Lipoprotein Alterations in the Spontaneously Hypertensive Rat Fed Diets Deficient in Selenium and Vitamin E (43731)

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Abstract. **Both vitamin E and selenium (Se) are antioxidant nutrients that play important roles in preventing *in vivo* lipid peroxidation. In this investigation, Se and vitamin E were found to influence lipoprotein levels in the spontaneously hypertensive rat (SHR). Four-week-old inbred SHRs were fed a basal (B) diet with 1% cholesterol deficient in both selenium and vitamin E (B+cho diet) or identical diets to which either vitamin E (B+E+cho) or selenium (B+Se+cho) or both micronutrients were added (B+Se+E+cho). Plasma-cho and lipoprotein-cho levels were measured after 6, 12, 16, and 18 weeks of feeding the experimental diets. Rats fed the B+cho diet for at least 12 weeks had plasma-cho levels about twice that observed for rats fed the B+E+Se+cho diet. Plasma-cho levels for rats in the two Se deficient groups (B+cho and B+E+cho) were, however, similar at any time point. Se deficiency was associated with increased plasma-cho, very low density lipoprotein-cho (VLDL-cho) and low-density lipoprotein-cho (LDL-cho). Vitamin E supplementation interacted with Se deficiency to increase plasma VLDL-cho levels. Neither vitamin E alone nor the interaction between vitamin E and Se consistently influenced LDL-cho levels. The percent cholesteryl ester in LDL from rats fed the Se-deficient diets (B+cho or B+E+cho) was at least twice that observed for rats fed the B+E+Se+cho diet. Plasma lipid peroxidation products were highly elevated in rats fed the B+cho diet compared with values for the B+E+cho or the B+E+Se+cho fed rats (which were not significantly different). These results suggest that dietary Se deficiency increases plasma-cho, VLDL-cho, and LDL-cho levels by a mechanism that may be unrelated to its role as an antioxidant nutrient.** [P.S.E.B.M. 1994, Vol 206]

Considerable evidence suggests that antioxidants play an important role in preventing atherosclerosis by inhibiting oxidative modifications of low-density lipoprotein (1–5). *In vitro* studies have shown that oxidatively modified LDL (OM-LDL) is rapidly taken up by macrophages which subsequently transform into cells closely resembling the "foam cells" observed in early atherosclerotic lesions. LDL modified by lipid peroxidation has been observed in human plasma (2, 3, 6), and in both plasma (3) and the arterial plaques (3, 7) of receptor-deficient Watanabe rabbits. Vitamin E (8, 9) and selenium (10) are antioxidant nutrients that inhibit *in vivo* lipid peroxidation and should, therefore, inhibit the oxidative modifications of LDL. Vitamin E is transported by lipoproteins, and it is the major lipid-soluble antioxidant in plasma. Dietary supplementation with vitamin E inhibits the *in vitro* susceptibility of human LDL to copper-catalyzed oxidation (11). Selenium is a cofactor for glutathione peroxidase which converts small organic hydroperoxides (or H_2O_2) to the corresponding alcohols (or H_2O). A second selenium dependent glutathione peroxidase has been described that reduces phospholipid hydroperoxides and thereby prevents membrane peroxidation (12). In humans, low

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Received September 7, 1993. [P.S.E.B.M. 1994, Vol 206]
Accepted February 15, 1994.

0037-9727/94/2062–0130$10.50/0

plasma and platelet levels of glutathione peroxidase (13) and low dietary intake of vitamin E (14) have recently been associated with increased risk of coronary heart disease. Vitamin E and glutathione peroxidase act synergistically in preventing *in vivo* lipid peroxidation (8). Despite the potential importance of vitamin E and Se in preventing lipid peroxidation, there are very few nutritional studies focussing on the overall influence of these micronutrients on lipid and lipoprotein metabolism. We previously found that Fischer 344 rats fed diets deficient in vitamin E and Se develop elevated levels of LDL-cholesterol (15). In this investigation, we explored the hypothesis that plasma lipid peroxidation, lipoprotein levels, and lipoprotein composition could be altered by *in vivo* lipid peroxidation in vitamin E and/or Se deficient spontaneously hypertensive rats (SHRs). *In vitro* evidence suggest that OM-LDL has an altered receptor mediated catabolism. LDL oxidatively modified by incubation with cultured endothelial cells is rapidly taken up by the "scavenger" or acetyl-LDL receptor on macrophages (16). In contrast, native-LDL is not recognized by the acetyl-LDL receptor (17). *In vitro* lipid peroxidation of LDL results in the formation of malondialdehyde (MDA) and other aldehydes capable of covalently reacting with the lysine residues of LDL. Haberland *et al.* (18) reported that LDL with less that 15% of its lysine residues modified with MDA (<15% LDL) had a decreased uptake by both the apoB,E receptor and the scavenger receptor. However, covalently modifying more that 15% of the LDL lysine residues dramatically increased its uptake by the scavenger receptor. It was plausible, therefore, to hypothesize that a partially oxidized form of LDL (analogous to <15% MDA-LDL) could accumulate *in vivo* until further oxidation converted it to OM-LDL. The SHR present a particularly interesting model for studying the influence of antioxidants on lipoproteins and atherosclerosis. It is known, for example, that SHRs have lower tissue levels of vitamin E than normotensive WKY rats given the same oral dose of vitamin E (19). In humans, hypertension accelerates atherosclerosis, and SHRs exhibit a reduced atheroresistance (20, 21).

Materials and Methods

Experiment 1. *Animals and diets.* Four week old male inbred SHRs weighing 40–50 g were obtained from Charles River's Breeding Laboratories (Wilmington, MA) and randomly divided into four dietary groups (six rats/group). The B + cho group was fed a basal (B) diet deficient in both Se and vitamin E (less than 3 mg of vitamin E/kg of diet and less than 0.03 ppm Se) to which 1.0% cholesterol (cho) was added. The B + cho diet also contained torula yeast (35%), sucrose (42%), tocopherol stripped corn oil (15%), vitamin mix with no vitamin E (2.8%), Draper mineral mix (4%), and methionine (0.25%). The basal diet has been previously described (15), and, with the exception of vitamin E and Se, it contains all the necessary nutrients proposed by the National Research Council (22). The B + E + cho, B + Se + cho, and B + E + Se + cho diets were identical to the B + cho diet except for the addition of vitamin E (50 mg of all-racemic-α-tocopherol/kg diet), Se (0.4 ppm as sodium selenite), or both micronutrients (50 mg of all-racemic-α-tocopherol/kg and 0.4 ppm Se), respectively. The diets were prepared in 2.0-kg batches every 3 weeks (or less) by slowly mixing the constituents to avoid heating, and stored at 4°C in airtight plastic bags from which all possible air was excluded during closure. The drinking water was deionized water with 3 ppm chromium added. The rats were maintained on a 12-hr light:dark cycle (3:00 AM–3:00 PM dark, 3:00 PM–3:00 AM light) while housed in suspended wire-bottomed cages. Rats fed the B + cho diet consumed the least amount of food per day, and these rats were fed *ad libitum*. The amount of food consumed by the B + cho rats was recorded in 2-day intervals (any uneaten food discarded to minimize rancidity), and this amount of food was provided to the rats fed the B + E + cho, B + Se + cho, and B + E + Se + cho diets. The glass and stainless steel feeders were obtained from Hazelton Systems (Aberdeen, MD). All dietary supplies were purchased from United States Biochemical Co. (Cleveland, OH).

Blood collection, glutathione peroxidase, and vitamin E determination. The rats were anesthetized in the middle of their dark cycle with 2,2-dichloro-1,1-difluoroethyl methyl ether (Pitman Moore, Inc., Washington Crossing, NJ) and approximately 1.3 ml of blood collected from the orbital sinus at weeks 6, 12, 16, and 18 on diet. Blood samples were collected into 1.7 ml Eppendorf microfuge tubes containing 15μl of Na_4EDTA (150 mg/ml) as an anticoagulant and centrifuged at 15,600*g* for 10 min. Plasma and red blood cells (RBCs) were separated and plasma was immediately assayed for glutathione peroxidase, vitamin E, plasma-cho, VLDL-cho, LDL-cho, and high-density lipoprotein cholesterol (HDL-cho) as described below. RBCs were resuspended in 0.155 *M* sodium bicarbonate containing 0.1 m*M* CaEDTA and sedimented at 15,600*g*. This wash procedure was repeated two times, followed by the addition of distilled water to the packed RBCs which were then placed in a freezer overnight to insure complete lysis. Lysed RBCs were spun for 15 min at 15,600*g* and aliquots of the supernatant assayed for glutathione peroxidase as described below.

Glutathione peroxidase is a selenoenzyme and its activity in rat plasma and RBCs is a reliable measure

of Se status as measured by neutron activation analysis (23). Glutathione peroxidase activity was measured in RBCs and plasma by the coupled method of Paglia and Valentine (24) using cumene hydroperoxide as a substrate. Plasma vitamin E levels were measured using reverse phase HPLC with an Altex-Ultrasphere-ODS (Beckman Instruments, Inc., Fullerton, CA) 4.6 mm ID × 25 cm column (25). A McPherson Model FL-750 spectrofluorescence detector was utilized with 294 nm excitation and 324 nm emission. Tocol (a generous gift of Hoffman LaRoche Chemical Co.) was used as an internal standard.

Cholesterol determination. Plasma-cho, VLDL-cho, LDL-cho, and HDL-cho were determined enzymatically by a modification of the method of Allain *et al.* (26) in which 2.0% triton X-100 was present to activate cholesteryl ester hydrolase (27). The assay measures unesterified cholesterol plus cholesteryl esters. Reagent I contains phenol (14 m*M*), 4-aminoantipyrine (0.82 m*M*), polyethylene glycol-400 (0.17 m*M*), triton X-100 (0.2%), and sodium cholate (3 m*M*) in 50 m*M* pH 6.7 phosphate buffer. Reagent I was stable for greater than 12 months when stored at −20°C. To insure uniformity, large amounts of reagent I were prepared and stored at −20°C. When required, a 100-ml aliquot of reagent I was thawed and cholesterol oxidase (11.7 units), cholesteryl esterase (3.3 units) and peroxidase (6700 units) were added to constitute the final assay reagent (reagent II) which remained stable at least 1 week at 4°C. A 20-μl lipoprotein aliquot was incubated for 60 min at 37°C with 500 μl of reagent II and the absorbance measured at 500 nm. Cholesterol standards were assayed in parallel with each set of lipoprotein-cho determinations.

Rat lipoprotein separation by heparin-Mn^{+2} precipitation method. Plasma lipoproteins were assayed by a modification of the heparin-Mn^{+2} precipitation method (28). A 75 μl aliquot of NaBr with a density of 1.006 g/ml was added to a 100-μl aliquot of plasma. After centrifugation (2.5 hr, 16,000*g*) in a Beckman Ti42.2 rotor with 200 μl capacity tubes, 30 μl of VLDL was removed from the top of the tube with a Beckman microtube fractionator and enzymatically assayed for cholesterol. The remaining infranate in the microtube was mixed and total HDL-cho and LDL-cho content measured in duplicate 20 μl aliquots. Heparin and Mn^{+2} were added to the remaining infranate to give a final concentration of 185 U/ml and 92 m*M*, respectively. The LDL precipitate was pelleted at 10,000*g* and HDL-cho measured in duplicate 30 μl aliquots of supernatant. Cholesterol standards were assayed in parallel.

Lipoprotein Separation by the Single Discontinuous Gradient Vertical Spin Method. *Experiment 2.* Four-week-old male SHRs weighing 40–50 g were randomly divided into B + cho (seven rats), B + E + cho (six rats) or B + E + Se + cho (six rats) dietary groups. The diets were prepared as described previously except that all-rac-α-tocopheryl acetate was used instead of the free all-rac-α-tocopherol. The rats were fed *ad libitum*. Plasma and RBCs were separated and the plasma analyzed for vitamin E and glutathione peroxidase activity as described above. Plasma levels of lipid peroxidation products were measured using a sensitive fluorescent thiobarbituric acid (TBA) assay (29). RBC-glutathione peroxidase levels were also measured. Plasma lipoproteins were separated for compositional analyses (after 16 weeks of being fed the experimental diets) by the single continuous gradient vertical spin (SVS) method described by Chung *et al.* (30). Plasma (1.0 ml) obtained from individual rats was adjusted to a density of 1.3 g/ml, underlayered with 4.0 ml of 0.154 *M* NaCl in 5 ml Dupont polyalymer ultracentrifuge tubes and centrifuged for 45 min at 369,548*g* at 10°C using a Dupont TV 865 rotor in a Sorvall Ultracentrifuge OTD-65B. After centrifugation, the tubes were placed in an Haakebuchler auto densiflow IIC dispenser and about 18 fractions were collected per tube. The index of refraction of each fraction was measured using a Fischer Scientific refractometer and used to calculate the density. The lipoprotein fractions were pooled based on density: VLDL 1.014–1.016, LDL 1.020–1.062 and HDL 1.062–1.200 g/ml. VLDL-cho could not, however, be accurately determined because a small amount of VLDL was lost from the top of the tube during removal of the cap.

Lipid analysis of pooled LDL and HDL fractions. Triglyceride (TG) was determined enzymatically using a kit from Sigma Chemicals (St. Louis, MO) (31). Phospholipids were assayed by the method of Dittmer *et al.* (32). Cholesterol levels were determined for individual fractions and pooled LDL and HDL samples using the enzymatic cholesterol assay described above. Protein determinations were made by a modification of the Lowry assay (33, 34).

Statistical analyses. The measurements obtained in this investigation were analyzed by a one-way analysis of variance (ANOVA) followed by Scheffe's test. In some cases, two-way ANOVA were also used with dietary Se and vitamin E as factors.

Results

Experiment 1. Table I provides the plasma-cholesterol levels as well as VLDL-cho, LDL-cho, and HDL-cho levels after 6, 12, 16, and 18 weeks of feeding SHRs the experimental diets. SHRs fed the diet deficient in both vitamin E and Se (B + cho diet) for at least 12 weeks consistently had plasma-cholesterol levels about twice that observed for rats fed the diet supplemented with both these nutrients (B + E + Se + cho diet). Moreover, plasma-cholesterol levels for rats fed the two Se deficient diets (B + cho

Table I. Plasma Cholesterol and Lipoprotein Cholesterol Levels of SHR Rats Fed Diets Supplemented or Deficient in Selenium (Se) and/or Vitamin E (E)

Time (weeks)	Diet	Lipoprotein-cholesterol (mg/dl)			
		Plasma (mg/dl)	VLDL	LDL	HDL
6	B + cho	86 ± 2.8[a]	11 ± 1.3[a,b]	44 ± 2.6[a,c]	30 ± 2.4[a]
	B + E + cho	83 ± 4.4[a,b]	16 ± 3.0[a]	51 ± 7.2[a]	21 ± 1.5[a]
	B + Se + cho	68 ± 3.5[a,b]	10 ± 1.2[a,b]	28 ± 2.7[b,c]	29 ± 4.3[a]
	B + E + Se + cho	60 ± 6.0[b]	5 ± 1.6[b]	20 ± 1.4[b]	36 ± 3.9[a]
12	B + cho	113 ± 7.0[a]	14 ± 2.3[a]	48 ± 8.0[a]	51 ± 4.3[a]
	B + E + cho	91 ± 5.1[a]	15 ± 2.9[a]	33 ± 2.9[a,b]	43 ± 6.4[a,b]
	B + Se + cho	74 ± 1.2[a,b]	11 ± 1.1[a,b]	27 ± 1.9[a,b]	37 ± 2.8[a,b]
	B + E + Se + cho	47 ± 6.4[b]	3 ± 0.4[b]	9 ± 2.0[b]	32 ± 2.9[b]
16	B + cho	102 ± 9.0[a]	10 ± 1.4[b]	52 ± 5.4[a]	41 ± 9.4[a]
	B + E + cho	111 ± 2.9[a]	23 ± 2.4[a]	37 ± 3.0[a,b]	51 ± 1.7[a]
	B + Se + cho	90 ± 5.5[a,b]	17 ± 3.2[a,b]	28 ± 6.7[a,b]	45 ± 8.4[a]
	B + E + Se + cho	58 ± 7.3[b]	7 ± 1.2[b]	21 ± 1.8[b]	30 ± 5.8[a]
18	B + cho	111 ± 10.8[a]	11 ± 1.8[b]	61 ± 5.5[a]	39 ± 5.5[a]
	B + E + cho	147 ± 10.3[a]	42 ± 2.5[a]	57 ± 4.8[a]	48 ± 5.5[a]
	B + Se + cho	65 ± 3.5[b]	8 ± 1.9[b]	31 ± 4.5[b]	27 ± 1.7[a]
	B + E + Se + cho	55 ± 4.5[b]	8 ± 0.7[b]	19 ± 2.4[b]	27 ± 5.3[a]

Note. Lipoproteins were separated by the heparin-Mn^{+2} method and cholesterol determined by an enzymatic assay (see Materials and Methods). Data are reported as mean ± SEM (n = 6). Values in any column not sharing a common letter are significantly different ($P < 0.05$) at a given time point.

and B + E + cho) were similar at all time points. Two-way ANOVA with Se and vitamin E as factors indicated that Se was a factor ($P < 0.005$) consistently associated with increased plasma-cholesterol levels at all time points. At Week 16 and 18, two-way ANOVA also indicated that dietary Se and vitamin E interact ($P < 0.05$) to influence plasma-cholesterol levels. In particular, rats fed the vitamin E-supplemented diets showed a greater increase in plasma-cholesterol levels with dietary Se deficiency than did rats fed the vitamin E-deficient diets.

VLDL-cho was found to be significantly higher (by Scheffe's test for multiple comparisons) at every time point for rats fed the diet deficient in Se alone (B + E + cho group) compared with values obtained for rats fed the diet supplemented with both vitamin E and Se (B + E + Se + cho group). Two-way ANOVA indicated that: (i) Se deficiency (B + cho or B + E + cho diets) was consistently associated ($P < 0.05$) with increased VLDL-cho levels at all time points; (ii) Se deficiency interacts with vitamin E supplementation to increase VLDL-cholesterol levels ($P < 0.001$ at Week 16 and 18, $P < 0.07$ at Week 12, $P < 0.01$ at Week 6).

LDL-cho levels for rats fed the Se-deficient diets (B + cho and B + E + cho diets) were similar but were higher in rats fed the B + cho diet compared with rats fed the B + E + Se + cho diet. Two-way ANOVA showed that Se deficiency was associated ($P < 0.005$) with increased LDL-cho levels at all time points. Neither vitamin E alone nor the interactions between vitamin E and Se were found to be consistently important factors influencing LDL-cho levels. HDL-cho levels were, for the most part, not influenced by vitamin E and/or Se deficiencies. The exception was Week 12 when rats fed the B + cho diet had more HDL-cho than rats fed the B + E + Se + cho diet.

After 8 weeks of feeding the experimental diets, plasma vitamin E levels in the B + cho, B + E + cho, B + Se + cho, and B + E + Se + cho groups were 0, 2.1 ± 0.3, 0.4 ± 0.2, and 2.6 ± 0.1 μg/ml of plasma, respectively. Rats fed the vitamin E-deficient diets (B + cho and B + Se + cho) had significantly lower ($P < 0.01$) plasma vitamin E levels than rats fed the vitamin E supplemented diets (B + E + cho and B + Se + E + cho). As expected, plasma and RBC glutathione peroxidase activities (Table II) were significantly decreased at all time points for the Se-deficient rats, (B + cho and B + E + cho) compared with the Se-supplemented rats, (B + Se + cho and B + E + Se + cho). The mean body weights of SHRs were measured and no significant differences in mean body weights were observed until Week 20 when the B + E + cho rats (205 ± 14.2 g) weighed less compared with the B + E + Se + cho (301 ± 19.0 g).

Experiment 2. Experiment 1 showed, in general, no differences in the lipoprotein cholesterol levels of the B + E + Se + cho group compared with the B + Se + cho group at any time point. Therefore, in Experiment 2 only three dietary groups were used. SHRs were maintained on the B + cho or B + E + cho or B + E + Se + cho diet for 22 weeks. All the values reported in this experiment were at Week 16.

Figure 1 shows the cholesterol content (cholesterol plus cholesteryl esters) of each plasma fraction

Table II. Plasma and Red Blood Cell (RBC) Glutathione Peroxidase Activities for SHRs Fed Diets Supplemented or Deficient in Selenium (Se) and/or Vitamin E (E)

Diet	Weeks on diet	Glutathione peroxidase activity	
		Plasma	RBC
B+cho	8	0.1 ± 0[a]	72 ± 3[a]
B+E+cho	8	0.1 ± 0[a]	54 ± 2[a]
B+Se+cho	8	3 ± 1.2[b]	577 ± 9[b]
B+E+Se+cho	8	2 ± 0.4[a,b]	555 ± 237[b]
B+cho	12	0.1 ± 0[a]	67 ± 10[a]
B+E+cho	12	0.1 ± 0[a,b]	56 ± 6[a]
B+Se+cho	12	3 ± 0.4[a,b]	698 ± 57[b]
B+E+Se+cho	12	5 ± 1.5[b]	907 ± 169[b]
B+cho	16	0.1 ± 0[a]	55 ± 13[a]
B+E+cho	16	0[a]	60 ± 10[a]
B+Se+cho	16	6 ± 0.3[b]	687 ± 142[b]
B+E+Se+cho	16	4 ± 0.5[b]	741 ± 109[b]
B+cho	20	0.1 ± 0[a]	135 ± 30[a]
B+E+cho	20	0[a]	61 ± 6[a]
B+Se+cho	20	6 ± 0.2[b]	880 ± 50[b]
B+E+Se+cho	20	6 ± 0.6[b]	1247 ± 296[c]

Note. Plasma and RBC glutathione peroxidase activities (mean ± SEM, $n = 6$) are given as nanomoles of NADPH oxidized per min per μl of plasma and nanomoles of NADPH oxidized per min per mg of hemoglobin, respectively. Values in any column not sharing a common letter are significantly different ($P < 0.05$) at any time point.

obtained by the SVS method as a function of solution density. Figure 1A shows that dietary deficiency of both vitamin E and Se (B+cho group) caused a significant increase in cholesterol in the LDL density range (1.016–1.063 g/ml) and an altered distribution of cholesterol in the HDL range compared with values obtained for the B+E+Se+cho group. The cholesterol content of fractions in the HDL density range were more variable than in the LDL density range. Figure 1B shows that a deficiency in dietary Se alone (B+E+cho) caused a generalized increase ($P < 0.05$) in the cholesterol content at all fraction densities.

The LDL-cho and HDL-cho values were obtained from the fractions pooled at the indicated density ranges at Week 16 (Fig. 1A). As shown in Table III, LDL-cho in the B+cho group were about twice that observed in the B+E+Se+cho group. Similarly, rats fed the B+E+cho diet had significantly higher LDL-cho compared with rats fed the B+E+Se+cho diets, but significantly less LDL-cholesterol than rats fed the B+cho diets.

The lipid composition of HDL and LDL isolated by the SVS method is provided in Figure 2A and 2B, respectively. There was a significant increase in the percent of unesterified cholesterol (C) in LDL from the B+cho group compared with values for either the B+E+cho or B+E+Se+cho group. The percent of cholesteryl ester (CE) in LDL from rats fed the two Se-deficient diets (i.e., the B+E+cho or B+cho

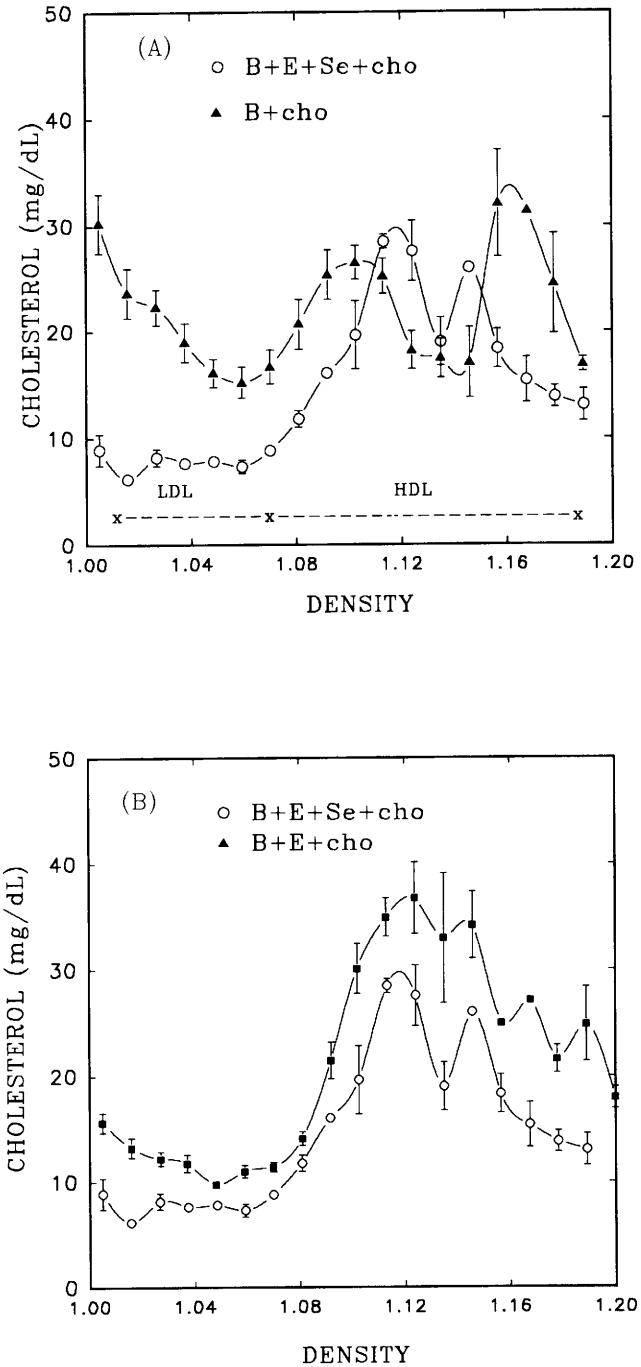

Figure 1. Density distribution of cholesterol (unesterified plus esterified) in plasma fractions after SVS centrifugation. (A) plasma fractions from rats fed the B+E+Se+ cho diet ($n = 6$) or the B+cho diet ($n = 7$) for 16 weeks; (B) plasma fractions from rats fed the B+E+Se+cho ($n = 6$) diet or the B+E+cho diet ($n = 6$) for 16 weeks. Values are mean ± SEM and n is the number of independent experiments.

groups) was at least twice that observed for rats fed the B+E+Se+cho diet. The percent TG of LDL from the B+E+Se+cho group was higher ($P < 0.05$) compared with values from either the B+cho or B+E+cho group. For HDL lipids, the percentage FC in the B+E+Se+cho group was significantly less compared with the B+cho group. No other differ-

Table III. Low-Density Lipoprotein (LDL) and High-Density Lipoprotein (HDL) Cholesterol Levels in SHR Rats Fed Diets Supplemented or Deficient in Selenium (Se) and Vitamin E (E) for 16 Weeks

Dietary group	*n*	LDL	HDL
		(mg cholesterol/dl of Plasma)	
B+cho	7	45 ± 3.6[a]	62 ± 1.4[a]
B+E+cho	6	32 ± 3.3[b]	65 ± 2.1[a,b]
B+E+Se+cho	6	23 ± 1.0[c]	46 ± 5.6[b]

Note. Lipoproteins were isolated from plasma by the single continuous gradient vertical spin method. Data are reported as mean ± SEM. Values in any column not sharing a common letter are significantly different ($P < 0.05$).

ences in HDL lipids were observed between any of the dietary groups.

For Experiment 2 the SHRs were allowed to feed *ad libitum*. At Week 16, there were no differences in either mean body weight or weight gain in the three dietary groups. Food consumption (g/day/kg body wt) in both the B+cho (68 ± 0.4) and B+E+cho groups (64 ± 2.2) was greater compared with the B+E+Se+cho group (56 ± 1.0). Before Week 16, there were no difference in food consumption in the three dietary groups.

Plasma and RBC glutathione peroxidase (GSH-PX) activities, as well as plasma vitamin E levels, were measured for rats fed the experimental diets for 16 weeks, and the results were very similar to those reported above for Experiment 1. Rats fed the B+cho had less ($P < 0.05$) plasma vitamin E (0 ± 0 μg/ml) compared with rats fed either the B+E+cho (1.9 ± 0.1 μg/ml) or the B+E+Se+cho diets (1.5 ± 0.0 μg/ml). Plasma GSH-PX activities were decreased ($P < 0.05$) in both the B+cho (0.1 ± 0.05) and the B+E+cho (0.1 ± 0.05) groups compared with the B+E+Se+cho group (6.1 ± 0.5) but similar in the B+cho and B+E+cho groups. RBC GSH-PX activities were also significantly decreased at Week 16 in both the B+cho (37 ± 5.1) and the B+E+cho (29 ± 5.1) groups compared with values from the B+E+Se+cho group (1050 ± 90) but were similar in the B+cho and B+E+cho groups.

Plasma levels of thiobarbituric acid reactive substances (TBARS) in the B+cho group was significantly higher compared with either the B+E+cho or B+E+Se+cho groups ($P < 0.01$) which were similar (Table IV).

Discussion

In this investigation, dietary Se deficiency (B+cho or B+E+cho diets) was consistently (Week 6–18) associated with increased plasma-cho levels, as well as increased plasma levels of LDL-cho and

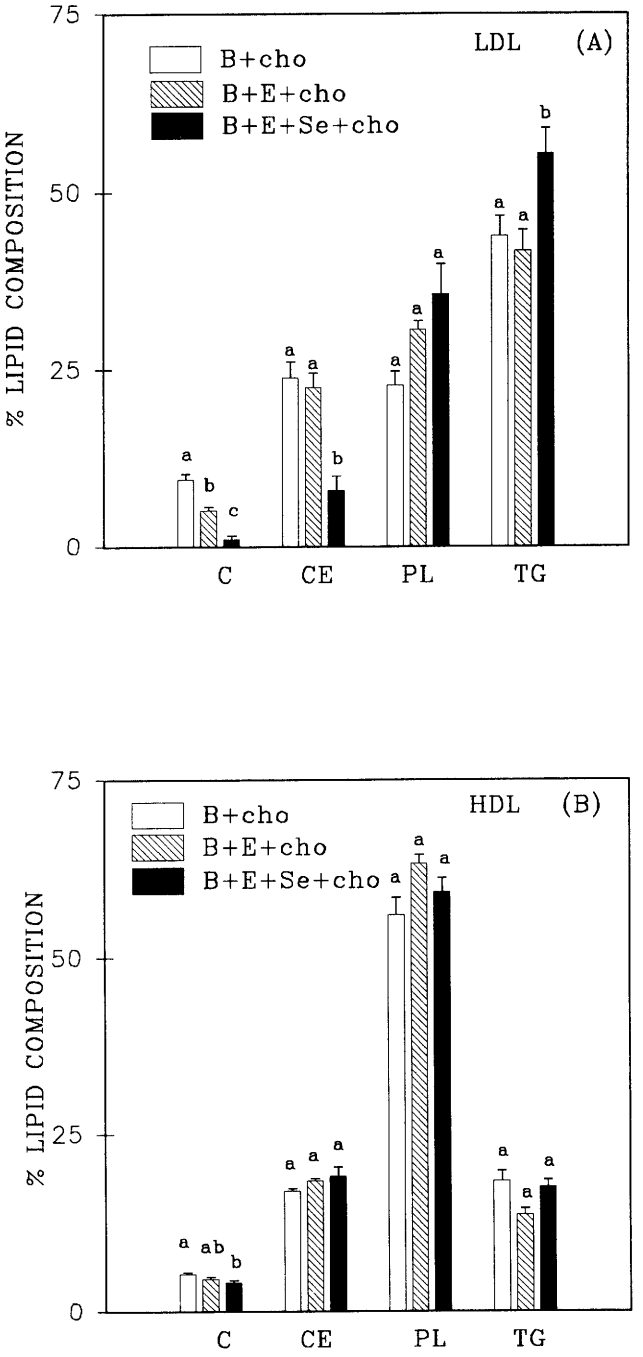

Figure 2. Percent lipid composition of lipoproteins isolated by the SVS method from plasma obtained from rats fed the B+cho diet ($n = 7$), the B+E+cho diet ($n = 6$), and the B+E+Se+cho diet ($n = 6$) for 16 weeks. (A), LDL; (B), HDL. Values are mean ± SEM and n is the number of independent experiments. Means not sharing a common letter are significantly different. ($P < 0.05$).

VLDL-cho (Table I). Moreover, at Week 16 and 18, Se deficiency interacted with vitamin E supplementation to increase plasma-cholesterol and VLDL-cho levels. The data in Figure 2A indicate that Se deficiency increased the CE content of LDL but decreased the TG. The ratio of surface (PL and Cho) lipids to core lipids (TG and CE) was 0.48, 0.52, and 0.57 for LDL isolated

Table IV. Plasma Thiobarbituric Acid Reactive Substances (TBARS) in SHRs Fed Diets Supplemented or Deficient in Selenium (Se) and/or Vitamin E (E) for 16 Weeks

Diet	*n*	nmol TBARS/ml plasma
B + cho	7	136 ± 22[a]
B + E cho	6	4 ± 0.3[b]
B + E + Se + cho	6	4 ± 0.6[b]

Note. Values are given as mean ± SEM. Values not sharing a common letter are significantly different at $P < 0.01$.

from the B + cho, B + E + cho, and B + E + Se + cho rat plasma, respectively (calculated from the data in Fig. 2A). These data suggest that Se deficiency was also associated with slightly smaller LDL particles. HDL levels and composition did not undergo major changes as a result of Se and/or vitamin E deficiency. Rats fed the B + cho diet did, however, have somewhat higher levels of HDL-cho than rats fed the B + E + Se + cho diet (Fig. 2A and Table III). The free cholesterol content was slightly higher in HDL from rats fed the B + cho diet compared with the value observed for rats fed the B + E + Se + cho diet (Fig. 2B).

There are several possible explanations for the observed effects of dietary Se and vitamin E deficiency on lipoprotein-cho levels. Vitamin E and Se are antioxidant nutrients and dietary deficiency of these micronutrients act synergistically to increase *in vivo* lipid peroxidation (8). The data in Table II indicate that both plasma and RBC Se-dependent glutathione peroxidase activities are dramatically decreased in rats fed the Se-deficient diets. Rats fed the vitamin E-deficient diets also had very low plasma tocopherol levels. Table IV shows that a combined deficiency of vitamin E and Se markedly increased plasma TBARS levels. We found, however, that Se deficiency acted synergistically with vitamin E supplementation to increase plasma cholesterol and VLDL-cho levels. Moreover, plasma TBARS for rats fed the diet deficient in Se alone (the B + E + cho diet) were not significantly different from values obtained for rats fed the diet supplemented with both vitamin E and Se (the B + E + Se + cho diet). Nevertheless, Se deficiency alone increased plasma levels of LDL-cho and VLDL-cho without causing a significant increase in plasma TBARS. These data cast doubt on the hypothesis that all the alterations in lipoprotein levels and composition observed in this study could be attributed to *in vivo* oxidative modification of lipoproteins.

The use of TBARS to determine levels of lipid hydroperoxides is, however, limited by the potential lack of specificity of the thiobarbituric acid (TBA) reaction in biological samples (35). Nevertheless, the data in Table IV parallel those reported by Hafeman and Hoekstra (8) who evaluated *in vivo* oxidative stress in rats by measuring the evolution of ethane, a decomposition product of peroxidizing n-3 unsaturated fatty acids. These workers found very little ethane evolution in rats fed a basal torula yeast based diet containing vitamin E (B + E) or both vitamin E and Se (B + E + Se). However, rats fed the basal diet (B diet) deficient in both vitamin E and Se had very high levels of ethane evolution.

We have recently conducted a series of pilot experiments (unpublished) to determine if the expression of apoB,E receptors or acetyl-LDL receptors in SHRs was influenced by dietary vitamin E and/or Se deficiency. Our preliminary results indicate that the plasma clearance of either native-LDL or acetyl-LDL is similar in rats fed the B + E + cho and B + E + Se + cho diets. It is unlikely, therefore, that Se deficiency could cause alterations in the expression of apoB,E or acetyl-LDL receptors.

Alternatively, Se deficiency could increase the hepatic synthesis and secretion of VLDL-cho. We recently explored this possibility using a recirculating rat liver perfusion system. Livers from SHR rats fed the Se deficient diet (B + E + cho) secreted more VLDL and showed a marked decrease in fatty acid oxidation (36). The biochemical basis of this altered hepatic fatty acid metabolism is not yet known but could be due to hypothyroidism caused by Se deficiency. It is interesting that Type I iodothyronine 5′-deiodinase (5′-DI) is a Se-containing microsomal enzyme (37) that converts thyroxine (T4) into the more metabolically active 3,3′,5-tri-iodothyronine (T3). The liver is known to have a very high activity of 5′-DI, and Se deficiency produces a profound drop in hepatic 5′-DI activity (38–40). Arthur *et al.* (38) found a 30% drop in the circulating levels of T3 in rats fed a Se-deficient diet (<0.005 mg/kg diet). Moreover, the hepatic activity of 5′-DI is inhibited by propylthiouracil (PTU). Keyes, Wilcox, and Heimberg (41) reported that livers from rats treated with PTU showed increased secretion of VLDL and decreased fatty acid oxidation; i.e., results very similar to those were recently reported with Se-deficient SHR livers (36). Hypothyroidism, caused by decreased levels of 5′-DI, might contribute to the increased plasma levels of VLDL-cho and LDL-cho observed in Se deficient rats. We are actively exploring this hypothesis.

This research was supported by Grant HL44591 (W.L.S.) from the National Institutes of Health, National Heart, Lung & Blood Institute, Grant 91-01249 (W.L.S.) from the United States Department of Agriculture, and a Grant-in-Aid from the Tennessee Affiliate of the American Heart Association (W.L.S.)

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