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# Vitamin C level and dietary fat saturation alter hepatic cholesterol homeostasis and plasma LDL metabolism in guinea pigs

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*This study addressed the independent and interactive effects of vitamin C status and dietary fat saturation on hepatic cholesterol and LDL metabolism in guinea pigs. Animals were fed adequate (500 mg/kg) or marginal (50 mg/kg) vitamin C with diets high in polyunsaturated (POLY), monounsaturated (MONO), or saturated (SAT) fatty acids during 6 weeks. Plasma cholesterol, triacylglycerol (TAG), and apo B concentrations were higher in animals fed vitamin C-deficient compared with Vit C-adequate diets ($P < 0.02$). SAT fat intake also resulted in more elevated plasma cholesterol and apo B concentrations ($P < 0.001$). The susceptibility of LDL to oxidation was highest in animals fed POLY diets and marginal levels of vitamin C ($P < 0.001$). Intake of adequate levels of vitamin C significantly reduced TBARS formation by 64% in animals fed POLY diets indicating an interactive effect between POLY fat intake and the level of vitamin C. Hepatic Acyl CoA cholesterol acyltransferase (ACAT) activity was higher by SAT fat intake ($P < 0.01$) and marginal intake of vitamin C ($P < 0.05$), whereas cholesterol 7α-hydroxylase activity was lower in vitamin C-deficient groups ($P < 0.01$). LDL fractional catabolic rates (FCR) were 42 to 67% faster and LDL apo B flux slower in animals fed POLY and MONO diets compared with the SAT group ($P < 0.001$). Animals fed marginal levels of vitamin C had higher LDL apo B flux ($P < 0.05$). These data suggest that the higher plasma LDL concentrations induced by SAT diets are caused by both slower LDL FCR and higher LDL apo B flux, whereas the hypercholesterolemia and hypetriglyceridemia induced by vitamin C deficiency with POLY and MONO diets is mainly caused by alterations in hepatic cholesterol homeostasis, which result in higher LDL apo B flux.* (J. Nutr. Biochem. 8:414–424, 1997) © *Elsevier Science Inc. 1997*

**Keywords:** vitamin C; fat saturation; LDL oxidation; LDL receptor; cholesterol 7α-hydroxylase; ACAT; guinea pig

## Introduction

The role of apo B containing lipoproteins as risk factors for cardiovascular disease is documented clearly in epidemiological studies.[1,2] In addition, elevated plasma triacylglycerol (TAG) levels may be involved in the pathogenesis of atherosclerosis.[3] Dietary fat saturation has a major effect on plasma lipid levels and hepatic cholesterol and lipoprotein metabolism.[4] Intake of saturated (SAT) fat results in higher concentrations of plasma LDL cholesterol in man[5,6] and several studied animals.[7–9] In addition to the well known effects of dietary fatty acids on plasma lipoprotein concentrations,[4] there is recent interest on the effects of dietary fat saturation on LDL susceptibility to oxidation. Intake of polyunsaturated (POLY) fat diets results in higher susceptibility to LDL oxidation compared with monounsaturated (MONO) or SAT fat intake.[10] Because of the hypothesized significance of oxidized LDL in the initiating events of the atherogenic process, the effects of diet on LDL oxidation have turned into a critical question.

Vitamin C, probably the most important anti-oxidant

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identified in mammalian systems[11] cannot be synthesized by humans nor by some animals including guinea pigs.[12] Epidemiological studies have demonstrated a negative correlation between vitamin C consumption and plasma cholesterol concentrations.[13] The Basel protective study documented that men with low plasma carotene and ascorbate levels had significant higher risk for coronary heart disease.[14] In addition, increases in plasma TAG concentrations caused by vitamin C deficiency have been reported for guinea pigs.[15] Further in guinea pigs fed for 6 months with vitamin C-deficient diets, lesions in the intima with lipid accumulation, and degenerative changes in the aorta have been observed.[16] Although studies in guinea pigs have addressed the potential mechanisms of hypercholesterolemia and hypertriglyceridemia induced by vitamin C deficiency,[16,17] the specific and interactive effects of fat saturation and vitamin C status on LDL metabolism remains poorly understood.

There is evidence from clinical data and in studies conducted in guinea pigs[12,18] that suboptimal intakes of vitamin C may enhance the potential of LDL oxidation by simultaneous decrease of this antioxidant and other important antioxidant defenses such as vitamin E and glutathione peroxidase.[19] The role of vitamin C status and dietary fat saturation on LDL oxidation needs to be further clarified.

The present studies were undertaken to determine the independent and interactive effects of vitamin C status and dietary fat saturation on hepatic cholesterol and lipoprotein metabolism. The effects of these dietary manipulations were addressed at different sites of action such as the synthesis of lipoproteins, modifications of LDL in the intravascular compartment including susceptibility to oxidation and LDL catabolism. Guinea pigs were chosen as the animal model due to similarities to humans in terms of plasma lipoprotein profile (high LDL relative to HDL),[20] the fact that they cannot synthesize Vitamin C,[12] distribution of hepatic cholesterol pool[21] and the well documented response to dietary fat saturation.[8,9]

## Methods and materials

### *Materials*

Reagents were obtained from the following sources: DL-hydroxy-[3-$^{14}$C]methylglutaryl coenzyme A (1.81 GBq/mmol), DL-[5-$^{3}$H]mevalonic acid (370 GBq/mmol), cholesteryl [1,2,6,7-$^{3}$H]oleate (370 GBq/mmol), [$^{14}$C] cholesterol, aquasol and liquifluor were purchased from New England Nuclear (Boston, MA USA); [1-$^{14}$C]oleoyl coenzyme A (1.8 GBq/mmol) and DL-3-hydroxy-3-methylglutaryl coenzyme A from Amersham (Arlington Heights, IL USA) cholesteryl oleate, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and NADP from Sigma (St. Louis, MO USA); malonaldehyde bis (diethyl acetal) from Aldrich (Milwaukee, WI USA); enzymatic cholesterol kits, cholesterol oxidase, cholesterol esterase, and hydroperoxidase were purchased from Boehringer Mannheim (Indianapolis, IN USA). Quick-seal ultracentrifugation tubes were obtained from Beckman (Palo Alto, CA USA); halothane from Halocarbon Lab., Inc. (Hackensack, NJ USA).

**Table 1** Composition of experimental diets

| Nutrient | Diet composition | |
|---|---|---|
| | gm/100 gm | % calories |
| Protein (casein/soy)* | 22.4 | 23.0 |
| Fat mix† | 15.1 | 35.1 |
| Starch | 11.9 | 12.6 |
| Sucrose | 27.7 | 29.3 |
| Fiber (cellulose/guar gum)‡ | 13.6 | |
| Mineral mix§ | 8.2 | |
| Vitamin mix§ | 1.1 | |
| Vitamin C¶ | 0.05 or 0.005 | |
| Cholesterol | 0.04 | |

*casein: soy 60:40.
†Fat mix was either polyunsaturated, monounsaturated, or saturated (see *Table 2*).
‡Cellulose:guar gum 1:4.
§Mineral and vitamin mixes ares formulated to meet NRC specified requirements for guinea pigs.
¶Adequate diets contained 500 mg/Kg and marginal diets 50 mg/kg vitamin C.

### *Diets*

Diets were prepared and pelleted by Research Diets Inc. (New Brunswick, NJ USA). The six diets had the same composition except for the dietary fat mix and the levels of vitamin C as indicated in *Table 1*. The fat mix was either high in SAT, MONO, or POLY fatty acids. Dietary fatty acid composition for the three types of fat mix is indicated in *Table 2*. The fat mixes were formulated in such a way that the type of fatty acids under evaluation represented 50% of total and calculations were based on documented values for fatty acid composition of the tested oils. For example, the POLY diet was postulated to contain 50% linoleic acid, 25% MONO, and 25% SAT fatty acids. Oils used for the different diets were as follows: SAT: 22% olive, 48% palm kernel, and 30% safflower oil; MONO: 50% olive, 26% palm, 62% safflower, and 5% soybean oil, and for POLY: 7% olive, 26% palm, 62% safflower, and 5% soybean oil. After the fatty acids of the mixtures were analyzed by gas chromatograph as described previously,[8,9] minor discrepancies to the postulated values were obtained. The following compositions were: POLY diet: 52.7% linoleic, 23.5% MONO, and 23.3 total SAT fatty acids; the MONO diet contained 44.9% oleic acid, 24.9% linoleic, and 23.2% SAT fatty acids, and the SAT diet had 49.4% SAT fatty acids with 31.7% contribution from lauric + myristic acids; 19.9% POLY and 26.4% MONO (*Table 2*). Fat represented 35.1% of total energy. Cholesterol content was 0.04% (w/w), which is equivalent to 0.25 times the daily endogenous cholesterol synthesis in guinea

**Table 2** Fatty acid composition of fat mixes (gm/100 gm)

| Fatty acid | Diet* | | |
|---|---|---|---|
| | Polyunsaturated | Monounsaturated | Saturated |
| Lauric (12:0) | 0 | 0.1 | 23.8 |
| Myristic (14:0) | 0.5 | 0.3 | 7.8 |
| Palmitic (16:0) | 16.2 | 16.7 | 9.2 |
| Stearic (18:0) | 7.1 | 6.5 | 8.6 |
| Oleic (18:1) | 23.5 | 44.9 | 19.9 |
| Linoleic (18:2) | 52.7 | 24.9 | 26.4 |
| Other | 0 | 6.6 | 4.3 |

*Values are the average of two determinations.

pigs.[20] Three of the diets contained adequate levels of vitamin C (500 mg/kg), whereas the other three had marginal levels of vitamin C (50 mg/kg) (*Table 1*).

### *Animals*

Male guinea pigs weighing 450 to 500 gm were assigned randomly to one of six dietary groups for 6 weeks. A total of 48 animals were used, 24 for LDL turnover experiments (four from each dietary group) and 24 for determination and characterization of lipoproteins and measurement of hepatic enzyme activity (6 from the POLY, 12 from the MONO, and 6 from the SAT group). They were housed in a light cycle room (0700 to 1900) and had access to diets and water ad libitum. Animals used for the isolation and characterization of lipoproteins were killed by heart puncture after halothane anesthesia. Animals used for the LDL kinetic studies were fasted overnight before surgery (insertion of catheter in carotid vein) and were killed by an excess of halothane vapors. All animal experiments were conducted in accordance with U.S. Public Health Service/U.S. Department of Agriculture guidelines and experimental procedures were approved by the University of Arizona Institutional Animal Care and Use Committee.

### *Isolation and characterization of VLDL and LDL*

Plasma total and lipoprotein cholesterol were determined by enzymatic methods.[21] VLDL (d = 1.006 kg/L) and LDL (d = 1.019–1.09 kg/L) were isolated by ultracentrifugation at 120,000 × g using a Ti50 rotor. Composition was determined by measuring protein,[23] TAG, phospholipids, and cholesterol as reported previously.[9] The number of component molecules of LDL was calculated assuming one apo B per particle with a molecular weight of 412,000 as reported for guinea pigs.[24] The molecular weight of TAG, cholesterol, esterified cholesterol, and phospholipids were calculated as 885.4, 386.6, 646, and 734, respectively, as reported previously.[25] LDL diameters were calculated according to Van Heek and Zilversmith.[26] The molecular weight of LDL was calculated by adding the molecular weight of all LDL components, including apo B.

### *In Vitro determination of LDL oxidation susceptibility*

LDL from individual guinea pigs isolated by ultracentrifugation were dialyzed in EDTA-free phosphate buffered saline (PBS: 10 mM $NaPO_4$ buffer, pH 7.4 containing 0.15 M NaCl). Copper-mediated oxidation of LDL was performed by adding 0.5 mM $CuCl_2 \cdot 2H_2O$ solution to 0.2 mg protein/ml LDL. To determine oxidation kinetics, samples were incubated for different time points in a 37°C water bath. The effect of dietary treatments on the extent of oxidation was measured by incubating samples for 3 hr at 37°C. The lipid peroxide content for oxidized LDL was determined by analyzing thiobarbituric acid-reactive substances (TBARS) expressed as malonaldehyde (MDA) equivalents.[27] The TBARS assay was conducted by adding 2 mL of TBARS reagent (26 mM TBA, 0.92 trichloracetic acid in 0.25 N HCl) to 550 μL incubation mixture and heating in a boiling water bath for 15 min. After removing the tubes from the water bath, 2.5 mL of n-butanol were added. After shaking briefly, the phases were separated by centrifugation at 1,500 × g for 15 min. The pink color developed in the organic layer was read in a spectrophotometer at 532 nm.

### *Hepatic cholesterol and triacylglycerol determination*

Hepatic lipids were determined as described by Carr et al.[28] Briefly 1 g of liver is resuspended in chloroform:methanol (2:1) for lipid extraction overnight. After mixing with acidified water, phases are separated and aliquots are evaporated and resuspended in ethanol to measure total and free cholesterol and triacylglycerol by enzymatic methods. Cholesteryl ester is calculated by subtracting free from total hepatic cholesterol.

### *Hepatic HMG-CoA reductase assay*

Microsomal HMG-CoA reductase (EC 1.1.1.34) activity was measured as described previously.[25] Briefly, 200 μg of microsomal protein was incubated with 7.5 nmol (0.33 Gbq)/nmol) of [3-$^{14}$C]HMG-CoA, 4.5 μmol glucose-6-phosphate, 3.6 μmol EDTA, 0.45 μmol NADP, and 0.3 IU glucose 6-phosphate dehydrogenase for 15 min at 37°C to a final volume of 0.05 mL. [$^3$H]mevalonic acid was used as an internal recovery standard (0.024 GBq per assay). HCl 10 mol/L (0.025 mL per assay) was used to stop the reaction and 1.2 mg/mL of unlabeled mevalonate were added to increase recovery. Samples were further incubated at 37°C for 30 min. After incubation, microsomal protein was precipitated by centrifugation in microfuge for 1 min, and an aliquot of the supernatant was applied to silica gel thin layer chromatography (TLC) plates (Alltech, Deerfield, IL USA). Plates were developed in acetone:benzene (1:1), the area containing mevalonate (Rf 0.6 to 0.9) was scraped and mixed with 5 mL aquasol and radioactivity was measured using a scintillation counter. HMG-CoA reductase activity was expressed as picomoles of [$^{14}$C]mevalonate produced per min per mg microsomal protein. Recoveries of [$^3$H] mevalonate were between 70 and 80%.

### *Hepatic ACAT assay*

ACAT (EC 2.3.2.26) activity was determined by preincubating microsomal protein 0.8 to 1.0 mg per assay with 84 g/L albumin, an amount of albumin equivalent to the molar ratio of the substrate (1:1 albumin:$^{14}$C-oleoyl CoA)[29] and buffer (50 mmol/L $KH_2PO_4$, 1 mol/L sucrose, 50 mmol/L KCl, 30 mmol/L EDTA, and 50 mmol/L NaF) to a final volume of 0.18 mL. After 5 min at 37°C, 20 μL (500 μmol/L) oleoyl-[1-$^{14}$C]coenzyme A (0.15 GBq/pmol) was added, and the reaction proceeded for 15 min at the same temperature. The reaction was stopped by addition of 2.5 mL chloroform:methanol (2:1). A [$^3$H] cholesteryl oleate recovery standard (0.045 GBq per assay) was added, and the sample was mixed and allowed to stand overnight at RT. The aqueous phase was removed, and after evaporation of the organic phase to dryness, samples were resuspended in 150 μL chloroform containing 30 μg unlabeled cholesteryl oleate. Samples were applied to 20 × 20 cm silica gel TLC plates and developed in hexane: diethyl ether (9:1 vol/vol). Cholesteryl oleate was visualized with iodine vapors and scraped from the TLC plates, and 5 mL Liquifluor was added and counted in a scintillation counter. Recoveries of [$^3$H] cholesteryl oleate were between 75 and 90%.

### *Hepatic cholesterol 7α-hydroxylase assay*

Cholesterol 7α-hydroxylase (EC 1.14.13.7) activity was assayed by the method of Shefer and Mosbach[30] as modified by Jelinek et al.[31] using [$^{14}$C] cholesterol as substrate, except that cholesterol was delivered as cholesterol: phosphotidylcholine liposomes (1:8 by weight) prepared by sonication and an NADPH-regenerating system (glucose-6-phosphate dehydrogenase, NADP, and glucose 6-phosphate) was included in the assay as a source of NADPH. After addition of glucose-6-phosphate dehydrogenase (0.3 I.U.), samples were incubated for an additional 30 min. The reaction was stopped by addition of 5 mL chloroform-methanol 3:1 and 1 mL acidified water (5% sulfuric acid). Tubes were mixed, the top layer was discarded and samples were dried under nitrogen. Samples and 7α- and 7β-hydroxycholesterol standards each were dissolved

**Table 3** Weight gain per day and plasma lipids of guinea pigs fed POLY, MONO, and SAT diets in combination with adequate (AA+) and marginal (AA−) levels of ascorbic acid*

| Diet | Weight gain (gm/day) | Plasma lipids (mmol/L) | | Apo B (mg/L) |
|---|---|---|---|---|
| | | Cholesterol | Triacylglycerol | |
| POLY/AA+ | 8.1 ± 1.0[a] | 1.29 ± 0.41[b] | 0.91 ± 0.19[b] | 530 ± 90[b] |
| POLY/AA− | 6.0 ± 1.1[b] | 1.58 ± 0.36[b] | 1.94 ± 1.14[a] | 560 ± 81[b] |
| MONO/AA+ | 7.8 ± 0.5[a] | 1.29 ± 0.23[b] | 1.05 ± 0.30[b] | 690 ± 120[b] |
| MONO/AA− | 6.3 ± 1.1[b] | 1.81 ± 0.44[b] | 1.93 ± 0.98[a] | 1030 ± 140[a] |
| SAT/AA+ | 8.9 ± 2.1[a] | 2.63 ± 1.24[a] | 0.96 ± 0.10[b] | 1060 ± 92[a] |
| SAT/AA− | 7.1 ± 2.1[b] | 3.31 ± 0.67[a] | 1.82 ± 0.70[a] | 1150 ± 171[a] |
| Two-way ANOVA | | | | |
| Fat saturation | N.S. | $P < 0.0001$ | N.S. | $P = 0.006$ |
| Vitamin C status | $P = 0.014$ | $P = 0.019$ | $P < 0.0001$ | $P < 0.0001$ |
| Interaction | N.S. | N.S. | N.S. | $P = 0.039$ |

*Values are the mean ± S.D. for $n = 12$ guinea pigs for animals fed MONO diets and $n = 7$ for animals fed POLY and SAT diets.
Values in the same column with different superscripts are significantly different as determined by two-way ANOVA and Tukey's T-test as post hoc test.
N.S., not significant.

in 100 μL of chloroform, applied to silica gel TLC plates, and developed with ethyl acetate-toluene 3:2. The plate was placed on XAR-5 film with intensifying screen overnight and placed in iodine vapors to mark the 7α- and 7β-hydroxycholesterol standards. Using the film as a guide, the location of the [$^{14}$C]7α-hydroxycholesterol spots was determined, scraped from the plate, and counted in a liquid scintillation counter.

### *Plasma lipoprotein isolation and labeling*

Human LDL was isolated by sequential ultracentrifugation between densities 1.019 and 1.063 kg/L and methylation was carried out as described by Weisgraber et al.[32] Pooled LDL from animals fed POLY, MONO, and SAT diets with adequate (AA+) or marginal (AA−) levels of vitamin C were separated by sequential ultracentrifugation in a L8-M ultracentrifuge (Beckman) at 125,000 × g at 15°C for 19 hr using a Ti50 rotor at a density range of 1.02 to 1.09 kg/L. Lipoproteins were dialyzed against 0.09% NaCl and 0.01% EDTA for 24 hr. Purity of LDL preparations was checked by electrophoresis (data not shown). Lipoproteins were iodinated according to Goldstein et al.[33] with $^{125}$I for guinea pig LDL and $^{131}$I to label human methylated LDL to determine total and nonreceptor-mediated FCR, respectively. Receptor-mediated clearance was calculated by subtracting receptor-independent from total FCR. Labeled lipoproteins were used within 2 days of iodination to minimize possible changes because of radiation oxidation.[34]

### *In vivo LDL kinetics*

Guinea pigs were fasted during the first 10 h of the experiment before surgery. Animals were injected with both isotopes through an indwelling catheter via the carotid artery and plasma samples were taken at 0, 0.5, 1, 2.5, 5, 10, 22, and 28 hr. The plasma disappearance of radiolabeled guinea pig LDL and human LDL was followed by counting plasma samples directly in the gamma counter and LDL FCR were determined by use of a two-pool model as described by Matthews.[35] Apo B protein mass was measured by radial immunodiffusion[36] using guinea pig apo B antibodies raised in sheep and purified by use of antigen affinity purification column[37] as described previously.[38] LDL apo B pool size was calculated by multiplying apo B concentration in mg/dL × plasma volume adjusted to 1 kg weight per animal. Plasma volume was assumed to be 4.5% of guinea pig weight as has been reported.[39] LDL Apo B flux was calculated on the assumption of steady state by multiplying FCR ($h^{-1}$) × apo B pool size (mg/kg). Results for LDL apo B flux are expressed as mg/kg-h.

### *Statistical analysis*

Two-way analysis of variance (ANOVA) was used to determine differences in plasma cholesterol, TAG concentrations, lipoprotein composition, hepatic lipids, LDL oxidation, hepatic enzyme activity, and LDL kinetic parameters due to dietary fat saturation, vitamin C status, and the interaction. Tukey's T-test was used as post-hoc analysis. Linear regression was used to identify significant correlations between variables. $P < 0.05$ was considered significant. Statistical analysis of the kinetic model data were best fitted using a two-pool model (JANA, SCI Software, Lexington, KY USA).

## Results

### *Plasma lipids*

Although the initial weight was the same for all animals, guinea pigs fed the vitamin C-deficient diets gained significantly less weight than animals fed vitamin C-adequate diets (*Table 3*). This lower weight gain was present in animals fed POLY, MONO, and SAT diets and is associated with marginal intake of vitamin C in guinea pigs. No effect from dietary fat saturation was observed in this parameter (*Table 3*).

Plasma cholesterol concentrations were 103% higher ($P < 0.0001$) in guinea pigs fed SAT fat compared to those fed MONO and POLY diets in the vitamin C adequate groups and 109 and 83% higher by consumption of SAT fat compared to intake of POLY and MONO diets in the vitamin C-deficient groups. Marginal levels of vitamin C intake also resulted in higher plasma cholesterol concentrations ($P < 0.02$) (*Table 3*). Plasma TAG concentrations were not affected by dietary fat saturation, whereas there was a clear hypertriglyceridemic effect caused by inadequate levels of vitamin C intake (*Table 3*). Consistent with the findings of plasma cholesterol, apo B concentrations

**Table 4** Plasma lipoprotein cholesterol of guinea pigs fed POLY, MONO, and SAT diets in combination with adequate (AA+) and marginal (AA−) levels of ascorbic acid*

| Diet | Plasma lipoprotein cholesterol (mmol/L) | | |
|---|---|---|---|
| | VLDL | LDL | HDL |
| POLY/AA+ | 0.07 ± 0.03 | 0.83 ± 0.44$^{c}$ | 0.43 ± 0.18 |
| POLY/AA− | 0.11 ± 0.08 | 1.36 ± 0.23$^{b}$ | 0.42 ± 0.04 |
| MONO/AA+ | 0.03 ± 0.01 | 0.92 ± 0.25$^{c}$ | 0.32 ± 0.06 |
| MONO/AA− | 0.13 ± 0.05 | 1.53 ± 0.22$^{b}$ | 0.35 ± 0.16 |
| SAT/AA+ | 0.21 ± 0.29 | 3.06 ± 1.07$^{a}$ | 0.25 ± 0.06 |
| SAT/AA− | 0.13 ± 0.01 | 3.01 ± 0.40$^{a}$ | 0.59 ± 0.39 |
| Two-way ANOVA | | | |
| Fat saturation | N.S. | $P < 0.0001$ | N.S. |
| Vitamin C status | N.S. | $P = 0.05$ | N.S. |
| Interaction | N.S. | N.S. | N.S. |

*Values are the mean ± S.D. for $n = 6$ guinea pigs for animals fed MONO diets and $n = 3$ for animals fed SAT and POLY diets.
Values in the same column with different superscripts are significantly different as determined by two-way ANOVA and Tukey's T-test as post hoc test.
N.S., not significant.

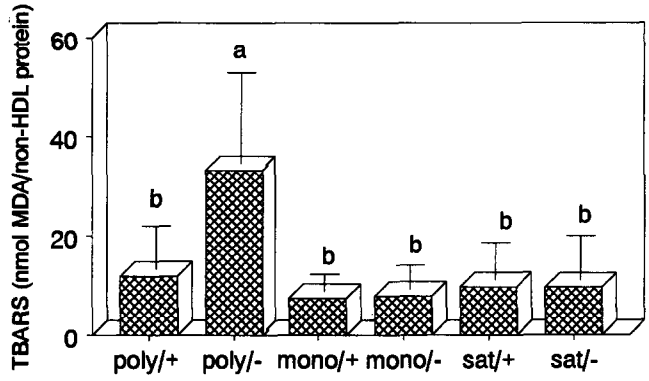


**Figure 1** TBARS expressed as nmol of malonaldehyde formed per non-HDL protein in guinea pigs fed POLY, MONO, and SAT with adequate (aa+) or marginal (aa−) levels of vitamin C. Two-way ANOVA indicated a fat effect ($P < 0.001$), a vitamin C status effect ($P < 0.02$), and an interaction ($P < 0.05$). Different superscripts indicate significantly different.

were highest in animals fed SAT fat and in those with marginal intake of vitamin C ($P < 0.01$).

The hypercholesterolemic effects of fat saturation and vitamin C status were limited to plasma LDL cholesterol concentrations because neither VLDL or HDL were affected (*Table 4*). Animals fed SAT diet had higher plasma LDL cholesterol concentrations than guinea pigs fed MONO or POLY diets ($P < 0.0001$). There were no significant differences in plasma LDL cholesterol levels between animals fed MONO and POLY diets. Guinea pigs fed marginal levels of vitamin C had higher plasma LDL cholesterol concentrations for all three dietary fats tested (*Table 4*).

The number of molecules of the different components of LDL were calculated and differences caused by dietary fat saturation were observed. The number of cholesteryl ester molecules was higher for animals fed SAT compared with MONO or POLY diets ($P < 0.05$), indicating that SAT fat intake results in larger cholesteryl ester enriched LDL. No vitamin C effects were observed in this parameter (*Table 5*). In contrast, the number of free cholesterol molecules was reduced in LDL derived from animals fed diets with marginal levels of vitamin C. The number of TAG molecules was increased in animals fed POLY diets indicating that LDL derived from these animals, specially from the vitamin C marginal group, is cholesteryl-ester poor and TAG enriched. When LDL molecular weights were calculated based on the component molecules of the particles, LDL isolated from guinea pigs fed the SAT diet had higher molecular weight and larger diameters than LDL from animals fed MONO or POLY diets (*Table 5*). No effect attributable to vitamin C status was observed for LDL molecular weight or diameter.

LDL susceptibility to oxidation measured by TBARS formation after three hours of incubation in the presence of $Cu^{2+}$ was higher for animals fed POLY + vitamin C-deficient diets (*Figure 1*) ($P < 0.01$). Two-way ANOVA indicated that guinea pigs fed POLY diets exhibited LDL with greater susceptibility to oxidation than LDL from animals fed MONO or SAT diets ($P < 0.05$), whereas

**Table 5** Number of cholesteryl ester (CE), free cholesterol (FC), phospholipids (PL), triacylglycerol (TAG) molecules, LDL molecular weight and diameter of guinea pigs fed POLY, MONO, and SAT diets in combination with adequate (AA+) and marginal (AA−) levels of ascorbic acid*

| Diet | Number of molecules | | | | M.W. × $10^{-6}$ (μg/mol) | Diameter A |
|---|---|---|---|---|---|---|
| | CE | FC | PL | TAG | | |
| POLY/AA+ | 476 ± 165$^{b}$ | 63 ± 15$^{a}$ | 178 ± 16 | 173 ± 78$^{a}$ | 1.30 ± 0.27$^{b}$ | 210 ± 52 |
| POLY/AA− | 542 ± 137$^{b}$ | 41 ± 22$^{ab}$ | 164 ± 22 | 215 ± 117$^{a}$ | 1.37 ± 0.14$^{b}$ | 255 ± 36 |
| MONO/AA+ | 498 ± 131$^{b}$ | 33 ± 18$^{b}$ | 163 ± 20 | 111 ± 30$^{b}$ | 1.22 ± 0.11$^{b}$ | 218 ± 49 |
| MONO/AA− | 597 ± 93$^{b}$ | 18 ± 8$^{b}$ | 135 ± 23 | 100 ± 20$^{b}$ | 1.26 ± 0.08$^{b}$ | 260 ± 36 |
| SAT/AA+ | 834 ± 241$^{a}$ | 33 ± 32$^{ab}$ | 153 ± 35 | 114 ± 45$^{b}$ | 1.55 ± 0.16$^{a}$ | 323 ± 87 |
| SAT/AA− | 753 ± 80$^{a}$ | 21 ± 13$^{ab}$ | 174 ± 18 | 70 ± 11$^{b}$ | 1.43 ± 0.10$^{a}$ | 276 ± 27 |
| Two-way ANOVA | | | | | | |
| Fat saturation | $P = 0.02$ | N.S. | N.S. | $P = 0.009$ | $P = 0.03$ | $P = 0.03$ |
| Vitamin C status | N.S. | $P = 0.048$ | N.S. | N.S. | N.S. | N.S. |
| Interaction | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. |

*Values are the mean ± S.D. for $n = 6$ guinea pigs for animals fed MONO diets and $n = 3$ for animals fed SAT and POLY diets.
Values in the same column with different superscripts are significantly different as determined by two-way ANOVA and Tukey's T-test as post hoc test.
N.S., not significant.

**Table 6** Hepatic lipids of guinea pigs fed POLY, MONO, and SAT diets in combination with adequate (AA+) and marginal (AA−) levels of ascorbic acid*

| Diet | Hepatic lipids (μg/g) | | |
|---|---|---|---|
| | Free cholesterol | Esterified cholesterol | Triacylglycerol |
| POLY/AA+ | 2.90 ± 0.46[b] | 0.15 ± 0.12[b] | 2.75 ± 1.08[b] |
| POLY/AA− | 3.27 ± 0.59[b] | 0.28 ± 0.03[b] | 3.20 ± 1.55[b] |
| MONO/AA+ | 3.24 ± 0.39[b] | 0.41 ± 0.33[b] | 2.24 ± 0.53[b] |
| MONO/AA− | 3.13 ± 0.28[b] | 0.28 ± 0.18[b] | 4.19 ± 2.32[b] |
| SAT/AA+ | 3.73 ± 0.80[ab] | 1.15 ± 0.90[ab] | 5.63 ± 2.69[ab] |
| SAT/AA− | 4.60 ± 0.85[a] | 2.36 ± 1.52[a] | 8.28 ± 5.03[a] |
| Two-way ANOVA | | | |
| Fat saturation | $P = 0.0013$ | $P = 0.0003$ | $P = 0.009$ |
| Vitamin C status | $P = 0.04$ | N.S. | N.S. |
| Interaction | N.S. | N.S. | N.S. |

*Values are the mean ± S.D. for $n = 6$ guinea pigs for animals fed MONO diets and $n = 3$ for animals fed SAT and POLY diets.
Values in the same column with different superscripts are significantly different as determined by two-way ANOVA and Tukey's T-test as post hoc test.
N.S., not significant.

vitamin C deficiency ($P < 0.05$) augmented LDL oxidation susceptibility. An interaction between POLY intake and vitamin C deficiency ($P < 0.01$) was observed for this parameter.

### *Hepatic lipids*

Hepatic-free cholesterol concentrations were highest in animals fed SAT fat ($P < 0.01$) while vitamin C deficiency also resulted in higher levels of free cholesterol in liver (*Table 6*). Hepatic cholesteryl ester concentrations and TAG were affected by dietary fat saturation only, with highest values observed for animals fed SAT diets (*Table 6*). However, a significant correlation was found between hepatic and plasma TAG concentrations indicating that the higher levels of plasma TAG induced by vitamin C deficiency also result from the increased availability of hepatic TAG to be packed into nascent VLDL (*Figure 2*).

### *Hepatic enzymes*

No effects attributable to dietary fat saturation or vitamin C status were observed in the activity of hepatic HMG-CoA reductase, however, hepatic ACAT was increased by SAT fat intake and by vitamin C deficiency in the POLY and SAT groups (*Table 7*). These increases in ACAT activity were significantly correlated with the higher availability of free cholesterol substrate for this enzyme (*Figure 3*). A positive correlation ($r = 0.79$, $P < 0.01$) was observed between ACAT activity and hepatic-free cholesterol concentrations for animals in all dietary groups.

In addition, hepatic cholesterol 7α-hydroxylase activity was 58%, 22%, and 53% lower in animals fed vitamin C marginal diets with POLY, MONO, and SAT fat, respectively ($P < 0.01$), whereas no effect attributable to fat saturation was observed (*Table 7*). These alterations in hepatic enzyme activity induced by dietary fat saturation and vitamin C deficiency have an effect on LDL turnover and flux as will be addressed below.

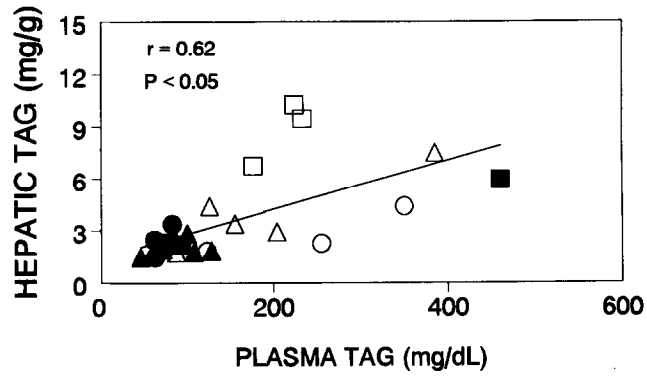


**Figure 2** Correlation ($r = 0.62$, $P < 0.05$) between plasma triacylglycerol (TAG) levels and hepatic TAG concentrations of guinea pigs fed POLY, MONO, and SAT with adequate (aa+) or marginal (aa−) levels of vitamin C. POLY/AA+ (●), POLY/AA− (○), MONO/AA+ (▲), MONO/AA− (△), SAT/AA+ (■), or SAT/AA− (□).

### *LDL Kinetics*

Both dietary fat saturation and vitamin C status significantly altered LDL kinetic parameters. Guinea pigs fed the POLY diets had the smallest LDL apo B pool size, MONO fat intake resulted in intermediate values and animals fed SAT diets had the highest values for apo B pool size (*Table 8*). There was no effect of vitamin C status on LDL apo B pool size. Total and receptor-mediated FCR were 42% and 67% faster in animals fed POLY and MONO diets, respectively, compared with those fed SAT fat ($P < 0.001$) (*Table 8*). No effects of vitamin C deficiency were observed for this parameter. In contrast, LDL apo B flux, calculated by multiplying pool size × FCR as assumed in a steady state, was affected by fat saturation and levels of vitamin C intake. Animals fed SAT diet and marginal levels of vitamin C had higher values for LDL apo B flux than those fed POLY or

**Table 7** Activities of hepatic HMG-CoA reductase, ACAT, and cholesterol 7α-hydroxylase of guinea pigs fed POLY, MONO, and SAT diets in combination with adequate (AA+) and marginal (AA−) levels of ascorbic acid*

| Diet | Hepatic enzymes (pmol/min$^{-1}$ · mg$^{-1}$) | | |
|---|---|---|---|
| | HMG CoA reductase | ACAT | Cholesterol 7α-hydroxylase |
| POLY/AA+ | 4.4 ± 2.4 | 13.6 ± 1.9[c] | 3.8 ± 3.0[ab] |
| POLY/AA− | 4.3 ± 3.9 | 23.8 ± 6.5[bc] | 1.6 ± 0.4[b] |
| MONO/AA+ | 3.1 ± 1.5 | 30.8 ± 16.1[b] | 4.4 ± 0.7[a] |
| MONO/AA− | 2.1 ± 0.6 | 21.7 ± 4.3[bc] | 3.9 ± 1.3[ab] |
| SAT/AA+ | 3.3 ± 3.1 | 29.6 ± 5.9[b] | 5.8 ± 2.8[a] |
| SAT/AA− | 2.4 ± 1.0 | 46.0 ± 8.5[a] | 2.7 ± 0.5[b] |
| Two-way ANOVA | | | |
| Fat saturation | N.S. | $P = 0.0003$ | N.S. |
| Vitamin C status | N.S. | $P = 0.01$ | $P = 0.01$ |
| Interaction | N.S. | N.S. | N.S. |

*Values are the mean ± S.D. for $n = 6$ guinea pigs for animals fed MONO diets and $n = 3$ for animals fed SAT and POLY diets.
Values in the same column with different superscripts are significantly different as determined by two-way ANOVA and Tukey's T-test as post hoc test.
N.S., not significant.

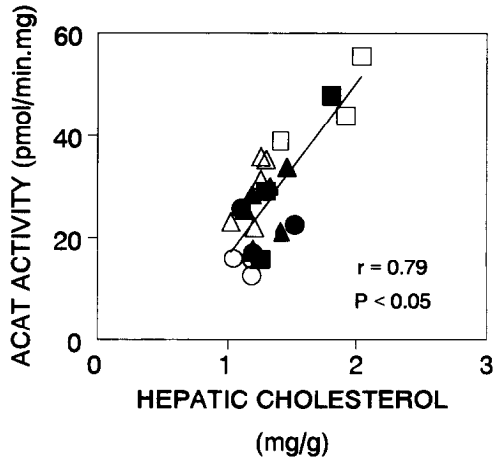


**Figure 3** Correlation between hepatic cholesterol concentrations and ACAT activity ($r = 0.79$, $P < 0.05$) of guinea pigs fed POLY, MONO, and SAT with adequate (aa+) or marginal (aa−) levels of vitamin C. POLY/AA+ (●), POLY/AA− (○), MONO/AA+ (▲), MONO/AA− (△), SAT/AA+ (■), or SAT/AA− (□).

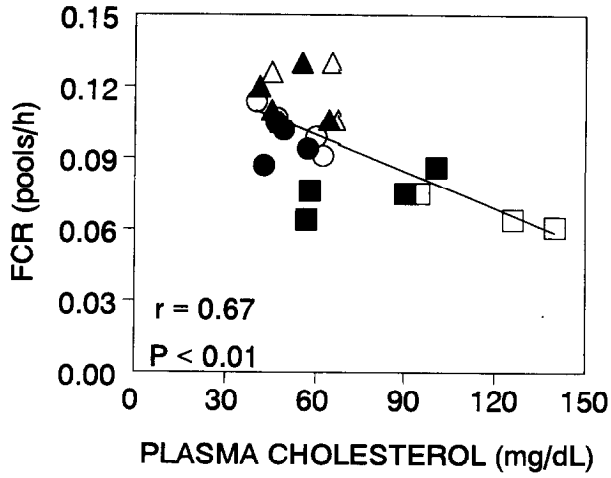


**Figure 4** Correlation between plasma cholesterol concentrations and LDL fractional catabolic rates of guinea pigs fed POLY, MONO, and SAT with adequate (aa+) or marginal (aa−) levels of vitamin C. POLY/AA+ (●), POLY/AA− (○), MONO/AA+ (▲), MONO/AA− (△), SAT/AA+ (■), or SAT/AA− (□). (FCR)

MONO ($P < 0.001$) diets or adequate levels of vitamin C ($P < 0.05$) (*Table 8*). These results suggest that the hypercholesterolemia induced by SAT intake results from decreases in LDL turnover rates associated with increased LDL apo B flux, whereas the hypercholesterolemia and hypertriglyceridemia resulting from vitamin C deficiency are associated with increased LDL apo B flux only.

There was a significant negative correlation between plasma cholesterol concentrations and FCR ($r = 0.67$, $P < 0.05$) (*Figure 4*) indicating that the LDL receptor has a very significant contribution to the observed differences in plasma cholesterol caused by the different dietary treatments.

## Discussion

Hepatic cholesterol homeostasis is maintained by the fine balance between synthesis, secretion, flux and catabolism regulated by specific enzymes.[40] This balance can be perturbed by metabolic alterations and external agents including dietary factors. In addition, the liver plays a major role in determining plasma lipoprotein levels and composition as a result of hepatic cholesterol alterations in metabolism influencing the synthesis of VLDL and the catabolism of apo B containing lipoproteins.[4]

In these studies we have demonstrated that the independent and interactive effects of dietary fat saturation and vitamin C levels have significant effects on plasma cholesterol and triacylglycerol concentrations, on LDL composition and susceptibility to oxidation, and on the mechanisms regulating synthesis and catabolism of lipoproteins. Animals fed SAT fat with vitamin C deficiency had the highest plasma cholesterol levels coupled with high plasma TAG concentrations, a lipid profile associated with increased risk for cardiovascular disease.

**Table 8** LDL kinetic parameters of guinea pigs fed POLY, MONO, and SAT diets in combination with adequate (AA+) and marginal (AA−) levels of ascorbic acid*

| | LDL metabolic parameters | | | |
|---|---|---|---|---|
| Diet | Pool size (mg/Kg) | Total FCR (pools/h) | Receptor-mediated FCR (pools/h) | Apo B flux (mg/kg · h) |
| POLY/AA+ | 23 ± 4$^{c}$ | 0.098 ± 0.008$^{a}$ | 0.060 ± 0.003$^{a}$ | 2.2 ± 0.2$^{c}$ |
| POLY/AA− | 23 ± 3$^{c}$ | 0.102 ± 0.010$^{a}$ | 0.064 ± 0.006$^{a}$ | 2.4 ± 0.1$^{c}$ |
| MONO/AA+ | 29 ± 5$^{bc}$ | 0.116 ± 0.010$^{a}$ | 0.071 ± 0.002$^{a}$ | 3.0 ± 0.4$^{b}$ |
| MONO/AA− | 36 ± 5$^{b}$ | 0.116 ± 0.013$^{a}$ | 0.071 ± 0.017$^{a}$ | 4.4 ± 0.9$^{a}$ |
| SAT/AA+ | 47 ± 3$^{a}$ | 0.075 ± 0.008$^{b}$ | 0.043 ± 0.004$^{b}$ | 3.5 ± 0.6$^{ab}$ |
| SAT/AA− | 52 ± 10$^{a}$ | 0.066 ± 0.007$^{b}$ | 0.029 ± 0.005$^{b}$ | 3.6 ± 0.6$^{a}$ |
| Two-way ANOVA | | | | |
| Fat saturation | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| Vitamin C status | N.S. | N.S. | N.S. | $P = 0.02$ |
| Interaction | N.S. | N.S. | N.S. | N.S. |

*Values are the mean ± S.D. for $n = 4$ guinea pigs.
Values in the same column with different superscripts are significantly different as determined by two-way ANOVA and Tukey's T-test as post hoc test.
N.S., not significant.

### *Vitamin C deficiency, dietary fat saturation and hepatic cholesterol metabolism*

Vitamin C deficiency apparently altered hepatic cholesterol pools by increasing the concentration of free cholesterol. When the effects of marginal intake of vitamin C were examined in the regulatory enzymes of hepatic cholesterol metabolism in the preset studies, a decrease in cholesterol 7α-hydroxylase activity, an increase in hepatic ACAT and no changes in total HMG-CoA reductase activity were observed.

Studies in guinea pigs have demonstrated that increases in hepatic cholesterol might be a result of reductions in cholesterol 7α-hydroxylase activity and decreases in bile acid production.[41] Conversion of cholesterol to bile acids represents the most important regulatory pathway of cholesterol catabolism.[42] It is postulated that vitamin C deficiency is associated with reductions in hepatic microsomal P-450 leading to reductions of cholesterol 7α-hydroxylase activity.[43] In the present studies, the increases in hepatic cholesterol levels could be associated with lower activity of cholesterol 7α-hydroxylase. In addition, bile from guinea pigs fed vitamin C deficient diets has a much lower phospholipid to cholesterol ratio (Fernandez and Lounsbury, unpublished observations) suggesting that these higher concentrations of cholesterol in bile might be related to alterations in the second important route of cholesterol elimination.[42]

Total HMG-CoA reductase activity (determined in microsomes isolated in the absence of NaF) was not altered by vitamin C status demonstrating that augmentation of cholesterol synthesis is not a significant contributor to the elevation of plasma and liver cholesterol observed by marginal intake of vitamin C. Other studies have demonstrated that the active form of the reductase (measured in microsomes isolated in the presence of NaF) is lower in guinea pigs fed Vitamin C deficient or excess diets.[43] Further, cholesterol synthesis measured by incorporation of tritiated water into sterols was also increased in guinea pigs fed vitamin C deficient diets.[43] These studies suggest that ascorbate may influence HMG-CoA reductase activity by modulating the phosphorylation state of the enzyme. It is suggested that this transient inhibition may be mediated through free radical generation during ascorbate metabolism.[43] Although these reported effects on the active form of reductase in liver might not have a significant contribution to the elevated plasma cholesterol levels, the synthesis of cholesterol in extra-hepatic tissues which is of much greater importance in guinea pigs[40,44] might have.

Vitamin C deficiency also resulted in increased esterification of hepatic cholesterol via ACAT activity, which can be correlated with increased incorporation of cholesteryl ester into nascent VLDL and higher synthesis rates of apo B by the liver.[45]

Dietary fat saturation also altered hepatic cholesterol metabolism independently of vitamin C status. SAT fat intake resulted in significant accumulation of hepatic cholesterol and TAG. The increased hepatic ACAT activity attributable to SAT fat intake has been reported previously in guinea pigs fed SAT diets[46] and has been correlated with increased incorporation of cholesteryl ester into VLDL and higher plasma LDL cholesterol concentrations[46] as was confirmed by the present studies.

Carnitine plays a major role in the transport of long chain fatty acids to the mitochondria for β-oxidation and ascorbate is an essential cofactor in its synthesis.[47] The hypertriglyceridemia observed in Vitamin C deficient animals might be a result of suppression of carnitine synthesis. In the case of SAT fat intake, an increase in hepatic TAG concentrations was observed, which might be related to suppression of carnitine synthesis as has been observed in rats fed high cholesterol diets.[48] What it is very clear from these studies is that alterations in hepatic cholesterol metabolism induced by both dietary fat saturation and Vitamin C deficiency significantly affect plasma lipid levels and lipoprotein metabolism.

### *Vitamin C deficiency, dietary fat saturation and lipoprotein metabolism and LDL susceptibility to oxidation*

SAT fat intake and vitamin C deficiency increased plasma cholesterol concentrations and this hypercholesterolemia was specific to LDL. In addition, marginal intakes of vitamin C resulted in hypertriglyceridemia in guinea pigs. Both of these lipid profiles are a result of hepatic alterations in cholesterol and possibly TAG metabolism. Animals fed MONO and POLY diets had similar plasma cholesterol concentrations whether given with marginal or adequate levels of vitamin C. These results are somewhat different from our previous report in which MONO diets consistently induced elevated plasma cholesterol in guinea pigs when compared with POLY intake.[8,49] A likely explanation for these discrepancies could be related to the source and composition of the olive oil used for these studies compared to the earlier ones.[8,49] For the present studies, the olive oil used was acquired from a different vendor and differences in other components of the oil, most likely squalene, might have accounted for the observed discrepancies. The results presented here for the effects of POLY and MONO diets on plasma lipid levels are more consistent with what has been reported for human studies.[6,50]

Animals fed SAT fat exhibited LDL containing more cholesteryl ester than those from guinea pigs fed POLY or MONO diets. In addition, LDL from SAT fed animals had higher molecular weight and a larger diameter. These large, cholesteryl ester enriched particles derived from diets high in SAT fat have been postulated to be atherogenic in African green monkeys.[45] LDL from animals fed POLY diets contained more TAG, less cholesteryl ester and were smaller in size, characteristics that make these particles similar to small LDL subfractions, which are more susceptible to oxidation.[51] In agreement with this observation, LDL from POLY-fed guinea pigs had higher susceptibility to oxidation as evidenced by the increased number of TBARS generated after incubation in the presence of $Cu^{2+}$. A protective effect of vitamin C to LDL oxidation was observed, because intake of adequate levels of vitamin C in combination with POLY fat rendered LDL particles less susceptible to oxidation. In agreement with our observations, lipid peroxide values in liver, kidney, heart, and brain in guinea pigs have been shown to be reduced with vitamin

C supplementation[52] and clinical studies have demonstrated that vitamin C prevents initiation of lipid peroxidation in LDL and that it also protects partially oxidized LDL against further oxidation modification.[53] From these reported studies and our observations we can conclude that adequate vitamin C diets effectively protect against LDL oxidation even in the presence of dietary components known to induce free radical formation.[10]

In addition to the modifications observed in LDL composition and susceptibility to oxidation, vitamin C status and dietary fat saturation significantly altered the parameters of plasma LDL turnover. In agreement with our previous observations, SAT fat intake reduced LDL turnover rates and increased LDL apo B flux.[49,54] Previous reports in guinea pigs have shown that animals fed MONO diets had similar FCR than those fed SAT diets[8,49] but as discussed earlier the faster LDL FCR in animals fed MONO in the present studies can be explained by the different source of olive oil used for preparing the MONO diets. In addition, the faster LDL FCR for the MONO group compared to the SAT group is in agreement with the lower plasma LDL cholesterol concentrations observed in guinea pigs fed MONO diets in the current studies.

Downregulation of LDL receptors has been reported previously by SAT fat intake in guinea pigs[8] and in hamsters.[7] The novelty of this study was to assess whether there was an interactive or an independent effect of vitamin C status and fat saturation on LDL receptor to determine its contribution to the elevated plasma cholesterol levels.

In the present study no effect of vitamin C deficiency on plasma LDL turnover rates was observed. In contrast to our observations, Ginter and Jurcovicoca[55] have reported decreased plasma LDL cholesterol clearance in guinea pigs fed vitamin C-deficient diets. The differences among studies could be explained in terms of the amount of vitamin C used in the deficient diets and possibly in the saturation of the fatty acids. Ginter and Jurcovicoca[55] fed their guinea pigs with a vitamin C-free diet for 2 weeks and supplemented them with 0.5 mg per day of vitamin C, a level substantially lower than the marginal vitamin C diets used in the present study. This higher vitamin C depletion in guinea pigs might have had a more dramatic effect on hepatic cholesterol homeostasis and downregulation of the LDL receptor. In addition, it can be argued that the effects of dietary fat saturation on LDL kinetics are so pronounced that any vitamin C deficiency effect on LDL turnover rates was obscured by the significant contribution of the type of fat to this parameter.

Auslinkas et al.[56] have also shown increases in the number of LDL receptors on cultured aortic smooth muscle cells when supplemented with physiological concentrations of ascorbate. However, the studies by Auslinkas et al.[56] suggest that vitamin C might have an effect on the rate at which the newly synthesized LDL receptor is recycled during the process of endocytosis. It is possible that vitamin C deficiency might be impairing this process in guinea pigs, although it could not be detected under our experimental conditions. Studies measuring LDL receptor number in hepatic membranes could clarify whether vitamin C status alters LDL receptor expression in guinea pigs.

Increases in LDL apo B flux can explain in part the hypercholesterolemic action of marginal intake of vitamin C. The higher rates of LDL apo B flux may be the result of increased conversion of VLDL to LDL or increased synthesis of VLDL by the liver. Guinea pigs fed with marginal levels of Vitamin C presented elevated plasma TAG, and one can speculate that there is higher synthesis of apo B by the liver, which would increase VLDL apo B flux and consequently plasma LDL levels through the delipidation cascade. Since vitamin C deficiency was also correlated with increases in plasma apo B concentrations, this hypothesis seems plausible and would explain the higher LDL apo B flux observed in animals fed marginal levels of vitamin C. In addition, the intravascular processing of VLDL could be accelerated by vitamin C deficiency and increase the rates of LDL formation. Studies by Bobek and Ginter[57] have demonstrated that the activity of tissue lipoprotein lipase, the major enzyme catabolizing plasma TAG, is inhibited in the heart and stimulated in the adipose tissue by high levels of vitamin C. What are the implications of these findings in how vitamin C deficiency affects conversion rates of VLDL to LDL still remains to be elucidated.

From these studies we conclude that vitamin C status and dietary fat saturation independently affect LDL kinetic parameters which, in turn, affect plasma cholesterol concentrations. The alterations induced by these dietary treatments in the liver are of significant importance in the elevations of plasma cholesterol and TAG levels observed by SAT fat intake and vitamin C deficiency. In addition, some beneficial interactive effects were observed between the level of vitamin C and the degree of saturation of the fatty acids. For example, the anti-oxidant properties of vitamin C interact with the susceptibility to oxidation of LDL derived from POLY-fed animals, decreasing the atherogenic potential of small TAG enriched LDL generated from POLY intake.

Although both high intake of SAT fat and consumption of vitamin C-depleted diets resulted in an undesirable plasma lipid profile, the combination of these two dietary insults had a more detrimental effect from increases in both cholesterol and TAG concentrations. In contrast, animals fed MONO and POLY diet with adequate levels of vitamin C presented lower concentrations of plasma cholesterol and TAG, associated with decreased risk for coronary heart disease. In addition, animals fed MONO diets did not exhibit LDL particles with increased susceptibility to oxidation even in the cases of low vitamin C intake indicating a protective effect against free radical formation by MONO intake.

## References


1 Shaefer, E.J., Lichtenstein, A.H., Lamon-Fava, S., McNamara, J., and Ordovas, J.M. (1995). Lipoproteins, nutrition, aging and atherosclerosis. *Am. J. Clin. Nutr.* **61,** 726S–740S

2 Stamler, J., Wentforth, D., and Neaton J.D. (1986). Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA* **256,** 2823–2828

3 Bradley, W.A. and Gianturco, S.H. (1994). Triglyceride-rich lipoproteins and atherosclerosis: pathophysiological considerations. *J. Int. Med.* **361,** 33–39

4 McNamara, D.J. (1992). Dietary fatty acids, lipoproteins and cardiovascular disease. *Adv. Food Nutr. Res.* **36,** 253–361

5 Sheperd, J. Packard, C.J., Grundy, S.M. Gotto, A.M., and Taunton, O.D. (1989). Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. *J. Lipid Res.* **21,** 91–98

6 Mattson, F.H. and Grundy, S.M. (1985). Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid Res.* **26,** 194–202

7 Spady, D.K. and Dietschy, J.M. (1988). Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. *J. Clin. Invest.* **81,** 300–309

8 Fernandez, M.L. and McNamara, D.J. (1991). Regulation of cholesterol and lipoprotein metabolism in the guinea pig mediated by dietary fat quality and quantity. *J. Nutr.* **121,** 934–943

9 Fernandez, M.L., Lin, E.C.K., and McNamara, D.J. (1992). Regulation of guinea pig plasma low density lipoprotein kinetics by dietary fat saturation. *J. Lipid Res.* **33,** 97–109

10 Holvoet, P. and Collen, D. (1994). Oxidized lipoproteins in atherogenesis and thrombosis. *FASEB J.* **8,** 1279–1284

11 Frei, B., England, C., and Ames, B.N. (1989). Ascorbate is an outstanding antioxidant in human blood plasma. *Proc. Natl. Acad. Sci. USA* **86,** 6377–6381

12 Sauberlich, H.E. (1978). Pharmacology of Vitamin C. *Annu. Rev. Nutr.* **14,** 371–391

13 Cerna, O. and Ginter, E. (1978). Blood lipids and vitamin C status. *Lancet* **121,** 1055–1056

14 Gey, K.F., Moser, U.K., Jordan, P., Stahelin, H.B., and Ludin, E. (1993). Increased risk of cardiovascular disease at suboptimal plasma concentrations of essential antioxidants: an epidemiological update with special attention to carotene and vitamin C. *Am. J. Clin. Nutr.* **57,** 787S–797S

15 Ha, T.Y., Otsuka, M., and Arawaka, N. (1990). The effects of graded doses of ascorbic acid on the tissue carnitine and plasma lipid concentrations. *J. Nutr. Sci. Vitaminol.* **36,** 225–227

16 Ginter, E. (1978). Marginal vitamin C deficiency, lipid metabolism and atherogenesis. *Adv. Lipid Res.* **16,** 167–215

17 Ginter, E. (1975). Ascorbic acid in cholesterol and bile acid metabolism. *Ann. NY Acad. Sci.* **258,** 410–416

18 Oliver, M. (1995). Antioxidant nutrients, atherosclerosis, and coronary heart disease. *Br. Heart J.* **73,** 299–301

19 Meister, A. (1994). Glutathione-ascorbic acid antioxidant system in animals. *J. Biol. Chem.* **269,** 9397–9400

20 Lin, E.C.K., Fernandez, M.L., and McNamara, D.J. (1994). Dietary fat type and cholesterol quantity interact to affect cholesterol metabolism in guinea pigs. *J. Nutr.* **122,** 2019–2029

21 Angelin, B., Olivecrona, T., Reihner, E., Rudling, M., Stahlberg, D., Eriksson, M., Ewerth, S., Henriksson, P., and Einarsson, K. (1992). Hepatic cholesterol metabolism in estrogen-treated men. *Gastroenterology* **103,** 1657–1663

22 Allain, C.C., Poon, L.C., Chan, C.G.S., Richmond, W., and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20,** 470–475

23 Markwell, M.A.K., Haas, S.M., Bieber, L.L., and Tolbert, N.E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87,** 206–210

24 Chapman, J.M., Mills, G.L., and Ledford, J.F. (1975). The distribution and partial characterization of the serum apolipoproteins in the guinea pig. *Biochem. J.* **149,** 423–436

25 Fernandez, M.L., Sun, D-M., Montano, C., and McNamara, D.J. (1995). Carbohydrate-fat exchange and regulation of hepatic cholesterol and plasma lipoprotein metabolism in the guinea pig. *Metabolism* **44,** 855–864

26 Van Heek, M. and Zilversmit, D.B. (1991). Mechanisms of hypertriglyceridemia in the coconut oil/cholesterol-fed rabbit. *Arterioscler. Thromb.* **11,** 918–927

27 Puhl, H., Waeg, G., and Esterbauer, H. (1994). Methods to determine oxidation of low-density lipoproteins. *Methods Enzymol.* **233,** 425–441

28 Carr, T., Andressen, C.J., and Rudel, L.L. (1993). Enzymatic determination of triglyceride, free cholesterol and total cholesterol in tissue lipid extracts. *Clin. Chem.* **20,** 470–475

29 Smith, J.L., de Jersey, J., Pillay, S.P., and Hardie, I.R. (1986). Hepatic acyl-CoA:cholesterol acyltransferase. Development of a standard assay and determination in patients with cholesterol gallstones. *Clin. Chim. Acta* **158,** 271–282

30 Shefer, S., Hauser, S., and Mosbach, E.H. (1968). 7α-hydroxylation of cholestanol by rat liver microsomes. *J. Lipid Res.* **9,** 328–333

31 Jelinek, D.F., Andersson, D., Slaughter, C.A., and Russell D. (1990). Cloning and regulation of cholesterol 7α-hydroxylase, the rate limiting enzyme in bile acid biosynthesis. *J. Biol. Chem.* **265,** 8190–8197

32 Weisgraber, K.H., Innerarity, T.L., and Mahley, R.W. (1978). Role of the lysine residues of plasma lipoprotein in high affinity binding to cell surface receptors on human fibroblasts. *J. Biol. Chem.* **253,** 9053–9062

33 Goldstein, J.L., Basu, S.K., and Brown, M.S. Receptor-mediated endocytosis of low density lipoprotein in cultured cells. *Methods Enzymol.* **98,** 241–260, 1983

34 Khouw, A.S., Parhasarathy, S., and Witztum, J.L. (1993). Radioiodination of low density lipoprotein initiates lipid peroxidation: protection by use of antioxidants. *J. Lipid Res.* **34,** 1483–1496

35 Mattehws, C.M.E. (1957). The theory of the tracer experiments with $^{131}$I-labelled plasma lipoproteins. *Phys. Med. Biol.* **2,** 36–53

36 Ishida, B.Y. and Paigen, B. (1992). Silver-enhanced radial immunodiffusion assay of plasma lipoproteins. *J. Lipid Res.* **33,** 1073–1077

37 Stoffel, W. and Demant, T. (1981). Selective removal of apo B-containing serum lipoproteins from blood plasma. *Proc. Natl. Acad. Sci. USA* **12,** 611–615

38 Fernandez, M.L. (1995). Distinct mechanisms of plasma LDL lowering by dietary fiber in the guinea pig: specific effects of pectin, guar gum and psyllium. *J. Lipid Res.* **36,** 2394–2404

39 National Research Council. *Nutrient Requirements for Laboratory Animals. Nutrient Requirements of the Guinea Pig.* National Academy of Sciences, Washington, D.C. USA

40 Dietschy, J.M., Turley, S.D., and Spady, D.K. (1993). Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species including humans. *J. Lipid Res.* **34,** 1637–1658

41 Holloway, D.E., Peterson, F.J., Prigge, W.F., and Gebjard, R. (1981). Influence of dietary ascorbic acid upon enzymes of sterol biosynthesis in the guinea pig. *Biochim. Biophys. Acta* **102,** 1283–1289

42 Wilson, M.D. and Rudel, L.L. (1994). Review of cholesterol absorption with emphasis on dietary and biliary cholesterol. *J. Lipid Res.* **35,** 943–955

43 Greene, Y.J., Harwood, H.J., and Stacpoole, P.W. (1985). Ascorbic acid regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol synthesis in guinea pig liver. *Biochim. Biophys. Acta* **834,** 134–138

44 Fernandez, M.L., Yount, N.Y., and McNamara, D.J. (1990). Whole body and hepatic cholesterol synthesis rates in the guinea pig. Effects of dietary fat quality. *Biochim. Biophys. Acta* **1044,** 340–348

45 Carr, T.P., Parks, S.J., and Rudel, L.L. (1992). Hepatic ACAT activity in African green monkeys is highly correlated to plasma LDL cholesteryl ester enrichment and coronary artery atherosclerosis. *Arterioscler Thromb.* **12,** 1274–1283

46 Fernandez, M.L. and McNamara, D.J. (1994). Dietary fat saturation and chain length modulate guinea pig hepatic cholesterol metabolism. *J. Nutr.* **124,** 331–339

47 Hulse, J.D., Ellis, S.R., and Henderson, L.M. (1978). Carnitine biosynthesis. *J. Biol. Chem.* **253,** 1654–1661

48 Fungwe, T.V., Cagen, L.M., Cook, G.A. Wilcox, H.G., and Heimberg, M. (1993). Dietary cholesterol stimulates hepatic biosynthesis of triglyceride and reduces oxidation of fatty acids in the rat. *J. Lipid Res.* **34,** 933–941

49 Fernandez, M.L., Lin, E.C.K., and McNamara, D.J. (1992). Differential effects of saturated fatty acids on low density lipoprotein metabolism in the guinea pig. *J. Lipid Res.* **33,** 1833–1842

50 Grundy, S.M. (1989). Monounsaturated fatty acids and cholesterol metabolism: implications for dietary recommendations. *J. Nutr.* **119,** 529–533

51 Kraus, R.M. and Burke, D.J. (1982). Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J. Lipid Res.* **23,** 97–104

52 Kaplan, B. (1995). Correlations between lipid peroxide and glutathione levels in vitamin C supplemented fasting guinea pigs. *Horm. Metab. Res.* **27,** 415–420

53 Retsky, K.L. and Frer, B. (1995). Vitamin C prevents metal ion-dependent initiation and propagation of lipid peroxidation in human low-density lipoprotein. *Biochim. Biophys. Acta* **1257,** 279–287

54 Fernandez, M.L., Lin, E.C.K., and McNamara, D.J. (1992). Differential effects of saturated fatty acids on low density lipoprotein metabolism in the guinea pig. *J. Lipid Res.* **33,** 1833–1842

55 Ginter, E. and Jurcovicoca, L. (1987). Chronic vitamin C deficiency lowers fractional catabolic rate of low density lipoprotein in guinea pigs. *Ann. NY Acad. Sci.* **498,** 473–475

56 Auslinkas, T.H., Westhuysen, D.R., and Koetze, G.A. (1983). Ascorbate increases the number of low density lipoprotein receptors in cultured arterial smooth muscle cells. *Atherosclerosis* **47,** 159–171

57 Bobeck, P. and Ginter, E. (1978). Serum triglycerides and post-heparin lipolytic activity in guinea pigs with latent vitamin C deficiency. *Experentia* **34,** 1554–1555