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Modulation of carbon tetrachloride-induced oxidative stress by dietary fat in rats☆

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Abstract

Oxidative stress is believed to be involved in the pathophysiology of a number of chronic diseases including atherosclerosis, diabetes, and cataracts and to accelerate the aging process. The aim of this study was to elucidate the role of various dietary fats in the *in vivo* modulation of CCl_4 induced oxidative stress using rat as a model. Rats were raised on diets enriched with saturated (Beef Tallow), n-9 (Sunola oil), n-6 (Safflower oil) or n-3 (Flaxseed oil) fatty acids and exposed to elevated oxidative stress by administration of CCl_4. Plasma concentration of 8-iso-$PGF_{2\alpha}$, antioxidant micronutrients and antioxidant enzymes were measured to examine changes to oxidative stress subsequent to the administration of CCl_4. The fatty acid profiles of plasma and RBC membranes reflected the fats fed in the different diets. CCl_4 administration had no significant effect on fatty acid composition of plasma or RBC lipids. Plasma 8-iso-$PGF_{2\alpha}$ concentrations were elevated by CCl_4 administration regardless of the dietary fat fed. Within the induced oxidative groups the 8-iso-$PGF_{2\alpha}$ concentrations were highest in Safflower oil followed by Sunola oil, Tallow and finally Flaxseed oil. Induction of oxidative stress by CCl_4 administration was associated with a significant reduction in Vitamin A content reaching a significantly lower concentration ($P < 0.05$) in the Tallow and Flaxseed oil groups. Vitamin E concentrations were significantly lower ($p = 0.01$) in the Safflower oil and the Flaxseed oil than in the Tallow diet group following CCl_4 administration. Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSHPx) activities were not affected by dietary fat manipulation. The results of this study indicate that dietary fat can modulate lipid peroxidation and antioxidant defenses when exposed to a pro-oxidant challenge.

Keywords: Oxidative stress; Antioxidant; Dietary fat; 8-iso-$PGF_{2\alpha}$; Carbon tetrachloride; Rat

1. Introduction

The human body is constantly exposed to free radicals and oxygen derived species from endogenous and exogenous sources. Free radicals are formed in the energy/respiratory pathways of the body, as inflammatory mediators in the immune system and in other biochemical pathways that are an essential aspect of cellular metabolism [1]. Exogenous sources include air pollutants, oxides of nitrogen, tobacco smoke, car exhausts, irradiation and the diet [1]. Complex antioxidant defense mechanisms against the damage that free radicals cause have evolved, but these defenses are not completely efficient and the presence of free radicals can result in damage to DNA molecules, proteins and lipids [2]. Oxidative stress is caused when the pro-oxidant challenge overwhelms the antioxidant defenses and potentially leads to cellular damage [1]. Oxidative stress in the human body is proposed to be involved in the pathophysiology of many chronic human diseases such as atherosclerosis [3], diabetes, kidney disease, [4], cataracts [5], septic shock, and possibly to accelerate the aging process [6].

The type of fat eaten in the diet may affect the level of oxidative stress. Dietary saturated fats are known to increase plasma concentration of total cholesterol and LDL cholesterol [7]. A diet rich in polyunsaturated fat, while associated with reduced plasma cholesterol concentration [8], is thought to elevate the oxidative stress experienced in the body [9], and in this manner may influence chronic illness. Monounsaturated fats are known to be neutral in their influence on plasma cholesterol concentration, and are less vulnerable to oxidative damage than polyunsaturated fatty acids ([10], [7]). Indeed dietary monounsaturated fats have

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been shown to reduce the oxidisability of LDL by Cu^{2+} ions *in vitro* ([11], [12]). Evidence of *in vivo* effects of dietary fats on oxidative stress is limited.

The aim of the current study was to elucidate the role of various dietary fats in modulating oxidative stress *in vivo.* To examine this, rats were raised on diets enriched with saturated (Tallow), n-9 (Sunola oil), n-6 (Safflower oil) and n-3 (Flaxseed oil) fatty acids, and were exposed to elevated oxidative stress by CCl_4 administration. Isoprostane concentrations, antioxidant defense molecules and antioxidant enzymes were measured to establish oxidative stress effects.

2. Materials and methods

Forty female weanling Wistar rats (Animal Resource Center, Murdoch WA, Australia) weighing between 94–121g (110 ± 0.85) were randomly assigned to four diet groups (n = 10). The diets differed only in the type of fat used; however the fat content remained constant (20% w/w). The rats were fed ad libitum for four weeks. The animals were given fresh food and the left over food discarded on a daily basis. The amount of food consumed and body weights of the rats were recorded. The University of Newcastle's Animal Care and Ethics Committee approved the study.

The rats were individually caged in polycarbonate cages with high-topped wire lids and housed in a facility with alternating 12 hr light/dark cycles. The diets were prepared using commercially supplied, nutritionally balanced powdered food (Glen Forrest Stockfeeders, Perth WA, Australia) and 20% (w/w) fat was added from four different fat types (Tallow and Safflower oil, Safflower oil, Sunola oil, and Flaxseed oil) (Meadowlea Foods, Australia), finally the Vtiamin E content of the diets was standardized to 900IU/kg of diet. The Tallow diet served as the reference diet. The diet contained sucrose (44g/100g), starch maize (18g/100g), cellulose (7g/100g), casein (25g/100g), methionine (0.38g/100g), AIN 93G mineral mix (4.2g/100g), and AIN 93 G vitamin mix (1.2g/100g). Fat content and fatty acid composition of the diets is detailed in Table 1. At the completion of the four week feeding regime, seven rats from each diet group (n = 7) were administered a sublethal dose of CCl_4 (2mLs/Kg body weight in a 1:1 dilution with corn oil) by gavage to induce lipid peroxidation [13]. After four hours these animals were anesthetized with isoflurane (Veterinary Medical Supplies, Newcastle, NSW, Australia), a heart puncture performed and the animal was euthanaised using CO_2**.** Three rats from each diet group served as their respective controls. Since none of the parameters determined were significantly different in the control groups, the data for all the animals (n = 12) was pooled which served as a control group. The control animals were also anesthetized with isoflurane, had a heart puncture performed and euthanaised by CO_2. Blood was collected in ethylenediaminetetraacetic acid (EDTA) coated tubes with reduced glutathione added (1mg/ml blood) and centrifuged at 3000 rpm, at 4°C for 10 min. The plasma and the Red Blood Cells (RBC) pellet were separated for analysis and stored at -70°C.

Table 1
Fat content and fatty acid composition of the experimental diets

Oils	Diet g/Kg diet			
	Beef tallow	Safflower	Sunola	Flaxseed
Beef tallow	180			
Safflower	20	200		
Sunola			200	
Flaxseed				200
Fatty acids			(%)	
C14:0	3.1	0.3	0.2	0.2
C16:0	24.8	7.5	4.2	6.0
C16:1n-7	2.5	0.2	0.1	0.1
C18:0	14.0	2.9	2.2	4.4
C18:1n-9	33.57	15.4	80.1	21.0
C18:1n-7	2.1	1.3	0.3	1.0
C18:2n-6	10.7	69.7	10.1	14.8
C18:3n-6	0.2	0.0	0.0	0.1
C18:3n-3	0.6	0.6	0.2	50.7
C20:0	0.3	0.4	0.4	0.2
C20:1n-9	0.4	0.3	0.3	0.3
C20:2n-6	0.1	0.1	0.0	0.0
C22:0	0.2	0.4	1.4	0.3
C22:1n-9	0.1	0.5	0.0	0.0
C24:0	0.1	0.1	0.3	0.1
C24:1n-9	0.0	0.2	0.0	0.0

2.1. Antioxidant and micronutrient analysis

Plasma concentration of free vitamin A and E were determined by reverse phase High Performance Liquid Chromatography (HPLC) using a program variable wavelength UV-visible detector [14]. Samples were thawed, mixed with ethanol to precipitate proteins, vortexed, and reconstituted with hexane and injected into the HPLC column (lab-packed Whatman ODS 3 (5micron) 300 x 3.5 mm ID). The eluting solvent had a flow rate of 1 ml/min. Vitamin A was measured at 310 nm and Vitamin E was measured at 450nm. Plasma concentrations of zinc and selenium were analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Samples were diluted in an ammonium EDTA based diluent in a Quantitative Application; platinum and rhodium were used as internal standards in the diluent. Calibration was by Addition Calibration in a pooled plasma base.

2.2. 8-iso-$PGF_{2\alpha}$ assay

An aliquot of plasma was taken and a known amount of tritiated prostaglandin ($PGF_{2\alpha}$) (Amersham, Arlington Heights, IL, USA) was added, to determine recovery rate after the purification procedure. Ethanol was added to pre-

cipitate proteins and the sample was centrifuged at 1500 rpm, at 4°C for 10 min. The supernatant was decanted and an equal volume of 15% KOH was added, then incubated at 40°C for one hour to cleave the esterified isoprostane molecules. After incubation the pH was lowered to 3 (Caymen Chemical Ann Arbor, MI, USA). The sample was first passed through a C18 Sep Pak reverse phase cartridge (Waters, Milford, MA, USA), and eluted with ethyl acetate: heptane (1:1). The sample was further purified by passing through a Silica Sep Pak cartridge (Waters) and eluted with ethyl acetate: methanol (1:1). The solvent was evaporated using N_2, and the sample reconstituted in assay buffer. A small amount of the sample was then analyzed with an 8-isoprostane enzyme immunoassay Kit (Cayman Chemical, Ann Arbor, MI, USA). Absorbance values were measured using a plate reader at 405nm wavelength, and the raw data corrected using the recovery rates.

The assay was validated by adding known amounts of 8-isoprostane to aliquots of purified plasma, the concentration of these samples was determined using the EIA kit. A strong correlation (0.99) was obtained between the known amounts of pure 8-isoprostane added and the concentration determined by EIA. The antiserum used in the assay has 100% cross-reactivity with 8-isoprostane, 20% with 8-isoprostane $F_{3\alpha}$, 0.2% with $PGF_{2\alpha}$, $PGF_{3\alpha}$, PGE_1 and PGE_2 and 0.1% with 6-keto-$PGF_{1\alpha}$. Detection limit is 4 pg/ml (Caymen).

2.3. Glutathione peroxidase

Whole blood samples were collected into EDTA tubes, and centrifuged at 8500g, at 4°C for 10 min. The plasma was discarded and the cells were washed in 10 volumes of cold buffer (50mM TRIS-HCL, pH 7.5, containing 5 mM EDTA, and 1mM dithiothreitol), centrifuged at 8500g, at 4°C for 10 min and the supernatant discarded. RBC were lysed by 4 volumes of cold deionized water and again centrifuged at 8500g, at 4°C for 10 min. Supernatant was collected and stored at -70°C for analysis. RBC cellular glutathione Peroxidase (GSHPx) activity was measured using a GPx-340 colorimetric assay (Bioxytech; OXIS International, Portland, OR, USA) to obtain values in units per milliliter.

2.4. Superoxide dismutase

The RBC pellet was thawed and 4 volumes of cold deionized water added and vortexed. Ice cold extraction reagent (ethanol/chloroform, 62.5/37.7 (v/v)) added to an aliquot of the suspension and vortexed, then centrifuged at 3000 g, at 4°C for 5 min. The upper aqueous phase is used for analysis. The erythrocyte Zn/Cu SOD was analyzed using a SOD-525 Spectrophotometric assay kit (Oxis Health Products, Inc, Portland, OR, USA), to obtain values in units per milliliter (Oxis). The hemoglobin (Hb) concentration of the sample was measured before the extraction procedure using Kit no. 525 for total Hb (Sigma), to allow the Zn/Cu SOD activity to be expressed as units per milligram of Hb.

2.5. Fatty acid analysis

Erythrocyte pellets were collected and stored in an EDTA coated tube at -70°C. The samples were thawed and the cells lysed and membranes soluablilized using the method of Tomoda et al [15]. The erythrocyte membranes were then methylated using the method of Leparge and Roy [16]. A small portion of the suspended membrane was added to a methanol: Toluene (4: 1) mixture containing an internal standard and BHT. Acetyl chloride was and incubated at 100°C. Potassium carbonate is added to stop the reaction and the mixture is centrifuged at 3000 rpm, at 5°C for 10 min. Fatty acid methyl esters in Toluene phase were analyzed using 30m x 0.25mm (DB-225) fused carbon silica column, coated with cyanopropylphenyl as previously described [17]. The injector and detector port temperature is 700°C. The oven temperature begins at 170°C for two minutes then increases 10°C per minute to 220°C and this is maintained throughout the run time of 30 min. The sample fatty acid peaks were identified by comparison with authentic standard mixture.

Plasma samples were methylated using the same method as for the erythrocyte membranes by the method Leparge and Roy [16] and analyzed on the GC using the same conditions described above. A known amount of C21: 0 was added to the methanol: toluene mixture and was used as the internal standard. This enabled quantitative analysis of plasma fatty acids expressed as microgram per mL.

The plasma unsaturated index (or double bond index) was calculated by multiplying the amount (μg/ml) of unsaturated fatty acid by the number of double bonds in the fatty acid, the values then summed to provide a number which represents the fatty acid unsaturated index [18].

2.6. Statistics

Normally distributed data is presented as mean ± SEM. Results were analyzed using Minitab version 12 for Windows (Minitab Inc., State college, PA, USA). Data tested for normality using the Anderson Darling normality test and for homogeneity of variance. Data was $\log_{10}$ transformed if not homogenous. Statistical comparisons were performed using ANOVA with significance level of 0.05. Differences were considered significant when $p < 0.05$.

3. Results

The average body weight gain of rats in all diet groups did not differ significantly (133 ± 24g; $p = 0.1$) at completion of the study. This indicates that dietary fat manipulation had no significant effect on body weight gain. The

Table 2
The fatty acid composition of the plasma (ug/mL) in rats fed different fat-supplemented diets

Fatty acid	Bf tallow	Safflower	Sunola μg/mL[1]	Flaxseed	P value
C14:0	6.9 ± 0.9[a]	4.6 ± 0.7[ab]	3.3 ± 0.2[b]	4.0 ± 0.5[ab]	<0.005
C16:0	202.5 ± 14.6[a]	193.9 ± 13.8[ab]	143.7 ± 5.5[bc]	130.1 ± 5.5[c]	<0.001
C16:1n-7	11.5 ± 0.6[a]	6.8 ± 0.8[b]	4.7 ± 0.4[b]	6.1 ± 0.4[b]	<0.001
C18:0	262.7 ± 19.2[a]	256.7 ± 16.0[a]	240.7 ± 15.5[ab]	175.7 ± 12.3[b]	<0.01
C18:1n-9	158.1 ± 15.0[a]	69.9 ± 8.9[b]	181.8 ± 10.1[a]	77.2 ± 7.8[b]	<0.001
C18:1n-7	20.5 ± 1.7[a]	15.2 ± 1.6[b]	14.1 ± 1.0[b]	10.1 ± 0.6[b]	<0.001
C18:2n-6	102.9 ± 6.3[ac]	247.9 ± 34.1[b]	54.6 ± 4.4[c]	143.2 ± 6.7[a]	<0.001
C18:3n-6	3.5 ± 0.4	5.7 ± 0.8			
C18:3n-3				76.1 ± 12.8	
C20:0	11.4 ± 1.0	10.9 ± 0.6	11.0 ± 0.9		
C20:3n-6				5.5 ± 0.7	
C20:4n-6	435.4 ± 31.0[a]	481.8 ± 27.6[a]	430.8 ± 21.3[c]	122.0 ± 7.3[b]	<0.001
C20:5n-3				169.3 ± 31.4	
C22:0	6.4 ± 0.5[a]	6.1 ± 0.3[ac]	9.2 ± 0.4[b]	4.5 ± 0.3[c]	<0.001
C22:5n-3				11.1 ± 1.45	
C22:6n-3	27.1 ± 2.6[a]	11.5 ± 0.4[b]	14.4 ± 0.6[bc]	20.2 ± 2.3[c]	<0.001
C24:0	23.0 ± 1.6	25.6 ± 2.5	28.9 ± 2.6	24.1 ± 1.2	0.3
C24:1n-9	19.7 ± 1.5[ac]	22.9 ± 2.6[c]	14.5 ± 0.8[ab]	9.8 ± 0.4[b]	<0.001
Total FA	1291.2 ± 85.4[ab]	1358.9 ± 94.9[a]	1149.8 ± 57.2[ab]	977.0 ± 73.2[b]	<0.05
Σ SFA	513.0 ± 34.8[a]	497.9 ± 30.2[a]	434.9 ± 23.4[ab]	331.5 ± 20.2[b]	<0.001
Σ MUFA	209.7 ± 17.3[a]	114.9 ± 10.8[b]	215.0 ± 11.2[a]	99.9 ± 9.4[b]	<0.001
Σ n-6 PUFA	541.4 ± 35.2[a]	735.4 ± 54.5[c]	485.4 ± 25.5[a]	268.9 ± 12.3[b]	<0.001
Σ n-3 PUFA	27.2 ± 2.6[a]	11.5 ± 0.4[a]	14.4 ± 0.6[a]	276.7 ± 42.4[b]	<0.001
Unsaturated Index	2329.5 ± 159.3	2623.9 ± 171.1	2133.9 ± 105.9	2136.8 ± 222.1	0.1

[1] The results are presented as mean ± standard error of the mean.
[a,b,c] The values without common superscript are significantly different.

Table 3
Fatty acid composition of the rat RBC membranes (%) in all diet groups

Fatty acid	Bf tallow	Safflower	Sunola Ave %[1]	Flaxseed	P-value
C14:0	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6
C16:0	17.4 ± 0.3[a]	20.1 ± 0.6[b]	17.9 ± 0.3[c]	18.1 ± 0.3[a]	<0.001
C16:1n-7	0.4 ± 0.1[a]	0.3 ± 0.0[ab]	0.4 ± 0.1[a]	0.2 ± 0.0[b]	<0.05
C18:0	21.4 ± 0.6[a]	22.6 ± 0.3[ab]	21.3 ± 0.5[a]	23.3 ± 0.5[b]	<0.01
C18:1n-9	10.8 ± 0.7[a]	6.1 ± 0.4[b]	14.6 ± 0.3[c]	8.4 ± 0.3[a]	<0.001
C18:1n-7	2.1 ± 0.10.1[a]	2.1 ± 0.1[b]	1.7 ± 0.1[c]	1.6 ± 0.1[cd]	<0.001
C18:2n-6	6.5 ± 0.4[a]	10.4 ± 0.3[b]	4.0 ± 0.1[c]	9.3 ± 0.2[d]	<0.001
C18:3n-6	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.531
C18:3n-3	0.2 ± 0.1[a]	0.1 ± 0.0[a]	0.1 ± 0.0[b]	1.6 ± 0.0[c]	<0.001
C20:0	2.2 ± 1.1	0.6 ± 0.2	1.1 ± 0.3	0.5 ± 0.2	0.170
C20:1n-9	0.3 ± 0.2	0.1 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	0.157
C20:2n-6	0.3 ± 0.1[a]	0.6 ± 0.1[c]	0.4 ± 0.0[ab]	0.5 ± 0.0[bc]	<0.001
C20:3n-6	0.5 ± 0.0[ac]	0.4 ± 0.0[a]	0.3 ± 0.0[c]	1.2 ± 1.3[b]	<0.001
C20:4n-6	25.1 ± 1.0[a]	26.1 ± 0.4[a]	26.2 ± 0.7[a]	11.5 ± 1.1[b]	<0.001
C20:5n-3	0.3 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.1[a]	7.7 ± 0.4[b]	<0.001
C22:0	1.0 ± 0.1[a]	0.8 ± 0.0[bd]	0.7 ± 0.0[c]	0.7 ± 0.0[d]	<0.001
C22:1n-9	0.6 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.563
C22:2n-6	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.220
C22:5n-3	1.1 ± 0.1[a]	0.6 ± 0.2[b]	0.6 ± 0.1[c]	3.0 ± 0.2[d]	<0.001
C22:6n-3	3.2 ± 0.3[a]	1.5 ± 0.1[bc]	2.1 ± 0.1[c]	3.9 ± 0.2[d]	<0.001
C24:0	1.8 ± 0.2[a]	2.3 ± 0.1[a]	2.0 ± 0.2[a]	3.9 ± 0.4[b]	<0.001
C24:1n-9	1.6 ± 0.2[a]	1.2 ± 0.1[a]	2.5 ± 0.3[b]	1.7 ± 0.2[a]	<0.005
Total FA	98.0 ± 0.4	97.5 ± 0.5	97.6 ± 0.4	97.8 ± 0.6	0.870
Σ SFA	44.3 ± 0.8[a]	47.1 ± 0.7[b]	43.6 ± 0.2[a]	47.0 ± 0.4[b]	<0.000
Σ MUFA	15.8 ± 1.0[a]	9.9 ± 0.5[b]	19.9 ± 0.5[c]	12.3 ± 0.5[b]	<0.001
Σ n-6 PUFA	33.1 ± 1.0[a]	38.2 ± 0.5[b]	31.2 ± 0.8[a]	22.3 ± 0.5	<0.001
Σ n-3 PUFA	4.8 ± 0.3[a]	2.3 ± 0.3[b]	2.9 ± 0.1[b]	16.2 ± 0.5[c]	<0.001
Unsaturated Index	163.5 ± 3.8	157.3 ± 1.9	156.4 ± 2.4	152.9 ± 2.1	0.058

[1] The results are presented as mean ± standard error of the mean.
[a,b,c] The values without common superscript are significantly different.

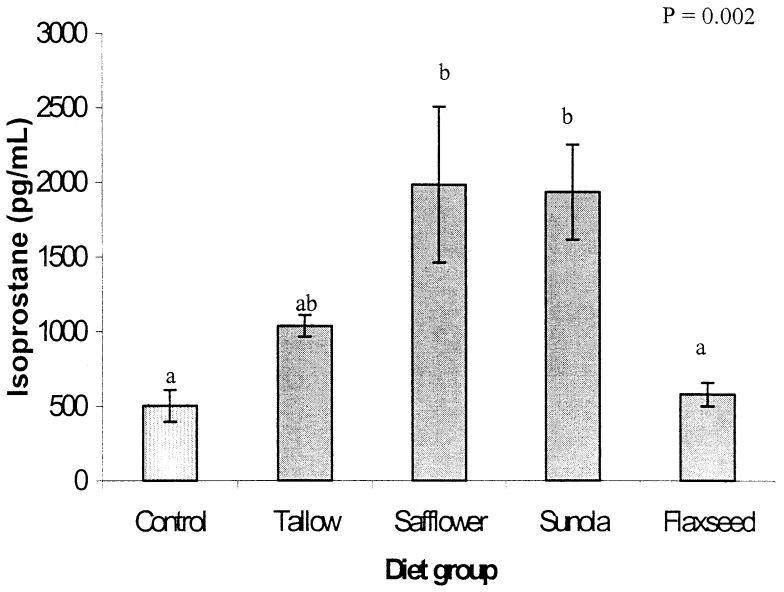


Fig. 1. The values without common superscript are significantly different 8-epi $PGF_{2\alpha}$(pg/ml) concentration in the plasma of rats fed various fat supplemented diets following four hours of CCl_4administration.

animals on different diets consumed similar amounts (18 ± 0.4 g/day) of food throughout the study period.

The lipid content and fatty acid content of the diets is detailed in Table 1. The Tallow diet was enriched with saturated fatty acids (42.4%) and sufficient C18:2n-6 (10.7%) to prevent essential fatty acid deficiency. The Safflower oil diet contained high concentration of C18:2n-6 (69.7%). The Sunola diet had the highest percentage of C18:1n-9 (80.1%). The Flaxseed oil diet had the highest percentage of C18: 3n-3 (50.7%).

The quantitative analysis of the plasma fatty acids is detailed in Table 2. In comparison to the Tallow diet the Safflower diet group had significantly higher concentrations of C18:2n-6 and a reduction in C18:1n-9 and C22:6n-3 concentrations. The Sunola diet group contained significantly higher concentrations of C18:1n-9 and lower concentrations of C18:2n-6 and C22:6n-3. The Flaxseed oil diet group had significantly high concentrations of not only the C18:3n-3 but also the elongated and desaturated product C20:5n-3. This diet also resulted in a significant reduction in the C20:4n-6 content of the plasma.

The erythrocyte pellet membrane fatty acid results also reflect the dietary intake of the different diet groups and are outlined in Table 3. In comparison to the reference diet the Safflower diet group had high concentrations of C18:2n-6 and the highest concentrations of C20:4n-6. The Sunola diet group had the highest concentrations of C18:1n-9. The Flaxseed oil diet group had the highest concentrations of C18:3n-3, C20:5n-3 and C22:6n-3 and correspondingly low concentrations of C20:4n-6. There was no significant effect of dietary fat on either the plasma ($p = 0.11$) or the RBC ($p = 0.06$) unsaturated index.

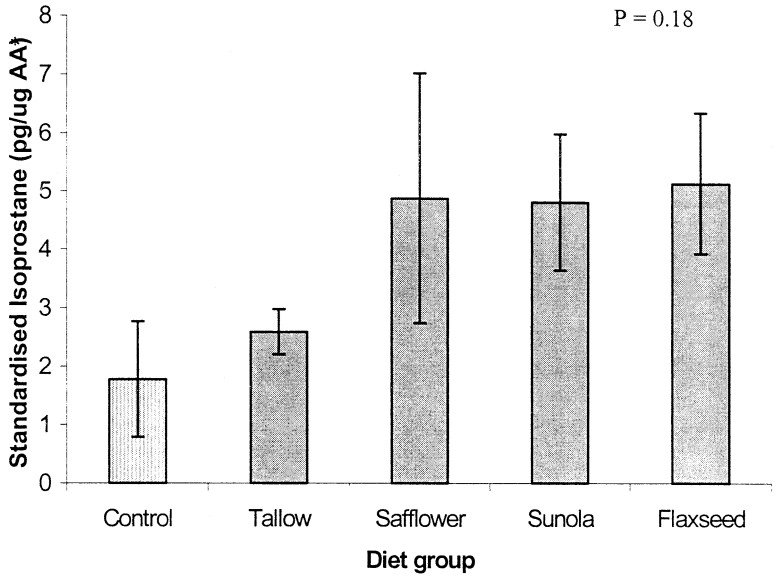
Plasma 8-iso-$PGF_{2\alpha}$ concentrations (Figure 1) were elevated in all the diet groups post CCl_4 administration (Tallow 1037.2; Safflower 1984.2; Sunola 1936.6; Flaxseed 578.4 pg/ml) when compared to the control animals (501.7 ± 105.1 pg/ml). The Flaxseed oil diet group had the lowest and Safflower oil diet group had the highest 8-iso-$PGF_{2\alpha}$ concentrations among the CCl_4 administered animals. Sunola oil diet group didn't differ significantly in comparison to the Tallow group but showed a trend towards increased isoprostane concentrations.

The standardization of plasma 8-iso-$PGF_{2\alpha}$ concentration to the plasma C20:4n-6 concentration (Figure 2) showed no significant difference ($P = 0.6$) in oxidative stress as measured by 8-iso-$PGF_{2\alpha}$ between the diet groups in this study.

Plasma concentrations of Vitamin E were lowered by all unsaturated fat diets (Table 4). Flaxseed oil was most effective in reducing plasma Vitamin E followed by the Safflower oil group. Sunola oil diet had no significant effect on plasma concentrations of Vitamin E when compared to the reference diet.

Vitamin A concentrations (Figure 3) in the plasma were significantly reduced following four hours of CCl_4 administration in the Tallow and Flaxseed oil diet groups, the trend in Vitamin A reduction was independent of the type of dietary fat used.

The plasma selenium concentrations showed no effect of diet, similarly the concentrations of GSHPx did not show a significant variation in between any of the diet groups ($P = 0.110$; $P = 0.382$ respectively). SOD and Zinc concentrations (not shown here) were also measured and showed no significant results when dietary fat was manipulated ($P = 0.110$). However administration of CCl_4 reduced SOD ac-



* AA = Arachidonic Acid

Fig. 2. Plasma 8-epi $PGF_{2\alpha}$(pg/ml) concentrations standardized against plasma Aracidonic Acid in rats fed various fat supplemented diets following four hours of CCl_4administration.

tivity regardless of dietary fat fed in comparison to the control group without CCl_4 administration (Table 4).

4. Discussion

This study concerned the regulation of *in vivo* oxidative stress by the alteration of dietary fat. Non-enzymatic, free radical catalyzed peroxidation of arachidonic acid to form 8-iso-$PGF_{2\alpha}$ was used as an *in vivo* marker of oxidative stress [5,13]. In this study CCl_4 was used to initiate lipid peroxidation which is an established model of inducing oxidative stress *in vivo* [13].

Previous studies examining the contribution of dietary fats to oxidative stress involved feeding diets varying in type of fat, followed by isolation of LDL particles and determining oxidisability by introduction of copper [11]. We now provide evidence that dietary fat type makes a substantial contribution to the extent of oxidative stress measured by an *in vivo* marker of lipid peroxidation, when animals are challenged with a pro-oxidant (CCl_4).

Dietary fats were incorporated into the plasma and the red blood cell lipids. Flaxseed oil diet additionally produced changes in the C18:2n-6 and C20:4n-6 concentrations which are consistent with our previously published observations on dietary n-3 polyunsaturated fatty acids [19]. Despite the differences in the unsaturation of the dietary lipids fed to the rats, the unsaturation index of the plasma lipids were not affected, suggesting existence of homeostatic mechanisms for the maintenance of the overall degree of unsaturation in the fatty acyl chains of cells and tissue membranes ([20], [21]). Four hours of CCl_4 administration failed to induce any changes in the fatty acid profiles or unsaturation index of plasma and red blood cell membranes (baseline fatty acid composition not presented).

Administration of CCl_4 in rats, induced oxidative stress as indicated by the increase in plasma 8-iso-$PGF_{2\alpha}$ concentration compared to the control. Morrow et al [13] stated a

Table 4
The concentration of antioxidant micronutrients (μmol/L) and antioxidant enzymes (units of activity) in rat plasma in all the diet groups

Antioxidant micronutrient (μmol/L)	Diets[1]					
	Control	Beef tallow	Safflower	Sunola	Flaxseed	P-value
Vitamin E	28.3 ± 1.3^{a}	30.5 ± 2.6^{a}	24.3 ± 1.6^{ab}	28.3 ± 2.1^{a}	19.2 ± 2.2^{b}	0.002
Selenium	5.2 ± 0.2	5.5 ± 0.3	5.7 ± 0.1	4.8 ± 0.2	5.0 ± 0.2	0.110
Superoxide dismutase ([SOD]/mg Hb)	2.9 ± 0.1	2.0 ± 0.1	2.6 ± 0.3	2.3 ± 0.3	2.7 ± 0.2	0.110
Glutathione Peroxidase ([GSHPx])	9.5 ± 0.5	8.7 ± 1.6	9.1 ± 1.3	8.6 ± 1.6	11.6 ± 0.8	0.382

[1]The results are presented as mean ± standard error of the mean.
The values without common superscript are significantly different.
n = 7 in all diet groups.

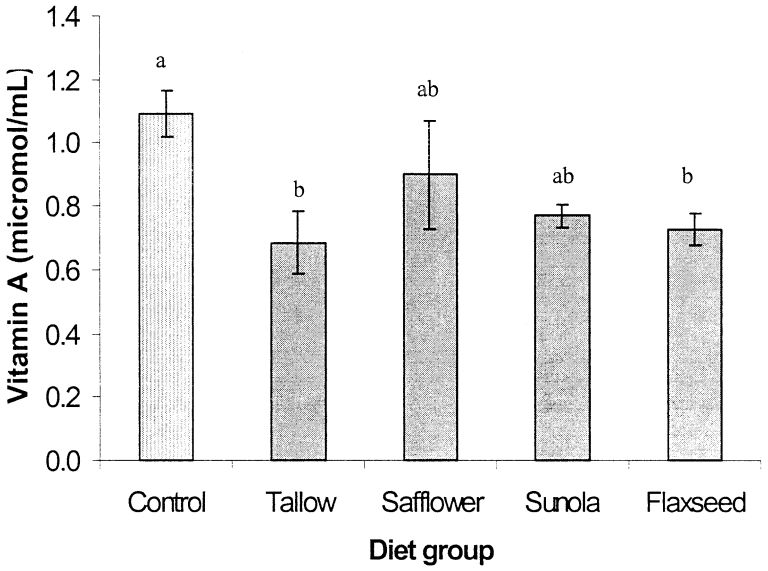


Fig. 3. The values without common superscript are significantly different Vitamin A (μmol/L) concentration in the plasma of rats fed various fat supplemented diets following four hours of CCl_4 administration.

50 fold increase in isoprostane concentration when oxidative stress was induced in rats using CCl_4, as compared to the 15–30 fold increase reported in the present study. The intragastric method of delivering the CCl_4 in studies by Morrow et al [13] compared to the gavage method of delivery in this study may account for the differences in the rate and the amount of CCl_4 absorption. Nevertheless, the elevation in plasma isoprostanes indicates that lipid peroxidation has occurred confirming CCl_4 administration as a relevant model for inducing oxidative stress in rats.

Total 8-iso-$PGF_{2\alpha}$ concentrations in the plasma were lower in the Flaxseed oil diet group. This is consistent with our previous observation that n-3 polyunsaturated fatty acid (PUFA) reduces concentrations of pro-aggretory 8-iso-$PGF_{2\alpha}$ [22]. This effect is in addition to already established thromboxane A_2 lowering potential of n-3 PUFA. The Flaxseed oil diet feeding resulted in lowering of vitamin E concentration and although not significantly different, higher activities of SOD and GSHPx enzymes. These observations are in agreement with previous studies, which demonstrated depletion of vitamin E in plasma([23], [24]) and upregulation in the activity ([25], [26], [27]) and the expression of SOD ([28], [23]) and in some cases GSHPx following dietary enrichment with n-3 polyunsaturated fatty acids. Together with already published data, the results presented suggest that despite increasing demands for exogenous antioxidants, n-3 PUFA stimulated endogenous antioxidant defenses. These results are suggestive of the concept that a combination of dietary antioxidants and n-3 PUFA may not only be required for efficient combating of oxidative stress but may also reduce the concentration of both the pro-aggregatory compounds TxA_2/8-iso-$PGF_{2\alpha}$ concentrations for the prevention of thrombosis, which is a major manifestation of atherosclerosis.

8-iso-$PGF_{2\alpha}$ concentrations were highest in the Safflower oil group and, although not statistically significant, this group had lower vitamin E concentration in comparison with the Tallow diet. As no changes in SOD and GSHPx activities were noticed following the feeding of the Safflower oil diet, it appears that n-6 PUFA cause maximum elevation of oxidative stress following exposure to a pro-oxidant like CCl_4. The Sunola oil diet rich in MUFA caused a non-significant increase in 8-iso-$PGF_{2\alpha}$ concentration when challenged with a pro-oxidant compared to the Tallow diet, however, no depletion of vitamin E concentration and no alterations in antioxidant enzyme activities. It would seem that monounsaturated fats may mitigate oxidative stress without any of the detrimental effects such as elevation of cholesterol concentrations, which is evident of saturated fats.

One of the major criticisms of the 8-iso-$PGF_{2\alpha}$ as a measure of oxidative stress is that it is derived from the free-radical oxidation of arachidonic acid, therefore any dietary fat that modulates the concentration of plasma C20:4n-6 may possibly effect the concentration of 8-iso-$PGF_{2\alpha}$ produced. This study indicated Flaxseed oil in the diet lowered the plasma concentration of 8-iso-$PGF_{2\alpha}$. The standardization of plasma 8-iso-PGF2α results to plasma C20:4n-6 concentrations did not radically change the conclusions of this study. The standardized figures indicate that the amount of oxidative stress in the Flaxseed oil diet is not significantly higher compared to the other diet types. Although the Flaxseed oil is a more highly polyunsaturated oil compared to the other oils in the study, and the feeding of this diet produces lower concentration of plasma C20:4n-6, it does not produce a concomitant increase in oxidative stress compared to the other dietary fat as measured by

standardized 8-iso-PGF2α in a model of induced oxidative stress.

An interesting observation not directly related to antioxidant defense or oxidative stress, was a reduction in plasma concentration of vitamin A following CCl_4 administration for four hours. This effect appears to be independent of the dietary fat type, which cannot be explained on the basis of existing knowledge in this area. There is some evidence in the literature to suggest that vitamin A could act as an antioxidant ([29]; [30])however, the mechanisms by which vitamin A could behave as an antioxidant is not clear. We have recently demonstrated that in Cystic Fibrosis patients, who experience massive oxidative stress, also have reduced-vitamin A concentrations in addition to being deficient in other antioxidants (vitamin E, C, β-carotenoids) [31]. Alternatively it is likely that vitamin A is rapidly oxidized following administration of CCl_4 in rats [32]. This aspect of CCl_4 induced oxidative stress deserves further investigation.

In conclusion, the results presented in this paper indicate that dietary fat has an effect on the level of lipid peroxidation as measured by total 8-iso-$PGF_{2\alpha}$ concentrations in the plasma. Other antioxidant defense mechanisms measured also showed that the type of dietary fat has a profound effect on the concentration of oxidative stress indices.

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