Malondialdehyde Excretion by Subjects Consuming Cod Liver Oil vs a Concentrate of n-3 Fatty Acids

L.A. Piché, H.H. Draper* and P.D. Cole
Department of Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Urinary malondialdehyde (MDA), an indicator of lipid peroxidation in the diet and in the tissues, was determined in human adults consuming a supplement of n-3 fatty acids derived from a pharmaceutical grade of cod liver oil (CLO) without added antioxidants vs a concentrate of n-3 acids containing dodecyl gallate and vitamin E. MDA excretion increased immediately in the subjects consuming CLO but remained unchanged in those ingesting the concentrate for 50 days. The increase in the subjects taking CLO was attributable to MDA in the oil. The results indicate that consuming unstabilized fish oils as a source of n-3 fatty acids may entail exposure to potentially toxic products of lipid peroxidation.
Lipids 23, 370–371 (1988).

Fish oils and concentrates of n-3 fatty acids from fish oils have been widely promoted for the prevention of heart attacks. In as much as feeding fish oils is a classical method of inducing vitamin E deficiency in experimental animals, the question arises whether long-term ingestion of such oils may jeopardize the vitamin E status of humans. Long-term feeding of cod liver oil (CLO) to rats results in a significantly greater excretion of malondialdehyde (MDA) in the urine after a subsequent fast than is seen in rats fed corn oil, indicating an increase in peroxidation of fatty acids in vivo (1). This increase is inhibited partially but not entirely by feeding a high level of dietary antioxidants.

The possible presence in fish oils of peroxides and their degradation products including malondialdehyde, a mutagen (2) and reported carcinogen (3), is a further consideration in their long-term ingestion. Feeding a diet containing CLO to rats results in an immediate, marked increase in urinary MDA, reflecting a high level of this compound in the diet (4). MDA excretion in humans has been reported to increase following a meal of rancid foods (5).

In this study, the effect on MDA excretion by human adults of ingesting a commercial CLO product without added antioxidants vs a commercial fish oil concentrate containing a mixture of antioxidants was investigated.

METHODS

Experiment 1. Six adults consumed 30 ml of CLO (10 ml with each meal) for 14 days in conjunction with a free choice diet. The oil was a pharmaceutical product (Life Brand, Shoppers Drug Mart, Toronto, Ontario), labeled as a "natural source" containing no preservatives. On the basis of its reported content of eicosapentaenoic and docosahexaenoic acids (6% each) (6), this product provided about 1.5 g of each acid per day. After being opened, the bottles were kept at 4 C. Morning urine samples, one collected on the day before CLO ingestion began and the other on the last day of ingestion, were analyzed for MDA by the high performance liquid chromatography (HPLC) method of Draper et al. (1).

Experiment 2. Seven adults consumed a low-MDA diet (i.e. one devoid of meats and high-fat foods) and abstained from alcoholic beverages for three days, during which time consecutive morning urine samples were collected. For a further three days they consumed 30 ml of CLO, as in Experiment 1, in conjunction with a low-MDA diet, and three additional morning urine samples were collected for MDA analysis.

Experiment 3. Seven adults consumed 10 capsules per day of a concentrate of n-3 acids from fish oil (MaxEPA, Seven Seas Health Care Ltd., Kingston-upon-Hull, U.K.) for 50 days. The gelatin-coated capsules contained 100 ppm dodecyl gallate and provided 1.9 g eicosapentaenoic acid, 1.2 g docosahexaenoic acid and 10 IU of dl-α-tocopheryl acetate per day. Two consecutive 24-hr urine samples were obtained for MDA analysis immediately before supplementation began and on days 49 and 50. One day before and during each urine collection period, the subjects consumed a low-MDA diet.

RESULTS AND DISCUSSION

In Experiment 1, ingestion of CLO was associated with an increase in MDA excretion in all six subjects (Table 1). The mean increase of 37.5%, from 24.5 ± 3.5 μg to 34.7 ± 2.5 μg MDA (mean ± SEM), was significant ($P < 0.01$) using a two-tailed paired t-test.

In Experiment 2, CLO ingestion again was associated with an increase in MDA excretion in all subjects (Table 2). The mean increase of 54.3%, from 31.7 μg to 49.1 μg MDA/sample was highly significant ($P < 0.001$).

MDA excretion was unaffected by MaxEPA ingestion. Urinary MDA was 148 ± 15 μg/24 hr before

TABLE 1

Effect of Consuming a Pharmaceutical Grade of Cod Liver Oil[a] on MDA Excretion in the Urine (Experiment 1)

	MDA (μg)[b]	
Subject	−CLO	+CLO
1	29	36
2	12	31
3	35	38
4	31	44
5	18	26
6	22	33
$\bar{x}$	24.5	34.7[c]
SEM	3.5	2.5

[a]30 ml per day for 14 days.
[b]Morning void.
[c]$P < 0.01$.

*To whom correspondence should be addressed.

Abbreviations: CLO, cod liver oil; HPLC, high performance liquid chromatography; MDA, malondialdehyde.

TABLE 2

Effect of Consuming Cod Liver Oil[a] on Urinary MDA Excretion (Experiment 2)

Subject	MDA (μg)[b]	
	−CLO	+CLO
1	59	82
2	21	41
3	15	39
4	43	74
5	32	38
6	35	47
7	17	23
$\bar{x}$	31.7	49.1[c]
SEM	5.9	8.0

[a]30 ml per day for three days.
[b]Mean of three morning urine samples taken on consecutive days.
[c]$P < 0.001$.

TABLE 3

MDA Excretion by Subjects Consuming MaxEPA (μg/24 hr)

Subject	Diet	
	Free choice[a]	+MaxEPA[b]
1	130	160
2	216	201
3	153	118
4	154	113
5	123	109
6	113	133
$\bar{x}$	148	139[c]
SEM	15	15

[a]Mean of two 24-hr samples taken on consecutive days.
[b]After 50 days of supplementation.
[c]$P > 0.05$.

MaxEPA consumption and 139 ± 15 μg/24 hr after its consumption for 49–50 days (Table 3). This finding indicates that the fish oil concentrate was not a significant source of MDA in the diet or of MDA generated as a result of enrichment of the tissues with fatty acids of the n-3 series. It further indicates that the increase in MDA excretion seen during consumption of CLO was due to MDA present in the oil. Repeated extraction of a solution of CLO in hexane with 10% $NaHCO_3$ solution yielded sustained amounts of MDA, showing that the oil was undergoing continuous oxidative decomposition and further indicating that it was the source of the increase in MDA in the urine of the subjects consuming this product.

The proportion of ingested MDA excreted in the urine by humans is unknown but, judging from the results of studies on animals, it probably is small. Following stomach intubation with ^{14}C-MDA, rats excreted about 10% more ^{14}C in the urine than after intubation with ^{14}C-acetate (7). Sixty to 70% of the radioactivity was recovered in expired $^{14}CO_2$ within 12 hr.

Protracted consumption of n-3 fatty acids must be presumed to increase the vitamin E requirement. There is a need not only for an antioxidant capable of stabilizing these acids during storage but for a biologically active antioxidant capable of preventing their oxidation in vivo. Whether the normal diet provides enough vitamin E to meet the additional need for a biologically active antioxidant arising from consumption of n-3 fatty acids in unsupplemented fish oils is problematical. The results of this study indicate that the amounts of antioxidants present in MaxEPA are sufficient to suppress the oxidation of n-3 fatty acids in this product both in the capsules and in the tissues. They further indicate that ingestion of unstabilized fish oils entails a risk of exposure to potentially toxic products of n-3 fatty acid peroxidation. This is a particular consideration in jurisdictions, such as Canada, where the use of concentrates of n-3 fatty acids currently is prohibited.

ACKNOWLEDGMENTS

This work was supported by the Natural Sciences and Engineering Council of Canada and the National Cancer Institute of Canada.

REFERENCES

1. Draper, H.H., Polensek, L., Hadley, M., and McGirr, L.G. (1984) *Lipids 19,* 836–843.
2. Basu, A.K., and Marnett, L.J. (1983) *Carcinogenesis (Lond.) 4,* 331–333.
3. Shamberger, R.J., Andreone, T.L., and Willis, C.E. (1974) *J. Natl. Cancer Inst. 53,* 1771–1773.
4. Piché, L.A. (1987) *Factors Affecting Malondialdehyde Levels in Rat and Human Urine and the Identification of N-ε-*(2-Propenal) Lysine as a Digestion Product of Food Proteins Exposed to Peroxidized Lipids, pp. 38–44, Ph.D. Thesis, University of Guelph, Ontario, Canada.
5. Jacobson, E.A., Newmark, H.L., Bird, R.P., and Bruce, W.R. (1983) *Nutr. Reports Int. 28,* 509–517.
6. Ahmed, A.A., and Holub, B.J. (1984) *Lipids 19,* 617–624.
7. Siu, G.M., and Draper, H.H. (1982) *Lipids 17,* 349–355.

[Received August 10, 1987; Revision accepted November 2, 1987]