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Plasma levels of 24S-hydroxycholesterol reflect the balance between cerebral production and hepatic metabolism and are inversely related to body surface

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Abstract We have previously presented evidence that most of the 24S-hydroxycholesterol present in the circulation originates from the brain and that most of the elimination of this oxysterol occurs in the liver. Plasma 24S-hydroxycholesterol levels decline by a factor of about 5 during the first decades of life. The concentration of the enzyme cholesterol 24S-hydroxylase in the brain is, however, about constant from the first year of life, and reduced enzyme levels thus cannot explain the decreasing plasma levels during infancy. In the present work we tested the hypothesis that the plasma levels of 24S-hydroxycholesterol may reflect the size of the brain relative to the capacity of the liver to eliminate the substance. It is shown here that the age-dependent changes in absolute as well as cholesterol-related plasma level of 24S-hydroxycholesterol closely follow the changes in the ratio between estimated brain weight and estimated liver volume. The size of the brain is increased only about 50% whereas the size of the liver is increased by about 6-fold after the age of 1 year. Liver volume is known to be highly correlated to body surface, and in accordance with this the absolute as well as the cholesterol-related plasma level of 24S-hydroxycholesterol was found to be highly inversely correlated to body surface in 77 healthy subjects of varying ages ($r^2 = 0.74$). Two chondrodystrophic dwarves with normal size of the brain but with markedly reduced body area had increased levels of 24S-hydroxycholesterol when related to age but normal levels when related to body surface. It is concluded that the balance between cerebral production and hepatic metabolism is a critical determinant for plasma levels of 24S-hydroxycholesterol at different ages and that endocrinological factors are less important. The results are discussed in relation to the possibility to use 24S-hydroxycholesterol in the circulation as a marker for cholesterol homeostasis in the brain.—Bretillon, L., D. Lütjohann, L. Stähle, T. Widhe, L. Bindl, G. Eggertsen, U. Diczfalusy, and I. Björkhem. Plasma levels of 24S-hydroxycholesterol reflect the balance between cerebral production and hepatic metabolism and are inversely related to body surface. *J. Lipid Res.* 2000. 41: 840–845.

24S-hydroxycholesterol is one of the major oxysterols in human circulation. We have shown that most of this compound originates from the brain, and that there is a continuous flux of it from the brain into the circulation (1, 2). This flux is likely to be an important part of the cholesterol turnover in the brain, and the flux of 24S-hydroxycholesterol from a rat brain seems to be similar to the rate of synthesis of cholesterol in that organ (3). As judged from the arteriovenous concentration difference, the liver seems to be the major eliminator of circulating 24S-hydroxycholesterol (2).

There is a strong age-dependent variation in the levels of circulating 24S-hydroxycholesterol (1). Newborns have very low circulating levels of 24S-hydroxycholesterol (D. Lütjohann, I. Björkhem, S. Locatelli, C. Dame, J. Schmolling, K. von Bergmann, and H. Fahnenstich, unpublished observation) but these levels increase rapidly after birth and are highest at an age of about 1–2 years (1). The levels decline by a factor of about 5 during the first two decades of life and then remain about constant.

24S-hydroxycholesterol has a high affinity for the LXR α receptor (4) and the possibility has been discussed that this oxysterol is an important part of a signalling system from the brain (5). In addition it is evident that plasma levels of 24S-hydroxycholesterol may reflect turnover of cholesterol in the brain and may be used as a marker for disturbances in this turnover. Very recently we showed that a specific population of patients with Alzheimer disease and vascular dementia had slightly but significantly higher levels of 24S-hydroxycholesterol than controls (6). In view of these findings, it was considered to be important to define the factors affecting plasma levels of 24S-hydroxycholesterol and, in particular, to explain the mechanism behind the dramatic decrease in these levels during infancy and puberty. This decrease may be due to reduced

Supplementary key words liver volume • cholesterol 24S-hydroxylase • brain size • brain cholesterol homeostasis

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enzymatic activity, reduced levels of the enzyme cholesterol 24S-hydroxylase in the brain, reduced levels of substrate (free cholesterol) for the enzyme, or increased metabolism of the oxysterol in the liver.

In a very recent work, antibodies towards cholesterol 24S-hydroxylase were prepared and used for immunoassay of the enzyme in human brain (7). The levels of the enzyme in the brain were found to be about constant from the age of 1.5 years and onwards. Decreasing enzyme levels are thus not the explanation for the decreasing plasma levels of the oxysterol during infancy.

In the present work results are presented suggesting the balance between cerebral production and hepatic metabolism to be the most important determinant for plasma levels of 24S-hydroxycholesterol. The age-dependent changes in the plasma levels of the oxysterol were found to closely follow the age-dependent changes in the ratio between estimated brain weight and liver volume. This was found to be valid also in chondrodystrophic dwarves with normal size of the brain but with reduced body size. In consonance with this, the circulating levels of 24S-hydroxycholesterol were found to be inversely correlated to body surface.

MATERIALS AND METHODS

Subjects

A total of 205 adults of both sexes (120 women, 85 men, aged 21 to 86 years from the Swedish population) are included in the study presented in Table 1 and Fig. 1. All these adults regard themselves as healthy and had no medications. The samples were taken in the morning in the fasting state.

Some of the adults ($n = 29$) presented in Table 1 were defined with respect to body height and weight and are included in the study shown in Figs. 4 and 5. In addition, three healthy adults with exceptional body height were recruited for the study presented in these figures. Table 1, Figs. 4 and 5 also included data from 48 infants and children of both sexes from 1 to 18 years of age. The plasma samples had been collected for diagnostic purposes and ethical permission was obtained to use the excess of these plasma samples for the present study. In most cases, the infants could only be defined with respect to sex and age. All these infants were treated at the hospital due to a medical or surgical illness. Samples from a few apparently healthy well-defined infants who were hetero- or homozygotes with respect to sitosterolemia were also included ($n = 3$ and 2 , respectively), as well as 9 well-defined infants with short-bowel syndrome, Ullrich-Turner syndrome, atrium-septum defect, chronic diarrhea, or Mb Crohn. The levels of 24S-hydroxycholesterol from the latter infants were similar to those of infants of the same age with an undefined medical or surgical illness. The weight and height of the infants and children could only be defined in the latter two groups ($n = 14$). The brain weight and the liver volume of the above 78 subjects (30 adults and 48 infants and children) were calculated using literature data (8–10).

Two chondrodystrophic dwarves were included in the study. The male dwarf was 48 years of age, had a length of 150 cm and a weight of 70 kg. He had been operated in the back due to spinal stenosis a few days before collection of the blood sample, but was otherwise healthy. The female dwarf was 54 years of age, had a length of 123 cm and a weight of 52 kg. She was bound to a

wheel-chair due to spinal stenosis at the time of the collection of the blood sample, but was otherwise healthy.

The study was done in accordance with the principles of the Declaration of Helsinki, and ethical permission for collection of plasma samples was obtained from the Ethical Committee of Huddinge Hospital.

Analyses and analytical methods

Serum concentrations of cholesterol were measured by standard enzymatic procedures (CHOP-method, Boehringer Inc., Mannheim, Germany). Levels of 24S-hydroxycholesterol were assayed by isotope dilution mass spectrometry using racemic [23, 23, 24-²H₃]deuterium-labeled 24-hydroxycholesterol and the instrumentation and conditions previously described (1–3, 6, 11). The intra- and interassay coefficient of variation of this method is about 4% and 8%, respectively.

Turnover models and statistics

Previous data suggest that the production of 24S-hydroxycholesterol is of cerebral origin while the elimination is hepatic (2). Assuming that the production is proportional to the brain size, the elimination is proportional to the liver size, and assuming that the volume of distribution does not affect the plasma concentration, due to the steady-state conditions, we get:

$$\begin{aligned} dC_p/dt &= k_p \cdot BrW - k_e \cdot C_p \cdot LW = 0 \\ \text{thus, } C_p &= k_p \cdot BrW / k_e \cdot LW \end{aligned}$$

where C_p is the plasma concentration of 24S-hydroxycholesterol, BrW is the brain weight, LW is the liver weight, and k_p and k_e are proportionality constants for formation and elimination, respectively. Literature data were used to estimate the brain weight (8) and the liver volume (9, 10). In the latter case, volume was substituted for weight assuming a density of 1.0.

Ordinary linear regression was used to analyze the relation between 24S-hydroxycholesterol and BrW/LW .

RESULTS

In **Table 1** results of measurements of plasma levels of 24S-hydroxycholesterol and cholesterol are presented from 48 infants and 205 adults of both sexes, covering an age interval from 1 to 86 years. The adults are all subjectively healthy. Most of the infants have some type of non-neurological disease, and the samples had been collected for routine diagnostic purposes. For ethical reasons most of the infants could only be defined with respect to age and sex.

Figure 1 shows the relation between plasma levels of 24S-hydroxycholesterol and cholesterol in the whole material of adults subjects ($n = 205$). In accordance with a previous study with fewer subjects (2), there was a high correlation between the two parameters with a r^2 value of 0.44. In view of the high correlation, the levels of 24S-hydroxycholesterol are presented below both as absolute concentration (ng/mL) and as the ratio between the level of 24S-hydroxycholesterol and that of cholesterol (ng/mg).

The results presented in Table 1 confirm the previously reported decline in both absolute and cholesterol-related plasma levels of 24S-hydroxycholesterol during infancy and puberty. A noteworthy finding was that the levels during decades 6 and 7 were slightly but significantly higher than the levels during decades 3–5. There was no consis-

TABLE 1. Absolute and cholesterol-related plasma levels of 24S-hydroxycholesterol in 253 human subjects (48 infants and 205 adults of both sexes)

Age Group	Age yr	n			24S-Hydroxycholesterol ng/mL	24S-Hydroxycholesterol/Cholesterol ng/mg
		Total	Female	Male		
1	1-5	12 ^d	6	2	385 ± 64	391 ± 73
2	6-9	11 ^d	2	8	258 ± 31	240 ± 29
3	10-18	25 ^d	14	10	192 ± 12 ^a	157 ± 9 ^b
4	19-30	37	20	17	77 ± 3 ^c	65 ± 2 ^c
5	31-40	30	13	17	76 ± 4	59 ± 2 ^a
6	41-50	47	28	19	77 ± 4	56 ± 2
7	51-60	49	36	13	87 ± 3 ^a	58 ± 2
8	61-70	20	11	9	105 ± 7 ^b	67 ± 2 ^b
9	71-86	22	12	10	101 ± 5	64 ± 3

Results are expressed as means ± SEM.

^a $P < 0.05$; ^b $P < 0.01$; ^c $P < 0.001$; significantly different from the corresponding value of the previous age.

^d The reason for the differences observed between the total number of subjects and the number of females plus males is that some infants can only be defined with respect to age.

tent significant sex difference in any of the different age groups.

In the previous work (2) we showed that the brain is the major producer and that the liver is the major eliminator of 24S-hydroxycholesterol. Given the fact that the concentration of the enzyme is about constant after the age of 1.5 years (7), the ratio between the size of the brain and the size of the liver is likely to be of importance for the levels of 24S-hydroxycholesterol in the circulation.

Figure 2 shows the age-dependent changes in the brain weight and liver volume reported from autopsy studies (8), and studies with computed tomography scanning (9) or ultrasound technique (10). The ratio between brain weight and liver volume decreases with a factor of 4 during infancy and puberty, followed by a slight increase during the later decades of life. It should be mentioned that there are some sex-related differences in brain weight and liver volume, whereas the ratio between the two parameters is affected very little by sex (in general the difference is less than 10%).

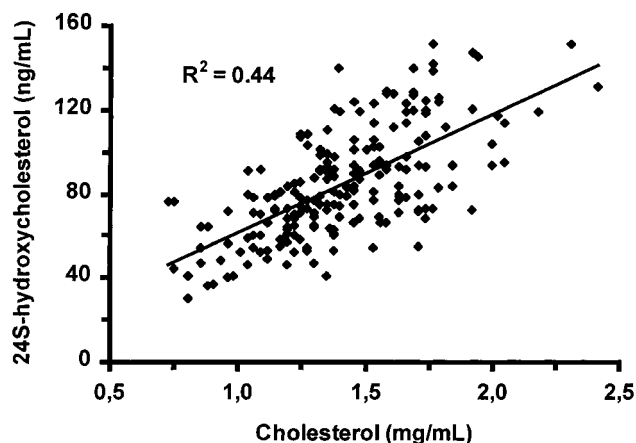


Fig. 1. Relation between plasma levels of 24S-hydroxycholesterol and cholesterol in adult subjects (from 21 to 86 years old, 120 women, 85 men).

Figure 3 shows the levels of absolute (A) and cholesterol-related (B) levels of 24S-hydroxycholesterol from the age of 1 year and onwards. The ratio between brain weight and liver volume for the different ages, based on literature data (8–10), is also indicated in the figure. The levels of 24S-hydroxycholesterol closely follow the ratio between brain weight and liver volume during infancy and puberty as well as during later decades of life.

If the ratio between the brain and the liver is the major determinant for plasma levels of 24S-hydroxycholesterol, endocrinological factors during puberty should be less important. Adult chondrodystrophic dwarves have a normal head size but a markedly reduced body size. Except for reduced levels of receptors for growth hormone in the skeleton, they are normal from an endocrinological point of view. As indicated in Fig. 3B, two chondrodystrophic dwarves of opposite sexes had highly significant increased cholesterol-related levels of 24S-hydroxycholesterol (101 ng of 24S-hydroxycholesterol per milligram of cholesterol for both of them). This finding supports the contention that the high levels of 24S-hydroxycholesterol during infancy and puberty are due to the relation between the size of the brain and the size of the body (including the liver) rather than due to endocrinological factors.

A very high correlation between liver size and body surface area during the first three decades of life has been reported (9). An inverse correlation between levels of 24S-hydroxycholesterol and body surface area would therefore be expected. The absolute (results not shown) as well as the cholesterol-related plasma levels of 24S-hydroxycholesterol (Fig. 4) have been plotted against body surface area in 77 subjects of different ages from 1 to 86 years. There was a high inverse correlation in both cases ($r^2 = 0.65$ and 0.74 , respectively). The plasma cholesterol-related levels of 24S-hydroxycholesterol and the estimated body surface area of the two dwarves have also been indicated.

Body surface area is well correlated to body height (12, 13). Thus an inverse correlation between levels of 24S-hydroxycholesterol and body length would also be expected. The correlation (r^2) between absolute levels of

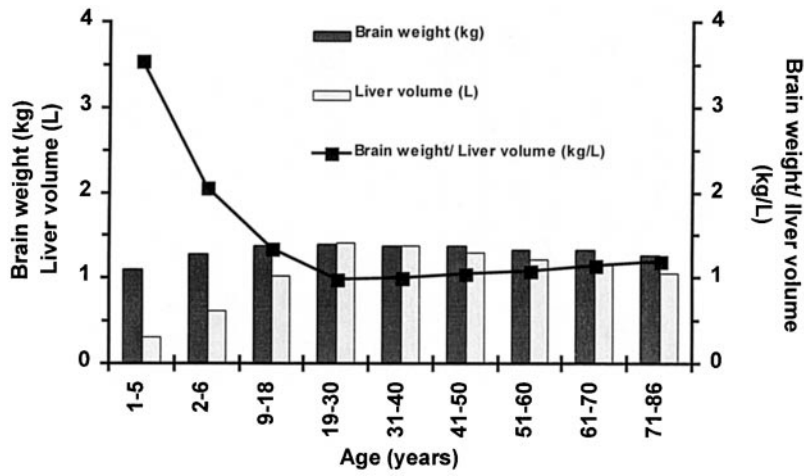


Fig. 2. Age-related changes in brain weight (adapted from ref. 8) and liver volume (adapted from refs. 9 and 10).

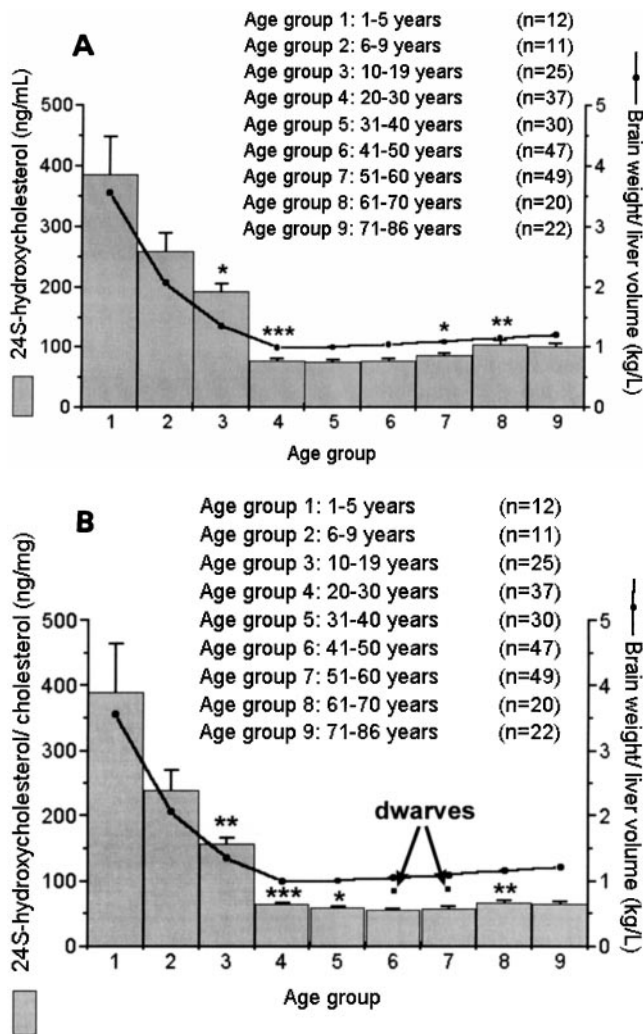


Fig. 3. A: Absolute and B: cholesterol-related levels of 24S-hydroxycholesterol in human subjects at different ages (48 infants and 205 adults). Results are expressed as means \pm SEM. Estimated ratio between brain weight and liver volume is indicated (cf. Materials and Methods); *, **, and ***: significantly different from the corresponding value of the previous age at $P < 0.05$, 0.01 , and 0.001 , respectively.

24S-hydroxycholesterol and body length was found to be 0.62 and the corresponding correlation for cholesterol-related levels of 24S-hydroxycholesterol and body length was found to be 0.71 (results not shown). The corresponding correlations to body weight were found to be 0.64 and 0.73, respectively (results not shown).

DISCUSSION

The background to this study was our previous result that there are arteriovenous concentration differences over the liver and the brain, indicating that the brain produces and the liver eliminates 24S-hydroxycholesterol (2). This model predicts that the plasma level of 24S-hydroxycholesterol should vary with the ratio between brain

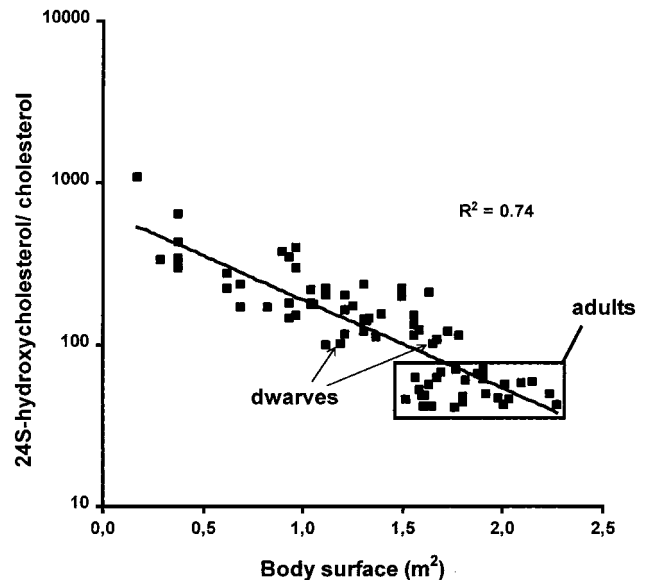


Fig. 4. Relation between plasma cholesterol-related levels of 24S-hydroxycholesterol and body surface in 77 subjects (48 infants and 29 adults of both sexes). Values of cholesterol-related levels of 24S-hydroxycholesterol have been plotted in a logarithmic scale.

weight and liver weight, as shown above. The results obtained in this study are in accordance with the prediction and support our hypothesis.

The high correlation between circulating levels of 24S-hydroxycholesterol and cholesterol observed in the present work is in accordance with previous observations in smaller populations (2, 6). The possibility has been discussed that part of the explanation for this may be that there is a small flux of cholesterol from the circulation over the blood-brain barrier into the brain (2). The distribution of 24S-hydroxycholesterol in the lipoproteins is, however, similar to that of cholesterol (14) and changes in the cholesterol pool are thus likely to lead to changes in the distribution volume for the oxysterol and consequently also in the concentration of this oxysterol. The plasma concentration of the structurally similar oxysterol 27-hydroxycholesterol has also been found to be highly correlated to the plasma level of cholesterol (15). A consequence of the high correlation between plasma levels of 24S-hydroxycholesterol and cholesterol is that the ratio between 24S-hydroxycholesterol and cholesterol is likely to be a better marker for the cerebral production than the absolute concentration of the oxysterol.

The evident age-dependent changes in the circulating levels of 24S-hydroxycholesterol seem to be unique, as all other oxysterols measured in the circulation vary little with age (1). It should be pointed out that production of 24S-hydroxycholesterol occurs in an organ that increases little in size after the age of 2 years (about 30%) whereas elimination of 24S-hydroxycholesterol involves an organ that increases in size up to 6-fold during this period.

If the capacity of the liver to metabolize 24S-hydroxycholesterol is proportional to the size of the organ, this is a likely explanation for the age-dependent variations. The blood flow through the liver must be of importance for the clearance rate, and it is known that there is a good correlation between hepatic blood flow and the size of the liver (10). During the last decades of life, the liver size decreases more than the brain size (cf. Fig. 2). If the metabolism of 24S-hydroxycholesterol is proportional to liver size, slightly increased levels of 24S-hydroxycholesterol would be expected during the last decades of life. That such an increase could be demonstrated lends further support for the contention that the balance between cerebral production and hepatic metabolism is most important for the circulating levels of this oxysterol.

The enzymes involved in hepatic metabolism of 24S-hydroxycholesterol have not been defined in detail. The relatively long half-life of 24S-hydroxycholesterol in the circulation, 10–14 h (2), suggests that the enzymes involved are less effective than those involved in the metabolism of other oxysterols. We have shown that the cholesterol 7 α -hydroxylase (CYP7A) present in human liver has at least some activity towards 24S-hydroxycholesterol (M. Norlin, A. Toll, I. Björkhem, and K. Wikvall, unpublished observation), but the quantitative importance of this specific cytochrome is not known. Oxysterol 7 α -hydroxylase (CYP7B) does not seem to have any activity towards 24S-hydroxycholesterol (M. Norlin, A. Toll, I. Björkhem and

K. Wikvall, unpublished observation). It is noteworthy that treatment of patients with ketoconazol, which is an inhibitor of CYP7A and also other species of cytochrome P-450, causes an increase in the levels of 24S-hydroxycholesterol (I. Björkhem and U. Diczfalusy, unpublished observation). It is well documented that cholestasis causes increased levels of 24S-hydroxycholesterol in the circulation (16, 17), and it is evident that the metabolic capacity of the liver is a critical factor for the circulating levels of 24S-hydroxycholesterol.

There was a high inverse correlation between body surface area and plasma levels of 24S-hydroxycholesterol ($r^2 = 0.74$, Fig. 4). Because there is a high correlation between body surface area and liver volume (10), such a correlation would be expected. In this connection it is interesting that chondrodystrophic dwarves, with normal size of the brain but with markedly reduced body size, had high cholesterol-related levels of 24S-hydroxycholesterol in the circulation. This finding supports the contention that the ratio between size of the brain and size of the liver is the most important determinant for circulating levels of 24S-hydroxycholesterol.

In previous works (1, 2), we discussed the possibility that the high levels of 24S-hydroxycholesterol in the circulation of infants and children may be secondary to a high turnover of brain cholesterol during this period of life. The results presented here suggest that most of the age-dependent variation in levels of 24S-hydroxycholesterol reflects a balance between a relatively constant production of the compound in the brain and an age-dependent elimination of the compound in the liver (Fig. 5). To our knowledge, there are few other examples of this. The continuous relatively constant production of creatinine in the muscles and the elimination of this compound by the kidneys has some similarity with the mechanism studied here.

Plasma levels of 24S-hydroxycholesterol are not subjected to diurnal variations (1) and with the exception of changes due to liver disease (16, 17), neurological disorders (6), and drugs, the levels seem to be relatively stable

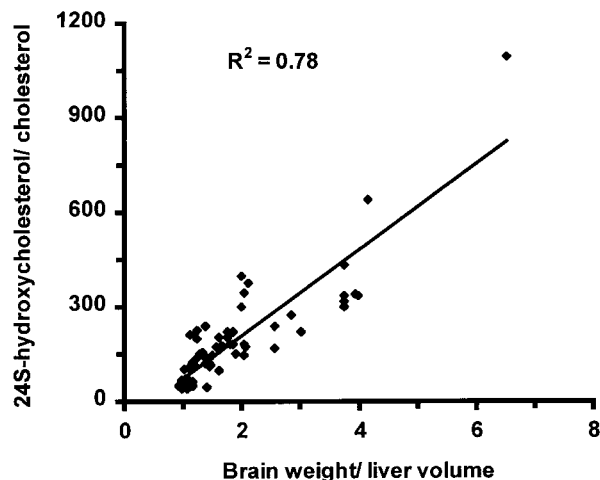


Fig. 5. Relation between plasma cholesterol-related levels of 24S-hydroxycholesterol and estimated brain weight to liver volume ratio in 77 healthy subjects.

in adults. In view of this it seems less likely that 24S-hydroxycholesterol is of major regulatory importance in extracerebral tissues. It is evident, however, that plasma levels of cholesterol and body surface area should be taken into account when evaluating circulating levels of 24S-hydroxycholesterol in different populations. ■■

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