Free Rad. Res., Vol. 33, pp. 115–127
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Measurement of Plasma F_2-Isoprostanes as an Index of Lipid Peroxidation Does not Appear to be Confounded by Diet

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Accepted by Prof. C. Rice-Evans

(Received 26 August 1999; In revised form 7 December 1999)

F_2-isoprostanes (F_2-IPs) are formed by the free radical-catalysed oxidation of arachidonic acid. The measurement of F_2-IPs, especially 8-epi-$PGF_{2\alpha}$, is recognised as a reliable marker of lipid peroxidation and is currently used as a sensitive index of oxidative stress *in vivo*. The majority of 8-epi-$PGF_{2\alpha}$ present in the circulation occurs in association with lipoproteins which are synthesised in the liver. Since lipoproteins are derived from dietary fatty acids and triglycerides, it is possible that 8-epi-$PGF_{2\alpha}$ generated in polyunsaturated fatty acid-rich food (during initial processing/packaging or during meal preparation) may become incorporated within these lipoproteins during synthesis. In view of the growing use of 8-epi-$PGF_{2\alpha}$ as a marker of lipid peroxidation *in vivo* in nutritional or clinical studies, it is therefore important to investigate the possibility that the circulating levels measured could be confounded by the presence of 8-epi-$PGF_{2\alpha}$ in food. In this study we evaluated the levels of 8-epi-$PGF_{2\alpha}$ present in several popular fast-foods, using a combination of solid phase extraction and gas chromatography–mass spectrometry. Fast-foods were selected to represent meals prepared from vegetable-, chicken-, fish- and meat-derived ingredients. Total (free + esterified) 8-epi-$PGF_{2\alpha}$ levels ranged from 0.09 to 0.73 pmol/g (122–644 pmol/mmol arachidonic acid), with the highest levels present in beef-derived meals. Further investigation of hamburgers and cheeseburgers revealed 8-epi-$PGF_{2\alpha}$ levels of 1.83 ± 0.24 and 0.84 ± 0.03 nmol/mmol arachidonic acid, respectively. Lower concentrations of vitamin E were found in the hamburgers. The postprandial contribution to plasma 8-epi-$PGF_{2\alpha}$ levels following ingestion of 100 g portions of these fast-foods would therefore be expected to be no greater than the low picomole range, and would be unlikely to influence the normal endogenous levels of 8-epi-$PGF_{2\alpha}$ and those produced during oxidative stress.

Keywords: F_2-Isoprostanes, 8-epi-$PGF_{2\alpha}$, lipid peroxidation, plasma, diet, fast-foods

INTRODUCTION

Isoprostanes (IPs) are a complex group of prostaglandin-like molecules which are produced by the free radical-catalysed peroxidation of specific polyunsaturated fatty acids (PUFAs). Peroxidation of arachidonic acid, for example, produces

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several compounds including the F_2-IP series, which can be divided into four major regioisomeric groups comprising a total of 64 isomers. Morrow *et al.*[1,2] reported the presence of F_2-IPs *in vivo* and subsequently showed that levels of one of the isomers, 8-epi-$PGF_{2\alpha}$, increased by up to 50-fold in established animal models of oxidative injury.

Circulating levels of 8-epi-$PGF_{2\alpha}$ are higher in subjects with non-insulin dependent diabetes mellitus (NIDDM) compared to healthy controls.[3] Several studies have also demonstrated elevated F_2-IPs in humans with hypercholesterolemia,[4] diabetes,[5] hepatic cirrhosis,[6] Alzheimer's disease,[7] cardiovascular[8] and respiratory[9] diseases. The high plasma and tissue levels of 8-epi-$PGF_{2\alpha}$ in animal models of diabetes (STZ-diabetic and Zucker obese rats) are decreased following dietary supplementation with antioxidants.[10,11] Similar observations have been reported in human studies.[4,5,12] It is now recognised that the measurement of 8-epi-$PGF_{2\alpha}$ represents a sensitive and reliable index of lipid peroxidation both *in vitro* and *in vivo*, and can also provide a valuable approach for monitoring the effects of antioxidant supplementation. However, its application to nutritional studies requires an investigation of the possibility that it could be confounded by the presence of F_2-IPs in food, as can happen with other biomarkers of lipid peroxidation, especially with malondialdehyde.[13]

Consumption of fast-foods represents an increasing percentage of the daily nutritional intake in developed and developing societies, specially among the young.[14–16] The cooking methods used in popular commercial fast-food outlets involve heating, dehydration and thermolysis in the presence of oil, and consequently enhance the rate of lipid peroxidation in food. Staprans *et al.*[17] have shown that feeding oxidised linoleic acid to rats resulted in the accumulation of the oxidised fatty acid in circulating lipoproteins and extrahepatic tissue. More recently, it has been demonstrated that dietary supplementation of oxidised lipids in humans can lead to elevated postprandial levels of these compounds for an extended period, increasing exposure of the vasculature to potentially atherogenic material.[18] Thus, if F_2-IPs are generated in food during the manufacturing process and/or preparative stages they might become incorporated within lipoproteins in the circulation and eventually accumulate in tissue. Increased levels of F_2-IPs disrupt the physico-chemical integrity of cell membranes[19] and can exert significant pharmacological activity. Indeed, 8-epi-$PGF_{2\alpha}$ is a potent vasoconstrictor acting on the renal and pulmonary vascular beds,[1,20] modulates platelet aggregation[21] and is involved in the activation of intracellular signalling.[22] It has also been suggested that F_2-IPs may represent a new class of mediators acting in parallel with classical prostaglandins, via receptors that are distinct but closely related to the thromboxane A_2 receptor.[21]

The aim of this study was to determine the levels of F_2-IPs present in several popular fast-foods, using a GC-MS assay developed for the measurement of 8-epi-$PGF_{2\alpha}$ in biological fluids and tissue. The combination of a solid-phase and immunoaffinity extraction procedure to isolate 8-epi-$PGF_{2\alpha}$ from the food samples and analysis by GC-MS, allows accurate quantitative determinations of this F_2-IP. The mechanisms involved in lipid peroxidation however, generate other products such as lipid hydroperoxides, which can provide an additional measure of oxidation in food samples. Fatty acids, total cholesterol and vitamin E were also measured since they can influence the peroxidation process. The data in the present paper therefore enable an overall assessment of the extent of lipid peroxidation in fast-foods.

MATERIALS AND METHODS

Materials

The following fast-foods were obtained from shops in the London EC1 area: Kentucky chicken

filet burger (Chkn Flt Bg), French fries (Frch Frs), döner kebab (Dnr Kb), fried cod filet (Fr Cod Flt), stir-fried vegetables (SF Vg), stir-fried vegetables + chicken (SF Vg + Chkn), stir-fried vegetables + beef (SF Vg + Bf) and McDonald's Big Mac (McD BgMc). *N,O-bis*(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Pierce Chemical Company (Rockford, IL, USA). Pentafluorobenzyl bromide (PFB-Br), *N,N*-di*iso*-propylethylamine (DIPEA), butylated hydroxytoluene, trisodium citrate, triphenylphosphine, sodium chloride, potassium hydroxide, cholesterol reagents, xylenol orange (3,3′-*bis*[*N,N*-Di(carboxymethyl)-aminomethyl]-*o*-cresolsulfonephthalein sodium salt) and thimerosal were obtained from Sigma-Aldrich (Gillingham, UK). Tetradeuterated 3,3′,4,4′-8-*epi*-$PGF_{2\alpha}$ (8-epi-$PGF_{2\alpha}$-d_4) was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Vitamin E standards (D-α-, D-γ- and D-δ-tocopherol) were obtained from ICN Biomedicals (Thame, UK). Sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$), disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$) and hydrochloric acid were obtained from BDH (Poole, UK). All commercially available solvents were of HPLC grade. Sep-Pak C_{18} (500 mg) cartridges were obtained from Waters (Watford, UK).

Sample Preparation

Food samples were carefully cut into small portions before weighing. In the case of the chicken filet burger and the Big Mac, ingredients such as the bun, onions, pickle, lettuce, salad cream, mustard and tomato sauce were removed prior to extraction. Although most of the cheese present in the Big Mac was removed, a small part of the cheese (especially the surface which comes in direct contact with the hot meat patty) had melted over the meat and was therefore combined with the meat sample used for extraction.

A food sample (9–16 g) was snap frozen in liquid nitrogen (2–3 min) and pulverised in a stainless steel percussion mortar (Biomedix, Pinner, UK) held on dry ice. The sample was homogenised (1–2 min) in an ice-cold solution (20 mL) of $CHCl_3$/MeOH (2 : 1), containing butylated hydroxytoluene (BHT, 0.005%) and triphenylphosphine (1 mM). The homogenate was then sealed under nitrogen and allowed to stand for 1 h at room temperature. Following this period, 4 mL of NaCl (0.9% w/v) were added to the homogenate and vortex-mixed for 15 s. The solution was then centrifuged at 1120× *g* for 15 min, and the chloroform layer isolated and evaporated under nitrogen. The residue was reconstituted in ethanol (6 mL) containing BHT (0.005%) and stored at −30°C until analysis. Aliquots of this reconstituted solution were used for analysis.

Analysis of Total 8-Epi-$PGF_{2\alpha}$

Hydrolysis and Solid-Phase Extraction of 8-Epi-$PGF_{2\alpha}$

An aliquot of the extracted solution (1.0 mL) was dried under nitrogen and reconstituted in ethanol (100 µL). Tetradeuterated 8-epi-$PGF_{2\alpha}$ (8-epi-$PGF_{2\alpha}$-d_4) was then added as an internal standard (2 ng in 20 µL ethanol) and the sample was hydrolysed using KOH (0.5 mL, 1.0 M) for 30 min at 40°C. Hydrolysis was terminated by the addition of 1.0 M HCl (0.5 mL) and the pH was adjusted to 1.5 with 0.1 M HCl (2 mL). Isolation of 8-epi-$PGF_{2\alpha}$ was carried out by sequential solid-phase extraction (SPE) of the hydrolysed sample using (i) a commercially available C_{18} cartridge and (ii) an immunoaffinity cartridge specific for 8-epi-$PGF_{2\alpha}$. SPE on the C_{18} cartridge was carried out as described earlier[23] and the final eluate dried under nitrogen. After reconstituting in ethanol (100 µL), the sample was diluted to 20 mL with phosphate buffer (0.05 M, pH 7.4, containing 0.005% thimerosal as an antibacterial) and applied to the immunoaffinity cartridge, pre-conditioned with phosphate buffer (16 mL). The cartridge was washed with water (20 mL) to remove non-retained components and 8-epi-$PGF_{2\alpha}$ was eluted using acetone/water (95/5, 5.5 mL). All the SPE steps were programmed into

an ASPEC XL sample processor (Gilson Medical Electronics, Villiers-le-Bel, France) and run automatically.

GC-MS of 8-Epi-PGF$_{2\alpha}$

The final eluate from the immunoaffinity extraction was dried under nitrogen and the sample converted to a PFB/TMS derivative. GC-NICI-MS was carried out on a bench top unit consisting of a GC 8000 interfaced to a Trio 1000 MS (Fisons Instruments, Manchester, UK) using ammonia as reagent gas. The GC-MS assay for 8-epi-PGF$_{2\alpha}$ in biological samples has a limit of detection of about 10 pg/mL with and intra- and inter-assay coefficient of variation of 8.2% and 10.7%, respectively. Analysis was performed using selected ion recording (SIR) of the carboxylate anion $[M-181]^-$ at *m/z* 569 for 8-epi-PGF$_{2\alpha}$ and *m/z* 573 for 8-epi-PGF$_{2\alpha}$-d$_4$. Quantitative determination was based on the peak area ratio of 8-epi-PGF$_{2\alpha}$ against the internal standard.[3]

Lipid Hydroperoxides

Lipid hydroperoxides were measured using the ferrous oxidation – xylenol orange (FOX-2) assay.[24] Briefly, an aliquot of food extract (100 µL) was transferred to an Eppendorf tube, followed by the addition of FOX-2 reagent (900 µL). The mixture was incubated at room temperature for 40 min and the absorbance of the supernatant measured by spectrophotometry at 560 nm.

Cholesterol

Total cholesterol was assayed by an enzymatic method (Sigma Diagnostics, cholesterol procedure No. 401) coupled with absorbance measurements at 500 nm.

Vitamin E

Vitamin E (α- and γ-tocopherol) was measured by reverse-phase HPLC. An aliquot of food extract (0.5 mL) was transferred to a glass tube, followed by addition of the internal standard D-δ-tocopherol (500 ng in 50 µL ethanol) and 0.5 M potassium hydroxide (200 µL). The sample was incubated for 30 min at 37°C. Following incubation, water (0.5 mL) was added and the sample extracted twice with hexane (1.0 mL). The pooled hexane extracts were evaporated under nitrogen at room temperature and the residue reconstituted in acetonitrile (50 µL). An aliquot (25 µL) was injected automatically (WISP 715 autoinjector, Waters Inc., USA) on to a Hypersil ODS column (200 × 3 mm ID, particle size 5 µm; Chrompack, Middelburg, Netherlands), using an isocratic mobile phase of acetonitrile/tetrahydrofuran/water (80/14/6, v/v) at a flow rate of 0.7 mL/min. Quantitative determination of α- and γ-tocopherol (α- and γ-TOH) was based on the peak height ratio of these isomers against the internal standard, using fluorescence detection at λ_{EX} 295 nm and λ_{EM} 340 nm.

Fatty Acids

Total fatty acids were analysed as methyl esters by gas chromatography, using a modified method of Ohta *et al.*[25] Briefly, an aliquot of food extract (300 µL) was transferred to a glass tube followed by heptadecanoic acid (100 µg in 50 µL dichloromethane) as an internal standard. Water (100 µL) and concentrated hydrochloric acid (10 µL) were added and the sample extracted twice with a $CHCl_3$/MeOH (2/1) mixture (800 µL). The combined chloroform extracts were dried under nitrogen and the residue was derivatised with 14% boron trifluoride–methanol (0.5 mL) for 30 min at 60°C. Following derivatisation, water (0.3 mL) was added and the sample extracted with hexane (0.5 mL). The solvent was dried under nitrogen, reconstituted in hexane (30 µL) and an aliquot (3 µL) injected on to a capillary column (Supelcowax-10, 30 m × 0.53 mm ID × 1.0 µm D_f, Supelco Inc., Bellefonte, USA). Quantitative determination was based on the peak height ratio of

each fatty acid against the internal standard, using an HP 6890 GC with an autoinjector and flame ionisation detection (Hewlett Packard, Bracknell, UK).

Statistics

Three measurements were carried out on each sample and the data expressed as the mean ± SE. Data were analysed using GraphPad InStat (V2.04a). Student's unpaired *t*-test was applied to determine differences between the means. Differences were considered statistically significant at the 5% level.

RESULTS

As shown in Table I, total fatty acid profiles for the fast-foods were established for palmitic (C16 : 0), stearic (C18 : 0), oleic (C18 : 1, *cis*-9), linoleic (C18 : 2, *cis*-9,12), linolenic (C18 : 3, *cis*-9,12,15), arachidonic (C20 : 4, *cis*-5,8,11,14) and docosahexaenoic (C22 : 6, *cis*-4,7,10,13,16,19) acids. Arachidonic acid content ranged from 232 to 369 μg/g. Levels present in the chicken-based meals were not significantly different (353 ± 18 and 369 ± 22 μg/g, chicken filet burger and stir-fried vegetables + chicken, respectively), indicating that the animal tissue represents the major source of C20 : 4 in these samples. The three stir-fried meals analysed in this study (mixed vegetables, vegetables + chicken and vegetables + beef) came from the same outlet and were bought at the same time, suggesting that the mixed vegetables used in these preparations were derived from the same stock. Differences in C20 : 4 may therefore be associated with the presence of chicken (59.1% increase vs mixed vegetables) or beef (49.6% increase vs mixed vegetables) in the samples. Levels of C20 : 4 in two deep-fried fast-foods obtained from different outlets were not significantly different (275 ± 23 and 253 ± 32 μg/g, in French fries and fried cod filet, respectively),

TABLE I Levels of total fatty acids, lipid hydroperoxides, total cholesterol, total 8-epi-$PGF_{2\alpha}$ (normalised to total arachidonic acid) and vitamin E (normalised to total cholesterol) in fast-foods. Data are expressed as the mean ± SE of three measurements made on each fast-food

Lipid peroxidation marker	Chicken filet burger	French fries	Doner kebab	Fried cod filet	Stir-fried meals			McDonald's Big Mac
					Mixed veg	Chkn + veg	Beef + veg	
Total fatty acids (μg/g)								
16 : 0	938 ± 32	306 ± 26	1215 ± 85	1591 ± 80	184 ± 28	804 ± 26	688 ± 25	853 ± 32
18 : 0	179 ± 30	69 ± 9	573 ± 39	700 ± 42	247 ± 17	159 ± 26	650 ± 28	617 ± 22
18 : 1	519 ± 20	339 ± 22	833 ± 26	1026 ± 75	275 ± 25	441 ± 29	845 ± 30	799 ± 36
18 : 2	563 ± 30	407 ± 30	381 ± 19	590 ± 38	356 ± 15	706 ± 32	471 ± 28	474 ± 26
18 : 3	79 ± 15	136 ± 12	181 ± 15	73 ± 25	209 ± 32	104 ± 15	219 ± 18	184 ± 20
20 : 4	353 ± 18	275 ± 23	296 ± 18	253 ± 32	232 ± 22	369 ± 22	347 ± 32	244 ± 30
22 : 6	106 ± 14	59 ± 26	155 ± 19	390 ± 30	108 ± 28	80 ± 15	106 ± 24	129 ± 36
Hydroperoxides (nmol/g)	2.96 ± 0.06	1.94 ± 0.06	2.93 ± 0.13	7.39 ± 0.21	5.46 ± 0.03	4.7 ± 0.03	5.79 ± 0.05	8.96 ± 0.09
Total cholesterol (μmol/g)	0.88 ± 0.05	0.2 ± 0.01	1.42 ± 0.25	0.64 ± 0.03	0.22 ± 0.02	0.92 ± 0.03	1 ± 0.01	0.81 ± 0.03
8-epi-$PGF_{2\alpha}$ (nmol/mmol 20 : 4)	0.17 ± 0.01	0.28 ± 0.02	0.23 ± 0.01	0.24 ± 0.01	0.12 ± 0.01	0.17 ± 0.01	0.64 ± 0.01	0.6 ± 0.01
Vitamin E (nmol/μmol cholesterol)								
γ-TOH	2.55 ± 0.05	15.2 ± 0.1	0.53 ± 0.04	3.01 ± 0.05	1.9 ± 0.06	0.94 ± 0.02	1.42 ± 0.04	1.04 ± 0.09
α-TOH	4.71 ± 0.06	21.6 ± 0.3	0.62 ± 0.23	1.68 ± 0.02	4.6 ± 0.34	2.72 ± 0.09	2.83 ± 0.01	1.63 ± 0.01

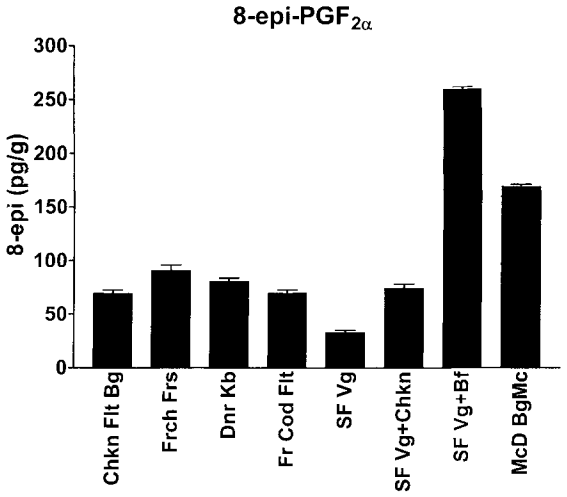

FIGURE 1 Total 8-epi-$PGF_{2\alpha}$ levels in fast-foods. Data are expressed as the mean ± SE of three measurements made on each fast-food.

which may also suggest that the cooking oil could influence the fatty acid profile in some types of preparations. Although vegetable cooking oil does not contain C20 : 4, a small amount may be present when mixed animal and vegetable oils are used. Earlier use of vegetable oil for cooking meat-containing products may also result in leaching of C20 : 4 into the oil. Levels of C22 : 6 in fish were significantly higher compared to the other samples analysed, as expected.

The levels of total (free + esterified) 8-epi-$PGF_{2\alpha}$ in the food samples are shown in Figure 1. Levels of total 8-epi-$PGF_{2\alpha}$ normalised to the total C20 : 4 concentration for each sample are shown in Table I. Absolute F_2-IP concentrations ranged from 33 to 260 pg/g (0.09–0.73 pmol/g; normalised range 122–644 pmol/mmol C20 : 4). The F_2-IP content in six of the samples ranged from 33 to 91 pg/g (0.09–0.26 pmol/g). However, stir-fried vegetables + beef and the beef burger (McDonald's Big Mac) contained the highest levels, 260 ± 2 and 169 ± 3 pg/g, respectively, even when normalised to arachidonic acid (0.64 ± 0.01 and 0.60 ± 0.01 nmol/mmol C20 : 4, respectively). The 8-epi-$PGF_{2\alpha}$ levels in the chicken-based samples (chicken filet burger and stir-fried vegetables + chicken) were not significantly different, either in terms of absolute (70 ± 3 and 74 ± 4 pg/g, respectively) or normalised (169 ± 7 and 172 ± 9 pmol/mmol C20 : 4, respectively) concentrations. This suggests that the total F_2-IP contribution was derived primarily from the chicken tissue and was not influenced by the cooking technique. Absolute levels of 8-epi-$PGF_{2\alpha}$ were 2.3- and 7.9-fold higher in the chicken- and beef-containing meals (1.4- and 5.3-fold, respectively, for normalised 8-epi-$PGF_{2\alpha}$) compared to the vegetable meal, indicating a prevalence of F_2-IPs in food derived from animal origin. Although absolute concentrations of 8-epi-$PGF_{2\alpha}$ in the fish (fried cod filet, 70 ± 3 pg/g) and lamb (döner kebab, 81 ± 3 pg/g) were not significantly different when compared to the corresponding levels in chicken-based samples, the normalised levels varied significantly compared to the average normalised value in chicken-derived meals (237 ± 6 vs 171 ± 8, $P < 0.01$ and 234 ± 9 vs 171 ± 8, $P < 0.01$ pmol/mmol C20 : 4, in fish and lamb vs chicken, respectively).

Lipid hydroperoxides ranged from 1.94 to 8.96 nmol/g (Table I), the highest concentration being found in the Big Mac. The high levels detected in fish tissue may partly represent the contribution from the heated oil (180°C) used during deep-frying. However, although French fries (obtained from a different fast-food outlet) were also subjected to deep-frying, the hydroperoxide concentrations were 3.2-fold lower compared to the fish. Hydroperoxides in the stir-fried meals were between 4.7 and 5.7 nmol/g. Moreover, a significant difference was observed between stir-fried vegetables + chicken and the chicken burger (4.7 ± 0.01 and 3.0 ± 0.06 nmol/g, respectively, $P < 0.01$), again suggesting that the cooking medium may contribute to this index of lipid peroxidation. Total cholesterol varied between 0.2 and 1.4 μmol/g, with the lowest values in French fries and stir-fried vegetables (Table I). Higher levels were detected in the fish-, chicken- and meat-based samples.

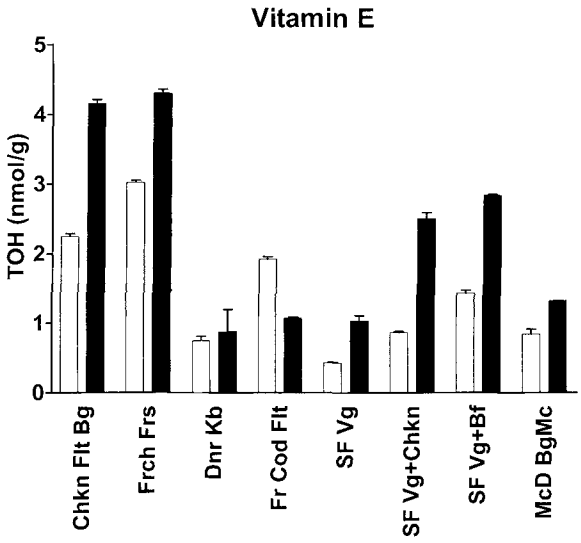

FIGURE 2 Vitamin E in fast-foods. γ-TOH, open bars; α-TOH, solid bars. Data are expressed as the mean $\pm$ SE of three measurements made on each fast-food.

The levels of α- and γ-TOH in the food samples are shown in Figure 2. The concentrations of α- and γ-TOH normalised to total cholesterol are shown in Table I. The majority of samples contained a higher amount of α-TOH (range 0.87–4.3 nmol/g; 0.62–21.6 nmol/μmol cholesterol) compared to γ-TOH (range 0.43–3.0 nmol/g; 0.53–15.2 nmol/μmol cholesterol). However, in the fish-based meal the absolute and normalised γ-TOH concentrations were both approximately 1.8-fold higher than the corresponding α-TOH concentrations. French fries contained significantly higher normalised γ- and α-TOH concentrations (15.2 ± 0.14 and 21.6 ± 0.27 nmol/μmol cholesterol, respectively), representing a 9.3- and 8.1-fold increase in γ- and α-TOH, respectively, compared to the average content of these isomers in the other fast-foods analysed.

Following our initial observations that high levels of 8-epi-$PGF_{2\alpha}$ were present in beef-derived products, a separate experiment was carried out to study the variation of F_2-IPs and other markers of lipid peroxidation in beef burgers. One hamburger and one cheeseburger were purchased from each of three different McDonald's outlets (A–C) in the central London (EC2, EC3 and EC4) area. The food samples were extracted and analysed in triplicate, as described earlier. For both hamburgers and cheeseburgers, ingredients such as the bun, pickle, onions, mustard and tomato sauce were removed prior to extraction of the meat. Hamburgers (total weight 93 ± 0.3 g, $n = 3$) consisted of a single beef patty (30.8 ± 0.5 g) together with the ingredients listed above (62.3 ± 0.8 g). Cheeseburgers (total weight 104.8 ± 0.9 g, $n = 3$) contained a slice of processed cheese in addition to the beef patty and other ingredients used to prepare hamburgers. Although most of the cheese (9.8 ± 0.7 g) was removed with the other ingredients (62.2 ± 0.9 g) before extraction, a small part of the cheese that had melted on the beef patty was included in the sample used for analysis. The beef patty obtained from cheeseburgers weighed 32.8 ± 0.3 g ($n = 3$). Since the beef patty is similar to the one used for preparing hamburgers, the difference in weight (about 2 g) is due to the amount of melted cheese present. This suggests that the melted cheese represents approximately 6.1% of the total weight of the meat patty, with the remaining 93.9% being beef.

As shown in Figure 3, the fatty acid profiles of both burgers were similar. Levels of C20:4 in hamburgers and cheeseburgers were not significantly different (392 ± 22 and 310 ± 31 μg/g, respectively). The absolute and normalised 8-epi-$PGF_{2\alpha}$ levels varied significantly in hamburgers obtained from different outlets (Table II). Less variation was observed among cheeseburgers, with the absolute (0.302 ± 0.01 ng/g) and normalised (0.837 ± 0.03 nmol/mmol C20:4) levels of 8-epi-$PGF_{2\alpha}$ about 2.8- and 2.2-fold lower, respectively, compared to hamburgers (Figure 4). 8-epi-$PGF_{2\alpha}$ was not detected in the cheese, suggesting that the presence of melted cheese on the beef patty resulted in dilution of the beef in the sample analysed. Since beef represented approximately 93.9% of the sample analysed, the dilution factor due to the cheese was about 1.07. Recalculation of 8-epi-$PGF_{2\alpha}$ in

cheeseburgers, taking the dilution into account, gives an absolute concentration of 0.322 ± 0.01 ng/g, which is still significantly lower compared to hamburgers (0.836 ± 0.11 ng/g). Interestingly, absolute and normalised 8-epi-$PGF_{2\alpha}$ in the cheeseburgers were 1.8 and 1.4 times higher, respectively, compared to those initially detected in the Big Mac (McDonald's Big Mac, Figure 1 and Table I). Although most of the cheese had also been removed from the beef patty in the Big Mac prior to analysis, a small amount melted over the meat was present in the sample analysed. These differences in 8-epi-$PGF_{2\alpha}$ levels may therefore reflect variation in the sample composition, since preparation procedures are similar. Lipid hydroperoxides in cheeseburgers (1.52 ± 0.27 nmol/g) were significantly lower compared to hamburgers (2.95 ± 0.61 nmol/g, $P < 0.05$, Figure 4), although considerable variation was observed between samples from different outlets (Table II). The levels of lipid hydroperoxides may be influenced by the quality of oil and other ingredients used during food preparation, and may explain the higher concentrations observed in the initial experiment (Table I). The absolute and normalised concentrations of α- and γ-TOH were significantly higher in cheeseburgers, suggesting that the tocopherol increment may be associated with the cheese used in this preparation (Figure 5). Indeed, levels of α- and γ-TOH in the cheese were 9.54 ± 0.3 and 1.23 ± 0.1 nmol/g ($n = 3$), respectively. The amounts of α- and γ-TOH in the cheeseburgers were comparable to those detected earlier in the Big Mac, which also included a slice of cheese among the ingredients that were removed before analysis (Figure 2). Total cholesterol in cheeseburgers was significantly higher compared to hamburgers (Figure 6), and may also be associated with the presence of processed cheese.

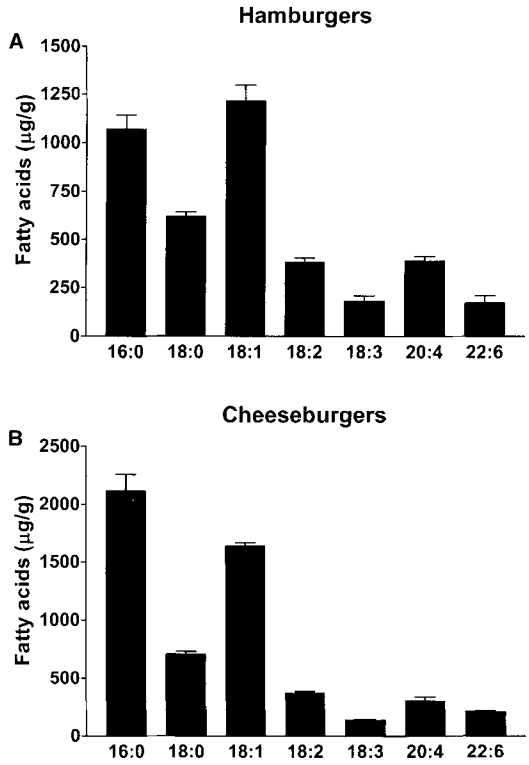

FIGURE 3 Total fatty acid profile in hamburgers (A, $n = 3$) and cheeseburgers (B, $n = 3$). Three measurements were made on each of the six burgers. Data are expressed as the mean ± SE for each burger type.

TABLE II Variation of 8-epi-$PGF_{2\alpha}$ and lipid hydroperoxides in hamburgers and cheeseburgers. One hamburger and one cheeseburger were purchased from each of three different McDonald's outlets (A–C) in the central London area. 8-epi-$PGF_{2\alpha}$ values are expressed as absolute concentrations and normalised to the total arachidonic acid concentration for each burger. Data are expressed as the mean ± SE of three measurements made on each of the six burgers

Lipid peroxidation marker	Hamburgers			Cheeseburgers		
	A	B	C	A	B	C
8-epi-$PGF_{2\alpha}$ (ng/g)	0.77 ± 0.1	0.59 ± 0.02	1.15 ± 0.02	0.29 ± 0.02	0.28 ± 0.02	0.33 ± 0.01
8-epi-$PGF_{2\alpha}$ (nmol/mmol 20:4)	1.66 ± 0.21	1.43 ± 0.05	2.31 ± 0.04	0.88 ± 0.05	0.89 ± 0.06	0.77 ± 0.02
Hydroperoxides (nmol/g)	4.34 ± 0.24	1.12 ± 0.12	3.38 ± 0.04	1.19 ± 0.04	2.36 ± 0.12	1.00 ± 0.14

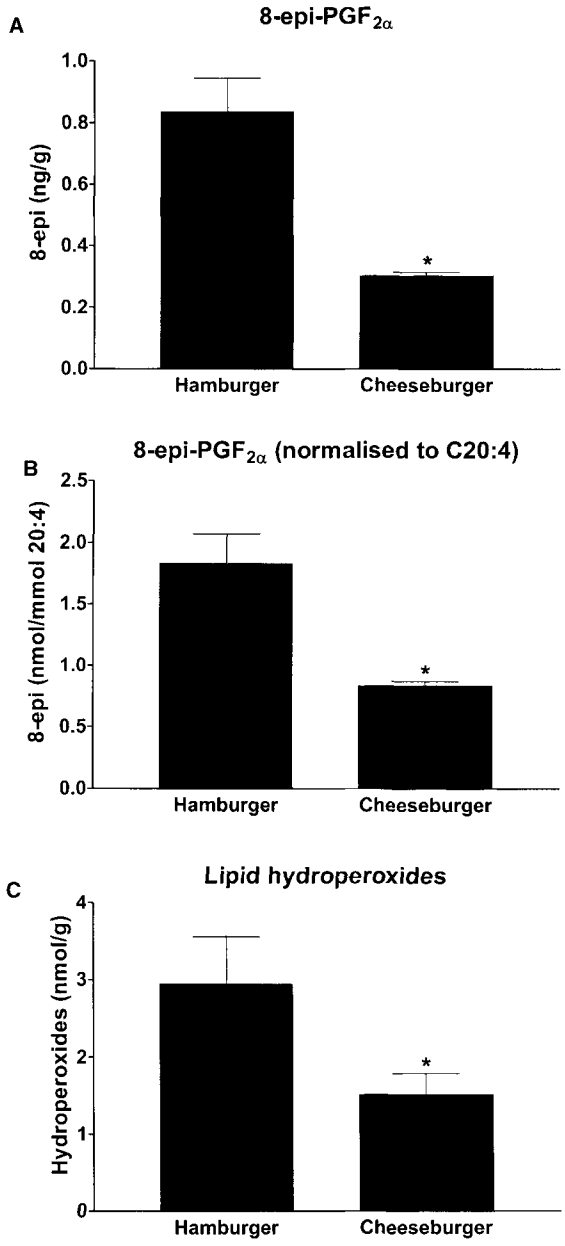

FIGURE 4 8-epi-$PGF_{2\alpha}$ and lipid hydroperoxides in hamburgers ($n=3$) and cheeseburgers ($n=3$). 8-epi-$PGF_{2\alpha}$ values are expressed as absolute concentrations (A) and normalised to the total arachidonic acid concentration for each burger type (B). Lipid hydroperoxide levels in the burgers are shown in (C). Three measurements were made on each of the six burgers. Data are expressed as the mean ± SE for each burger type. $^{*}P<0.05$.

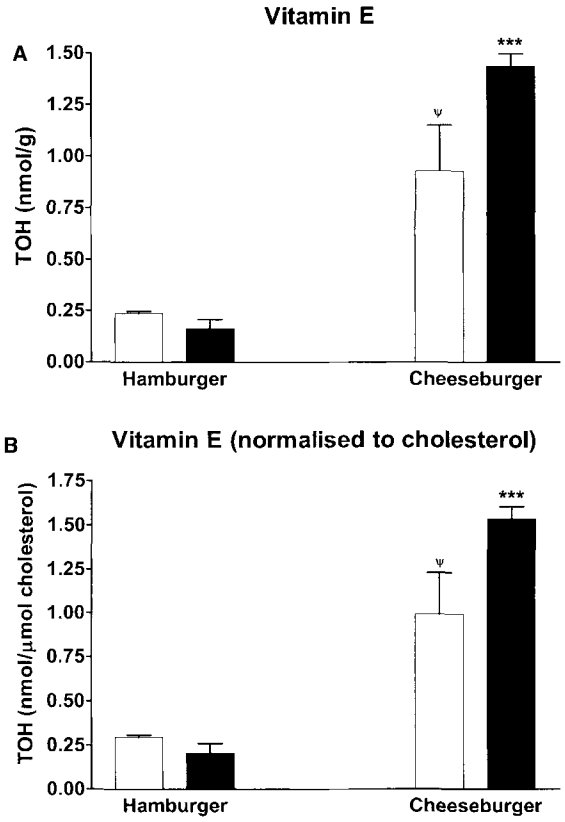

FIGURE 5 Vitamin E in hamburgers ($n=3$) and cheeseburgers ($n=3$). α- and γ-TOH values are expressed as absolute concentrations (A) and normalised to the total cholesterol concentration (B). γ-TOH, open bars; α-TOH, solid bars. Three measurements were made on each of the six burgers. Data are expressed as the mean ± SE for each burger type. The statistically significant differences in γ- and α-TOH between the burger types are marked with: Ψ $P<0.05$ vs hamburger γ-TOH; $^{***}P<0.0001$ vs hamburger α-TOH.

DISCUSSION

IPs are formed during the free radical-mediated oxidation of PUFAs such as C20:4, C20:5 and C22:6. The measurement of F_2-IPs (derived from C20:4), in particular 8-epi-$PGF_{2\alpha}$, constitutes a sensitive index of lipid peroxidation both *in vitro* and *in vivo*. In addition, 8-epi-$PGF_{2\alpha}$ has pharmacological properties and may play an important role as a signalling mediator in diseases associated with elevated oxidative stress. F_2-IPs are

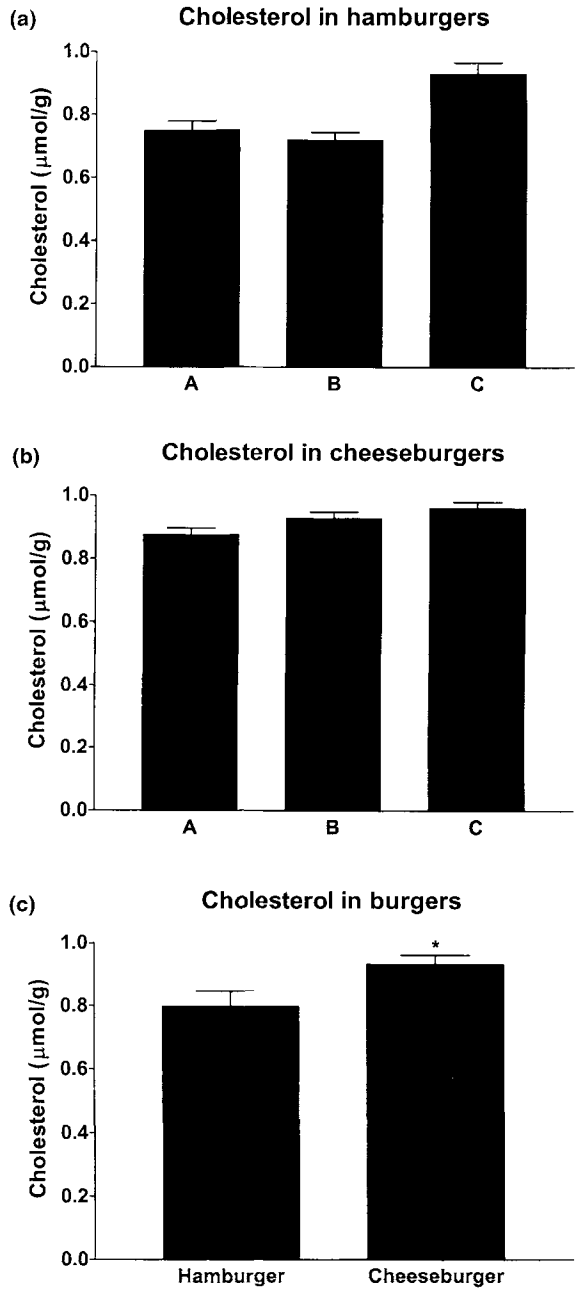

FIGURE 6 Total cholesterol in hamburgers (a) and cheeseburgers (b). One hamburger and one cheeseburger were purchased from each of three different McDonald's outlets (A–C) in the central London area. Data are expressed as the mean ± SE of three measurements made on each of the six burgers. Total cholesterol levels in the hamburgers ($n = 3$) and cheeseburgers ($n = 3$) are also expressed as the mean ± SE for each burger type (c). *$P < 0.05$.

initially formed esterified to phospholipids and can be released as free components via the action of phospholipases. The majority of esterified F_2-IPs present in the circulation occurs in association with lipoproteins (LDL, HDL) which are synthesised in the liver. A small proportion of these F_2-IPs may also result from the normal turnover of membrane phospholipids. Since lipoproteins are partly derived from dietary fatty acids and triglycerides, it is possible that F_2-IPs generated in C20 : 4-rich food (during initial processing and packaging, or during meal preparation) may become incorporated within these lipoproteins during synthesis, unless they undergo metabolism in the GI tract.[26] Consequently, diet-derived F_2-IPs could conceivably contribute to the levels determined in plasma. Our initial aim in this study was to evaluate this possibility by measuring the 8-epi-$PGF_{2\alpha}$ content in some foods currently available.

A selection of fast-foods was chosen to represent meals prepared from plant- (French fries, stir-fried mixed vegetables), fish- (fried cod filet), chicken- (chicken filet burger, stir-fried vegetables + chicken) or meat- (döner kebab, stir-fried vegetables + beef, McDonald's Big Mac) derived ingredients. The total levels of 8-epi-$PGF_{2\alpha}$ in the fast-foods selected were in the low picogramme/g range, with amounts in 100 g portions ranging from 3.3 to 26 ng (9–73 pmol). Variations were observed in terms of both absolute and normalised 8-epi-$PGF_{2\alpha}$ concentrations, suggesting that F_2-IP formation may be dependent on the relative concentrations of PUFAs present in the samples. Significantly higher levels of 8-epi-$PGF_{2\alpha}$ were present in the beef-derived meals (even when corrected for their arachidonic acid content) and may partly reflect the longer meat processing time (from slaughter to customer) involved with large animals. These levels could also be influenced by the release of iron and haemoglobin during meat cooking, which is pro-oxidant for lipids.[27] Extended storage can influence the rate of autoxidation of fatty acids, further contributing to F_2-IP formation in C20 : 4-rich non-frozen food.

Could the levels of 8-epi-$PGF_{2\alpha}$ we have measured confound plasma concentrations? For 100 g portions of the fast-foods ingested, let us assume that all was absorbed. The distribution of 8-epi-$PGF_{2\alpha}$ over the total blood volume (5 L) would result in postprandial contributions ranging from 0.66 to 5.2 pg/mL (1.9–14.7 pM). In the case of burgers, complete absorption of 8-epi-$PGF_{2\alpha}$ from the beef patty in hamburgers and cheeseburgers would result in postprandial levels of 5.1 pg/mL (14.4 pM) and 1.9 pg/mL (5.4 pM), respectively. Consequently, the 8-epi-$PGF_{2\alpha}$ contribution from the fast-foods examined in this study would be expected to be minimal in comparison with the normal range of endogenous total (sum of free + esterified) levels (250–385 pg/mL; 703–1087 pM) and those produced during oxidative stress.[28] This estimate is based on the assumption that all F_2-IPs in the food are absorbed and become assimilated within lipoproteins. However, complete absorption seems unlikely. Hydrolysis occurring during the enzymatic breakdown of dietary fat (triglycerides, phospholipids, cholesterol esters) via the action of lipase and phospholipase A_2,[29] may result in a fraction of the esterified 8-epi-$PGF_{2\alpha}$ present in food being actively absorbed as the free compound. With a half-life of a few minutes,[30] free 8-epi-$PGF_{2\alpha}$ is rapidly metabolised and excreted in the urine, implying that the total postprandial 8-epi-$PGF_{2\alpha}$ contribution from fast-foods is likely to be even lower.

Other markers of lipid peroxidation (lipid hydroperoxides, vitamin E, PUFAs, cholesterol) were monitored to assess the oxidant status of fast-foods. Depletion of PUFAs and vitamin E occur during lipid peroxidation and represent useful markers for determining the susceptibility to oxidation. In addition PUFAs, especially C20 : 4, are measured in order to standardise F_2-IPs and enable comparisons between samples of different origins. Similarly, vitamin E is often associated with the total cholesterol content and the normalised vitamin E : cholesterol ratio allows easier comparison. The lipid hydroperoxide content in 100 g portions of the fast-foods ingested would be expected to range from 194 to 896 nmol, resulting in postprandial levels of 39–179 nM. For hamburgers and cheeseburgers, total absorption of the hydroperoxides in the beef patty would represent an intake of 91 and 47 nmol, respectively, with corresponding postprandial levels of 18 and 9 nM, respectively. Again, these levels would be expected to be minimal when compared to normal endogenous levels (0.1–1.0 μM). Although the overall contribution to the different indices of lipid peroxidation remained small, several conclusions can be drawn based on their inter-relationship. The variation in lipid hydroperoxides observed in the food samples may partly be attributed to the type and quality of oil used, and the differences in cooking time. French fries, for instance, are fried at 180°C for a few minutes whereas fish filets are exposed to the heated oil for longer, which may account for the higher hydroperoxide concentrations in the fish tissue. Antioxidants such as butylated hydroxyanisole (BHA), BHT and sodium sulphite are routinely added to frozen potato products as stabilising agents during commercial processing (blanching, part-frying and freezing) and may contribute to the lower hydroperoxide levels observed in French fries.[31] The elevated levels of both α- and γ-TOH in French fries may be related to the type of oil used and the significant absorption of the oil into the food during frying, as a result of the large surface area : volume ratio of the fries.[32] Although vegetable frying oils contain vitamin E (15–49 mg α-TOH equivalents/100 g) and could contribute to the levels measured in some of the foods, a wide variation in α- and γ-TOH can occur due to the quality of the oil and the number of repeat fryings to which the oil has been subjected.[33] In this context, the concentration of α-TOH was significantly higher in the chicken filet burger compared to the stir-fried vegetables + chicken, suggesting differences in the type of oil used. Cholesterol data indicate that the majority of the total cholesterol assayed comes from animal tissue. The low levels observed in the plant-derived foods (French fries and stir-fried

vegetables) may reflect the use of mixed animal and vegetable oils for frying, as previously reported.[34,35] However, frying is nowadays mostly done in vegetable oils, which suggests that the cholesterol detected in the plant-derived samples may come from meat-, chicken- or fish-derived foods fried earlier in the same oil. Earlier frying of meat-containing products may also influence the arachidonic acid levels measured in plant-derived preparations. For example, French fries (obtained from a Kentucky Fried Chicken outlet) may have been cooked in oil previously used for frying chicken. The fried cod filet (obtained from a fish and chips outlet) was also cooked in the same oil used to prepare fried chicken pieces.

In conclusion, based on our initial data the overall contribution of fast-foods to total plasma 8-epi-$PGF_{2\alpha}$ is likely to be minimal, compared to normal endogenous levels and those present in situations associated with increased oxidant stress. Although lipid hydroperoxides can also be absorbed in the GI tract, the postprandial contributions are still expected to remain low compared to normal endogenous levels. Further development of feeding experiments with food known to be rich in 8-epi-$PGF_{2\alpha}$ will enable us to establish the role, if any, of the contribution of dietary F_2-IPs and their metabolites as indicators of oxidative stress. We also need to further refine our methods to determine levels of other IPs based on the F_3- and F_4-series, especially for fish. For instance, food prepared using genetically modified ingredients may be enriched in specific ways (e.g. with C22 : 6) that could enhance lipid peroxidation during initial processing, storage or meal preparation, and possibly confound measurements of F_4-IPs.[36,37] These experiments will also provide crucial information needed for the recommendation of a healthy diet.

Acknowledgements

We are grateful to Dr. Kevin Moore for use of the GC-MS at the Department of Medicine, Royal Free and University College Medical School, University College London, UK. This work has been supported by a grant from the Ministry of Agriculture, Fisheries and Food, UK (MAFF project code AN04/23).

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