IN VIVO CHOLESTEROL OXIDATION RESPONSES TO DIETARY VITAMIN E

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INTRODUCTION

Atherosclerosis is the number one public health problem in the United States. A large body of recent research points to the possible role of lipid peroxidation in the initiation of this disease. Researchers have demonstrated that the lipid or protein moieties in the lipoprotein fractions such as β-VLDL (1), LDL (2), and HDL (3) can be altered by incubation with endothelial cells or with copper ions. The oxidatively-modified lipoproteins may be taken up by scavenger receptors on the monocyte-derived macrophages at a faster rate than the unoxidized counterparts. Because these receptors are not down-regulated by cellular cholesterol, the macrophage can become ladened with lipids resulting in the formation of fatty streaks which are seen in the arotic endothelium during early atherosclerosis.

Lipid peroxidation is quantified frequently by following lipid oxidaton products such as conjugated dienoic acids and malendialdehyde (MDA). Conjugated diene value increases with the formation of lipid hydroperoxides causing the polyunsaturated fatty acids (PUFA) to lose their methylene interrupted double bonds. The newly-formed conjugated double bonds are then monitored at 233 nm (4). This index measures only

the initial stage of lipid oxidation. MDA is the degradation product of long chain polyunsaturated lipid peroxides. It reacts with thiobarbituric acid to produce a red adduct. However, formation of this adduct is highly dependent on sample preparation conditions as well as the presence of interfering substances in the sample matrices (5), making it difficult to compare results across studies and across samples.

Certain lipid oxidation products are shown to be more biologically active than native cholesterol. Evaluating these compounds as an index of lipid peroxidation would offer additional information such as cytotoxic effect or cholester ol-lowering effect of the experimental treatment. Although still controversial, cholesterol oxides are shown by many researchers to be more atherogenic than purified cholesterol. This topic is extensively reviewed recently by Smith (6) and Peng and Morin (7) that their biological effects will not be discussed here. In this study, a group of cholesterol oxidation products were quantified and the effect of vitamin E on in vivo cholesterol oxidation was examined. The findings of two recent prospective studies linked vitamin E supplementation to lower incidence of coronary heart disease in men (8) and women (9). Therefore, the overall objective of this study was to extablish in vivo oxidation of cholesterol by feeding a diet high in PUFA and purified cholesterol to vitamin E-deficient and-sufficient animals. Eight commonly-encountered cholesterol oxides were quantified in the liver and the aorta as an index of cholesterol oxidation resulting from in vivo oxidative stresses.

MATERIALS AND METHODS

Materials. Reagents were obtained from the following sources: cholesterol oxides standards and internal standards for gas liquid chromatography (cholesterol, 5 α—cholestane, 19-hydroxycholesterol, 5,6 α-

epoxy-5 α—cholestan-3 β—ol, 22-hydroxycholesterol, 7-ketocholesterol, 5α-cholestane-3β ,5,6β -triol, and 25-hydroxycholesterol) from Sigma Chemical Co., St. Louis, MO; 7α -hydroxycholesterol, a cholesterol oxide standard, from Research Plus, Bayonne, NJ; 2' ,7'-dichlorofluorescein from J.T. Baker Chemical Co., Phillipsburg, NJ; silica gel Redi-Plate, 0.25 mm thick, 20 x 20 cm, from Fisher Scientific, St. Louis, MO; all solvents used were either HPLC- or Optima-grade from Fisher Scientific.

Animal Study. Thirty female Wistar rats (SASC Inc., St. Louis, MO) weighing between 230-245 g were randomly assigned to three different dietary groups with and without cholesterol and vitamin E: no cholesterol and no vitamin E (Basal), with 1% (wt/wt) cholesterol (CH), and with 1% cholesterol and 100 IU vitamin E/kg diet (CH+E). The basal diet consisted of 20% vitamin-free casein (ICN, Cleveland, OH), 0.3% dl-methionine (ICN), 15% cornstarch (Best Foods, Englewood Cliffs, NJ), 44.5% dextroes (Sigma Chemical Co.), 5% alphacel (ICN), 1% AIN-76 vitamin mixture (vitamin E omitted, USB), 8% vitamin E-Stripped corn oil (USB), and 2% cod liver oil (USB). Cholesterol (purity grade, 95%+, Sigma Chemical Co.) was purified by recrystallizing in methanol just prior to diet preparation. The purity of the resultant cholesterol was checked by capillary gas liquid chromatography (GC). A 97-98% purity can often be achieved. None of the retention times of the contaminants matched the retention times of the eight authentic cholesterol oxide standards. Diets were prepared every two weeks and amount sufficient for one day's feeding were packaged separately and stored at -20℃ to minimize unnecessary exposure to room temperature. Unconsumed feed was discarded and fresh feed was given daily. Once a week, the glass containers were washed and rinsed thoroughly. Food and water were provided ad libitum to rats housed in separate hanging cages. The CH+E group was gavage-fed daily d-α-tocopheryl succinate-saline solution equivalent to 100 IU vitamin E/kg diet.

Animals in the other two groups were gavage-fed daily with an equal volume of saline solution. All procedures relating to the use of animals were in compliance with the National Institutes of Health Guidelines on the care and use of animal subjects and were approved by the university's Animal Care and Use Protocol Review Committee.

Urine was collected every other week for urinary TBARS assay. After 10 weeks, food was removed from the cages and the animals were terminated the following day. Rats were anesthetized with ketamine hydrochloride (85 mg/kg body weight) and xylazine (15 mg/kg body weight). Livers were perfused with cold 0.15 M KCl solution, blotted dry, and dropped into liquid N_2. Aortas were carefully removed and dissected free of adventitious tissue. All samples were stored in -70℃ freezer until analyses. All analyses were performed within three months of sample collection.

Urinary thiobarbituric acid reactive-substance (TBARS). The procedure of Ohkawa et al. (10) was used to determine the TBARS in animals fed the three experimental diets. One ml of urine was used for the analysis and TBARS values were expressed as mmole of urinary creatinine.

Hepatic total lipid and vitamin E. Total lipid was analyzed by the procedure of Folch et al. (11). Total lipid values were used to equalize hepatic vitamin E content. The method of Zaspel and Csallany (12) was used to determine hepatic vitamin E. The procedure was modified slightly in that a fluorescence detector was used in place of a UV detector and that (+)-delta-tocopherol (Sigma Chemical Co.,) was added to the 0.1 g liver as the internal standard.

Total cholesterol and cholesterol oxides. The procedure of Park and Addis (13) as modified by Yan and White (14) with an added lipid fractionation step as described by Horrocks and Sun (15) were used. The following describes briefly the procedure with all the modifications as used

in this study. To 0.2 g liver or 0.1 g of aorta, 0.01 % butylated hydroxytoluene, 19-OH cholesterol and 5 α -cholestane were added. The sample was homogenized in methylene chloride : methanol (2 : 1) and the homogenate was centrifuged at 5000 g for 15 min. The supernatant was transferred to another centrifuge tube and deionized water added to back wash the lipid extract. This mixture was centrifuge again to separate the water layer. The organic layer was dried by rotary-evaporation. The lipid extract was saponified for 18-22 hr in 1 N KOH at room temperature. Water was added and the unsaponifiable was repeatedly extracted with diethyl ether. The combined ether fraction was back-washed until it was neutral. The solvent was removed and separated into its subclasses on a thin-layer chromatographic (TLC) Redi-Plate using the solvent system hexane : diethyl ether : acetic acid (85 : 15 : 2). The TLC plate was sprayed with 0.1% 2',7'-dichlorofluorescein to locate the different lipid bands under UV light. Total cholesterol was determined by combining the 5 α cholestane band and the free cholesterol band whereas the different cholesterol oxides and 19-hydroxycholesterol remained close together around the origin and were scraped as one single band. The lipid was eluted from the silica gel with chloroform : methanol (1 : 1). After the mixture was allowed to stand, 0.1% NaOH was added to remove the dye and the silica gel. The solvent was removed from the organic layer and dried under nitrogen. The lipid was dissolved in pyridine and derivatized in Sylon BTZ (Supelco, Inc., Bellefonte, PA). The silylated samples were chromatographed on a DB-1 column (0.1 micron thick, 15 m long, 0.25 μ m diam.) installed in Varian 3400 GC equipped with a FID detector and capillary column injector port. Peaks in the samples were identified by comparing their retention time with those of the authentic cholesterol and cholesterol oxides standards.

Statistical analyses. All results are presented as group means $\pm$ SEM.

Differences among dietary groups were analyzed by the general linear model of SAS (SAS Institute, Cary, NC). Group differences were considered statistically significant at a level of $P < 0.05$.

RESULTS

Weight gain. Female Wistar rats with initial body weight ranging from 230 to 245 g were randomly assigned into three dietary groups. Three weeks into the study, animals in the Basal group gained less weight than the CH and the CH+E group (Fig. 1). This difference reached significance between Basal and CH ($P < 0.01$) and between Basal and CH+E ($P < 0.05$) at week 7. There was no difference in body weight between CH and CH+E. This trend, however, was not observed in a different study done in our lab at a later date (unpublished observation). Animals in the latter study were fed 10% vitamin E-stripped corn oil instead of the 2% cod liver oil and 8% vitamin E-stripped corn oil. It is not known if the inclusion of cod liver oil and the omission of dietary cholesterol contributes to the differences in weight gain seen in this study or if there were more "bad eaters" in the Basal group.

Liver Morphology. Liver wet weights (g/100g body weight) are shown in Table 1. Animals maintained on the cholesterol supplemented diets (CH and CH+E) had significantly larger livers than those fed the Basal diet ($P < 0.05$). No differences in liver weight were found between the two cholesterol-fed groups. Livers were examined histologically for abnormality since they appeared to be small (mean weight = 2.99 ± 0.19 g/100g) and bright red for the Basal group while they were significantly larger (mean weight = 4.60 ± 0.30 and 4.86 ± 0.30 g/100g) and pale in both the CH and CH+E groups, respectively. Histological examinations showed mild hepatic lipidosis in animals fed the Basal diet and moderate to severe

lipidosis in those fed the CH and CH+E diets.

Hepatic lipids and vitamin E. Total lipids, total cholesterol and hepatic vitamin E are shown in Table 1. Total lipid values were determined to equalize hepatic vitamin E levels as well as to quantify the hyperlipidosis effect of dietary cholesterol. Total lipid values from all three dietary groups were significantly different from one another ($P < 0.05$). Animals from the Basal group had the lowest total hepatic lipid (0.07 ± 0.01 g/g) whereas animals from the CH+E group had the highest (0.24 ± 0.02 g/g). Adding 1% cholesterol to the basal diet resulted in an almost two-fold increase in hepatic total lipid. Vitamin E added to the basal diet with cholsterol (CH+E) led to a further increase in total lipid content.

Hepatic cholesterol was significantly different between the Basal group and the other two cholesterol-supplemented groups. Supplementing the diet with cholesterol resulted in a dramatic increase of between 21- to 24-fold in the cholesterol content of the liver. Addition of vitamin E to the cholestrol-containing diet did not affect liver cholesterol content.

No differences in liver vitamin E content were noted between the Basal and CH groups when the vitamin E levels were expressed either as nmole/g liver or nmole/g total lipid. Hepatic vitamin E content was significantly higher in the group fed the CH+E diet as compared to those fed either the Basal or CH.

Urinary TBARS. Urinary TBARS were determined biweekly throughout the study as an index of lipid peroxidation. Urinary creatinine levels were also determined so as to equalize differences in urine dilution. Although MDA values were shown not to be affected by keeping samples at room temperature for up to 24 hr or by prolonged storage in the freezer(16), in this study TBARS were determined in freshly collected samples. No differences were noted in TBARS between the Basal and CH groups while the CH+E group excreted significantly less TBARS throughout the ten

week period as compered to either of other two groups.

Hepatic cholesterol oxides. The levels of total cholesterol oxides were significantly different ($P < 0.05$) among all three diet groups, lowest in the Basal group and highest in the CH group. Total cholesterol oxides in the CH+E group were elevated but lower than those found in the CH group. A comparison of the levels of the seven commonly-occurring cholesterol oxidation products studied are shown in Table 3. Among animals fed the Basal diet, cholesterol oxidation products from C-7 of the cholesterol molecule (7α -and 7β -hydroxycholesterol and 7-ketocholesterol) were found in the highest amounts, followed by the C-5 oxidation products (5α ,6α-epoxycholesterol and cholestanetriol). Side chain cholesterol oxidation prducts (22-and 25-hydroxycholesterol) were present at very low levels. The addition of cholesterol to the Basal diet resulted in a significant ($P<0.05$) over all increase in the formation of cholesterol oxides with the exception of cholestanetriol, the most atherogenic cholesterol oxidation product. Adding vitamin E to the cholesterol-containing diet resulted in a reduction in cholesterol peroxidation. This is illustrated by significantly lower levels of hepatic total oxides. Among the oxides, 7-ketocholesterol, 7β -hydroxycholesterol, and 5β ,6β -epoxycholesterol were significantly reduced with vitamin E supplementation.

With hepatic vitamin E not significantly different and urinary TBARS values not consistently different between the Basal and the CH groups, one would expect little differences in the level of cholesterol oxidation products seen in the livers of the animals fed the three different diets. Yet, we observed large differences in total cholesterol oxides among all three diet groups. This indicates that tissue vitamin E levels as well as tissue TBARS values are not good predictors of cholesterol peroxidation.

Aortic cholesterol and cholesterol oxides. The differences in total cholesterol levels were less than 30% between diets with and without added

cholesterol (Table 4). The level of cholesterol oxides detected in the aortas followed a similar trend. Animals fed the Basal diet had consistently lower levels of all oxides. The differences were not statistically significant.

DISCUSSION

Using the rat as an animal model to study the effect of hypercholesterolemic diets on human atherosclerosis has been criticized. Unlike humans, rats carry the majority of their cholesterol in the high-density lipoprotein fraction. Nevertheless, many studies involving dietary modifications of different fat sources including oils from a variety of marine sources have been carried out in rats. Although gross atherogenic lesions are not frequently demonstrated in rats fed diets considered highly atherogenic to humans, susceptibility to oxidative stress has been shown in these animals through dietary manipulations. The levels of cholesterol oxidation products found in the liver, aorta, plasma and milk of rats have been shown to be affected by dietary manipulations (17,18). Dietschy and coworkers (19) used humans and different animal species including the rat to study the kinetic characteristics and mechanisms of regulation of receptor-dependent and receptor-independent LDL transport in the liver. The receptor-independent LDL transport and their preferential uptake of modified-LDL has been implicated as a possible cause leading to the development of atherosclerosis (20). It is therefore, not necessary to rely on the development of gross atherogenic lesions in an animal model to demonstrate oxidative susceptibility through dietary manipulations.

Urinary TBARS were determined to establish a general level of in vivo lipid peroxidation in these animals. The determination of TBARS, is useful in predicting the extent of PUFA oxidation since these substances are

predominantly breakdown products of polyunsaturated fatty acids containing five to six double bonds. TBARS however, do not reflect cholesterol peroxidation as shown in Table 2. Although addition of 1% purified cholesterol to the rat diet substantially raised the total cholesterol content of the liver, no differences in TBARS were seen between rats fed the Basal and those fed the CH diets. Basal and CH diets differ only in cholesterol content and should have identical fatty acid profiles. The similarity in TBARS excretion despite the differences in liver total cholesterol and cholesterol oxides between the Basal and CH groups strongly suggests the limited use of TBARS in predicting overall lipid peroxidation. The protective effect of dietary tocopherol on lipid peroxidation is reflected in the consistently lower TBARS in rats given vitamin E (CH+E) as compared to the Basal and CH groups for the duration of the study.

The level of cholesterol oxides in the liver may depend on factors such as hepatic cholesterol concentration, hepatic vitamin E concentration and the type of lipid present in the diet. In this study, we were able to demonstrate the antioxidant effect of vitamin E on in vivo cholesterol oxidation. Bjorkhemet al. (21) recently described the effect of another antioxidant, butylated hydroxytoluene (BHT), on lowering the levels of plasma 5α ,6α -epoxycholesterol and 7-ketocholesterol in BHT-cholesterol-fed animals as compared to cholesterol-fed animals despite the hypercholesterolemic effect of BHT on these rabbits. These results support the contention that vitamin E and similar antioxidant nutrients may play an important role in delaying in vivo cholesterol oxidation.

The amount of cholesterol oxides formed would undoubtedly depend on the availability of the starting substrate. Animals in the Basal group grew steadily over the 10-week period even though their weight became significantly lower just prior to the termination of the study. These

animals must have met their cholesterol needs for growth and maintenance solely through endogenous synthesis. It seems unlikely for cholesterol synthesis to occur in amounts far beyond meeting the body's needs to result in its accumulation in the liver. This is clearly demonstrated by the low levels of total hepatic cholesterol (Table 1) and cholesterol oxides (Table 3) found in this group of rats. The CH and CH+E groups given 1% purified cholesterol had high total hepatic cholesterol oxides. It is doubtful that the cholesterol oxides found in the liver of these animals were totally of dietary origin since we had taken precautionary measures to minimize lipid oxidation in the diets. The high level of cholesterol oxides found in the liver of the cholesterol-fed animals were likely the result of in vivo cholesterol oxidation. Adding 1% cholesterol to the diet inevitably led to cholesterol accumulation in the liver as cholesteryl esters. Halminski et al. (22) fed MaxEPA to Sprague-Dawley rats for ten days and found significant increases in liver and plasma eicosapentaenoic and docosahexaenoic acid values as compared to the palm oil- and safflower oil-fed animals. When animals are maintained on a vitamin E-deficient diet, these long chain PUFAs become susceptible to lipid peroxidation. Free radicals and lipid hydroperoxides generated in the process will attack the cholesterol moieties due to their close proximity to these fatty acids in the phospholipid and triglyceride fractions. It is quite possible that such oxidation are amplified by adding fish oil to the diets tested here. Rogers and Adelstein (23) had observed that MaxEPA increased intimal foam cell formation in rabbits fed an atherogenic diet but not the nonatherogenic diet. Consequently, a diet high in very-long-chain PUFAs could potentially co-oxidize cholesterol leading to the formation of the more biologically-active cholesterol oxides.

The most abundant cholesterol oxide found in all three test diets was 7 α -hydroxycholesterol. Smith (24) suggests that cholesterol oxides can have

both biological as well as oxidative origins. This is especially true for 7α-hydroxycholesterol. One route for decreasing cholesterol pool size is through excretion of cholesterol directly into the bile and the formation of bile acids (25). The first step of bile acid biosynthesis is the formation of 7α -hydroxycholesterol from cholesterol. Therefore it is not surprising to find a high level of 7α -hydroxycholesterol in the liver of cholesterol-fed animals. Yet not all the 7α -hydroxycholesterol found in the liver is derived from bile acid synthesis since the addition of tocopherol in the diet (CH+E) resulted in a lower (though not significant) level of this oxide. A similar observation was noted in a separate study done in our laboratory whereby vitamin E-stripped corn oil was used as the sole source of dietary lipid. Results from that study showed a significant reduction in hepatic 7 α -hydroxycholesterol with dietary tocopherol supplementation (unpublished observations).

Oxidation of cholesterol in vitro generally favors the three most susceptible sites with the allylic C-7 being the most reactive, followed by the double-bond at C-5 and with side-chain oxidation the least frequent (26). A similar pattern seems to hold for in vivo cholesterol oxidation as seen from our results. The C-7 oxides which include 7α -hydroxy, 7β -hydroxy, and 7-ketocholesterol account for over 90% of the total oxides found in all groups of animals. A high level of dietary cholesterol seems to maintain if not heighten the amount of C-7 oxidation. One of the C-5 oxides, 3α ,5β ,6 α -cholestanetriol, considered to be highly atherogenic, was elevated by cholesterol feeding thought not in proportion to the amount of cholesterol fed. The side-chain oxide, 25-hydroxycholesterol, was increased approximately 200-fold by feeding 1% cholesterol. This oxide has been shown to be a potent inhibitor of 3-hydroxy-3 methyl glutaryl CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis (27). Supplementing the diet with 100 IU vitamin E/kg diet did not significantly

reduce the amount of 25-hydroxycholesterol in the liver.

Aortic total cholesterol and cholesterol oxides were determined to establish the effect of feeding a large amount of cholesterol on rat aorta. The difficulty of developing atherogenic lesions in the rat has been a strong criticism for using the rat as a model to study human atherogenesis. Our data show no differences on the levels of total cholesterol and total cholesterol oxides in the aorta of rats fed 1% cholesterol as compared to those fed the basal diet for 10 weeks. The low accumulation of cholesterol and cholesterol oxides in the aorta of these rats may explain the difficulty observed in producing atherogenic lesions in rats fed atherogenic diets. One can speculate from these findings that other animal models like the rabbit, may be as susceptible to atherogenesis as humans as a result of their capability of accumulating cholesteryl esters in the aorta which can become oxidized under oxidative stresses. In fact, rabbits fed an atherogenic diet showed a 50% increase in aortic total cholesterol and 3.7-fold increase in aortic total oxides (28). Supplementation with vitamin E resulted in a significant decrease in aortic cholesterol and lower levels of total oxides in the aortas. It is interesting to note that rat aorta does not respond to dietary cholesterol challenge even though hepatic cholesterol and cholesterol oxides are sufficiently elevated.

With the implication of oxidatively-modified LDL for initiating the atherogenic process, it is important to systematically study not only the role of cholesterol oxidation in this process but also the relationship between dietary cholesterol and in vivo cholesterol oxidation under various oxidative stresses. Results from this study show the limited use of TBARS determination for cholesterol oxidation. Specific investigations of cholesterol oxidation can be performed appropriately by direct quantification of the commonly-encountered cholesterol oxides.

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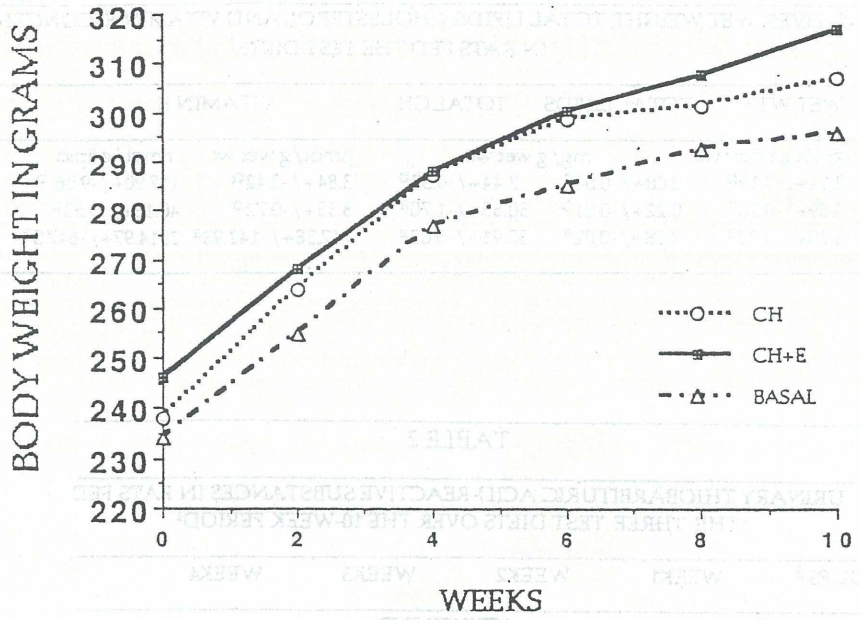

Figure 1. Mean body weights of rats fed the three test diets: Basal (no cholesterol, no vitamin E); CH (1% cholesteerol); CH+E (1% cholesterol, 100 IU vitamin E/kg diet).

TABLE 1

MEAN LIVER WET WEIGHT, TOTAL LIPIDS, CHOLESTEROL AND VITAMIN E CONCENTRATIONS IN RATS FED THE TEST DIETS[1]

DIET GROUP[2]	WET WT	TOTAL LIPIDS	TOTAL CH	VITAMIN E	
	g/100g body wt.	mg/g wet wt.		nmol/g wet wt.	nmol/g lipid
BASAL	3.14+/-0.19^{b}	0.08+/-0.01^{c}	3.44+/-0.33^{b}	3.84+/-0.42^{b}	117.90+/-9.06^{b}
CH	4.89+/-0.30^{a}	0.22+/-0.01^{b}	50.65+/-1.70^{a}	8.55+/-0.72^{b}	40.13+/-3.53^{b}
CH+E	5.20+/-0.30^{a}	0.28+/-0.02^{a}	52.91+/-5.03^{a}	742.38+/-142.93^{a}	2914.97+/-647.3^{a}

TABLE 2

URINARY THIOBARBITURIC ACID-REACTIVE SUBSTANCES IN RATS FED THE THREE TEST DIETS OVER THE 10-WEEK PERIOD[1]

DIET GROUPS[2]	WEEK1	WEEK2	WEEK3	WEEK4
	(μmol/mmol CREATININE)			
BASAL	4.22+/-0.18^{b}	7.83+/-0.93^{a}	6.37+/-0.32^{a}	5.00+/-0.27^{a}
CH	5.68+/-0.40^{a}	6.79+/-0.39^{a}	4.58+/-0.18^{b}	4.66+/-0.29^{a}
CH+E	3.91+/-0.39^{b}	4.37+/-0.22^{b}	3.52^{+}+/-0.37^{c}	.28^{+}+/-0.26^{b}

[1] Values are means +/- SEM. Values with different superscripts in the same column are significantly different at $p<0.05$

[2] Basal: diet with 2% cod liver oil + 8% vitamin E-stripped corn oil; CH: basal diet + 1% purified cholesterol; CH+E: d-α-tocopherol succinate equivalent to 100IU vitamin E/kg diet gavage-fed daily + CH

TABLE 3

CHOLESTEROL OXIDE CONCENTRATION(ug/g wet wt.) IN LIVER OF RATS FED THE THREE TEST DIETS[1]

DIET GROUPS[2]	BASAL	CH	CH+E
CHOLESTEROL OXIDES[3]			
7αOH	1.83+/-0.22^{b}	156.2+/-22.70^{a}	145.91+/-9.00^{a}
7βOH	1.60^{+}+/-0.28^{c}	39.99+/-5.77^{a}	16.28+/-3.07^{b}
7keto	2.83+/-0.43^{b}	51.08+/-8.40^{a}	18.38^{+}+/-4.20^{a}
α epox	0.55+/-0.11^{c}	5.94+/-0.94^{a}	3.72+/-0.65^{b}
triol	0.02+/-0.02^{b}	1.97+/-0.60^{a}	0.37+/-0.20^{a}
22OH	0.04+/-0.02^{b}	0.52^{+}+/-0.56^{a}	0.53+/-0.19^{a}
25OH	0.01+/-0.01^{b}	2.48+/-0.21^{a}	2.82+/-0.16^{a}
TOTAL OXIDES	6.86+/-0.84^{c}	258.45+/-31.20^{a}	183.33+/-22.00^{b}

TABLE 4

CHOLESTEROL OXIDE CONCENTRATION(ug/g wet wt.) AND TOTAL CHOLESTEROL CONCENTRATION (mg/g wet wt) IN AORTA OF RATS FED THE THREE TEST DIETS[1]

DIET GROUPS[2]	BASAL	CH	CH+E
CHOLESTEROL OXIDES[3]			
7αOH	8.86+/-2.51	9.13+/-2.59	5.37+/-1.16
7βOH	8.04+/-2.55	7.76+/-2.43	3.62+/-0.14
7keto	21.97+/-6.80	19.32+/-6.60	12.56+/-4.10
α epox	1.50+/-0.75	0.86+/-0.45	0.62+/-0.50
TOTAL OXIDES	37.93+/-11.92	37.06+/-11.97	22.96+/-6.80
TOTAL CHOLESTEROL	2.51+/-0.16[b]	3.37+/-0.36[a]	3.39+/-0.28[a]

1 Values are means +/- SEM. Values with different superscripts in the same row are significantly different at $p<0.05$

2 Basal: diet with 2% cod liver oil + 8% vitamin E-stripped corn oil; CH: basal diet + 1% purified cholesterol; CH+E: d-a-tocopherol succinate equivalent to 100IU vitamin E/kg diet gavage-fed daily + CH

3 7αOH: 7α-hydroxycholesterol; 7βOH: 7β-hydroxycholesterol; 7keto: 7 ketocholesterol; α-epox: 5α,6α-epoxycholesterol.