Modulating Influence of Dietary Lipid Intake on the Prostaglandin System in Adult Men

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We evaluated the effect of moderate dietary changes on the prostaglandin system by measuring the urinary excretion of 7α-hydroxy-5,11-dioxo-tetranorprostane-1,16-dioic acid (PGE-M). In a crossover design, twenty-four free-living male subjects in good health (24 to 54 years of age) were fed two diets: (i) Regular (R) diet, 41% energy (en%) from fat, P/S 0.59, M/S 0.96; (ii) Experimental (E) diet, 19 en% from fat, P/S 1.31, M/S 1.48. Diet R contained 13.9 g/day of neutral detergent fiber (NDF) and about 600 mg/day of cholesterol per 3200 kcal; Diet E contained 35.5 g/day NDF and about 280 mg/day cholesterol. Each controlled-diet period lasted ten weeks. The menu cycle was 7 days, and all diets were calculated to provide adequate amounts of essential nutrients. The PGE-M excretion rates were determined in 24-hr urine by stable-isotope dilution gas chromatography-mass spectrometry in the selected ion-monitoring mode. Low-fat Diet E, with an intake of 6.6 en% from polyunsaturates, was associated with an average 14.2% reduction in PGE-M daily output, compared to high-fat Diet R with a 9.3 en% from polyunsaturates (P = 0.046). These results support the view that dietary lipid changes can significantly alter the *in vivo* production of E-series prostaglandins. We cannot conclude, however, if this apparent diet effect was brought about by the change in linoleate intake alone or was the result of complex biochemical interactions among individual fatty acids, both saturated and unsaturated.
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The molecular basis of nutritionally-induced changes in cardiovascular parameters is a subject of considerable interest. According to the "eicosanoid hypothesis," the putative vascular effects of dietary polyunsaturates result from associated alteration of the endogenous production of prostaglandins. Thus, a quantifiable diet/prostaglandin link is of interest to clinicians, nutritional biochemists and epidemiologists. Progress in this area has been hindered by the limited availability of highly sensitive and specific analytical methods for the quantification of appropriate markers in complex biological matrices.

Biosynthesis of eicosanoids ultimately depends on the availability of their immediate precursors: arachidonate (AA, 20:4ω6), dihomo-gamma-linolenate (DGLA, 20:3ω6), and eicosapentaenoate (EPA, 20:5ω3). Only EPA can be introduced in significant amounts through the diet or through fish oil supplementation. AA and DGLA, the precursors of diene and monoene prostaglandins (PG), respectively, are derived from elongation and desaturation of linoleate (LA, 18:2ω6) which is plentiful in a conventional "Western" diet. That lipid intake should influence PG biosynthesis *in vivo* seems, in general, a reasonable expectation. Direct dietary effects have indeed been demonstrated with arachidonate (1) and eicosapentaenoate (2–5). In contrast, the consequences of dietary LA manipulations on the PG system are much less predictable (6,7). It is probably so because many enzymes are involved in the conversion of linoleate to DGLA and AA. Such enzymes are influenced by several factors, nutritional as well as hormonal (8,9). It has been shown recently in a rat study that *ex vivo* PG biosynthesis can be modulated by dietary alterations involving fatty acids which are non-PG precursors, e.g., saturated fatty acids (10). These observations have enhanced interest in unraveling the complex relationships among dietary fat, eicosanoid production and physiologic effects.

Because present dietary modifications recommended to the general public for the prevention of atherothrombotic disorders and cancer include reduction of total fat and replacement of saturated fats with polyunsaturates, we deemed it relevant to evaluate the comparative effects of two types of diets on the activity of the prostaglandin system in a group of twenty-four healthy male volunteers. As a chemical marker of such activity we selected the whole-body turnover of E-series prostaglandins (E_1 + E_2) which was measured by the urinary excretion rate (μg/24 hr) of 7α-hydroxy-5,11-dioxo-tetranorprostane-1,16-dioic acid (PGE-M). Prominent among the physiological properties of PGE_2 is its powerful influence on myocardial and coronary circulation and on peripheral microvessels (11). Thus, any agent that can modify the PGE_2 biosynthetic level is expected to produce an effect on important cardiovascular variables. The two diets considered in this study differ in their percent of energy (en%) from fat, P/S and M/S ratios. Could such dietary manipulation have a quantifiable effect on the prostaglandin system? Based on present knowledge, and given the acute scarcity of similar studies with human subjects, the answer to this question is not obvious. Cholesterol and fiber intakes also differ in the two diets but, for the purpose of this study, they are not regarded as significant variables. This is part of a larger study in which the two diets must approximate, in all their variables, a typical American diet and a diet recommended for prevention of heart attack and cancer.

MATERIALS AND METHODS

Subjects. Twenty-four male volunteers between 24 and 54 years of age (mean 36.1 ± 8.6, SD) were recruited in the area immediately surrounding the Beltsville Agricultural Research Center. The subjects ultimately selected were free from known metabolic abnormalities as determined by a complete physical examination and laboratory tests. None of them had been on a special dietary regimen, e.g. vegetarian, over the previous year, nor had they taken prescription medications, vitamins, aspirin, aspirin-

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Abbreviations: AA, arachidonate; DGLA, dihomo-gamma-linolenate; en%, energy %; EPA, eicosapentaenoate; E, experimental; LA, linoleate; NDF, neutral detergent fiber; PGE-M, 7α-hydroxy-5,11-dioxo-tetranorprostane-1,16-dioic acid; PG, prostaglandin; R, regular.

containing drugs or nonsteroidal anti-inflammatory drugs ten days before the beginning of the study and during the study. Nutrient supplements were also forbidden for the duration of the study. The subjects continued their normal life and normal activities throughout the experiment and were informed of the purpose of the study, procedures to be followed, and samples to be collected. All the procedures were approved by the Human Studies Committees of the U.S. Department of Agriculture, the National Institutes of Health and the Georgetown University Medical School.

Controlled diets. Two diets designated as E (experimental) and R (regular) were formulated from commonly available foods. Diet E contained about 19% of energy (en%) as fat, 35.5 g/day of neutral detergent fiber per 3200 kcal, and a P/S ratio of 1.31. This ratio is more than twice that found in typical Western Diets, and is characteristic of low-risk populations. It has been recommended for prevention of coronary artery disease. Diet R contained about 41 en% as fat, 13.9 g/day of neutral detergent fiber per 3200 kcal, and a P/S ratio of 0.59. This ratio approximates that observed in earlier studies (12) among free-living subjects in the Beltsville area. Tables 1 and 2 show the nutrient contents of two typical diets. The relative distribution of polyunsaturates to saturates and monounsaturates (P:S:M) was approximately 1.31:1:1.48 in the experimental diet and 0.59:1:0.96 in the regular diet. The mean daily cholesterol intake was 280 mg/day on Diet E (3200 kcal) and 600 mg/day on Diet R, with proportional adjustments for different caloric intakes. Total energy input was adjusted to maintain a constant body weight for the duration of the study.

TABLE 1

Composition of a Typical 3200-Kcal Diet: Mean Daily Intake of Nutrients[a]

Nutrient	Diet E	Diet R
Carbohydrate		
g/day	537.6	366.7
en%	67.2	45.8
Lipid		
g/day	67.2	144.7
en%	18.9	40.7
Protein		
g/day	136.8	118.4
en%	17.1	14.8

[a]Calculated from a 7-day menu cycle.

TABLE 2

Fatty Acid Content of a Typical 3200-Kcal Diet[a]

Fatty acid	Diet E	Diet R
Total saturated		
g/day	18.1	51.6
en%	5.1	14.5
Oleic (18:1ω9)		
g/day	26.6	49.7
en%	7.5	14.0
Linoleic (18:2ω6)		
g/day	21.7	30.6
en%	6.1	8.6
α-Linolenic (18:3ω3)		
g/day	2.0	1.9
en%	0.6	0.6

[a]Minor fatty acid constituents: arachidonic (20:4ω6), 0.12 g/day; eicosapentaenoic (20:5ω3), 0.02–0.1 g/day.

We used a seven-day menu cycle to provide variety and maintain acceptability of the diets. All nutrients for which food data are available were provided by the diets in amounts to meet the Recommended Dietary Allowances (13). Nutrient compositions of the diets were calculated by using the Lipid Nutrition Laboratory food database, which provided the latest data on food composition from the USDA, together with data provided by the food industry, the Nutrient Coding Center in Minneapolis, and analysis. The controlled-diet meals were prepared in the Human Studies Facility of the Beltsville Human Nutrition Research Center (BHNRC). On weekdays, all meals were eaten in the Center's dining facility. Meals for Saturdays, Sundays and holidays were prepacked and distributed for home consumption. No food other than what was provided by the study was permitted, and alcohol consumption was not allowed during the controlled-diet periods. Consumption of coffee, tea, and water was unrestricted.

Experimental design. The study employed a crossover design with two phases involving controlled diets: after a pre-diet free-choice period (baseline) lasting two weeks, twelve subjects were placed on the E Diet and the other twelve on the R Diet for ten weeks (phase 1); phase 2 was another ten-week controlled-diet period (switchover from the diet in phase 1). After phase 1 there was a ten-day holiday break during which the volunteers consumed self-selected diets. Subjects in the two groups were balanced on the basis of their body mass index.

Urine collection. Twenty-four-hour urine was collected in silanized glass bottles and kept on ice during the collection period, three times during week 2 of the pre-diet free-choice period and three times during week 10 of phases 1 and 2. After the 24-hr collection was completed, the volumes were measured, portions were taken (*vide infra*), and either analyzed immediately or stored at −22°C for a few days.

Measurement of PGE-M. Two-percent portions of each 24-hr collection, in a given week, were pooled. A 20-ml aliquot of the resulting mixture was analyzed for 7α-hydroxy-5,11-dioxo-tetranorprostane-1,16-dioic acid (PGE-M) to assess the mean daily turnover of prostaglandins $E_1 + E_2$ *in vivo* during the 72-hr period. We used stable isotope dilution mass spectrometry in the selected ion-monitoring mode. Procedures and instrumentation have been described (14). When storage of urine specimens lasted more than one day, PGE-M values were adjusted to compensate for decay according to a first-order rate constant determined in our laboratory (15). PGE-M excretion rates are all expressed as μg/24 hr.

Statistical methods. Twenty-four-hour PGE-M excretion rates and lipid intakes were evaluated by linear regression and by paired t tests with the computer methodology of the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Multiple linear regression with backwards elimination was used to evaluate the potential influence of height, weight, and baseline PGE-M

levels on the differences observed between individual PGE-M levels on Diet E and Diet R.

RESULTS

One subject withdrew for personal reasons at the end of phase 1 and another withdrew later for health reasons leaving 22 subjects for analysis. Before conducting further statistical analyses, we first determined that the order in which the subjects went on the controlled diets (R or E first) did not have an effect on the results. The mean of PGE-M urinary excretion was 13.44 ± 1.63 (SEM) μg/24 hr after ten weeks on controlled Diet R and 11.53 ± 1.24 (SEM) μg/24 hr after ten weeks on Diet E. Paired t testing indicated a significant reduction in the urinary marker after ten weeks on the experimental diet (difference = 1.91 ± 0.90 (SEM) μg/24 hr, P = 0.046). Values for individuals are shown graphically in Figure 1 for each of the 22 individuals who completed both study diets. The mean PGE-M excretion rates of subjects who switched from Diet E to Diet R were 11.61 and 13.57 μg/24 hr, respectively; those of subjects who switched from Diet R to Diet E were 13.28 and 11.44 μg/24 hr, respectively. In multiple regression models, weight was shown to have a significant effect on the difference in PGE-M levels between diets: lighter weight individuals (weight range 56.3–75.8 kg) showed greater reduction (16% vs 1%) in PGE-M levels on Diet E compared to Diet R than heavier individuals (weight range 77.4–108.2 kg).

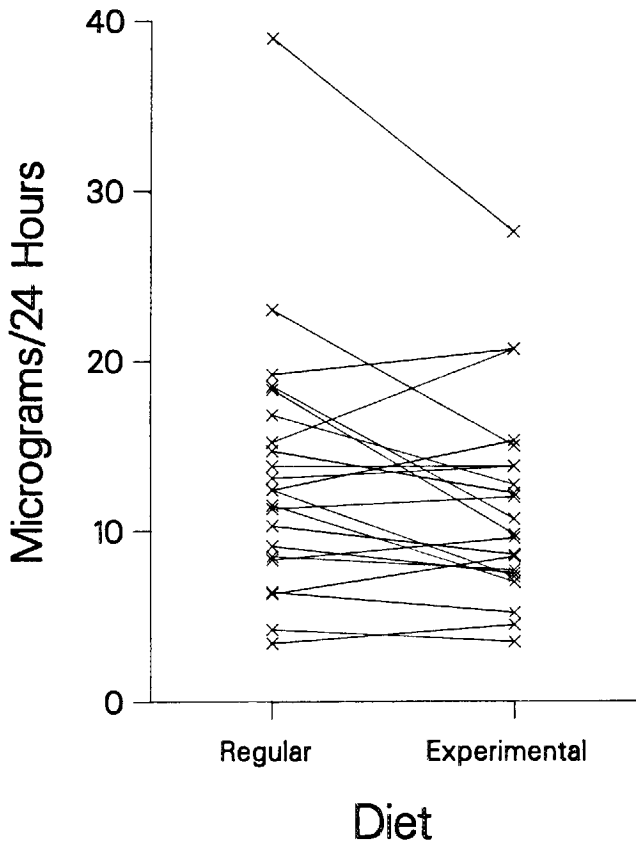

FIG. 1. PGE-M urinary excretion rates of the 22 individuals who completed both study diets.

DISCUSSION

This study was conducted to determine if relatively minor dietary modifications can significantly influence the systemic production of E-series prostaglandins as measured by the urinary excretion rate of 7α-hydroxy-5,11-dioxo-tetranorprostane-1,16-dioic acid (PGE-M). Because plasma fatty acids do not represent the PG precursor pool (6,7,17–19), we omitted their determination. The most direct and meaningful means of assessing *in vivo* prostaglandin production is by measurement of their urinary catabolites (20). The PGE-M excretion rate provides a measure of the aggregate endogenous synthesis of prostaglandins E_2 and E_1 (21). This methodology neglects the contribution from kidney and seminal vesicles to whole-body turnover of PGE. The rationale for disregarding such contribution has been discussed (22).

Pioneering studies by Zöllner *et al.* (23), Nugteren *et al.* (24) and Adam *et al.* (25,26) demonstrated the possibility of nutritionally altering the endogenous biosynthesis of primary prostaglandins in humans. Those studies, however, involved very substantial variations of the levels of linoleate intake. Similarly, a more recent study by Adam and Wolfram (27) compared the effects of linoleate intake of 0 and 20% energy on the excretion of tetranorprostanedioic acid, an analytical artifact which has been proposed as an index of systemic turnover of primary PG of both the E and F series (28). In contrast, the objective of our study was to compare the effect of a typical American diet (R) with that of a diet which has been recommended for the prevention of coronary artery disease. The main finding here is that—while all the subjects were presumably in a PUFA nondeficient status—variations in lipid intake profile (see Table 3) brought about a measurable and statistically significant change in the biosynthetic level of E prostaglandins. In a recent human diet study from this laboratory (22) we could not detect an effect on the PGE-M excretion rate when two levels of linoleate intake (10 and 30 g/day) were considered in a crossover design in which the energy from fat was maintained constant at 35%. That preliminary study, however, was conducted with only four volunteers. Moreover, the analytical method we used then to measure PGE-M (29) did not afford the same level of accuracy as the method used in this study (14).

We must consider the possibility that the apparent diet effect on the prostaglandin system observed in this study may have not been brought about solely by the different linoleate intakes in the two diets. As shown in Table 3, the intake of monounsaturates in Diet R is twice that in Diet E, while the level of saturates in Diet R is three times that in Diet E. The intake of polyunsaturates (93% of which is linoleate) in the "regular" diet is only 1.4 times

TABLE 3

Energy Percent Contributed by Various Lipid Classes[a]

Lipid class	Diet E	Diet R
Polyunsaturates (P)	6.6	9.3
Saturates (S)	5.0	16.0
Monounsaturates (M)	7.4	15.4

[a]Calculated from data of Table 2.

the level in the "experimental" diet, namely 9.3 en% versus 6.6 en%. It would indeed be extraordinary if the only driving force behind the modulation of the endogenous PGE production were such a modest change in 18:2ω6 intake. Results of whole animal nutritional studies and of biochemical studies performed at the enzyme or subcellular levels have led to the hypothesis, now widely accepted, that competitive inhibition may occur among all dietary unsaturated fatty acids, i.e., those of the ω3, ω6 and ω9 groups, at each step of their metabolic transformation (6,9). Specifically, each of the most important members of dietary unsaturates—linoleic, oleic and α-linolenic—inhibits the elongation and desaturation of the other (30,31). Interactions among saturated and unsaturated fatty acids present in the diet and in the metabolic pool have also been demonstrated (32). The nature of our study does not permit us to conclude whether we are observing the direct and simple effect of linoleate intake or the cumulative effect of complex interactions. Perhaps the more important point to keep in mind is that a diet very similar to the typical American diet is associated with a higher biosynthetic rate of E-series prostaglandins compared to a diet with fewer fatty calories and a higher P/S ratio. The lowering of systolic blood pressure in conjunction with enhanced intakes of polyunsaturates observed in several studies (33,34) may have been brought about at least in part by alteration of the eicosanoid system, possibly involving the E prostaglandins. The effect of a nutritionally-induced alteration of the thromboxane to prostacyclin ratio in those studies cannot be ruled out.

Dietary fiber has been shown to affect cholesterol absorption and metabolism (35). But, to our knowledge, there is no report suggesting a fiber-modulating influence on the prostaglandin system. The effect of dietary fiber in this study was probably insignificant. Similarly, a direct correlation between dietary cholesterol and eicosanoid synthesis *in vivo* has not been demonstrated.

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