Subchronic Toxicity Studies of SALATRIM Structured Triacylglycerols in Rats. 3. Triacylglycerols Composed of Stearate, Acetate, Propionate, and Butyrate

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SALATRIM 234CS lot A018 and SALATRIM 234CA lot A019 are structured triacylglycerols having lower caloric availability (4.5–6.0 kcal/g) than corn oil (9.0 kcal/g). Rats were fed both SALATRIM fats at 2%, 5%, and 10% or corn oil at 10% in the diet for 13 weeks. Body weight and feed consumption varied predictably with caloric density of the diets. Ophthalmologic observations, serum and liver concentrations of fat-soluble vitamins, clinical pathology, organ weights, and necropsy findings were unaffected by treatment. Small variations in bone mineral concentrations of 10% SALATRIM and corn oil rats were considered to be related to the levels of unsaturated fatty acids in these diets. A slightly increased incidence of renal mineralization was noted in mid- and high-dose SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 females. This finding was considered to be possibly related to a general mineral effect indicated by the changes in bone minerals. Overall, neither SALATRIM fat produced biologically significant effects.

INTRODUCTION

Americans consume approximately 37% of their daily total caloric intake as fat. The recommendation to decrease dietary fat consumption from 37% to 30% of total caloric intake (National Research Council, 1989; DHHS Publication 88-50210, 1988) has stimulated the agricultural and food industries to use available technologies to alter dietary fats to meet this goal. Dietary fats contain a variety of saturated, monounsaturated, and polyunsaturated fatty acids. Most common fatty acids contain 16–18 carbon atoms (long-chain fatty acids). The fatty acid composition of dietary fats can be altered to provide triacylglycerols with the physical properties of triacylglycerols normally found in the food supply but with less utilizable energy content. SALATRIM is a family of structured triacylglycerols that provide 4.5–6.0 kcal/g compared to 9 kcal/g for corn oil (Finley et al., 1994). SALATRIM triacylglycerols are composed of stearic acid (C18) and a variety of short-chain fatty acids (C2–C4). SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 are members of this family of triacylglycerols. Each is produced by interesterification among triacetin, tripropionin, tributyrin, and a common hydrogenated vegetable fat with a high concentration of stearic acid. The resulting triacylglycerols contain a preponderance of acetic, propionic, butyric, and stearic acids esterified to glycerol. The stearic acid sources for SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 are hydrogenated canola oil and hydrogenated cottonseed oil, respectively.

SALATRIM triacylglycerols appear to be hydrolyzed in the gastrointestinal tract like typical dietary fats to produce monoacylglycerols (primarily monostearin), diacylglycerols, and acetic, propionic, butyric, and stearic acids (Hayes et al., 1994a). Short-chain fatty acids and monoacylglycerols are absorbed and enter normal metabolic pathways (Bugaut, 1987; Jensen et al., 1982; Rombeau et al., 1990), whereas unabsorbed stearate is excreted in the feces as free stearic acid or calcium and magnesium soaps (Benzonana and Desneulle, 1968; Bliss et al., 1972; Gacs and Barltrop, 1977; Mattson et al., 1979; Sammons and Wiggs, 1960). Poor absorption of stearate and the lower number of calories provided by metabolism of short-chain fatty acids compared to long-chain fatty acids result in lower caloric availability for members of the SALATRIM family compared with dietary fats such as corn oil.

Short-term feeding studies in rats have been conducted with other fats in which one or two long-chain fatty acids in the triglyceride molecule were replaced with acetic acid with no reported adverse effects (Mattson et al., 1956).

Ambrose et al. (1958a) presented data from studies in which rats were fed fats, termed acetostearins. These fats were produced from hydrogenated lard and triacetin and appear to be almost identical. One of the acetostearins produced decreased reproductive performance and histopathologic changes in the reproductive organs, while the other, almost identical, acetostearin did not produce these changes. Ambrose et al. (1958b) reported the reproductive effects were related to a decrease in vitamin E levels in the rats. Because the results of feeding almost identical acetostearins were strikingly different, the relevance of these findings to SALATRIM fats cannot be ascertained. However, unlike the acetostearins investigated by Ambrose et al. (1958a,b), SALATRIM fats have not been shown to deplete vitamin E.

Because of their similarity to dietary fats and their predictable metabolism (Hayes et al., 1994d), members of the SALATRIM family should have no potential to produce toxicological effects. Thirteen-week subchronic toxicity studies in rats were conducted with three other members of the SALATRIM family (Hayes et al., 1994b,c). Each of these fats contained stearate, while each differed in the predominant short-chain fatty acid. These studies

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Table 1. Total Fatty Acid Profile for SALATRIM 234CA Lot A019[a]

fatty acid		
name	designation	wt %
stearic (octadecanoic)	C18:0	46.802 ± 5.213
butyric	C4:0	11.71 ± 0.56
propionic	C3:0	9.12 ± 0.36
acetic	C2:0	8.22 ± 0.17
palmitic (hexadecanoic)	C16:0	3.059 ± 0.333
arachidic (eicosanoic)	C20:0	2.077 ± 0.126
oleic (9-octadecenoic)	C18:1	0.927 ± 0.076
behenic (doconsanoic)	C22:0	0.601 ± 0.046
lignoceric (tetracosanoic)	C24:0	0.297 ± 0.023
linoleic (9,12-octadecadienoic)	C18:2	0.055 ± 0.004
myristic (tetradecanoic)	C14:0	0.053 ± 0.004
lauric (dodecanoic)	C12:0	<0.014

[a] Data represent the mean ± standard deviation for triplicate determinations.

Table 2. Total Fatty Acid Profile for SALATRIM 234CS Lot A018[a]

fatty acid		
name	designation	wt %
stearic (octadecanoic)	C18:0	39.848 ± 0.453
palmitic (hexadecanoic)	C16:0	11.663 ± 0.134
butyric	C4:0	10.67 ± 0.85
propionic	C3:0	7.84 ± 1.64
acetic	C2:0	6.82 ± 2.34
myristic (tetradecanoic)	C14:0	0.503 ± 0.028
arachidic (eicosanoic)	C20:0	0.245 ± 0.009
behenic (docosanoic)	C22:0	0.106 ± 0.005
lignoceric (tetracosanoic)	C24:0	0.075 ± 0.005
oleic (9-octadecenoic)	C18:1	0.251 ± 0.026
linoleic (9,12-octadecadienoic)	C18:2	0.068 ± 0.004
lauric (dodecanoic)	C12:0	0.024 ± 0.001

[a] Data represent the mean ± standard deviation for triplicate determinations.

demonstrate that irrespective of the short-chain fatty acids esterified to the glycerol backbone, none of the fats caused toxicologic effects in rats. The study reported here was designed to evaluate the effect of different hydrogenated vegetable fats as the source of stearic acid in SALATRIM triacylglycerols containing approximately equal amounts of the three short-chain fatty acids.

MATERIALS AND METHODS

This 13-week subchronic toxicity study with rats was conducted at Hazleton Wisconsin, Inc., Madison, WI, from January 31, 1992, through May 8, 1992.

Test and Control Materials. SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 were provided by Nabisco Foods Group (NFG), East Hanover, NJ. Analytical determinations conducted at NFG show that these SALATRIM fats are mixed triacylglycerols composed predominantly of various combinations of two short-chain fatty acids (acetic, propionic, or butyric) and one stearic acid esterified to glycerol.

Total fatty acid profile analyses for these two fats were conducted at EPL Bio-Analytical Services, Inc. (EPL-BAS), Decatur, IL, and the results are presented in Tables 1 and 2. Total fatty acid profile data for the higher molecular weight groups (≥C12:0) were obtained by saponification of the triacylglycerol mixture with methanolic sodium hydroxide followed by esterification with methanolic boron trifluoride. Methyl esters of the fatty acids were quantitated by gas chromatography. For lower molecular weight groups (<C12:0), profile data were obtained by saponification with methanolic sodium hydroxide followed by acidification with concentrated hydrochloric acid. The fatty acids were quantitated by gas chromatography. Standard curves were constructed by bracketing the concentration level of the analyte. External standards were used, and quantitation was based upon peak height.

Free fatty acid (FFA) concentration and peroxide value (PV) analyses were conducted by NFG. FFA concentrations were determined by titration using AOCS Official Method Ca 5a-40 (AOCS, 1990a). The FFA concentrations and PVs for the two SALATRIM triacylglycerol mixtures were low. For SALATRIM 234CA lot A019, the FFA concentration and PV were 0.07 ± 0.01 wt % and 0.33 ± 0.05 mequiv of peroxide/kg, respectively. The results for SALATRIM 234CS lot A018 were 0.19 ± 0.03 wt % and 0.87 ± 0.04 mequiv of peroxide/kg, respectively.

Commercially available Mazola corn oil was used as the control fat.

Dosing and Diets. Rats were fed either SALATRIM 234CA lot A019 or SALATRIM 234CS lot A018 at 0%, 2%, 5%, and 10% or corn oil at 10% of the diet by weight for at least 13 weeks. The high dose represents the highest concentration believed to be acceptable to avoid excessive dilution of micronutrients in the diet. The SALATRIM fats and corn oil were mixed with powdered NIH-07 Rat and Mouse Ration 5018 (Purina Mills, Inc.) and fed *ad libitum* except when rats were fasted overnight before blood collection and necropsy. Test diets were prepared biweekly and divided into two portions. The first portion was fed during the first of the two weeks, and the second portion was stored frozen (−5 to −20 °C) until fed during the second week. Drinking water was provided *ad libitum* during all phases of the study. Diets were analyzed for content of either corn oil (by gravimetric analysis) or the SALATRIM fats (by supercritical fluid chromatography). Homogeneity was evaluated for the 2% and 10% SALATRIM diets and the 10% corn oil diet mixed for week 1. Stability of the SALATRIM fats in the diets for up to 15 weeks when frozen (−5 to −20 °C) was assessed by analysis of samples of the 2% and 10% diet mixtures.

Animals. Crl:CD®BR VAF/Plus rats were from Charles River Laboratories, Inc. (Portage, MI). Five rats per sex were used for serum viral antibody analysis and the remaining rats acclimated for 2 weeks before study initiation. At initiation of treatment, rats were 5–6 weeks old, and the body weight distributions were 150–201 g for males and 114–158 g for females. Animal husbandry complied with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication 86-23, 1985). Rats were identified by ear tags and housed singly in stainless steel, wire-bottom cages in an animal room set to maintain 22 ± 3 °C (66–78 °F) and 50 ± 20% relative humidity, with a 12-h light/12-h dark cycle.

Randomization into treatment groups was unrestricted except that the body weight of each rat considered for assignment to the study could not vary by more than 2 standard deviations from the mean body weight of all rats of the same sex. In addition, group mean body weights for each sex could not differ statistically ($p \leq 0.05$).

Experimental Design. Rats were divided into nine groups: (1) untreated controls that received basal diet only; (2) treated groups that received SALATRIM 234CA lot A019 at either 2%, (3) 5%, or (4) 10% of the diet by weight; (5) treated groups fed SALATRIM 234CS lot A018 at either 2%, (6) 5%, or (7) 10% of the diet by weight; (8) controls fed corn oil at 10% of the diet by weight; and (9) an untreated group that served as a sentinel for health and viral serology at study termination. Each group, except the sentinel group, contained 20 rats per sex. The sentinel group contained 5 rats per sex.

Antemortem Data Collection. Rats were observed twice daily for mortality, moribundity, and signs of toxicity. For all rats except the sentinel group, physical examinations were conducted weekly. Opthalmic examinations were conducted before initiation of treatment and during week 13. Body weight was recorded on the first day of treatment, weekly thereafter, and at necropsy. Individual feed consumption was measured weekly during treatment.

After 13 weeks of treatment, blood and urine were collected from 10 rats per sex per group, with the exception of the sentinel group, for a standard battery of hematology, serum and urine chemistry, and urinalysis determinations. The variables evaluated were the same as in a previous subchronic rat study with SALATRIM 4CA lot A006 (Hayes et al., 1994b). Blood was obtained from an additional 10 rats per sex per group to determine serum concentrations of fat-soluble vitamins. Rats were fasted for approximately 16 h before blood collection and necropsy. Blood was collected from the retro-orbital plexus after ketamine

anesthesia. Samples for hematology were collected with 10% EDTA anticoagulant, plasma for the prothrombin assay was prepared from blood collected with 3.8% sodium citrate anticoagulant, and serum for the clinical chemistry determinations was prepared from blood collected without anticoagulant. Urine was collected via metabolism cages during the fasting period before blood sampling.

Hematology variables were determined using a Coulter Counter S-Plus IV whole blood automated analyzer. Prothrombin time was measured using a Coag-A-Mate X2 coagulation analyzer. Differential leukocyte count and blood cell morphology slides were prepared using a Geometric Data Hemastainer and read manually. Serum and urine chemistry variables were determined using a Hitachi 704 random access chemistry analyzer except that low-density lipoprotein cholesterol (Friedewald et al., 1972) and urinary fractional clearance of calcium, phosphorus, sodium, potassium, and chloride (Duncan and Prasse, 1986) were calculated. Globulin was calculated by subtraction of serum albumin from total protein. Urinalysis was conducted manually and with the Ames Multistix. Serum *trans*-retinol (vitamin A) and α-tocopherol (vitamin E) concentrations were determined by high-pressure liquid chromatographic methods (Driskell et al., 1982), and serum 25-hydroxy vitamin D (vitamin D) was measured by radioimmune assay using commercially available reagents from Incstar Corp. (Stillwater, MN).

Prior to study initiation and after 13 weeks of treatment, blood was collected from the retro-orbital plexus of each of five nonfasted sentinel rats per sex and used for serum viral antibody analysis. The antibody profile included pneumonia virus of mice, Sendai virus, Kilham rat virus, rat coronavirus/sialodacryoadenitis virus, Toolan H-1 virus, Theiler's mouse encephalomyelitis virus, Reovirus type III, mouse adenovirus, lymphocytic choriomeningitis virus, and *Mycoplasma pulmonis*. At the pretest evaluation, Hantaan virus, encephalitozoon cuniculi, *Bacilis piliformis*, and cilia-associated respiratory bacillus antibodies were also determined.

Postmortem Data Collection. At terminal necropsy, following 13 weeks of treatment, all rats in groups 1–8 were subjected to gross necropsy. Adrenals, brain, kidneys, liver, and testes were weighed. After the liver of each rat selected for serum vitamin chemistry was weighed, the left medial lobe was removed and frozen in liquid nitrogen and stored frozen at −70 °C until analyzed for vitamins A and E using high-performance liquid chromatographic methods (Kayden et al., 1983). The entire femur not used for histopathology from each rat selected for clinical pathology was removed and stored frozen at −20 °C. Defatted dry weight and percent ash of femurs were determined. Each femur was assayed for barium, calcium, copper, iron, magnesium, phosphorus, potassium, sodium, strontium, and zinc concentrations by inductively coupled plasma spectrometry.

A complete set of tissues was collected from all rats and fixed in standard fixatives. The tissues were the same as those collected in the previous subchronic study with SALATRIM 4CA lot A006 (Hayes et al., 1994b). Tissues from the control rats and rats fed 10% SALATRIM 234CA lot A019 and 10% SALATRIM 234CS lot A018 were subjected to histopathology. Macroscopic lesions (if any), lungs, liver, and kidneys from all rats (except sentinel) were subjected to histopathology. Tissues for histopathological examination were embedded, sectioned, stained with hematoxylin and eosin, and examined by light microscopy.

Statistical Analyses. Statistical analyses were conducted for the following: body weights, cumulative body weight gains; food consumption; serum chemistry; hematology (except red blood cell morphology); urine pH, volume, and specific gravity; urine chemistry; serum and liver vitamin concentrations; organ weights; organ-to-body weight percentages; organ-to-brain weight ratios; and bone mineral analyses. Levene's test (Levene, 1960) was used to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations were used to stabilize the variance (Draper and Hunter, 1969). When necessary, the following transformations were conducted in sequence until homogeneity of variance was achieved: $\log_{10}$, square, square root, reciprocal, angular, and rank. Analysis of variance (ANOVA) (Winer, 1971) was performed on the homogeneous or transformed data. If ANOVA was significant, the Games and Howell modified Tukey–Kramer test (Games and Howell, 1976) was used for pairwise comparisons between groups. Group comparisons were evaluated at the 5% two-tailed probability level. All statistically significant differences cited are based on comparisons with the untreated control group (group 1).

RESULTS

Diet Analysis. Pretest analysis of 2% and 10% SALATRIM fats and 10% corn oil diets for homogeneous dispersion of the fats indicated that the diet mixing procedures were appropriate. The 5% SALATRIM diets were not assayed for homogeneity because it was presumed these diets would be homogeneous if the 2% and 10% SALATRIM diets were homogeneous.

The stability of SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 was determined for 2% and 10% test diets stored at room temperature and under freezer conditions. The diets demonstrated acceptable stability when stored frozen for up to 15 weeks. For the 2% diet preparations, the concentrations were at least 85% of theoretical levels after 8 days of room temperature storage. For the 10% diets, the concentrations were at least 90% of theoretical levels after 8 days of room temperature storage. Diets fed during the study were not maintained at room temperature for longer than 1 week.

Analysis of each diet preparation fed during the study indicated the diets contained the proper amount of fats. Because SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 diets were analyzed by supercritical fluid chromatography, the analyses also confirmed that the fats in the diets were the appropriate SALATRIM fats.

Compound Consumption. Daily SALATRIM 234CA lot A019 consumption for the 2%, 5%, and 10% groups averaged 1.4, 3.5, and 6.9 g/kg in males and 1.7, 4.0, and 7.9 g/kg in females, respectively, during the 13-week study. Daily SALATRIM 234CS lot A018 consumption for the 2%, 5%, and 10% groups averaged 1.4, 3.4, and 6.7 g/kg in males and 1.6, 4.0, and 7.6 g/kg in females, respectively. Daily consumption of corn oil averaged 6.1 and 7.0 g/kg in males and females, respectively.

Antemortem Observations. No treatment-related effects were noted during daily observations and weekly physical examinations. Ophthalmologic findings were comparable in treated and control rats. All rats survived to the scheduled sacrifice except for one 10% SALATRIM 234CS lot A018 male that was sacrificed during week 12 because of a fractured calvarium. This was considered to be unrelated to treatment.

Mean body weights and body weight gains for male rats in the groups receiving the SALATRIM fats were comparable to those of untreated control rats, while those receiving corn oil showed slight, although not statistically significant, increases in body weight gain. Body weights and body weight gains of females were generally similar, although slightly higher weights and weight gains were observed in females fed corn oil and 10% SALATRIM 234CS lot A018. Cumulative body weight gain data are presented in Figure 1 and 2. Feed consumption (g/kg per day) for the 10% corn oil males and females was significantly lower than that of controls at all intervals. Feed consumption (g/kg per day) for the groups given SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 generally was similar to that of the control group for each sex except that significantly lower consumption was noted during weeks 8–11 for females given 10% SALATRIM 234CS lot A018. The reason for the marked increase in week 2 feed consumption of females fed 5% SALATRIM 234CS lot A018 and the subsequent significant decrease during week 3 is unknown. It was apparently unrelated

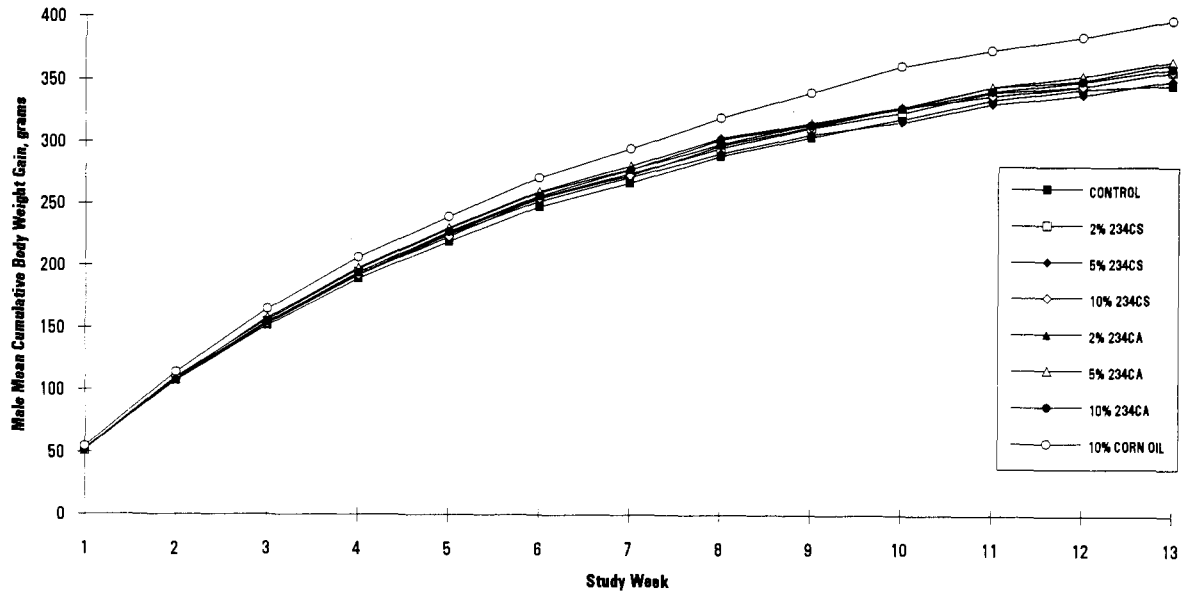

Figure 1. Male mean cumulative body weight gain (g) vs study week. Data points represent the means of 20 rats. Standard deviations are not shown for the sake of clarity of the figure. In most cases, the SD was less than 20% of the mean.

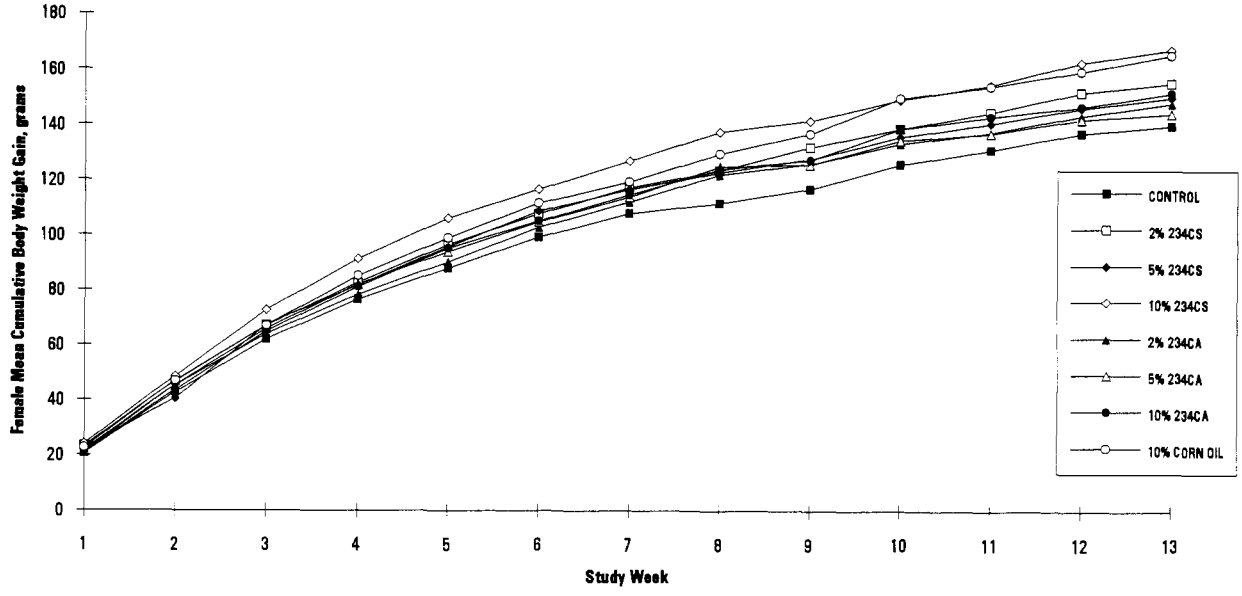

Figure 2. Female mean cumulative body weight gain (g) vs study week. Data points represent the means of 20 rats. Standard deviations are not shown for the sake of clarity of the figure. In most cases, the SD was less than 25% of the mean.

to treatment since feed consumption in this group at all other study weeks was comparable with that of the control group. Feed consumption data (g/kg per day) are presented in Figures 3 and 4.

Serology. Viral antibody titers were negative at initiation and termination of the study.

Clinical Pathology. Hematology revealed no treatment-related effects when values for rats fed SALATRIM or corn oil were compared with controls. Selected serum chemistry data are presented in Table 3. Statistically significant lower aspartate aminotransferase levels were noted for females fed 2% and 10% 234CA lot A019 and for females fed 2%, 5%, and 10% 234CS lot A018. Serum aspartate aminotransferase was not affected for males in any treatment group. No other changes in serum chemistry variables, including serum lipids, were noted for rats fed the SALATRIM fats.

In a completed subchronic study with two other SALATRIM fats, slightly, but statistically significantly, increased urinary phosphorus clearance was noted in rats fed 10% SALATRIM compared with controls (Hayes et al., 1994c). Although not statistically significant, a similar slight increase in urinary phosphorus clearance was observed with a third SALATRIM fed to rats at 10% (Hayes et al., 1994b). In this study with SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018, slightly, but not statistically significantly, increased urinary phosphorus clearance was again noted in 10% SALATRIM-treated rats. No other changes in urinary mineral clearance or other urinalysis variables were observed in rats fed either SALATRIM fat. Urinary mineral clearance data are presented in Table 4. Serum mineral concentration data also are presented in these tables for the purpose of comparison.

Vitamin Chemistry. Serum and liver concentrations of fat-soluble vitamins are presented in Figures 5–7. Serum and liver concentrations of vitamin E in SALATRIM-treated rats were comparable to those of control rats (Figure 6). Mean serum vitamin A was significantly higher than control in male rats fed 2% SALATRIM 234CA lot

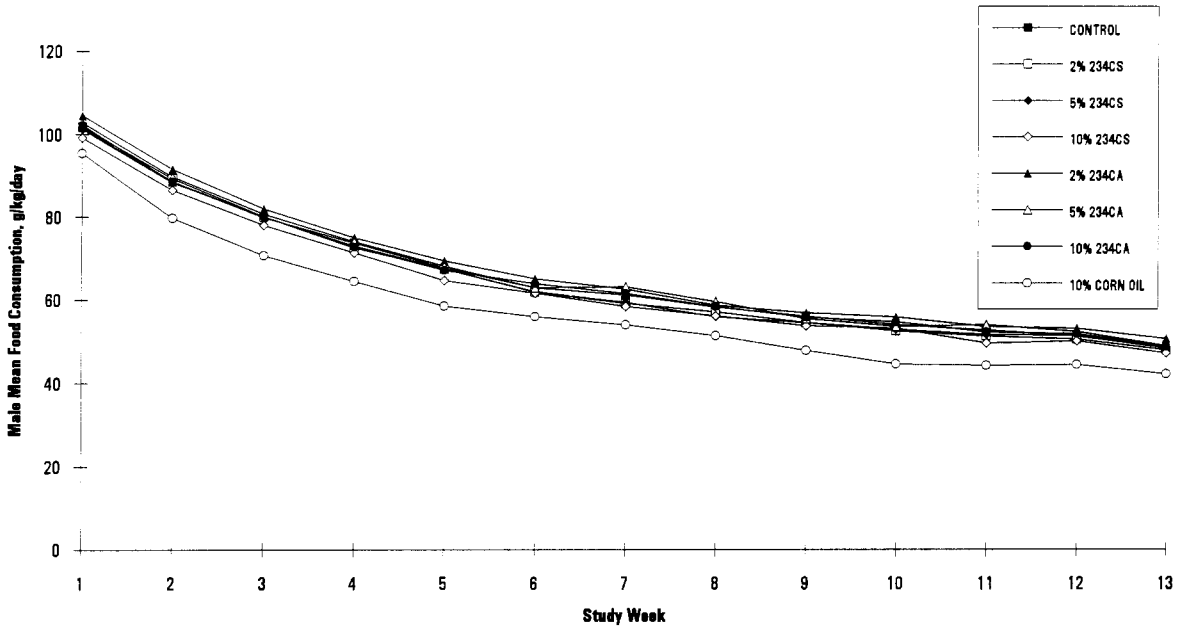

Figure 3. Male mean daily food consumption relative to body weight (g/kg) vs study week. Data points represent the means of 13–20 rats. Standard deviations are not shown for the sake of clarity of the figure. In most cases, the SD was less than 10% of the mean.

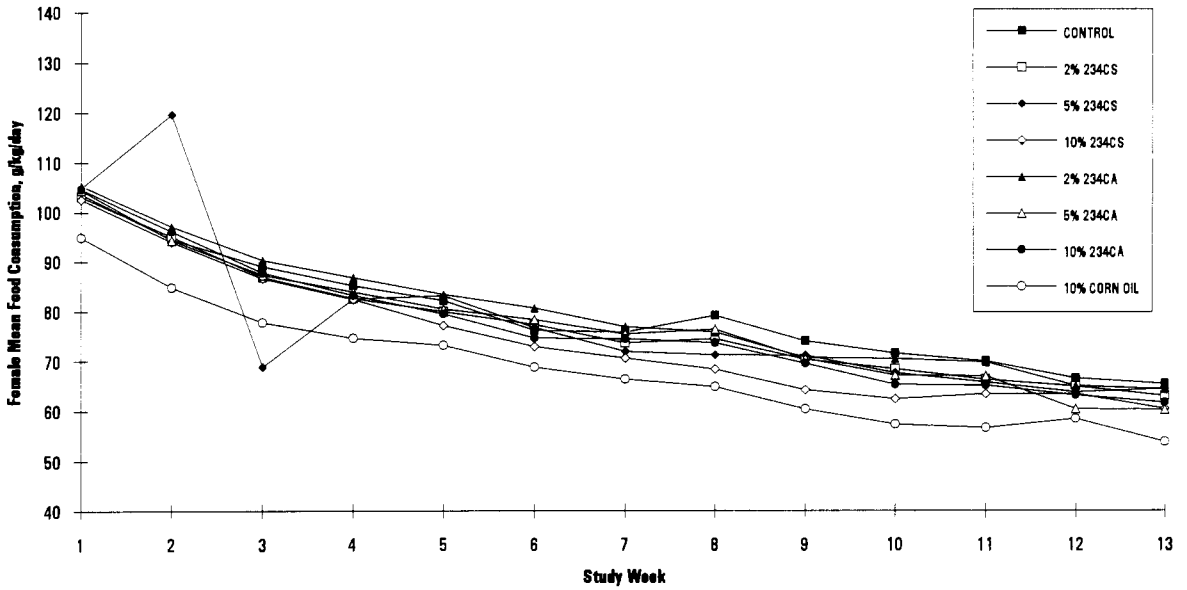

Figure 4. Female mean daily food consumption relative to body weight (g/kg) vs study week. Data points represent the means of 10–20 rats. Standard deviations are not shown for the sake of clarity of the figure. In most cases, the SD was less than 15% of the mean. The weeks 2 and 3 data were reviewed in an attempt to explain the values for the 5% SALATRIM 234CS lot A018 group. No explanation for the apparently aberrant value was determined.

A019 and 10% corn oil. Mean liver vitamin A was significantly lower than controls in 10% SALATRIM 234CA lot A019 males and 10% SALATRIM 234CS lot A018 and 10% corn oil males and females (Figure 5). Mean serum 25-hydroxy vitamin D concentrations were lower than control in females fed 2% and 10% SALATRIM 234CA lot A019, 2% and 10% SALATRIM 234CS lot A018, and 10% corn oil. These lower values were statistically significant for the 2% SALATRIM 234CA lot A019 and 2% and 10% SALATRIM 234CS lot A018 groups. No differences in serum 25-hydroxy vitamin D levels were detected in males (Figure 7). Prothrombin time, an indicator of vitamin K status, was unaffected by exposure to the SALATRIM fats and corn oil.

Bone Mineral Analyses. Bone mineral data are presented in Table 5. No differences in defatted femur weights or in bone concentrations of barium, calcium, copper, iron, magnesium, phosphorus, potassium, or strontium were noted between treated and control rats of either sex in this study. Mean sodium content in bone was significantly lower in females fed 2% SALATRIM 234CA lot A019, and mean zinc content in bone was significantly higher in males fed 2% SALATRIM 234CA lot A019 and in females fed 10% of both SALATRIM fats than in controls.

Organ Weights, Macroscopic and Microscopic Pathology. In general, there were no differences between the organ weights of rats fed either of the SALATRIM fats and untreated control rats. In the 10% SALATRIM 234CS lot A018 females, body weight collected at necropsy, absolute liver weight, and liver-to-brain weight ratio were greater and brain-to-body weight ratio was lower than controls. The organ weight differences were considered to be related to the increased terminal body weight and were not considered to be toxicologically significant because (1) these organ weight changes occurred in one

Table 3. Summary of Selected Serum Chemistry Data—Week 14[a]

	treatment							
		SALATRIM 234CA lot A019			SALATRIM 234CS lot A018			
	control	2%	5%	10%	2%	5%	10%	10% corn oil
Males								
aspartate aminotransferase (IU/L)	136 ± 26	135 ± 37	142 ± 42	114 ± 16	140 ± 25	157 ± 52	127 ± 24	149 ± 22
alanine aminotransferase (IU/L)	44 ± 9	41 ± 4	44 ± 7	43 ± 6	47 ± 5	47 ± 12	47 ± 6	49 ± 12
cholesterol (mg/dL)	74 ± 34	66 ± 13	81 ± 23	67 ± 15	68 ± 18	63 ± 17	61 ± 10	102 ± 27
HDL[b] (mg/dL)	62 ± 28	55 ± 10	70 ± 21	57 ± 15	58 ± 18	52 ± 16	51 ± 9	89 ± 25
LDL[c] (mg/dL)	1 ± 2	0 ± 1	0 ± 1	1 ± 2	0 ± 0	2 ± 3	1 ± 2	0 ± 1
triglycerides (mg/dL)	82 ± 47	80 ± 27	117 ± 70	84 ± 50	78 ± 34	71 ± 33	88 ± 46	97 ± 46
Females								
aspartate aminotransferase (IU/L)	173 ± 36	119 ± 26[e]	136 ± 36	117 ± 19[e]	123 ± 18[d,e]	122 ± 17[e]	110 ± 35[e]	142 ± 37
alanine aminotransferase (IU/L)	46 ± 16	33 ± 4	40 ± 10	38 ± 4	37 ± 8[d]	37 ± 6	38 ± 10	36 ± 10
cholesterol (mg/dL)	85 ± 31	91 ± 13	83 ± 14	84 ± 20	83 ± 19[d]	80 ± 20	89 ± 19	103 ± 14
HDL[b] (mg/dL)	75 ± 30	81 ± 10[d]	75 ± 10	78 ± 22[d]	77 ± 20[d]	75 ± 20	82 ± 19	96 ± 19[d]
LDL[c] (mg/dL)	4 ± 4	3 ± 4[d]	5 ± 6	1 ± 2[d]	0 ± 1[d]	1 ± 2	1 ± 3	1 ± 3[d]
triglycerides (mg/dL)	36 ± 14	32 ± 8	28 ± 10	45 ± 23	60 ± 53[d]	30 ± 8	52 ± 21	60 ± 31

[a] Data represent the mean ± standard deviation for 10 rats unless otherwise noted. [b] HDL, high-density lipoprotein cholesterol. [c] LDL, low-density lipoprotein cholesterol. [d] Mean based on nine rats. [e] Significantly different from control group ($p \leq 0.05$).

Table 4. Summary of Serum Mineral Analyses and Urine Mineral Clearance Data[a]

	treatment							
		SALATRIM 234CA lot A019			SALATRIM 234CS lot A018			
	control	2%	5%	10%	2%	5%	10%	10% corn oil
Males								
serum minerals								
calcium (mg/dL)	10.0 ± 0.3	9.9 ± 0.2	10.2 ± 0.3	9.8 ± 0.3	10.1 ± 0.3	9.8 ± 0.3	9.8 ± 0.2	10.0 ± 0.3
inorg phosphorus (mg/dL)	6.8 ± 0.5	7.2 ± 0.8	7.2 ± 0.6	6.6 ± 0.6	7.1 ± 0.3	6.9 ± 0.7	6.8 ± 0.4	7.0 ± 0.6
sodium (mmol/L)	146 ± 3	147 ± 3	147 ± 2	147 ± 4	148 ± 3	147 ± 2	148 ± 4	147 ± 3
potassium (mmol/L)	5.1 ± 0.4	5.2 ± 0.4	5.0 ± 0.3	5.0 ± 0.4	5.2 ± 0.3	5.3 ± 0.6	5.0 ± 0.3	5.1 ± 0.2
chloride (mmol/L)	105 ± 2	105 ± 2	105 ± 3	106 ± 3	105 ± 2	107 ± 2	106 ± 2	105 ± 2
urine mineral clearance								
calcium (%)	0.22 ± 0.08	0.26 ± 0.06	0.28 ± 0.13[b]	0.19 ± 0.06	0.21 ± 0.13	0.21 ± 0.05[b]	0.18 ± 0.06	0.26 ± 0.17[c]
phosphorus (%)	15.0 ± 1.8	17.5 ± 4.5	16.7 ± 3.7[b]	18.0 ± 3.2	14.8 ± 3.8	18.3 ± 4.6[b]	18.4 ± 2.8	15.6 ± 2.7[c]
sodium (%)	0.18 ± 0.08	0.21 ± 0.14	0.21 ± 0.07[b]	0.20 ± 0.09	0.20 ± 0.09	0.21 ± 0.07[b]	0.19 ± 0.08	0.15 ± 0.06[c]
potassium (%)	15.6 ± 3.2	16.7 ± 3.9	18.4 ± 5.5[b]	13.0 ± 2.9	14.9 ± 4.9	14.6 ± 4.0[b]	15.5 ± 3.4	13.2 ± 1.7[c]
chloride (%)	0.18 ± 0.14	0.30 ± 0.32	0.23 ± 0.25[b]	0.33 ± 0.28	0.24 ± 0.21	0.17 ± 0.16[b]	0.37 ± 0.14[b]	0.31 ± 0.32[c]
Females								
serum minerals								
calcium (mg/dL)	10.0 ± 0.3	10.1 ± 0.6	10.0 ± 0.3	10.2 ± 0.4	10.2 ± 0.3[b]	9.9 ± 0.2	10.3 ± 0.5	10.1 ± 0.5
inorg phosphorus (mg/dL)	6.6 ± 0.6	6.3 ± 1.0	6.5 ± 0.6	5.8 ± 1.0	6.0 ± 0.6[b]	5.9 ± 0.5	6.0 ± 0.5	5.8 ± 1.0
sodium (mmol/L)	147 ± 3	149 ± 3	148 ± 2	150 ± 3	147 ± 4[b]	146 ± 3	147 ± 5	146 ± 2
potassium (mmol/L)	4.9 ± 0.4	4.9 ± 0.6	4.7 ± 0.4	4.8 ± 0.5	4.6 ± 0.5[b]	4.6 ± 0.2	4.6 ± 0.4	4.9 ± 0.3
chloride (mmol/L)	107 ± 3	109 ± 2	108 ± 3	109 ± 2	107 ± 3[b]	108 ± 2	105 ± 3	107 ± 2
urine mineral clearance								
calcium (%)	1.17 ± 1.18[d]	0.60 ± 0.21[d]	0.56 ± 0.25[c]	0.58 ± 0.27	1.01 ± 0.59[b]	0.79 ± 0.48[c]	0.78 ± 0.37[d]	0.69 ± 0.37
phosphorus (%)	24.3 ± 7.0[d]	26.9 ± 7.6[d]	24.2 ± 4.4[c]	30.6 ± 8.5	27.6 ± 4.6[b]	27.2 ± 5.8[c]	28.7 ± 5.0[d]	27.5 ± 7.1
sodium (%)	0.58 ± 0.52[d]	0.25 ± 0.06[d]	0.35 ± 0.13[c]	0.40 ± 0.21	0.41 ± 0.21[b]	0.32 ± 0.12[c]	0.38 ± 0.27[d]	0.36 ± 0.41
potassium (%)	22.4 ± 6.5[d]	21.6 ± 5.6[d]	17.7 ± 4.4[c]	18.3 ± 5.9	21.0 ± 3.9[b]	22.8 ± 7.6[c]	18.8 ± 6.1[d]	19.9 ± 6.5
chloride (%)	1.17 ± 1.31[d]	0.43 ± 0.30[d]	0.67 ± 0.42[c]	0.59 ± 0.22	0.94 ± 0.73[b]	0.72 ± 0.39[c]	0.81 ± 0.60[d]	0.94 ± 1.57

[a] Data represent the mean ± standard deviation for 10 rats except where otherwise noted. [b] Means based on $n = 9$. [c] Means based on $n = 8$. [d] Means based on $n = 7$. [e] Significantly different from control group ($p \leq 0.05$).

sex only, (2) no effects on the brain and liver were detected during the histopathologic examination of these females, and (3) no similar organ weight or histopathologic effects were associated with SALATRIM 234CA lot A019 in this study and no such effects were noted in previous subchronic studies with three other SALATRIM fats (Hayes et al., 1994b,c).

Body weights collected at necropsy for rats of both sexes in the 10% corn oil group were significantly increased compared with those of untreated controls. Absolute liver weights and liver-to-brain weight ratios were significantly higher for 10% corn oil-fed males than for untreated controls. A few statistically significant decreases in organ weights relative to body weights of 10% corn oil-treated rats and controls also were noted. The differences in organ weights relative to body weight were considered to be reflective of the significantly increased body weights of corn oil-treated rats. The increases in liver weight noted in corn oil-treated males also may have been reflective of the increased terminal body weight but also may have been related to the mottled livers and hepatocellular vacuolation that were observed in these males.

Macroscopically, no treatment-related effects were observed in SALATRIM-treated rats. Microscopically, an increased incidence of mineralization was noted in the kidneys of females fed 5% and 10% SALATRIM 234CA lot A019 when compared with the incidence in control females (Table 6). A slightly higher incidence of renal mineralization also was noted for females fed 5% and 10% SALATRIM 234CS lot A018 compared with controls. Except for the 10% SALATRIM 234CA lot A019-treated groups, the mineralization was similar in appearance in all groups. In the 10% SALATRIM 234CA lot A019

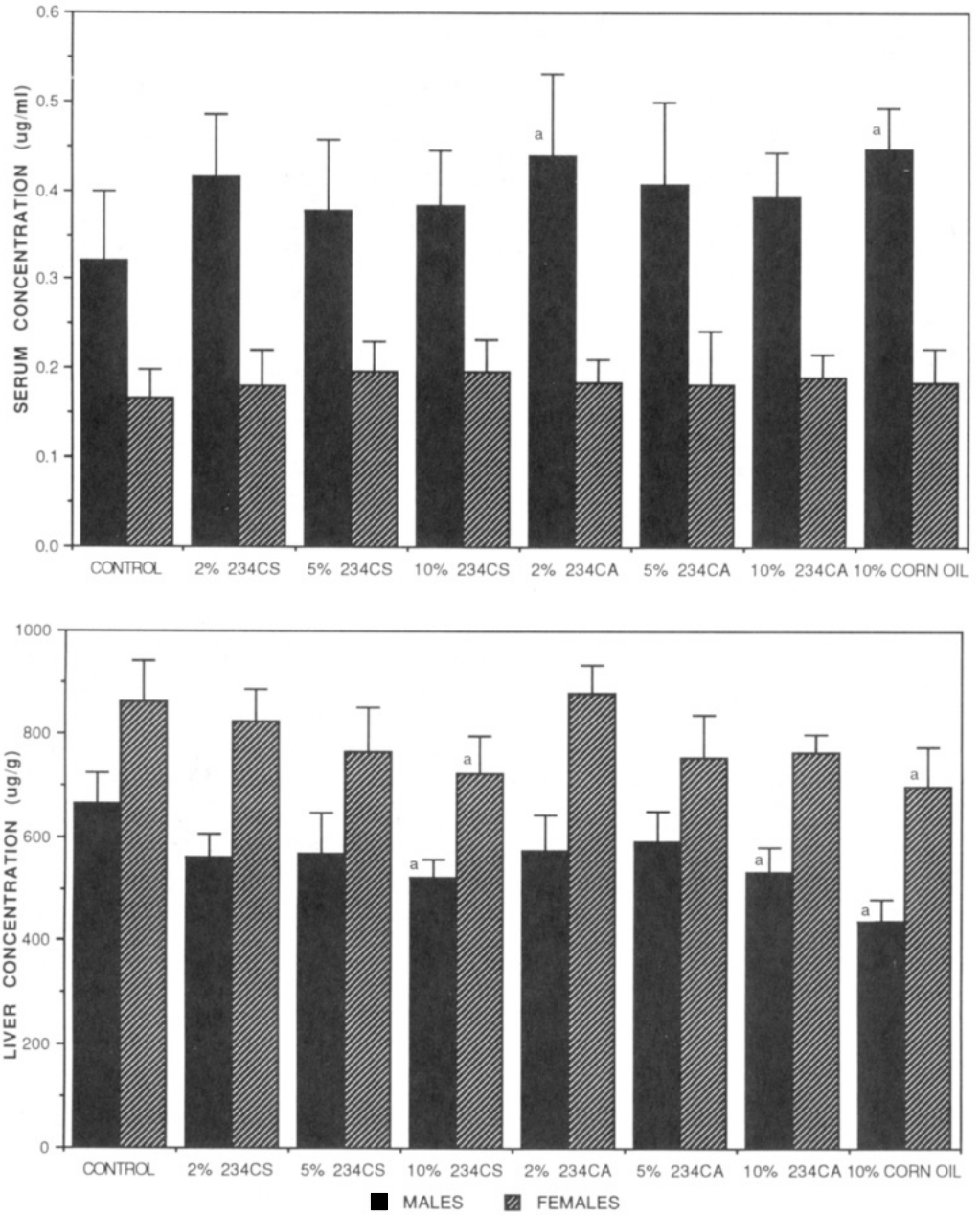

Figure 5. Male and female concentration of vitamin A (*trans*-retinol) in serum (μg/mL) and liver (μg/g) vs treatment at study week 14. Data represent mean ± standard deviation of the mean for 10 rats. Data significantly different from control group ($p \leq 0.05$) are noted with an "a".

females, the severity of this mineralization was slightly greater than in other groups.

Mottled livers and hepatocellular vacuolation were noted for males in the 10% corn oil group. No hepatic effect was observed in SALATRIM-treated rats.

DISCUSSION

On the basis of the similarity to fats normally found in the food supply, their predictable metabolism, and the results of toxicity testing with three other SALATRIM triacylglycerols (Hayes et al., 1994b,c), SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 were not expected to cause toxicological effects. This 13-week subchronic toxicity study with rats was conducted to test this hypothesis. These members of the SALATRIM family were fed at dietary concentrations of 0%, 2%, 5%, and 10% of the diet. A high-fat control group received 10% dietary corn oil.

There were no significant differences between the body weights of controls and those of SALATRIM-treated rats. Relative (g/kg per day) feed consumption values were significantly lower than controls during a few weeks in 10% SALATRIM 234CS lot A018 females and continuously during the study in 10% corn oil males and females. The lower feed consumption for these rats is predictable on the basis of total caloric consumption.

Hematology data revealed no treatment-related effects for rats fed the SALATRIM fats or corn oil compared to controls. The significantly lower serum aspartate aminotransferase levels noted in this study for females fed SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 were considered to be due to normal biological variation. There was no definite dose relationship, the males in these groups were not similarly affected, other liver enzymes were unaffected, and no treatment-related pathology was noted in the livers. Furthermore, clinical significance is usually only assigned to increases in serum aspartate aminotransferase. The high dietary concentrations of triacylglycerols (the SALATRIM fats and corn oil) fed in this study did not produce effects on serum

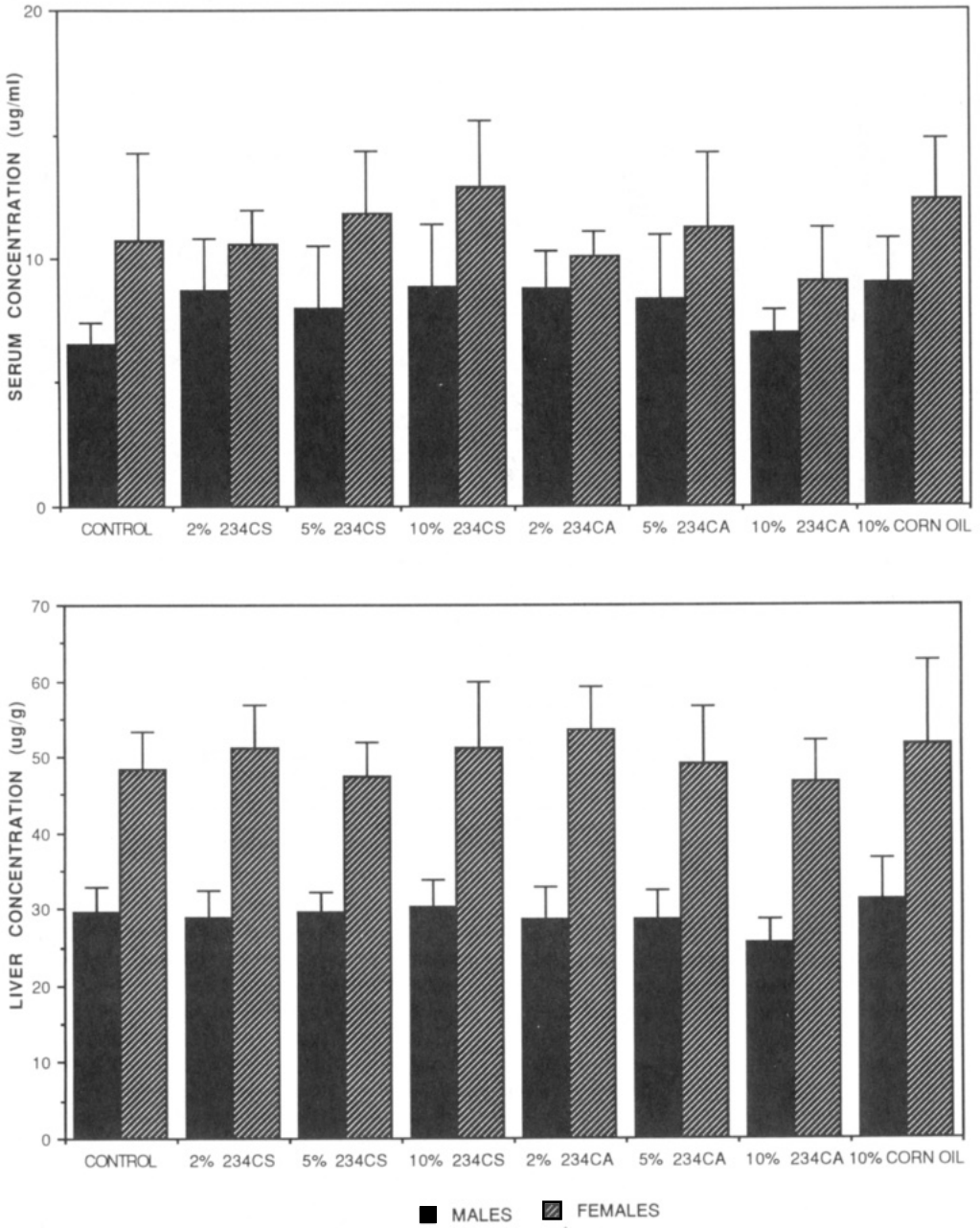

Figure 6. Male and female concentration of vitamin E (α-tocopherol) in serum (μg/mL) and liver (μg/g) vs treatment at study week 14. Data represent mean ± standard deviation of the mean for 10 rats. Data significantly different from control group ($p \leq 0.05$) are noted with an "a".

triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol.

Slight, although not statistically significant, increases in urinary phosphorus clearance were noted in rats fed 10% SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 compared with controls. Slight increases in urinary phosphorus clearance were also noted in rats fed diets containing 10% of three other SALATRIM fats during previous studies (Hayes et al., 1994b,c).

Serum 25-hydroxy vitamin D concentrations was lower in females fed 2% and 10% SALATRIM 234CA lot A019, 2% and 10% SALATRIM 234CS lot A018, and 10% corn oil. However, no effect on serum 25-hydroxy vitamin D levels was detected for males in this study or for males and females fed three other SALATRIM fats in previous studies (Hayes et al., 1994b,c). Serum vitamin A was significantly higher than control in males fed 2% SALATRIM 234CA lot A019 or 10% corn oil. Liver vitamin A was significantly lower than control in males fed 10% SALATRIM 234CA lot A019 and in males and females fed 10% SALATRIM 234CS lot A018 and 10% corn oil. The magnitude of change in serum or liver vitamin A for males and females fed 10% corn oil, compared to controls, exceeded that of the groups fed the two SALATRIM fats. No significant effects on serum or liver vitamin A were noted in the previous studies with other SALATRIM fats (Hayes et al., 1994b,c). Serum and liver concentrations of vitamin E, another fat-soluble vitamin, in males and females fed SALATRIM 234CA lot A019, SALATRIM 234CS lot A018, or corn oil were comparable to those of controls in this study, and no significant effects were noted with three other SALATRIM fats evaluated previously (Hayes et al., 1994b,c). Similarly, prothrombin time, an indicator of vitamin K status, was unaffected by SALATRIM and corn oil in this study and in the previous studies (Hayes et al., 1994b,c). Overall, it appears that SALATRIM fats do not substantially alter fat-soluble vitamin absorption.

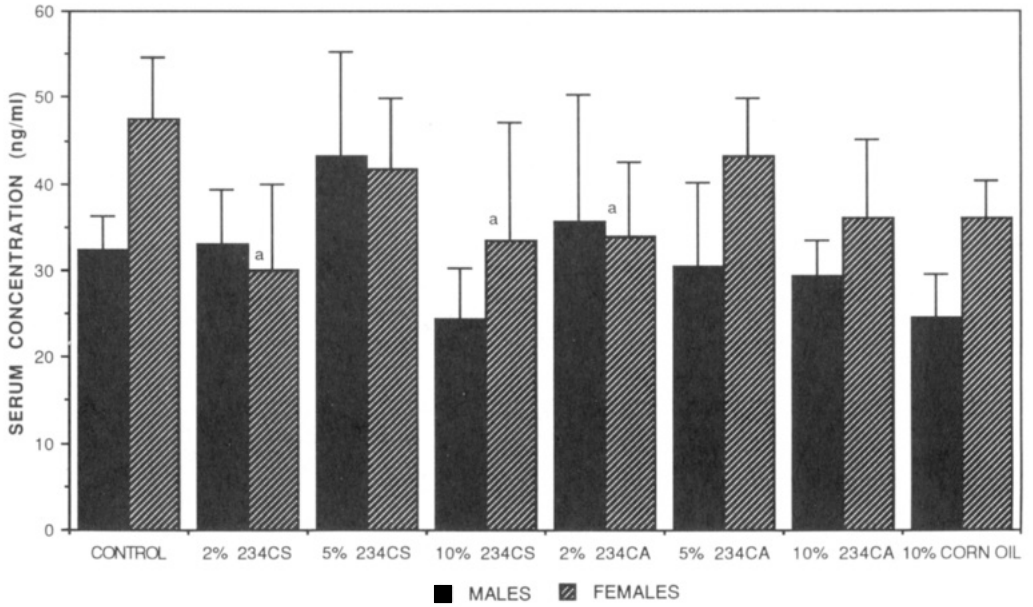

Figure 7. Male and female concentration of vitamin D (25-hydroxy vitamin D) in serum (ng/mL) vs treatment at study week 14. Data represent mean ± standard deviation of the mean for 10 rats. Data significantly different from control group ($p \leq 0.05$) are noted with an "a".

Table 5. Summary of Bone Mineral Analyses[a,b]

	treatment							
		SALATRIM 234CA lot A019			SALATRIM 234CS lot A018			
	control	2%	5%	10%	2%	5%	10%	10% corn oil
Males								
defatted femur wt (mg)	772.1 ± 55.8	742.4 ± 77.1	750.5 ± 58.5	753.2 ± 59.5	790.3 ± 80.6	757.5 ± 93.6	733.9 ± 75.2	771.6 ± 77.4
ash (%)	67.6 ± 1.1	67.4 ± 1.2	67.4 ± 0.9	67.5 ± 1.5	67.6 ± 0.6	67.5 ± 0.9	67.5 ± 0.8	66.9 ± 1.2
calcium (mg/g)	240 ± 4	241 ± 7	239 ± 7	240 ± 6	240 ± 6	241 ± 7	243 ± 5	238 ± 9
copper (μg/g)	9.5 ± 0.8	9.3 ± 0.7	9.4 ± 0.7	9.4 ± 0.9	9.7 ± 0.6	9.1 ± 0.4	9.5 ± 0.8	9.2 ± 0.8
iron (μg/g)	58.3 ± 12.4	54.8 ± 14.2	50.7 ± 11.6	47.7 ± 9.6	67.4 ± 23.0	59.9 ± 10.3	50.3 ± 11.5	72.6 ± 13.5
magnesium (mg/g)	4.51 ± 0.34	4.64 ± 0.22	4.66 ± 0.12	4.66 ± 0.09	4.58 ± 0.21	4.60 ± 0.18	4.69 ± 0.23	4.56 ± 0.17
phosphorus (mg/g)	117 ± 2	118 ± 3	117 ± 3	117 ± 3	118 ± 2	117 ± 3	118 ± 2	116 ± 4
sodium (mg/g)	4.10 ± 0.29	4.11 ± 0.15	4.11 ± 0.23	4.04 ± 0.33	4.15 ± 0.15	4.14 ± 0.12	4.14 ± 0.14	4.14 ± 0.18
strontium (μg/g)	62.0 ± 6.7	59.6 ± 4.7	59.6 ± 4.3	66.0 ± 3.8	60.3 ± 3.1	62.6 ± 2.7	66.6 ± 4.7	63.3 ± 4.5
zinc (μg/g)	246 ± 11	267 ± 17[c]	263 ± 14	262 ± 16	258 ± 25	257 ± 17	260 ± 11	233 ± 13
Females								
defatted femur wt (mg)	514.3 ± 60.4	516.5 ± 26.3	519.5 ± 50.1	477.1 ± 61.6	512.0 ± 37.1	501.9 ± 22.5	527.0 ± 47.6	508.3 ± 54.8
ash (%)	68.3 ± 0.9	68.1 ± 0.7	68.3 ± 0.8	67.9 ± 1.2	68.3 ± 0.7	68.0 ± 0.7	68.4 ± 0.6	67.6 ± 0.9
calcium (mg/g)	249 ± 6	250 ± 5	252 ± 5	249 ± 7	249 ± 4	246 ± 7	247 ± 6	249 ± 9
copper (μg/g)	9.9 ± 0.6	10.3 ± 0.8	9.9 ± 1.0	10.3 ± 0.9	10.0 ± 0.8	9.5 ± 0.7	9.9 ± 0.8	9.7 ± 0.7
iron (μg/g)	73.8 ± 18.8	59.5 ± 12.2	67.6 ± 16.2	62.9 ± 11.8	65.1 ± 24.8	60.0 ± 12.3	63.1 ± 13.8	66.0 ± 19.3
magnesium (μg/g)	4.74 ± 0.16	4.76 ± 0.19	4.75 ± 0.12	4.78 ± 0.18	4.82 ± 0.17	4.74 ± 0.12	4.84 ± 0.11	4.82 ± 0.19
phosphorus (mg/g)	120 ± 2	121 ± 2	121 ± 2	120 ± 3	120 ± 2	119 ± 3	120 ± 2	120 ± 3
sodium (mg/g)	4.25 ± 0.24	3.92 ± 0.24[c]	4.07 ± 0.17	4.05 ± 0.22	4.05 ± 0.25	4.08 ± 0.15	4.13 ± 0.13	4.03 ± 0.13
strontium (μg/g)	62.2 ± 2.6	61.8 ± 3.2	64.5 ± 2.7	63.0 ± 4.4	62.2 ± 3.0	60.4 ± 2.6	62.9 ± 3.5	63.8 ± 6.1
zinc (μg/g)	262 ± 14	279 ± 18	284 ± 12	296 ± 15[c]	278 ± 19	284 ± 11	287 ± 16[c]	269 ± 21

[a] Data represent the mean ± standard deviation for 10 rats. [b] Barium and potassium concentrations were also determined. No treatment-related effects were noted. [c] Significantly different from control group ($p \leq 0.05$).

Distinct sex differences, unrelated to treatment, were noted in serum and liver concentrations of fat-soluble vitamins in this study. Serum concentrations of vitamin A were higher in males than in females. Serum concentrations of vitamins D and E and liver concentrations of vitamins A and E were generally higher in females than in males. The same sex differences were noted in the previous studies with three other SALATRIM fats (Hayes et al., 1994b,c).

The significantly lower mean sodium content noted in femurs of females fed 2% SALATRIM 234CA lot A019 is not thought to be treatment-related because there was no dose–response in females and the males were not similarly affected. Additionally, the sodium content in femurs of females fed 10% SALATRIM 4CA lot A006 in a previous study was significantly higher than in controls (Hayes et al., 1994b). There was no change in the sodium content in femurs of males and females in previous testing with SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015 (Hayes et al., 1994c) or with SALATRIM 234CS lot A018 in this study.

Compared to controls, mean zinc concentrations in the femurs were slightly, but significantly, higher than those of controls in males fed 2% SALATRIM 234CA lot A019 and in females fed 10% SALATRIM 234CA lot A019 and 10% SALATRIM 234CS lot A018. These effects are consistent with previous findings for SALATRIM fats. Zinc content in femurs of males fed 10% SALATRIM 4CA lot A006 and of females fed 10% SALATRIM 4CA lot A006, 10% SALATRIM 23CA lot A014, and 10% SALATRIM 32CA lot A015 were also significantly higher than in femurs of controls (Hayes et al., 1994b,c). In the

Table 6. Summary of Renal Mineralization Incidence and Severity Data—Week 14[a]

	treatment							
		SALATRIM 234CA lot A019			SALATRIM 234CS lot A018			
	control	2%	5%	10%	2%	5%	10%	10% corn oil
				Males				
total incidence	0	0	0	1	0	0	0	0
severity of mineralization[b]								
0	20	20	20	19	20	20	20[c]	20
1	0	0	0	1	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
				Females				
total incidence	8	11	15	18	7	12	12	10
severity of mineralization[b]								
0	12	9	5	2	13	8	8	10
1	7	9	13	13	7	9	9	10
2	0	0	0	0	0	0	3	0
3	1	2	2	4	0	3	0	0
4	0	0	0	1	0	0	0	0
mean severity[d]	1.3 ± 0.7	1.4 ± 0.8	1.3 ± 0.7	1.6 ± 1.0	1.0 ± 0.0	1.5 ± 0.9	1.3 ± 0.5	1.0 ± 0.0

[a] Twenty rats were evaluated in each group. [b] 0 = normal; 1 = minimal; 2 = slight; 3 = moderate; 4 = moderately severe; 5 = severe. [c] One rat was sacrificed moribund during week 12. [d] Data represent the mean ± standard deviation for rats in which mineralization was noted.

earlier studies, femur strontium content was significantly higher for males and females fed 10% SALATRIM 4CA lot A006 and for females fed 10% SALATRIM 23CA lot A014 compared to controls. In the subchronic studies with SALATRIM 4CA lot A006, SALATRIM 23CA lot A014, and SALATRIM 32CA lot A015, femur zinc was significantly lower in 10% corn oil fed rats compared to controls (Hayes et al., 1994c). In the SALATRIM 4CA lot A006 study, strontium was higher in the corn oil group than in controls (Hayes et al., 1994b).

Lukaski and Johnson (1992) concluded that diets containing high levels of polyunsaturated fatty acids can depress zinc status, as well as the status of other minerals, in the rat. The authors indicated that high linoleate diets have been reported to significantly depress zinc concentrations in the tibia. In the previous subchronic studies with three other SALATRIM fats, and in this study with SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018, the dietary content of polyunsaturated fatty acids was greatest in the 10% corn oil diets, because corn oil contains predominantly unsaturated fatty acids. Polyunsaturates were lower in the untreated control diets and were lowest in the 10% SALATRIM diets, because the predominant long-chain fatty acid in SALATRIM fats (stearic acid) is saturated. It is not surprising that the zinc concentration in the femurs of rats from these studies varied with the dietary concentration of polyunsaturated fatty acids and it seems probable that the other mineral-related changes were related to dietary fatty acid concentration. The mineral-related changes in rats fed diets containing SALATRIM and corn oil were not considered to be toxicological effects, and they appeared to be directly related to the high level of unsaturated fatty acids in the corn oil diets and the lower level of unsaturated fatty acids in the SALATRIM diets.

Histopathological examination of tissues from rats consuming SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 indicated no treatment-related changes except for the incidence of renal mineralization in the female 5% and 10% groups. With the exception of a slightly greater severity in the 10% SALATRIM 234CA lot A019 females, the appearance of renal mineralization in the treated animals was similar to that observed in controls. In one of the previous subchronic studies with SALATRIM fats, a slight increase in the incidence and/or severity of renal mineralization was noted in females fed SALATRIM 23CA lot A014, SALATRIM 32CA lot A015, and corn oil (Hayes et al., 1994c). In another SALATRIM rat study, no renal effects were reported with SALATRIM 4CA lot A006 (Hayes et al., 1994b).

Focal mineralization in the renal medulla is a common histopathologic finding in rats, especially in females, and most pathologists attach little pathologic significance to this finding (Casey et al., 1978; Morrissey, 1986; Greaves and Faccini, 1984). Suggested or demonstrated causes of renal mineralization include calcium, phosphorus, chloride, magnesium, protein, and lipid imbalances (Montgomery and Seely, 1990). The increase in renal mineralization noted in female rats in this study may be another indication of a change in mineral balance related to the fatty acids consumed in this high-fat diet and of no toxicologic significance.

Hepatocellular vacuolation noted microscopically for males of the 10% corn oil group also was noted in both previous subchronic studies (Hayes et al., 1994b,c) and is thought to be intracytoplasmic fat vacuoles resulting from the higher absorption of long-chain fatty acids in the 10% corn oil group. This change would not be expected with the SALATRIM fats because the level of long-chain fatty acids available for absorption is considerably lower than that for corn oil.

Overall, these data indicate that SALATRIM 234CA lot A019 and SALATRIM 234CS lot A018 are well tolerated in rats when fed for as long as 13 weeks at average daily doses in males as high as 6.9 and 6.7 g/kg, respectively, and in females as high as 7.9 and 7.6 g/kg, respectively. The data confirm the hypothesis, based on the scientific literature, structure/activity relationships, and results of testing with similar SALATRIM fats, that these fats do not produce toxicologically significant effects in rats.

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