The effect of decreased plasma cholesterol concentration on circulating mevalonate metabolism in rats

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Abstract Circulating mevalonate is metabolized by two mechanisms: the sterol pathway leading to cholesterol and the shunt pathway resulting in CO_2 production. The kidney is the chief site of circulating mevalonate metabolism by both pathways. The present study investigated the effect of plasma cholesterol concentration on circulating mevalonate metabolism. 4-Aminopyrazolo(3,4-*d*)pyrimidine and Triton WR 1339 were utilized to induce "functional hypocholesterolemia". An enhancement of both renal total nonsaponifiable lipid synthesis (36–43%) and cholesterol synthesis (42%) from circulating mevalonate was observed when "functional hypocholesterolemia" was induced by either compound. Hepatic total nonsaponifiable lipid synthesis from circulating mevalonate was not enhanced in the Triton-treated animals, but 4-aminopyrazolo(3,4-*d*)-pyrimidine treatment increased accumulation of total labeled nonsaponifiable lipids and cholesterol. No increase in labeled total nonsaponifiable lipids or cholesterol in the carcass was observed after treatment with either compound. "Functional hypocholesterolemia" reduced the shunt pathway of circulating mevalonate metabolism by approximately 30%. This reduction occurred in both the renal and extrarenal shunt pathways. These data indicate that plasma cholesterol concentration regulates the in vivo metabolism of circulating mevalonate in that hypocholesterolemia reduces the shunt pathway and stimulates sterologenesis, an effect chiefly localized to the kidneys.—**Feingold, K. R., M. H. Wiley, G. MacRae, and M. D. Siperstein.** The effect of decreased plasma cholesterol concentration on circulating mevalonate metabolism in rats. *J. Lipid Res.* 1981. **22:** 990–997.

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Mevalonate, an essential intermediate in cholesterogenesis, is synthesized from β-hydroxy-β-methylglutaryl (HMG) CoA by a reaction catalyzed by the enzyme, HMG CoA reductase. Inasmuch as the primary feedback control of cholesterogenesis in both hepatic (1–4) and extrahepatic tissues (5) is located at the site of mevalonate synthesis, the metabolic fate of this compound has been studied extensively. Circulating mevalonate is metabolized chiefly by two mechanisms: the sterol pathway, which ultimately leads to cholesterol, and the shunt pathway, which diverts mevalonate away from cholesterol and results in the production of CO_2 (6–12). A quantitatively minor portion of the mevalonate is metabolized to isoprene compounds, such as dolichols, ubiquinone, and isopentenyl adenine (13–15).

This and other laboratories have demonstrated that the kidneys are the chief site of circulating mevalonate metabolism by both the sterol and shunt pathways (9, 12, 16). Other studies have revealed sex differences in the metabolism of circulating mevalonate. Female rats oxidize circulating mevalonate by the shunt pathway at twice the rate of males; male rats, however, convert significantly more circulating mevalonate to total nonsaponifiable lipids. Nephrectomy completely abolishes the sex difference in the oxidation of circulating mevalonate to CO_2. Further studies have demonstrated that the sex differences in circulating mevalonate metabolism are mediated by sex steroid hormones (17). Recently, a sex difference in the human shunt pathway of circulating mevalonate metabolism, similar to that in rats, has been demonstrated; premenopausal women oxidize 68% more circulating mevalonate to CO_2 than age-matched males (18).

In addition to the alterations mediated by sex hormones, insulin and thyroxine also profoundly affect circulating mevalonate metabolism. Insulin deficiency in rats affects both pathways of circulating mevalonate metabolism: it increases sterol synthesis and decreases mevalonate oxidation to CO_2 (19). Thyroxine primarily alters the renal conversion of

Abbreviation: 4-APP, 4-aminopyrazolo(3,4-*d*)pyrimidine.

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circulating mevalonate to cholesterol; hypothyroidism induces a decrease and hyperthyroidism an increase in cholesterogenesis (20). Thus, polypeptide and thyroid as well as steroid hormones modulate the metabolism of circulating mevalonate.

Recently, it has been demonstrated that de novo extrahepatic cholesterol synthesis is markedly influenced by plasma cholesterol levels. In guinea pigs, the infusion of lipoproteins resulted in an inhibition of hepatic and extrahepatic sterol synthesis (21). In rats, a reduction in plasma cholesterol concentration enhanced the activity of HMG CoA reductase and simultaneously increased de novo cholesterogenesis (5, 22–24). In the present study we examined the effect of alterations in plasma cholesterol concentrations on the metabolism of circulating mevalonate. Plasma cholesterol levels were altered by using either 4-aminopyrazolo(3,4-*d*)pyrimidine (4-APP) or Triton WR 1339. 4-APP causes a profound and prompt decrease in lipoprotein secretion by the liver and therefore a marked lowering of plasma cholesterol concentration (25–27). This decrease in plasma cholesterol concentration results from the reduction in the cholesterol carried by both the low density lipoprotein and high density lipoprotein fractions (24). In addition, the rapidity of this decrease in plasma cholesterol levels is dependent on the dose of 4-APP administered. For example, 24 hr after the administration of 4-APP (20 mg/kg), plasma cholesterol concentrations are 50% less than those observed after 4-APP treatment at a dose of 10 mg/kg (24). Triton WR 1339, a nonionic detergent, is thought to "trap" triglycerides and cholesterol in the plasma. This trapping of cholesterol in the vascular compartment induces a state of "functional hypocholesterolemia"[2] because the circulating cholesterol is unavailable for utilization by tissues (28–32). Both of these compounds have been shown by others to stimulate de novo extrahepatic sterologenesis (5, 22–24).

METHODS

Materials

R,S-[5-^{14}C]mevalonate (15 mCi/nmol) was purchased from Research Products, Inc., and the [1α-2α-^{3}H]cholesterol (31 Ci/nmol) used as an internal standard was purchased from New England Nuclear. Thin-layer Polygram Sil G plates were purchased from Brinkmann Instruments. RP-1 radioautography film was purchased from Kodak; Triton WR 1339 was purchased from Ruger Chemical Co., Inc. (Irvington, NJ), and 4-APP from Sigma Chemical Co. Female Sprague-Dawley rats were obtained from Simonson Animal Vendors. The counting solution used for $^{14}CO_2$ samples contained 300 ml of Beckman Bio-Solv III, 1,000 ml of Packard scintillation-grade toluene, 100 ml of glass-distilled water, and 6.0 g of 2,5-diphenyloxazole (PPO) (Amersham/Searle). The thin-layer strips were counted in a solution containing 0.133 g of 1,4-bis(2-(phenyloxazolyl))benzene (Amersham/Searle), 9.33 g of PPO, 1,333 ml of scintillation-grade toluene, and 666 ml of Triton X-100 (Beckman Instruments).

Procedures

For 3 consecutive days, the rats (200 g) were injected intraperitoneally with either 4-APP dissolved in a 0.9% NaCl–25 mM phosphate buffer (pH 4) or saline-phosphate buffer alone. In other experiments the rats were injected intraperitoneally with 100 mg/100 g of Triton WR 1339 in 0.9% NaCl at 24, 16, and 1 hr before study; control animals were injected with saline alone. All groups of animals were fed Simonson Rat Chow ad libitum and maintained on a reverse 12-hr light cycle. Beginning with drug administration, all animals were fasted and allowed free access to drinking water containing 5% glucose and 0.45% NaCl. Nephrectomies were performed immediately before study.

On the day of study, between 8:00 and 9:00 AM, the rats were anesthetized with diethyl ether and injected, via the tail vein, with 5 μCi (1.05 μmol) of potassium *R,S*-[5-^{14}C]mevalonate in 0.4 ml of 0.9% saline solution. The animls were placed in 2-liter widemouth Erlenmeyer flasks fitted with 2-hole stoppers. Air was drawn through the bottles at the rate of 2 liters/min, and $^{14}CO_2$ was collected continuously in gas-washing bottles containing 180 ml of 1 N NaOH. To ensure complete trapping of CO_2, a second gas-washing bottle also containing 180 ml of 1 N NaOH was connected in tandem with the primary collecting bottle. At 6 hr, 0.2-ml samples of the 1 N NaOH were added to 10 ml of scintillation counting solution, and the amount of $^{14}CO_2$ was determined on a Beckman LS-330 scintillation counter. At the time indicated the animals were killed and weighed; the livers and kidneys were saponified by refluxing overnight in approximately 50 ml of a solution of 90% KOH–water–70% ethyl alcohol 1:2:5. The remaining carcass was saponified in approximately 600 ml of the KOH ethanol solution. The flasks containing the livers and kidneys were cooled and an internal standard of [^{3}H]cholesterol was added before extraction of the nonsaponifiable material three times with 25 ml of

[2] The effect of Triton WR 1339 administration on HDL transport is unknown and it is therefore possible that HDL cholesterol transport to and from cells is not inhibited.

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TABLE 1. Plasma cholesterol concentrations

Group	Amount
	mg/dl ± SE
Control (N = 7)	55 ± 6.7
10 mg 4-APP (N = 4)	<10
20 mg 4-APP (N = 4)	<10
Triton (N = 6)	313 ± 35

petroleum ether. The flasks containing the carcasses were thoroughly mixed, the volume was measured, and 50-ml aliquots were transferred to smaller flasks. The internal standard was added to these flasks and the nonsaponifiable material was extracted three times with 25 ml of petroleum ether. The petroleum ether extract was dried and dissolved in 1 ml of chloroform, and 100 μl was applied to thin-layer chromatographic plates. The plates were developed in ethyl acetate–benzene 1:5 for 50 min, radioautographed, and then the areas corresponding to standards of cholesterol, lanosterol, and squalene were cut out and placed in scintillation vials containing 10 ml of counting solution. The determination of total nonsaponifiable lipids was derived from the sum of the cholesterol, lanosterol, and squalene bands obtained from the thin-layer plates. The gain and discriminator settings of the scintillation counter were adjusted so that less than 0.2% of the ^{3}H counts were recorded in the ^{14}C window and approximately 10% of the ^{14}C counts were recorded in the ^{3}H window. The amount of [^{3}H]cholesterol added as an internal standard was adjusted so that ^{3}H counts were approximately five times greater than the ^{14}C counts. Calculations were corrected for spillover of ^{3}H and ^{14}C and for background. Because only the *R* isomer of mevalonate is metabolized, all calculations assume that half the administered mevalonate is inactive.

Determination of plasma cholesterol

Plasma cholesterol levels were determined by the method of Ham (33). Briefly, 0.1 ml of plasma was saponified at 37°C for 1½ hr in a KOH–ethyl alcohol solution and then extracted with petroleum ether. The petroleum ether was evaporated and 2 ml of a solution consisting of 80 g of *p*-toluenesulfonic acid, made up to 1 liter with glacial acetic acid, and 100 ml of H_2SO_4 was added to the tube. The tube was incubated in a boiling water bath for 5 min, then cooled; absorption was determined at 475 nm with a Beckman Model 24 Spectrophotometer.

RESULTS

The plasma cholesterol concentrations of the four experimental groups of animals are shown in **Table 1.** In all rats administered either 10 mg/kg or 20 mg/kg of 4-APP for 3 days, plasma cholesterol levels were reduced to below 10 mg/dl, an effect similar to that observed by other investigators (5, 22–24). The administration of 20 mg/kg of 4-APP for 3 days did not significantly alter renal function (Control: BUN 16 ± 2, creatinine 0.3 ± 0.1; 4-APP:BUN 21 ± 2 mg/dl, creatinine 0.5 ± 0.1 mg/dl, $P > 0.1, P > 0.1$).

Table 2 shows the effects of 3 days' administration of either 10 mg or 20 mg/kg 4-APP on circulating mevalonate metabolism by both the sterol and shunt pathways. In the kidneys, mevalonate incorporation into total nonsaponifiable lipids is increased 21% in the 10 mg/kg-treated and 43% in the 20 mg/kg-treated 4-APP rats. This difference between the 4-APP-treated animals and controls is statistically significant (control 136 ± 7; 10 mg/kg 4-APP, 165 ± 9, $P < 0.05$; control, 136 ± 7; 20 mg/kg 4-APP, 195 ± 9 nmol of mevalonate incorporated into total nonsaponifiable lipids in

TABLE 2. Effects of 4-APP on circulating mevalonate metabolism

	Body Weight	Kidney Weight	Liver Weight	$^{14}CO_2$	Total Nonsaponifiable Lipids		
					Kidney	Liver	Carcass
	g	*g*	*g*		*nmol mevalonate metabolized in 6 h*		
Control (N = 8)	185 ± 4	1.67 ± 0.05	5.82 ± 0.19	106 ± 8	136 ± 7	21.2 ± 1.8	56 ± 6 (N = 4)
4-APP (10 mg) (N = 4)	183 ± 3	1.66 ± 0.07	7.50 ± 0.31[a]	96 ± 4	165 ± 9[b]	31.0 ± 1.8[c]	N/A
4-APP (20 mg) (N = 4)	186 ± 3	1.76 ± 0.06	9.43 ± 0.32[a,d]	78 ± 6[b,e]	195 ± 9[a,f]	44.8 ± 1.8[a,d]	36 ± 2[g]

[a] Difference between control $P < 0.001$.
[b] Difference between control $P < 0.05$.
[c] Difference between control $P < 0.01$.
[d] Difference between 10 mg 4-APP $P < 0.01$.
[e] Difference between 10 mg 4-APP $P < 0.05$.
[f] Difference between 10 mg 4-APP $P < 0.10$.
[g] Difference between control $P < 0.02$.

Control females and females administered 4-APP for 3 days were injected intravenously with 5 μCi 1.05 μmol of [5-^{14}C]mevalonate. The $^{14}CO_2$ expired was trapped in 1 N NaOH and at 6 hr the animals were killed. The organs and carcass were weighed and then separately saponified in a KOH–ethanol solution. ^{14}C-Labeled total nonsaponifiable lipids were assayed after extraction with petroleum ether and thin-layer chromatography. The results are the mean ± SE.

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6 hr, $P < 0.001$.) Furthermore, the higher dose of 4-APP, demonstrated by others to cause a more rapid reduction in plasma cholesterol concentrations (24), induced a greater increase in total nonsaponifiable lipid synthesis from mevalonate (20 mg/kg 4-APP, 195 ± 9; 10 mg/kg 4-APP, 165 ± 9 nmol, $P < 0.10$). These data demonstrate that a lowering of plasma cholesterol concentration induces an increased renal synthesis of total nonsaponifiable lipids from circulating mevalonate, and suggest that the longer the duration of reduced plasma cholesterol concentration the greater the enhancement of renal sterol synthesis.

In the liver, 4-APP administration resulted in an increased accumulation of labeled total nonsaponifiable lipids (control, 21.2 ± 1.8; 10 mg/kg 4-APP, 31.0 ± 1.8, $P < 0.001$; control, 21.2 ± 1.8; 20 mg/kg 4-APP, 44.8 ± 1.8 nmol, $P < 0.001$). In the carcass, the labeled total nonsaponifiable lipid is reduced in the 4-APP-treated rats (control, 56 ± 6; 20 mg/kg 4-APP, 36 ± 2, $P < 0.02$).

Because of the possible influence of either transport or metabolism of the newly synthesized sterols during the 6-hr time period studied, liver and carcass nonsaponifiable lipid content was analyzed 1 hr after the administration of labeled mevalonate. In rats administered 20 mg/kg 4-APP for 3 days, hepatic conversion of circulating mevalonate to total nonsaponifiable lipids is 96% greater than in controls. This 1-hr value is very similar to the percentage increase in hepatic total nonsaponifiable lipid synthesis observed in 4-APP animals at 6 hr (111%) and suggests that 4-APP administration stimulates hepatic sterologenesis from circulating mevalonate. In the carcass the incorporation of circulating mevalonate into total nonsaponifiable lipids in the 4-APP-treated animals is reduced 22% at 1 hr while at 6 hr it was decreased 36%. It is apparent that in contrast to the effect on the liver and kidneys, 4-APP administration inhibits carcass sterologenesis. This 1-hr study demonstrates that neither the transport nor metabolism of newly synthesized sterols accounts for the alterations in sterologenesis observed in the 4-APP-treated animals.

The shunt pathway of circulating mevalonate metabolism, as reflected by $^{14}CO_2$ production, is unchanged by 10 mg/kg of 4-APP administration. However, 20 mg/kg of 4-APP for 3 days reduced shunt activity by 27% (control, 106 ± 8; 20 mg/kg 4-APP, 78 ± 6; $P < 0.05$). As noted above, the difference in rapidity of induction of hypocholesterolemia described by others possibly explains this discrepancy. Perhaps the duration of hypocholesterolemia in the 10 mg/kg-treated animals was not sufficient to reduce the shunt pathway of circulating mevalonate metabolism.

TABLE 3. Effects of 4-APP on circulating mevalonate metabolism

	Control (N = 4)	4-APP (N = 4)	P
	g		
Weight			
Body	207 ± 6	221 ± 7	not significant
Kidney	1.62 ± 0.08	1.69 ± 0.04	not significant
Liver	5.79 ± 0.16	9.76 ± 0.31	<0.001
	nmol		
Mevalonate metabolized in 6 hr			
CO_2	266 ± 19	190 ± 18	<0.05
Kidney			
Cholesterol	181 ± 13	257 ± 7	<0.01
Total NS[a] lipids	352 ± 23	477 ± 17	<0.01
Liver			
Cholesterol	29 ± 1.6	55 ± 10	<0.05
Total NS lipids	38 ± 3	87 ± 10	<0.01
Carcass			
Cholesterol	94 ± 7	53 ± 2	<0.01
Total NS lipids	137 ± 10	99 ± 5	<0.02

[a] NS, nonsaponifiable.

Control females and females administered 20 mg/kg of 4-APP for 3 days were injected intravenously with 5 μCi (2.10 μmol) of [5-^{14}C]mevalonate.

To confirm these changes in circulating mevalonate metabolism induced by 20 mg/kg of 4-APP administration, an additional group of animals was administered twice the dose of mevalonate (2,000 nmol) as substrate. As shown in **Table 3**, the conversion of circulating mevalonate to total nonsaponifiable lipids was again increased in renal tissue (control 352 ± 23; 20 mg/kg 4-APP, 477 ± 17, $P < 0.01$). Additionally, the incorporation of circulating mevalonate into cholesterol in the kidneys was also stimulated in the animals treated with 4-APP (control, 181 ± 13; 4-APP, 257 ± 7, $P < 0.01$). The magnitude of the percentage increase over control of total nonsaponifiable lipid and cholesterol synthesis in the kidneys was similar (total nonsaponifiable lipids, 36% increase; cholesterol, 42% increase). These data confirm the earlier results that demonstrated an increase in total nonsaponifiable lipid synthesis in renal tissue of animals with 4-APP-induced hypocholesterolemia, and further demonstrate that cholesterol synthesis from circulating mevalonate is similarly increased. In the liver, the increased accumulation of labeled total nonsaponifiable lipids was again observed in the 4-APP-treated animals. In the 4-APP-treated liver, as noted for the kidneys, the increase of labeled cholesterol paralleled the increase in total nonsaponifiable lipids. Both [^{14}C]cholesterol and total nonsaponifiable lipids were reduced in the carcass of the 4-APP-treated animals and it is evident that 4-APP-induced hypocholes-

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terolemia in contrast to the liver and kidney does not stimulate carcass sterologenesis from circulating mevalonate.

The shunt pathway of circulating mevalonate metabolism was reduced 29% in the 4-APP-treated animals (control, 266 ± 19; 4-APP, 190 ± 18; $P < 0.05$). These results, derived from using twice the concentration of mevalonate, confirm that a reduction in plasma cholesterol concentration induces an increase in renal sterologenesis from circulating mevalonate and a decrease in the shunt pathway.

The majority of circulating mevalonate is metabolized in the kidneys by either the sterol or shunt pathway (9, 12, 16); thus, it is possible, despite the large excess of mevalonate used in these studies, that alterations in circulating mevalonate metabolism by peripheral tissue could be masked because of limited available substrate. To investigate this possibility, we studied circulating mevalonate metabolism in control and 4-APP-treated animals immediately after nephrectomy (**Table 4**). In the control rats, nephrectomy caused major changes in circulating mevalonate metabolism, similar to those reported previously by this laboratory (12). In the nephrectomized controls, the shunt pathway was reduced by two-thirds whereas nonsaponifiable lipid accumulation from mevalonate in both the liver and carcass was markedly increased. In nephrectomized 4-APP-treated animals, a similar marked reduction in the shunt pathway was observed, but the remaining extrarenal shunt pathway of circulating mevalonate metabolism was still less than in controls (controls, 31.5 ± 1.7; 4-APP, 20.2 ± 1.1; $P < 0.01$). The magnitude of the difference in the shunt pathway activity between control and 4-APP-treated animals was reduced after nephrectomy (before, 28 nmol; after, 11 nmol). This suggests that the shunt pathway in both renal and extrarenal tissue is decreased by hypocholesterolemia. In the liver, the accumulation of ^{14}C-labeled total nonsaponifiable lipids was slightly greater in the 4-APP-nephrectomized animals, but this difference was not significant. Carcass sterologenesis in nephrectomized 4-APP-treated animals was much less than in the nephrectomized controls (control, 188 ± 8; 4-APP, 128 ± 6 nmol; $P < 0.001$). This demonstrates that even under optimal conditions, 4-APP-induced hypocholesterolemia does not induce an increase in carcass sterol synthesis from circulating mevalonate.

As shown in Table 1, the Triton WR 1339-treated animals had grossly lipemic sera and markedly elevated plasma cholesterol concentrations (Triton WR 1339, 313 mg/dl ± 35; control 55 mg/dl ± 6.7), which indicates the trapping of lipoproteins within the vascular compartment. The effects of Triton WR 1339 administration on circulating mevalonate metabolism are shown in **Table 5.** Renal incorporation of circulating mevalonate into total nonsaponifiable lipids increased 40% (control, 102 ± 2; Triton, 143 ± 8; $P < 0.01$), a value similar to that obtained after 4-APP treatment. This Triton-induced stimulation of renal sterologenesis from circulating mevalonate persists even when the data are presented per gram of kidney (control, 61.1 ± 1.9 nmol/g; 4-APP, 75.6 ± 3.9 nmol/g; $P < 0.02$). In the liver, the accumulation of labeled total nonsaponifiable lipids was slightly reduced (control, 14.6 ± 1.2; Triton, 9.7 ± 0.9; $P < 0.01$). In the carcass, sterologenesis from circulating mevalonate was similar in controls and Triton-treated animals (control, 36.5 ± 3.7; Triton, 39.7 ± 1.6; $P > 0.10$). The shunt pathway was reduced 31% in the Triton-treated animals (control, 89 ± 4; Triton, 61 ± 6; $P < 0.01$). Using Triton WR 1339, an agent that induces "functional hypocholesterolemia" by an entirely different mechanism than 4-APP, we have observed similar alterations in renal metabolism of circulating mevalonate; the shunt pathway is reduced and the sterol pathway is stimulated in the kidneys.

DISCUSSION

Circulating mevalonate is metabolized primarily by two mechanisms: the sterol pathway, which ultimately leads to isoprene compounds and cholesterol, and the

TABLE 4. Circulating mevalonate metabolism in nephrectomized animals

	Weight			Total Nonsaponifiable Lipids	
	Body	Liver	CO_2	Liver	Carcass
	g		*nmol mevalonate metabolized in 6 hr*		
Controls (N = 7)	177 ± 4	5.23 ± 0.23	31.5 ± 1.7	98 ± 5	188 ± 8
4-APP (N = 5)	191 ± 4	6.83 ± 0.32	20.2 ± 1.1	116 ± 11	128 ± 6
	$P < 0.05$	$P < 0.01$	$P < 0.01$	NS	$P < 0.001$

Control females and females administered 20 mg/kg of 4-APP for 3 days were nephrectomized and then injected intravenously with 5 μCi (1.05 μmol) of [5-^{14}C]mevalonate. The results are the mean ± SE.

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TABLE 5. Effects of Triton WR 1339 on circulating mevalonate metabolism

	Body Weight	Kidney Weight	Liver Weight	$^{14}CO_2$	Total Nonsaponifiable Lipids		
					Kidney	Liver	Carcass
		g			*nmol mevalonate metabolized in 6 hr*		
Control (N = 4)	173 ± 5	1.68 ± 0.08	5.30 ± 0.15	89 ± 4	102 ± 2	14.6 ± 1.2	36.5 ± 3.7
Triton (N = 6)	178 ± 3	1.89 ± 0.06	6.44 ± 0.12	61 ± 6	143 ± 8	9.7 ± 0.9	39.7 ± 1.6
	NS	$P < 0.1$	$P < 0.001$	$P < 0.01$	$P < 0.01$	$P < 0.01$	NS

Control females and females administered 100 mg/100 g of Triton WR 1339 at 24, 16, and 1 hr prior to study were injected intravenously with 5 μCi (1.05 μmol) of [5-^{14}C]mevalonate. The results are the mean ± Se.

shunt pathway, which results in the oxidation of mevalonate to CO_2 (6–12). The kidneys are the chief site of circulating mevalonate metabolism by both pathways (9, 12, 16). Additionally, sex steroid, polypeptide, and thyroid hormones can modulate the metabolism of circulating mevalonate (17, 19, 20).

The present study was initiated to determine if plasma cholesterol concentrations also influence circulating mevalonate metabolism. Recent studies in guinea pigs and rats have demonstrated that de novo extrahepatic cholesterogenesis is regulated by plasma cholesterol levels (5, 21–24). After either 4-APP administration, which profoundly reduces plasma cholesterol concentration by inhibiting lipoprotein secretion by the liver (25–27), or Triton WR 1339 treatment, which prevents utilization of lipoproteins by peripheral tissue leading to "functional hypocholesterolemia" (28–32), de novo cholesterogenesis in extrahepatic tissue was markedly enhanced (5, 22–24). Besides stimulating sterologenesis, a reduction of plasma cholesterol concentrations with 4-APP treatment greatly increased HMG CoA reductase activity (5). This stimulation of HMG CoA reductase activity appeared to be related to both the plasma cholesterol concentration and to the duration of hypocholesterolemia (5). These results suggest that the low basal rates of de novo cholesterol synthesis and HMG CoA reductase activity normally seen in the extrahepatic tissue of rats are due to inhibition by plasma cholesterol levels. These observations correlate with those in tissue culture experiments showing receptor-mediated suppression of cholesterol synthesis by lipoproteins (34).

The present study demonstrated that plasma cholesterol concentrations also regulate the metabolism of circulating mevalonate. When "functional plasma cholesterol" concentrations were reduced by administering either 4-APP or Triton WR 1339, total nonsaponifiable lipid and cholesterol synthesis from circulating mevalonate was enhanced. Moreover, this stimulation of sterologenesis from mevalonate induced by hypocholesterolemia was chiefly localized to the kidneys, the major site of circulating mevalonate metabolism. In two separate studies, the kidneys of animals administered 20 mg/kg of 4-APP exhibited a 36% or 43% increase in mevalonate incorporation into total nonsaponifiable lipids. Triton WR 1339-treated animals demonstrated a similar increase; renal incorporation of mevalonate into total nonsaponifiable lipids was 40% greater than in controls. In addition to these increases in total nonsaponifiable lipid synthesis, 4-APP-treatment also enhanced mevalonate incorporation into cholesterol by 42%. These data demonstrate that "functional hypocholesterolemia" stimulates both total nonsaponifiable lipid and cholesterol synthesis from circulating mevalonate in the kidneys.

In animals administered 10 mg/kg 4-APP, renal total nonsaponifiable lipid synthesis from circulating mevalonate increased only 21%. Other investigators have reported that higher doses of 4-APP lead to a more rapid reduction of plasma cholesterol levels, even though the ultimate plasma cholesterol concentrations are similar (24); therefore, it is possible that in the 10 mg/kg 4-APP animals, the decreased enhancement was due to a shorter duration of hypocholesterolemia. This suggests that the duration of hypocholesterolemia may be important in determining the magnitude of enhancement of sterologenesis. It is also possible that the 20 mg/kg dose of 4-APP caused a slightly lower plasma cholesterol concentration than the 10 mg/kg dose and that this lower concentration of plasma cholesterol explains the greater enhancement of sterol synthesis observed in the 20 mg/kg animals.

From the results of our studies, the effect of "functional hypocholesterolemia" on hepatic sterologenesis from circulating mevalonate was uncertain. The accumulation of ^{14}C-labeled total nonsaponifiable lipids was not increased in the livers of Triton WR 1339-treated animals. In the animals administered 4-APP, however, an increased hepatic accumulation of ^{14}C-labeled total nonsaponifiable lipids and cholesterol was observed. Whether this elevation of total nonsaponifiable lipids and cholesterol represents an enhancement of hepatic sterologenesis by hypocholesterolemia or a pharmacologic effect of 4-APP cannot be definitely answered by our data. 4-APP-

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treatment in nephrectomized animals in comparison to nephrectomized controls did not result in an enhancement of hepatic total nonsaponifiable lipid synthesis. This lack of stimulation is probably because nephrectomy alone maximally stimulates hepatic nonsaponifiable lipid synthesis from circulating mevalonate and therefore 4-APP treatment is without a significant additive effect.

No increase in labeled carcass total nonsaponifiable lipids or cholesterol was observed in either the 4-APP- or Triton WR 1339-treated animals. Neither does 4-APP-treatment increase sterologenesis after nephrectomy, a condition which stimulates carcass incorporation of mevalonate into total nonsaponifiable lipids. It is possible, of course, that carcass sterologenesis from mevalonate would have been enhanced if the duration of hypocholesterolemia had been extended. Unfortunately, using either 4-APP or Triton WR 1339 for longer durations is limited by increasing drug toxicity.

The finding that hypocholesterolemia enhances the post-mevalonate steps of sterologenesis in renal tissues of animals in vivo agrees with observations of others on the general regulatory scheme of sterol synthesis. It is well known that cholesterol ingestion inhibits hepatic sterol synthesis and that this inhibition occurs primarily at the conversion of HMG CoA to mevalonate, a reaction catalyzed by HMG CoA reductase (1–4). However, cholesterol intake of extended duration inhibits the post-mevalonate steps of hepatic sterol synthesis as well (35, 36). In tissue culture, the addition of lipoproteins to the media (as noted earlier) causes a rapid reduction in sterologenesis, an effect due primarily to an inhibition of HMG CoA reductase (37). Again, exposing cells to media containing lipoproteins for a longer duration also inhibits the post-mevalonate steps of sterol synthesis (38). In tissue culture the removal of lipoprotein from the media causes a rapid increase in HMG CoA reductase activity (within 10 hr) and a delayed increase (within 30 hr) in the activity of squalene synthetase, an enzyme in the post-mevalonate sterol pathway (39). In conjunction with the data of others showing that a reduction in plasma cholesterol concentration leads to an increase in extrahepatic de novo cholesterogenesis and HMG CoA reductase activity (5, 22, 24), we interpreted our experiments as demonstrating that the post-mevalonate steps of sterologenesis are also stimulated by hypocholesterolemia. This represents the first evidence of the regulation of the post-mevalonate sterol pathway by plasma cholesterol concentrations in intact extrahepatic tissue. Moreover, the data in our experimental model indicated that this regulation occurred chiefly in renal tissue.

Our study also demonstrated that "functional hypocholesterolemia" reduced the shunt pathway of circulating mevalonate metabolism by approximately 30% (27 to 31%). Studies of nephrectomized animals further demonstrated that both the renal and extra-renal shunt pathways were reduced after 4-APP administration. After nephrectomy, as expected, this pathway of mevalonate metabolism was markedly reduced in both control and 4-APP-treated animals. In addition, the shunt pathway was still 36% lower in the nephrectomized 4-APP-treated animals than in the nephrectomized controls, indicating that the extra-renal shunt pathway is decreased by hypocholesterolemia. This difference in the extra-renal shunt pathway of circulating mevalonate metabolism was not sufficient, however, to account for the entire difference observed in intact animals (before nephrectomy, 28 nmol difference; after nephrectomy, 11 nmol difference); rather it indicated that both the renal and extra-renal shunt pathways are reduced by hypocholesterolemia.

It is recognized that throughout this study the doses of mevalonate administered would result in 10 to 40 times the plasma levels of mevalonate that have been shown to be physiologic (9, 40). We have previously demonstrated that these doses of mevalonate accurately portray the relative activity of the shunt and sterol pathways at physiologic concentrations (12).

In summary, these data indicate that plasma cholesterol concentrations regulate the in vivo metabolism of circulating mevalonate in that hypocholesterolemia stimulates renal sterologenesis and reduces the shunt pathway.

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