

# Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation

Ingemar Björkhem,<sup>1,\*</sup> Dieter Lütjohann,<sup>2,\*</sup> Ulf Diczfalusy,<sup>\*</sup> Lars Ståhle,<sup>†</sup> Gunvor Ahlborg,<sup>§</sup> and John Wahren<sup>\*\*</sup>

Divisions of Clinical Chemistry,<sup>\*</sup> Clinical Pharmacology,<sup>†</sup> and Clinical Physiology,<sup>§</sup> Karolinska Institute, Huddinge University Hospital, SE-141 86, Huddinge, Sweden, and Division of Clinical Physiology,<sup>\*\*</sup> Karolinska Institute, Karolinska Hospital, Stockholm, Sweden

**Abstract** We have previously demonstrated that the brain contains about 80% of the 24S-hydroxycholesterol in the human body and that there is a net flux of this steroid from the brain into the circulation (Lütjohann, D. et al. 1996. *Proc. Natl. Acad. Sci. USA.* 93: 9799–9804). Combining previous data with new data on 12 healthy volunteers, the arteriovenous difference between levels of this oxysterol in the internal jugular vein and in a peripheral artery was found to be  $-10.2 \pm 2.8$  ng/ml (mean  $\pm$  SEM) corresponding to a net flux of 24S-hydroxycholesterol from the brain of about 6.4 mg/24 h. The arteriovenous difference between levels of 24S-hydroxycholesterol in the hepatic vein and a peripheral artery of 12 other volunteers was found to be  $7.4 \pm 2.2$  ng/ml, corresponding to a hepatic uptake of about 7.6 mg/24 h. The concentrations of 24S-hydroxycholesterol in the renal vein were about the same as those in a peripheral artery, indicating that a renal elimination is not of importance. Intravenously injected deuterium-labeled racemic 24-hydroxycholesterol was eliminated from the circulation of two human volunteers with half-lives of 10 h and 14 h, respectively. A positive correlation was found between the levels of circulating cholesterol and 24S-hydroxycholesterol. The results are consistent with a cerebral origin of most of the circulating 24S-hydroxycholesterol and suggest that the liver is the major eliminating organ. It is concluded that conversion into 24S-hydroxycholesterol is a quantitatively important mechanism for elimination of cholesterol from human brain. The possibility is discussed that circulating levels of 24S-hydroxycholesterol can be used as a marker for pathological and/or developmental changes in the brain.—Björkhem, I., D. Lütjohann, U. Diczfalusy, L. Ståhle, G. Ahlborg, and J. Wahren. **Cholesterol homeostasis in human brain: turnover of 24S-hydroxycholesterol and evidence for a cerebral origin of most of this oxysterol in the circulation.** *J. Lipid Res.* 1998. 39: 1594–1600.

**Supplementary key words** brain cholesterol • cytochrome P-450 • myelin • isotope dilution-mass spectrometry

In a recent study we showed that about 80% of the content of 24S-hydroxycholesterol in the human body is

present in the brain (1). By measuring the levels of 24S-hydroxycholesterol in serum samples from the internal jugular vein and the brachial artery, a net flux could be demonstrated from the brain into the circulation. Based on measurements in eight subjects, the net flux of 24-hydroxycholesterol into the circulation was calculated to be about 4 mg/24 h. In view of the very low rate of cholesterol synthesis in the brain of adult primates (2), it was suggested that this flux may be of importance for cholesterol homeostasis in the brain. It was also shown that the levels of 24S-hydroxycholesterol in the circulation are markedly age-dependent with levels that were five times higher during the first decade of life than after the second decade. The levels of 24S-hydroxycholesterol in the cerebrospinal fluid, corrected for cholesterol, were higher and varied also with age in parallel with the levels in the circulation (1).

From the above information, it is evident that at least part of the 24S-hydroxycholesterol in the circulation originates from the brain. Using an  $^{18}\text{O}_2$ -inhalation technique it was recently shown that there is a continuous flux of newly synthesized 24S-hydroxycholesterol from rat brain into the circulation and that this flux is of the same magnitude as the rate of synthesis of cholesterol in the brain (3). The results of that study suggested that conversion of cholesterol into 24S-hydroxycholesterol may be the most important mechanism for elimination of excess cholesterol from rat brain. A prerequisite for the efficacy of this mechanism is an efficient transport of 24S-hydroxycholesterol over the blood-brain barrier. It has been shown that side-chain hydroxylated cholesterol species can be transferred in lipophilic membranes at a rate that is orders of magnitude higher than that of cholesterol itself (4).

<sup>1</sup>To whom correspondence should be addressed.

<sup>2</sup>Present address: Department of Clinical Pharmacology, Rheinische Friedrich-Wilhelms Universität, Bonn, Germany.

The microsomal fraction of brain homogenates from cows and rats is able to convert cholesterol into 24S-hydroxycholesterol (3, 5, 6). Very recently we showed that the NADPH-dependent cholesterol 24S-hydroxylase present in the microsomal fraction of rat brain has a capacity that would allow for the observed flux of 24S-hydroxycholesterol from the brain under *in vivo* conditions (3). Significant cholesterol 24S-hydroxylase activity could not be found in any other organs or tissues of rats. Next to the brain the adrenals contain the highest levels of 24S-hydroxycholesterol. It is not known whether human adrenals have a capacity to synthesize 24-hydroxycholesterol or whether this organ can contribute to the circulating levels of 24S-hydroxycholesterol. Purified sterol 27-hydroxylase from pig liver seems to have a very low capacity to synthesize 24S-hydroxycholesterol (7). At present the possibility cannot be completely excluded that the human liver can also produce small amounts of 24S-hydroxycholesterol.

If the brain is a major source of circulating 24S-hydroxycholesterol, this oxysterol could have a potential as a marker for pathological or developmental changes in the brain.

In order to evaluate cerebral production as well as hepatic and renal elimination of 24S-hydroxycholesterol, we have now measured the net fluxes of this steroid across the brain in more subjects and also determined the fluxes through the splanchnic area and the kidneys in healthy subjects. The kinetics for elimination of deuterium-labeled 24-hydroxycholesterol from the circulation has also been defined.

## MATERIAL AND METHODS

### Materials

Tetra-deuterium-labeled racemic 24-hydroxycholesterol used as internal standard and for the *in vivo* experiment (shown in Fig. 4) was synthesized as described previously (8).

### Studies on healthy volunteers

Blood samples for determination of the levels of 24-hydroxycholesterol in the internal jugular vein and brachial artery were collected from 12 healthy male volunteers (aged 20–35 years) in the fasting basal state. Eight of these subjects participated in the previous investigation (1) and the results obtained from them have also been reported (1). The blood samples were taken from catheters inserted percutaneously. A thin Teflon catheter was introduced into the brachial artery and a Cournand catheter no. 7 was introduced into a peripheral vein, with the tip positioned in the internal jugular vein at the level of the orbita. Blood samples collected from the hepatic vein and the brachial artery were also obtained using the same technique from another 12 healthy male volunteers, 21–34 years of age. The samples from the hepatic vein were collected through a Cournand catheter that had been introduced percutaneously into a median antecubital vein and advanced to a right-sided hepatic vein under fluoroscopic control (9). In 6 of these subjects the hepatic plasma flow was estimated by infusion of indocyanine green at a constant rate (9). Blood samples from the renal vein and the brachial artery were obtained by the same procedure from 7 healthy male volunteers,

21–32 years of age. Four of these subjects were the same as those above.

Deuterium-labeled 24-hydroxycholesterol, 200  $\mu\text{g}$  dissolved in ethanol and mixed with human serum albumin and sodium chloride solution (0.9%, w/v), was administered intravenously to a healthy volunteer, 41 years of age, weighing 95 kg. In another experiment 400  $\mu\text{g}$  of the steroid was administered to another healthy volunteer, 56 years of age, weighing 93 kg. Blood samples were taken before and at specific time points after the administration (cf. Fig. 3).

Blood samples for determination of levels of cholesterol and 24S-hydroxycholesterol were also collected from 31 healthy normocholesterolemic volunteers, 14 males and 17 females, 28–57 years of age, mean 37 years.

All experiments involving human volunteers were reviewed and approved by the ethics committees at the Huddinge Hospital and the Karolinska Hospital.

### Analytical methods

Levels of 24-hydroxycholesterol were assayed by isotope dilution–mass spectrometry with the use of deuterium-labeled 24-hydroxycholesterol and the same instrumentation and conditions as described previously (3, 8). The coefficient of variation of this method in the range of concentrations measured was about 4% (8).

Dilution of administered  $^2\text{H}_4$ -labeled racemic 24-hydroxycholesterol was determined by selected monitoring of the ions at  $m/z$  413 and  $m/z$  416 ( $M-90-43$  ion in the mass spectrum of trimethylsilyl ether of unlabeled and deuterium-labeled 24-hydroxycholesterol, respectively). It should be noted that one of the four deuterium atoms in the molecule was lost in the generation of this ion. Under the conditions used with a content of trideuterium-labeled molecules ranging down to 1%, the content of deuterium could be measured with a coefficient of variation of less than 4%. All calculations were performed with use of computer-based measurements of area of the different ion tracings. In some cases cholesterol was also measured in plasma using an isotope dilution method (3).

### Kinetic calculations

Standard methods for linear regression and pharmacokinetic methods were used to calculate the half-life of 24-hydroxycholesterol (10).

## RESULTS

**Figure 1** shows the results of previous (1) and present measurements of 24S-hydroxycholesterol in the internal jugular vein and a peripheral artery. This is an expansion of the previous study and data from eight of the 12 subjects have thus been presented previously (1). Eleven of the 12 volunteers had higher levels of the oxysterol in the internal jugular vein than in the peripheral artery. One of the subjects had the same concentration of 24S-hydroxycholesterol in the two vessels. The average arteriovenous difference between the levels of the oxysterol in the two vessels was found to be  $-10.2 \pm 2.8$  ng/ml (mean  $\pm$  SEM). The results are consistent with a net flux of 24S-hydroxycholesterol from the brain into the circulation ( $P = 0.004$ , two-tailed paired *t*-test). This flux was estimated to be  $6.4 \pm 1.8$  mg/24 h, assuming a constant cerebral plasma flow of 450 ml/min (11).

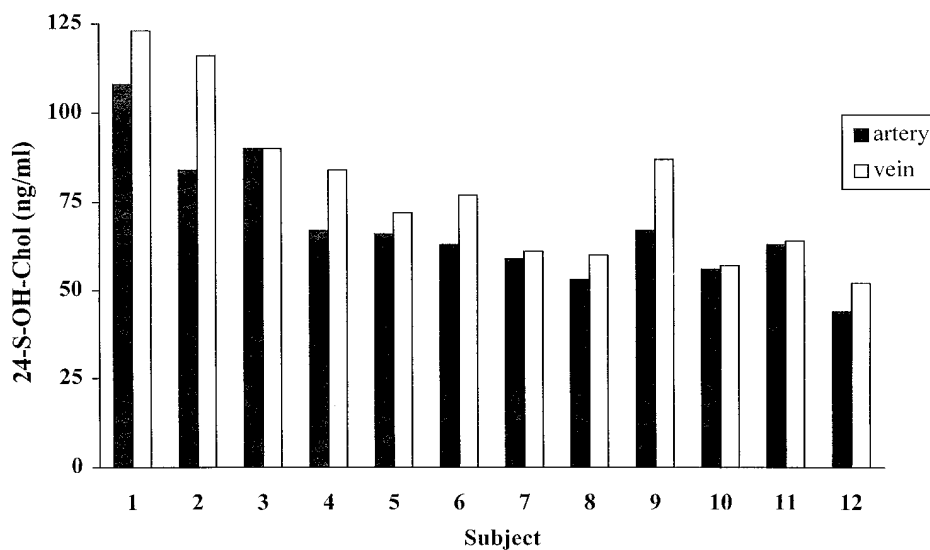


Fig. 1. Arterial-venous concentration difference in levels of 24S-hydroxycholesterol. Filled bars, arterial concentrations; open bars, venous concentrations. Data from eight of the patients have been presented previously in ref. 1.

Figure 2 and Table 1 show the results of the measurements of 24S-hydroxycholesterol in the hepatic vein and in a peripheral artery. With three exceptions, there were higher levels of 24S-hydroxycholesterol in the peripheral artery than in the hepatic vein, demonstrating a net uptake of the oxysterol in the liver. In two of the three exceptions, the levels of 24S-hydroxycholesterol in the hepatic vein were almost identical to those in the peripheral artery. The arterio-venous difference was significant from a statistical point of view ( $P = 0.006$ , two-tailed paired  $t$ -test) and was found to be  $7.4 \pm 2.2$  ng/ml (mean  $\pm$  SEM). The hepatic plasma flow was measured in six of the subjects and found to be  $0.69 \pm$

$0.04$  L/min. Using this figure for plasma flow, the net uptake of 24S-hydroxycholesterol in the splanchnic area was estimated to be  $7.6 \pm 2.2$  mg/24 h, which is similar to, and not significantly different from, the net flux of 24S-hydroxycholesterol from the brain into the circulation.

In seven subjects the plasma level of 24S-hydroxycholesterol was measured in the renal vein and in a peripheral artery. Of the seven subjects studied, four had higher levels of the oxysterol in the renal vein and three had higher levels in the peripheral artery. The arterio-venous difference,  $-3 \pm 4$  ng/ml, was not significantly different from zero.

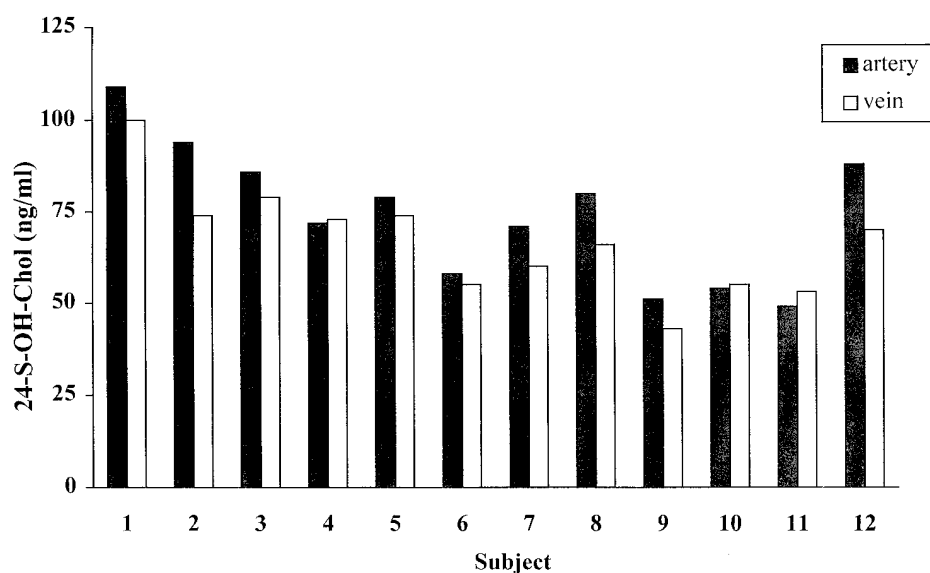


Fig. 2. Arterial hepatic venous concentration difference in levels of 24S-hydroxycholesterol. Filled bars, arterial concentrations; open bars, venous concentrations.

TABLE 1. Arterial-venous difference (A-V) and uptake of 24S-hydroxycholesterol in the splanchnic region

Subject No.	Concentration in		A-V Difference	Plasma Flow	Uptake in Splanchnic Region <sup>a</sup>
	Artery	Vein			
	ng/ml		ng/ml	L/min	mg/24 h
1	49	53	-4	nd	-4.0
2	51	43	+8	0.677	7.80
3	54	55	-1	0.558	-0.8
4	88	70	+18	0.767	19.9
5	79	74	+5	0.636	4.6
6	58	55	+3	0.845	3.7
7	71	60	+11	0.669	10.6
8	80	66	+14	nd	13.9
9	86	79	+7	nd	7.0
10	94	74	+20	nd	19.9
11	109	100	+9	nd	9.0
12	72	73	-1	nd	-1
Mean ± SEM	74.3 ± 5.3	66.8 ± 4.3	7.4 ± 2.2	0.692 ± 0.041	7.6 ± 2.2

<sup>a</sup>The uptake was calculated from the hepatic plasma flow measured and the concentration difference. In cases where the hepatic plasma flow was not measured, it was assumed to be 0.692 L/min; nd, not determined.

Using the extracerebral body content of 24S-hydroxycholesterol (4.5 mg), estimated from measurements of this oxysterol in different organs and tissues obtained at autopsy (1), and the arterial concentration of the compound (74 ng/ml), the volume of distribution could be calculated to be 60.6 L. The hepatic clearance is 4.14 L/h. From these data, the terminal half-life of intravenously administered 24-hydroxycholesterol could be expected to be 609 min (10).

In order to study the elimination of 24S-hydroxycholesterol from the circulation, deuterium-labeled 24-hydroxycholesterol was administered intravenously to a healthy volunteer. The basal plasma levels of 24S-hydroxycholesterol and cholesterol in this subject were 77 ng/ml and 1.95 mg/ml, respectively. The material injected was a racemic mixture of 24S- and 24R-hydroxycholesterol and the amount of <sup>2</sup>H<sub>4</sub>-labeled 24S-hydroxycholesterol administered containing 3 atoms of deuterium in the fragment (M-90-43) was calculated to be 200 μg. As shown in Fig.

3, there was a rapid decline in atoms percent excess deuterium during the first hour, reflecting the distribution phase with exchange with tissue 24-hydroxycholesterol. The dilution of the deuterium-labeled 24S-hydroxycholesterol followed first order kinetics between 2 and 8 h after the administration. The terminal half-life was calculated to be 603 min. Due to incomplete separation between the two isomers of 24-hydroxycholesterol, it was not possible to evaluate whether there were differences between the two stereoisomers with respect to the rate of elimination.

In another experiment with another volunteer in which the administered amount of deuterium-labeled 24-hydroxycholesterol was 400 μg, the terminal half-life was calculated to be 840 min (data not shown).

From the degree of dilution of the administered 24-hydroxycholesterol, the pool of 24S-hydroxycholesterol in equilibrium with the administered material was calculated to be about 9 mg and 10 mg, respectively. From the measured half-lives, the rate of elimination of 24-hydroxycho-

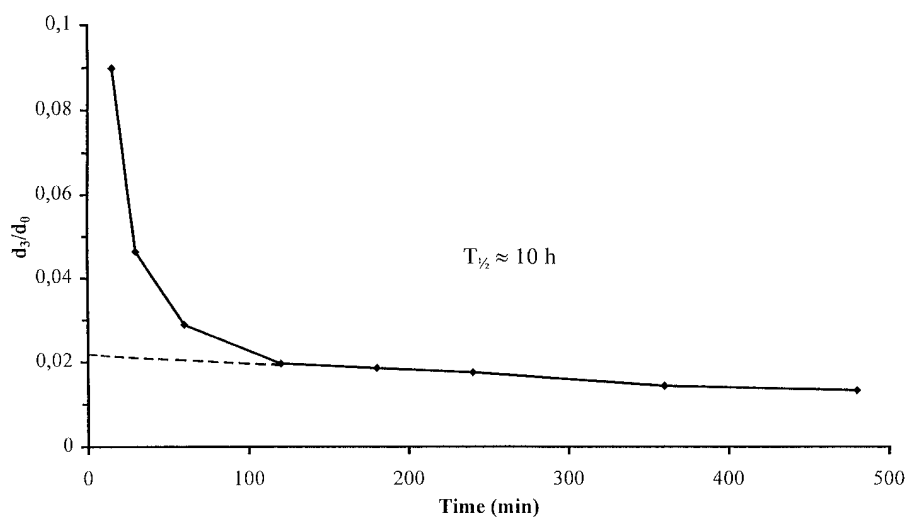
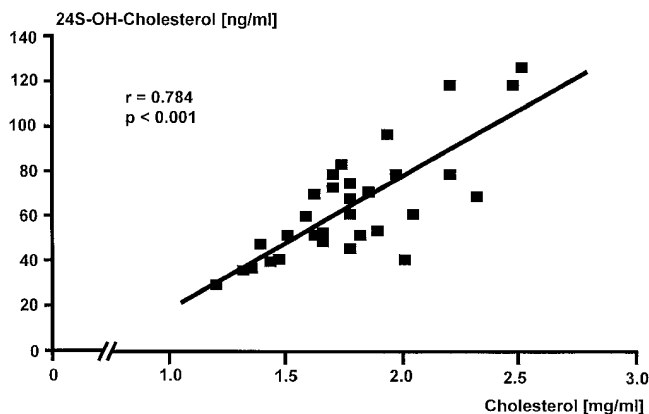


Fig. 3. Kinetics of racemic deuterated 24-hydroxycholesterol in human circulation.



**Fig. 4.** Correlation between cholesterol and 24S-hydroxycholesterol in the plasma of healthy normocholesterolemic volunteers.

lesterol in the two subjects studied was estimated to be about 11 mg/24 h and 9 mg/24 h, respectively. This is of the same magnitude as the uptake of 24S-hydroxycholesterol measured in the splanchnic region ( $7 \pm 2$  mg/24 h, cf. above).

**Figure 4** shows that there is a positive correlation between levels of 24S-hydroxycholesterol and cholesterol in the circulation of healthy normocholesterolemic volunteers ( $r = 0.78$ ,  $P < 0.001$ ). In accordance with previous work (8), no gender difference was seen.

## DISCUSSION

The following results obtained here are consistent with a cerebral origin of most of the 24S-hydroxycholesterol present in human circulation: 1) a net flux of 24S-hydroxycholesterol from the human brain into the circulation; 2) a net uptake of 24S-hydroxycholesterol in the splanchnic region of the same magnitude as the flux from the brain; 3) apparent absence of a renal elimination of 24S-hydroxycholesterol; and 4) a terminal half-life of a single dose of deuterium-labeled 24-hydroxycholesterol that is consistent with data on whole-body content of 24S-hydroxycholesterol, its steady state plasma level, and the hepatic clearance.

### Flux of 24S-hydroxycholesterol from the brain

The net flux of 24S-hydroxycholesterol from the brain into the circulation, based on measurements in 12 healthy volunteers, was estimated to be  $6.4 \pm 1.8$  mg/24 h. In the previous work based on measurements in 8 of the volunteers, the net flux was calculated to be about 4 mg/24 h (1). The cerebral plasma flow was never measured and the calculation is based on the assumption that the cerebral plasma flow is about 0.45 ml/min (11).

The difference between the present result (about 6 mg/24 h) and that obtained in the previous work (about 4 mg/24 h) may be due to a combination of the analytical imprecision and the larger number of subjects used here. It should be emphasized that the mean arteriovenous difference was only about 10% and the analytical coefficient

of variation was 4%. Under such conditions a relatively large variation can be predicted and measurements on relatively many subjects are necessary.

It has been calculated (12) that a transport of cholesterol from the human brain by an apolipoprotein E-dependent mechanism could account for a removal of 1–2 mg cholesterol per day. The 24S-hydroxylase-mediated mechanism thus appears to be more important than the apolipoprotein E-mediated mechanism. The two different mechanisms may together have a capacity to remove about 8 mg cholesterol from the human brain per 24 h. As the adult human brain has been reported to contain about 30 g cholesterol (13), the half-life for elimination of this cholesterol by these two mechanisms would be about 5 years. In this connection it is of interest that the half-life of brain cholesterol in adult rats has been reported to be between 2 and 6 months by different groups (3, 14, 15).

It should be pointed out that only plasma levels of 24S-hydroxycholesterol were considered here. In principle, part of the 24S-hydroxycholesterol in the circulation may be transported in erythrocytes. The concentration of 24S-hydroxycholesterol in erythrocytes was found to be about 10% of that in plasma (data not shown).

We have shown that the rate of elimination of cholesterol from the brain of rats by the 24S-hydroxylase mechanism is of the same magnitude as the rate of cholesterol synthesis in this organ (2). There is no information about the rate of synthesis of cholesterol in the human brain, but experiments in other primates (2) suggest that it is very low. Brain cholesterol is efficiently but not entirely protected from exchange with circulating lipoproteins by the blood–brain barrier (2, 14). In accordance with this, most recent studies have favored the contention that the majority of brain cholesterol is synthesized locally and not derived from the circulation (13). If the blood–brain barrier is equally effective in both directions to prevent flux of cholesterol, the importance of the present oxidative mechanism may be restricted to balance cholesterol synthesis. There is, however, also a possibility that the present oxidative mechanism compensates both for the local synthesis and for a flux of cholesterol from the circulation into the brain. The magnitude of the latter flux is not known.

### Uptake of 24S-hydroxycholesterol in the splanchnic region

The average uptake of 24S-hydroxycholesterol in the splanchnic region was found to be similar or slightly higher than the average flux of 24S-hydroxycholesterol from the brain, about 7 mg/24 h. The splanchnic area includes both the intestine and the liver. It seems most likely that uptake occurs in the liver rather than in the intestine. In a previous work we measured the uptake of another side-chain hydroxylated oxysterol, 27-hydroxycholesterol, in both the intestine and the liver (16). The uptake in the liver was found to be about 5- to 6-times higher than in the intestine.

The present results are consistent with the brain as the major producer and the liver as the major eliminator of 24S-hydroxycholesterol. As there was no significant differ-

ence between the levels of 24S-hydroxycholesterol in the renal vein and in a peripheral artery, a renal elimination of 24S-hydroxycholesterol seems less likely, particularly considering that the renal blood flow is smaller than the hepatic blood flow. In accordance with this we have never found a significant excretion of 24S-hydroxycholesterol or its possible metabolites in the urine of healthy volunteers (unpublished observation). In patients with cholestasis, however, a significant such excretion of unconjugated and sulfated 24S-hydroxycholesterol seems to occur (D. Lütjohann, unpublished observation and ref. 17).

If the liver is the major eliminator of 24S-hydroxycholesterol, injected labeled 24S-hydroxycholesterol can be expected to be eliminated at a rate corresponding to the measured uptake of this compound by the liver.

Assuming a plasma flow through the liver of about 1000 L/24 h, an extracerebral pool of 24S-hydroxycholesterol of about 4.5 mg (cf. ref. 1), a plasma concentration of 24S-hydroxycholesterol of 74 ng/ml, and an uptake of 10% of this in the liver, the half-life of 24S-hydroxycholesterol in the extracerebral compartment should be about 10 h (cf. Fig. 5). In accordance with this theoretical calculation, deuterium-labeled 24-hydroxycholesterol administered to two healthy volunteers was found to be eliminated from the circulation with a  $T_{1/2}$  of about 10 h and 14 h, respectively. The difference observed between the two subjects may in part be due to the analytical variation.

Figure 5 summarizes the present state of knowledge about concentrations of 24S-hydroxycholesterol in the brain and in the other tissues (1), the flux from the brain, and the uptake in the liver. In similarity with other side-chain oxidized cholesterol species (18), 24S-hydroxycholesterol may be converted into bile acids in the liver. There is, however, no information about the mechanism of this conversion. At least a small part of the 24S-hydroxycholesterol reaching the liver may be eliminated as such or as sulfate into the bile. Presence of unmetabolized or sulfated 24S-hydroxycholesterol in feces is thus well documented from previous work (19).

#### Role of the cholesterol 24S-hydroxylase in the brain. Relation between levels of 24S-hydroxycholesterol and cholesterol in the circulation

Brain is not completely isolated by the blood-brain barrier (20). If the role of the microsomal 24S-hydroxylase in the human brain would be exclusively to compensate for the local synthesis, no relationship would be expected between circulating levels of cholesterol and levels of 24S-hydroxycholesterol. However, a clear positive correlation between these two levels was found here. A possible explanation is that there is some flux of cholesterol over the blood-brain barrier and that this flux is dependent upon the concentration of lipoprotein-bound cholesterol in the circulation. An important role of the cholesterol 24S-hydroxylase may then be to prevent accumulation of cholesterol in the brain caused by such a transfer, and the relationship between 24S-hydroxycholesterol and cholesterol in the circulation may be due to this. However, further work is needed to confirm this hypothesis. At the present

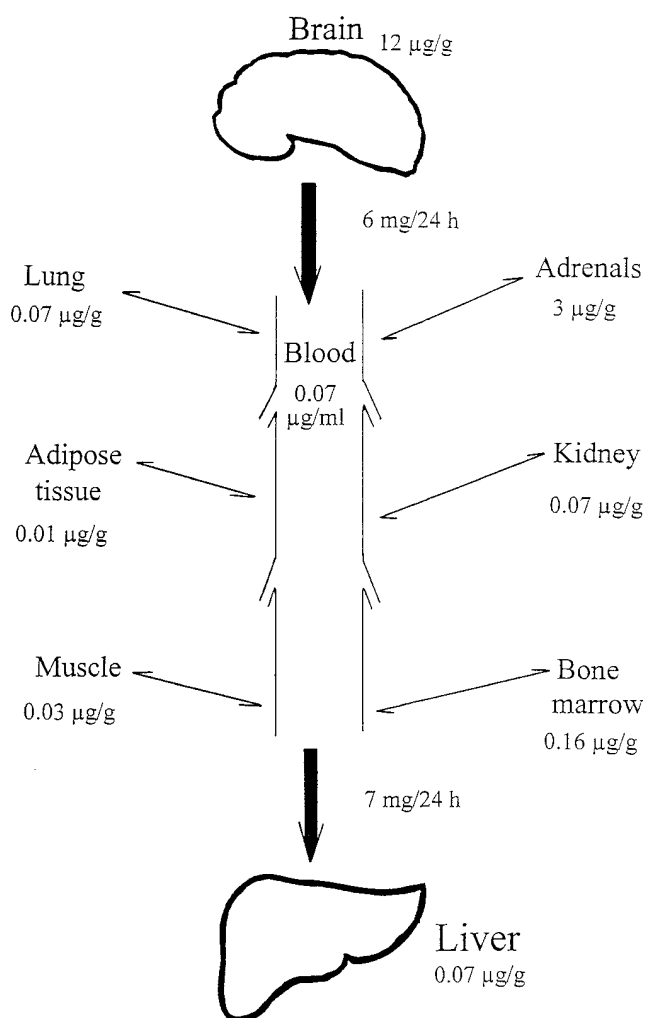


Fig. 5. Pools and fluxes of 24S-hydroxycholesterol in humans. Data on concentration of 24S-hydroxycholesterol in the different organs are from ref. 1.

state of knowledge the possibility cannot be excluded that the preferred substrate for the cerebral 24S-hydroxylase is cholesterol from the circulation newly transferred over the blood-brain barrier.

#### Can 24S-hydroxycholesterol be used as a marker for disturbances in turnover of cholesterol in the brain?

Our finding of an average uptake of 24S-hydroxycholesterol in the splanchnic region that is similar to the average flux of 24S-hydroxycholesterol from the brain, together with the absence of a renal elimination, suggests that most of the 24S-hydroxycholesterol in the circulation is derived from the brain (Fig. 5). In view of the experimental variations, a smaller contribution from other sources cannot be excluded. Adrenals contain relatively high concentrations of 24S-hydroxycholesterol (1). The total amounts of 24S-hydroxycholesterol in these organs are, however, less than 1% of the total content in the body (1), and in view of this it seems unlikely that the adrenals are important sources of circulating 24S-hydroxycholesterol. Human liver contains only very low levels of 24S-hydroxycholesterol

(1), and in view of the net uptake of 24S-hydroxycholesterol in the liver demonstrated here, it seems unlikely that a significant part of circulating 24S-hydroxycholesterol is derived from the liver.

If most of the circulating 24S-hydroxycholesterol is produced in the brain, and formation of 24S-hydroxycholesterol is an important mechanism for elimination of brain cholesterol, circulating levels of this oxysterol may reflect turnover of cholesterol in this organ. In our previous work we found a marked age-dependency in the circulating levels of 24S-hydroxycholesterol such that much higher levels are observed in young individuals than in adults. We have suggested that this may be secondary to a higher rate of cholesterol turnover in the brain in the early phases of life (1).

The above findings and considerations would make it possible to use serum 24S-hydroxycholesterol as a marker for disturbed turnover of cholesterol in the brain. The levels of 24S-hydroxycholesterol in the circulation may, however, also be dependent to some extent on the transporting capacity of the lipoproteins in the circulation and/or factors of importance for the activity of the 24S-hydroxylase in the brain. The hepatic clearance will also directly affect the plasma levels of this oxysterol. Another oxysterol in the circulation, 27-hydroxycholesterol, originates mainly from extracerebral and extrahepatic sources of cholesterol (1, 18). In a recent study we found that 24S-hydroxycholesterol and 27-hydroxycholesterol have similar distribution in circulating lipoproteins (21). In view of this, it is possible that the ratio between 24S-hydroxycholesterol and 27-hydroxycholesterol in the circulation may be a better marker for cholesterol turnover in the brain than the absolute levels of 24S-hydroxycholesterol.

Whether or not the levels of circulating 24S- and 27-hydroxycholesterol are affected in connection with various neurological disorders is now under study in our laboratory. ■■

This work was supported by grants from the Swedish Medical Research Council, Hjärt-Lungfonden and Ostermans Foundation. The skillful technical assistance of Manfred Held and Anita Lövgren is gratefully acknowledged.

Manuscript received 6 March 1998 and in revised form 13 April 1998.

## REFERENCES

1. Lütjohann, D., O. Breuer, G. Ahlborg, I. Nennesmo, Å. Sidén, U. Diczfalusy, and I. Björkhem. 1996. Cholesterol homeostasis in hu-

man brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation. *Proc. Natl. Acad. Sci. USA.* **93**: 9799–9804.

2. Spady, D. K., and J. M. Dietschy. 1983. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster and rat. *J. Lipid Res.* **24**: 303–315.

3. Björkhem, I., D. Lütjohann, O. Breuer, A. Sakinis, and Å. Wennmalm. 1997. Importance of a novel oxidative mechanism for elimination of brain cholesterol. *J. Biol. Chem.* **272**: 30178–30184.

4. Lange, Y., J. Ye, and F. Strelbel. 1995. Movement of 25-hydroxycholesterol from the plasma membrane to the rough endoplasmic reticulum in cultured hepatoma cells. *J. Lipid Res.* **36**: 1092–1097.

5. Dhar, A. K., J. I. Teng, and L. L. Smith. 1973. Biosynthesis of cholest-5-ene-3 $\beta$ ,24-diol (cerebrosterol) by bovine cerebral cortical microsomes. *J. Neurochem.* **21**: 51–60.

6. Lin, Y., and L. L. Smith. 1974. Biosynthesis and accumulation of cholest-5-ene-3 $\beta$ ,24-diol (cerebrosterol) in developing rat brain. *Biochim. Biophys. Acta.* **348**: 189–196.

7. Lund, E., I. Björkhem, C. Furster, and K. Wikvall. 1992. 24-, 25- and 27-Hydroxylation of cholesterol catalyzed by a purified preparation of 27-hydroxylase from pig liver. *Biochim. Biophys. Acta.* **1166**: 177–182.

8. Dzeletovic, S., O. Breuer, E. Lund, and U. Diczfalusy. 1995. Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. *Anal. Biochem.* **225**: 73–80.

9. Ahlborg, G., and A. Juhlin-Dannfelt. 1994. Effect of beta-receptor blockade on splanchnic and muscle metabolism during prolonged exercise in men. *J. Appl. Physiol.* **76**: 1037–1042.

10. Rowland, M., and T. Tozer. 1989. Clinical Pharmacokinetics. Second Edition. Lea & Fabiger, Malvern, PA.

11. Kety, S. S., and C. F. Schmidt. 1948. The nitrous oxide method for the quantitative determination of cerebral blood flow in man: theory, procedure and normal values. *J. Clin. Invest.* **27**: 476–483.

12. Pitas, R. E., J. K. Boyles, S. H. Lee, D. Foss, and R. W. Mahley. 1987. Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E containing lipoproteins. *Biochim. Biophys. Acta.* **917**: 148–161.

13. Snipes, G. J., and U. Suter. 1997. Cholesterol and myelin. *Subcell. Biochem.* **28**: 173–204.

14. Serougne-Gautheron, C., and F. Chevallier. 1973. Time course of biosynthetic cholesterol in the adult rat brain. *Biochim. Biophys. Acta.* **316**: 244–250.

15. Andersson, M., P. G. Elmberger, C. Edlund, K. Kristensson, and G. Dallner. 1990. Rates of cholesterol, ubiquinone, dolichol and dolichol-P biosynthesis in rat brain slices. *FEBS Lett.* **269**: 15–18.

16. Lund, E., O. Andersson, J. Zhang, A. Babiker, G. Ahlborg, U. Diczfalusy, K. Einarsson, J. Sjövall, and I. Björkhem. 1996. Importance of a novel oxidative mechanism for elimination of intracellular cholesterol in humans. *Arterioscler. Thromb. Vasc. Biol.* **16**: 208–212.

17. Meng, L. J., W. J. Griffiths, H. Nazer, Y. Yang, and J. Sjövall. 1997. High levels of (24S)-24-hydroxycholesterol 3-sulfate, 24-glucuronide in the serum and urine of children with severe cholestatic liver. *J. Lipid Res.* **38**: 926–934.

18. Björkhem, I. 1992. Mechanism of degradation of the steroid side-chain in the formation of bile acids (review). *J. Lipid Res.* **33**: 455–471.

19. Gustafsson, J-Å., and J. Sjövall. 1969. Identification of 22-, 24-, and 26-hydroxycholesterol in the steroid sulfate fraction of faeces from infants. *Eur. J. Biochem.* **8**: 467–472.

20. DeVries, H. 1995. Characteristics of blood-brain barrier endothelial cells in response to inflammatory stimuli. Thesis. Coostar Europe Ltd, Badhoevedorp, The Netherlands.

21. Babiker, A., and U. Diczfalusy. 1998. Transport of side-chain oxidized oxysterols in the human circulation. *Biochim. Biophys. Acta.* In press.