Proc. Natl. Acad. Sci. USA
Vol. 76, No. 9, pp. 4654–4657, September 1979
Medical Sciences

Cholesterol homeostasis in the rat with a portacaval anastomosis

(sterol balance/hydroxymethylglutaryl-CoA reductase/tissue deposition/bile acid synthesis)

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ABSTRACT **Studies were undertaken to determine the effect of portacaval anastomosis on cholesterol homeostasis in rats fed sucrose/lard under conditions of normal body growth. Four to 6 weeks after portacaval shunt surgery, we found decreases in plasma cholesterol and triglyceride concentrations, total liver weight, and hepatic microsomal protein concentration. Measurements of hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) activity showed decreases in specific activity and total liver activity in portacaval shunt rats, but the enzyme diurnal rhythm remained. Decreased reductase activity in shunted rats was not due to an altered K_m for D-HMG-CoA, nor was an enzyme inhibitor found in the livers of the portacaval shunt animals. Sterol balance measurements in rats with shunts showed a 22% decrease in whole body cholesterol synthesis rate compared to controls. These metabolic studies, coupled with postmortem data, showed diminished bile acid synthesis, unchanged fecal neutral steroid excretion, and decreased net tissue accumulation of cholesterol during growth. The decreased whole body cholesterol synthesis rate ultimately led to a diminished total carcass cholesterol concentration in the rats with shunts.**

The surgical procedure of portacaval anastomosis (PCA) has provided an effective therapeutic approach in homozygous familial hypercholesteremia in man, as judged by a decrease in plasma cholesterol level and clinical amelioration in the signs and symptoms of premature coronary artery disease. Since the original report in 1973 by Starzl and coworkers (1), more than 30 other patients with this lethal inborn error of metabolism have been subjected to PCA (2); medical procedures (diets or drugs) have usually been ineffective in controlling the rapid progression of atherosclerotic heart disease in this condition. Starzl *et al.* (3) have presented evidence, obtained in experiments on dogs, that the diversion of portal blood around the liver leads to a decrease of liver mass and of hepatic cholesterol synthesis.

Various experiments to test this hypothesis have produced conflicting results. Decreased hepatic cholesterol synthesis has been reported to occur in dogs (3), pigs (4), and rats (5) after PCA. In contrast, studies in baboons (6), dogs (7), and rats (8, 9) have shown hepatic cholesterol synthesis to be unchanged or possibly even increased after portal diversion. Bilheimer *et al.* (10) reported that PCA in a 6-year-old girl with homozygous familial hypercholesteremia resulted in a 59% decrease in whole body cholesterol synthesis as measured by sterol balance methods, whereas in a 12-year-old boy Meinertz *et al.*§ found an increased whole body cholesterol synthesis rate.

In an attempt to resolve some of these uncertainties, we undertook studies of whole body cholesterol synthesis rates and hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (mevalonate:NADP oxidoreductase, EC 1.1.1.34) activity in PCA rats fed a purified high-carbohydrate diet that allows normal body growth to occur after institution of a PCA (11–13). Normal body growth is essential to the proper interpretation of sterol balance data and of enzyme activities, a prerequisite that has not been met in most of the studies thus far reported (8, 14, 15). Our results support the original hypothesis of Starzl *et al.* (3) that, in the rat, PCA results in decreases in specific activities and total liver activities of hepatic HMG-CoA reductase as well as decreased rates of whole body cholesterol synthesis as measured by sterol balance methods.

MATERIALS AND METHODS

DL-3-Hydroxy-3-methyl-[3-^{14}C]glutaryl coenzyme A [26.5 mCi/mmol (1 Ci = 3.7 × 10^{10} becquerels)], [1,2-^{3}H]cholesterol (40 Ci/mmol), and [24-^{14}C]cholic acid (40 mCi/mmol) were purchased from New England Nuclear. DL-3-HMG-coenzyme A was purchased from P-L Biochemicals; NADP, glucose-6-phosphate dehydrogenase, and glucose 6-phosphate (sodium salt) were obtained from Sigma. All other reagents and solvents were analytical grade; solvents were glass-distilled prior to use.

Animals. Male Sprague–Dawley rats weighing 200–250 g were purchased from Holtzman (Madison, WI), and housed in a light-cycled room (12 hr/cycle). Animals were fed ad lib. a diet containing, by weight, 58.5% sucrose, 21.6% casein, 5.0% lard, 10.4% cellulose, 3.5% AIN-76 mineral mix, and 1.0% Teklad no. 40060 vitamin mix (diet 72276, Teklad Test Diets, Madison, WI) (11–13). End-to-side PCA was performed in all cases by one of us (K.D.G.E.) using the surgical procedure of Herz *et al.* (15); 4 weeks postoperatively the animals were housed individually in metabolic cages (Hazelton Systems, Cincinnati, OH) that allowed separate quantitative recoveries of feces and urine as well as measurements of food intake. At the end of the balance study, rats were exsanguinated from the abdominal aorta, and shunt patency was verified. Control rats were not operated on; initial experiments showed no differences between such rats and sham-operated rats in the variables being measured.

Sterol Balance Measurements. Three-day pools of feces were dried, powdered, and extracted as described by Cohen *et al.* (16). Neutral steroids were isolated and measured by the method of Miettinen *et al.* (17); fecal bile acids were isolated and measured by the method of Grundy *et al.* (18) as modified by Cohen *et al.* (16). Total liver and carcass cholesterol contents were measured as described (19). All determinations were corrected for procedural losses by use of radiolabeled internal recovery standards. Daily net tissue cholesterol accumulation was expressed as the difference in total body cholesterol (carcass + liver + blood + intestinal contents) measured at 4 and again at 6 weeks postoperatively. Whole body cholesterol synthesis

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Abbreviations: PCA, portacaval anastomosis; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A.

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§ Meinertz, H., Faergeman, O., Nilhausen, K., McNamara, D. & Ahrens, E. H., Jr. (1978) Abstract, European Lipoprotein Club, Cologne, West Germany, Aug. 31–Sept. 3, 1978.

Table 1. Changes induced in rats by PCA* (4–5 weeks postoperatively)

	Control rats	PCA rats	*P*
Body growth rate,† g/day	2.68 ± 0.44 (6)	2.90 ± 0.46 (12)	NS
Plasma cholesterol, mg/dl	72 ± 11 (14)	48 ± 8 (19)	<0.001
Plasma triglyceride, mg/dl	221 ± 78 (14)	34 ± 10 (19)	<0.001
Liver weight, g	12.64 ± 2.07 (14)	8.01 ± 1.28 (19)	<0.001
(Liver weight/body weight) × 100	3.27 ± 0.19 (14)	2.01 ± 0.21 (19)	<0.001
Hepatic microsomal protein, mg/g liver	11.29 ± 1.80 (17)	9.83 ± 1.93 (23)	<0.02
Hepatic cholesterol, mg/g liver	2.20 ± 0.26 (13)	2.54 ± 0.40 (12)	<0.02
Hepatic cholesterol, mg/liver	30.23 ± 5.23 (13)	22.45 ± 4.78 (12)	<0.001

* Data were obtained 4–5 weeks postoperatively and are shown as mean ± SD with number of measurements in parentheses. Control and PCA rats were matched for initial body weight. Control rats were not operated on because initial experiments showed no significant differences between these and sham-operated rats in the measurements listed in this table.

† Measured between 0 and 5 weeks after PCA.

was calculated by: total neutral and acidic steroid excretion plus net tissue accumulation minus dietary intake of cholesterol.

HMG-CoA Reductase Assay. Hepatic HMG-CoA reductase activity was measured in isolated microsomes prepared and assayed as described (20, 21). Microsomal protein concentration was measured by the procedure of Lowry *et al.* (22).

Statistical Analysis. Statistical analysis was carried out by using a Hewlett–Packard 97 calculator programmed for the Student *t* test of unpaired means (23) as supplied in the Hewlett–Packard Stat Pac I. Linear regression analysis of Lineweaver–Burk kinetics was performed by using a two-variable linear regression program on a Hewlett–Packard 9810A calculator.

RESULTS

Table 1 confirms the previously reported (11–13) normal growth rate achieved by PCA rats fed a purified high carbohydrate diet. There were significant decreases in plasma cholesterol and triglyceride concentrations and relative liver weight, as reported (11, 12, 24), and in liver microsomal protein concentrations in PCA rats. Although hepatic cholesterol concentration increased in the PCA rat, total hepatic cholesterol content decreased by 26% due to the decreased liver mass.

Sterol Balance Data. Table 2 demonstrates the decreased rate of whole body cholesterol synthesis in PCA rats. Fecal excretion of acidic steroids was significantly decreased, but fecal output of neutral steroids was unchanged in PCA rats. Because both control and PCA rats excreted negligible quantities of bile acids in their urine [≈1.3% of total output (unpublished observations)], the decreased fecal excretion of bile acids in PCA rats represented actual decreases in bile acid synthesis in these animals. An unexpected finding was the 87% decrease in net tissue accumulation of cholesterol in PCA rats despite growth rates that were the same in control and PCA animals, an observation set out in greater detail in Table 3.

Reductase Activities. In PCA rats there were marked decreases in HMG-CoA reductase activities, whether expressed in terms of microsomal protein or per whole liver (Table 4). Total hepatic reductase activity per liver was decreased 74% in PCA rats, although specific activity was decreased only about 50%. In addition, PCA rats exhibited significant diurnal rhythms of reductase specific activity, with 5- to 7-fold increases occurring in the mid-dark period compared to the mid-light period.

Kinetic analysis of the specific activities of hepatic HMG-CoA reductase in control and PCA rats (Table 4) demonstrated that the enzyme from both sets of animals exhibited similar K_m values for D-HMG-CoA (25). Nevertheless, the enzyme from livers of PCA rats showed a 72% decrease in calculated maximal velocity. Mixing experiments were performed to test for the presence of inhibitors of hepatic HMG-CoA reductase in the livers of PCA rats. These tests failed to demonstrate any *in vitro* inhibitory activity in the PCA rat liver with either whole liver homogenates or isolated microsomes mixed with enzyme protein from control animals. Thus, the decreases in hepatic HMG-CoA reductase activities in PCA rats resulted from decreased enzyme contents.

DISCUSSION

Previous studies of the metabolic response of cholesterol metabolism to PCA in the rat required the use of pair-fed, semi-starved controls to compensate for the postoperative body weight loss in the PCA animal (5, 8, 9). This weight loss has also been observed in dogs, swine, monkeys, and baboons (26). The well-documented response of rat hepatic HMG-CoA reductase activity to fasting (27) and the changes in body tissue rates of

Table 2. Sterol balance data in control and PCA rats*

Measurements	Control rats (n = 8)	PCA rats (n = 7)	*P*
Fecal acidic steroid excretion	18.74 ± 2.75	13.85 ± 3.64	<0.02
Fecal neutral steroid excretion	22.74 ± 2.24	25.27 ± 3.59	NS
Net tissue cholesterol accumulation†	9.61 ± 1.81	1.27 ± 4.86	<0.001
Dietary cholesterol intake	1.92 ± 0.10	1.99 ± 0.12	NS
Cholesterol synthesis‡	49.15 ± 5.01	38.41 ± 4.77	<0.001

* Sterol balance measurements were performed 4–6 weeks after PCA; three 3-day fecal pools were analyzed for each rat. Data are shown as mean ± SD, in mg/kg of body weight per day.

† Net accumulation on a daily basis over the period 4–6 weeks after PCA.

‡ Shown in mg/kg per day and calculated by: fecal neutral and acidic steroids + net tissue cholesterol accumulation − cholesterol intake.

Table 3. Changes in carcass cholesterol concentration in PCA rats

Time after PCA, weeks	Carcass cholesterol, mg/g		P
	Control rats	PCA rats	
4	1.60 ± 0.08 (3)	1.52 ± 0.03 (3)	NS
6	1.57 ± 0.10 (4)	1.40 ± 0.05 (4)	<0.05

Carcass cholesterol = total body cholesterol − cholesterol content of liver, exsanguinated blood, and contents of intestine. Data are shown as mean ± SD with number of measurements in parentheses.

cholesterol synthesis from acetate in rats fed subcaloric diets (28) suggest that interpretation of studies comparing semi-starved controls and PCA rats is seriously confounded.

The present study circumvented these difficulties by utilizing animals fed a purified high-carbohydrate diet that allows normal body growth rates in both control and PCA rats (11–13); however, the diet itself causes a 68% decrease in hepatic HMG-CoA reductase specific activity as compared to animals fed a cereal-based diet (29). Similar results have been reported by Reiser *et al.* (30) who found that rats fed a purified diet exhibited a 63% decrease in hepatic reductase levels and a 44% decrease in total daily fecal steroid excretion. The present study deals only with comparisons of sterol metabolism in control and PCA rats fed the same diet. Despite the reduction in cholesterol synthesis in the control rats fed the purified diet, the results in the PCA rat 4–6 weeks postoperatively clearly demonstrate a further significant decrease in both whole body cholesterol synthesis and hepatic HMG-CoA reductase activity. The observed 22% decrease in whole body cholesterol synthesis rate in PCA rats is similar to that reported by Bilheimer *et al.* (10) in studies of sterol balance in a 6-year-old girl with homozygous familial hypercholesteremia prior to and after PCA.

It should be noted that the total daily fecal steroid excretion was not significantly different in control and PCA rats; the major difference was in the net daily tissue accumulation of cholesterol which decreased by 87% in the PCA rats. Total fecal bile acid output was also significantly decreased in the PCA rats whereas fecal neutral steroid excretion was unchanged, presumably due to the fact that intestinal synthesis of cholesterol is the major contributor to fecal neutral steroid excretion in the rat (31, 32).

The decreasing carcass cholesterol concentrations in PCA rats suggests that substantial tissue efflux or redistribution of cholesterol occurs within a relatively short time after surgery (4–6 weeks). In this respect, the rat fed the purified diet is a particularly useful animal model for studies on cholesterol metabolism because tissue efflux of cholesterol is one of the most striking observations in humans with PCA for treatment of homozygous familial hypercholesteremia (2). Furthermore, these results emphasize the necessity of measuring body cholesterol pool sizes for correct interpretation of the sterol balance results obtained after an experimental manipulation such as PCA; if we had not discovered that carcass cholesterol content of PCA rats was decreasing at 4–6 weeks postoperatively, the sterol balance data considered alone would have led us to the erroneous conclusion that whole body cholesterol synthesis was unchanged in the PCA rat.

The observed decrease in total sterol balance is concordant with the demonstrated decrease in total HMG-CoA reductase activity in the livers of PCA rats. Nevertheless, hepatic HMG-CoA reductase from PCA rats exhibits a normal diurnal rhythm and substrate affinity as shown by a K_m for D-HMG-CoA identical to that in control animals. Moreover, the observed decrease in activity is not the result of the presence of an *in vivo* inhibitor of the enzyme in the livers of these animals, as judged by *in vitro* mixing experiments. Decreased hepatic HMG-CoA reductase activity is the probable cause of the reduced carcass cholesterol concentration in PCA rats, because the rate of new synthesis of cholesterol in the liver may not be adequate to satisfy the continuing need in the liver for formation of biliary cholesterol and bile acids (33). However, the present studies do not rule out the possibility that reduction in extrahepatic cholesterol synthesis may also contribute to the lower carcass cholesterol contents of PCA rats.

Finally, these studies support the observation by Starzl *et al.* (3) in dogs that diversion of portal blood past the liver results in decreased hepatic cholesterol synthesis. The present studies have extended this observation by demonstrating in PCA rats that decreased hepatic cholesterol synthesis leads to diminished tissue accumulation of cholesterol during growth. This was revealed by cholesterol pool size measurements combined with sterol balance data.

We gratefully acknowledge the excellent technical assistance of Jeffrey C. Strachan. This work was supported in part by U.S. Public Health Service Grants HL 06222 from the National Heart, Lung, and

Table 4. HMG-CoA reductase activities* in control and PCA rats (5 weeks postoperatively)

	Control rats	PCA rats	P
Kinetics:†			
K_m (μM D-HMG-CoA)			
Test 1	4.03	2.75	—
Test 2	3.92	3.40	—
V_{max} (pmol/min/mg)			
Test 1	39.65	8.92	—
Test 2	29.85	10.51	—
Diurnal rhythm:‡			
Mid-light cycle			
nmol/min/mg	0.023 ± 0.014 (10)	0.010 ± 0.002 (13)	<0.01
nmol/min/liver	2.975 ± 1.997 (10)	0.809 ± 0.205 (13)	<0.01
Mid-dark cycle			
nmol/min/mg	0.105 ± 0.032 (6)	0.057 ± 0.033 (8)	<0.02
nmol/min/liver	21.263 ± 9.524 (6)	5.361 ± 3.587 (8)	<0.001

* Enzyme specific activities are expressed per mg of microsomal protein.
† Kinetic constants were determined from Lineweaver–Burk plots by using the procedure of Langdon and Counsell (25).
‡ Values represent mean ± 1 SD; number of samples is shown in parentheses.

Blood Institute, FR-00102 from the General Clinical Research Centers Branch, Division of Research Resources, and General Research Support Grant 2160-191 from the National Institutes of Health, by the Minot M. Shaw Memorial Fund (2160-558) and the Brian Piccolo Fund (1160-015), by a Grant-in-Aid from the National Dairy Council, by grants from the Herman Goldman Foundation and Weight Watchers Foundation, Inc., and by a Career Scientist Award to D.J.M. from the Irma T. Hirschl Charitable Trust.

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