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The greater atherogenicity of nonpurified diets versus semipurified diets in hamsters is mediated via differences in plasma lipoprotein cholesterol distribution, LDL oxidative susceptibility, and plasma α-tocopherol concentration

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In the current study, plasma lipid, lipoprotein cholesterol and α-tocopherol concentrations, LDL oxidative susceptibility, and early aortic atherosclerotic responses of feeding a hypercholesterolemic nonpurified or semipurified diet containing similar levels of macro- and certain micro-nutrients were compared in hamsters. Although plasma total cholesterol (TC) concentrations in the hamsters fed the semipurified versus nonpurified diet were significantly higher ($P < 0.05$), non-high density lipoprotein cholesterol (non-HDL-C) concentrations were 41% greater in hamsters fed the non-purified diet ($P < 0.05$). This greater plasma non-HDL-C concentration, coupled with significantly lower plasma concentrations of high-density lipoprotein cholesterol (HDL-C) ($P < 0.05$), resulted in a greater plasma non-HDL-C/HDL-C and TC/HDL-C ratios for the nonpurified versus the semipurified diet ($P < 0.05$). Despite comparable amounts of dietary vitamin E, plasma α-tocopherol concentrations were 233% higher in the semipurified versus nonpurified diet ($P < 0.05$). Resistance of LDL to oxidation, as measured by lag phase of conjugated diene formation, was reduced nearly 50%, and the rate of formation of conjugated dienes was 105% higher in the nonpurified versus the semipurified diet, respectively ($P < 0.05$). Early aortic atherosclerosis was fivefold greater in hamsters fed the nonpurified versus semipurified diet. These results suggest that, despite similar dietary concentrations of many of the macro- and micro-nutrients, ingestion of hypercholesterolemic nonpurified diets by hamsters is associated with a more atherogenic lipoprotein profile, greater LDL oxidative susceptibility, lower plasma α-tocopherol levels, and greater early aortic atherosclerosis compared to semipurified diets. (J. Nutr. Biochem. 9:591–597, 1998) © *Elsevier Science Inc. 1998*

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Introduction

The oxidation of lipoproteins, particularly low-density lipoprotein (LDL), has been implicated in the initiation and development of atherosclerosis.[1] Support for this hypothesis is derived from the immunochemical demonstration of oxidized LDL in atherosclerotic lesions,[2] isolation of oxidized LDL from lesion areas,[3] and the identification of circulating auto-antibodies specific for epitopes on oxidized

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LDL from human plasma.[4] This association between the oxidation of LDL and the development of atherosclerotic lesions has prompted investigations of the potential anti-atherosclerotic properties of antioxidants. There have been several studies both in vitro[5] and in vivo[6–8] that have demonstrated that LDL enriched with tocopherol, following dietary supplementation, exhibits greater resistance to ex vivo oxidation. In addition, several epidemiological studies[9–11] and one intervention study[12] suggest that the risk of cardiovascular disease is inversely related to circulating α-tocopherol levels and/or vitamin E intake.

The effects of vitamin E supplementation on diet-induced atherosclerosis in various animal models have been equivocal. As described in a recent review by Lynch and Frei,[13] previous studies in rabbits[14] and cockerels[15] indicated either no effect or an increase in atherosclerosis with vitamin E supplementation. However, other studies in rabbits[16] and rats[17] demonstrated decreased atherosclerosis with vitamin E supplementation. More recent studies in vitamin E-supplemented rabbits[18] do not allow one to determine whether the reduction in atherosclerosis was the result of the hypocholesterolemic and/or antioxidant activity of vitamin E. Although the review by Lynch and Frei[13] would suggest that the more recent studies, in general, support the notion that vitamin E inhibits the development of atherosclerosis, the evidence is still equivocal. For example, studies by Morel et al.[19] in cholesterol-fed rabbits, Willingham et al.[20] and Kleinveld et al.[21] in Watanabe heritable hyperlipidemic rabbits, and Parker et al.[22] in one cohort of hypercholesterolemic hamsters could not show a vitamin E protective effect on atherosclerosis. On the other hand, in one study in monkeys,[23] vitamin E appeared to lessen the severity and rate of atherosclerosis, whereas in a cohort of hamsters in one study[22] and in a second study in hamsters[24] the anti-atherosclerotic properties of vitamin E were demonstrable without any significant alterations in blood lipids.

Review of both positive and negative studies of vitamin E supplementation and atherosclerosis suggest that, in general, animals fed semipurified diets (SP) show greater inhibitory effects of vitamin E on atherosclerosis. This notion receives some support as the negative studies[19–22] versus the positive investigations[22–24] of vitamin E effects on atherosclerosis occurred in animals fed nonpurified (NP) and SP diets, respectively. Thus, these observations raised the possibility that components in a NP diet might reduce the efficiency of vitamin E absorption or that alternatively, a pro-oxidant in NP diets could oxidize a portion of the dietary vitamin E, perhaps at the level of the intestine, reducing its availability, and thus rendering LDL more susceptible to oxidation.

The possibility that LDL from animals fed NP diets could be more susceptible to ex vivo oxidation is also supported by the rabbit studies of Parthasarathy et al.[25] This possibility coupled with the observation from our laboratory[26] and others[27] that hamsters and rabbits[28,29] fed NP versus SP diets transport more cholesterol in the lower density lipoprotein fractions led us to hypothesize that hamsters fed the NP diet would develop more atherosclerosis than those fed SP diets and that this finding would be associated with not only more elevated plasma LDL-C, but also LDL more susceptible to ex vivo oxidation owing to reduced circulating concentrations of plasma α-tocopherol. To test this hypothesis, Purina chow was analyzed for many micro- and macro-nutrients, and a SP diet was blended to closely mimic the NP diet, especially the vitamin E levels and fatty acid composition. Hamsters were then fed these two diets for 11 weeks, and various blood lipids and α-tocopherol concentrations, ex vivo LDL oxidation, and early aortic atherosclerosis measurements were performed.

Methods and materials

Animals and diets

Thirty male F_1B Golden Syrian hamsters, 7 weeks old, were obtained from BioBreeders Inc. (Fitchburg, MA). The animals were individually housed under standard conditions in hanging wire mesh cages and had unrestricted access to drinking water and feed consisting of chow pellets (Harlan Teklad, No. 8604W, Madison, WI) for 2 weeks. At the end of this acclimatization period, animals were deprived of food for 16 hours and plasma harvested from blood collected via the retro-orbital sinus for plasma cholesterol analysis. Hamsters were then divided into two groups with similar plasma total cholesterol (TC) concentrations and body weight. Hamsters were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication no. 85-32, rev. 1985). Also, the animals were maintained in AAALAC (American Association for the Accreditation of Laboratory Animal Care) accredited facilities, in an environmentally controlled atmosphere (20°C) on a 12/12 h light/dark cycle. The Institutional Animal Care and Use Committee of the University of Massachusetts Lowell approved all animal procedures.

For 11 weeks, the two groups had free access to either a NP diet supplemented with a 10% (wt/wt) oil blend (69.3% edible coconut oil, 18.5% corn oil, 12.2% olive oil) and 0.05% cholesterol, or a SP diet containing the same oil blend and 0.05% cholesterol. Prior to the start of the study, the following components were analyzed in the Purina chow 5001 to allow formulation of the SP diet: fat content, fatty acid composition, protein, carbohydrate, fiber, vitamin E, and a range of minerals (iron, copper, zinc, manganese, and selenium). The compositions of the NP (including the blended oil) and the SP diets are presented in *Table 1*. The carbohydrate composition of the Purina chow 5001 was estimated by the manufacturer to be 84.4% starch, 0.6% glucose, 0.8% fructose, 9.8% sucrose and 4.4% lactose. The carbohydrate source of the semipurified diet was corn starch. The fiber content of the Purina chow 5001 was estimated to be 80% insoluble fiber. Solka-Floc (an insoluble fiber) was used as the fiber source in the semipurified diet. Diets were made in one large batch and stored frozen at −20°C throughout the course of the study. Animals were replenished with their respective diets on a daily basis.

Dietary vitamin E

The acetate concentrations [*d*]-α-tocopherol, [*d*]-γ-tocopherol, and [*d*]-α-tocopheryl in the diets were determined by high-performance liquid chromatography (HPLC). Samples of diet were treated with a mixture of 600 mg trypsin (200 FIP U/g, Merck, Darmstadt, Germany) and 60 mg pepsin (2500 FIP U/g, Merck, Darmstadt, Germany) at 30°C for 30 min to release gelatin-stabilized α-tocopheryl acetate. Tocopherols were extracted with diethyl ether. The solvent was evaporated in a stream of nitrogen, and the residue was dissolved in chloroform: 2-propanol (1:1, v/v). The samples were analyzed on a reverse-phase C-18 column

Table 1 Composition of diets fed hamsters for 11 weeks

	Semipurified g/kg Diet		Nonpurified g/kg Diet
Casein[1]	213.0	Protein[2]	213.0
Fat[3]	47.0	Fat	47.0
Blended oil[4]	91.0	Blended oil	91.0
Corn starch	445.0	Carbohydrate	445.0
Solka-floc	48.0	Fiber	48.0
Vitamin mix[5]	2.9	Vitamin[7]	24.0
Mineral mix[6]	14.5	Minerals[8]	63.0
Water	139.0	Moisture	69.0
Cholesterol	0.50	Cholesterol	0.50

[1]Amino acid composition of casein (g/100 g protein): arginine, 3.7; histidine, 2.8; lysine, 7.5; leucine, 8.4; isoleucine, 5.6; cystine, 0.5; methionine, 2.5; tyrosine 5.6.
[2]Amino acid composition of Purina chow 5001 (g/100 g protein): arginine, 5.9; cystine 1.37; glycine, 5.1; histidine, 2.35; isoleucine, 5.04; leucine, 7.26; lysine 6.07; methionine, 1.84; phenyalanine, 4.4; tyrosine, 2.91; threonine, 3.89; tryptophan, 1.24; valine, 5.17; serine, 5.17; aspartic acid, 12.1; glutamic acid, 19.4; alanine, 6.15; proline, 6.62; taurine, 0.09.
[3]Fatty acid composition of Purina chow 5001 (% total fatty acids): <C14:0, 0.1; C14:0, 0.3; C16:0, 21.1; C18:0, 9.6; C18:1, 34.8; C18:2, 32.1; C18:3, 0.5; C20:0, 0.5; C20:1, 0.3; C22:0, 0.4.
[4]Fat composition of blended oil: 69.3% edible coconut oil, 18.5% corn oil, and 12.2% olive oil. Fatty acid composition of blended oil (% total fatty acids): <C14:0, 45.2; C14:0, 0.3; C16:0, 9.4; C16:1, 0.1; C18:0, 2.3; C18:1, 17.7; C18:2, 12.5; C18:3, 0.3; C20:0, 0.2; C20:1, 0.1; C22:0, 0.1.
[5]Composition of semipurified diet vitamin mixture (mg/kg diet): choline (50%), 1735; myo-inositol, 87; vitamin B-12, 45; thiamin, 5.2; calcium pantothenate, 17.4; nicotinamide, 17.4; riboflavin, 5.2; pyridoxine, 5.5; folic acid, 0.87; vitamin K-3, 3.49; biotin, 0.17; vitamin A, 2.61; vitamin D-3, 0.22; vitamin E, 23.0; corn starch, 697.
[6]Composition of semipurified diet mineral mixture (mg/kg diet) calcium, 407; phosphorous, 879; potassium, 2314; magnesium, 460; sulphur, 34; sodium, 830; chlorine, 575; iron, 261; zinc, 13.8; manganese, 58; copper, 11.6; iodine, 0.15; selenium, 0.20; corn starch, 791.
[7]Composition of nonpurified diet vitamin mix (mg/kg diet): carotene, 4.15; vitamin K-3, 0.46; thiamin, 13.8; riboflavin, 7.4; niacin, 114.2; pantothenic acid, 22.2; choline, 2077; folic acid, 5.4; pyridoxine, 5.5; biotin, 0.18; vitamin B-12, 0.02; vitamin A, 6.1; vitamin D-3 (added), 0.11; vitamin E, 30.3.
[8]Composition of nonpurified diet mineral mix (mg/kg diet): calcium, 8674; phosphorous, 6117; phosphorous (nonphytate), 3652; potassium, 10,043; magnesium, 1917; sulphur, 2557; sodium, 3652; chlorine, 5935; fluoride, 16.0; iron, 181; zinc, 639; manganese, 58.7; copper, 16.4; cobalt, 0.55; iodine, 0.73; chromium, 1.83; selenium, 0.18.

(250 × 4.6 mm, Merck, Darmstadt, Germany) and eluted with methanol: acetonitrile (1:1, v/v). Analysis of stored diets at the end of the study showed no loss in vitamin E content of either diet.

Dietary fat

Dietary fat content was determined gravimetrically following a Soxtec extraction with dichloromethane.

Dietary cholesterol

The cholesterol content of the experimental diets was determined by gas–liquid chromatography (GC) with flame ionization detection (Carlo Erba, HRGC, MEGA 2, Rodano, Italy) using 5 α-cholestane (Sigma, St. Louis, MO USA) as an internal standard. Dietary fat was first extracted into dichloromethane. Following saponification of cholesterol esters by ethanolic potassium hydroxide, the unsaponified component was extracted into ethanol. Free cholesterol was derivatized by reaction with *N*,*O*-bis-(trimethylsilyl)-trifluoracetamid (BSTFA, Merck, Darmstadt, Germany) to form the trimethylsilyl ester, which was injected onto a column (50 m, 0.32 mm, Chrompack, Middelburg, The Netherlands) coated with 0.20 μm CP-Sil 19-CB fused silica.

Dietary fatty acid analysis

Fatty acids were extracted from diets by heptane-ether (1:1, v/v) after addition of C17:0 as internal standard. After isolation, fatty acid methyl esters were prepared by reaction with methanolic HCl. The fatty acid methyl esters were purified on a silica column (Silica Gel 60, Merck, Darmstadt, Germany) by elusion with heptane-ether 9:1 (v/v) and subsequently analyzed by GC (Carlo Erba, HRGC, MEGA 2, Rodano, Italy) using a WCOT (50 m, 0.25 mm) fused silica capillary column coated with 0.20 μm CP Sil 88 (Chrompack Middelburg, The Netherlands).

Dietary fiber analysis

Total dietary fiber was determined in Purina chow 5001 as the residue remaining following incubation of the diet with α-amylase, amyloglucosidase, and protease (Bioquant, Merck, Darmstadt, Germany) and precipitation of digestible matter with ethanol.

Dietary protein analysis

Nitrogen was analyzed in Purina chow 5001 by the Kjeldahl method.

Dietary mineral analysis

Copper, iron, manganese, and zinc contents in the diets were analyzed by plasma emission spectrometry (Perkin-Elmer Plasma 1000, Norwalk, CT USA) after extraction with HCl (final concentration 2 mol/L). The selenium content of the diets was determined by atomic absorption spectrophotometry.

Blood collection

After an overnight of food deprivation, hamsters were tranquilized with a mixture of 50% oxygen and 50% carbon dioxide. Blood samples were collected from the retro-orbital sinus into heparinized capillary tubes. Samples were kept on ice until centrifugation. Plasma was prepared from blood by centrifugation at 1500 *g* at 4°C for 15 min and frozen at −80°C until analysis. Four plasma pools of three or four animals per pool were produced for measuring plasma α-tocopherol and LDL oxidation levels.

Plasma lipid determination

Plasma total cholesterol (TC) and triglycerides (TG) were measured enzymatically using previously reported methods.[30] Plasma

high-density lipoprotein cholesterol (HDL-C) was also determined enzymatically following phosphotungstate-Mg^{+2} precipitation of very low and low-density lipoproteins as we have previously reported.[30] The concentration of plasma non-HDL-C was calculated as the difference between the measured plasma TC and HDL-C. Lipid determinations in our laboratory are standardized by participation in the Center for Disease Control–National Heart, Lung and Blood Institute Standardization Program.

Plasma α-tocopherol measurements

Plasma α-tocopherol (AT) levels were determined by treating 300 μL of plasma with an equal volume of ethanol containing the internal standard tocopheryl acetate and butylated hydroxytoluene (15 mg/L). The samples were agitated in a vortex mixer and AT extracted twice with hexane. The hexane extracts were combined followed by evaporation and reconstitution with HPLC mobile phase. The HPLC conditions used were a modification[31] of the method of Kaplan et al.[32] Accuracy and precision of plasma AT measurements are monitored by participation in the National Institute of Standards and Technology (NIST) Lipid Soluble Vitamin Quality Assurance Program.

Isolation and oxidation of LDL

Plasma LDL was isolated from plasma by single near-vertical spin discontinuous density gradient ultracentrifugation as previously described.[33] Protein concentration of the isolated LDL was determined by a modified Lowry method.[34] LDL oxidation was measured as conjugated diene production by the method of Frei and Gaziano.[35] Conjugated diene formation was monitored every 10 min as the change in 234 nm wavelength absorption as described by Esterbauer et al.[36] Parameters of the conjugated diene assay measured included lag phase (resistance to oxidation), propagation phase (rate of oxidation), and maximum dienes formed.

Aortic morphometric analysis

The quantitation of aortic fatty streak area (early atherosclerosis) in the hamster has been previously described.[37] Units of measurement of fatty streak area are expressed in $\mu m^2/mm^2$ of aortic tissue.

Body weight and food consumption rate

Animal body weights were measured weekly. At this time, individual animals were inspected for diarrhea, feed rejection, or any other signs of illness. Food consumption rates were measured twice per week.

Statistical analysis

SigmaStat software (Jandel Scientific, San Rafael, CA USA) was used for all statistical evaluations. Significant mean differences between the two diet groups for the variables were determined by unpaired Student's *t*-test. Pearson's product-moment correlation coefficients were used to evaluate linear relationships between variables. Values are expressed as means ± SEM, and statistical significance was set at $P < 0.05$.[38]

Results

The fat content of the NP and SP diet was determined to be 13.5% and 13.4% by weight, respectively. Results of the fatty acid analyses of the dietary lipids are reported in *Table 2* and those for the dietary mineral and vitamin E analyses in *Table 3*. The fatty acid profiles were similar for both diets. While the vitamin E levels were fairly similar in both diets, iron levels were higher in the NP diet, and zinc levels were higher in the SP diet. Daily food consumption for the NP- and SP-fed hamsters did not differ throughout the course of the study ($12.7 + 0.1$ vs. $12.3 + 0.2$ g · animal^{-1} · d^{-1}, respectively) although NP-fed hamsters gained significantly more weight during the 11-week period than did the SP-fed hamsters (4.2 ± 0.2 vs. 2.3 ± 0.3 g/wk, respectively). However, none of the significant differences observed in the various biochemical variables and the degree of atherosclerosis between the two groups were significantly correlated with degree of weight gain (data not shown). Although plasma TC concentrations were significantly higher for the SP diet vs. the NP diet (*Table 4*), the non-HDL-C levels were 41% higher in the NP vs. SP fed hamsters ($P < 0.05$). On the other hand, plasma HDL-C concentrations were significantly higher in the SP vs. NP-fed hamsters ($P < 0.05$). This resulted in a significantly greater non-HDL-C/HDL-C and TC/HDL-C ratios in the NP vs. SP fed hamsters ($P < 0.05$) (*Table 4*).

Despite reasonably similar levels of vitamin E in both diets, plasma AT levels obtained from five plasma pools of SP-fed hamsters were up to twofold higher in the SP vs. the NP-fed hamsters (*Table 5*). This difference did not change when the plasma AT was normalized to the plasma TC concentrations in the respective animals (*Table 5*). γ-Tocopherol was not detected in the plasma samples from either dietary treatment.

The lag phase of conjugated diene formation of LDL was

Table 2 Fatty acid composition of dietary lipids

Fatty acid	g/100 g Fatty acids	
	Nonpurified	Semipurified
6:0	0.3	0.3
8:0	3.3	3.5
10:0	2.9	2.9
12:0	21.7	22.2
14:0	9.2	8.4
16:0	12.4	13.5
16:1	0.9	0.2
18:0	4.4	5.0
18:1	22.8	23.8
18:2	18.1	19.2
18:3	1.1	0.4
20:0	0.3	0.3

Table 3 Dietary mineral and vitamin E concentrations (mg/kg)

	Nonpurified	Semipurified
[*d*]-α-Tocopherol	4.1	7.5
[*d*]-α-Tocopherol acetate	15.6	17.0
Tocopherol equivalents	14.5	19.0
Fe	97.8	169.0
Cu	7.0	8.7
Zn	54.0	17.0
Mn	35.3	41.5
Se	0.2	0.2

Table 4 Plasma lipid and lipoprotein cholesterol concentrations and aortic fatty streak area (AFSA) in hamsters fed nonpurified or semipurified hypercholesterolemic diets

	Nonpurified	Semipurified
Total cholesterol (mmol/L)	6.01 ± 0.39	6.81 ± 0.26*
Non-HDL-C (mmol/L)	3.81 ± 0.39	2.82 ± 0.13*
HDL-C (mmol/L)	2.20 ± 0.05	3.99 ± 0.13*
TC/HDL-C (mol/mol)	2.79 ± 0.20	1.72 ± 0.10*
Non-HDL-C/HDL-C (mol/mol)	1.79 ± 0.20	0.72 ± 0.10*
Triglycerides (mmol/L)	2.90 ± 0.33	2.21 ± 0.11*
AFSA ($\mu m^2/mm^2$)	8015 ± 1850	1357 ± 202*

Values are mean ± SEM; $n = 15$.
*Significantly different from nonpurified diet at $P < 0.05$.

50% lower ($P < 0.05$) and the propagation phase was 100% greater ($P < 0.05$) in the four plasma pools analyzed from the hamsters fed the NP vs. the SP diet, respectively (*Table 5*). No difference in maximum conjugated dienes formed was observed between diets.

Early aortic atherosclerosis as defined by accumulation of aortic macrophage-derived foam cells was five times higher in the NP diet vs. the SP diet (*Table 5*). There were no significant correlations among plasma lipids, lipoprotein cholesterol, and early atherosclerosis for both diet treatments treated individually or combined (data not shown).

Discussion

The present study was undertaken to test the hypothesis that hamsters fed the NP diet would have a more atherogenic lipoprotein profile (>non-HDL-C/HDL-C) or (>TC/HDLC), lower plasma AT concentrations, and LDL more susceptible to ex vivo oxidation, which subsequently would result in more early atherosclerosis than hamsters fed the SP diet. This hypothesis came from observations that hamsters fed a NP diet have more plasma non-HDL-C than HDL-C compared to hamsters fed SP diets,[26,27] LDL from NP-fed rabbits are more susceptible to ex vivo oxidation,[25] and, in general, the lack of effect of vitamin E supplementation on atherosclerosis reported in animals fed NP diets as compared to the positive effects of vitamin E supplementation on atherosclerosis observed in animals fed SP diets.[19–24]

Table 5 Plasma α-tocopherol concentrations and susceptibility of low density lipoprotein to copper-induced oxidation in hamsters

	Nonpurified	Semipurified
α-Tocopherol (mmol/L)	0.018 ± 0.001	0.060 ± 0.003*
α-Tocopherol/total cholesterol (mol/mol)	0.003 ± 0.0004	0.009 ± 0.002*
Lag phase (min)	89 ± 5	171 ± 6*
Propagation phase (nmol · min^{-1} · mg^{-1} LDL protein)	9.77 ± 1.03	4.77 ± 0.54*
Maximum dienes formed (nmol/mg LDL protein)	750 ± 12	748 ± 21

Values are mean ± SEM; $n = 4$ pools of 3 or 4 animals/pool.
*Significantly different from nonpurified diet at $P < 0.05$.

However, because none of these studies directly compared NP vs. SP diets with similar micro- and macro-nutrients on the parameters mentioned above, data to support our hypothesis is equivocal. Thus, these studies were undertaken in which a SP diet was formulated with similar nutrient content to a NP diet, especially with regard to vitamin E and fatty acid composition.

For reasons that still remain to be explained, the controlled studies described here confirmed the previous finding that hamsters[26,27] and rabbits[28,29] fed NP diets have a more atherogenic lipoprotein profile (>non-HDL-C/HDL-C and TC/HDL-C) than do those fed the SP diet. Because in the hamster studies, both dietary fatty acids and cholesterol were similar for both treatment groups, we can only conclude that some unidentified component(s) in NP diets induces this different lipoprotein profile.

In the rabbit studies,[28,29] in which the greater atherogenicity of the NP diet was attributed to a more atherogenic lipoprotein profile, other possible mechanism(s) such as LDL vitamin E and oxidizability were not measured. Although there were no significant correlations between any of the biochemical variables and body weight (data not shown), it is still possible that the greater body weights for the NP vs. SP-fed hamsters may have contributed to this difference.

The finding that plasma AT levels were reduced in hamsters fed the NB vs. SP diets, despite similar vitamin E intake, is also unexplained. We have speculated that a component in nonpurified diets either binds vitamin E and thereby reduces its absorption or this unknown component acts as a pro-oxidant, thereby oxidizing dietary available vitamin E resulting in concentrations at which antioxidant efficiency is reduced. It is also possible that oxidative differences in the digestion process may arise following consumption of NP vs. SP diets or that nutrient-nutrient interactions, which could influence the bioavailability of dietary components, are different between the two base diets.

Although the AT content of LDL was not measured directly owing to inadequate sample quantity, plasma AT concentrations generally reflect LDL AT concentrations. Thus, the increased LDL susceptibility to ex vivo oxidation is presumably associated with decreased LDL AT in the NB- vs. SP-diet-fed hamsters. The decreased plasma antioxidant levels and increased LDL oxidation susceptibility activity in the NP- vs. SP-fed hamsters is not readily explained, as one would expect the NP diet to contain more plant-derived antioxidant material than a SP diet.

Despite efforts to provide a similar mineral content for the two diet treatments, the concentration of iron was greater in the SP diet. However, this should have resulted in lower plasma AT concentrations and more LDL susceptibility to ex vivo oxidation in the SP group, as iron is considered to be a pro-oxidant and populations that accumulate tissue iron are more susceptible to atherosclerosis.[39] Reduced plasma AT concentrations and antioxidant activity of hamsters fed the NP diet suggest that the higher zinc content in the NP diet did not appear to be of any consequence, as this mineral in normal amounts is thought to possess antioxidant function[40] and may actually be protective in the development of atherosclerosis.[41]

Similarly, while we were able to match the level of dietary protein between the two treatment groups, the amino acid composition, and in particular the lysine:arginine ratio was twofold greater in the SP (casein)-fed animals compared to the NP-fed hamsters. However, as reported in the studies of Kritchevsky et al.,[42] in rabbits where a greater dietary lysine:arginine ratio was associated with more atherosclerosis, the hamster studies reported in this communication revealed just the opposite.

Because the NP diet has more plant-derived antioxidant material, vitamin A levels were higher in the nonpurified diet. However, a recent study in cholesterol-fed rabbits suggested that pro-vitamin A carotenoids inhibited the development of atherosclerosis.[43] Thus, their study would suggest that the higher levels of vitamin A in the NP diet should have resulted in reduced rather than an enhanced degree of foam cell formation as was observed in the present study.[43] Similarly, the higher niacin content of the NP diet should have been associated with lower plasma non-HDL-C concentrations. Consequently, the higher vitamin content of the NP diet should have resulted in protective rather than atherogenic effects.

Despite the lack of a significant association between plasma non-HDL-C and early aortic atherosclerosis, possibly due to insufficient animal numbers, the higher plasma non-HDL-C concentrations of NP-fed hamsters relative to SP-fed hamsters could have contributed to the greater atherogenicity of the former. Numerous reviews have described the evidence relating the association between hypercholesterolemia and, in particular, elevated plasma LDL-C concentrations and the development of atherosclerosis.[44]

The higher plasma HDL-C concentrations of hamsters fed the SP diet may also have contributed to the reduced early aortic atherosclerosis despite the lack of a significant association between plasma HDL-C and aortic foam cell coverage. This possibility is supported by the putative protective effect of HDL and its major apoprotein apo A-1 in transgenic mouse models[45–47] and the recent hamster studies of Stein et al.,[24] in which the resistance to aortic atherosclerosis was postulated to be the result of the role of HDL in reverse cholesterol transport rather than in prevention of peroxidation.

In conclusion, this study indicates that ingestion of hypercholesterolemic NP diets compared to SP diets, similar in concentration of known micro- and macro-nutrients, is associated with the production of an atherogenic lipoprotein profile, reduced plasma AT concentrations, more oxidatively susceptible LDL, and more early aortic atherosclerosis. The explanation for these findings remains to be elucidated.

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