Subchronic Toxicity Studies of SALATRIM Structured Triacylglycerols in Rats. 1. Triacylglycerols Composed of Stearate and Butyrate

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SALATRIM 4CA lot A006 belongs to a family of structured triacylglycerols with lower caloric availabilities (4.5–6.0 kcal/g) than corn oil (9 kcal/g). The predominant fatty acids esterified to glycerol in this fat are butyric and stearic. Rats were fed 2%, 5%, and 10% dietary SALATRIM 4CA lot A006 for 13 weeks. A 10% corn oil diet was fed as a reference for the high-fat SALATRIM diets. Body weight and feed consumption varied with dietary caloric density, as expected. Serum concentrations and urinary clearance of minerals were unaffected by treatment. Variations in bone minerals of rats fed 10% SALATRIM and corn oil were considered to be directly related to the levels of unsaturated fatty acids in these high-fat diets. Necropsy, clinical pathology, and histopathology revealed no adverse effects related to SALATRIM. Increased hepatocellular vacuolation occurred with 10% corn oil. Neither fat altered fat-soluble vitamin concentration in the serum and liver. Overall, the SALATRIM fat produced no toxicologically significant effects.

INTRODUCTION

SALATRIM 4CA lot A006 is a member of a family of structured triacylglycerols termed SALATRIM. These triacylglycerols provide lower caloric availability than other fats, 4.5–6.0 kcal/g compared to 9 kcal/g for corn oil (Finley et al., 1994). SALATRIM triacylglycerols are composed of a glycerol backbone esterified with stearic acid and short-chain fatty acids, i.e., acetic, propionic, and/or butyric. SALATRIM 4CA lot A006 is produced by interesterification between tributyrin and hydrogenated canola oil. Therefore, it is composed of mixed triacylglycerols with a preponderance of butyric and stearic acids esterified to glycerol.

When SALATRIM 4CA lot A006 is hydrolyzed by pancreatic lipase *in vitro*, stearic acid, butyric acid, and mono- and diacylglycerols are produced (Hayes et al., 1994a). Several investigators have shown that short-chain fatty acids and 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 stearate (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 short-chain fatty acids compared to those of long-chain fatty acids are responsible for the lower caloric availability of members of the SALATRIM family compared with fats such as corn oil.

Feeding studies of 8–12-weeks duration have been conducted with rats exposed to 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 detected.

Ambrose et al. (1958a) presented data from studies in which rats were fed four fats and an apparent acylglycerol mixture. Two of the fats, termed acetostearins, were produced from hydrogenated lard and triacetin and appear to be almost identical. The acylglycerol mixture was a partially acetylated hydrogenated lard. The additional two fats were produced from steamed lard and triacetin and cottonseed oil and triacetin. These fats were termed aceto-oleins. 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. The acylglycerol mixture produced reproductive changes similar to those produced by the first acetostearin. One of the aceto-oleins produced discoloration in the rat uterus, similar to the acetostearin, but did not affect reproductive performance and had no effect on the rat testes. Ambrose et al. (1958b) reported that 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 fats consumed in the human diet and their predictable metabolism (Hayes et al., 1994d), SALATRIM fats should not produce toxicological effects. To test this hypothesis, a 13-week subchronic toxicity study in rats was conducted. High dietary concentrations of SALATRIM 4CA lot A006 were used to maximize the potential for producing toxicity. The dietary concentration was limited to 10% by weight of the diet because higher doses may have a potential to produce marginal micronutrient deficiency by dilution. Since the high lipid content of the diet could possibly alter fat-soluble vitamin absorption, the diet was supplemented with the fat-soluble vitamins A, E, D, and K.

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Table 1. Total Fatty Acid Profile for SALATRIM 4CA Lot A006[a]

fatty acid name	fatty acid designation	wt %
stearic (octadecanoic)	C18:0	58 ± 4
butyric	C4:0	21 ± 2
palmitic (hexadecanoic)	C16:0	2.75 ± 0.08
arachidic (eicosanoic)	C20:0	1.51 ± 0.05
behenic (docosanoic)	C22:0	0.64 ± 0.02
oleic (9-octadecenoic)	C18:1	0.56 ± 0.02
lignoceric (tetracosanoic)	C24:0	0.299 ± 0.008
linoleic (9,12-octadecadienoic)	C18:2	0.124 ± 0.003
lauric (dodecanoic)	C12:0	0.019
11-eicosanoic	C20:1	0.017 ± 0.001
palmitoleic (9-hexadecanoic)	C16:1	0.002 ± 0.000

[a] Data represent the mean ± standard deviation for triplicate determinations with the exception of lauric acid, which represents the mean of two determinations.

MATERIALS AND METHODS

This 13-week subchronic toxicity study with rats was conducted at Hazleton Wisconsin Inc., Madison, WI, from January 1991 through April 1991.

Materials. SALATRIM 4CA lot A006 was provided by Nabisco Foods Group (NFG), East Hanover, NJ. This SALATRIM fat (also known as TAG A7200 lot A006) is a mixture of triacylglycerols composed predominantly of dibutyrylstearoylglycerol. Chemical characterization of this SALATRIM fat was conducted at EPL Bio-Analytical Services, Inc., Decatur, IL. A total fatty acid profile for SALATRIM 4CA lot A006 is presented in Table 1. Total fatty acid profile data were obtained by saponification of the acylglycerol mixture with methanolic sodium hydroxide followed by esterification with methanolic boron trifluoride. Methyl esters of the fatty acids were quantified by gas chromatography. Standard curves were constructed using a stepped, point-to-point calibration bracketing the concentration level of the analyte. An internal standard, stearyl stearate, was used, and quantitation was based upon peak height. A titratable acid value of 0.220 ± 0.000 wt % was obtained by using AOCS Official Method Ca 5a-40 (AOCS, 1990a). This indicates that the free fatty acid concentration in the acylglycerol mixture was low. The peroxide content of the fat was 0.45 ± 0.05 mequiv of peroxide/kg according to AOCS Official Method Cd 8-53 (AOCS, 1990b).

Commercially available Mazola corn oil was used as the reference fat. Vitamins A (retinyl palmitate), D (cholecalciferol), E (D,L α-tocopherol acetate), and K (menadione sodium bisulfite) were supplied by Teklad, Inc. Other reagents used in this study were from commercial suppliers.

Dosing and Diets. Rats were fed SALATRIM 4CA lot A006 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 avoid excessive dilution of micronutrients. Test diets were prepared weekly. SALATRIM 4CA lot A006 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 or necropsy. Drinking water was provided *ad libitum* during all phases of the study. At weeks 1, 2, 3, 4, 7, 10, and 13, diets were analyzed for corn oil or SALATRIM. Homogeneity was assessed before study initiation for the 2% and 10% SALATRIM and 10% corn oil diets and was reevaluated for the 2% SALATRIM diet at week 1. Homogeneity of the 2% SALATRIM diet was marginally acceptable at week 1, and the diet preparation method was revised. Diet homogeneity was evaluated for all dose levels at week 2. Stability of SALATRIM in the diets for up to 18 days at room temperature and for up to 15 weeks when frozen (−5 to −20 °C) was evaluated by analysis of the 2% and 10% diet mixtures. To prevent potential effects on serum and liver fat-soluble vitamin concentrations in the rats, diets were supplemented with vitamins A (retinyl palmitate), E (D,L-α-tocopheryl acetate), D (cholecalciferol), and K (menadione sodium bisulfite). Vitamin supplementation concentrations for the different dose levels are presented in Table 2. Dietary vitamin A, E, and D supplementation was assessed by homogeneity determinations and analysis of vitamins A, E, and D at weeks 1, 7, and 13. If vitamins A, E, and D were homogeneous and at the intended concentrations, it was assumed vitamin K was properly and homogeneously added to the diet.

Table 2. Experimental Design and Vitamin Supplementation

treatment	dietary concn (%)	dietary vitamin supplementation (mg/kg of finished diet)[a] A	D	E	K	no. of rats male	female
control[b]	NA[c]	0.0	0.0	0.0	0.0	30	30
vitamin control	NA	8.0	12.0	300	2.5	30	30
SALATRIM	2	0.0	4.0	60	0.5	30	30
SALATRIM	5	4.0	8.0	150	1.25	30	30
SALATRIM	10	8.0	12.0	300	2.5	30	29[d]
corn oil	10	0.0	0.0	0.0	0.0	30	30
Sentinel[b]	NA	0.0	0.0	0.0	0.0	5	5

[a] Values calculated from the potency of the vitamin concentrate and the target supplement level. Vitamin A (retinyl palmitate, 500 000 IU/g); vitamin D (cholecalciferol, 500 000 IU/g); vitamin E (D,L-a-tocopheryl acetate, 500 IU/g); vitamin K (menadione sodium bisulfite). Vitamin supplementation was to prevent potential effects on serum and liver levels of fat-soluble vitamins in SALATRIM-treated rats. [b] Basal diet only. [c] Not applicable. [d] This group was initiated with 30 animals. During week 3, one of the rats was discovered to be missexed and was removed from the study.

Animals. Crl:CD®BR VAF rats were from Charles River Laboratories, Inc. (Portage, MI). Five rats per sex were 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*. The remaining rats were acclimated for 2 weeks before study initiation. Rats were 5–6 weeks old and weighed 134–172 (males) and 115–142 g (females) at initiation of 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 72 ± 3 °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. The study design is presented in Table 2. Rats were divided into seven groups: (1) controls that received basal diet with neither test material nor vitamin supplementation; (2) vitamin-supplemented controls that received the same vitamin supplementation as the highest vitamin supplementation in diets containing SALATRIM; (3) treated groups that received the SALATRIM at either 2%, (4) 5%, or (5) 10% of the diet by weight and vitamin supplementation based upon the percentage of the SALATRIM in the diet; (6) a reference group fed corn oil at 10% of the diet by weight; and (7) an untreated, unsupplemented group that served as a sentinel for health and viral serology at study termination.

Antemortem Data Collection and Interim Sacrifice. Rats were observed twice daily for mortality, moribundity, and signs of toxicity. Physical examinations were conducted weekly. Ophthalmic examinations were performed 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. Rats found either moribund or dead were subjected to gross necropsy and histopathology.

After the initial 4 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, and subjected to clinical pathology as outlined in Table 3. Most hematology measurements were conducted using a Coulter Counter S-Plus IV whole blood

Table 3. Clinical Pathology

Hematology	
red blood cell count	platelet count
hemoglobin	prothrombin time
hematocrit	white blood cell count
mean corpuscular volume	differential blood cell count
mean corpuscular hemoglobin	blood cell morphology
mean corpuscular hemoglobin concentration	reticulocyte count smear (made but not examined)
Serum Chemistry	
glucose	aspartate aminotransferase
urea nitrogen	alanine aminotransferase
creatinine	alkaline phosphatase
total protein	γ-glutamyltransferase
albumin	calcium
globulin	inorganic phosphorus
albumin/globulin ratio	sodium
total bilirubin	potassium
cholesterol	chloride
high-density lipoprotein cholesterol	triglycerides
low-density lipoprotein cholesterol	
Urinalysis	
volume	protein
appearance	ketones
pH	occult blood
specific gravity	urobilinogen
glucose	microscopic examination of sediment
bilirubin	
Urine Chemistry	
urine calcium	fractional clearance of
urine phosphorus	calcium
urine sodium	sodium
urine chloride	potassium
urine creatinine	chloride

automated hematology analyzer. Prothrombin time was determined using a Coag-A-Mate X2 coagulation analyzer, and the 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.

Rats were fasted overnight before blood sampling. Blood was collected from the retro-orbital plexus after ketamine anesthesia. Hematology samples were collected with 10% EDTA anticoagulant, plasma for the prothrombin assay were prepared from blood collected with 3.8% sodium citrate anticoagulant, and serum for the clinical chemistry determinations were prepared from blood collected without anticoagulant. Urine was collected via metabolism cages during the fasting period before blood sampling. The ten rats per sex per group used for clinical pathology were sacrificed and necropsied. Hepatic (Kayden et al., 1983) and serum (Driskell et al., 1982) concentrations of *trans*-retinol (vitamin A) and α-tocopherol (vitamin E) were determined using high-performance liquid chromatographic methods, and the serum concentration of 25-hydroxy vitamin D (vitamin D) was determined by radioimmune assay using commercially available reagents from Incstar Corp. (Stillwater, MN). Adrenals, brain, kidneys, liver, and testes were weighed. Tissues listed in Table 4 were collected from all rats and fixed in 10% phosphate-buffered formalin. Those from the nonsupplemented control group and 10% SALATRIM group were subjected to histopathology. For all other groups, only macroscopic lesions (if any), lungs, liver, kidney, and one adrenal were subjected to histopathology. Tissues for histopathological examination were embedded, sectioned, stained with hematoxylin and eosin, and examined by light microscopy.

Postmortem Data Collection after 13 Weeks. Blood was obtained for clinical pathology immediately before sacrifice from 10 rats per sex per group through the retro-orbital plexus as described previously (Table 3). Blood was obtained from an additional 10 rats per sex per group to determine serum concentrations of vitamins A, E, and D and from the 5 sentinel rats per sex for serum viral antibody analysis.

Table 4. Tissues Collected for Histopathology

adrenals	muscle (thigh)
aorta	ovaries
bone marrow smear[a]	pancreas
brain	pituitary
cecum	prostate
colon	rectum
duodenum	salivary gland (submandibular)
epididymides	sciatic nerve
esophagus	seminal vesicles
eyes	skin
femur and bone marrow	spinal cord (cervical, thoracic, lumbar)
heart	spleen
ileum	sternum and bone marrow
jejunum	stomach
kidneys	testes
lacrimal gland	thymus
lesions	thyroid with parathyroid
liver	trachea
lungs	urinary bladder
lymph nodes (mandibular and mesenteric)	uterus
mammary gland (females)	

[a] Smears prepared for possible examination, if deemed necessary.

All rats were subjected to gross necropsy. Terminal sacrifice procedures were the same as those after 4 weeks of treatment except femurs from the nonsupplemented control group, SALATRIM groups, and the 10% corn oil group were analyzed. Defatted dry weight and percent ash of the femurs were determined. Each femur was assayed for calcium, copper, iron, magnesium, phosphorus, sodium, strontium, and zinc concentrations by inductively coupled plasma spectrometry.

Statistical Analyses. Statistical analyses were conducted for the following: body weights, cumulative body weight gains; feed 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. Groups comparisons were evaluated at the 5% two-tailed probability level. All differences cited are based on comparisons with the nonsupplemented control group. If a statistical difference was noted, a comparison with the vitamin-supplemented control group was conducted.

RESULTS

Four-week interim clinical pathology and necropsy data provided no evidence of adverse effects and are discussed but generally not presented here.

Diet Analysis. Prestudy homogeneity data indicated that the 10% fat diets met the homogeneity specification, but the range was somewhat high for the 2% SALATRIM diet. The 2% diet homogeneity analysis was repeated at week 1 and confirmed the data obtained during the prestudy analysis. The diet mixing procedure was modified, and the week 2 SALATRIM diets were analyzed. The modified diet mixing method resulted in the SALATRIM diets having acceptable homogeneity.

The stability of SALATRIM 4CA lot A006 was determined in test diets stored at room temperature and under

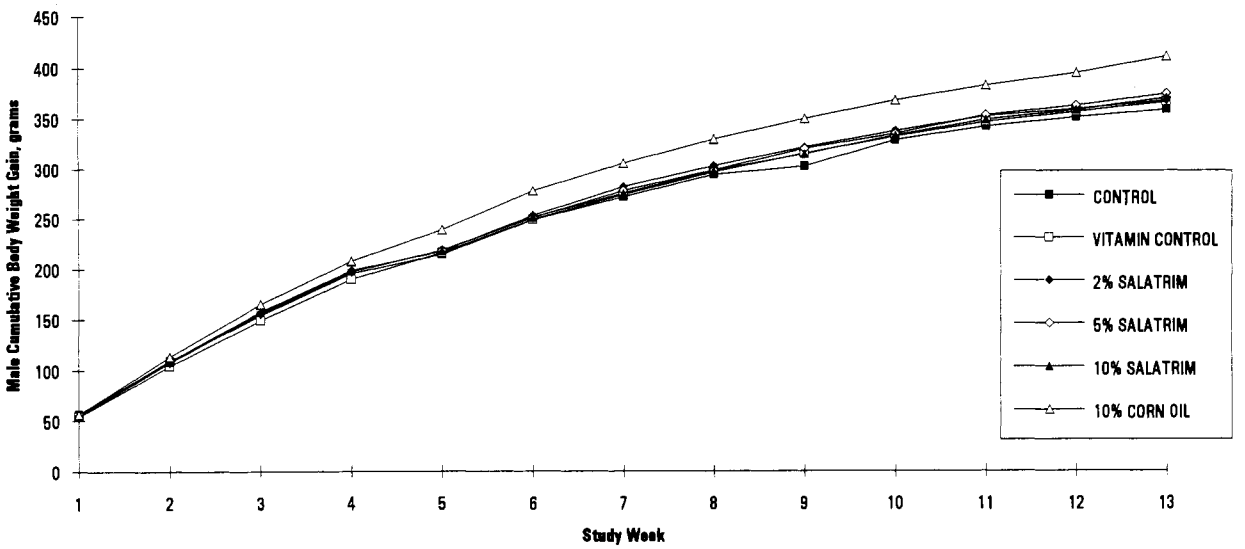

Figure 1. Male mean cumulative body weight gain (g) vs study week. Data points represent the means of 19–30 rats. Standard deviations (SD) are not shown for the sake of clarity of the figure. In most cases, the SD was less than 15% of the mean. Lower values at week 5 are considered to be the result of fasting of the rats prior to clinical laboratory studies.

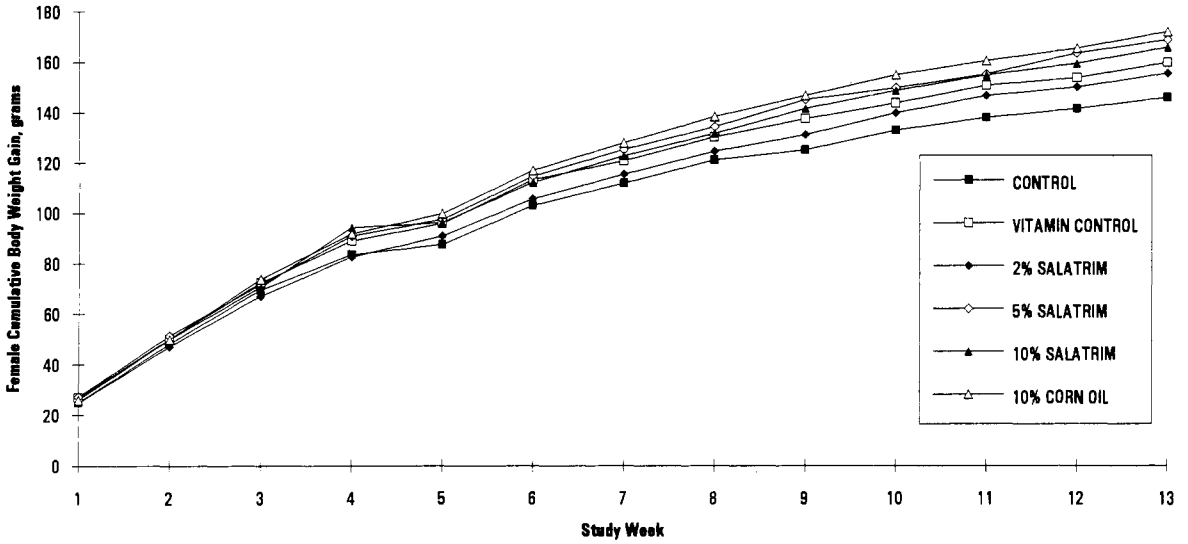

Figure 2. Female mean cumulative body weight gain (g) vs study week. Data points represent the means of 19–30 rats. Standard deviations (SD) are not shown for the sake of clarity of the figure. In most cases, the SD was less than 20% of the mean. Lower values at week 5 are considered to be the result of fasting of the rats prior to clinical laboratory studies.

freezer conditions. The data indicate that the fat has acceptable stability in rodent diet at room temperature for up to 18 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 SALATRIM and corn oil diets at weeks 1, 2, 3, 4, 7, 10, and 13 indicated that the diets contained the proper amounts of fat. Because the SALATRIM diets were analyzed by supercritical fluid chromatography, the analyses also confirmed that the fat in these diets was SALATRIM 4CA lot A006.

Analysis of dietary vitamin A, D, and E content on weeks 1, 7, and 13 indicated that proper vitamin supplementation was achieved in the diets.

Compound Consumption. Daily SALATRIM 4CA lot A006 consumption for the 2%, 5%, and 10% groups through the 4-week data collection and interim sacrifice averaged 1.8, 4.6, and 8.8 g/kg in males and 1.9, 4.9, and 9.3 g/kg in females. Overall daily SALATRIM consumption for the 2%, 5%, and 10% groups during the 13-week study averaged 1.4, 3.4, and 6.4 g/kg in males and 1.5, 3.9, and 7.3 g/kg in females. Overall daily consumption of corn oil averaged 5.8 and 6.8 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. No treatment-related deaths occurred. One control male was sacrificed in a moribund condition due to a fracture of the maxilla, and one male given 5% SALATRIM died on test after blood collection.

In both sexes, mean body weight trends and mean body weight gains in nonsupplemented and vitamin-supplemented control rats and SALATRIM-treated rats were similar. Mean body weights in corn oil-treated males were significantly higher than those of controls during a few weeks of the study. Mean body weight gains of the corn oil males were significantly greater than those of controls for most weeks of the study. This was also true for body weight gain in 10% corn oil females at several study intervals. Cumulative body weight gain data are presented in Figures 1 and 2.

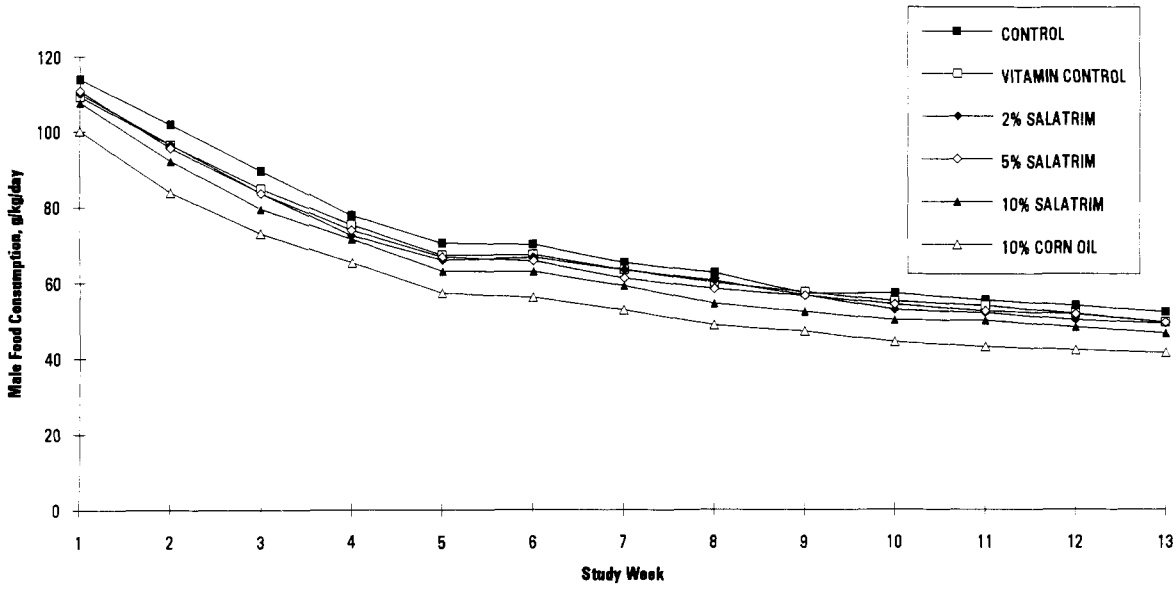

Figure 3. Male mean daily food consumption relative to body weight (g/kg) vs study week. Data points represent the means of 15–30 rats. Standard deviations (SD) are not shown for the sake of clarity of the figure. In most cases, the SD was less than 10% of the mean. Lower values at week 5 are considered to be the result of fasting of the rats prior to clinical laboratory studies.

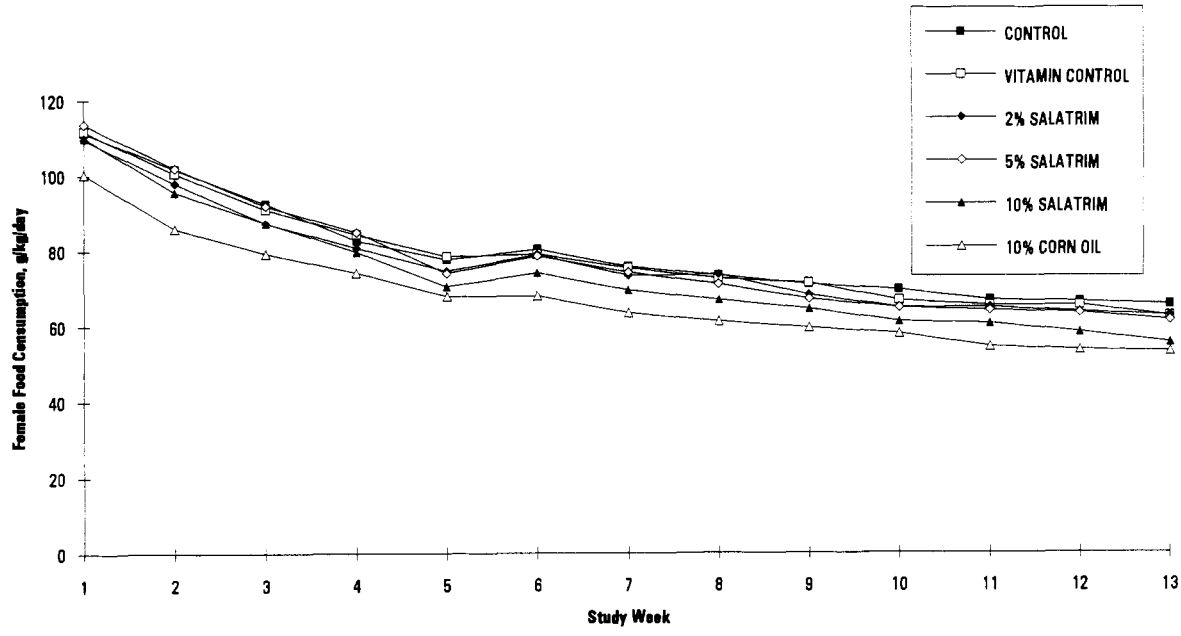

Figure 4. Female mean daily food consumption relative to body weight (g/kg) vs study week. Data points represent the means of 13–29 rats. Standard deviations (SD) are not shown for the sake of clarity of the figure. In most cases, the SD was less than 10% of the mean. Lower values at week 5 are considered to be the result of fasting of the rats prior to clinical laboratory studies.

Feed consumption (grams per week) during most weeks of the study was significantly lower than controls for 10% SALATRIM males and 10% corn oil males and females. Daily feed consumption relative to body weight (grams per kilogram) was lower for both sexes given 10% SALATRIM and 10% corn oil. For males given 2% and 5% SALATRIM, feed consumption relative to body weight was lower than that of untreated controls at several study weeks but was comparable to that of vitamin controls throughout the study. Feed consumption relative to body weight data are shown in Figures 3 and 4.

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

Clinical Pathology. Hematology, serum and urine chemistry, and urinalysis data revealed no treatment-related effects when animals treated with SALATRIM 4CA lot A006 or corn oil were compared with controls. Occasional statistically significant differences between SALATRIM-treated and corn oil-treated groups and the nonsupplemented control group were not substantiated when the treated group was compared with the vitamin-supplemented control group. Mean serum concentrations of triglycerides, cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol are presented in Table 5.

Vitamin Concentrations and Mineral Determinations. At weeks 5 and 14, serum concentrations of fat-soluble vitamins A, E, and D and liver concentrations of vitamins A and E in SALATRIM-treated rats were comparable to, or in some cases, greater than those of control rats fed basal diet with no vitamin supplementation. Serum and liver vitamin concentrations for corn oil-treated rats were generally similar to those of non-supplemented controls. Serum and liver concentrations of vitamins A and E and serum concentration of vitamin D at week 14 are shown in Figures 5–7. Prothrombin times,

Table 5. Summary of Serum Lipid Data—Week 14[a]

	treatment					
	control	10% corn oil	vitamin control	2% SALATRIM	5% SALATRIM	10% SALATRIM
			Males			
no. analyzed	9	10	10	10	9	10
cholesterol (mg/dL)	61 ± 17	86 ± 17[b]	79 ± 20	71 ± 18	61 ± 12	81 ± 15
HDL[c] (mg/dL)	47 ± 16	67 ± 16	62 ± 15	53 ± 15	46 ± 11	65 ± 14
LDL[d] (mg/dL)	2 ± 2	5 ± 4	3 ± 4	4 ± 3	1 ± 4	3 ± 3
triglycerides (mg/dL)	76 ± 45	75 ± 28	91 ± 45	75 ± 23	103 ± 43	75 ± 19
			Females			
no.	9	9	7	10	9	8
cholesterol (mg/dL)	90 ± 13	95 ± 12	96 ± 14	97 ± 20	91 ± 19	90 ± 21
HDL (mg/dL)	73 ± 11	76 ± 12	80 ± 17	82 ± 17	79 ± 21	73 ± 17
LDL (mg/dL)	10 ± 4	10 ± 4	8 ± 5	7 ± 4	4 ± 4	8 ± 6
triglycerides (mg/dL)	34 ± 8	46 ± 22	40 ± 17	39 ± 13	45 ± 14	41 ± 10

[a] Data represent mean ± standard deviation [b] Significantly different from control ($p \leq 0.05$). [c] HDL, high-density lipoprotein cholesterol. [d] LDL, low-density lipoprotein cholesterol.

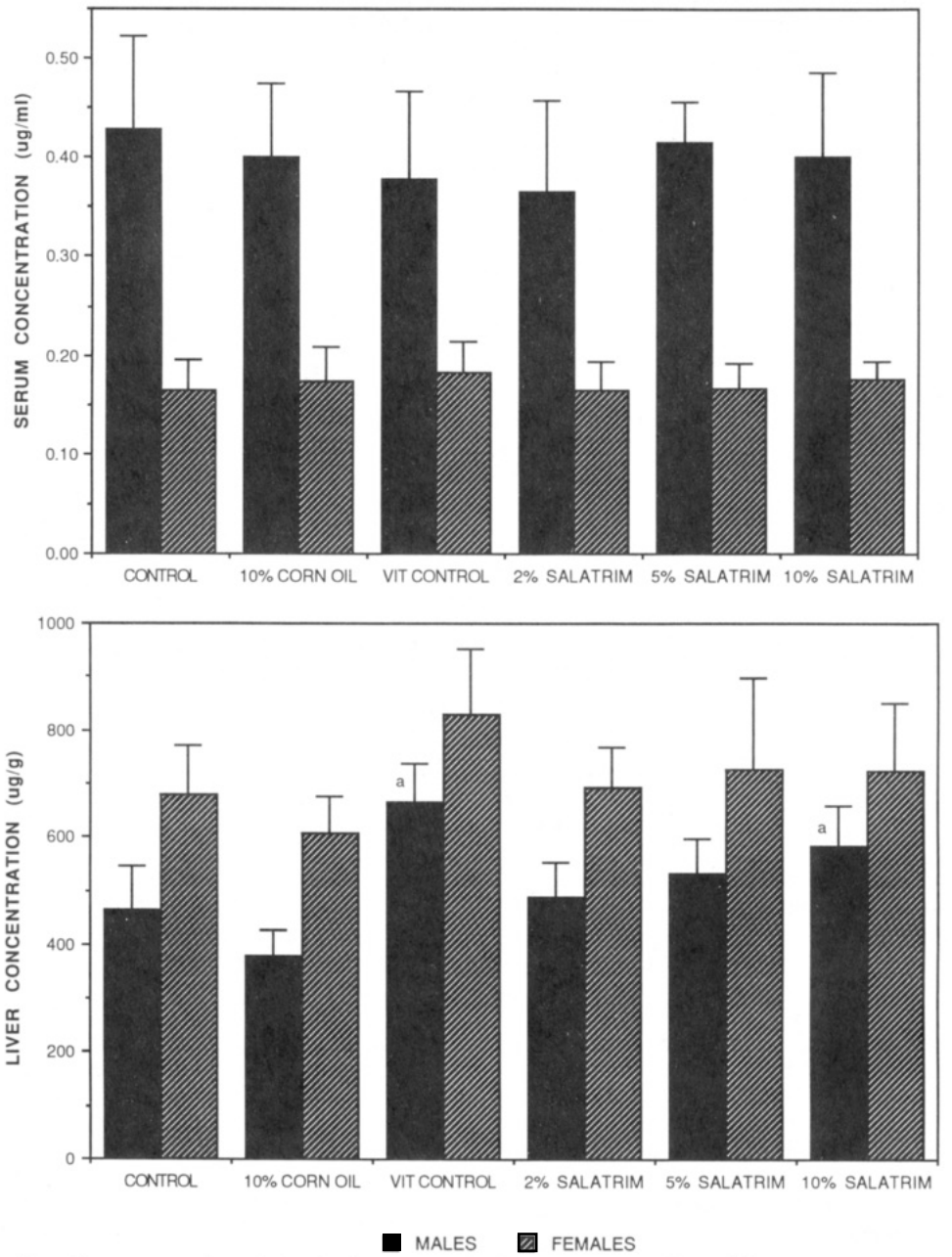

Figure 5. Male and female concentration of vitamin A (*trans*-retinol) in serum (μg/mL) and liver (μg/g) vs treatment at study week 14. Data represent mean ± standard deviation of the mean for 9–10 rats. Data significantly different from untreated control group ($p \leq 0.05$) are noted with an "a".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

an indicator of vitamin K status, were not different between groups.

Comparison of serum and liver vitamin concentrations from the 10% SALATRIM group with the vitamin-

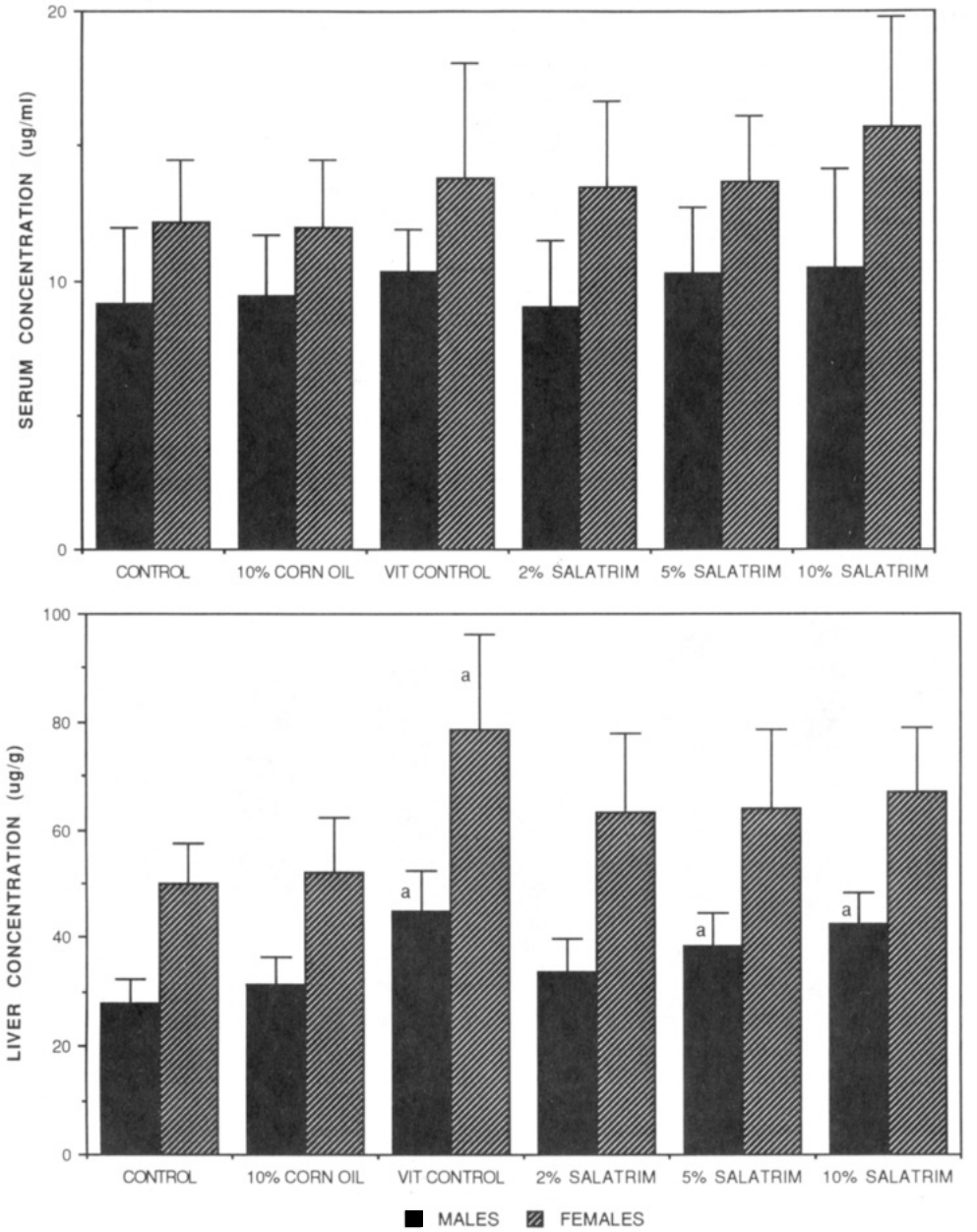

Figure 6. Male and female concentration of vitamin E (α-tocopherol) in serum (μg/mL) and liver (μg/g) vs treatment at study week 14. Data represent mean ± standard deviation of the mean for 9–10 rats. Data significantly different from untreated control group ($p \leq 0.05$) are noted with an "a". Data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

supplemented control group, each of which received the same dietary vitamin supplementation, revealed no significant differences for vitamins A and E, with the exception of serum vitamin E in females at week 5. In that single case, vitamin E concentration in the 10% SALATRIM females was significantly greater than that in the vitamin-supplemented controls. Serum concentrations of vitamin D were significantly lower for the 10% SALATRIM males at 5 weeks and the 10% SALATRIM females at 14 weeks compared with the vitamin-supplemented controls (Figure 7). However, serum vitamin D was significantly greater for the 10% SALATRIM males and females at both 5 and 14 weeks when compared with that of the nonsupplemented controls.

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 higher in females than in males.

Results of mineral analyses of femurs from the control group without dietary vitamin supplementation, the 10% SALATRIM 4CA lot A006 group, and the 10% corn oil group are summarized in Table 6. At 10% SALATRIM, strontium and zinc concentrations were higher in both sexes, while sodium was higher only in females compared with the nonsupplemented control group. In the 10% corn oil group, strontium was higher in both sexes, while zinc was lower only in males compared with nonsupplemented controls. Femur concentrations of the other minerals were similar among all three groups. Serum concentrations of calcium, inorganic phosphorus, sodium, potassium, and chloride and urinary clearance of these minerals were comparable among all groups at weeks 5 and 14. Data for week 14 are summarized in Table 7.

Organ Weights, Macroscopic and Microscopic Pathology. No treatment-related organ weight changes were observed in rats treated with SALATRIM 4CA lot A006 or corn oil at the 5- and 14-week necropsies. Macroscopic findings in all groups at both necropsy

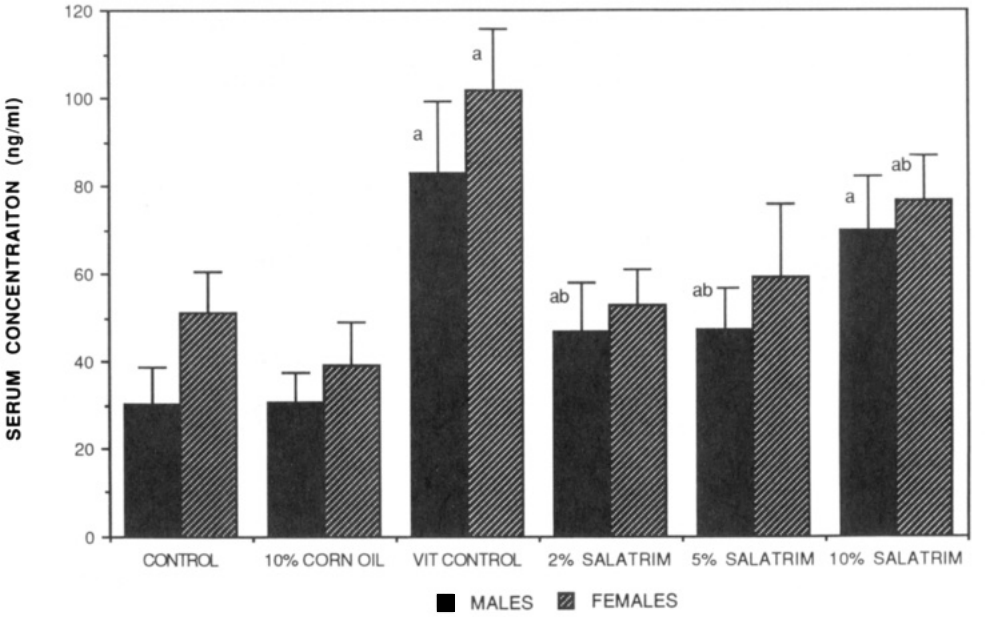

Figure 7. Male and female concentration of vitamin D (25-hydroxy vitamin D) in serum (ng/mL) vs treatment at study week 14. Data represent mean ± standard deviation of the mean for 9–10 rats. Data significantly different from untreated control group ($p \leq 0.05$) are noted with an "a". Data significantly different from vitamin control group ($p \leq 0.05$) are noted with a "b".

Table 6. Summary of Bone Mineral Analyses—Week 14[a]

	treatment		
	control	10% corn oil	10% SALATRIM
	Males		
no. analyzed	12	13	11
absolute defatted femur wt (mg)	728 ± 79	754 ± 34	734 ± 84
ash (%)	66.4 ± 0.9	66.3 ± 1.1	66.8 ± 0.6
calcium (mg/g)	225 ± 6	221 ± 4	222 ± 5
copper (μg/g)	11.9 ± 0.7	11.3 ± 0.5	11.2 ± 0.5
iron (μg/g)	66.3 ± 11.0	66.6 ± 16.5	57.0 ± 20.2
magnesium (mg/g)	4.36 ± 0.20	4.38 ± 0.15	4.49 ± 0.16
phosphorus (mg/g)	112 ± 2	112 ± 1	112 ± 2
sodium (mg/g)	4.40 ± 0.15	4.36 ± 0.09	4.37 ± 0.19
strontium (μg/g)	45.9 ± 1.9	49.4 ± 2.2[b]	54.1 ± 4.1[b]
zinc (μg/g)	243 ± 12	220 ± 12[b]	263 ± 14[b]
	Females		
no. analyzed	11	10	11
absolute defatted femur wt (mg)	496 ± 52	528 ± 96	515 ± 63
ash (%)	67.1 ± 1.2	67.8 ± 0.7	66.7 ± 1.3
calcium (mg/g)	228 ± 5	228 ± 4	227 ± 4
copper (μg/g)	11.8 ± 0.5	11.7 ± 0.6	11.8 ± 0.8
iron (μg/g)	70.9 ± 17.0	75.7 ± 25.3	75.6 ± 19.5
magnesium (mg/g)	4.63 ± 0.27	4.78 ± 0.15	4.64 ± 0.22
phosphorus (mg/g)	114 ± 2	115 ± 2	113 ± 2
sodium (mg/g)	4.23 ± 0.16	4.37 ± 0.12	4.41 ± 0.14[b]
strontium (μg/g)	46.9 ± 3.2	50.5 ± 3.0[b]	50.5 ± 3.3[b]
zinc (μg/g)	251 ± 17	244 ± 12	288 ± 14[b]

[a] Data represent mean ± standard deviation. No analyses were conducted for the vitamin control and 2% and 5% SALATRIM groups. [b] Significantly different from control ($p \leq 0.05$).

intervals were typical for rats of this age with the exception of an increased incidence of mottled liver in 10% corn oil males. Microscopically, no treatment-related findings were noted in SALATRIM-treated rats and the only significant finding for corn oil-treated rats was an increased incidence of hepatocellular vacuolation in both sexes (Table 8).

DISCUSSION

The general processes of triacylglycerol digestion and absorption are similar for all fats. However, there are some noteworthy differences in metabolism of certain fats and fatty acids. The stomach and proximal small intestine are the primary sites for these processes. Initial hydrolysis of triacylglycerols by lingual lipase (Abrams et al., 1984; Carey et al., 1983; Hamosh, 1984; Hamosh and Burns, 1977; Jensen et al., 1982; Nelson et al., 1977; Van Dyke, 1989) and possibly gastric lipase (Cohen et al., 1971; Gargouri et al., 1986) occurs in the stomach and accounts for 10–40% of total triacylglycerol hydrolysis. Products of hydrolysis are diacylglycerols and free fatty acids. Short- and medium-chain fatty acids are hydrolyzed more rapidly than long-chain fatty acids. The preferential, rapid hydrolysis of short- and medium-chain fatty acids allows diffusion into the gastric mucosa (Aw and Grigor, 1980; Bugaut, 1987; Fernando-Warnakulasuriya et al., 1981; Greenberger and Skillman, 1969; Mattson and Volpenhein, 1964; Staggers et al., 1981) for cytosolic absorption and transport without reesterification. They enter the portal circulation and are transported to the liver as a ready

Table 7. Summary of Serum Mineral Analyses and Urine Mineral Clearance Data—Week 14[a]

	treatment					
	control	10% corn oil	vitamin control	2% SALATRIM	5% SALATRIM	10% SALATRIM
			Males			
serum minerals						
no. analyzed	9	10	10	10	9	10
calcium (mg/dL)	9.2 ± 0.4	9.6 ± 0.5	9.6 ± 0.3	9.6 ± 0.4	9.3 ± 0.4	9.6 ± 0.4
inorg phosphorus (mg/dL)	7.3 ± 0.4	7.3 ± 0.7	7.2 ± 0.3	7.1 ± 0.5	6.9 ± 0.5	6.9 ± 0.3
sodium (mmol/L)	148 ± 3	147 ± 4	147 ± 3	147 ± 2	148 ± 2	148 ± 2
potassium (mmol/L)	4.9 ± 0.2	5.0 ± 0.2	4.8 ± 0.2	5.0 ± 0.2	4.9 ± 0.2	4.9 ± 0.2
chloride (mmol/L)	107 ± 3	106 ± 4	106 ± 3	107 ± 3	108 ± 3	108 ± 2
urine mineral clearance						
no. analyzed	8	10	10	10	9	10
calcium (%)	0.31 ± 0.14	0.26 ± 0.17	0.43 ± 0.19	0.31 ± 0.13	0.24 ± 0.07	0.29 ± 0.20
phosphorus (%)	14.9 ± 2.1	12.4 ± 2.1	13.2 ± 3.5	13.9 ± 1.4	16.0 ± 2.8	16.7 ± 2.4
sodium (%)	0.17 ± 0.09	0.15 ± 0.04	0.15 ± 0.04	0.17 ± 0.05	0.15 ± 0.05	0.24 ± 0.25
potassium (%)	19.2 ± 4.8	14.8 ± 2.3	15.6 ± 1.9	16.6 ± 2.7	16.0 ± 2.9	23.8 ± 30.1
chloride (%)	0.35 ± 0.09	0.36 ± 0.16	0.38 ± 0.13	0.38 ± 0.11	0.36 ± 0.14	0.41 ± 0.30
			Females			
serum minerals						
no. analyzed	9	9	7	10	9	8
calcium (mg/dL)	9.6 ± 0.5	10.0 ± 0.5	10.1 ± 0.7	9.7 ± 0.4	9.7 ± 0.6	9.7 ± 0.4
inorg phosphorus (mg/dL)	6.8 ± 0.5	6.2 ± 0.5	6.4 ± 0.4	6.4 ± 0.5	6.1 ± 0.7	6.3 ± 0.5
sodium (mmol/L)	146 ± 3	147 ± 2	147 ± 2	147 ± 2	148 ± 3	147 ± 2
potassium (mmol/L)	4.6 ± 0.2	4.5 ± 0.3	4.4 ± 0.4	4.4 ± 0.4	4.5 ± 0.4	4.3 ± 0.2
chloride (mmol/L)	108 ± 3	108 ± 2	107 ± 3	109 ± 3	108 ± 3	108 ± 2
urine mineral clearance						
no. analyzed	7	7	5	9	7	6
calcium (%)	0.84 ± 0.37	0.70 ± 0.22	1.64 ± 1.29	1.25 ± 0.64	0.73 ± 0.42	0.70 ± 0.36
phosphorus (%)	20.8 ± 3.6	17.8 ± 1.9	21.0 ± 3.3	23.4 ± 5.2	25.1 ± 11.2	23.1 ± 4.5
sodium (%)	0.24 ± 0.06	0.18 ± 0.07	0.34 ± 0.24	0.34 ± 0.08	0.27 ± 0.11	0.25 ± 0.08
potassium (%)	18.9 ± 5.0	15.5 ± 4.1	22.3 ± 1.3	21.4 ± 4.2	21.8 ± 5.3	18.2 ± 5.4
chloride (%)	0.52 ± 0.14	0.37 ± 0.15	0.71 ± 0.53	0.68 ± 0.16	0.55 ± 0.26	0.49 ± 0.18

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

Table 8. Summary Incidence of Microscopic Hepatocellular Vacuolation

	treatment					
	control	vitamin control	2% SALATRIM	5% SALATRIM	10% SALATRIM	10% corn oil
			Males			
week 5						
no. examined	10	10	10	10	10	10
no. with vacuolation	0	0	0	0	0	5
week 14						
no. examined	20[a]	20	20	20[b]	20	20
no. with vacuolation	0	1	1	0	0	18
			Females			
week 5						
no. examined	10	10	10	10	10	10
no. with vacuolation	1	0	0	0	0	4
week 14						
no. examined	20	20	20	20	19[c]	20
no. with vacuolation	1	0	0	0	0	4

[a] One rat was sacrificed in a moribund condition during week 10. [b] One rat died after blood collection at week 5. [c] At week 3, one rat in this group was discovered to be missexed and was removed from the study.

energy source. Short-chain fatty acids are the preferred energy source for the gastric mucosa (Bugaut, 1987).

A significant amount of stearic acid is likely to be hydrolyzed from the glycerol backbone by lingual lipase and partitioned back into lipid droplet (droplet of di- and triacylglycerols) and carried in the GI tract through the pylorus into the duodenum (Carey et al., 1983). In the small intestine-, di- and triacylglycerols in lipid droplets are hydrolyzed by pancreatic lipase in conjunction with colipase and bile salts (Abrams et al., 1984; Borgstrom, 1986; Brockerhoff, 1970; Brockerhoff and Jensen, 1974; Carey et al., 1983; Fernando-Warnakulasuriya et al., 1981; Mattson and Volpenhein, 1964; Patton, 1981; Staggers et al., 1981; Van Dyke, 1989). Stearic acid is poorly absorbed after release as a fatty acid, and a portion forms insoluble salts with calcium and magnesium that are excreted in the feces (Benzonana et al., 1968; Bliss et al., 1972; Gacs and Barltrop, 1977; Mattson et al., 1979; Sammons and Wiggs, 1960), while another portion is excreted as free stearate.

SALATRIM 4CA lot A006 presumably is hydrolyzed by lingual and pancreatic lipase to release monostearin, glycerol, and free butyric and stearic acids. As with other triacylglycerols, the butyric acid not metabolized by the mucosal cells will enter the portal circulation and bind to albumin. In the liver, the butyric acid will be metabolized to ketone bodies, CO_2 and water with resultant energy production (Bugaut, 1987; Rombeau et al., 1990). A substantial portion of stearic acid in SALATRIM fats should be hydrolyzed and excreted in the feces as soaps and free stearate like 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 stearate is poorly absorbed compared with other long-chain fatty acids and because short-chain fatty acids

provide fewer calories than long-chain fatty acids, SALATRIM fats have a lower caloric value than most fats (Finley et al., 1994). On the basis of their similarity to fats normally found in the food supply and their predictable metabolism, SALATRIM fats are not expected to cause toxicological effects. This 13-week rat subchronic toxicity study with SALATRIM 4CA lot A006 was conducted to test this hypothesis.

There were no statistically significant differences between the body weights of control (nonsupplemented and vitamin-supplemented) and SALATRIM-treated males and females. The male corn oil group showed a trend toward increased body weight when compared with control and SALATRIM-treated rats. The higher body weight of the corn oil group was likely caused by increased calorie consumption. As with the males, the female corn oil group generally showed the highest weight gain. The untreated control group showed the lowest body weight gain with the other dietary groups falling between these extremes. When feed consumption and caloric availability of this SALATRIM fat and corn oil are considered, changes in body weight and feed consumption follow a generally predictable pattern based upon total caloric consumption, with the trends being clearest in males.

The high dietary fat concentrations (SALATRIM and corn oil) fed in this study did not produce effects on serum triglycerides, low-density lipoprotein, high-density lipoprotein, and total cholesterol. Statistically significantly higher serum cholesterol in corn oil-treated males compared with untreated controls was considered to be unrelated to corn oil because a similar value was observed in the vitamin-supplemented control males.

Serum and liver concentrations of vitamins A, E, and D indicate SALATRIM 4CA lot A006 has little or no effect on fat-soluble vitamin absorption. Vitamin-supplemented controls generally had significantly higher concentrations of vitamins A and E in the liver and vitamin D in the serum than did the nonsupplemented controls. Vitamin concentrations in the 10% SALATRIM-treated group and the vitamin-supplemented control, which both received the same dietary vitamin supplementation, were similar. At the 5-week period, male serum vitamin D was significantly lower than the vitamin-supplemented control in the 10% SALATRIM group but was not different at the 14-week period. Female data indicated no difference at 5 weeks, but a lower serum vitamin D concentration compared to the vitamin-supplemented control was found at week 14. These differences in vitamin D do not appear to be biologically significant because of the inconsistencies in the noted changes. On the basis of the results of these vitamin analyses, SALATRIM 4CA lot A006 does not appear to significantly alter fat-soluble vitamin absorption and the vitamin supplementation used in this study was unnecessary. In subsequent subchronic rat studies with four other members of the SALATRIM family in which no vitamin supplementation was used, this conclusion was confirmed (Hayes et al., 1994b,c).

While serum concentrations of calcium, inorganic phosphorus, sodium, potassium, and chloride and urinary clearance of these minerals were comparable among all groups, strontium and zinc in the femurs from the 10% SALATRIM males and females and sodium concentration in the femurs from the 10% SALATRIM females were significantly higher than those in nonsupplemented control rats. Increased bone strontium and decreased bone zinc were noted in the 10% corn oil group. In a subsequent subchronic study (Hayes et al., 1994b), increased bone concentrations of strontium and zinc, increased urinary phosphorus clearance, and slightly increased renal mineralization were associated with exposure to two other SALATRIM fats, SALATRIM 23CA lot A014 and SALATRIM 32CA lot A015. In that study, decreased bone zinc was noted in corn oil-treated rats. In a third subchronic study, increased bone zinc and renal mineralization were noted in rats fed another two SALATRIM fats (Hayes et al., 1994c).

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. They indicated that high linoleate diets have been reported to significantly depress zinc concentrations in the tibia. In subchronic rat studies with SALATRIM fats, the dietary content of polyunsaturated fatty acids was greatest in the 10% corn oil diets, because corn oil contains predominantly unsaturated fatty acids. Polyunsaturated fatty acids were lower in the untreated control diets and were lowest in the 10% SALATRIM diets, because the predominant long-chain fatty acid in SALATRIM (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 because they appeared to be directly related to the high level of unsaturated fatty acids in the corn oil diet and the lower level of unsaturated fatty acids in the SALATRIM diet.

Histopathological examination of the tissues from rats consuming SALATRIM 4CA lot A006 indicated no changes compared to the controls. An increased incidence of hepatocellular vacuolation was noted in the corn oil group. These vacuoles may be fat vacuoles resulting from higher absorption of long-chain fatty acids in the 10% corn oil group than in the 10% SALATRIM group. This change would not be expected with 10% SALATRIM because the amount of long-chain fatty acids available for absorption from SALATRIM is considerably lower than for corn oil.

These data indicate SALATRIM 4CA lot A006 does not elicit toxicologically significant effects when fed to rats for as long as 13 weeks at average daily doses as high as 6.4 g/kg for males and 7.3 g/kg for females. The data support the hypothesis, based upon the scientific literature and structure/activity relationships, that SALATRIM fats are similar to dietary triacylglycerols in their lack of toxicological effects on rats.

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