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Determination of DNA adducts of malonaldehyde in humans: effects of dietary fatty acid composition

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The effects of dietary fatty acid composition on the endogenous formation of DNA adducts of malonaldehyde (MA), the major product of lipid peroxidation, were investigated in humans. A group of 59 healthy individuals of both sexes and different ages was initially fed a milk fat-based diet rich in saturated fatty acids for 14 days. Following this initial period, after which the group was considered homogeneous with respect to diet, 30 randomly chosen subjects were given a sunflower oil-based (rich in polyunsaturated fatty acids) (SO) diet and the remaining 29 individuals a low erucic acid rapeseed oil-based (rich in monounsaturated fatty acids) (RO) diet for 25 days. The fatty acid composition of plasma lipid fractions and the level of DNA adducts of MA in total white blood cells were then determined at the end of the SO and RO dietary periods. DNA adduct levels were measured by ^{32}P-post-labelling using reversed-phase HPLC with on-line detection of radioactivity. Higher concentrations of polyunsaturated fatty acids in plasma triglycerides and higher levels of DNA adducts of MA were found in the subjects on the SO diet when compared with those in the RO dietary group. A large inter-individual variation in adduct levels was observed. The average adduct level in the SO diet group was 7.4 ± 8.7 adducts/10^7 nucleotides (n = 23). This level was 3.6-fold higher than that found in individuals in the RO diet group ($P < 0.001$). Our results, in conjunction with the mutagenic and carcinogenic properties of MA, thus suggest the interaction of lipid peroxidation products such as MA with DNA as one pausible mechanism explaining the involvement of dietary fat in carcinogenesis.

Introduction

On the basis of the epidemiological and experimental evidence available to date a consensus has emerged that diet might be involved in the aetiology of 30–60% of cancers of the developed world (1). Furthermore, the concept that dietary fat affects the incidence of cancer and influences tumour development is widely accepted. Also, it has been agreed that one of the most important measures to bring about a substantial reduction in cancer incidence is a reduction in the consumption of total fat (1–6). Nevertheless, the mechanisms by which high dietary fat intake modulates cancer development are far from understood. Thus it has been proposed that the tumourigenic effect of high dietary fat rich in polyunsaturated fatty acids (PUFA*) takes place via increased synthesis of prostaglandins, which in turn exert an inhibitory effect on the host immune system (7,8). It has also been proposed that the effect of dietary fat on cancer occurs through the activation of pro-carcinogens to ultimate carcinogens by fat oxidation products such as lipid hydroperoxides (for a review see 9). Furthermore, the direct interaction of DNA with lipid hydroperoxides and/or other highly reactive compounds formed during lipid peroxidation has also been proposed as a plausible mechanism for the association between high dietary fat intake and carcinogenesis (9–12).

High levels of PUFA in the diet result in high levels of polyunsaturated membrane lipids and, as a consequence, in high concentrations of substrates for lipid peroxidation reactions (13–16).

Malonaldehyde (MA) is the major reactive aldehyde resulting from the peroxidation of PUFA constituents of biological membranes (9). Also, MA is a by-product of prostaglandin biosynthesis (17). MA is not only a product of normal metabolism, but is also present in a variety of fat-containing foodstuffs (18). Moreover, MA is carcinogenic in rats (19), mutagenic in a number of bacterial and mammalian mutational assays (9,20) and readily reacts with deoxynucleosides to produce adducts (21). Thus MA is an endogenously produced genotoxic agent that may contribute to the development of human cancer.

Several analytical techniques, including ^{32}P-postlabelling, GC-MS and LC-MS, are available at present and have been used for the quantitation of these adducts *in vivo* (11,12,22–27). Furthermore, relatively high endogenous levels of DNA adducts of MA have been detected in healthy individuals (1–10 adducts/10^7 nucleotides) (25,26). On the basis of the available evidence so far obtained it is therefore likely that the interaction of lipid peroxidation products such as MA with DNA constitutes an important mechanism explaining the carcinogenic effects of high dietary fat intake.

To further investigate this mechanism DNA samples isolated from blood obtained from a group of 59 healthy individuals of both sexes and of different ages who participated in a carefully controlled dietary intervention study (28) were analysed for adducts of MA by ^{32}P-postlabelling.

Materials and methods

Chemicals

Inorganic chemicals and organic solvents used for lipid extraction and fatty acid analysis were of analytical grade. All other chemicals and biochemicals

*Abbreviations: PUFA, polyunsaturated fatty acids; MA, malonaldehyde; SFA, saturated fatty acids; SO, sunflower oil-based; MUFA, monounsaturated fatty acids; RO, rapeseed oil-based; TWBC, total white blood cells; TG, plasma triglycerides; MN, micrococcal nuclease; SPD, spleen phosphodiesterase; MA–3′-dGMP, malonaldehyde–2′-deoxyguanosine-3′-monophosphate adducts; LDL, low density lipoprotein.

used for the isolation and hydrolysis of DNA, as well as those employed for enrichment of the adducts from unmodified nucleotides in DNA samples and adduct analysis by ^{32}P-postlabelling, were of analytical grade and used without further purification and have been described elsewhere (22).

Subjects, diets and study design

A more detailed account of the study design and the participating subjects has been reported elsewhere (28). Briefly, the subject population comprised 59 healthy volunteers, 29 males and 30 females. The age of the individuals was between 18 and 65 years, with a median age of 25 years. Exclusion criteria for the study were serum cholesterol >7.4 mM, hypertension, anaemia, glycosuria or proteinuria. None of the subjects were daily smokers. In addition, the volunteers were asked to maintain their lifestyle, as well as their patterns of physical activity, during the experiment. The study protocol, which had been approved by the ethics committees of the Finnish National Public Health Institute and the Faculty of Forestry and Agriculture at the University of Helsinki, Finland, was carefully explained to the volunteers, who then gave their written consent.

The dietary study was started only after an initial period of 14 days during which the participating subjects were given a baseline diet (milk fat-based diet) rich in saturated fatty acids (SFA). At the end of this initial baseline diet period the group of participants was considered homogeneous with regard to diet. After this period 30 randomly chosen subjects were given a high linoleic acid sunflower oil (*Helianthus annuus*, rich in PUFA)-based (SO) diet for 25 days. The other 29 volunteers were given a low erucic acid (~0.5%), high oleic acid Finnish turnip/rapeseed oil [*Brassica rapa* subsp. *oleifera DC*, rich in monounsaturated fatty acids (MUFA)]-based (RO) diet, also for 25 days. The dietary interevention had a cross-over design (28). However, only blood samples obtained after the dietary periods indicated above were available for isolation of DNA and analysis of adducts. The diets were planned to provide the same total fat, cholesterol and nutrient content throughout the dietary periods. The differences between the diets were solely in their fatty acid composition. The diets contained mixed solid foods customary in the Finnish diet covering ~90% of the energy intake per day and were supplied to the subjects. In addition to this food, the individuals were free to choose foods that were free of fat and cholesterol to cover the remaining 10% of their daily energy intake. These food items, as well as any leftovers of the supplied food, were recorded by the subjects in their diaries, for calculation of their contribution to the daily intake of nutrients. The food for each subject was weighed according to energy intake and adjusted to maintain the actual body weight of the subjects throughout the study.

Blood sampling

Blood samples (10 ml) were taken from overnight fasted subjects into EDTA-containing (0.1 ml/10 ml, 3.8 mM) tubes by venepuncture on day 22 of the dietary period. The blood samples were centrifuged at 1000 *g* for 10 min and the plasma was separated within 1 h after venepuncture and thereafter quickly frozen to −70°C until analysis of the plasma fatty acid composition. The remaining blood cells were immediately frozen and kept at −70°C for 2 years until isolation of DNA from total white blood cells (TWBC) for analysis of DNA adducts.

Fatty acid analysis

Lipids were extracted from 0.5 ml plasma by a modification of the method of Folch *et al.* (29). The analysis of the plasma triglyceride (TG) fatty acid composition has been described in detail elsewhere (30). Briefly, TG were separated by TLC, the fatty acids were transesterified with acidic methanol, free cholesterol was removed by elution through an aluminium oxide column and the composition of the fatty acids was determined by gas chromatography. The temperature programme was run between 180 and 230°C. The composition of the fatty acid methyl esters (as percentages) was calculated for C14.0–C22:0. Before quantitation the individual fatty acids were identified by co-chromatography with authentic fatty acid standards.

Isolation of DNA

The isolation of DNA from TWBC was carried out as previously described (26). The DNA thus obtained was dissolved in distilled water and its concentration and purity were assayed using a Beckman DU 640 spectrophotometer. The ranges of A_{230}/A_{260} and A_{260}/A_{280} in the DNA samples were 0.4–0.5 and 1.7–1.8 respectively. The UV spectrum at 220–300 nm was also taken in order to detect the presence of shoulders. RNA contamination was assayed by reversed-phase HPLC using previously described ammonium formate/methanol gradients (22), after enzymatic hydrolysis of aliquots of DNA (10 μg) to deoxynucleosides according to Martin and Garner (31). RNA contamination levels, on the basis of the concentration of cytidine and adenosine in the samples, were <0.1%.

DNA hydrolysis, DNA adduct enrichment and measurement by reversed-phase HPLC with on-line detection of radioactivity

DNA samples (10 μg) isolated from TWBC were enzymatically hydrolysed to 3′-monophosphate nucleotides by digestion with micrococcal nuclease (MN) and spleen phosphodiesterase (SPD) as previously described (32).

The MN/SPD-digested DNA solutions were then further hydrolysed at pH 5.3 by adding 1 μl nuclease P1 solution (2.5 U/μl in 1.5 mM $ZnCl_2$, 0.25 U/μg DNA) and incubating the mixture at 37°C for 20 min to remove normal nucleotides. After this enzymatic treatment only traces of 2′-deoxyguanosine-3′-monophosphate are detected in the samples by ^{32}P-postlabelling (32). The digested material was then evaporated to dryness and redissolved in 1 μl distilled water and used for the ^{32}P-postlabelling reaction.

Recovery of the MA–2′-deoxyguanosine-3′-monophosphate adducts (MA–3′-dGMP) after hydrolysis with nuclease P1 was determined by a series of experiments where unmodified calf thymus DNA samples (10 μg) hydrolysed by MN and SPD were mixed with 10, 50, 100 or 200 fmol authentic MA–3′-dGMP standard synthesized according to Vaca *et al.* (22) and subjected to hydrolysis with nuclease P1. The adduct levels were then measured by ^{32}P-postlabelling (22). The concentration and purity of the calf thymus DNA used were assayed as described above. Recoveries of the added standard, calculated after subtraction of the radioactivity on TLC plates obtained for calf thymus DNA samples alone (background), were 40–60%. Highest recoveries were obtained when the concentration of the standard added was 10 fmol. Adduct levels obtained in the human DNA samples were corrected accordingly.

Human TWBC DNA samples (10 μg) or samples of the MA–3′-dGMP standard (10 fmol) were ^{32}P-postlabelled as previously described (22). The analysis of ^{32}P-labelled MA–DNA adducts was, however, carried out by reversed-phase HPLC with on-line detection of radioactivity as follows. The labelled samples (3′,5′-bisphosphates) were 3′-dephosphorylated with nuclease P1 (0.5 U/μ g DNA, 5 U/μl in 1.5 mM $ZnCl_2$) at 37°C for 5.5 h, after adjustment of the pH of the mixture to 5.0 with 0.5 μl 0.2 M ammonium acetate, pH 5.0. The samples were then diluted to 30 μl with distilled water and analysed on Beckman HPLC System Gold equipment consisting of a programmable solvent module (model 126), a 166 UV detector and a 171 radioisotope detector supplied with a home made 50 μl Teflon tube as flow cell. The samples were injected onto a 150×2.0 mm (5 μm) Phenomenex Kromasil C18 reversed phase column (Torrance, CA). The counting efficiency of the on-line detector was 25%. A gradient between methanol (solvent A) and 0.2 M ammonium formate containing 20 mM phosphoric acid, pH 4.5, (solvent B) was used for elution of the adducts as follows: 0–25 min, 0% A, isocratic; 25–73 min, 0–3% A; 73–76 min, 3–100% A; 76–82 min, 100% A, isocratic. A split flow system was used. Thus the actual flow rate through the analytical column was 0.2 ml/min. The column was equilibrated after every run with solvent B for at least 15 min. The adducts were quantitated by using a calibration curve (peak area versus adduct concentration) obtained through the analysis of known amounts of the standard. Background radioactivity was determined by injecting a sample containing all the ingredients of the hydrolysis and labelling procedures and water instead of DNA. Subtraction of background radioactivity for quantitation of the adducts was done automatically for each sample by the peak integration function of the Beckman HPLC System Gold programme.

The efficiency of phosphorylation of MA–3′-dGMP adducts by T_4 polynucleotide kinase was determined for each experiment using labelled MA–3′-dGMP standard (10 fmol) and analysing the samples by TLC [determined as 3′,5′-bisphosphate according to Vaca *et al.* (22)] and by reversed-phase HPLC (determined as MA–5′-dGMP) as described above.

The limit of detection of the reversed-phase HPLC method with on-line detection of radioactivity was established through a series of experiments where aliquots of unmodified calf thymus DNA samples (10 μg) hydrolysed by MN and SPD and labelled as described above were mixed with 0.1, 0.5, 1.0 and 5.0 fmol labelled MA–3′-dGMP standard and thereafter subjected to 3′-dephosphorylation with nuclease P1 and analysis by reversed-phase HPLC. Only background radioactivity was observed at the retention time corresponding to that of the standard when labelled calf thymus DNA samples alone were analysed. The lowest detection limit so far achieved was 4 adducts/10^8 nucleotides, using 10 μg DNA samples.

Statistical analysis

The statistical comparisons were made using Student's *t*-test for two population means.

Results

Fat intake and fatty acid composition of the diets

The mean daily intake of nutrients during the SO and RO experimental diets, based on double portion analyses, is shown

Table I. Mean daily intake of nutrients according to duplicate portion analysis and calculated contribution from freely selected items

Nutrient	SO diet	RO diet
Total energy (MJ)	10.6	10.5
Protein (% of total energy)	14.5	15.1
Fat (% of total energy)	37.7	37.8
Saturates	12.7	12.4
C12–C16[a]	8.0	8.1
Stearic acid (C18:0)	3.8	3.4
Monounsaturates	10.2	16.2
Oleic acid (C18:1)	9.6	15.0
Erucic acid (C22:1)	0.1	0.2
Polyunsaturates	13.3	7.6
Linoleic acid [C18:2 (*n*-6)]	12.7	5.5
α-Linolenic acid [C18:3 (*n*-3)]	0.4	2.1
Cholesterol (mg/day)[b]	315	360
Carbohydrates (% of total energy)	48.4	47.7
Monosaccharides and disaccharides[c]	20.2	20.1
Polysaccharides	28.2	27.6
Dietary fibre (mg/day)[b,d]	30	29
Alcohol (% of total energy)	1.0	1.1
Vitamin A (retinol, equivalents/day)[c]	590	575
Vitamin E (mg α-tocopherol/day)[c]	27.2	13.7
Selenium (μg/day)[c]	108	118
Vitamin E/PUFA[e]	2.0	1.8
Vitamin E/MUFA[f]	2.7	0.8
PUFA/SFA[g]	1.0	0.6

[a]Saturated fatty acids with chain lengths of 12, 14 or 16 carbon atoms.
[b]For a subject of average energy intake.
[c]Does not include small amounts from freely chosen foods.
[d]Including the natural sugars in fruit, milk, etc.
[e]The ratio between vitamin E (α-tocopherol) and PUFA intake.
[f]The ratio between vitamin E (α-tocopherol) and MUFA intake.
[g]The ratio between PUFA and SFA.

in Table I. The intake of SFA was 12.7 and 12.4% of the total energy intake (energy %) on the SO and RO diets respectively. The intake of MUFA, which was on average 10.2 energy % in the SO diet group, was 59% higher in the RO diet group. On the other hand, PUFA intake was 75% higher in the SO than in the RO diet group.

Both diets were very similar with regard to SFA composition. Major differences were, however, obtained in the concentrations of oleic acid (C18:1), linoleic acid [C18:2 (*n*-6)] and α-linolenic acid [C18:3 (*n*-3)]. Thus the RO diet had a 56% higher content of C18:1 and a 425% higher content of C18:3 (*n*-3) than the SO diet. On the other hand, the content of C18:2 (*n*-6) and the PUFA:SFA ratio were 131 and 67% higher in the SO than in the RO diet group (Table I).

Fatty acid composition of TG of subjects after the SO and RO dietary periods

Several differences with regard to the fatty acid composition of TG, the lipid fraction that best reflected the fatty acid composition of the diets, were seen between the dietary groups (Table II). Thus the individuals given the RO diet had 19 and 38% higher proportions of C16:0 and C18:1 fatty acids, respectively and also a 37% higher plasma concentration of MUFA in their plasma TG than individuals on the SO diet. On the other hand, the subjects that received the SO diet exhibited a 122% higher proportion of C18:2 (*n*-6) in the TG fraction than those in the RO diet group. Similarly, their total plasma PUFA concentration and the ratio PUFA:SFA were 88 and 117% higher, respectively, than in subjects on the RO diet.

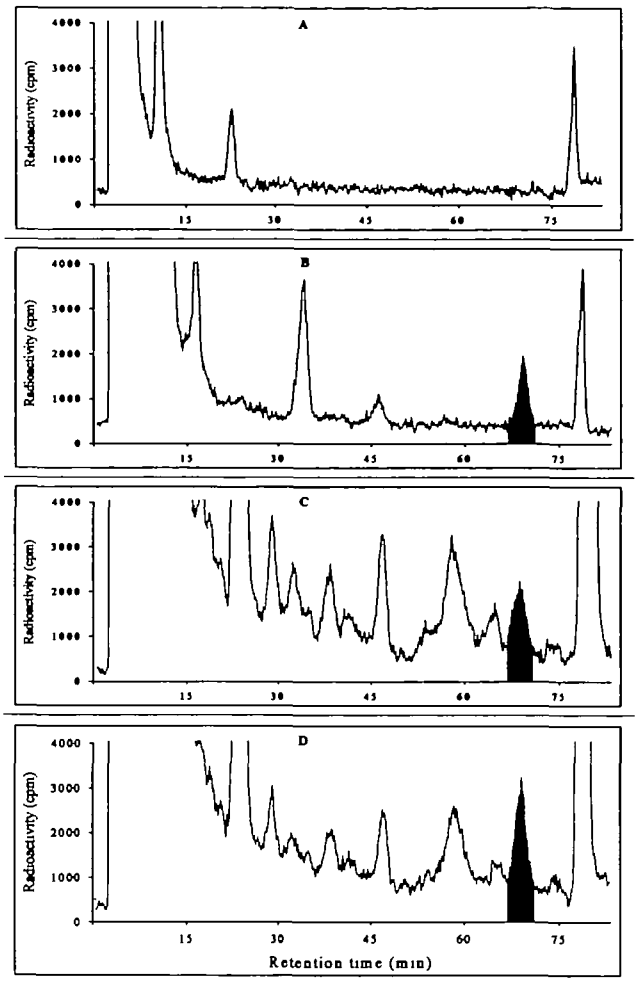

Fig. 1. Reversed-phase HPLC profiles of ^{32}P-postlabelled DNA adducts of MA in human samples. DNA was enzymatically digested with MN, SPD and nuclease P1 and analysed by reversed-phase HPLC with on-line detection of radioactivity as described in Materials and methods. (**A**) Reagent blank (control); (**B**) MA–3′-dGMP adduct standard (10 fmol); (**C**) human TWBC DNA sample (10 μg); (**D**) the same human TWBC DNA sample as in (C) spiked with MA–3′-dGMP adduct standard (10 fmol). The adduct peaks are shaded black.

DNA adducts of MA in TWBC

Representative reversed-phase HPLC chromatographic profiles of the analysis of ^{32}P-postlabelled DNA adducts of MA in human DNA samples are shown in Figure 1. As can be seen, an adduct peak exhibiting an identical retention time to that obtained for the MA–3′-dGMP standard (69 min) was found (cf. Figure 1B and C). In addition, and for the sake of comparison, the chromatographic profile of a reagent blank sample (to show the background radioactivity during the runs) and the profile of the human DNA sample shown in Figure 1C spiked with 10 fmol standard are also shown (cf. Figure 1A and D).

The levels of DNA adducts of MA found in TWBC DNA samples from individuals that received the SO and RO diets are shown in Table III. Although the total number of individuals that participated in the controlled dietary study was 59, the results shown are from the analysis of DNA samples from 23 subjects in the SO diet group and from 26 individuals in the RO diet group. Not enough DNA for analysis of adducts of MA could be isolated from the blood samples of the remaining

Table II. Composition of TG fatty acids of subjects after the SO and RO dietary periods

Fatty acid	SO[a] (% of total fatty acids) (mean ± SD)	RO[b] (% of total fatty acids) (mean ± SD)	Difference
C14:0	1.93±0.36	2.85±0.70	$P < 0.001$
16:0	21.78±1.88	25.98±2.78	$P < 0.001$
C16:1	3.49±1.14	4.66±1.03	$P < 0.001$
C18:0	4.00±0.70	4.05±0.73	Not significant
C18:1[c]	30.52±2.80	42.06±1.90	$P < 0.001$
C18:2 (*n*-6)	34.37±4.36	15.59±2.50	$P < 0.001$
C18:3 (*n*-3)	0.97±0.34	2.44±0.76	$P < 0.001$
C20:3 (*n*-6)	n.d.[d]	n.d.	
C20:4 (*n*-6)	1.52±0.44	0.99±0.17	$P < 0.001$
C20:5 (*n*-3)	0.22±0.09	0.38±0.12	$P < 0.001$
C22:6 (*n*-3)	1.22±0.26	1.06±0.27	$P < 0.05$
SFA	27.71±1.84	32.88±3.57	$P < 0.001$
MUFA	34.01±3.70	46.72±2.21	$P < 0.001$
PUFA	38.29±4.31	20.41±3.01	$P < 0.001$
PUFA (*n*-3)/PUFA (*n*-6)	0.07±0.02	0.23±0.57	Not significant
PUFA/SFA	1.39±0.23	0.64±0.17	$P < 0.001$

[a] $n = 23$ volunteers.
[b] $n = 26$ volunteers.
[c] Sum of 18:1 (*n*-9) and 18:1 (*n*-7).
[d] n.d., not determined.

Table III. Effects of consumption of the SO or RO diets on the level of DNA adducts of MA in human TWBC[a]

Group	Adduct levels						Difference
	SO			RO			
	Mean ± SD	*n*[b]	Range	Mean ± SD	*n*[b]	Range	
All subjects	7.4±8.7	23	0.4–28.7	1.6±1.9	26	0.2–7.9	$P < 0.001$
All males	4.3±5.1	12	0.4–17.2	1.1±1.6	13	0.2–5.4	$P < 0.05$
All females	10.6±10.8	11	1.2–28.7	2.0±2.2	13	0.2–7.9	$P < 0.01$
Males <30 years	4.9±5.8	9	0.4–17.2	1.4±1.9	8	0.2–5.4	Not significant
Males >30 years	2.6±0.9	3	1.6–3.2	0.6±0.9	5	0.2–2.3	$P < 0.05$
Females <30 years	8.6±10.7	6	1.2–28.7	2.4±2.4	10	0.2–7.9	$P < 0.05$
Females >30 years	13.1±11.7	5	3.5–28.0	0.5±0.6	3	0.2–1.2	Not significant

[a] Adduct levels are expressed as number of adducts/10^7 nucleotides. DNA samples (10 μg) were isolated from human TWBC and the adduct levels analysed by ^{32}P-postlabelling and reversed-phase HPLC as described in Materials and methods.
[b] n = number of subjects. Parallel samples (2–6) of DNA of each individual were analysed.

10 subjects in the study. At least two parallel samples of DNA from each individual were analysed.

Following the initial period of 14 days on the baseline diet the group of individuals was considered homogeneous with respect to diet. This was indicated by the similarity between the cholesterol ester, triglyceride and phospholipid fatty acid composition in plasma from subjects who received the SO diet in the subsequent phase of the dietary study and that of the individuals who became the RO diet group (33). Furthermore, the homogeneity of the group was reflected by the levels of DNA adducts of MA. Thus the adduct levels, expressed as mean values ± SD, were 1.1 ± 2.3 ($n = 46$, range 0.2–12.6), 1.2 ± 2.7 ($n = 23$, range 0.2–12.6) and 1.0 ± 2.0 ($n = 23$, range 0.2–6.8)/10^7 nucleotides for the whole group of individuals in the study, the group of individuals that received the SO diet in the next phase of the study and the group of subjects that became the RO diet group, respectively. No statistical differences between these adduct levels were obtained.

As can be seen in Table III, a large inter-individual variation in adduct levels was found (see the Range for each group). The highest adduct levels were found in TWBC DNA from female individuals in both dietary groups. The adduct level found in subjects in the SO diet group was 7.4 ± 8.7 adducts/10^7 nucleotides (mean ± SD, $n = 23$). This adduct level was 3.6-fold higher than that for subjects on the RO diet, when considering all individuals (both sexes and all ages).

When only male or only female subjects in both dietary groups were considered 2.9- and 4.3-fold higher adduct levels were found for the SO diet group. In addition, when different age intervals in the SO diet group were compared adduct levels in male subjects >30 years and in female subjects <30 years were 3.3- and 2.6-fold higher than in the corresponding age intervals in the RO diet group. The differences between adduct levels in the age intervals males <30 years and females >30 years were not statistically different when the two dietary groups were compared. Nor were statistically significant differences in adduct levels obtained when comparisons between both sexes and the various age intervals were made within each dietary group (Table III).

Discussion

The present study aimed at investigating in humans our earlier proposed mechanism (9,10) to explain the involvement of

dietary fat in carcinogenesis, i.e. the interaction of DNA with reactive compounds endogenously formed during the peroxidation of fatty acids of biological membranes.

It is known that the fatty acid composition of lipids in animal tissues can be modulated by the dietary fatty acid composition (34,35). In the present study the difference between the diets with regard to total PUFA content was accurately reflected in the difference in PUFA content of the plasma TG (cf. Tables I and II). This similarity of diet and plasma TG fatty acid composition has also been observed by others (36,37). A more detailed account on the effects of fatty acid composition of the SO and RO diets on plasma fatty acid composition of the individuals who participated in the dietary trial has been presented elsewhere (33).

High concentrations of substrate (PUFA), in particular *n*-6 and *n*-3 PUFA, result in endogenous formation of high concentrations of peroxidation products such as MA (13–15). MA arises from fragmentation of cyclic peroxides and endoperoxides formed during autoxidation of PUFA (38).

In a controlled dietary study (39) a diet rich in PUFA induced a significantly higher tendency for low density lipoprotein (LDL) peroxidation than a diet rich in MUFA. When considering the subjects who participated in our dietary trial a higher, although not statistically significant, plasma level of MA (measured as thiobarbituric acid-reactive substances) was also found in the subjects in the SO diet group compared with those in the RO diet group (16). The rate and extent of oxidation of LDL were not measured in the present work. However, in a dietary intervention study similar to that reported here an increased susceptibility to oxidation was found in LDL isolated from subjects given the SO diet in our study, when compared with LDL from individuals in the RO diet group (16). A detailed account of the effects of the SO and RO diets on lipoprotein levels has been given elsewhere (28).

A pyrimidopurinone produced from deoxyguanosine through reaction with one molecule of MA is the major DNA adduct of MA at physiological pH (21,22,40). This adduct has been shown to be quite persistent in mouse liver (half-life 12.5 days) (23) and to induce base pair substitutions (G→T transversions and C→T and A→G transitions) and frameshift mutations using a recombinant M13 phage (41).

The ^{32}P-postlabelled DNA adducts of MA were analysed by reversed-phase HPLC with on-line detection of radioactivity and not by TLC as in our previous studies (22,23,26). Although the HPLC method is time consuming and less sensitive than TLC, it provides a better separation of the adducts from other materials also present in DNA samples and simplifies quantitation. When using this procedure the enrichment of adducts by reversed-phase HPLC prior to phosphorylation which was carried out in our previous studies (22,23,26) was omitted.

The results presented in Table III show that higher and statistically different levels of DNA adducts of MA were found in TWBC DNA from individuals on the SO diet than in those from subjects in the RO diet group. These results are consistent with the concentrations of substrates for lipid peroxidation (PUFA) detected in the plasma TG of the individuals in each group (cf. Tables II and III) and with the relative ease of oxidation exhibited by LDL from individuals in the two dietary groups as discussed above. A higher tendency for oxidation would result in higher concentrations of MA, thus leading eventually to higher adduct levels in individuals in the SO diet group.

A high inter-individual variation with regard to adduct levels was observed (see the Range for each group in Table III). Factors such as dietary antioxidants, the level of physical activity and dietary iron are known to influence the extent of intracellular oxidative stress. Plasma α-tocopherol levels were similar in the individuals from both dietary groups (6.4 and 6.9 mg/ml after the RO and SO diets, respectively) (16). However, neither iron status nor physical activity were controlled in the present study. The effect of these factors on DNA adduct levels of MA deserves further consideration. Furthermore, genetically determined inter-individual differences in the rate and extent of intracellular oxidation processes and/or differences in repair rates of DNA adducts formed would have eventually determined the individual adduct levels (42,43). We have recently reported on similar inter-individual variations in adduct levels of MA in human TWBC and breast tissue DNA (26).

An accumulation of lipid peroxidation products accompanies ageing (44,45). Therefore it seemed relevant to make comparisons with regard to adduct levels of MA between individuals in the various age intervals. When different age intervals in the SO diet group were considered female subjects exhibited higher adduct levels than males. However, due to the large inter-individual variation in adduct levels and the limited number of subjects who participated in the study, the differences between males and females in the various age intervals on each diet were not statistically significant.

In conclusion, this is the first report showing that endogenous levels of DNA adducts of MA in humans can be influenced by dietary fatty acid composition. A high PUFA content in dietary fats results in a high concentration of plasma PUFA, which after peroxidation leads to high concentrations of DNA adducts of MA in TWBC. Considering the persistence of these adducts *in vivo* (23) and their mutagenic properties (41), they could be considered as fully capable of initiating the tumourigenic process. The above results thus indicate that direct interaction of DNA with oxidative degradation products of fat could be one of the plausible mechanisms explaining the carcinogenic effects of dietary fats. Additional studies involving a larger number of individuals of both sexes and different ages and the measurement of DNA adducts and the mutation spectrum in, for example, lymphocytes from individuals participating in controlled dietary trials are necessary to further elucidate the role of dietary fat, in particular that rich in PUFA, in carcinogenesis.

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