that long-term feeding diminished the hypertriglyceridaemic effect of both fats.

In general, LP increased with age in serum and tissues of rats[25] and humans[26]. We found this to be true in our study, except for serum CD levels, which declined after 9 months, even though they showed a transient rise in the group fed BF. LP products were higher in the groups with added fats as compared to the control group on basal diet, and this is consistent with the increased amount fatty acid susceptible to peroxidation in the high-fat diets. The cholesterol found

in BF can itself undergo peroxidation[1]. Oxidation of cholesterol was found to occur during processing of butter to ghee[27]. We found CD concentrations in all three organ systems studied, ie, serum, liver and heart, to be higher in the BF group as compared to the CO group. Animals fed diets enriched with CO + cholesterol produced more LP[28,29], but this could be due to peroxidation of cholesterol itself, rather than the CO. The differences was seen after 4 months in serum and liver, but only after 9 months in the heart, suggesting that lipid peroxidation products accumulate at a slower rate in the heart.

No difference was observed in serum, liver and heart MDA concentrations between CO and BF groups, despite the differences seen in CD concentrations. This could be because, MDA, even though it is sensitive and simple to measure, is however less specific. Other compounds can react with TBA to form complexes which react at the same wavelength as the MDA-TBA complex[30]. MDA may also be formed upon heating the sample with acid[31]. MDA is also formed as a by-product in the synthesis of prostaglandins from arachidonic acid[32].

GSHPx activity increased with age in the rats. This increase is attenuated in the serum, but enhanced in the liver and heart by adding CO or BF. Heart GSHPx activity was lower after 9 months compared to 4 months for all three groups, implying that the ageing heart is less able to withstand peroxidative stress. No difference in GSHPx activity was seen between the two groups, despite the difference in CD concentrations. Other natural antioxidants present in the body, such as superoxide dismutase and catalase may have a more important role to play in the LP process. The presence of natural elements in the oils studied could also affect the LP process seen; CO contains 13 ppm of the antioxidant vitamin E (tocopherol)[33]. Therefore, measuring the total antioxidant activity may be more meaningful than just a single enzyme.

Thus, the long period of feeding in this study saw a decline in serum TG and CD, and heart GSHPx activity; while HDL-chol, tissue LP products and GSHPx activity in the liver increased. Further studies on lipoprotein and TG metabolism, assays of other by-products of LP and other antioxidants, as well as determining the extent of atherosclerosis after BF and CO feeding will be useful.

In conclusion BF increased serum lipids and serum LP to a greater degree than CO, making it more atherogenic than CO. Tissue LP was also greater in the BF diet than the CO diet. Long-term feeding of rats with saturated oils adversely affected the serum lipids and LP. CD is a more specific indicator of LP as compared to MDA. GSHPx activity is not correlated to the degree of LP observed.

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椰子油或牛油長期喂養大鼠的血清脂類、脂類過氧化作用和谷胱甘肽過氧化物酶活性

摘要

作者研究了大鼠血清、肝臟和心臟的脂類、脂類過氧化作用（LP）和谷胱甘肽過氧化物酶（GSHPX）活性，從而測定了兩種飽和脂肪酸的相對致動脈粥樣硬化作用。他們選用 Rattus Norwegicus 雄鼠爲對象，喂以基本膳食（對照組），或強化 20%（w/w）牛油（BF）或椰子油（CO）的基本膳食 4 或 9 個月。結果發現，4 或 9 個月喂養後，牛油組大鼠的血清高密度脂蛋白膽固醇（HDL-C）和高密度脂蛋白膽固醇／低密度脂蛋白膽固醇比值均低於椰子油組。喂養 4 個月後，牛油組大鼠血清和肝臟的脂類過氧化作用較高；而喂養 9 個月後，心臟的脂類過氧化作用也較高。三組（對照、牛油、椰子油組）喂養 9 個月後大鼠，其血清低密度脂蛋白膽固醇較喂養 4 個月後高，但脂類過氧化作用則較低。比較肝臟和心臟丙醛（MDA）和脂類過氧化作用，9 個月喂養後均較 4 個月喂養後高。牛油組大鼠經 9 個月喂養後，其肝臟 GSHPX 活性較 4 個月喂養時增高。牛油組和椰子油組經 9 個月喂養後，心臟 GSHPS 活性較 4 個月喂養時減低。結論：從血清脂類和脂類過氧化作用看，牛油組較椰子油組具有較大的致動脈粥樣硬化作用。兩種油的喂養時問愈長，其血清脂類和脂類過氧化作用也愈高。

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Lipid serum, peroksidasi lipid dan aktiviti glutation peroksidase pada tikus yang diberi makan minyak kelapa atau minyak sapi jangkapanjang.

Kajian ini membandingkan aterogenisiti dua jenis lemak tepu dengan menentukan kesan mereka ke atas profil lipid serum, proses peroksidasi lipid dan aktiviti enzim antioksidan glutation peroksidase (GSHPx) di serum, hepar dan jantung tikus yang diberi lemak tersebut selama 4 atau 9 bulan. Tikus Rattus norwegicus jantan diberi diet asas atau diet asas yang ditambah dengan 208% berat/berat minyak kelapa (CO) atau minyak sapi (BF). Kolesterol lipoprotein ketumpatan tinggi (HDL-chol), dan nisbah HDL-chol:LDL-chol adalah lebih rendah pada kumpulan BF berbanding CO sesudah 4 dan 9 bulan kajian. Kepekatan dien terkonjugat (CD) serum dan hepar adalah lebih tinggi pada kumpulan BF berbanding CO. Kolesterol lipoprotein ketumpatan rendah (LDL-chol) adalah lebih tinggi, tetapi CD lebih rendah pada 9 bulan berbanding 4 bulan kajian untuk ketiga-tiga kumpulan. Malonaldehid (MDA) dan CD hepar dan jantung adalah lebih tinggi untuk kumpulan BF dan CO pada 9 bulan berbanding 4 bulan. Aktiviti GSHPx hepar adalah lebih tinggi pada 9 bulan berbanding 4 bulan kajian untuk kumpulan BF, tetapi kesan sebaliknya dicerap untuk GSHPx jantung kedua-dua kumpulan BF dan CO. Keputusan menunjukkan BF adalah lebih aterogenik daripada CO dari segi lipid serum dan peroksidasi lipid. Kesan kurang baik kedua-dua jenis lemak tepu ini menjadi lebih ketara pada jangkamasa rawatan yang lebih lama.

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