A Human Dietary Arachidonic Acid Supplementation Study Conducted in a Metabolic Research Unit: Rationale and Design

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ABSTRACT: While there are many reports of studies that fed arachidonic acid (AA) to animals, there are very few reports of AA feeding to humans under controlled conditions. This 130-d study was conceived as a controlled, symmetrical crossover design with healthy, adult male volunteers. They lived in the metabolic research unit (MRU) of the Western Human Nutrition Research (WHNRC) for the entire study. All food was prepared by the WHNRC kitchen. The basal (low-AA) diet consisted of natural foods (30 en% fat, 15 en% protein, and 55 en% carbohydrate), containing 210 mg/d of AA, and met the recommended daily allowance for all nutrients. The high-AA (intervention) diet was similar except that 1.5 g/d of AA in the form of a triglyceride containing 50% AA replaced an equal amount of high-oleic safflower oil in the basal diet. The subjects (ages 20 to 39) were within −10 to +20% of ideal body weight, nonsmoking, and not allowed alcohol in the MRU. Their exercise level was constant, and their body weights were maintained within 2% of entry level. Subjects were initially fed the low-AA diet for 15 d. On day 16, half of the subjects (group A) were placed on the high-AA diet, and the other group (B) remained on the low-AA diet. On day 65, the two groups switched diets. On day 115, group B returned to the low-AA diet. This design, assuming no carryover effect, allowed us to merge the data from the two groups, with the data comparison days being 65 (low-AA) and 115 (high-AA) for group B and 130 (low-AA) and 65 (high-AA) for group A. The main indices studied were the fatty acid composition of the plasma, red blood cells, platelets, and adipose tissue; *in vitro* platelet aggregation, bleeding times, clotting factors; immune response as measured by delayed hypersensitivity skin tests, cellular proliferation of peripheral blood mononuclear cells in response to various mitogens and antigens, natural killer cell activity, and response to measles/mumps/rubella and influenza vaccines; the metabolic conversion of deuterated linoleic acid to AA and the metabolic fate of deuterated AA in the subjects on and off the high-AA diet; and the production of eicosanoids as measured by excretion of 11-DTXB_2 and PGI$_2$-M in urine. The results of these studies will be presented in the next five papers from this symposium.
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Arachidonic acid (AA) is the immediate metabolic precursor of the major biologically active eicosanoids (1,2), such as prostaglandins, thromboxanes, prostacyclins, and leukotrienes as well as a large number of hydroxy eicosatetraenoic acids and their metabolic products. It is also a normal constituent of the human diet (3) if one is not a vegan. Lacto-ovo vegetarians would consume AA from egg yolks. The average amount of AA in Western diets is not certain but has been estimated at between 50 and 300 mg per day (4,5). Jonnalagadda *et al.* (6) reported that the U.S. diet contains approximately 100 mg/d of AA based on the USDA Nationwide Food Consumption Survey for 1987–1988. Sinclair and O'Dea (7) reported that the Australian diet contained between 80 and 100 mg/d of AA. It is likely that general food consumption surveys based on computerized food composition tables underestimate the amount of AA in the daily diet because most food composition data were generated by food analysis that either did not report values for AA or underreported the true amount in the product.

Because of its metabolic importance, there is considerable interest in the metabolic and physiological effects of dietary AA, but most of the data available currently come from animal experiments (8–12). Also, AA has a reputation as being a harmful substance if it is consumed in significant amounts. AA injected as the free acid intravenously in rabbits causes rapid disseminated intravascular coagulation and the death of the animals (13,14). In animal studies, large amounts of AA in the ester form do not seem to cause significant harm to the animals in the short term (8–12,15,16). Seyberth *et al.* (17) reported a human feeding study in which 6 g/d of AA were given to normal male subjects in the form of ethyl ester. Although the study was planned to continue for a month, it was

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Abbreviations: AA, arachidonic acid; FA, fatty acid; MRU, metabolic research unit; SD, stabilization diet.

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terminated after 3 wk because of markedly increased *ex vivo* ADP-induced aggregation (10 to 60%) by platelets from the volunteers. Ever since this 1975 report (17), feeding studies using AA in any form in humans have been avoided, presumably out of fear of adverse consequences.

Given the large number of animal feeding studies (8–12, 15,16) demonstrating little or no harmful effects of dietary AA when fed in the ester form, and the fact that even in the study by Seyberth *et al.* (17) there was no actual physical harm to the subjects coupled with the fact that AA is a normal constituent of the human diet, it seems that human feeding studies with AA could be conducted safely. More recently Sinclair and colleagues (18) investigated the effects of dietary AA in Australians using all natural food diets with modestly elevated amounts of AA (7,18) In addition, within the last decade a natural triglyceride produced by selected strains of algae (ARASCO®) that contained 50% AA became commercially available. Thus, because of the physiological and metabolic importance of eicosanoids in humans and the sparse availability of good data from human feeding studies using AA, we decided to mount a human feeding study in which we fed healthy male subjects 1.5 g of AA per day for 50 d. The study was conducted in a metabolic ward setting in which all aspects of the volunteers' lives could be strictly controlled. The major hypothesis to be tested was that dietary AA at this level would be metabolized differently from AA synthesized *in vivo* from linoleic acid and that it has significant influence on platelet function, immune response, and eicosanoid production.

The main physiological and biochemical parameters to be studied were platelet aggregation *in vitro,* blood lipid levels, the fatty acid composition of various tissues, the immune response as measured by response to several antigens, vaccines, and the delayed hypersensitivity skin tests, production of thromboxane and prostacyclin as measured by their metabolic products in urine, and the actual metabolism of dietary fatty acids (FA) as determined by feeding deuterium-labeled FA and measuring the metabolic products in blood over a time course of up to 72 h. This paper will describe the design of the study, the diets and the FA composition of the ARASCO® oil and the actual diets, and the timing of the blood draws. Subsequent papers in this symposium will present the results obtained in the study to date on platelet function, blood lipids, lipoproteins and apoproteins, tissue FA composition, effect on eicosanoid metabolism, immune response, and the metabolism of 20:3n6 in high- and low-AA diets.

STUDY DESIGN

The design was a single blind crossover, feeding study in which healthy volunteers lived in the metabolic unit (MRU) of the Western Human Nutrition Research Center (San Francisco, CA) for 130 d. A schematic of the design is presented in Figure 1. The physical parameters of the 10 male volunteers who completed the study are: age, yr, 31.6 ± 6.7; weight, kg, 72.7 ± 7.7; body mass index, kg/m^2, 23.5 ± 3.6; blood pressure, systolic, 115.9 ± 9.5; blood pressure, diastolic, 73.2 ± 7.8; no smokers. The study had a symmetrical design in which all subjects were fed a stabilization diet for 15 d after they entered the MRU. On day 16 of the study, six of the subjects, designated Group A, were switched to a diet containing 1.7 g/d of AA (210 mg from the basal diet and 1.5 g from ARASCO® oil). The remaining six subjects, designated Group B, continued to consume the basal diet which contained 210 mg of AA. On day 65 of the study, the two groups switched diets. On day 115, the study Group B was returned to the basal diet. The subjects were discharged from the MRU on day 130 of the study.

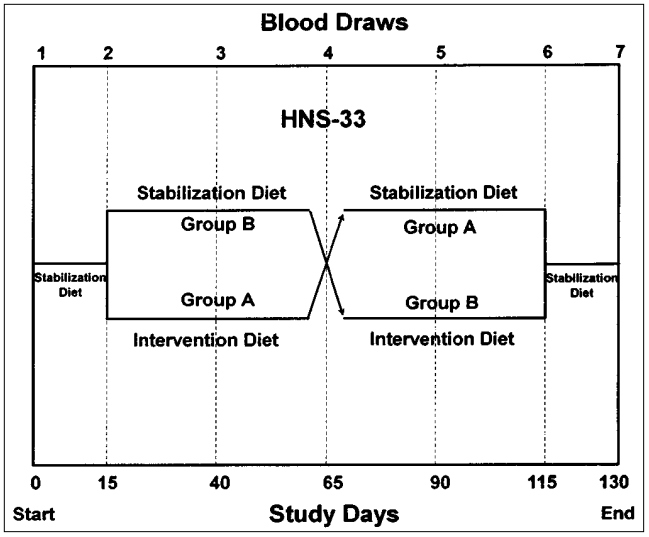

FIG. 1. A schematic plan of the crossover design for this feeding study showing the days of the blood drawn and dietary periods. Note that the values were compared for each group after 50 d on the intervention diet to those obtained after 65 d on the stabilization diet. Thus, the data for group A were comparing the data obtained at day 65 with those obtained at day 130, while in group B the data obtained from day 115 were compared to those obtained on day 65.

This design provided a 50-d intervention period and a 65-d control period for each subject. We assumed that a 65-d washout period for Group A was long enough to eliminate any carryover effect from the high-AA diet. This design allowed the two groups to be merged for statistical purposes, and for most analyses we used an n = 10 (two subjects dropped out of the study prior to completion).

MATERIALS AND METHODS

Source and purity of AA (ARASCO® oil). AA, in the form of a natural triglyceride manufactured and purified from algae (Martek Biosciences Corporation, Columbia, MD), was incorporated into salad dressings and yogurt desserts. The ARASCO® oil produced by the Martek Corporation meets all the requirements for an edible oil for human consumption and contains (wt%) 49.7% AA in the form of mixed triglycerides. The other major FA components of ARASCO® oil are 11.1%

16:0; 10.6% 18:0; 8.7% 18:1n-9; 6.5% 18:2n-6; 3.8% 18:3n-6; 0.9% 20:0; 2.5% 20:2n-6; 1.6% 22:0; and 1.2% 24:0. It is refined and purified by the standard methods used in the edible oil processing industry in the United States. It contains 0.025% α-tocopherol and 0.025% ascorbyl palmitate as antioxidants. The Western Human Nutrition Research Center has conducted human feeding studies with polyunsaturated, long-chain FA for over 10 yr. The oil was kept under nitrogen and at −20°C until it was needed for the food preparation. Open bottles were flushed with N_2 before they were resealed and returned to the freezer. Samples from the ARASCO® oil were taken periodically for analysis of their peroxide values and FA composition. As we fed the AA in a cold yogurt cocktail, it is unlikely that any oxidative degradation took place between the time the food was prepared and when it was eaten.

Subjects. The volunteers were recruited from the West Coast of the United States, and consisted of men between the ages of 20 and 39. Initially 12 volunteers were included in the study, but 2 were unable to complete the protocol. The volunteers were given complete physical examinations. Body weights had to be within −10 to +20% of ideal body weights using the Metropolitan Life Insurance Company tables (medium-frame values from the 1983 edition) (19). Evidence of existing illness or chronic disease was an exclusion criterion. Mild hypertension was not an exclusion criterion, but the group that was recruited tended to have blood pressures slightly below average for men in this age group. Smoking, excessive alcohol consumption, or evidence of narcotic abuse were also exclusionary.

Experimental protocol. All the volunteers were confined to the Nutrition Research Suite of the Western Human Nutrition Research Center for the duration of the study. As the subjects stayed within the confines of the Nutrition Suite except for occasional supervised outings, they had no opportunity to consume any food except that provided by the Center. Thus, compliance with the protocol was, of necessity, 100%. In addition, all food intake was monitored, portions were weighed, and subjects were required to consume everything provided them during their meals. (A rubber spatula was provided to ensure that all food was scraped from the plates and eaten.) Food spills were carefully monitored and recorded, and fluid intake, while *ad libitum,* was also measured precisely.

The protocol for this study was approved by the Institutional Review Boards of the University of California at Davis (Davis, CA) and the United States Department of Agriculture (USDA; Washington, D.C.). A crossover design was used so that the subjects acted as their own controls. The subjects were fed the stabilization diet (SD), containing about 30% of calories as fat, for 15 d. The SD had a target macronutrient composition of 30 en% fat, 15 en% protein, and 55 en% carbohydrate. On day 16 of the study, they were divided randomly into two groups. Group B remained on the SD for 50 d. Group A was placed on a diet in which 1.5 g/d of AA (in the form of a triglyceride containing 50% AA, ARASCO®) was added to the diet for 50 d. After 50 d, day 65 of the study, the groups switched diets for the next 50 d of the study. On day 115 of the study, Group B was returned to the SD. On day 130, all the volunteers were discharged from the study and returned to free living status in the community.

During the confinement period, there were blood draws on study days 2, 16, 40, 65, 90 115, and 130. For most analyses, values from samples obtained on days 65 and 130 for group A and days 65 and 115 for group B were pooled, and the data used for statistical analysis. Data from day 65 for Group A and day 115 for Group B were the end of the AA feeding periods, while data from day 130 for Group A and day 65 for group B were the baseline period.

Diets. The diets consisted of natural foods. No dietary supplements were given. The macronutrient composition of the diets is given in Table 1. (A complete description of the diets, listing all the major and minor nutrients, is available upon request.) A 5-d menu cycle was used throughout the study. Proximate analysis was made on five individual diet composite samples taken from each menu once during the study for both the SD and intervention diets. The results for the seven composite samples were averaged to find the actual composition of the diets. No alcohol was included in these diets. The nutrient composition of the diets was calculated from a computerized nutrient data bank using the data from the *USDA Handbook 8* (20), and adjusted to provide at least the recommended daily allowance for known essential nutrients (21).

Table 2 gives the measured FA composition of both diets. Inspection of Table 2 indicates that all the major FA were very similar except that the level of AA was elevated in the high-AA diet and the level of oleic acid was reduced. The 210 mg of AA found in the SD represents the level of AA found in the natural food used in this study. The theoretical level calculated from *Handbook 8* values was only 150 mg. We believe that this difference is caused by missing values and inaccurate analyses in *Handbook 8*. At the beginning of the

TABLE 1
Composition of Diets (% of total calories), Proximal Analysis[a]

	Intervention diet (high-AA)		Stabilization diet (low-AA)	
	Measured	Target value	Measured	Target value
Macronutrient energy distribution				
Protein	15.2	15.0	15.8	15.0
Fat	27.9	30.0	27.1	30.0
Carbohydrate	56.9	55.0	57.1	55.0
Cholesterol content, mean, mg/d	—	360	—	360
Fatty acid energy distribution				
Saturated	7.7	10	7.4	10
Monounsaturated	9.7	10	10.3	10
Polyunsaturated	7.7	10	7.0	10
P/S Ratio	1.0	1.0	0.9	1.0

[a]AA, arachidonic acid; P/S, polyunsaturated/saturated.

TABLE 2
Fatty Acid Composition (wt%) of Experimental Diets[a]

Fatty acid methyl esters	Intervention diet (high-AA)	Stabilization diet (low-AA)	*P* values
12:0 (laurate)	0.8 ± 0.4	0.5 ± 0.3	
14:0 (myristate)	2.5 ± 1.3	2.3 ± 1.3	
15:0	0.3 ± 0.1	0.3 ± 0.1	
16:0 (palmitate)	15.5 ± 3.2	15.6 ± 3.4	
16:1n-9	0.8 ± 0.3	0.8 ± 0.3	
17:0	0.3 ± 0.1	0.3 ± 0.1	
18:1 DMA[b]	0.2 ± 0.1	0.2 ± 0.1	
18:0 (stearate)	7.9 ± 1.5	7.9 ± 1.7	
18:1*t*, all isomers	6.3 ± 1.9	6.4 ± 1.9	
18:1n-9 (oleate)	30.7 ± 2.6	33.8 ± 2.8	
18:1n-7	1.5 ± 0.2	1.6 ± 0.2	
18:1n-5	1.5 ± 0.6	1.5 ± 0.7	
18:2*tt* and 19:0	0.6 ± 0.2	0.5 ± 0.2	
18:2n-6 (linoleate)	23.8 ± 4.1	23.8 ± 4.5	
18:3n-6	0.4 ± 0.1	0.4 ± 0.0	
18:3n-3 (α-linolenate)	1.9 ± 0.7	1.8 ± 0.5	
20:1n-11	0.4 ± 0.1	0.3 ± 0.0	
22:0 (behenate)	0.3 ± 0.0	0.3 ± 0.0	
20:4n-6 (arachidonate)	2.1 ± 0.1	0.2 ± 0.1	$P < 0.001$
Total	97.7 ± 0.3	98.5 ± 0.4	
Unknowns	2.3 ± 0.3	1.5 ± 0.4	

[a]Means ± SD ($n = 5$). See Table 1 for abbreviation.
[b]DMA, dimethylacetal.

study, each participant's caloric intake was estimated, and the caloric content of the meals calculated to maintain current body weight. As the estimates were subject to error, the participants were weighed every day, and the caloric value of their meals adjusted occasionally, if necessary, to maintain their weight within the limits of the study. Thus, the average weights of the participants did not vary significantly during the 130 d of the study. If all the intakes for each volunteer were averaged during the entire study, the mean energy intake was approximately 2800 Kcal/d.

Measurements. Blood was drawn between 7:00 and 8:00 a.m. after an overnight fast of at least 12 h. Blood was drawn into vacutainers with appropriate anticoagulants, using a Teflon catheter (Angiocath; Deseret Medical, Sandy, UT) for FA analysis, or syringes containing 1 mL of citrate solution for platelet studies.

Studies with deuterated AA. The first experiment involved deuterated AA fed to three subjects eating high-AA diet. A second group of three subjects eating the low-AA diet also received the deuterated AA. An equal amount of deuterated oleic acid was fed at the same time. Blood samples were drawn immediately before feeding the tracers and at 4, 6, 8, 12, 24, 48, and 72 h. A second experiment using different subjects (three from each diet group) from this study fed deuterated 18:2n-6 to subjects pre-fed the diets described in the first experiment above. Details of this studies are found in the paper by Emken *et al.* (22).

Physiological parameters examined. We determined the blood lipid concentrations, FA composition of the tissue samples, eicosanoid levels in urine, blood, and media from cultured cells. Bleeding times *in vivo* and platelet function *in vitro* were determined. Deposition of AA in the adipose tissue was followed using tissue biopsies. The volunteers' immune status was evaluated by determining the number of lymphocytes and their subsets, monocytes and granulocytes, lymphocyte proliferation in response to T and B cell mitogens, *in vitro* production of IL-1, IL-2, IL-2R, IL-6, and TNF and by determining their concentration through ELISA assays. Other indices of immune response tested included natural killer cell activity, delayed hypersensitivity skin response to a battery of seven recall antigens, and primary and secondary antibody responses to viral and bacterial antigens (23,24).

RESULTS

In this study we could detect very few physiological effects from dietary AA when consumed at the level of 1.7 g/d for 50 d. Plasma PL and cholesterol esters showed a marked increase in their content of AA, while the plasma triglyceride level exhibited only minimal incorporation of AA. Adipose tissue did not incorporate any significant amount of AA during this study. Blood lipid levels showed no changes nor did the apoproteins and lipoproteins. Perhaps most importantly we detected no changes in platelet aggregation or bleeding times contrary to the report by Seyberth *et al.* (17). Most indices of the immune response were not affected either. The detailed results to date from this study are presented in the next five papers from this symposium.

DISCUSSION

For several decades now polyunsaturated FA have been considered beneficial compared to saturated FA in the diet (25–27). This was particularly true between 1950 and 1970. Then, with greater knowledge of the role of eicosanoids in human physiology and the rise in interest in n-3 polyunsaturated FA, some investigators (28–30) questioned the wisdom of raising the level of n-6 FA in the diet above that commonly consumed in a Western diet. AA in particular was considered an undesirable dietary constituent, particularly as it was known to have adverse effects in animals (13,14), coupled with the report of Seyberth *et al.* (17) that indicated that AA in the human diet increased the tendency for platelets to aggregate. Conversely, more recent work in infant nutrition has suggested that newborn infants require dietary AA for optimal growth and development (31,32). Still, very little work has been reported in humans on AA feeding studies since the report of Seyberth *et al.* (17) in 1975. Sinclair and colleagues (18,33,34) have reported some studies on AA in human diets from Australia with selected food that increases the level of AA in the diet, although well below the levels used in the study by Seyberth *et al.* (17). Sinclair and colleagues (18,33,34) observed no adverse effects from their AA-enhanced diets.

Of course, none of these negative results from this study should be taken to mean that dietary AA has no metabolic in-

fluences in humans. We did detect significant changes in the production of urinary eicosanoids. In addition, we (22) found changes in the metabolism of the polyunsaturated FA in the presence of elevated dietary AA. Furthermore, while the amount of AA fed in this study was 5 to 10 times that in the typical Western diet (6,35), it was still well below that used in the study reported by Seyberth *et al*. (17). We cannot predict whether higher levels of dietary AA, or longer feeding period, will have major metabolic and physiological consequences. However, it is interesting that in 1961 Kingsburg *et al.* (36) fed 10 g/d of ethyl AA to two students for 11 d without any reported ill effects. The results from this study suggest that dietary AA fed at 1.5 g/d is not harmful to humans nor does it affect blood lipid levels [Kingsburg *et al.* (36) reported a cholesterol-lowering effect from 10 g of ethyl arachidonate] or platelet function while only having minimal effects on the normal immune response. Perhaps the most important implication from this work is the suggestion that the physiological control mechanisms for FA homeostasis in humans shunt dietary AA into specific metabolic pathways. These pathways then tightly control AA utilization and conversion to other compounds in the body. Whether these control mechanisms can be overwhelmed by increasing the amount of AA in the diet remains to be determined. The detailed results for this collaborative study effort are presented in the five other arachidonic acid symposium papers (22, 37–40).

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