Diet Induced Jejunal Lipodystrophy in the Cotton Top Marmoset (*Saguinus oedipus*)[1] (35821)

SAMUEL DREIZEN, BARNET M. LEVY, AND SOL BERNICK

University of Texas Dental Science Institute, Houston, Texas 77025; and Department of Anatomy, School of Medicine, University of Southern California, Los Angeles, California 90033

Of the various free ranging primates for which data are available, the marmoset is least susceptible to naturally occurring atherosclerosis (1). Marmosets are small New World primates indigenous to the forested areas of tropical America from Costa Rica to northern Bolivia and southern Brazil. In the wild they are arboreal and omnivorous, subsisting on a diet of fruit, tender vegetation, seeds, insects, bird eggs and nestlings. Their nutritional profile is distinguished by an inability to utilize vitamin D_2 and by an inordinately high daily requirement for vitamin D_3 (2). In the course of testing whether marmosets could be made vulnerable to atherosclerosis by dietary means, a novel syndrome of jejunal lipodystrophy, steatorrhea, and osteomalacia has been produced which is detailed in the present report.

Materials and Methods. Four (2 male and 2 female) adult, healthy, colony-conditioned cotton top marmosets (*Saguinus oedipus*) were fed the experimental diet shown in Tables I and II. The ration was prepared fresh daily. To facilitate ingestion, 4 ml of 25% ethyl alcohol were added to 120 g of diet and the mixture was kneaded to the consistency of a soft banana. The dough was molded into pellets and offered at a level of 30 g/animal/day. Marmosets given the identical diet except for replacement of the 5% cholesterol and the 23% coconut oil by 20% corn starch and 8% corn oil served as controls. Specifics of the preparation of the control diet have been published elsewhere (3). Each animal was housed in a hanging wire cage with an *ad libitum* supply of clean drinking water in a room maintained at 80°F and a minimum humidity of 60%.

Base line weights and serum cholesterol levels were measured immediately prior to the start of the experimental regimen. The cholesterol determinations were made on

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TABLE I. Composition of the High Cholesterol–High Fat Marmoset Diet.

Component	Amount (%)
Sucrose	40.6
Casein (vitamin free)	25.0
Coconut oil	23.0
Cholesterol	5.0
Salts IV[a]	4.0
Corn oil (Mazola)	2.0
Choline chloride	0.2
p-Aminobenzoic acid	0.1
Inositol	0.1

[a] Salt mixture (g): $CaCO_3$, 60.0; K_2HPO_4, 64.5; $CaHPO_4 \cdot 2H_2O$, 15.0; $MgSO_4 \cdot 7H_2O$, 20.4; NaCl, 33.5; $Fe(C_6H_5O_7)_2 \cdot 6H_2O$, 5.5; KI, 0.16; $MnSO_4 \cdot 4H_2O$, 1.0; $ZnCl_2$, 0.05; and $CuSO_4 \cdot 5H_2O$, 0.06.

TABLE II. Composition of Daily Vitamin Supplement per Marmoset.

Vitamin	Amount
Ascorbic acid	25.0 mg
Nicotinic acid	4.9 mg
Ca pantothenate	3.0 mg
Thiamine HCl	1.0 mg
Riboflavin	1.0 mg
Pyridoxine HCl	1.0 mg
Folic acid	0.1 mg
Biotin	25.0 μg
Vitamin B_{12}	1.0 μg
Vitamin E (α-tocopherol)	1.1 mg
Vitamin A acetate	360 IU
Vitamin D_3	600 IU

femoral vein blood by the method of Schoenheimer and Sperry (4). Each animal was inspected daily and weighed weekly. One marmoset from each group was killed by cardiac puncture exsanguination at the end of 24 and 34 weeks; the remainder after 42 weeks. The termination schedule was dictated by the occurrence of spontaneous fractures in the long bones of the test animals which interfered with ingestion or locomotion. Each heart blood specimen was analyzed for serum cholesterol, calcium, magnesium, inorganic phosphorus and alkaline phosphatase. Calcium was determined by flame photometry, magnesium by atomic absorption spectrophotometry, inorganic phosphorus by the method of Taussky and Shorr (5), and alkaline phosphatase by the Klein *et al.* procedure (6).

All structures removed at necropsy were fixed in 10% neutral formalin. The soft tissues were processed in paraffin, sectioned at 6 μ and stained with hematoxylin and erythrosin B. Following fixation, the skeletal material was Grenz-rayed on Kodak projector slide plates of medium contrast emulsion, decalcified in formic acid and prepared as above for microscopic examination.

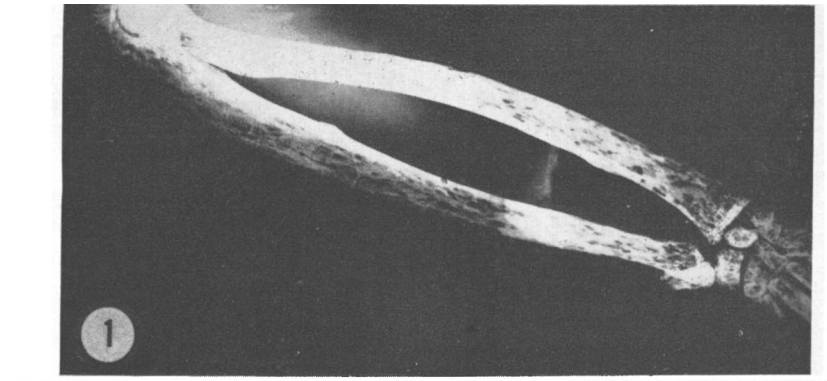
Results. Every marmoset given the cholesterol-enriched diet developed diarrhea by day 10, which persisted throughout the feeding period. The stools were copious, loose, light colored, and fatty in appearance. Fecal total lipid concentrations in random samples ranged from 26.1 to 33.1% of the dry weight when measured by the Sobel method (7). Despite the diarrhea, there were no significant weight changes in any of the animals consuming the test diet; the mean weekly weights paralleling that of the control group.

Radiographic, histologic, and biochemical evidence of osteomalacia was found in each marmoset confined to the cholesterol-coconut oil containing diet and in none of the control animals. In addition to gross fractures of the humerus, tibia and/or fibula, the radiographs demonstrated prominent and irregular areas of bone loss throughout the axial and appendicular skeleton (Fig. 1). These were represented by thinning of the cortical bone with marked lamellation, cortical microfractures, coarse and uneven trabecular networks and widening of the medullary canals. Histologically, the mineralized surfaces of the trabeculae and cortical bone were surrounded by broad bands of osteoid (Fig. 2). The cortical microfractures contained proliferations of osteoid devoid of bone mineral. All terminal serum calcium, phosphorus, alkaline phosphatase, and magnesium concentrations in cholesterol-coconut oil fed animals were consistent with osteomalacia; calcium ranging between 5.96 and 7.27 mg/100 ml, inorganic phosphorus between 4.01 and 4.21 mg/100 ml, alkaline phosphatase between 16.5 and 17.4 Bodansky units and magnesium between 1.42 and 1.60 mEq/liter. The corresponding mean values for the control group were 9.93 and 4.75 mg/100 ml, 11.9 Bodansky units, and 2.14 mEq/liter.

All of the osteomalacic marmosets showed striking structural changes in the small intestine which were confined to the jejunal villi and spared the duodenal and ileal counterparts. In the control animals, the jejunal villi (Fig. 3) were lined by a single layer of tall columnar epithelial absorptive cells interspersed among numerous mucus secreting goblet cells. The absorptive cells contained a cuticular striated free border, finely granular acidophilic cytoplasm and a basally located elongated nucleus. In the osteomalacic animals the cytoplasmic component of the absorptive cells was displaced by large clear vacuoles which distended the cells, compressed the nuclei and made them indistinguishable from the goblet cells (Fig. 4). Histochemically, the vacuolated absorptive cells were differentiated from the goblet cells by staining bright red (fat positive) with oil red O in frozen section.

Mean serum total cholesterol in the marmosets restricted to the high fat diet rose from 92.8 $\pm$ 11.5 to 258.0 $\pm$ 45.2 mg/100 ml during the feeding periods. In contrast the average pretest and terminal values for the control group were 94.3 $\pm$ 12.6 and 87.7 $\pm$ 10.8 mg/100 ml, respectively. No gross or microscopic manifestations of atherosclerosis were found in any of the hypercholesteremic or control animals.

Discussion. Available evidence indicates



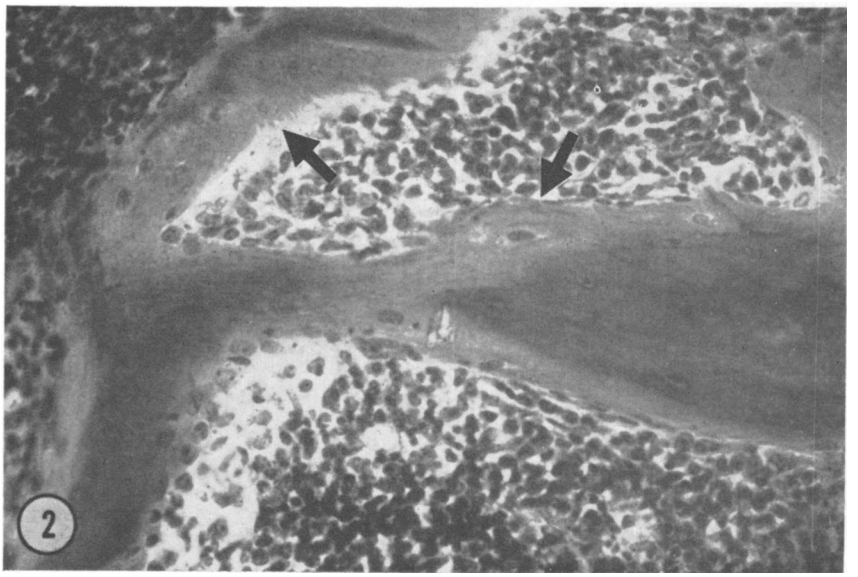


FIG. 1. Radiographic manifestations of osteomalacia in bones of upper extremity of marmoset fed test diet for 36 weeks.

FIG. 2. Osteoid seams (arrows) around bony trabeculae in marmoset given test diet for 36 weeks; ×200.

that the upper jejunum is the major site of absorption of ingested fatty acids (8, 9) and that dietary cholesterol is absorbed mainly in the distal half of the small intestine (10). Absorption of the products of fat digestion is largely confined to the villous tip. Electron microscopic studies of rat jejunum show that only the most apical cells in the villous become filled with minute lipid droplets in various stages of passage through the endoplasmic reticulum following a high fat feeding. Neighboring cells along the sides of the villous contain progressively diminishing amounts of fat (11). The massive intracellular accumulations of fat in almost all of the jejunal absorptive cells in the marmosets given the test diet and their absence in the control group is strongly suggestive of an impaired functional capacity in these cells.

The alimentary tract of the marmoset is featured by a relatively short small intestine which is approximately half the length of the same structure in rats of equal weight. Clogging of the absorptive surface of the jejunum by lipid deposits in the lining epithelium and the laxative effect of the high fat diet superimposed upon the short small intestine presumably precipitated a malabsorption syndrome in the test animals. In the presence of a nutritionally adequate diet, the absorptive

disorders which produce bone disease are almost invariably associated with steatorrhea (12). Failure to absorb fat is accompanied by a deficiency in calcium absorption as fat-soluble dietary vitamin D is a captive of the fat lost in the feces (13). Dietary calcium may also be lost to absorption by linkage with digested fats to form nonabsorbable

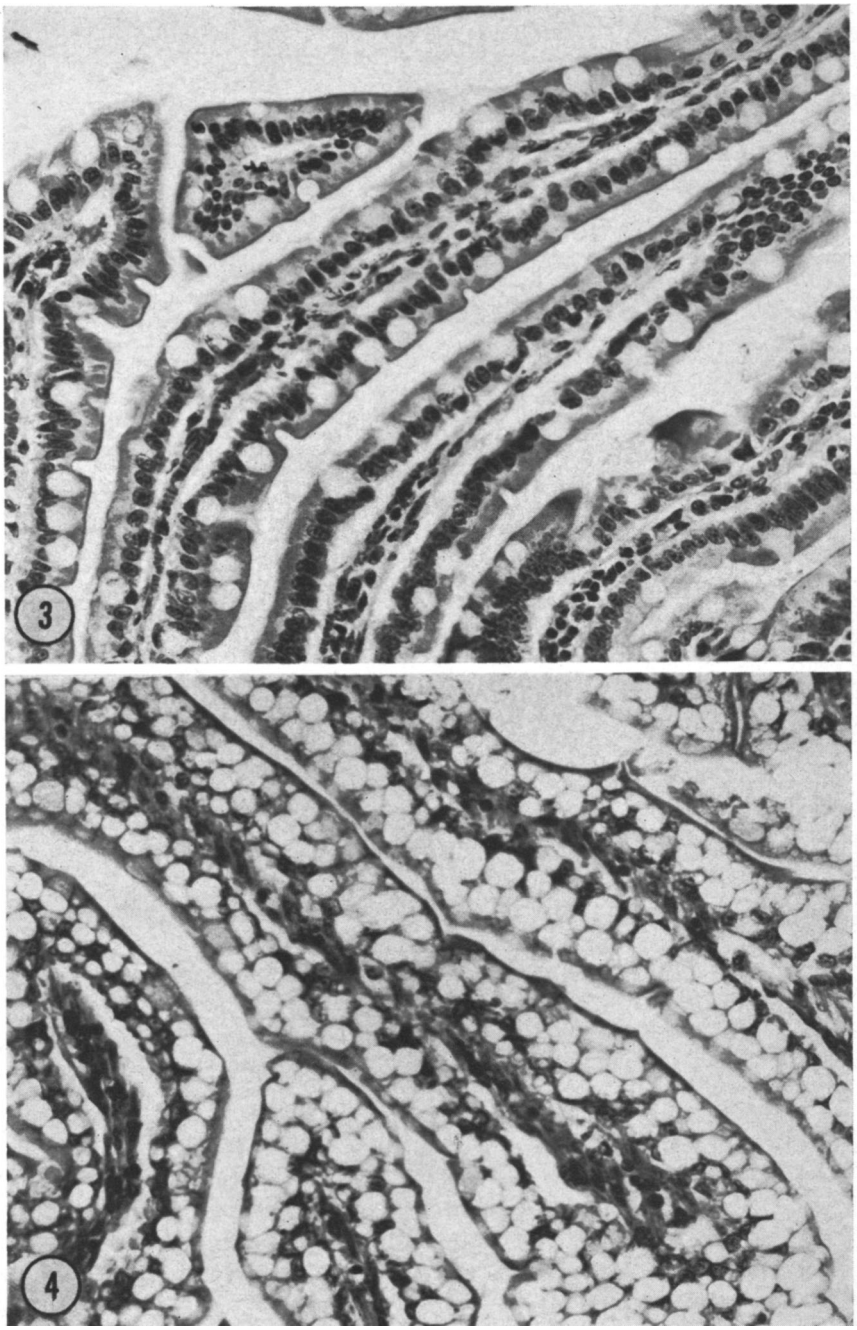


FIG. 3. Histologic appearance of jejunal villi in control animal; ×400.

FIG. 4. Histologic appearance of lipodystrophy in jejunal villi of marmoset consuming test diet for 42 weeks, ×400.

soaps. This sequence was reflected in the present study by the development of osteomalacia in each of the test animals. Their peculiarly high and specific vitamin D requirement rendered them highly susceptible to steatorrhea-induced osteomalacia even when adequate amounts of vitamin D_3 were incorporated in the experimental diet.

The absence of significant weight losses and the presence of diet-related hypercholesteremia and jejunal lipodystrophy in the test animals were all indicative of a localized rather than generalized absorptive defect. The jejunal block to the uptake of calcium and vitamin D did not extend to the absorption of cholesterol by the ileum. Despite the ensuing hypercholesteremia which reached a height of 423 mg/100 ml in 1 animal, there were no detectable atherosclerotic changes in any of the cholsterol fed marmosets. This primate thus appears to be highly resistant to both naturally occurring and experimental atherosclerosis.

Summary. A malabsorption syndrome denoted by jejunal lipodystrophy, steatorrhea, and osteomalacia has been produced in adult cotton top marmosets (*Saguinus oedipus*) fed a high cholesterol, high coconut oil containing diet. The diet included sufficient amounts of all presently known nutritional essentials for this primate. The lipodystrophy was marked by huge collections of neutral fat in the absorptive cells of the jejunal villi. The fat filled cells apparently created an absorptive block which contributed to the development of steatorrhea and osteomalacia.

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