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α-Linolenic acid prevents the hypercholesteremic effects of cholesterol addition to a corn oil diet

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The effects of three different diets to which cholesterol with or without cholic acid were compared for their hyperlipemic effect in C57BL/6J mice. The addition of 1% cholesterol and 0.5% cholic acid (CC) to a standard Laboratory Rodent Chow diet that contains 4.5% total mixed fat, had little effect on serum lipids. In contrast, addition of CC to a AIN76A diet that contains 5% corn oil and low levels of α-linolenic acid (18:3 ω3, α LNA) resulted in a 76% increase in total plasma cholesterol levels. The fat content of a semipurified (ANFAT) hypercholesteremic diet (ANFAT + CC) had to be raised to 18% lard to obtain a similar elevation in plasma total lipid levels. Neither dietary corn oil, ANFAT, nor CC alone caused an increase in plasma cholesterol and total lipid levels. The addition of α LNA to the AIN76A diet resulted in a decrease in plasma cholesterol and total lipid concentrations. We conclude that the addition of CC to AIN76A diet unmasked an α-linolenic acid insufficiency in this diet, leading to elevation of plasma cholesterol levels. The addition of α LNA restored the dietary lipid balance and reestablished a normocholesteremic level. (J. Nutr. Biochem. 8:140–146, 1997.) © *Elsevier Science Inc. 1997*

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Introduction

With today's health-conscious society, data concerning the effects of dietary elements on cholesterol levels, heart disease, cancer, and diabetes deserve and receive critical attention. Even though total fat content is extremely important, the complex interactions of the individual constituents fatty acids in the diet often have a significant effect on the host. However, this fact is frequently overlooked. In the United States, the average daily consumption of fat ranges from 70 to 110 g and constitutes approximately 38% of total dietary calories.[1,2] Estimates indicate that saturated, monounsaturated, and polyunsaturated fatty acids (PUFAs) supply 14%, 15%, and 7% of calories from fat, respectively. Based on extensive studies, several groups have recently recommended changes in the dietary fat intake.[3,4] The recommendations are to reduce the total intake of fat to 30% of calories and concurrently reduce the ingestion of saturated acids and PUFAs to <10%, and allow monounsaturated fatty acids to make up most of the remainder. Additionally, dietary cholesterol should be limited to 100 mg/1000 calories. The principal rationale for these recommendations is to reduce plasma cholesterol and thereby reduce the risk and incidence of coronary heart disease (CHD).[4]

The reported findings emphasize the need to reexamine the effects of dietary fatty acids and their interactions, rather than the total dietary fat content alone. In our attempts to use the American Institute of Nutrition's standard diet (AIN76A) as the control diet in our experiments with high-fat and cholesterol in mice, we discovered that AIN76A possesses a marked hypercholesteremic effect when cholesterol and cholic acid are present in the diet. Even though the AIN76A diet includes only a total fat content of 5% corn oil, the serum lipid levels were similar

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to mice fed a diet containing 3.6 times more total fat, in the form of 18% lard. It became evident that the fatty acid composition of the particular fat may be more consequential than the absolute dietary content.

The essential fatty acid (EFA), α-linolenic acid (18:3 ω3, α LNA) possesses various biochemical functions, including formation of the physiologically important icosanoids including leukotrienes and prostaglandins.[5,6] As the predominant PUFA in chloroplast lipids and an abundant fatty acid in certain plant seeds, linolenic acid is the single most important dietary source of ω3 fatty acids. Thus, a deficiency of α LNA directly leads to the absence of ω3 fatty acids, and indirectly to a deficiency in icosanoids.[7,8] As compared with corn-oil-based diets, diet rich in ω3 PUFAs, such as those from fish oil, inhibited the development of chemically induced tumors in rats and mice.[9,10] Additionally, long-chain ω3 fatty acids have been suggested to be beneficial in reducing the risk of certain chronic diseases, particularly cardiovascular disease.[11–13]

Dietary fatty acids influence the cholesterol and lipoprotein concentrations in blood, which are major risk factors in the development of CHD.[14,15] Even though the hypercholesteremic effect of saturated fatty acids and the hypocholesteremic effect of linoleic acid (18:2 ω6) have been well established,[16–18] the role of α LNA in regulating cholesterol levels has been unclear. Chan et al. has recently suggested that α LNA is as effective as linoleic acid in lowering blood cholesterol levels in normolipidemic men.[19]

Our experimental observation suggested that the addition of cholesterol and cholic acid to AIN76A diet, has a marked a hypercholesteremic effect, which is greater than the sum effect of the individual components. Indeed, the addition of cholesterol and cholic acid to AIN76A, which contains 5% corn oil as the sole source of fat, results in a total cholesterol increase equivalent to that obtained with 18% lard in the ANFAT + CC diet. The addition of either 0.5% cholic acid alone,[20,21] which functions as a solubilizer, or 1% cholesterol alone did not mediate an increase in plasma lipid concentration. We suspected that the level of α-linolenic (18:3 ω3) acid in AIN76A (corn oil) was inadequate when cholesterol and cholic acid was added to this diet. Indeed, the supplementation of pure α LNA to the corn oil diet (AIN76A) containing cholesterol and cholic acid abrogates this hypercholesteremic effect. The results confirm and extend the functional role of α LNA as an EFA to the maintenance of normal blood cholesterol levels.

Methods and materials

Animals

Male C57BL\6J mice 6 to 8 weeks of age (Jackson Laboratories, Bar Harbor, ME USA) weighing 19 to 23 g were housed in groups of four. Animals were kept in plastic cages with wood chip bedding and a filter bonnet cover, tap water supplied ad libitum. On arrival, all mice were fed a standard diet of Laboratory Rodent Chow #5001 (CHOW, Ralston Purina, St. Louis, MO USA). This diet is composed of 23% crude protein, 4.5% crude fat, ash up to 8.0%, and 2.5% minerals. Within 7 to 8 days, the mice were segregated into groups of 12 (four per cage) and fed their respective diets. Plasma lipid levels were monitored on a regular basis in 6 of the 12 animals of each group.

Table 1 Fatty acid profile[a] of corn oil, lard, and CHOW

Fatty Acid		Corn Oil	Lard	CHOW
Myristic	14:0	1.0 ± 0.9	1.7 ± 0.7	1.73
Palmitic	16:0	10.0 ± 2.0	19.3 ± 1.1	22.9
Stearic	18:0	3.5 ± 1.0	23.3 ± 1.9	7.67
Palmitoleic	16:1	0.9 ± 0.7	3.35[c]	1.91
Oleic	18:1	34.0 ± 15.0	44.1 ± 3.6	32.4
Linoleic	18:2	47.0 ± 13.0	6.7 ± 0.5	29.5
Linolenic	18:3	≤0.5[b]	1.0 ± 0.6	3.07

[a]Expressed as percentages (w/w) of total fatty acids present. Numbers represent the average content as reported from suppliers.
[b]ICN catalog number 901414, as provided by the company, and references 22–24.
[c]Reference 56, value given as 3.35 (1.60–4.80).

Diets

a) AIN76A = American Institute of Nutrition, semipurified diet.
b) AIN76A + 1% cholesterol
c) AIN76A + 1% cholesterol + 0.5% cholic acid (AIN76A CC)
d) CHOW
e) CHOW + 1% cholesterol
f) CHOW + 1% cholesterol + 0.5% cholic acid (CHOW CC)

Cholic acid serves as a choleretic, stimulating the liver to increase the output of bile to aid in the emulsification of fats.

Both AIN76A (ICN Biochemicals, Irvine, CA USA) and the custom-made ANFAT diet have similar concentrations of sucrose and protein. The major differences between these diets, however, is that AIN76A contains 5% corn oil and 15% starch, whereas the ANFAT diet contains 18% lard as the source of fat, but contains no starch. The fatty acid analysis of corn oil,[5,22,23] lard, and CHOW is presented in *Table 1*, whereas the composition of the AIN76A and ANFAT diets are given in *Table 2*. The lard-containing semipurified diet with cholesterol and cholic acid (ANFAT CC) has been used extensively.[24–27]

Additional mice were fed selected diets supplemented with α LNA (Sigma, St. Louis, MO USA). This was achieved by fortifying the selected diets, such that the final composition of α LNA was 3% of the total fat content. Respectively, we added an additional 0.15% to the 5% corn oil diet (AIN76A CC + α − LNA). Animals were monitored daily and food was supplied ad libitum for an average of 10 to 12 weeks.

Lipid determination

Blood samples were obtained from the tail vein, and plasma lipids were separated by thin-layer chromatography as previously described.[28,29] Chromatogram quantitation was performed with a densitometer (Bio Rad 620 Video densitometer, Bio Rad Laboratories, Richmond, CA USA) using corresponding reference lipids and internal standards. All lipid data were analyzed for statistical significance by the Tukey's studentized test[30] for individual variables in each diet group. Evaluation of the overall effect was performed with the Kruskal-Wallis test.[31]

Results

Plasma lipid levels

After 10 to 12 weeks, plasma lipid levels were obtained for each of the three base diets, AIN76A, ANFAT, and CHOW (*Table 3*, group 1, group 4, and group 7, respectively). There was no significant difference in the level of free cholesterol,

Table 2 Constituents of semipurified diets

	Constituents (% by weight)	
	(ANFAT)	AIN76A
Casein	20.0	20.0
Lard	18	none
Corn oil	none	5.0
Cholesterol	0.0	0.0
Cholic acid	0.0	0.0
Sucrose	52.6	50.0[a]
Inositol	0.1	0.0
Choline chloride	0.2	0.0
Choline bitartrate	0.0	0.2
Salts (Hegsted)	4.0	3.5
Corn starch	0.0	15.0
Cellulose	5.0	5.0
dl-Methionine	0.0	0.3
Vitamin mix*	0.1	1.0
Vitamin Mix* Vit D (cholecalciferol, 400,000 IU/g)	2.53	2.50
Folic acid	0.19	2.00
DL-tocopherol	253.00	200.00
Pyridoxine	2.53	7.00
Thiamine	3.16	6.00
Biotin	0.13	0.20
Riboflavin	3.16	6.00
Vit A (Retinyl Palmitate)	3.16	16.00
Menadione	16.45	0.05
Dextrose	632.50	9720.00
Niacin	50.00	30.00
Calcium pantothenate	31.63	16.00

[a]1.5% additional sucrose is added with the vitamin and mineral mixes.

cholesterol ester, total cholesterol, or total lipid levels in the plasma of animals fed these three diets (*Table 3*). The levels of free fatty acids (FFA) and triacylglycerols (TG) in the plasma of animals fed the CHOW diet (group 7) were 0.82 ± 0.11 and 0.61 ± 0.29 g/L, respectively. These were significantly higher than the FFA and TG levels of animals in group 4, fed ANFAT, 0.30 ± 0.12 and 0.40 ± 0.11, or AIN76A diet (group 1) 0.49 ± 0.02 and 0.17 ± 0.07 g/L, respectively.

In spite of the high animal fat content of ANFAT diet, the level of the individual lipids or the level of total lipid was not sgnificantly higher than the one observed in animals fed AIN76A or CHOW diet. This was meaningful because the ANFAT diet contains 18% lard, whereas AIN76A contains 5% corn oil and CHOW has only 4.5% mixed animal and plant fats. The results also show that the addition of 1% cholesterol alone to either AIN76A, ANFAT, or CHOW diets did not result in a significant increase in plasma lipids levels as compared with the respective base diet (*Table 3*, groups 2, 5, and 8, respectively).

The addition of 1% cholesterol and 0.5% cholic acid (CC) to AIN76A resulted in a marked and statistically significant elevation of total lipid levels (*Table 3*, group 3). This elevation was limited to an increase in plasma cholesterol, cholesterol esters, and total cholesterol in AIN76A CC-fed group. As reported previously,[24–27] the ANFAT CC (group 6) also displayed a significant elevation in plasma FFA, but a decrease in TG levels. Total lipid levels in AIN76A CC-fed group increased from 3.05 to 5.09 g/L, whereas in the ANFAT CC group, the increase was from 3.17 to 4.81 g/L. These elevations from baseline are statistically significant at $P \leq 0.05$. The elevation of plasma lipid levels observed in the group 3 fed AIN76A CC occurred even though the total fat content of this regime was 3.6 times lower than the total fat contained in the ANFAT CC diet.

In contrast, feeding CHOW + CC (group 9) did not mediate a significant increase in the concentrations of any

Table 3 Effect of diet on the plasma lipid concentrations[A] in the C57BL/6J mouse[B]

Group	Diet	Chol	ChEs	Total CHOL[C]	FFA	TG	Total Lipid[D]
1	AIN76A	0.62 ± 0.09	1.77 ± 0.18	1.68 ± 0.20	0.49 ± 0.02	0.17 ± 0.07[F]	3.05 ± 0.28
2	AIN76A + 1% CHOL	0.41 ± 0.14	1.37 ± 0.36	1.23 ± 0.42	0.48 ± 0.15	0.25 ± 0.03	2.51 ± 0.52
3	AIN76A + 1% CHOL + 0.5% Cholic acid	1.11 ± 0.20	3.09 ± 0.50	2.96 ± 0.53	0.64 ± 0.10[G]	0.25 ± 0.14[F]	5.09 ± 0.62
4	ANFAT[E]	0.71 ± 0.08	1.76 ± 0.19	1.76 ± 0.25	0.30 ± 0.12	0.40 ± 11[H]	3.17 ± 0.29
5	ANFAT + 1% CHOL	0.61 ± 0.05	1.55 ± 0.17	1.53 ± 0.21	0.17 ± 0.06	0.34 ± 0.09	2.67 ± 0.32
6	ANFAT + 1% CHOL + 0.5% Cholic acid	1.13 ± 0.19	2.92 ± 0.35	2.88 ± 0.42	0.59 ± 0.11[I]	0.17 ± 0.04[H]	4.81 ± 0.53
7	CHOW	0.52 ± 0.05	1.66 ± 0.18	1.52 ± 0.23	0.82 ± 0.11	0.61 ± 0.29[J]	3.61 ± 0.31
8	CHOW + 1% CHOL	0.44 ± 0.07	1.48 ± 0.20	1.33 ± 0.26	0.67 ± 0.12[L]	0.14 ± 0.09	2.73 ± 0.43
9	CHOW + 1% CHOL + 0.5% Cholic acid	0.53 ± 0.13	2.03 ± 0.60	1.74 ± 0.65	0.93 ± 0.11[GIL]	0.34 ± 0.24[J]	3.83 ± 0.71

CHOL-cholesterol, ChEs-cholesterol esters, FFA-free fatty acids, TG-triacylglycerols.
[A]Concentrations are in grams per litter.
[B]Data represent mean values ± SD, obtained from 6 of 12 (n = 6) mice from each group after 10 weeks on the respective, diet. All lipid determinations were performed following overnight fast, using triplicate 2 μL samples.
[C]Total cholesterol = (Chol + ChEs × 0.59, refs. 24, 27).
[D]Total lipid = the sum of the individual lipid components.
[E]Lower amounts of lard (i.e., 9%, 11%, and 13%) do not cause an increase in plasma cholesterol levels, as seen with 18% lard (data not presented). Except for the T.G. levels, all lipid levels in group 3, AIN76A + CC, are significantly higher that the respective controls, groups 1 or 2, $P < 0.05$. Similarly, except for T.G. levels, all lipid levels in group 6, ANFAT + CC, are significantly higher that the respective controls groups 4, or 5. Except for TG levels, all lipid levels in group 9, CHOW + CC, were not significantly different than the lipid concentrations of the respective control groups 7 and 8, and were significantly lower that either group 3 or 6, $P < 0.05$.
[F]The superscripts [F–L] indicate that values in groups with the same superscripts are statistically different from each other at $P < 0.05$.

Table 4 Effects of diet on food consuption and caloric intake

Diet	Food Consumption (grams/day)	Diet Energy Density KJ/Kg	Daily Consumption KJ/day	Body Weight (grams)
CHOW	2.64 ± .54[A]	8.49[A]	22.43	26.4 ± 4.5
AIN76A	1.40 ± .32[B]	17.65[B]	24.73	27.7 ± 4.1
ANFAT	1.12 ± .24[B]	21.61[B]	24.22	26.6 ± 3.6

The superscripts [A,B] indicate that values in groups with different superscripts are statistically different from each other at $P < 0.05$. Results are expressed as mean ± SD.

of the plasma lipids measured. Excluding FFA and TG, these concentrations were approximately 50% lower than either group 3 or group 6, which were fed cholesterol and cholic acid.

Dietary consumption

The results presented in *Table 4* show that, compared with the CHOW diet, daily food consumption of both the AIN76A was significantly lower ($P \leq 0.05$). On the other hand, no significant difference in the daily caloric consumption was seen. The higher caloric densities of AIN76A compensated for the decreased food intake. This conclusion is supported by the observation that, between the three dietary groups, there was no significant difference in body weights with a mean of 26.9 ± 3.9 g for all groups.

Effects of α LNA on the AIN76A CC diet

Analysis of the fatty acid content of corn oil in the AIN76A CC diet and lard in the ANFAT CC diet as presented in *Table 1* revealed that corn oil is low in α LNA (18:3 ω3). In comparison, the fat mixture in CHOW diets contained 3.07% α LNA. We, therefore, tested the effect of the addition of α LNA to AIN76A CC. The results are presented in *Table 5*. The addition of 0.15% (3% of total fat) α LNA to AIN76A CC + α LNA led to a significant reduction in plasma total lipid levels as compared with animals fed the same diet without α LNA (AIN76A CC). The reductions in plasma free cholesterol, cholesterol ester and total cholesterol were 36%, 24.8%, and 29.4%, respectively. These results demonstrate that, under the specific conditions where cholesterol and cholic acid are added to AIN76A, which contain 5% corn oil as the sole source of fat, the level of αLNA in this diet is insufficient to maintain normal cholesterol levels. The addition of α LNA to corn oils reduced the higher than expected hypercholesteremic effect of CC.

Discussion

The fat component of food performs many important functions. It provides a source of energy, acts as a carrier for fat-soluble vitamins and flavors, influences a wide range of membrane receptors and enzymes, and provides bioactive fatty acids.[5]

The normal diet provides a wide array of fatty acids: saturated (palmitic, stearic, myristic), monounsaturated (oleic), and polyunsaturated (linoleic, α-linolenic) acids. Once absorbed, dietary fatty acids perform many diverse metabolic, structural, and regulatory functions. The effect of dietary fats and fatty acids on plasma lipids, cholesterol, and heart disease has been the emphasis of many studies.[1,4,24-27]

This report presents results obtained with three different diets, AIN76A, ANFAT, and CHOW, to which cholesterol with or without cholic acid was added. The results illustrate that the total fat content of the respective diets was not the primary factor in determining the level of hyperlipemia.[32-35] This conclusion is reached because addition of 1% cholesterol and 0.5% cholic acid to the CHOW diet with a 4.5% total fat content, was not associated with a significant elevation of plasma total lipid levels, whereas the same addition of cholesterol and cholic acid to groups fed AIN76A containing 5.0% total fat resulted in a marked and significant increase in plasma total lipid levels.

The effects on plasma total lipids of these three diets are not identical, and consequently, the comparisons among the groups have some limitations; nevertheless, significant conclusions can be drawn from the results. As detailed in *Table 1*, the fatty acid composition of corn oil differ considerably. In corn oil, the concentrations of palmitic, stearic, and palmitoleic acid is, respectively 2, 6, and 20 times lower than the respective acids in lard; yet, AIN76A + CC is more

Table 5 Effects of α linolenic acid on concentration[A] of plasma lipids[B] in the inbred C57BL/6J mouse

Diet	CHOL	ChEs	Total CHOL[C]	FFA	T.G.	Total[E] Lipid
AIN76A CC[F]	1.03 ± 0.09	2.74 ± 0.14	2.65 ± 0.14	0.51 ± 0.03	0.28 ± 0.03	4.57 ± 0.21
AIN76A CC + α-LAN	0.66 ± 0.06	2.06 ± 0.16	1.87 ± 0.16	0.46 ± 0.06	0.29 ± 0.02	3.47 ± 0.25
ANFAT CC	1.14 ± 0.10	2.81 ± 0.17	2.81 ± 0.17	0.51 ± 0.04	0.33 ± 0.06	4.80 ± 0.26
ANFAT CC + α-LAN	1.11 ± 0.07	2.67 ± 0.14	2.69 ± 0.12	0.54 ± 0.04	0.36 ± 0.04	4.69 ± 0.30

CHOL-cholesterol, ChEs-cholesterol esters, FFA-free fatty acids, TG-triacylglycerols.
[A]Concentrations are in grams per litter.
[B]Data represent mean values ± SD, obtained from 6 of 12 (n = 6) mice from each group after 10 weeks on the respective, diet. All lipid determinations were performed after overnight fast, using triplicate 2 μL samples.
[C]Total Cholesterol = (Chol + ChEs × 0.59, refs. 25, 28).
[D]Lower amounts of Lard i.e., 9, 11, and 13% do not cause an increase in plasma cholesterol levels, as seen with 18% lard (Data not presented).
[E]Total lipid = the sum of the individual lipid components.
[F]Except for the FFA and TG levels, all lipid levels in animals fed, AIN76A CC + α-LAN, are significantly lower than animals fed AIN76A CC only, $P < 0.05$. Addition of α-LAN did not have any significant effect on the lipid levels of the group fed ANFAT CC.

hypercholesteremic. The singular most striking qualitative difference between corn oil and lard is the higher content of linoleic acid (LA, 18:2 ω6), 47.0% versus 6.7%, respectively (*Table 1*). In fact, mice in the AIN76A group consumed 32.9 mg/day of LA, whereas the animals in the ANFAT group only ingested 13.5 mg/day of LA. Because LA is found in a such a significantly higher level in corn oil than in lard, it was logical to assume that it contributed to the unusual elevation in plasma lipids. Two lines of evidence suggest otherwise. First, the fat component of CHOW contains 29.8% LA and animals consumed 35.3 mg/day, a level not markedly different than the total intake observed in animals fed corn oil (AIN76A). The high LA content of the CHOW diet, in turn, suggests that linoleic acid is not directly responsible for the elevation, because CHOW-fed animals did not develop hypercholesteremia when fed cholesterol and cholic acid. Second, there is a considerable data attributing a hypocholesteremic effect to LA.[12–14] Nevertheless, hypercholesteremia was observed. Consequently, we did not believe that the high level of LA in corn oil was responsible for either mediating the hypercholesteremic effects nor counteracting it.

Alternatively, we speculated that the higher level of α LNA in the CHOW diet, 3%, was involved with prevention of the hypercholesteremic effect observed in this group. The fact that AIN76A contained the lowest level of α LNA, as compared with either ANFAT or CHOW supported this thesis. The results demonstrate that the addition of α-Linolenic acid led to a significant reduction in plasma cholesterol levels when AIN76A CC diet was used. Plasma cholesterol and cholesterol ester levels were restored to levels similar to those obtained with AIN76A lacking additional cholesterol and cholic acid (*Tables 3* and *5*).

The data strongly suggest that the composition of the dietary lipid mixture plays an important role, in mediating the degree of dietary induced hyperlipemia. Additionally, the increases cannot be attributed solely to cholesterol and cholic acid because no elevations were observed in the supplemented CHOW-CC group. The increases were only seen after the addition of both cholesterol and cholic acid to the semipurified diets containing either corn oil, as in the AIN76A diet, or a high amount of total fat (18%) as in the ANFAT diet. It is of particular interest that lower amounts of lard (i.e., 9%, 11%, and 13%) added to ANFAT diet did not mediate the increase in cholesterol or total lipid levels as obtained with 18% lard (data not shown). It is evident from these results that, when cholesterol is present in the diet, corn oil has a higher hypercholesteremic effect, because a similar level of hyperlipemia was obtained by feeding 5% corn as with feeding 18% lard.

Other dietary factors may influence plasma lipids; the AIN76A diet contains 15% corn starch, and there is substantial evidence that starch has a lipogenic effect.[36–39] The independent effect of dietary starch on lipogenesis or the interaction between starch and cholesterol in the diets studied has not been evaluated here. It should be noted, however, that CHOW contains appreciable amounts of carbohydrates primarily in the starch form (data not shown) and, thus, based on the results obtained with these diets, it suggests that starch is not a major factor leading to hyperlipemia. Our studies imply that the hyperlipemic effect was caused in part by the insufficiency of α LNA in corn oil. It is evident that corn oil alone did not mediate a hypercholesteremic effect.[40] The addition of cholesterol and cholic acid, however, unmasked its lipogenic potential.

The hyperlipemic effect revealed by the lack of α LNA may be an additional characteristic of an EFA deficiency. Humans and other animals (unlike plants) lack the enzymes that can synthesize LA and/or α-LNA, and these EFAs must be provided in the diet. These two families of unsaturated fatty acids are desaturated and elongated in the liver.[41] In their absence, growth is impaired and structural and metabolic perturbations are evident.[42] The essentiality of LNA apparently depends on the extent to which it can be converted to eicosapentaenoic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6 ω3).[43–45] The multiple diverse effects of each of these ω3 PUFAs on various physiological phenomena are, in part, mediated by modulating the metabolism of arachidonic acid (AA). These include competitively reducing AA synthesis, diluting AA pools in membranes, and attenuating the biosynthesis of eicosanoids.[46–48] Because eicosanoids derived from AA may play a key role in atherogenesis[49,50] and because dietary ω3 PUFA seems to attenuate the actions of the icosanoids, the efficacy of α-LNA in reducing atherosclerosis and thrombosis is of interest. Renaud[51] and others[50,52,53] have reported that modest intakes of α-LNA, or other ω3 PUFAs, as part of a regular diet decreased CHD most probably by a reduction of thrombotic tendency. Our data suggest that, at least in this regard, α LNA may play a role in regulating the levels of plasma lipids, especially cholesterol and cholesterol esters, which certainly are contributors to atherosclerosis and heart disease.

Excess fat intake has been the single most targeted risk factor in heart disease. Because dietary PUFA (mostly LA) decreased this risk, it has been promoted as a replacement for saturated fatty acids. Currently in the United States, the intake of LA approximates more that 20 g per day, which is equivalent to 7% of calories from fat. Alternatively, there has been limited concern over dietary requirements of α LNA and little interest in promoting ω3 PUFA. Thus, there is a limited dietary intake of α LNA. Based on recent estimates,[5] the ratio of LA to α-LNA in the American diet is around 10:1. This disparity may be of paramount concern because the concurrent intake of ω6 PUFA greatly influences the metabolic efficacy of α LNA, because the desaturation-elongation pathways of each involve common enzymes.[54] Thus, a high intake of LA compared to α-LNA tends to inhibit the conversion of α LNA to EPA and DHA, thereby negating the potential benefits.

The results of our study demonstrate that the requirements of EFA, specifically αLNA, may be dependent on the overall dietary fat intake, particularly the specific composition of the individual fats in the diet. Consequently, the interactions of the various lipids in the diet may lead to a insufficiency in α LNA, even though the total fat percentage is relatively low as is seen with the AIN76A CC diet. Conceptually, our data suggest that corn oil lacks sufficient levels of α LNA. Therefore, the data suggest that a dietary lipid imbalance will lead to in vivo deregulation of lipid levels in the circulation. Supplementation of dietary α LNA

levels results in maintaining normal plasma lipid concentration in the presence of high dietary lipid consumption.

This report demonstrates that when large amounts of dietary fat are consumed, α LNA is essential for the conservation of normal plasma lipid concentrations. This requirement for α LNA, may vary with the specific composition, not just the total content, of the individual fats in the diet.

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