High Dietary ω-6 Polyunsaturated Fatty Acids Drastically Increase the Formation of Etheno-DNA Base Adducts in White Blood Cells of Female Subjects

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Abstract

Lipid peroxidation generates reactive aldehydes such as trans-4-hydroxy-2-nonenal and malonaldehyde, which form promutagenic exocyclic DNA adducts in human cells and may contribute to diet-related cancers. Using ultrasensitive detection methods, analysis of WBC DNA from volunteers in a dietary study revealed that high intake of ω-6 polyunsaturated fatty acids increased malonaldehyde-derived adducts in male and female subjects. In contrast, etheno adducts (1,N²-ethenedeoxyadenosine; 3,N⁴-ethenedeoxycytidine) were not elevated in males but were, on average, 40 times higher in females, displaying a huge intersubject variation in lipid peroxidation-derived DNA damage. Exocyclic DNA adducts are promising biomarkers for examining the hypothesis of possible links between increased intake of dietary ω-6 polyunsaturated fatty acids, DNA damage, and elevated cancer risk for breast, colon, and prostate.

Introduction

At least one-third of all human cancers may be associated with diet (1); dietary fat is thought to be one of the main risk factors because positive correlations between dietary fat intake and increased risk of breast, colon, and prostate cancer have been reported (2–4). Although the role of individual fatty acids in human cancer risk is still poorly understood, epidemiological data have linked a high dietary ω-6 PUFA intake (linoleic acid C18:2), especially in connection with a low ω-3 PUFA (docosahexaenoic acid C22:6) consumption, to an increased risk of breast and colon cancer (5, 6). This can be explained, on one hand, by the findings that ω-6 PUFAs enhance mammary tumorigenesis and tumor cell proliferation in experimentally induced mammary tumors, whereas ω-3 PUFAs can act as growth inhibitors of initiated cancer cells (5, 6). On the other hand, oxidative stress and enhanced peroxidation of PUFAs in cell membranes, often involving hydroxyl radicals, are widely hypothesized to stimulate the development of human malignant diseases (7), raising the question of whether the carcinogenic process could be initiated and/or accelerated through ω-6 PUFA lipid peroxidation-derived DNA damage (8, 9). Recent studies reported the presence of such DNA-damaging adducts in human tissues (10–13). As cell replication before repair of the adducts leads to mutations, DNA adduction is commonly regarded as the initiating step in carcinogenesis.

HNE, one of the major products of lipid peroxidation (14) formed after oxidation of linoleic acid and arachidonic acid (15), is readily oxidized by fatty acid hydroperoxides (16) to form 2,3-epoxy-4-hydroxynonanal, a bifunctional alkylating agent. It reacts with DNA bases to yield etheno adducts, such as 1,N²-ethenedeoxynanosine (17). Likewise, oxidation of rat liver microsomal membranes or of arachidonic acid with cumene hydroperoxide or Fe²⁺ yielded edA and edC in the presence of the respective deoxynucleotides (Ref. 18; see scheme for formation of etheno adducts from PUFA in Fig. 1). The relevance of the three etheno adducts in carcinogenesis is supported by the following: they are promutagenic DNA lesions (19); there is strong evidence that they initiate carcinogenesis by vinyl chloride and urethane (19); edA and edC are elevated in the liver of Long-Evans-Cinnamon rats (animal model for Wilson’s disease), a rat strain that develops spontaneous hepatitis and later hepatocellular carcinoma due to aberrant copper accumulation (20); and much higher hepatic levels of etheno adducts were detected in patients with metal storage diseases at elevated risk for liver cancer, e.g., Wilson’s disease and primary hemochromatosis, as compared to liver DNA from healthy individuals (21). To investigate the impact of a diet high in ω-6 PUFAs or MUFAs on DNA damage, samples from a carefully controlled dietary study (22) were analyzed for promutagenic exocyclic DNA adducts, edA, and edC.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: PUFA, polyunsaturated fatty acid; HNE, trans-4-hydroxy-2-nonenal; MUFAs, monounsaturated fatty acid; edA, 1,N²-ethenedeoxynanosine; edC, 3,N⁴-ethenedeoxycytidine; FA, fatty acid; MA-dG, malonaldehyde-deoxyguanosine adduct; TBARS, thiobarbituric acid reactive substances; TWBC, total WBC; HPLC, high-performance liquid chromatography.
Fatty Acids Enhance Etheno-DNA Adducts in total FAs] for 25 days. The mean daily intakes (% of energy) of polyunsaturates, and 13% (w-3) polyunsaturates of the (Brassica rapa) ssp. rapa (w-3) saturates, (65% w-6) polyunsaturates, and traces of PUFAs [FA content of the oil: 12% saturates, 23% monounsaturates, 65% ω-6 polyunsaturates, and traces of ω-3 polyunsaturates of the total FAs] for 25 days. The mean daily intakes (% of energy) of ω-6 PUFA and of ω-3 PUFA were 12.7 and 0.4 (PUFA group) and 5.5 and 2.1 (MUFA group), respectively. The analysis of plasma phospholipids showed a statistically significant 2.2-fold increase in the ω-6 PUFA level in the PUFA diet group, compared to the MUFA diet group (23). We have investigated etheno adduct levels in a subset of volunteers from the MUFA diet group (four males and seven females) and PUFA diet group (three males and six females) who were previously analyzed for the MA-dG adduct (Ref. 24; see Table 1 for age and gender distribution of volunteers).

**Adduct Measurements.** DNA was isolated from TWBCs by phenol-chloroform extraction method (13) and coded. A highly sensitive method (detection limit, ~4 etheno adducts/10¹⁰ parent nucleotides) combining immunoaffinity purification with ³²P-postlabeling was used to quantitate edA and edC in DNA of TWBCs in the form of their 5′-monophosphates (11). The method has been validated against RIA (25), and the coefficient of variation of the method was found to be 20% (26). In brief, ~20 µg of DNA were hydrolyzed to 3′-deoxyxynucleoside monophosphates using micrococcal endonuclease and spleen phosphodiesterase. Normal nucleotides were quantitated by HPLC, and edA and edC were purified on immunoaffinity columns prepared from monoclonal antibodies (EM-A-1 for edA and EM-C-1 for edC), labeled (equivalent to ~10 µg of DNA) with [³²P]ATP and T4-polyadenylate kinase to 5′-nucleotide monophosphates, and resolved on polyethyleneimine cellulose plates using two-directional chromatography (11, 20). The spots corresponding to edA, edC, and deoxyuridine 5′-monophosphates were cut from the TLC plates, and the radioactivity was determined by liquid scintillation counting. The absolute levels of adducts were determined by comparing the radioactivity obtained with known amounts of standard; the number of adducts per parent nucleotides were obtained from the ratio of the quantity of etheno adducts measured on TLC to the quantity of parent nucleotides in the sample derived from HPLC analysis (11). The etheno adducts in two samples were further analyzed by reverse-phase HPLC, along with authentic standards of edA and edC after labeling with ³²P using a radioactivity detector and gradient elution with triethylamine phosphate and methanol.

**Results**

The isolation of the adducts using antibody immunoaffinity columns (highly specific for the adducts analyzed; Ref. 27) and identical chromatographic properties (Fig. 2, A–C) compared to authentic standards revealed the presence of edA and edC in DNA of all human TWBCs for the first time. Two samples were analyzed by reverse-phase HPLC, which further confirmed the identity and the high levels of edA and edC detected (Fig. 2D). Mean edA and edC levels in males were similar between the two diet groups; however, in female subjects on PUFA diet, the respective means were ~40 times higher than those on the MUFA diet, with ranges of 0.13–90.15 edA/10⁷ dA and 0.06–71.62 edC/10⁷ dC (Table 1 and Fig. 3). Due to the huge interindividual variation and small number of subjects in the study groups, resulting in a large SD, this sex-dependent difference did not reach statistical significance. However, if one outlier with low adduct levels was omitted, the mean edA and edC levels in five of six women on PUFA were significantly

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*Fig. 1.* Scheme of the formation of etheno adducts from PUFAs, as exemplified for HNE (R = C₅H₁₁). FAOOH, fatty acid hydroperoxide; dR, deoxyribosyl. The same adducts could also be formed by other enals produced by lipid peroxidation (11).
Therefore, our results clearly indicate that the DNA adduction was due to consumption of a high ω-6 PUFA diet, which resulted in elevated levels of polyunsaturated membrane lipids, implying increased lipid peroxidation (23, 28), although commonly used markers for lipid peroxidation status in serum, such as MA, TBARS, and conjugated dienes, failed to reveal differences between the MUFA and PUFA diet groups (23). However, significantly higher levels of MA-dG adducts were detected previously in the TWBCs of subjects on PUFA diet versus MUFA diet, and the highest mean levels of MA-dG adducts were formed in the DNA of TWBCs of female volunteers in the PUFA dietary group (24).

The marked diet-related difference of etheno adducts in DNA of TWBC in some females on PUFA diet may be due to increased dietary ω-6 PUFA intake and hormonal metabolism acting synergistically, which is supported by experimental data: treatment of male Syrian hamsters with the steroidal hormones estradiol and diethylstilbestrol increased both lipid hydroperoxides and MA-DNA adducts in liver and kidney (29). Studies in vitro suggested generation of hydroxyl radicals from estrogens by redox cycling, involving the 4-hydroxylated metabolites and their quinones (30). High intake of ω-6 PUFA, linoleic acid, and arachidonic acid, has been shown to inhibit the detoxification of estrogens by 2-hydroxylation (31) and to elevate 16-α-hydroxylation, leading to metabolites with the potential of redox cycling, thereby possibly enhancing the generation of hydroxyl radicals. Whether these fatty acids also enhance the formation of 4-hydroxylated estrogens is not known.

There was a ~1000-fold variation in etheno adduct levels among female individuals on ω-6 PUFA diet; only one of the female subjects had similarly low levels as males or females on MUFA diet. This interindividual variation may be due to the following host factors: different detoxification rate of HNE, suggested generation of hydroxyl radicals from estrogens and MA-DNA adducts in liver and kidney (29). Studies in vitro and in vivo suggested generation of hydroxyl radicals from estrogens by redox cycling, involving the 4-hydroxylated metabolites and their quinones (30). High intake of ω-6 PUFA, linoleic acid, and arachidonic acid, have been shown to inhibit the detoxification of estrogens by 2-hydroxylation (31) and to elevate 16-α-hydroxylation, leading to metabolites with the potential of redox cycling, thereby possibly enhancing the generation of hydroxyl radicals. Whether these fatty acids also enhance the formation of 4-hydroxylated estrogens is not known.
6(X)

w-6 Fatty Acids Enhance Etheno-DNA Adducts in Females

Mean ± SD

<table>
<thead>
<tr>
<th></th>
<th>edA</th>
<th>edC</th>
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<tbody>
<tr>
<td>MUFA(M)</td>
<td>0.7±0.6</td>
<td>0.4±0.5</td>
</tr>
<tr>
<td>PUFA(M)</td>
<td>0.3±0.1</td>
<td>0.2±0.1</td>
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<tr>
<td>MUFA(F)</td>
<td>0.6±0.9</td>
<td>0.6±0.3</td>
</tr>
<tr>
<td>PUFA(F)</td>
<td>26.5±36.6</td>
<td>22.5±29.0</td>
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</tbody>
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Fig. 3. Columns, individual and mean levels of etheno adducts/10^7 parent nucleotides in human TWBCs (for sequence see Table 1). Individual volunteers on diets: MUFA, rapeseed oil; PUFA, sunflower oil, M, male; F, female.

and edC from DNA (33); differential induction of specific cytochrome P-450s that catalyze 4-hydroxylation or 16 α-hydroxylation of estradiol; indeed, a cytochrome P-450 isozone related to CYP1B1 that increases the 4-hydroxylation of estradiol 5-fold has been identified in human uterine myometrium (34).

On the basis of our results, two important questions can be addressed: Does a high dietary ω-6 PUFA intake also result in increased levels of edA and edC in tissues, such as breast, colon, and prostate, in which fat intake or hormones are suspected carcinogenic risk factors? Is there a synergism between hormonal metabolism and exocyclic DNA adduct formation in humans? Should such adducts be formed via hydroxyl radicals by the ω-6 PUFA pathway in these target organs, they may offer new etiological and mechanistic clues for tumor induction related to dietary fatty acids. Etheno adducts have been proven to be highly miscoding lesions in mammalian cells (35) and are thought to initiate the carcinogenic process through specific point mutations by the known or suspected human carcinogens vinyl chloride or urethane, respectively (19).

Fig. 2. Autoradiograms of edA, edC, and deoxyuridine (dU; internal standard) as 5′-monophosphates. A, standard; B, DNA sample from volunteer on rapeseed oil diet; C, DNA sample from volunteer on sunflower oil diet. D1, acetic acid (1 M, pH 3.5); D2, saturated ammonium sulfate at −24°C (pH 3.5); X, Y, and Z, unknown adducts retained by immunoaffinity columns. D, comparison of reverse-phase HPLC profile between standard 5′-monophosphates of edA and edC (UV) and 32P-postlabeled edA and edC (radioactive) of one DNA sample from the PUFA diet group. Undesignated spots or peaks are unremoved normal nucleotides from samples and impurities from commercial [32P]ATP.

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References


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