Salmon-rich diet inhibits arachidonate cyclooxygenation in healthy men

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Dietary long-chain omega-3 polyunsaturated fatty acids (PUFA) influence cardiovascular and immunological parameters. Eicosanoid-mediated processes are believed to be key metabolic components of the underlying mechanisms. With a view toward developing a biochemical basis to rationalize the phenomena observed, we compared the effect of a salmon (S, ω6/ω3 PUFA ratio = 3.6) and of a reference (R, ω6/ω3 = 19.5) diet on the biosynthesis of E prostaglandins in 10 male volunteers by measuring the major urinary metabolite, PGE-M, in 24-hr urine by gas chromatography-mass spectrometry. Energy contributions (en%) from proteins, carbohydrates, and fat were virtually identical in both diets: 19, 56, and 25%, respectively. The subjects were confined in a nutrition suite at the Western Human Nutrition Research Center for 100 days. During a stabilization period of 20 days, they were placed on the R diet. Then half were fed the S diet for 40 days while the others remained on the reference diet. The two groups switched diets for the last 40-day period. The menu cycle was 5 days, and all diets were calculated to provide adequate amounts of essential nutrients. Diet S was associated with an average 24% reduction in PGE-M daily output, in comparison to diet R (P = 0.001). This reduction in the synthetic rate of E prostaglandins was attained by incorporating less than 500 g/day of salmon in the diet. Omega-6 PUFA were maintained constant in both diets. An alteration of PGE synthesis of this size is likely to have clinically significant repercussions on cardiovascular and immune functions. Although these effects appear to occur in a predominantly favorable direction, other physiological systems (e.g., the renal system) might be affected in ways yet to be determined.

Keywords: omega-3 polyunsaturates; prostaglandins; in vivo synthesis; PGE-metabolite; urinary excretion

Introduction

The current epidemic of coronary artery disease and cancer in Western industrialized societies might be supportive of the hypothesis, put forward by Cameron and coworkers,[1] that the current population has an omega-3 fatty acid deficiency. This intriguing hypothesis is perhaps the most eloquent statement to date of a newly emerging concept of fatty acid essentiality. The old concept based on growth promotion, fertility, curing of dermal symptoms, and hypocholesterolemic properties, has been progressively deemphasized during the last 15–20 years. Although one might not endorse Cameron's view, the protective influence of dietary omega-3 polyunsaturated fatty acids (PUFA) against cardiovascular disorders now has a firm experimental foundation in studies with humans and animal models.[2-6] Populations whose diet has a consistently low w6/w3 fatty acid ratio display a quasi-immunity toward degenerative diseases affecting the vascular system.[3,6-8] Concurrently, a possible role of fatty acids of the α-linolenic family in inhibiting certain forms of tumors has been suggested.[9-12] Dietary omega-6 PUFA have been associated with tumor promotion while their omega-3 counterparts have been associated with tumor inhibition.[1,9,10,13] Eicosanoid-mediated processes are suspected of being key metabolic constituents by which omega-3 PUFA exert their influence in the prevention of both cancer and cardiovascular disease.[14]

Ingestion of omega-3 polyunsaturated fatty acids tends to depress prostanoid production in animals and humans. Much work has been done to evaluate the

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effect on thromboxane A_2 and prostacyclin biosynthesis,[15-18] undoubtedly because these eicosanoids have an important role in vascular biology. Prostaglandin E_2 (PGE_2), the second most bioactive eicosanoid, has been implicated in mechanisms of blood pressure regulation,[19,20] tumorigenesis,[10,21] and immune response modulation.[22-24] However, relatively little is known about the capacity of dietary omega-3 PUFA to modulate PGE_2 synthesis in humans in vivo, or the physiological consequences of such modulation. The present study was designed to determine how dietary omega-3 fatty acids influence PGE biosynthesis and to add to our knowledge about diet and PGE metabolism.

We measured the effect of a change of the w6/w3 fatty acid ratio from 19.5 to 3.6 on whole-body turnover of PGE by quantifying the excretion rate (μg/24 hr) of 11α-hydroxy-9,15-dioxo-2,3,4,5,20-pentanor-19-carboxy-prostanoic acid (PGE-M). This catabolite of E-series prostaglandins is regarded as an index of their endogenous synthesis.[25,26] The low w6/w3 ratio was attained by including in the diet about 450 g of salmon, which provided about 90% of the omega-3 PUFA.

Materials and methods

Subjects

Initially 12 male subjects, 31–65 years of age (mean 51.6 ± 12.1 SD) residing in the San Francisco area and other localities along the western coast of the United States, were selected from a cohort of volunteers who responded to advertisements. The candidates considered were nonsmoking, and with no known history of alcohol abuse; they had maintained a relatively stable body weight for the last 12 months and had a history of only moderate physical activity. Those who met these preliminary selection criteria were given a physical examination and were subjected to hematological and chemical screening. Those with test results within normal ranges were entered into the study. They were required not to ingest any drug formulation containing aspirin or other anti-inflammatory agents and to report any antibiotic and other medications prescribed by a physician during the study for evaluation for possible effects on variables under investigation. They were housed at the Human Nutrition Suite, Western Human Nutrition Research Center, ARS-USDA, Presidio of San Francisco, CA, USA, for the duration of the study (100 days). A staff physician was on call 24 hours a day. The subjects were fully informed of the purpose of the study, procedures to be followed, samples to be collected, risks, benefits, rights, and payment associated with the study. All the procedures were approved by the Human Studies Committee of the U.S. Department of Agriculture and the Letterman Army Medical Center.

Controlled Diets

Two diets, designated as R (reference) and S (salmon), consisted of natural foods that were either fresh, canned, or frozen. All nutrients for which food data are available were provided by the diets in amounts to meet the Recommended Daily Allowances (RDA).[27] No dietary supplements were given except α-tocopherol to bring the diets to 200% of the RDA for this vitamin. The S diet was identical to the reference diet except that salmon, about 450 g per day, was substituted isocalorically for servings of chicken or beef in the R diet. The salmon provided more than 90% of the omega-3 fatty acids in the diet and essentially all of the 20 and 22 carbon omega-3 polyunsaturated fatty acids. The omega-6 content of both diets was maintained constant. (More details of the diets can be found in reference 28. Also, a complete description of the diets, listing all the major and minor nutrients and sample menus, are available from one of the authors [Gary J. Nelson] upon request.) The menu cycle was five days long, and no food or food supplement other than what was provided by the study was permitted.

While it is not possible to characterize a natural food diet to the same extent as semi-synthetic and formula diets, great care was taken to ensure that all diets contained the same nutrients in identical amounts. *U.S. Department of Agriculture Handbook 8* (The Nutrient and Chemical Composition of Foods, U.S. Department of Agriculture, Beltsville, MD, USA) and in-house nutrient data banks were utilized to determine the nutrient composition of the foods used in both diets. Alcoholic beverages were not allowed. Consumption of coffee, tea, and water was unrestricted, but all fluid intakes were recorded. Intake of minerals and vitamins was kept constant to the extent allowed by use of commonly available foods. The fatty acid composition of salmon was determined by gas-liquid chromatography prior to construction of the diets. Thus, the actual fatty acid composition of the salmon was used to calculate the theoretical fatty acid composition of the S diet. The fatty acid composition of the R diet was calculated from food composition tables without prior analysis of foods. Proximate analyses were done on five individual diet composite samples taken on each menu once during the study for both the R and the S diets. The results for the five composite samples were averaged to find the actual composition of the diets. Energy contribution (en%) from proteins, carbohydrates, and lipids was virtually identical in both diets: 19%, 56%, and 25%, respectively. The nutrient composition of the diets was calculated from a computerized nutrient data bank and adjusted to provide at least the RDA for known essential nutrients.

Table 1 shows the target fatty acid distribution in the two diets, and *Table 2* the actual fatty acid compositions as measured from the food composite samples by gas-liquid chromatography. The amount of saturated and omega-6 fatty acids was kept constant in both diets. The R diet had less than 1% omega-3 fatty acids as α-linolenic acid and about 50% monounsaturates (oleic acid). The S diet had about 7.5% long-chain omega-3 polyunsaturated fatty acids and concomitant reduction in the level of monounsaturates.

As no two subjects ate the same amount of salmon

each day, the quantity of omega-3 fatty acids is best expressed in terms of en% per day rather than grams of omega-3 fatty acids consumed per day. Individuals who take fish oil capsules usually consume a set number of capsules per day; consequently, the dosage varies from individual to individual depending on their weight. In this study, each volunteer on the salmon diet received 2.0 en% from omega-3 fatty acids. The energy contribution of the 20 and 22 carbon omega-3 polyunsaturated fatty acids was distributed as follows: 20:5ω3, 0.63 en%; 22:5ω3, 0.21 en%; and 22:6ω3, 0.88 en%. For example, an individual in this study receiving 3000 kcal/day would have consumed about 2.1 g of 20:5ω3, 0.8 g of 22:5ω3, and 3.0 g of 22:6ω3. This adds up to a total of about 6 g/day of omega-3 polyunsaturated fatty acids.

Maintenance of body weights

At the beginning of the study, the energy intake of each participant was estimated, and the energy contents of diets were calculated to maintain current body weights. As the estimates were subject to error, the participants were weighed every day, and the energy values of their meals were adjusted daily, if necessary, to maintain their body weights. Thus, the average weights of the subjects did not vary significantly during the 100 days of the study. The mean energy intake for all subjects during the entire study was about 2800 kcal/day.

Experimental protocol

All subjects were intially fed diet R for a 20-day stabilization (period 1). Six were then placed on the S diet, while the other subjects continued on the reference diet for 40 days (period 2). During the final segment of the study (period 3), diets were switched for another 40-day period.

Urine collection

For prostaglandin analysis, 24-hr urine was collected in polyethylene bottles and kept refrigerated during the collection period. Urine was collected twice during the first week of period 1 and three times during the last week of periods 1, 2, and 3. After the 24-hr collections were completed, urine volumes were measured, and 2% portions of each 24-hr collection (in a given week) were pooled and stored with dry ice until analyses could be performed, typically within a week. Urine collections were complete. The subjects, who had no access to normal toilet facilities, were under constant supervision and received detailed instruction about urine collection.

Table 1 Target fatty acid distribution in the diets

Type of Fatty Acid	Distribution			
	Reference diet		Salmon diet	
	Weight%	En%	Weight%	En%
Saturated	25	7.5	25	7.5
Monoenoic	50	14.7	43	12.9
ω6 Polyenoic	25	7.5	25	7.5
ω3 Polyenoic	<1	0.3	7	2.1

Table 2 Major fatty acids (%) in actual diets

Fatty Acid	Reference diet x̄	Salmon diet x̄
14:0	1.1	1.2
16:0	18.0	16.8
16:1ω7	1.2	2.1
18:0	7.0	5.6
18:1ω9*trans*	6.4	6.2
18:1ω9*cis*	33.6	24.3
18:1ω7	2.0	2.1
18:1ω5	a	1.4
18:2ω6	21.2	23.0
18:3ω3	1.1	1.1
20:1ω11	a	0.8
20:1ω9	0.1	1.0
20:4ω6	0.2	0.6
20:5ω3	a	2.0
22:0	0.3	a
22:5ω3	a	0.7
22:6ω3	a	2.8
Sum of identified fatty acids[b]	97.0	96.6
Subtotals:		
Saturated	26.5	23.6
Monoenoic	43.2	37.9
Polyenoic, ω6	21.4	23.6
Polyenoic, ω3	1.1	6.7

[a] None detected.
[b] The listed components will not add to these totals as the diet samples contained many minor constituents that are not shown here; however, their contributions to the total recovery are included in the totals given on this line.

Measurement of PGE-M

Analyses of 11α-hydroxy-9,15-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanoic acid (PGE-M) were done on 20-ml aliquots of the 48- or 72-hr pools prepared as described above. This enabled us to assess the mean daily total synthesis of E prostaglandins during the 48- or 72-hour periods. Analytical procedures and instruments were described.[29] PGE-M excretion rates are expressed as μg/24 hr.

Statistical analysis

Twenty-four-hour PGE-M excretion rates and lipid intakes were evaluated by paired *t* tests and by nonparametric signed rank tests with the computer methodology of the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). *P* values less than 0.05 were considered statistically significant.

Results

Two subjects withdrew from the study. They were both scheduled to go on the salmon diet during period 3. Thus, we were left with a 6:4 subject distribution between the groups. Lower PGE-M excretion rates

Table 3 PGE-M urinary excretion rates (μg/24 hr) during the last week on the indicated diet

Subject	Diet		% Diff.[a]
	Reference (R)	Salmon (S)	
1	5.79	4.56	21.2
2	8.27	6.25	24.4
3	7.72	4.74	38.6
4	12.00	8.68	27.7
5	11.54	11.26	2.4
6	6.71	5.32	20.7
7	6.88	6.63	3.6
8	8.23	7.21	12.4
9	8.03	4.88	39.2
10	8.12	4.18	48.5

[a] Percent difference calculated as (R-S)/R × 100.

were associated invariably with the S diet, regardless of whether the subjects received the R or the S diet first (*Table 3*). Statistical analysis indicated that there was no detectable effect resulting from the sequential order in which the two diets were administered. The mean PGE-M urinary excretion was 8.33 ± 0.63 (SEM) μg/24 hr after 40 days on diet R, and 6.37 ± 0.70 (SEM) μg/24 hr after 40 days on diet S. Paired *t* tests indicated a significant reduction of the urinary marker after 40 days on the salmon diet (difference = 1.96 ± 0.42 (SEM) μg/24 hr, $P = 0.001$). Non-parametric signed-rank testing also indicated a significant PGE-M reduction on diet S ($P = 0.002$). *Table 3* shows the PGE-M excretion rates during the last week on the R and S diets.

Discussion

Fats constitute an important source of energy in the typical American diet. During the 1980s, the American Heart Association and the National Institutes of Health issued recommendations to the general public that they modify their diet with respect to type and amounts of fat intake for maintenance of good health.[30,31] This study quantifies the effect of a change in the fatty acid distribution in the diet on the eicosanoid system that could be relevant to major chronic diseases afflicting the general population, such as cancer and coronary artery disease.

Ten male volunteers, when subjected to a change in the ω6/ω3 fatty acid ratio in their diet from 19.5 to 3.6, synthesized an average of 24% (range 2.4–49) less E prostaglandins as measured by the urinary excretion of their major catabolite (PGE-M). In a recent human diet study conducted at the Beltsville Human Nutrition Center,[32] we found that a low fat (19 en%) diet with an intake of 6.6 en% from polyunsaturates was associated with an average 14% reduction in PGE-M daily output, in comparison to a high-fat (41 en%) diet with 9.3 en% from polyunsaturates. However, in that study we could not conclude if the biochemical marker responded simply to the change in linoleate intake or to alteration of the overall dietary lipid profile. In contrast, the major dietary manipulation in the present study was the replacement of a diet devoid of omega-3 fatty acids with one rich in omega-3 PUFA. While this work cannot rule out the possibility that the results observed here were caused by some unknown component of the salmon used in the S diet (or by absence of dietary components replaced by salmon), it is highly probable that such results are the effect of the increase in the omega-3 fatty acids in the S diet compared to the R diet. The findings of this study are in agreement with those of a more recent study from the Beltsville Center* in which the diet of 40 free-living volunteers was supplemented with 15 g/day of a fish oil concentrate containing 52% EPA + DHA. In a study of much shorter duration (4 weeks), Knapp and FitzGerald observed a nonsignificant trend toward lower PGE-M excretion rates in a group of eight volunteers receiving 50 ml/day of fish oil for four weeks.[5]

The ultimate question that must eventually be answered is: What is the biological impact of PGE synthetic reduction of a given magnitude? E prostaglandins are synthesized in nearly all mammalian tissues and their function depends on the synthetic site. Consequently, the impact of reduced PGE production is not easy to predict. And, we have no indication if changes in PGE synthesis, measured by variation in PGE-M output, are uniformly distributed to all tissues. Additionally, it has been demonstrated that reduction of PGE_2 synthesis brought about by long-chain omega-3 PUFA intake can be accompanied by in vivo synthesis of PGE_3.[33] The limited knowledge of the biological activities of trienoic primary prostaglandins adds another element of uncertainty to the overall evaluation of omega-3 fatty acid supplementation.

Research conducted during the last 10 years in several laboratories has demonstrated a role for prostaglandin E in the modulation of cardiovascular parameters[19,20,34-36] and of the immune response.[22,23,37-42] Evidence is also accumulating that PGE_2 may initiate or mediate the processes leading to the development of atheromas.[43] Recent studies indicate that omega-6 PUFA promote tumorigenesis while omega-3 polyunsaturates seem to inhibit it.[1,13] In both cases, the action appears to be linked to their ability to enhance (omega-6) or suppress (omega-3) the synthesis of PGE_2.[1,10] In view of the above, it is conceivable that, in addition to renal function,[44,45] both cardiovascular and immune systems are likely to be influenced by shifts in PGE production. Further research will determine if alterations of the PGE system attainable through realistic dietary modifications can reach a clinically significant level.

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