Subchronic Toxicity Studies of SALATRIM Structured Triacylglycerols in Rats. 2. Triacylglycerols Composed of Stearate, Acetate, and Propionate

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SALATRIM 23CA lot A014, SALATRIM 32CA lot A015, and SALATRIM 23SO lot A026 are members of a family of structured triacylglycerols having caloric densities of 4.5–6.0 kcal/g. Rats received 0%, 2%, 5% and 10% of the first two SALATRIM fats or 10% corn oil in the diet for 13 weeks. Body weight and feed consumption were unaffected. Minimally increased urinary phosphorus clearance occurred in 10% SALATRIM groups. Bone mineral variations and an increased incidence of renal mineralization occurred in 10% SALATRIM and corn oil fed rats. These changes appeared to be directly related to the quantity of unsaturated fatty acids in the high-fat diets. Measurements of serum and liver fat-soluble vitamin concentrations, necropsy, clinical pathology, organ weights, and histopathology revealed no SALATRIM-related effects. Corn oil produced hepatocellular vacuolation. In a supplementary short-term study, 10% dietary SALATRIM 23SO lot A026 produced no effect on selected serum transaminases. Overall, the SALATRIM fats produced no toxicologically significant effects.

INTRODUCTION

SALATRIM 23CA lot A014, SALATRIM 32CA lot A015, and SALATRIM 23SO lot A026 are members of a family of structured triacylglycerols that provide lower caloric availability than other fats such as corn oil, 4.5–6.0 kcal/g compared to 9 kcal/g (Finley et al., 1994a). SALATRIM fats are composed of a glycerol backbone esterified with long-chain fatty acids (LCFA), predominantly stearic acid, and short-chain fatty acids (SCFA). SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015 are produced by interesterification between triacetin, tripropionin, and hydrogenated canola oil. SALATRIM 23SO lot A026 is produced by interesterification between triacetin, tripropionin, and hyrogenated soy oil. The ratios of the SCFA are different, resulting in the fats having different chemical and physical properties. SALATRIM 23CA lot A014 and SALATRIM 23SO lot A026 are manufactured using more triacetin than tripropionin. Therefore, they contain significantly more acetic than propionic acid. SALATRIM 32CA lot A015 is manufactured using more tripropionin than triacetin; therefore, propionic acid predominates. Another member of the SALATRIM family of structured triacylglycerols, SALATRIM 4CA lot A006, was previously tested in a 13-week toxicity study with rats (Hayes et al., 1994b). This SALATRIM fat is produced from tributyrin and hydrogenated canola oil, resulting in triacylglycerols with a preponderance of butyric and steric acids esterified to glycerol.

When members of the SALATRIM family are hydrolyzed by pancreatic lipase *in vitro*, stearic acid, SCFA, and mono- and diacylglycerols are produced (Hayes et al., 1994a). SCFA and the monoacylglycerols are absorbed and enter normal metabolic pathways (Bugaut, 1987; Jensen et al., 1982; Rombeau et al., 1990), whereas stearic acid released upon lipolysis is poorly absorbed (Carey et al., 1983; Hashim and Babayan, 1978; Jensen et al., 1982). A portion of the stearate is believed to be excreted in the feces as calcium and magnesium salts along with free stearic acid (Benzonana and Desnuelle, 1968; Bliss et al., 1972; Gacs and Barltrop, 1977; Mattson et al., 1979; Sammons and Wiggs, 1960). The poor absorption of stearate and the lower number of calories provided by the SCFA compared to LCFA are responsible for the lower caloric availability of members of the SALATRIM family compared with fats such as corn oil.

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

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, members of the SALATRIM family should have no potential to produce toxicological effects. To test this hypothesis, 13-week subchronic toxicity studies in rats were conducted with various members of the SALATRIM family. In a study with SALATRIM 4CA lot A006, no toxicologically significant

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effects were noted at dietary concentrations up to 10% (Hayes et al., 1994b). The only treatment-related observations in that study were slight, but statistically significant, increases in the concentrations of bone strontium and zinc at 10% SALATRIM. This apparent nutritional effect was considered to be related to the specific fatty acids contained in these high-fat diets because effects on bone mineral concentrations were also noted in rats receiving 10% dietary corn oil. The 13-week subchronic rat study with SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015 reported here was conducted to further test the hypothesis that SALATRIM fats should have no potential to produce toxicological effects.

Recently, it has been reported that caprenin, a reduced-calorie fat, produced significant increases in serum alanine aminotransferase (ALT) in male and female rats in a 28-day repeated dosing study (Webb et al., 1991). Although SALATRIM differs from caprenin in chemical structure, it is possible that the increased serum ALT observed with caprenin could be a general feature of low-calorie fats. It is also possible that the increase in serum ALT seen with caprenin is a transitory effect that would have returned to baseline upon continued exposure.

No studies have been published on the effects of caprenin on serum hepatic enzymes in humans. Studies where very high doses of SALATRIM fats (up to 60 g/day) have been fed to humans for up to 7 days indicate that slight, but statistically significant, increases in serum transaminases were noted (Finley et al., 1994b). The increases do not appear to be biologically significant because they do not exceed the ranges of normal values found in humans. Furthermore, a longer term study in which SALATRIM fats were fed at 60 g/day for 28 days indicated that the increases return to normal and are not of clinical significance (Finley et al., 1994c).

Because the subchronic rat studies with SALATRIM fats were not designed to detect short-term, transitory changes in serum hepatic enzymes, a short-term (17-day) study in rats exposed to 10% dietary SALATRIM 23SO lot A026 was conducted. Serum levels of several hepatic enzymes were measured at 12 and 4 days prior to initiation of SALATRIM administration and at 3, 6, 9, 13, and 17 days after SALATRIM administration was begun.

High dietary concentrations of SALATRIM fats were used in these rat studies to maximize the probability of detecting any potential toxicity. The dietary concentration was limited to 10% by weight because higher doses may produce micronutrient deficiency by dilution of the diet.

MATERIALS AND METHODS

Materials. SALATRIM 23CA lot A014, SALATRIM 32CA lot A015, and SALATRIM 23SO lot A026 were provided by Nabisco Foods Group (NFG), East Hanover, NJ. Analytical determinations conducted at NFG show that SALATRIM 23CA lot A014 and SALATRIM 23SO lot A026 are mixtures of triacylglycerols composed predominantly of diacetylstearoylglycerol and that SALATRIM 32CA lot A015 is a triacylglycerol mixture composed predominantly of dipropionylstearoylglycerol.

Total fatty acid profile analyses for these three fats were conducted at EPL Bio-Analytical Services, Inc. (EPL-BAS), Decatur, IL, and the results are presented in Tables 1–3. Total fatty acid profile data for the higher molecule 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 quantified by gas chromatography. For lower molecular weight groups (<C12:0), profile data were obtained by saponification with sodium hydroxide followed by acidification with concentrated hydrochloric acid. The fatty acids were quantified by gas chromatography. Standard curves were constructed by bracketing the concentration level of the analyte.

Table 1. Total Fatty Acid Profile for SALATRIM 23CA Lot A014[a]

fatty acid		
name	designation	wt %
stearic (octadecanoic)	C18:0	57 ± 1
acetic	C2:0	21.1 ± 0.1
propionic	C3:0	2.58 ± 0.03
palmitic (hexadecanoic)	C16:0	2.37 ± 0.04
arachidic (eicosanoic)	C20:0	1.50 ± 0.03
behenic (docosanoic)	C22:0	0.668 ± 0.007
oleic (9-octadecenoic)	C18:1	0.572 ± 0.005
lignoceric (tetracosanoic)	C24:0	0.335 ± 0.001
linoleic (9,12-octadecadienoic)	C18:2	0.066 ± 0.001
lauric (dodecanoic)	C12:0	0.007 ± 0.000

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

Table 2. Total Fatty Acid Profile for SALATRIM 32CA Lot A015[a]

fatty acid		
name	designation	wt %
stearic (octadecanoic)	C18:0	51.6 ± 0.4
propionic	C3:0	21 ± 1
palmitic (hexadecanoic)	C16:0	3.39 ± 0.01
acetic	C2:0	1.70 ± 0.07
oleic (9-octadecenoic)	C18:1	1.55 ± 0.03
arachidic (eicosanoic)	C20:0	1.43 ± 0.01
behenic (docosanoic)	C22:0	0.583 ± 0.003
linoleic (9,12-octadecadienoic)	C18:2	0.35 ± 0.01
lignoceric (tetracosanoic)	C24:0	0.306 ± 0.003
lauric (dodecanoic)	C12:0	0.013 ± 0.000

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

Table 3. Total Fatty Acid Profile for SALATRIM 23SO Lot A026[a]

fatty acid		
name	designation	wt %
stearic (octadecanoic)	C18:0	57.823 ± 1.000
acetic	C2:0	23.39 ± 1.12
palmitic (hexadecanoic)	C16:0	7.543 ± 0.027
propionic	C3:0	2.92 ± 0.11
arachidic (eicosanoic)	C20:0	0.425 ± 0.023
behenic (docosanoic)	C22:0	0.252 ± 0.015
oleic (9-octadecenoic)	C18:1	0.289 ± 0.011
lignoceric (tetracosanoic)	C24:0	0.100 ± 0.006
myristic (tetradecanoic)	C14:0	0.084 ± 0.004
linoleic (9,12-octadecadienoic)	C18:2	0.018 ± 0.001
lauric (dodecanoic)	C12:0	0.016 ± 0.001

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

Free fatty acid concentration (by titration) and peroxide value analyses were conducted by NFG and EPL-BAS. Titratable acid values were obtained according to AOCS Official Method Ca 5a-40 (AOCS, 1990a). The peroxide values were obtained using AOCS Official Method Cd 8-53 (AOCS, 1990b). The free fatty acid concentrations and peroxide values for the three triacylglycerol mixtures were low. These results are presented in Table 4.

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

Animals. Crl:CD®BR VAF rats were from Charles River Laboratories, Inc. (Portage, MI). For the 13-week study, 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 distribution was 169–222 g for the males and 129–170 g for the females. For the 17-day study, the rats were approximately 9 weeks of age; weight distributions for the males and females were 247–299 and 182–220 g, respectively, at initiation of

Table 4. Free Fatty Acid and Peroxide Value Analyses for Three SALATRIM Fats[a,b]

	SALATRIM		
	23CA lot A014	32CA lot A015	23SO lot A026
free fatty acid (wt %)	0.86 ± 0.01	0.42 ± 0.03	0.44 ± 0.01
peroxide value (mequiv/kg)	0.3 ± 0.1	0.699 ● 0.001	0.95 ± 0.29

[a] Data represent the mean ± standard deviation for triplicate determinations. [b] Data for SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015 were generated at EPL Bio-Analytical Services, Inc., and data for SALATRIM 23SO lot A026 were generated at Nabisco Foods Group.

treatment. 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 ($p \leq 0.05$).

13-Week Study. The 13-week subchronic toxicity study with rats was conducted at Hazleton Wisconsin, Inc., Madison, WI, from November 1991 through February 1992.

Dosing and Diets. Rats were fed either SALATRIM 23CA lot A014 or SALATRIM 32CA lot A015 at 0%, 2%, 5%, and 10% of the diet by weight 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 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 or necropsy. Test diets were prepared weekly during weeks 1 and 14 and biweekly for all other weeks of the study. When diets were prepared biweekly, they were 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. Proper diet preparation was assessed by analysis for content of either corn oil (by gravimetric analysis) or the SALATRIM fats (by supercritical fluid chromatography). Homogeneity was evaluated using the 2% and 10% SALATRIM and 10% corn oil diets. Stability of the SALATRIM fats in the diets for up to 8 days at room temperature (after being frozen for 2 days) and for up to 15 weeks when frozen (−5 to −20 °C) was assessed by supercritical fluid chromatographic analysis of samples of the 2% and 10% diet mixtures.

Experimental Design. Rats in this subchronic study were divided into nine groups: (1) untreated controls that received basal diet only; (2) treated groups that received SALATRIM 23CA lot A014 at either 2%, (3) 5%, or (4) 10% of the diet by weight; (5) treated groups that received SALATRIM 32CA lot A015 at either 2%, (6) 5%, or (7) 10% of the diet by weight; (8) controls fed 10% corn oil by weight; and (9) an untreated group that served as a sentinel for health and viral serology at study termination. Groups 1–8 contained 20 rats per sex; the sentinel group (group 9) 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. Ophthalmic 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 a subgroup of 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 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.

Most 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, and differential leukocyte count and blood cell morphology slides were prepared using a Geometric Data Hamastainer and read manually. Clinical 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 serum total protein. Urinalysis was done 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 from five nonfasted sentinel rats of each sex was collected from the retro-orbital plexus 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, *Mycoplasma pulmonis*, Hantaan virus, and encephalitozoon cuniculi. At the pretest evaluation, *Bacilis piliformis* and cilia-associated respiratory bacillus antibodies were also determined.

Postmortem Data Collection. After 13 weeks of treatment, all rats except those serving as sentinels were subjected to gross necropsy. Adrenals, brain, kidneys, liver, and testes were weighed. The cecum of each rat was exposed, ligated at the distal ileum, proximal colon, and approximately the distal one-third of the blind end. The distal portion of the cecum was collected for histology. The remaining ligated portion of the cecum from each of the rats fed basal diet, 10% SALATRIM 23CA lot A014, 10% SALATRIM 32CA lot A015, and 10% corn oil (groups 1, 4, 7, and 8) was used to assess the effects of dietary SALATRIM fats on cecal microflora (Scheinbach et al., 1994). After the liver was weighed from rats in groups 1–8 that were selected for serum vitamin chemistry, the left medial lobe was removed, frozen in liquid nitrogen, and stored at −70 °C until analyzed for vitamins A and E (Kayden et al., 1983). The entire femur not used for histopathology from rats selected for vitamin analyses was removed and stored 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. Initially, these bone mineral determinations were conducted for the rats fed basal diet, diets containing 10% of both SALATRIM fats, and diet containing 10% corn oil. Subsequently, the determinations for these groups, except for dry weight and percent ash, were repeated using the acid digestates prepared previously and determinations were conducted for the groups of rats fed diets containing 2% and 5% SALATRIM.

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 rat study with SALATRIM 4CA lot A006 (Hayes et al., 1994b). All tissues from the control rats and

rats fed 10% SALATRIM were subjected to histopathology. Macroscopic lesions (if any), lungs, liver, and kidneys from all other 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; feed consumption (g per week and g/kg of body weight per day); serum chemistry; hematology (except red blood cell morphology); urine pH, volume, and specific gravity; urine chemistry; serum and liver fat-soluble 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). For most of the variables measured, the following transformations were conducted, when necessary, in sequence until homogeneity of variance was achieved: $\log_{10}$, square, square root, reciprocal, angular, and rank. For feed consumption expressed relative to body weight and for bone mineral results, the only transformation performed was the rank transformation. 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 differences cited are based on comparisons with the untreated control group (group 1).

17-Day Study. The short-term rat study was conducted at Hazleton Wisconsin, Inc., from July through August 1992.

Experimental Design. Twenty-four rats per sex were fed 10% dietary SALATRIM 23SO lot A026 by weight throughout the study. An additional 24 rats per sex were fed 10% dietary corn oil by weight, and 12 rats per sex received only the basal diet throughout the study. Diets containing SALATRIM or corn oil were prepared once prior to initiation of the study. The diets were stored frozen until fed to the rats. Diet preparation and feeding procedures were similar to those in the 13-week study discussed above.

The rats were observed twice daily, and body weights were recorded weekly. Using the same procedures as in the 13-week study, blood was collected and serum concentrations of aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl-transferase were determined at 12 and 4 days prior to initiation of the study and on days 3, 6, 9, 13, and 17 after initiation of the study. Following completion of the study, all rats were terminated using carbon dioxide and were discarded without further examination. Statistical analysis was conducted in the same fashion as for the 13-week study above.

RESULTS

13-Week Study. *Diet Analysis.* Pretest analysis of 2% and 10% SALATRIM 23CA lot A014, 2% and 10% SALATRIM 32CA lot A015, 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 23CA lot A014 and SALATRIM 32CA lot A015 was determined for the 2% and 10% test diets stored at room temperature and under freezer conditions. These analytical results indicate that these SALATRIM fats have acceptable room temperature stability in the diet for at least 8 days (after being frozen for 2 days). Diets fed during the study were not maintained at room temperature for longer than 1 week. The diets also demonstrated acceptable stability when stored frozen for up to 15 weeks.

Analysis of each diet preparation fed during the study indicated the diets contained the proper amounts of fats. Because SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015 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 23CA lot A014 consumption for the 2%, 5%, and 10% groups averaged 1.3, 3.4, and 6.6 g/kg in males and 1.5, 3.7, and 7.4 g/kg in females, respectively, during the 13-week study. Daily SALATRIM 32CA lot A015 consumption for the 2%, 5%, and 10% groups averaged 1.4, 3.2, and 6.4 g/kg in males and 1.5, 3.8, and 7.5 g/kg in females, respectively. Daily consumption of corn oil averaged 5.9 and 6.7 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.

In both sexes, mean body weights and body weight gains for rats in the groups receiving the SALATRIM fats were comparable to those of untreated control rats. A statistically significantly higher mean cumulative body weight gain was noted only for the week 12 interval in corn oil-treated females. Cumulative body weight gain data for males and females are presented in Figures 1 and 2.

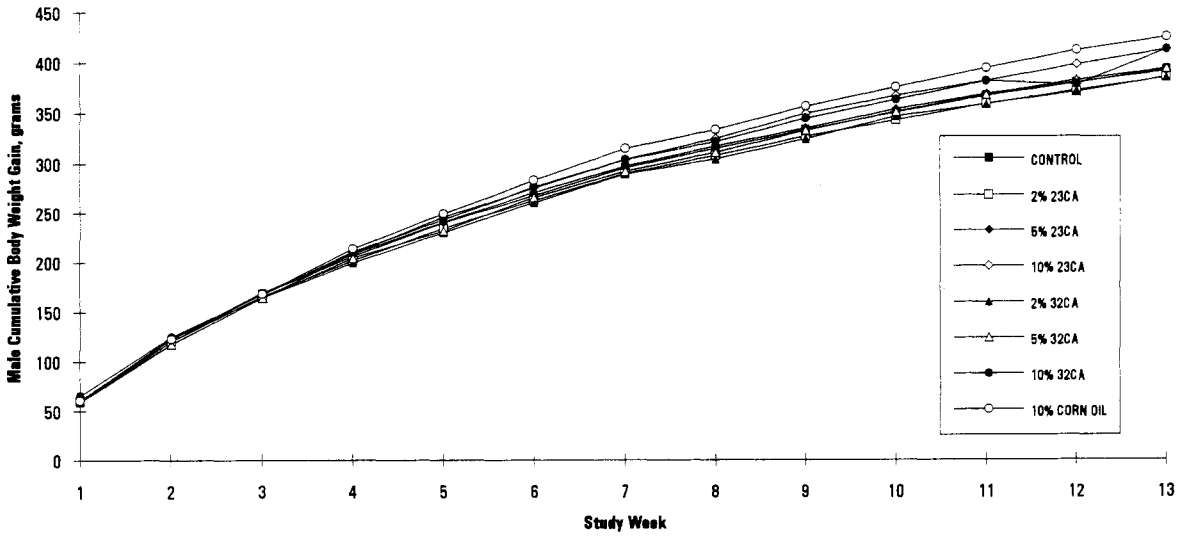

Figure 1. Male mean cumulative body weight gain (g) vs study week. Data points represent the means of 20 rats. Standard deviations (SD) are not shown for the sake of clarity of the figure. In most cases, the SD was less than 15% of the mean.

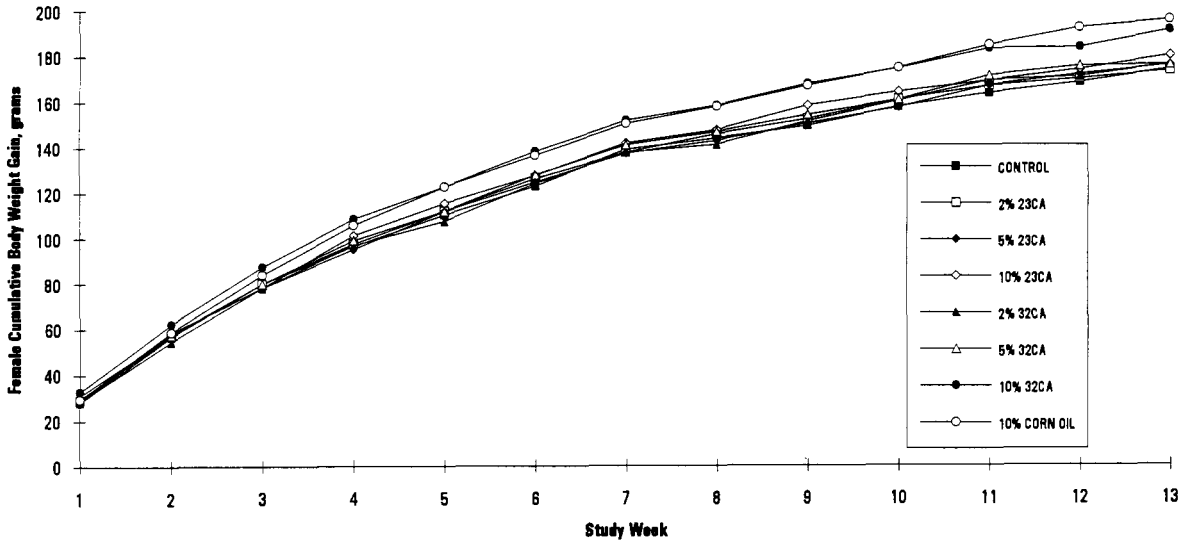

Figure 2. Female mean cumulative body weight gain (g) vs study week. Data points represent the means of 20 rats. Standard deviations (SD) are not shown for the sake of clarity of the figure. In most cases, the SD was less than 20% of the mean.

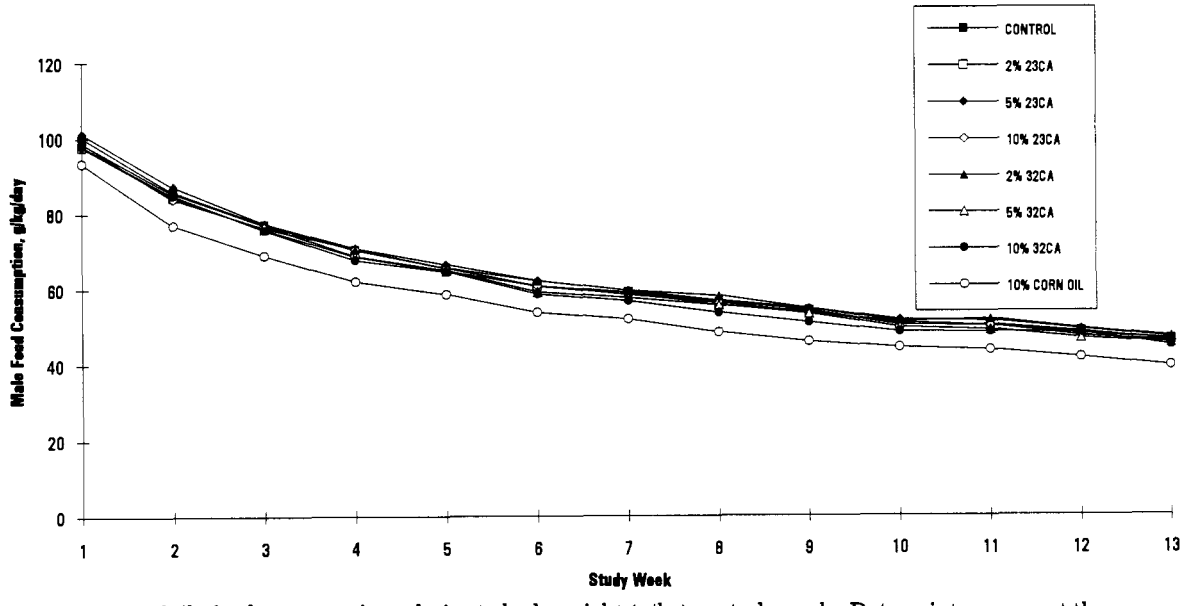

Figure 3. Male mean daily food consumption relative to body weight (g/kg) vs study week. Data points represent the means of 13–20 rats. Standard deviations (SD) are not shown for the sake of clarity of the figure. In most cases, the SD was less than 6% of the mean.

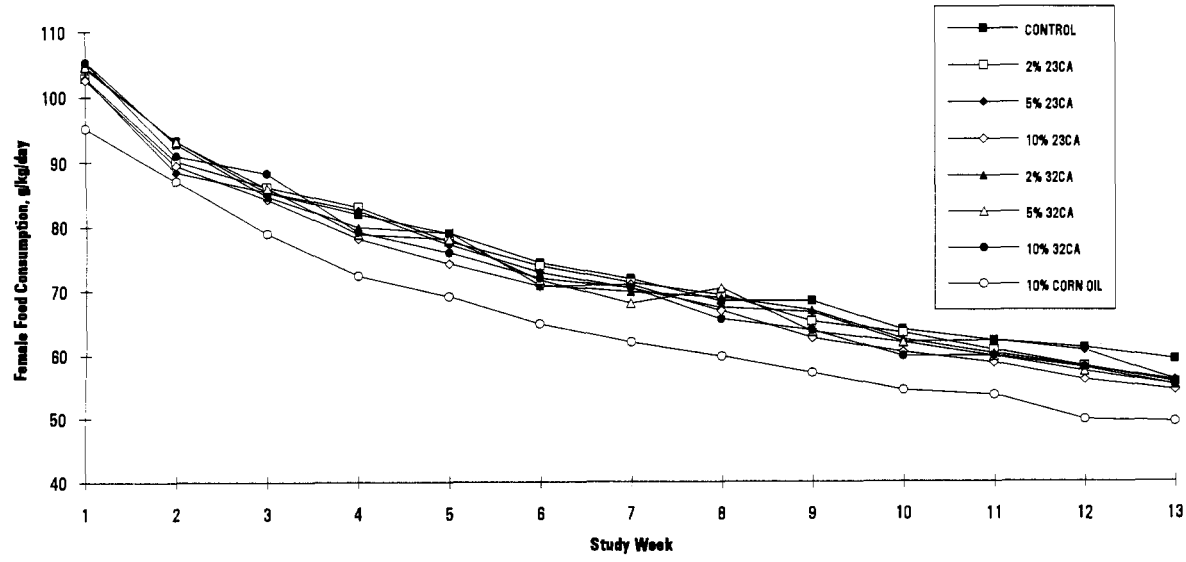

Figure 4. Female mean daily food consumption relative to body weight (g/kg) vs study week. Data points represent the means of 11–20 rats. Standard deviations (SD) are not shown for the sake of clarity of the figure. In most cases, the SD was less than 10% of the mean.

Table 5. Summary of Selected Serum Chemistry Data for SALATRIM 23CA Lot A014 and SALATRIM 32CA Lot A015—Week 14[a]

	treatment							
	control	2% 23CA	5% 23CA	10% 23CA	2% 32CA	5% 32CA	10% 32CA	10% corn oil
				Males				
total protein (g/dL)	6.8 ± 0.4	6.7 ± 0.3	6.6 ± 0.2	6.3 ± 0.4[e]	6.6 ± 0.3	6.5 ± 0.4	6.6 ± 0.3	6.8 ± 0.3
albumin (g/dL)	4.4 ± 0.2	4.4 ± 0.1	4.4 ± 0.2	4.2 ± 0.2	4.3 ± 0.2	4.4 ± 0.2	4.4 ± 0.3	4.4 ± 0.2
globulin (g/dL)	2.4 ± 0.3	2.3 ± 0.2	2.2 ± 0.2	2.1 ± 0.3[e]	2.3 ± 0.2	2.1 ± 0.2	2.2 ± 0.3	2.4 ± 0.2
cholesterol (mg/dL)	70 ± 18	68 ± 15	71 ± 16	82 ± 27	70 ± 16	73 ± 17	76 ± 23	88 ± 18
HDL[b] (mg/dL)	59 ± 14	56 ± 13	59 ± 18	69 ± 24	59 ± 16	60 ± 16	64 ± 20	76 ± 17
LDL[c] (mg/dL)	1 ± 2	1 ± 2	2 ± 3	1 ± 3	0 ± 1	1 ± 3	2 ± 3	1 ± 1
triglycerides (mg/dL)	86 ± 40	84 ± 38	83 ± 41	83 ± 21	91 ± 33	90 ± 49	104 ± 77	80 ± 39
				Females				
total protein (g/dL)	6.8 ± 0.3	6.6 ± 0.4	6.6 ± 0.4	6.5 ± 0.3	7.0 ± 0.3	6.9 ± 0.4	6.7 ± 0.4	7.0 ± 0.3
albumin (g/dL)	4.7 ± 0.3	4.6 ± 0.5	4.5 ± 0.4	4.6 ± 0.2	4.9 ± 0.2	4.9 ± 0.5	4.8 ± 0.4	5.1 ± 0.4
globulin (g/dL)	2.1 ± 0.2	2.0 ± 0.3	2.1 ± 0.3	1.9 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	1.9 ± 0.2
cholesterol (mg/dL)	78 ± 12	85 ± 14	82 ± 21	86 ± 14	86 ± 16	78 ± 18	95 ± 15	89 ± 26
HDL (mg/dL)	71 ± 13	73 ± 12[d]	75 ± 24[d]	79 ± 14	76 ± 14	71 ± 17	84 ± 16	78 ± 23
LDL (mg/dL)	2 ± 4	3 ± 3[d]	2 ± 2[d]	2 ± 2	4 ± 7	1 ± 1	2 ± 3	1 ± 2
triglycerides (mg/dL)	45 ± 20	37 ± 9	34 ± 13	43 ± 26	38 ± 15	43 ± 24	49 ± 16	54 ± 18

[a] Data represent mean ± standard deviation. Means based on 10 rats except where 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 ($p \leq 0.05$).

Table 6. Summary of Serum Mineral Analyses and Urine Mineral Clearance Data for SALATRIM 23CA Lot A014 and SALATRIM 32CA Lot A015[a]

	treatment							
	control	2% 23CA	5% 23CA	10% 23CA	2% 32CA	5% 32CA	10% 32CA	10% corn oil
				Males				
serum minerals								
calcium (mg/dL)	9.9 ± 0.4	9.9 ± 0.3	9.9 ± 0.4	9.6 ± 0.4	9.9 ± 0.3	9.8 ± 0.5	9.8 ± 0.4	10.0 ± 0.2
inorg phosphorus (mg/dL)	7.5 ± 0.7	7.1 ± 0.7	6.8 ± 0.4	7.0 ± 0.5	7.0 ± 0.7	6.8 ± 0.5	6.8 ± 0.5	6.8 ± 0.4
sodium (mmol/L)	145 ± 1	145 ± 1	146 ± 2	146 ± 2	146 ± 1	145 ± 1	146 ± 2	146 ± 1
potassium (mmol/L)	4.9 ± 0.3	4.8 ± 0.3	4.9 ± 0.3	4.9 ± 0.1	5.0 ± 0.2	4.9 ± 0.4	5.0 ± 0.3	5.0 ± 0.2
chloride (mmol/L)	102 ± 1	102 ± 1	102 ± 2	104 ± 3	102 ± 2	102 ± 2	103 ± 3	102 ± 2
urine mineral clearance								
calcium (%)	0.25 ± 0.14	0.24 ± 0.06	0.27 ± 0.18	0.22 ± 0.14	0.21 ± 0.05	0.21 ± 0.09	0.20 ± 0.03	0.18 ± 0.03
phosphorus (%)	14.0 ± 2.0	15.9 ± 2.1	17.6 ± 2.8	18.6 ± 3.5[b]	15.3 ± 2.1	16.6 ± 2.9	17.9 ± 1.5[b]	14.5 ± 1.6
sodium (%)	0.21 ± 0.06	0.23 ± 0.09	0.25 ± 0.13	0.27 ± 0.07	0.22 ± 0.05	0.21 ± 0.05	0.22 ± 0.04	0.19 ± 0.05
potassium (%)	19.3 ± 5.9	18.8 ± 2.3	17.7 ± 3.7	18.1 ± 2.2	17.0 ± 1.5	18.4 ± 3.8	18.2 ± 3.0	16.4 ± 2.1
chloride (%)	0.34 ± 0.16	0.40 ± 0.11	0.36 ± 0.22	0.39 ± 0.23	0.38 ± 0.17	0.37 ± 0.29	0.26 ± 0.14	0.48 ± 0.22
				Females				
serum minerals								
calcium (mg/dL)	10.0 ± 0.3	10.1 ± 0.3	9.9 ± 0.3	10.0 ± 0.3	10.1 ± 0.2	10.0 ± 0.4	10.1 ± 0.4	10.3 ± 0.3
inorg phosphorus (mg/dL)	7.0 ± 0.8	6.3 ± 1.1	6.2 ± 0.7	6.3 ± 0.4	6.2 ± 0.7	6.2 ± 0.7	6.0 ± 0.6	5.9 ± 0.7
sodium (mmol/L)	145 ± 1	145 ± 2	146 ± 2	146 ± 1	146 ± 2	146 ± 1	145 ± 1	145 ± 1
potassium (mmol/L)	4.7 ± 0.3	4.6 ± 0.3	4.9 ± 0.3	4.6 ± 0.4	4.9 ± 0.4	4.5 ± 0.3	4.6 ± 0.4	4.6 ± 0.3
chloride (mmol/L)	104 ± 2	104 ± 2	106 ± 1	105 ± 2	104 ± 2	104 ± 2	103 ± 3	103 ± 2
urine mineral clearance								
calcium (%)	0.55 ± 0.51	0.63 ± 0.43	0.55 ± 0.24	0.44 ± 0.19	0.66 ± 0.39	0.65 ± 0.33	0.59 ± 0.46	0.76 ± 0.40
phosphorus (%)	18.3 ± 5.1	19.6 ± 3.6	20.5 ± 2.5	22.2 ± 3.7	21.3 ± 5.8	21.1 ± 4.1	25.5 ± 3.4[b]	22.5 ± 6.0
sodium (%)	0.22 ± 0.08	0.27 ± 0.12	0.24 ± 0.05	0.31 ± 0.12	0.31 ± 0.15	0.28 ± 0.11	0.34 ± 0.12	0.20 ± 0.08
potassium (%)	20.0 ± 5.4	19.4 ± 2.8	17.9 ± 4.6	18.5 ± 4.9	19.6 ± 5.2	21.6 ± 3.8	21.2 ± 4.6	19.9 ± 5.0
chloride (%)	0.53 ± 0.21	0.62 ± 0.27	0.57 ± 0.21	0.63 ± 0.37	0.96 ± 0.58	0.49 ± 0.25	0.70 ± 0.27	0.53 ± 0.23

[a] Data represent mean ± standard deviation. Means based on 10 rats. [b] Significantly different from control ($p \leq 0.05$).

Generally, feed consumption (g per week and g/kg per day) was significantly lower than that of controls for the 10% corn oil males and females. Feed consumption for the groups given SALATRIM fats was similar to that of the control group, although sporadically lower consumption relative to body weight (g/kg/day) was noted occasionally for the males fed 10% SALATRIM 32CA lot A015 and for females fed 10% SALATRIM 23CA lot A014 (Figures 3 and 4).

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

Clinical Pathology. Hematology and urinalysis data revealed no treatment-related effects when rats in the groups receiving SALATRIM 23CA lot A014, SALATRIM 32CA lot A015, and corn oil were compared with untreated controls. Select serum chemistry data are presented in Table 5. Serum total protein and globulin of male rats in the 10% SALATRIM 23CA lot A014 group were slightly lower when compared with those of the untreated control groups. Total protein and globulin in females from this group and in rats of both sexes in the 10% SALATRIM 32CA lot A015 group were comparable with those of controls. No effect on these variables was observed in a previously conducted subchronic rat study with another SALATRIM fat, SALATRIM 4CA lot A006 (Hayes et al., 1994b). No treatment-related effect on any other serum chemistry variable, including serum lipids, was noted for any of the SALATRIM-treated groups in this study.

Compared to controls, a dose-related trend toward slightly increased urinary phosphorus clearance was noted

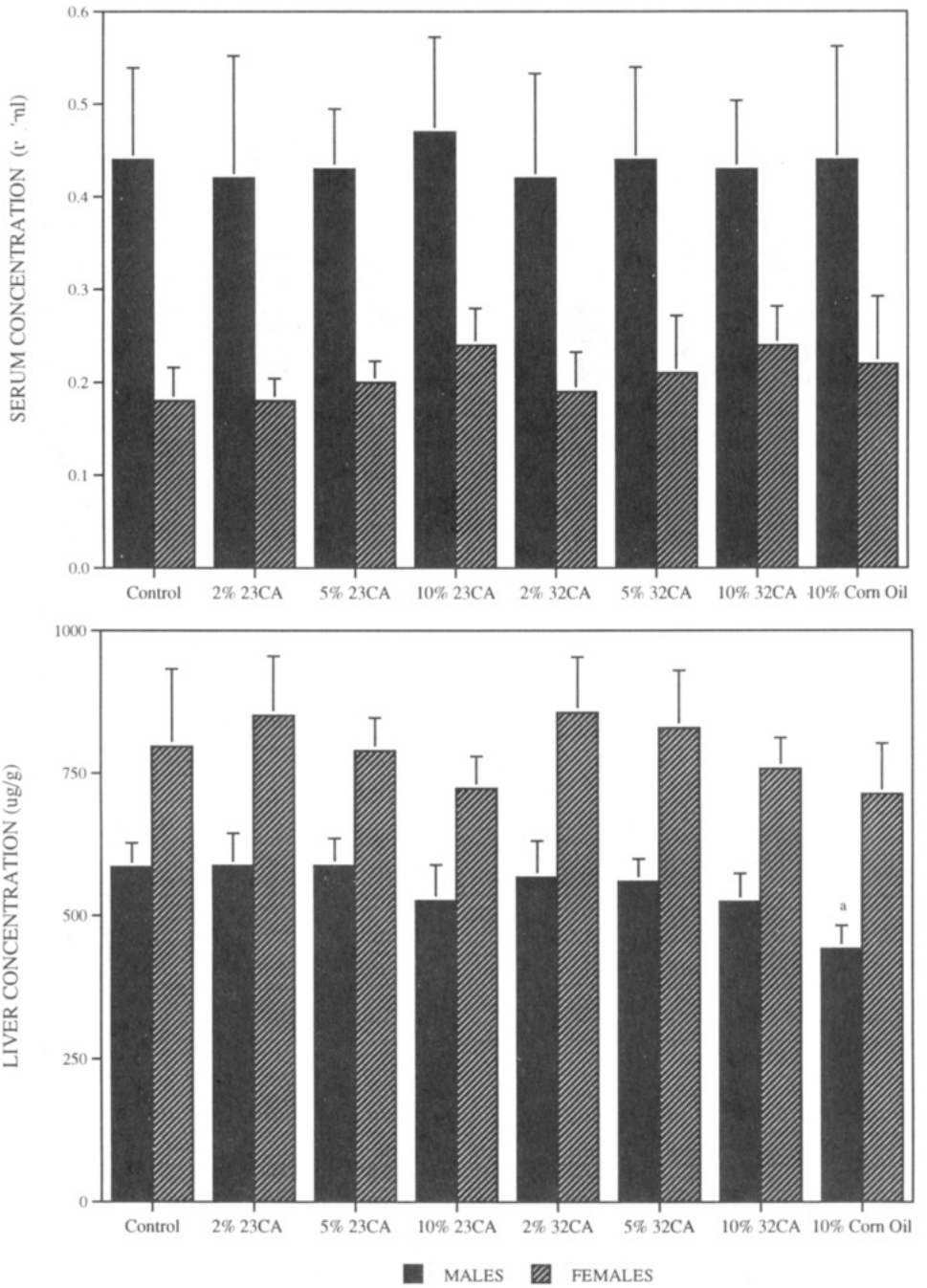

Figure 5. Male and female concentration of vitamin A (*trans*-retinol) in serum (μg/mL) and liver (μg/g) vs treatment at study week 14. Data represent mean ± standard deviation of the mean for 9–10 rats. Data significantly different from control group ($p \leq 0.05$) are noted with an "a".

for rats fed SALATRIM fats (Table 6). Statistically significant increased urinary phosphorus clearance was noted for 10% SALATRIM 23CA lot A014-treated males and for 10% SALATRIM 32CA lot A015-treated males and females. No other effects of SALATRIM exposure on urinary clearance of minerals were observed. In the study with SALATRIM 4CA lot A006 (Hayes et al., 1994b), urinary phosphorus clearance in control and SALATRIM-treated rats was statistically comparable, although the phosphorus clearance values for the SALATRIM-treated groups were slightly higher than control values in general. For example, phosphorus clearance values of the control and 10% SALATRIM 4CA lot A006 groups in that study were 14.9 ± 2.1% and 16.7 ± 2.4%, respectively, in males and 20.8 ± 3.6% and 23.1 ± 4.5%, respectively, in females.

Vitamin Chemistry. Serum concentrations of vitamins A, E, and D and liver concentrations of vitamins A and E in SALATRIM-treated rats were comparable to those of control rats (Figures 5–7). Mean liver vitamin A was significantly lower than control in 10% corn oil-treated males. Prothrombin time, an indicator of vitamin K status, was unaffected by exposure to the SALATRIM fats and corn oil. Consistent with the results in this study, no significant effects on serum concentrations of vitamins A, E, and D, liver concentrations of vitamins A and E, and prothrombin time were noted in rats fed up to 10% SALATRIM 4CA lot A006 in the diet during the previous subchronic study (Hayes et al., 1994b).

Distinct sex differences, unrelated to treatment, were noted in serum and liver concentrations of fat-soluble vitamins, as previously observed (Hayes et al., 1994b). Serum concentrations of vitamin A were higher in males than in females. Serum concentrations of vitamins D and

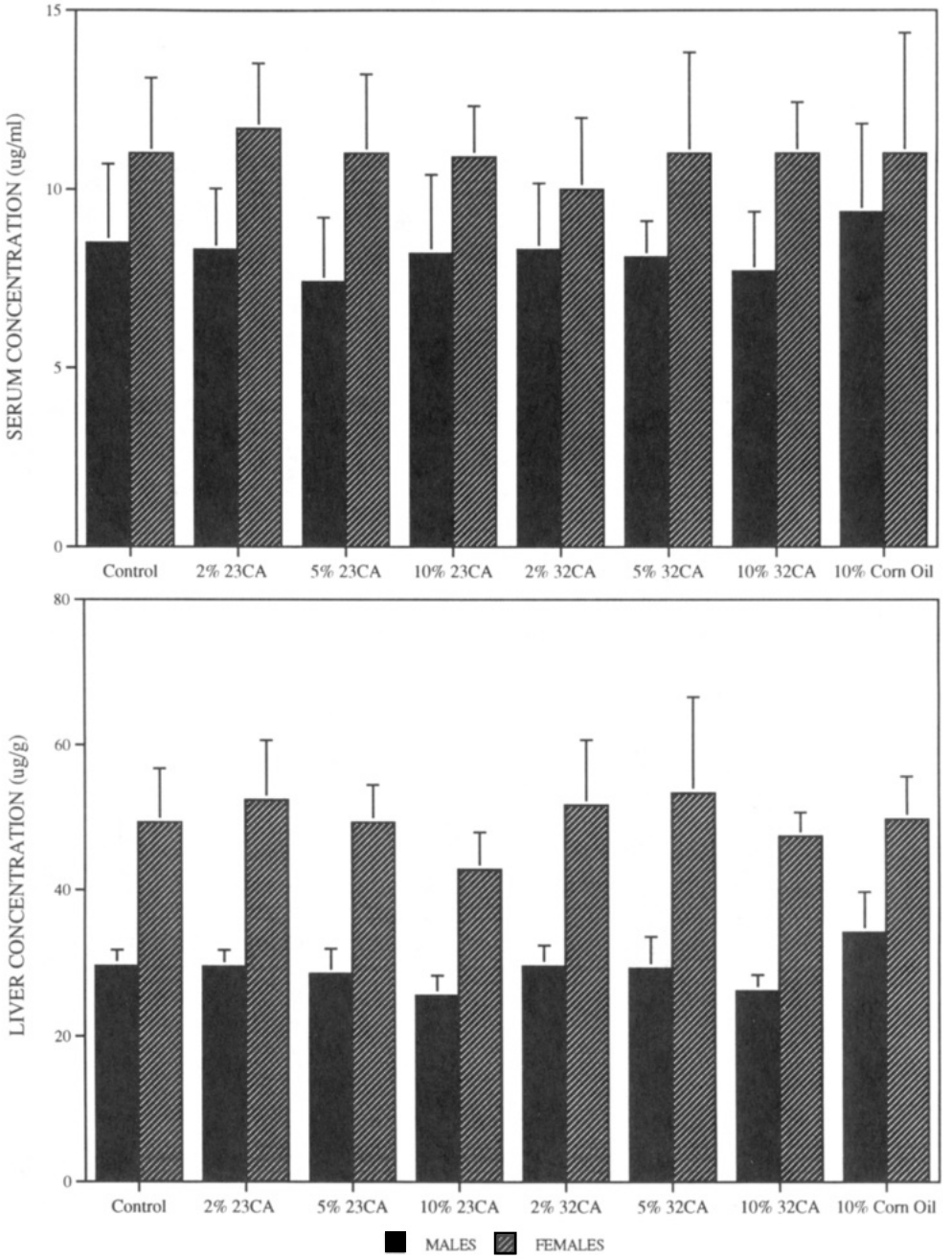

Figure 6. Male and female concentration of vitamin E (α-tocopherol) in serum (μg/mL) and liver (μg/g) vs treatment at study week 14. Data represent mean ± standard deviation of the mean for 9–10 rats. Data significantly different from control group ($p \leq 0.05$) are noted with an "a".

E and liver concentrations of vitamins A and E were generally higher in females than in males.

Bone Mineral Analyses. Mean strontium concentrations were significantly higher in femurs from 10% SALATRIM 23CA lot A014-treated females compared to controls (Table 7). Mean zinc concentrations were significantly higher in femurs from females fed 10% SALATRIM 23CA lot A014 and 10% SALATRIM 32CA lot A015 compared with control females. Mean zinc concentration was significantly lower in the 10% corn oil males than in controls. In the SALATRIM 4CA lot A006 study, bone strontium and zinc concentrations were significantly higher in rats for both sexes and bone sodium was higher in females in the 10% SALATRIM group compared with untreated controls (Hayes et al., 1994b). In that study, bone strontium was higher and bone zinc was lower in 10% corn oil-treated rats compared with controls.

Mean calcium and phosphorus concentrations and mean percent ash were slightly but statistically significantly lower for femurs in several of the groups fed 2% and 5% SALATRIM fats compared with untreated controls (Table 7). These differences were considered to be unrelated to treatment since, at both the initial analysis and the reanalysis, these variables were unaffected by exposure to either of the SALATRIM fats at 10% in the diet. The differences may have been the result of analytical variability.

Organ Weights, Macroscopic and Microscopic Pathology. There were no treatment-related differences between the organ weights of rats treated with SALATRIM 23CA lot A014, SALATRIM 32CA lot A015, or corn oil and untreated control rats.

Macroscopically, no treatment-related effects were observed in SALATRIM-treated rats. As shown in Table 8, a higher incidence of renal mineralization was noted microscopically in females of the groups receiving corn oil

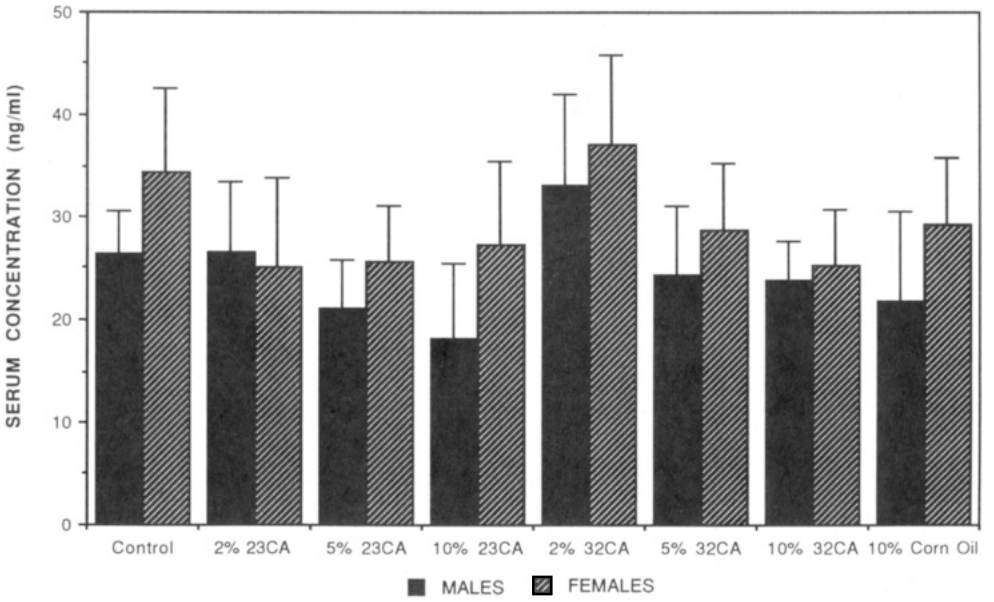

Figure 7. Male and female concentration of vitamin D (25-hydroxy vitamin D) in serum (ng/mL) vs treatment at study week 14. Data represent mean ± standard deviation of the mean for 10 rats. Data significantly different from control group ($p \leq 0.05$) are noted with an "a".

Table 7. Summary of Bone Mineral Analyses for SALATRIM 23CA Lot A014 and SALATRIM 32CA Lot A015[a]

	treatment							
	control	2% 23CA	5% 23CA	10% 23CA	2% 32CA	5% 32CA	10% 32CA	10% corn oil
				Males				
defatted femur wt (mg)	838.9 ± 136.7[b]	862.7 ± 102.4	869.9 ± 89.1	832.5 ± 82.7[b]	806.7 ± 112.1	837.3 ± 96.7	810.5 ± 71.0[b]	864.2 ± 57.4[b]
ash (%)	68.5 ± 0.7[b]	67.2 ± 1.0[c]	66.8 ± 0.9[c]	68.6 ± 0.6[b]	67.3 ± 0.6	67.0 ± 1.1[c]	68.1 ± 1.5[b]	68.4 ± 0.7[b]
calcium (mg/g)	250 ± 9	241 ± 7	239 ± 5[c]	251 ± 7	245 ± 7	242 ± 8	249 ± 9	250 ± 6
	261 ± 5[b]			267 ± 10[b]			261 ± 8[b]	265 ± 9[b]
copper (μg/g)	10.2 ± 0.4[b]			10.4 ± 0.4[b]			10.2 ± 0.3[b]	10.2 ± 0.2[b]
iron (μg/g)	50.6 ± 12.3	47.8 ± 18.7	50.0 ± 14.2	46.9 ± 8.4	48.4 ± 12.8	43.2 ± 13.9	48.2 ± 13.1	44.5 ± 8.3
	55.5 ± 12.6[b]			52.7 ± 9.5[b]			53.3 ± 13.2[b]	49.4 ± 9.7[b]
magnesium (mg/g)	4.43 ± 0.21	4.29 ± 0.06	4.26 ± 0.21	4.51 ± 0.22	4.21 ± 0.21	4.25 ± 0.16	4.46 ± 0.23	4.34 ± 0.12
	4.39 ± 0.21[b]			4.51 ± 0.20[b]			4.41 ± 0.20[b]	4.32 ± 0.16[b]
phosphorus (mg/g)	121 ± 3	116 ± 2[c]	116 ± 2[c]	122 ± 3	117 ± 2[c]	117 ± 2[c]	121 ± 4	122 ± 2
	122 ± 2[b]			125 ± 3[b]			122 ± 3[b]	123 ± 3[b]
sodium (mg/g)	4.57 ± 0.20	4.44 ± 0.15	4.49 ± 0.14	4.67 ± 0.22	4.51 ± 0.24	4.52 ± 0.25	4.61 ± 0.18	4.58 ± 0.21
	4.04 ± 0.14[b]			4.19 ± 0.20[b]			4.11 ± 0.17[b]	4.07 ± 0.22[b]
strontium (μg/g)	51.9 ± 3.7	51.5 ± 4.4	50.2 ± 3.4	56.5 ± 3.5	49.5 ± 5.4	51.8 ± 4.0	51.0 ± 4.1	51.6 ± 3.2
	57.2 ± 4.2[b]			62.2 ± 3.5[b]			56.6 ± 4.3[b]	57.0 ± 3.4[b]
zinc (μg/g)	269 ± 20	255 ± 13	266 ± 12	278 ± 14	255 ± 6	266 ± 12	277 ± 13	248 ± 16[c]
	273 ± 19[b]			287 ± 19[b]			281 ± 16[b]	255 ± 13[b]
				Females				
defatted femur wt (mg)	605.3 ± 52.9[b]	569.7 ± 72.3	602.8 ± 74.6	627.6 ± 72.8[b]	545.3 ± 80.9	590.3 ± 76.9	601.7 ± 39.7[b]	593.7 ± 66.7[b]
ash (%)	69.3 ± 0.8[b]	70.0 ± 0.9	68.0 ± 0.5[c]	69.0 ± 0.5[b]	68.2 ± 0.9[c]	68.0 ± 0.8[c]	69.3 ± 0.9[b]	69.2 ± 0.7[b]
calcium (mg/g)	265 ± 6	262 ± 4	255 ± 4[c]	264 ± 5	259 ± 7	255 ± 4[c]	264 ± 6	264 ± 6
	264 ± 7[b]			266 ± 6[b]			261 ± 3[b]	264 ± 4[b]
copper (μg/g)	10.5 ± 0.2[b]			10.6 ± 0.3[b]			10.6 ± 0.4[b]	10.5 ± 0.3[b]
iron (μg/g)	50.5 ± 14.0	43.8 ± 7.1	52.4 ± 9.9	59.9 ± 14.9	51.4 ± 16.1	51.5 ± 17.0	56.6 ± 12.2	52.5 ± 7.4
	55.5 ± 14.0[b]			65.6 ± 16.8[b]			61.0 ± 12.6[b]	56.9 ± 7.9[b]
magnesium (mg/g)	4.58 ± 0.22	4.58 ± 0.15	4.40 ± 0.17	4.62 ± 0.23	4.59 ± 0.19	4.45 ± 0.14	4.60 ± 0.12	4.62 ± 0.15
	4.52 ± 0.17[b]			4.58 ± 0.20[b]			4.55 ± 0.14[b]	4.57 ± 0.15[b]
phosphorus (mg/g)	124 ± 2	121 ± 1[c]	119 ± 1[c]	124 ± 2	120 ± 3[c]	119 ± 2[c]	123 ± 3	123 ± 2
	123 ± 2[b]			124 ± 2[b]			122 ± 2[b]	123 ± 2[b]
sodium (mg/g)	4.38 ± 0.18	4.52 ± 0.18	4.66 ± 0.14	4.50 ± 0.30	4.36 ± 0.16	4.59 ± 0.17	4.59 ± 0.28	4.51 ± 0.18
	3.94 ± 0.12[b]			4.07 ± 0.29[b]			4.12 ± 0.24[b]	4.07 ± 0.18[b]
strontium (μg/g)	48.9 ± 3.1	50.7 ± 2.3	49.2 ± 2.7	56.9 ± 4.0[c]	49.7 ± 2.3	50.8 ± 3.2	51.3 ± 2.8	52.9 ± 3.1
	55.1 ± 3.2[b]			63.0 ± 4.3[b,c]			56.9 ± 3.0[b]	58.8 ± 3.0[b]
zinc (μg/g)	284 ± 23	284 ± 17	291 ± 14	304 ± 12	286 ± 16	287 ± 17	313 ± 12[c]	271 ± 13
	284 ± 19[b]			309 ± 17[b,c]			313 ± 12[b,c]	275 ± 14[b]

[a] Barium, potassium, and copper concentrations were determined during the initial analysis (selected groups) and reanalysis (all groups). No treatment-related effects were noted. Data represent mean ± standard deviation for 10 rats, except for the 2% SALATRIM 32CA lot A015 group, for which the mean was for 9 rats. [b] Initial analysis. [c] Significantly different from control ($p \leq 0.05$).

and 5% and 10% SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015 when compared with untreated controls. The incidence was similar in each of these groups of triacylglycerol-treated females. The average severity of the mineralization was comparable among the groups of females, including untreated controls,

Table 8. Summary of Renal Mineralization Incidence and Severity Data—Week 14[a]

	treatment							
	control	2% 23CA	5% 23CA	10% 23CA	2% 32CA	5% 32CA	10% 32CA	10% corn oil
				Males				
total incidence	1	0	0	2	0	1	1	0
severity of mineralization[b]								
0	19	20	20	18	20	19	19	20
1	1	0	0	2	0	1	1	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
				Females				
total incidence	9	12	16	18	11	17	18	15
severity of mineralization[b]								
0	11	8	4	2	9	3	2	5
1	6	11	11	6	10	15	14	15
2	2	1	4	8	1	2	1	0
3	1	0	1	4	0	0	3	0
mean severity[c]	1.4 ± 0.7	1.1 ± 0.3	1.4 ± 0.6	1.9 ± 0.8	1.1 ± 0.3	1.1 ± 0.3	1.4 ± 0.8	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] Data represent the mean ± standard deviation for rats in which mineralization was noted.

and only in the 10% SALATRIM 23CA lot A014-treated group was this average slightly higher than the controls. No treatment-related renal mineralization was noted in any group of corn oil-treated or SALATRIM-treated males. No renal effect was observed in the SALATRIM 4CA lot A006 study (Hayes et al., 1994b).

Mottled livers were noted macroscopically in males and hepatocellular vacuolation was observed microscopically in males and females of the 10% corn oil group. These changes were not noted in the rats fed equivalent amounts of SALATRIM 23CA lot A014 or SALATRIM 32CA lot A015. Consistent with these findings, hepatocellular vacuolation was noted in corn oil-treated rats and was not observed in SALATRIM-treated rats in a previous subchronic study (Hayes et al., 1994b).

17-Day Study. Body weights and body weight gains of control rats and rats fed 10% dietary SALATRIM 23SO lot A026 or corn oil were similar. All rats except one control female and one 10% SALATRIM male survived the duration of the study.

Neither SALATRIM nor corn oil had any effect at any study interval on serum activity of aspartate aminotransferase, alanine aminotransferase, or γ-glutamyltransferase activities. Because no effect of SALATRIM or corn oil treatment was noted and because the values for these enzymes were similar in males and females, the means and standard deviations were calculated for all rats of both sexes at each dietary treatment, i.e., control, 10% SALATRIM, and 10% corn oil diets. These data are presented graphically in Figure 8.

DISCUSSION

The recommendation to decrease dietary fat consumption from 37% to 30% of total caloric intake (DHHS Publication 88-50210, 1988; NRC, 1989) has stimulated an interest to decrease fat in food. The agricultural and food industries continue to use available technologies to alter food fats to meet this goal.

SALATRIM 23CA lot A014, SALATRIM 32CA lot A015, and SALATRIM 23SO lot A026 are members of a family of structured triacylglycerols termed SALATRIM. These SALATRIM fats are composed of a glycerol backbone esterified with stearic acid and short-chain fatty acids (acetic and propionic). SALATRIM fats have caloric availabilities of 4.5–6.0 kcal/g (Finley et al., 1994a) compared with 9 kcal/g for corn oil.

SALATRIM fats are hydrolyzed by lingual and pancreatic lipase to release monostearin, glycerol, free short-chain fatty acids, and stearic acid. As with other triacylglycerols, the short-chain fatty acids not metabolized by the gut mucosal cells will enter the portal circulation and bind to albumin. In the liver, they will be rapidly oxidized to yield ketone bodies, CO_2, water, and energy (Buguat, 1987; Rombeau et al., 1990). A substantial portion of stearic acid in SALATRIM fats should be hydrolyzed and excreted in the feces as soaps or free stearic acid, as is stearic acid in other fats (Benzonana and Desnuelle, 1968; Bliss et al., 1972; Gacs and Barltrop, 1977; Mattson et al., 1979; Sammons and Wiggs, 1960). Because of this and because of the lower number of calories provided by short-chain fatty acids compared to long-chain fatty acids, SALATRIM fats have a lower caloric availability than most conventional fats and oils (Finley et al., 1994a).

On the basis of the similarity to fats normally found in the food supply, their predictable metabolism and the results of toxicity testing with SALATRIM 4CA lot A006 (Hayes et al., 1994b), SALATRIM fats are not expected to cause toxicological effects. This hypothesis was tested in the 13-week subchronic study with SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015 and the 17-day study with SALATRIM 23SO lot A026 that are the subject of this paper.

In the subchronic study, there were no significant differences between the body weights of control and SALATRIM-treated males and females. The female corn oil group had a significantly higher mean cumulative body weight gain at the week 12 interval compared with that of controls. In general, both absolute (g per week) and relative (g/kg per day) feed consumption values for the 10% corn oil males and females were significantly lower than those of controls. These differences in body weight gain and feed consumption for the corn oil-treated rats are predictable on the basis of total caloric consumption.

Evaluation of prothrombin time as an indicator of vitamin K status, serum concentrations of vitamins A, E, and D, and liver concentrations of vitamins A and E indicated the SALATRIM fats had no effect on fat-soluble vitamins in rats from the subchronic study. In the subchronic study with SALATRIM 4CA lot A006 (Hayes et al., 1994b), diets were supplemented with vitamins A, D, E, and K to prevent any potential effect on vitamin status in the rats. Analysis of fat-soluble vitamin status in those rats indicated that SALATRIM 4CA lot A006, like SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015, had no significant effect on vitamin status. These

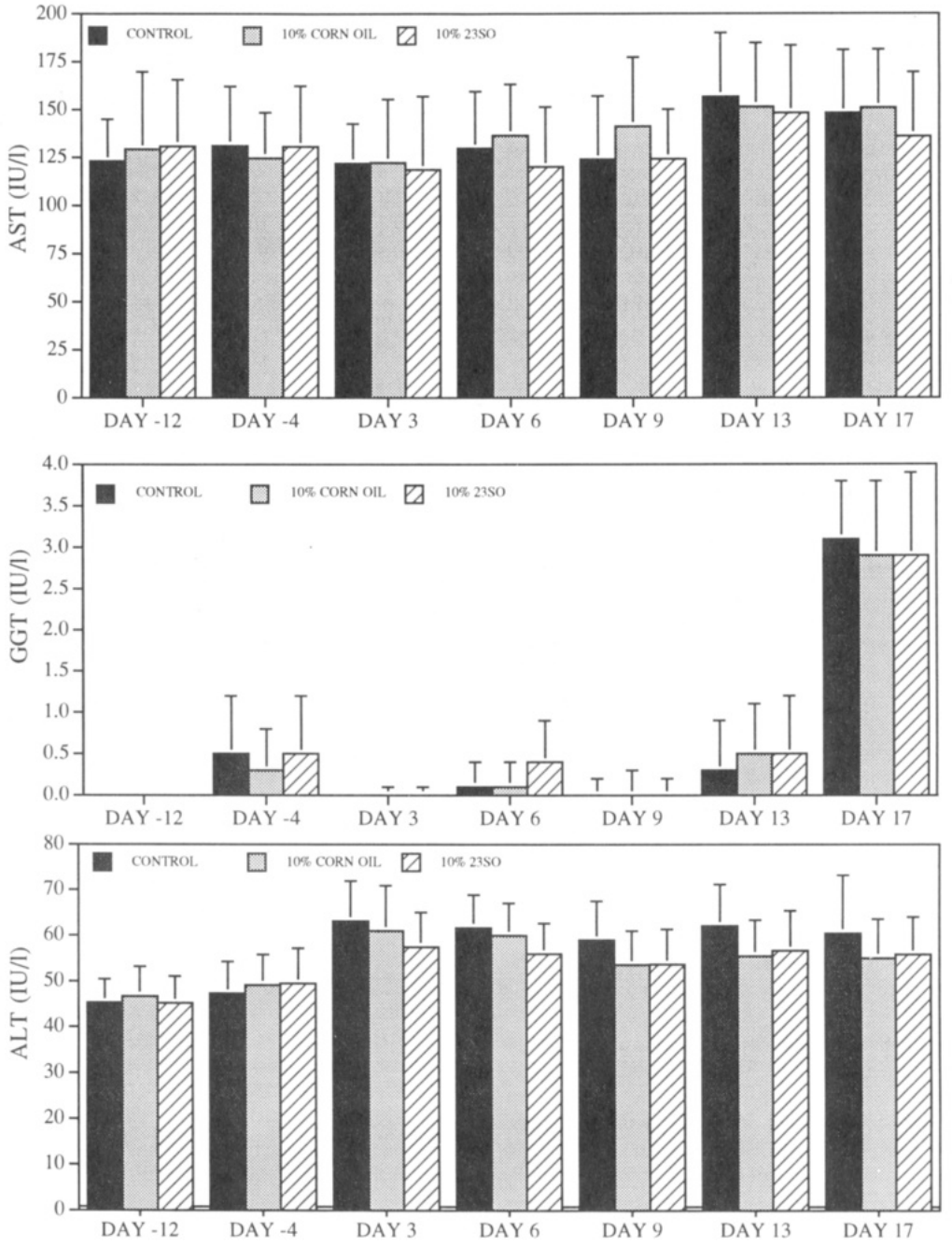

Figure 8. Serum activity (IU/L) of aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), and alanine aminotransferase (ALT) relative to treatment at various intervals in the short-term continuous-feeding study. Data represent the mean ± standard deviation of the mean for 22–24 control rats and 47–48 SALATRIM and corn oil rats. Data significantly different from control group ($p \leq 0.05$) are noted with an "a".

results show that dietary vitamin supplementation is unnecessary in toxicology studies with SALATRIM fats.

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 study with SALATRIM 4CA lot A006 (Hayes et al., 1994b).

During the clinical pathology determinations in the subchronic study, slightly lower total protein and globulin in males fed 10% SALATRIM 23CA lot A014 were noted. Total protein and globulin for females in this group and for rats of both sexes fed 10% SALATRIM 32CA lot A015 were comparable with those of controls. In addition, no effect was observed on these variables in the subchronic rat study with SALATRIM 4CA lot A006 conducted previously (Hayes et al., 1994b). Because statistically significantly lower total protein and globulin were noted only for one of three SALATRIM fats and only in one sex, the findings was considered to be incidental and biologically insignificant. The high dietary concentrations of triacylglycerols (both SALATRIM fats and corn oil) fed in this study did not produce effects on serum triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol.

No effect of SALATRIM exposure on serum hepatic enzymes was noted during this subchronic study with SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015 or during the previous subchronic rat study with SALATRIM 4CA lot A006 (Hayes et al., 1994b). In

addition, no indication of a transitory effect on these enzymes was noted in the supplemental short-term rat study with SALATRIM 23SO lot A026. The absence of such an effect in these SALATRIM studies indicates that the elevation in serum alanine aminotransferase noted in a 28-day rat feeding study with the low-calorie fat caprenin (Webb et al., 1991) was not reflective of a general effect of low calorie fats. Further, the absence of increased serum hepatic enzyme levels in the SALATRIM rat studies supports the conclusion of the SALATRIM human clinical study (Finley et al., 1994b). In this study, serum enzyme levels were minimally increased following human ingestion of SALATRIM but were well within the normal range for humans and, therefore, were considered to be biologically insignificant. In a subsequent human clinical study, these slight changes were determined to be reversible during longer term (28 day) SALATRIM exposure (Finley et al., 1994c).

It is possible that the reversible increase in serum enzyme activities in the SALATRIM clinical studies was related to high fat consumption. Transient increases in serum levels of alanine aminotransferase and aspartate aminotransferase activities associated with feeding of high-fat diets to rats have been reported (Krajcovicova-Kudlackova and Dibak, 1985). In those studies, aminotransferase activities of rats fed diets containing 30–36.5% fat were compared with diets containing 10–15% fat. The absence of effects on these enzymes in this subchronic study, the previous subchronic study with another SALATRIM (Hayes et al., 1994b), and the short-term study with SALATRIM 23SO lot A026 was possibly due to the lower *total* (SALATRIM plus basal diet fat) dietary fat concentrations. In the SALATRIM studies, the highest *total* dietary fat concentration was approximately 15%, which is equivalent to the concentration fed as one of the control levels by Krajcovicova-Kudlackova and Dibak (1985). At this concentration, it is not surprising that no effect on serum aminotransferase activity was noted.

Minimal effects on several mineral determinations were noted in the subchronic study. Increased urinary phosphorus clearance and increased bone concentrations of strontium and zinc were noted in rats fed SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015, and decreased bone zinc was noted in corn oil-treated rats. In the previous subchronic study, there was a suggestion of increased urinary phosphorus clearance in SALATRIM 4CA lot A006-treated rats, although the differences between treated and control groups were small and not statistically significant (Hayes et al., 1994b). Increases in bone zinc and strontium concentrations in rats receiving 10% SALATRIM 4CA lot A006 were observed. Increased bone strontium and decreased bone zinc were also noted for the 10% corn oil group in that study.

Lukaski and Johnson (1992) concluded that diets containing high levels of polyunsaturated fatty acids can depress zinc status in the rat, as well as the status of other minerals. They indicated that high linoleate diets have been reported to significantly depress zinc concentrations in the tibia. In the subchronic studies with SALATRIM 4CA lot A006, SALATRIM 23CA lot A014, and SALATRIM 32CA lot A015, 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 SALATRIM and corn oil-treated rats in both studies were not considered to be toxicological effects since 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.

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). In this subchronic study, a higher incidence of renal mineralization was noted in female rats fed 10% corn oil or 5% and 10% SALATRIM fats compared with untreated controls. The severity of mineralization was generally similar in all groups of females. The higher incidence of renal mineralization noted in females fed these triacylglycerols may be another indication of a change in mineral balance related to the fatty acid consumed in these high-fat diets.

With the exception of the renal mineralization in female rats, histopathological examination of the tissues from rats consuming the SALATRIM fats indicated no treatment-related changes. The increased incidence of hepatocellular vacuolation in the 10% corn oil-treated rats may have resulted from higher absorption of LCFA in this group compared with that of the 10% SALATRIM group. Such an effect would not be expected with SALATRIM, since the level of LCFA available for absorption is considerably lower than that for corn oil.

The data indicate that SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015 do not elicit toxicologically significant effects. The data show that dietary administration of SALATRIM does not produce any change in serum transaminases of rats. The data support the hypothesis, based on the scientific literature, structure/activity relationships, and results of a subchronic toxicology study with SALATRIM 4CA lot A006 (Hayes et al., 1994b), that SALATRIM fats do not produce toxicological effects in rats.

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