PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 502–507 (1979)

Portacaval Shunt and Whole-Body Cholesterol Metabolism in the Cholesterol-Fed African Green Monkey (40583)

JOHN S. PARKS, NOEL D. M. LEHNER, RICHARD W. ST. CLAIR, AND HUGH B. LOFLAND[1]

Arteriosclerosis Research Center, Departments of Pathology and Comparative Medicine, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina 27103

Interest in the portacaval shunt as a therapeutic measure for hypercholesterolemia was stimulated after Starzl *et al.* reported a dramatic serum cholesterol-lowering effect in a patient with homozygous familial hypercholesterolemia (1). This disorder, which includes elevated total serum cholesterol and low-density lipoprotein cholesterol concentrations (2), had characteristically been refractory to diet, drug, and surgical treatment (3–5). Starzl's patient, a 12-year-old girl, responded to the surgery by a decrease in serum cholesterol concentrations from 900 to 350 mg/dl (1). In addition to the serum cholesterol reduction, other ameliorative effects occurred such as resorption of tuberous and tendinous xanthomas, decreased aortic stenosis, and an improved general status of health (6).

Since Starzl's first report others have employed the same procedure, but with varying degrees of success. Some investigators have reported a 40–50% decline in serum cholesterol concentrations after portacaval shunt (6–8), while others have found less of an effect (20–50% lowering) on the long-term reduction of serum cholesterol concentrations in several patients (9–11). In a follow-up report, 14 homozygous familial hypercholesterolemic patients, who had portacaval shunts for 12 to 30 months, showed an average decrease in serum cholesterol of 30% (11).

Results from studies attempting to reproduce the cholesterol-lowering effect of portacaval shunt in animal models have been contradictory and highly variable. This is best exemplified by the studies done with rats in which portacaval shunt has been shown to increase (12), decrease (12–15), or have no effect (16, 17) on serum cholesterol concentrations. Studies with dogs and swine, however, tend to support the serum cholesterol-lowering effect of portacaval shunt (18–21). Direct comparisons of these studies are difficult because of the marked differences in cholesterol metabolism in these species and the broad range of dietary factors (level of dietary cholesterol and type of dietary fat) influencing the serum cholesterol concentrations.

This prompted us to investigate the effects of portacaval shunt on whole-body cholesterol metabolism in a nonhuman primate species, the African green monkey, in which we had previous knowledge of base-line whole-body cholesterol metabolism (22). In addition, the plasma lipoprotein concentrations and distribution are similar to those observed in human beings (23). In contrast to most other animal studies of portacaval shunt, which have been short term and in species other than nonhuman primates, this study was designed to investigate the effect of portacaval shunt on long-term whole-body cholesterol metabolism in nonhuman primates consuming a high-fat, high-cholesterol diet typical of that consumed by North American human beings.

Materials and methods. Experimental design. Seven juvenile male African green monkeys (*Cercopithecus aethiops*) of the vervet type were used in this study. The animals were fed a semipurified diet containing 0.62 mg cholesterol/Cal derived largely from egg yolk (22). Plasma cholesterol concentrations stabilized during the first 3 months while the animals were consuming the diet. After the animals had consumed the diet for 6 months whole-body cholesterol metabolism studies were conducted using both sterol balance techniques and kinetic analysis of the turnover of [4-^{14}C]cholesterol. These methods and the cholesterol metabolism data obtained on these monkeys prior to the surgical produc-

[1] Deceased.

0037-9727/79/090502-06$01.00/0

tion of the portacaval shunt have been described in detail elsewhere (22). One to five weeks after completion of these base-line (preoperative) sterol balance studies, four animals were selected for surgically induced portacaval shunt and three animals were sham operated. Sterol balance studies were repeated at 2, 3, 11, and 44 weeks after surgery. These periods were designated Post Op I, Post Op II, Post Op III, and Post Op IV, respectively. At 28–32 weeks after surgery each animal was injected with 5.22 μCi [4-^{14}C]cholesterol and kinetic analysis of the die-away of the radiolabeled cholesterol conducted as described previously (22).

This experimental design was adopted so that acute changes in whole-body cholesterol metabolism could be monitored immediately following surgery and before a new cholesterol steady state was reached; hence sterol balance measurements conducted in this study were selected to be those that did not require steady-state conditions. The serum cholesterol turnover curve analysis, however, does require the cholesterol steady state for valid interpretation, so these studies were done when plasma cholesterol concentrations and body weight had stabilized (28–32 weeks following surgery).

Surgical production of portacaval shunt. Prior to surgery the animals were fasted overnight, weighed, sedated with ketamine, intubated with an endotracheal tube, and anesthetized for surgery with halothane.[2] The abdomen was opened by a midline incision and the inferior vena cava and portal vein were clamped. The hepatic portal vein was transected and anastamosed to the side of the inferior vena cava. The veins were clamped for approximately 30 min during the operative procedure. The control animals received the same treatment except that no manipulation was performed on their portal circulation. After surgery, the animals were given 20,000 units of penicillin G and 2.5 mg streptomycin/kg and retained in a recovery room for a period of 1 week before being returned to their metabolism cages.

The patency of the portacaval shunts was inspected, following laparotomy, 3 months after the original surgery (immediately after the 11-week sterol balance study) and at the time of necropsy. All shunts were found to be patent at both times.

Results. Shown in Fig. 1 are the results of weekly serum cholesterol determinations, mean body weights, and the period of fecal collections for measurement of sterol balance parameters. The body weights of the animals quickly stabilized following surgery, except for animal 1369 in which body weight continued to decrease until the animal died. All animals, shunts and controls, had a transient decrease in serum cholesterol concentration during the first week after surgery, but then had variable increases of serum cholesterol concentrations above preoperative levels over the next 10 weeks. These increases were slight and were similar for the control monkeys and those that had a portacaval anastomosis. The variability in serum cholesterol concentration was large in both groups of animals.

Excretion of endogenous cholesterol was measured at four intervals after surgery by the sterol balance method (Table I). Results for endogenous neutral steroid excretion were similar for both control and portacaval shunt animals for all postoperative time periods. The mean values (±SEM) for endogenous neutral steroid excretion by the control animals in Post-Op periods I–IV were 9.1 ± 1.5, 9.2 ± 1.5, 9.4 ± 1.8, and 12.6 ± 0.3 mg/kg/day, respectively, while these values for the monkeys with portacaval shunts were 6.6 ± 1.3, 6.6 ± 0.7, 9.4 ± 1.3, and 14.9 ± 3.8 mg/kg/day.

Bile acid excretion (Table I) was also similar for both control and portacaval shunt animals. The mean values for bile acid excretion by the control animals in Post-Op periods I–IV were 4.2 ± 0.1, 4.0 ± 0.7, 4.9 ± 0.8, and 6.5 ± 2.4 mg/kg/day, respectively, while these values for the monkeys with portacaval shunts were 1.9 ± 0.8, 2.4 ± 0.1, 2.9 ± 0.6, and 5.4 ± 0.6 mg/kg/day.

The kinetic parameters describing the turnover of radiolabeled cholesterol for the monkeys after portacaval shunt or sham operations are listed in Table II. There was no difference between shunted and sham-operated control animals for any parameter derived from the serum cholesterol turnover

[2] The authors wish to gratefully acknowledge Drs. Howard G. Dawkins and Charles S. Turner who expertly performed the portacaval shunt and control operations.

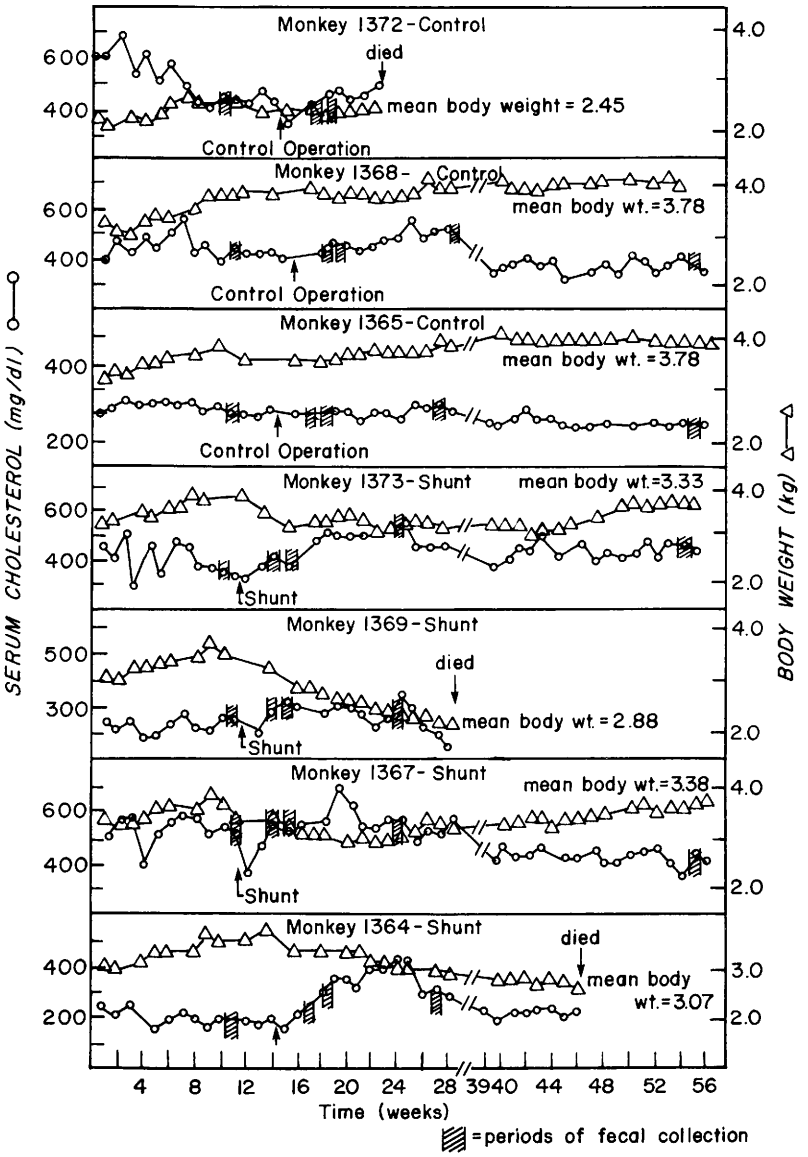

FIG. 1. Serum cholesterol concentrations and body weights of individual control and shunt animals during the entire experimental period. Preoperative cholesterol turnover analysis was performed during Weeks 1–12, while postoperative cholesterol turnover analysis was conducted Weeks 39–56 (28–32 weeks after surgery).

curves. Overlapping of data between shunt and control animals was found for M_A, fractional PR_A, T ½ first exponential, and the estimate of M_B (max). Although only four animals completed this phase of the study, these data nevertheless support the sterol balance data indicating little change in whole-body cholesterol metabolism as a result of portacaval shunt.

Cholesterol absorption was determined by feeding a diet of constant cholesterol specific activity until the serum cholesterol specific activity reached a plateau (isotopic steady-state method, Ref. (24)). Results are shown in Table III. By this method, there was no major difference between control and portacaval shunt monkeys in the proportion of plasma cholesterol derived from absorption.

TABLE I. INFLUENCE OF PORTACAVAL SHUNT ON CHARACTERISTICS OF STEROL BALANCE IN CHOLESTEROL-FED AFRICAN GREEN MONKEYS[a]

Animal number	Time period	Endogenous neutral steroid excretion (mg/kg/day)	Bile acid excretion (mg/kg/day)	Turnover[b] endogenous cholesterol (mg/kg/day)
1365 Control	Post-Op I[c]	10.3	4.3	14.6
	Post-Op II	12.0	4.9	16.9
	Post-Op III	11.2	4.1	15.3
	Post-Op IV	12.9	4.0	16.9
1368 Control	Post-Op I	6.2	3.9	10.1
	Post-Op II	6.8	4.3	11.1
	Post-Op III	7.5	5.7	13.2
	Post-Op IV	12.2	8.9	21.1
1372 Control	Post-Op I	10.9	4.3	15.2
	Post-Op II	8.7	2.7	11.4
1364 Shunt	Post-Op I	8.6	2.3	10.9
	Post-Op II	8.4	2.3	10.7
	Post-Op III	8.1	4.6	12.7
1367 Shunt	Post-Op I	8.7	3.9	12.6
	Post-Op II	6.2	2.6	8.8
	Post-Op III	9.3	2.8	12.1
	Post-Op IV	18.7	6.1	24.8
1369 Shunt	Post-Op I	5.7	1.0	6.7
	Post-Op II	5.2	2.1	7.3
	Post-Op III	7.2	2.1	9.3
1373 Shunt	Post-Op I	3.4	0.5	3.9
	Post-Op II	6.4	2.5	8.9
	Post-Op III	13.1	2.2	15.3
	Post-Op IV	11.0	4.8	15.8

[a] Data calculated from combined techniques of gas–liquid chromatography of total fecal neutral steroids and isotopic quantitation of fecal endogenous neutral steroids and bile acids.
[b] Endogenous neutral steroids and bile acids excreted.
[c] Post-Op I, II, III, and IV are fecal collection periods 2, 3, 11, and 44 weeks after surgery, respectively.

TABLE II. INFLUENCE OF PORTACAVAL SHUNT ON SERUM CHOLESTEROL TURNOVER IN CHOLESTEROL-FED AFRICAN GREEN MONKEYS[a]

Animal number	PR_A[b] (mg/kg/day)	M_A[c] (mg)	Fractional[d] PR_A	T 1/2 first[e] exponential (days)	T 1/2 second[f] exponential (days)	M_B (min)[g] (mg)	M_B (max)[h] (mg)
Control							
1365	20.0	318	0.0632	2.74	38.6	601	879
1368	20.3	456	0.0445	3.71	38.5	610	778
Portacaval shunt							
1367	23.0	521	0.0442	4.17	34.7	463	770
1373	28.3	424	0.0667	2.87	29.4	562	893

[a] Data were obtained 28–44 weeks after surgery by plotting serum cholesterol specific activity semilogarithmically against time after a single intravenous injection of [4-^{14}C]cholesterol. The data best fit the sum of two exponentials and the data were used as described for the preoperative turnover curves (22). At this time one control (1372) and two shunt monkeys (1364 and 1369) had died.
[b] Turnover rate of the rapidly misible pool (pool A) of cholesterol. Pool A is generally considered to be composed of the liver, intestine, and plasma.
[c] Mass of cholesterol in pool A.
[d] Fraction of pool A which is replaced with newly synthesized cholesterol each day.
[e] Half-life of the rapidly decaying exponential.
[f] Half-life of the slowly decaying exponential.
[g] Minimal estimate of the mass of pool B.
[h] Maximal estimate of the mass of pool B.

TABLE III. ABSORPTION OF DIETARY CHOLESTEROL AND SYNTHESIS OF CHOLESTEROL BY CHOLESTEROL-FED AFRICAN GREEN MONKEYS AFTER PORTACAVAL SHUNT OR CONTROL OPERATIVE PROCEDURES[a, b]

Animal number	% Ab.[c]	% Syn.[c]	mg/kg/day Chol. abs.	mg/kg/day Chol. syn.
Control				
1365	70.5	29.5	14.1	5.9
1368	86.2	13.8	17.5	2.8
Portacaval Shunt				
1367	91.2	8.8	21.0	2.0
1373	87.6	12.4	24.8	3.5

[a] Data were derived from the ratio of the specific activities of serum and dietary cholesterol after prolonged continuous feeding of a diet of constant [1,2-^{3}H]cholesterol specific activity.

[b] Data were derived 28–44 weeks after surgery. At this time one control (1372) and two shunt monkeys (1364 and 1369) had died.

[c] These values represent the percentage of total plasma cholesterol derived from absorption (Ab.) and synthesis (Syn.).

An estimate was made of the absolute rate of cholesterol absorption by multiplying the metabolic turnover rate of cholesterol (PR_A) derived from the die-away of radiolabeled cholesterol in the plasma by the percentage of serum cholesterol derived from the diet (Table III). Although there was a suggestion that there may have been more total cholesterol absorbed in animals with the portacaval shunt, it is impossible with the small number of animals to know whether this was a significant effect.

The rate of cholesterol synthesis, as determined by the isotopic steady-state method, is also included in Table III. There were no marked differences between control and portacaval shunt animals in either the percentage of plasma cholesterol derived from synthesis or in the absolute rate of whole-body cholesterol synthesis.

Discussion. Portacaval shunt had no effect on whole-body cholesterol metabolism in the cholesterol-fed African green monkeys of this study. There was no difference between shunt and control animals in cholesterol absorption, synthesis, or excretion, or in any of the kinetic parameters derived from the serum cholesterol turnover curves. Furthermore, there was no difference in serum cholesterol concentrations for up to 11 months following surgery. Since several studies in the literature have suggested a decrease in cholesterol synthesis in response to portacaval shunt, we determined hepatic HMG CoA reductase activity, the rate-limiting enzyme for cholesterol synthesis, in liver biopsies obtained prior to necropsy (1 year after operation) in the four animals remaining at the completion of the experiment.[3] Again the results were similar for the control and shunt animals, with reductase activities of 1.47 and 1.80 vs 1.34 and 2.41 nmol/hr/mg protein, respectively. These results are consistent with the sterol balance data indicating no difference in hepatic or whole-body cholesterol synthesis. However, our results are not inconsistent with the reports of other investigators that portacaval shunt lowers cholesterol synthesis in man and some experimental animals (8, 13, 18, 21). As shown in Table III, less than 20% of the total cholesterol turnover of the animals of this study was due to endogenous cholesterol synthesis. This reduction in cholesterol synthesis by dietary cholesterol is similar in magnitude to that described previously for African green monkeys with similar plasma cholesterol concentrations (22). Since hepatic cholesterol synthesis can account for only a portion of total endogenous synthesis, the level of hepatic cholesterol synthesis probably was too low for a change in either plasma cholesterol concentrations or whole-body cholesterol metabolism to be seen, even if portacaval shunt had altered hepatic cholesterol synthesis. Thus the lack of effect of portacaval shunt in cholesterol-fed animals indicates that the shunt does not alter the metabolism of dietary cholesterol, but probably acts to reduce the enhanced hepatic cholesterol synthesis seen in patients with familial hypercholesterolemia (8).

Summary. African green monkeys consuming a diet containing 0.62 mg cholesterol/Cal and 40% of calories as fat were characterized for whole-body cholesterol metabolism after control (sham) or portacaval shunt operations. Whole-body cholesterol metabolism was studied by three different methods: sterol

[3] The authors gratefully acknowledge Dr. Frank Hulcher for performing the enzyme assay. HMG CoA reductase assay was done according to the method of Brown, M. S., Dana, S. E., and Goldstein, J. L., J. Biol. Chem. **249**, 789–796 (1974).

balance analysis, serum cholesterol turnover analysis, and feeding radiolabeled cholesterol until a serum cholesterol isotopic steady state was reached. We found no difference between control and portacaval shunt animals for the following characteristics: serum cholesterol concentration, excretion of neutral steroids or bile acids, cholesterol absorption or synthesis, and kinetic parameters of serum cholesterol turnover. We have concluded that portacaval shunt in African green monkeys fed a diet containing high fat and a moderate amount of cholesterol had no effect on whole-body cholesterol metabolism.

We gratefully acknowledge the technical assistance of Mrs. Virginia Heaster and Mrs. Jean Phillips as well as the aid of Mrs. Linda Trust in the preparation of this manuscript. This work was supported by National Heart, Lung and Blood Institute Grant (SCOR) HL-14164 and by an Institutional National Research Service Award HL07115 and was in partial fulfillment for the master's degree (John S. Parks) in Comparative and Experimental Pathology from the Bowman Gray School of Medicine of Wake Forest University.

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Received January 8, 1979. P.S.E.B.M. 1979, Vol. 161.