Elimination of cholesterol as cholestenoic acid in human lung by sterol 27-hydroxylase: evidence that most of this steroid in the circulation is of pulmonary origin

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Abstract **Human alveolar macrophages have exceptionally high capacity to convert cholesterol into 27-hydroxycholesterol and cholestenoic acid by the sterol 27-hydroxylase mechanism. It is shown here that the human lung has a higher content of 27-hydroxycholesterol relative to cholesterol than any other organ. In order to evaluate the importance of the sterol 27-hydroxylase mechanism for cholesterol homeostasis in the lung, the production of cholestenoic acid by human lung was investigated. Removal of one lung reduced the level of cholestenoic acid in the circulation by 48 ± 4% ($P < 0.005$). The levels of cholestenoic acid in the pulmonary artery and in the pulmonary vein showed significant differences ($P < 0.002$) with higher levels in the pulmonary vein (108 ± 16 and 104 ± 16 ng/mL, respectively). This corresponds to a net flux of cholestenoic acid from the lung of about 14 mg/day, which is more than 80% of the reported removal of this oxysterol and its metabolites from the circulation by the liver per day. Bypassing the lung for 60 min led to a reduction in circulating cholestenoic acid (30%) that fits with a pulmonary origin when taking into account the half-life of cholestenoic acid. The level of circulating cholestenoic acid was found to be less in patients with different lung diseases. It is evident that most of the cholestenoic acid in the circulation is of pulmonary origin. The present results suggest that the sterol 27-hydroxylase in the lung is responsible for at least half of the total flux of 27-oxygenated cholesterol metabolites to the liver and that this enzyme system may be of importance for cholesterol homeostasis in the lung.**—Babiker, A., O. Andersson, D. Lindblom, J. van der Linden, B. Wiklund, D. Lütjohann, U. Diczfalusy, and I. Björkhem. **Elimination of cholesterol as cholestenoic acid in human lung by sterol 27-hydroxylase: evidence that most of this steroid in the circulation is of pulmonary origin.** ***J. Lipid Res.*** **1999.** 40: **1417–1425.**

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Cholesterol at the cellular level is removed mainly through the lipoprotein-dependent reverse cholesterol transport (RCT) which occurs by two different mechanisms: one aqueous diffusion mechanism and one specific apoprotein-mediated mechanism (1).

Recently a new oxidative mechanism for elimination of intracellular cholesterol in macrophages and endothelial cells by the enzyme sterol 27-hydroxylase was described (2–4). This mechanism involves conversion of cholesterol into 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid (cholestenoic acid). These products are more polar than cholesterol and are transported out of the cells more easily than unmetabolized cholesterol (5). The sterol 27-hydroxylase is widely distributed in different organs and tissues (2–7) and the above mechanism may therefore also be utilized for elimination of cholesterol from other types of cells than macrophages and endothelial cells. We have shown that there is a continuous flux of 27-oxygenated steroids from extrahepatic sources to the liver, where they are further oxidized into bile acids. The magnitude of this flux is such that it may be of importance for the overall cholesterol homeostasis (4).

How much different organs, tissues, and cells contribute to the flux of 27-oxygenated steroids to the liver has not been defined. The highest extrahepatic levels of sterol 27-hydroxylase mRNA have been reported in adrenals, intestine, and lung (7). When different types of cells were cultured under the same conditions, human alveolar macrophages were found to have considerably higher capacity to produce 27-oxygenated products than any other cells tested, and most of the product was cholestenoic acid (5).

The exceptionally high capacity of alveolar macrophages to produce cholestenoic acid and the high content of sterol 27-hydroxylase mRNA in the lung led us to specu-

Abbreviations: RCT, reverse cholesterol transport; GC–MS, gas chromatography–mass spectrometry; FEV1, forced expiratory lung volume; CTX, cerebrotendinous xanthomatosis.

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late that the lung may be the major source of circulating cholestenoic acid in humans.

In the present work we present evidence that this is the case, and that the sterol 27-hydroxylase may be of importance for cholesterol homeostasis in the lung. The effect of different pulmonary diseases on the production of cholestenoic acid has also been studied and we discuss the possibility that this steroid can be used as a marker for metabolically active lung tissue.

MATERIALS AND METHODS

Materials

Unlabeled norcholestenoic acid and deuterium-labeled 27-hydroxycholesterol were the same as those used in previous work (5).

Deuterium-labeled cholestenoic acid was synthesized as described previously (8) via standard Wittig-Horner reaction of 3β-tert-butyl dimethyl-siloxycholen-24-al with the exception that saturation of the delta 24-double bond was performed with $^2H_2$ gas instead of $^1H_2$ gas. With determination by mass spectrometry of the product as the methyl ester trimethylsilyl derivative, it was found that saturation of the double bond with deuterium gas under these catalytic conditions with PtO_2 as catalyst was associated with introduction of ^2H in some other undefined positions, yielding cholestenoic acid containing a mixture of mono-, di-, tri-, tetra-, penta-, and hexa-deuterium labeled species. The relative contribution of tetra- and penta-deuterium labeled molecules (25% and 23%, respectively) was highest and it was confirmed that there was no loss of deuterium label during exposure of the compound to aqueous or alkaline conditions. The final product was purified by aluminium oxide chromatography (as methyl ester) and was homogenous as judged by GC–MS of the methyl ester trimethylsilyl ether derivative.

Deuterium labeled 3β,7α-dihydroxy-5-cholestenoic acid was obtained from the above labeled cholestenoic acid by oxidation as described previously (9). This material had the same isotopic composition as the precursor. Deuterium-labeled 7α-hydroxy-3-oxo-chol-4-enoic acid was prepared from the above deuterium labeled 3β,7α-dihydroxy-5-cholestenoic acid by oxidation with 3β-hydroxysteroid dehydrogenase according to standard procedures. The labeled 7α-hydroxy-3-oxo-chol-4-enoic acid had the same isotopic composition as the precursor.

All other reagents and chemicals used were of high-purity standard commercial grade.

Collection of samples for determination of 27-hydroxycholesterol in different human organs

Post-mortem samples were collected from different organs of the same human corpse (6), washed with phosphate-buffered saline and then kept frozen at −20°C until analysis. 27-Hydroxycholesterol and cholesterol were determined as described previously (10).

Culturing alveolar macrophages

Alveolar macrophages were isolated and cultured as described previously (4).

Samples collected in connection with cardio-pulmonary bypass operations for measuring production of cholestenoic acid by the lung

Blood samples were collected for measuring cholestenoic acid in the radial artery from six patients during cardio-pulmonary bypass operations. The samples were drawn immediately before the application of the heart–lung machine, after bypassing the lung for 60 min, and then after removal of the machine when the lung had resumed its normal function. Blood was also collected for measuring cholestenoic acid in the pulmonary artery and pulmonary vein from the same patients before bypassing the lung. At the same time, cardiac output was measured using a Swan-Ganz catheter and applying the Fick principle.

Kinetic study

Deuterium-labeled 3β-hydroxy-5-cholestenoic acid, 1 mg dissolved in ethanol and mixed with human albumin (10%) and sodium chloride (0.9%, w/v), was administered intravenously to a healthy male volunteer, 56 years old, and weighing 93 kg. In another experiment, 1.15 mg of the steroid was administered to another healthy male volunteer, 34 years old, weighing 61 kg. Blood samples were collected before and at specific time points after the administration.

Samples from patients with lung diseases and patients undergoing pulmectomy

Blood samples for determination of cholestenoic acid and 27-hydroxycholesterol were collected from ten patients suffering from chronic lung diseases (tuberculosis and sarcoidosis). From another seven lung cancer patients, blood was collected before and 2–3 weeks after pulmectomy for measuring cholestenoic acid, 27-hydroxycholesterol, 3β,7α-dihydroxy-5-cholestenoic acid, and 7α-hydroxy-3-oxo-chol-4-enoic acid. Blood samples were also collected from patients with decreased forced expiratory lung volume (FEV1) due to different lung diseases. For reasons of comparison the levels of cholestenoic acid, 27-hydroxycholesterol, and cholesterol were determined in 22 healthy volunteers, nine males and 13 females, with a mean age of 38 years.

Analytical methods

Levels of 27-hydroxycholesterol and cholestenoic acid were determined by quantitative mass spectrometry with use of deuterium-labeled 27-hydroxycholesterol and norcholestenoic acid as internal standards, respectively, applying the same instrumentation and conditions as described previously (5). Under the conditions used, the coefficient of variation in the measurement of these two products is about 3% for both compounds. In a previous work (10) it was shown that the mean difference between expected and found value in a recovery experiment with 27-hydroxycholesterol was 2%. It was shown here that the corresponding figure for cholestenoic acid was 4%. Theoretically, the recovery of cholestenoic acid may be slightly different in arterial and venous blood. Recovery experiments where a fixed amount of unlabeled cholestenoic acid was added to arterial blood and venous blood from the same patient did not, however, show such a difference (results not shown).

The level of 27-hydroxycholesterol in 31 healthy volunteers determined by the present method has been reported to be 154 ± 43 ng/mL (10), whereas the level of cholestenoic acid by the above method was found to be 101 ± 25 ng/mL (mean ± SD).

The levels of 3β,7α-dihydroxy-5-cholestenoic acid were assayed with use of deuterium-labeled 3β,7α-dihydroxy-5-cholestenoic acid as internal standard. The internal standard, 150 ng, was added to serum, 0.5 mL, and the mixture was processed in the same way as for cholestenoic acid analysis. Gas chromatography–mass spectrometry was performed on the methyl ester–trimethylsilyl ether derivative, utilizing m/z 410 (M−2 × 90) for registration of the unlabeled compound and m/z 415 for registration of the penta-deuterium-labeled compound. A standard curve was obtained by analysis of standard mixtures of 150 ng of the internal standard and varying amounts of unlabeled 3β,7α-

dihydroxy-5-cholestenoic acid. The coefficient of variation was shown to be about 2.3% under the conditions used. The level of 3β,7α-dihydroxy-5-cholestenoic acid was found to be 28 ± 9 ng/mL (mean ± SD) when assayed by the present method in 16 healthy volunteers.

The levels of 7α-hydroxy-3-oxo-chol-4-enoic acid were measured with use of deuterium-labeled 7α-hydroxy-3-oxo-chol-4-enoic acid as internal standard. The internal standard, 300 ng, was added to serum, 0.5 mL, and the mixture was processed in the same way as for cholestenoic acid. Combined gas chromatography–mass spectrometry was performed on the methyl ester–trimethylsilyl ether derivative, utilizing *m/z* 426 (M–90) for registration of the unlabeled compound and *m/z* 431 for registration of the penta-deuterium-labeled compound. A standard curve was obtained by analysis of standard mixtures of the internal standard and varying amounts of unlabeled 7α-hydroxy-3-oxo-chol-4-enoic acid. The coefficient of variation was shown to be about 3.2% under the conditions used. In 16 healthy volunteers the level was found to be 66 ± 18 ng/mL (mean ± SD).

Kinetic calculations

Standard methods for linear regression and pharmacokinetic methods were used to calculate the half-life of deuterium-labeled cholestenoic acid (11).

Ethical aspects

All experiments involving human volunteers, as well as those in which samples were obtained from patients suffering from lung diseases or undergoing operations, were reviewed and approved by the Ethical Committee at the Huddinge University Hospital.

RESULTS

High content of 27-hydroxycholesterol and sterol 27-hydroxylase in the lung

The content of 27-hydroxycholesterol was determined in a number of tissues from the same deceased subject. As shown in **Table 1**, atheroma-containing arteries had the highest content (54 ng/mg wet weight) followed by tendons (2.7 ng/mg wet weight), lung (2.4 ng/mg wet weight), and adrenals (1.4 ng/mg wet weight). All other organs showed low levels in comparison with the above tissues. When the levels of 27-hydroxycholesterol were related to cholesterol content (ng 27-hydroxycholesterol/mg cholesterol), the lung had the highest ratio (6.8) followed by small intestine (3.8), tendon (2.2), and the arteries (1.7) (Table 1). As cholestenoic acid is more polar than 27-hydroxycholesterol, it was not expected to be retained in most organs. In accordance with this, only very low levels of cholestenoic acid were detected in the lung and there were no detectable levels in the other organs.

Production of cholestenoic acid by the lung in vivo

To estimate the production of cholestenoic acid by the lung, the level of cholestenoic acid was measured in the pulmonary artery and pulmonary vein of nine patients in connection with cardiac operations. The arterio-venous difference of cholestenoic acid was found to be 4.5 ± 1 ng/mL (mean ± SEM) ($P < 0.002$) (**Fig. 1**). The plasma flow was measured in six of these patients and the mean was found to be 3.0 L/min. From these data, the production of cholestenoic acid was calculated to be about 0.59 mg/h which is equivalent to about 14 mg/day ($P = 0.02$).

TABLE 1. Absolute and cholesterol-related levels of cholestenoic acid and 27-OH-cholesterol in tissues and organs of the human body

	Cholestenoic Acid	27-OH-Cholesterol	27-OH-Cholesterol/Cholesterol
	ng/mg wet weight		*ng/μg cholesterol*
Heart	not measured	0.11	0.34
Spleen	<0.2	0.10	0.13
Liver	not measured	0.30	0.39
Adrenals	<0.02	1.35	0.07
Kidney	<0.02	0.16	0.24
Thymus	<0.02	0.14	0.39
Duodenum	<0.02	0.09	0.31
Muscles	<0.02	0.14	1.32
Lung	0.04	2.36	6.79
Testes	<0.02	0.42	1.14
Fat tissue	<0.02	0.00	0.00
Artery (atheroma)	not measured[a]	54.23	1.70
Small intestine	not measured	0.58	3.82
Skin	<0.02	0.27	0.29
Bone marrow	<0.02	0.39	1.19
Tendons	<0.02	2.67	2.17

Measurements were performed in autopsy materials from one male patient, 72 years old, who died of cardiac insufficiency (6). The samples were collected 44 h post mortem.

[a] In another study, atheromas from 8 different patients were analyzed and found to contain 1.0 ± 0.3 ng/mg wet weight with a ratio between cholestenoic acid and 27-hydroxycholesterol of less than 0.03 in all cases. (U. Diczfalusy, S. Dzeletovic, and A. Babiker, unpublished observation).

For comparison purposes, 27-hydroxycholesterol and 24-hydroxycholesterol were measured in the pulmonary artery and the pulmonary vein. However, no significant arteriovenous difference was obtained (results not shown). It should be pointed out that variations in those measurements were relatively great.

In order to study the contribution of the lung for formation of cholestenoic acid in the body, circulating levels

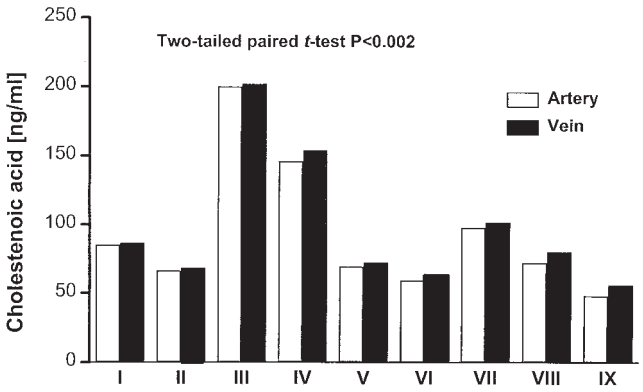

Fig. 1. Cholestenoic acid in pulmonary vessels. Comparison of human plasma levels of cholestenoic acid in the pulmonary artery and pulmonary vein in nine patients (I–IX). Samples were collected in connection with cardiac operation before the application of the heart–lung machine.

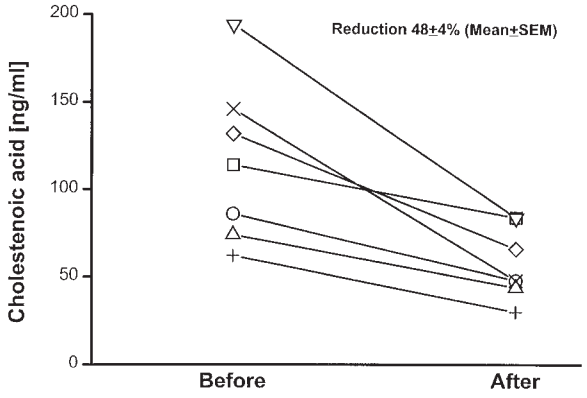

Fig. 2. Cholestenoic acid measured in human plasma before and after pulmectomy. Cholestenoic acid concentration in plasma of seven patients who underwent pulmectomy (removal of one lung). Two samples were taken from each patient, one before the operation and the other 2–3 weeks after pulmectomy.

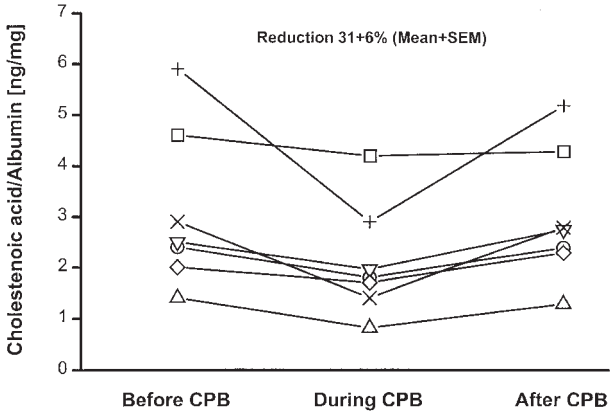

Fig. 3. Effect of lung bypass on circulating cholestenoic acid. Changes in the plasma levels of cholestenoic acid in seven patients during cardio-pulmonary bypass operations. Samples were taken from the same patient before bypassing of the lung, 60 min after application of the heart–lung machine, and after removal of the machine. The levels of cholestenoic acid were related to albumin concentration to correct for the hemodilution that accompanies the application of the heart-lung machine; CPB, cardio-pulmonary bypass.

of this oxysterol was measured in seven patients before and 2–3 weeks after removal of one lung (pulmectomy). **Figure 2** shows the result of this investigation. The concentration of cholestenoic acid in the plasma of these patients was reduced by half (48 ± 4%) after pulmectomy which was highly significant ($P < 0.005$).

In four of the above patients, circulating 3β,7α-dihydroxy-5-cholestenoic acid was also measured before and after pulmectomy. The pulmectomy caused a reduction of these levels by 53 ± 7% (mean ± SEM) which was significant ($P = 0.01$, results not shown). In three of the above mentioned patients, pulmectomy caused 31 ± 10% (mean ± SEM) reduction in the level of 7α-hydroxy-3-oxo-chol-4-enoic acid (results not shown).

Pulmectomy caused 18 ± 7% (mean ± SEM) reduction of the circulating levels of 27-hydroxycholesterol. Due to a relatively great variation in these measurements, the reduction was less significant from a statistical point of view ($P = 0.05$). In contrast, circulating levels of 24-hydroxycholesterol were not affected by pulmectomy ($P > 0.05$, results not shown).

To examine the effect of exclusion of the whole lungs from the body on the production of cholestenoic acid, the level of cholestenoic acid was measured in the radial artery before, 60 min after pulmonary bypass, and after removal of the heart–lung machine from patients undergoing cardio-pulmonary bypass operation. **Figure 3** shows that the level of cholestenoic acid was reduced by one third (31 ± 6%) after bypassing the lungs for 60 min, compared with the level before applying the heart–lung machine ($P = 0.03$). The concentration of cholestenoic acid increased after removal of the machine, when the lung had resumed its function, and returned to the original level. In order to evaluate the importance of the lung for production of cholestenoic acid from these data, the half-life of cholestenoic acid in the circulation must be known.

In order to study the elimination of cholestenoic acid from the circulation, deuterium-labeled cholestenoic acid was administered intravenously to a healthy volunteer. The amount of [$^2H_5$]cholestenoic acid injected was 1.15 mg. **Figure 4** shows that the dilution of the deuterium-labeled cholestenoic acid followed first order kinetics between 2 and 5 h after administration. The terminal half-life was calculated to be 105 min. In another experiment with another volunteer in which the administered amount of deuterium-labeled cholestenoic acid was 1 mg, the terminal half-life was found to be 80 min (data not shown). With a half-life of 90 min, exclusion of the source of production of cholestenoic acid would be expected to lead to a reduction by 37% in the circulating levels of this

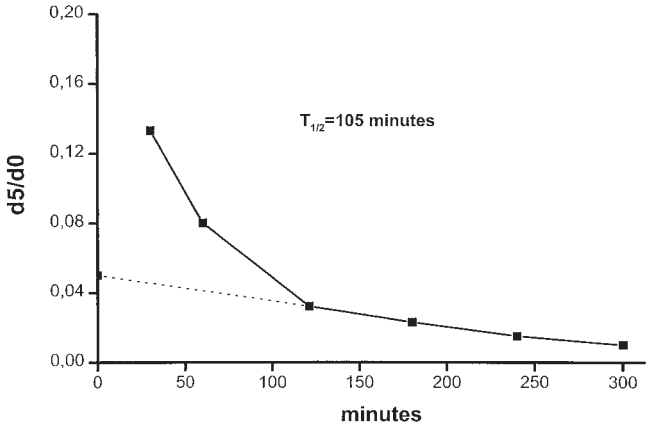

Fig. 4. Rate of dilution of deuterium-labeled cholestenoic acid in the circulation. Deuterium-labeled cholestenoic acid (1.15 mg of penta-deuterated molecules) was administered in a healthy volunteer, 34 years old, weighing 61 kg. Samples were collected at specified time points after injection. The ratio of deuterium-labeled cholestenoic acid to unlabeled compound was determined in each sample.

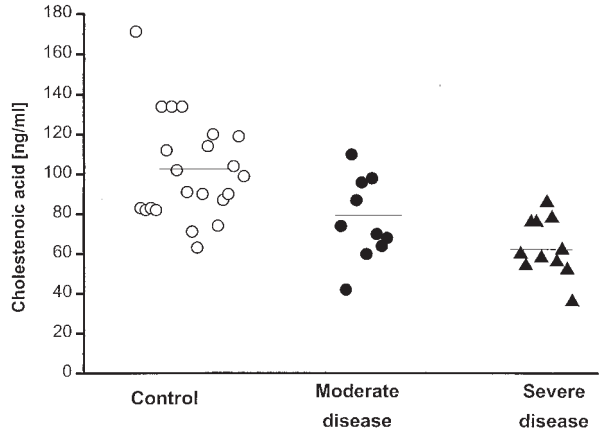

Fig. 5. Effect of pulmonary diseases on circulating cholestenoic acid. Samples were collected from 22 healthy volunteers, males and females, with a mean age of 38 years (open circles). Ten patients with either tuberculosis or sarcoidosis were taken to represent moderate lung diseases, all with forced expiratory lung volume greater than 1 L/second (closed circles). Eleven patients with advanced emphysema were taken to represent severe lung diseases (triangles). These patients had forced expiratory volume of less than 0.9 L/second with severe hypoxia and were under oxygen supplementation.

compound. This is in reasonable agreement with the findings above after exclusion of the lung.

Effect of pulmonary diseases on levels of circulating cholestenoic acid

To see whether different pulmonary diseases could have an effect on the circulating levels of cholestenoic acid, blood samples were collected from 22 patients with different lung diseases. As shown in **Fig. 5**, a group of patients with parenchymal/interstitial lung diseases with a moderate impairment of lung function had reduced levels of cholestenoic acid in the circulation, 77 ± 22 ng/mL (mean ± SD) as compared with the control group 101 ± 25 ng/mL (mean ± SD). This reduction was significant ($P < 0.001$). Another group of patients with respiratory insufficiency due to chronic airway disease and with markedly reduced lung function (FEV1 < 0.9 L) had even lower levels of cholestenoic acid in the circulation, 67 ± 20 ng/mL which was highly significant ($P = 0.0005$) as compared to the control group.

Correlation between circulating levels of cholestenoic acid and its precursors

It has been reported that there is some correlation between circulating cholesterol and circulating 27-hydroxycholesterol (12). If cholestenoic acid is produced in the lung and not from circulating 27-hydroxycholesterol, then a correlation between cholesterol and cholestenoic acid seems less likely.

The correlation between circulating levels of cholesterol and 27-hydroxycholesterol, between cholesterol and cholestenoic acid, and finally between 27-hydroxycholesterol and cholestenoic acid was studied in 22 volunteers. As shown in **Table 2**, significant positive correlation was found only between cholesterol and 27-hydroxycholesterol as reported previously (12). In 16 of the above volunteers, 3β,7α-dihydroxy-5-cholestenoic acid and 7α-hydroxy-3-oxo-chol-4-enoic acid were also measured. As reported previously (13) there was a positive correlation between cholestenoic acid and 3β,7α-dihydroxy-5-cholestenoic acid. However, no significant correlation was found between cholestenoic acid and 7α-hydroxy-3-oxo-chol-4-enoic acid.

TABLE 2. Correlation analysis of circulating levels of cholesterol and different 27-oxygenated metabolites

Analytes Studied	n	*r*	*P* Value
Cholesterol vs. 27-hydroxycholesterol	22	0.55	0.007
Cholesterol vs. cholestenoic acid	22	0.40	0.06
27-Hydroxycholesterol vs. cholestenoic acid	22	0.36	0.09
Cholestenoic acid vs. 3β,7α-dihydroxy-5-cholestenoic acid	16	0.72	0.001
Cholestenoic acid vs. 7α-hydroxy-3-oxo-chol-4-estenoic acid	16	0.37	0.14
3β,7α-dihydroxy-5-chol-estenoic acid vs. 7α-hydroxy-3-oxo-chol-4-estenoic acid	16	0.36	0.17

Samples were collected from 22 healthy volunteers, males and females, with a mean age of 38 years.

DISCUSSION

Methodological aspects

An accurate determination of the 27-oxygenated oxysterols in the circulation is a prerequisite for the present study. Mass spectrometry with use of deuterium-labeled internal standards (assay of 3β,7α-dihydroxy-5-cholestenoic acid and 7α-hydroxy-3-oxo-cholest-4-stenoic acid) or an internal standard lacking one methylene group (assay of cholestenoic acid) offers sufficient accuracy and precision. In spite of this, the very small arteriovenous difference in concentration of cholestenoic acid in pulmonary artery and vein (4.5 ± 1) requires a relatively large number of patients to be defined with certainty. It is not surprising that two of the nine patients studied had an arteriovenous difference less than 2 ng/mL (Fig. 1). The levels of 7α-hydroxy-5-cholestenoic acid and 7α-hydroxy-3-oxo-cholest-4-stenoic acid obtained in the present study are somewhat higher than reported in some previous studies (13, 14). Most probably this difference is due to the use of less suitable internal standards in the previous studies.

Sterol 27-hydroxylase and flux of 27-oxygenated metabolites of cholesterol to the liver

Sterol 27-hydroxylase is a cytochrome P-450 enzyme that has an important role in the degradation of the side-chain of cholesterol in connection with bile acid biosynthesis (15). The wide distribution of this enzyme suggests, however, that the enzyme may also have a role different from that of bile acid biosynthesis in the liver.

The primary product of the sterol 27-hydroxylase system (Cyp 27) is always 27-hydroxycholesterol when cholesterol is the substrate. In some cells with a high content of the enzyme in relation to available substrate cholesterol, the 27-hydroxycholesterol formed is however immediately

further oxidized into cholestenoic acid. The mechanism behind this three-step oxidation has recently been defined in detail in a reconstituted system containing highly purified human Cyp 27 (16). It was shown that after formation of 27-hydroxycholesterol and its release from cytochrome P-450, there is a competition between this oxysterol and cholesterol for re-entry into the enzyme active site for further oxidation. Under subsaturating conditions, the efficiency of oxidizing 27-hydroxycholesterol to cholestenoic acid is thus greater than the efficiency to oxidize cholesterol to 27-hydroxycholesterol.

Cholestenoic acid is able to leave the cells much faster than 27-hydroxycholesterol and cholesterol. This flux is independent of the presence of lipoproteins, but seems to be facilitated by albumin (5).

Cholestenoic acid may be further metabolized by oxysterol 7α-hydroxylase and 3β-hydroxysteroid dehydrogenase to yield 3β,7α-dihydroxy-5-cholestenoic acid and 7α-hydroxy-3-oxo-cholest-4-stenoic acid (13–15, 17–20). Both the above enzymes are known to be present in a number of different organs and tissues (21–26), and it seems likely that most of 3β,7α-dihydroxy-5-cholestenoic acid and 7α-hydroxy-3-oxo-cholest-4-stenoic acid in the circulation are derived from extrahepatic sources. The latter two metabolites are more polar than 27-hydroxycholesterol and are also taken up more efficiently by the liver (4). Under normal conditions, circulating levels of cholestenoic acid and its two metabolites are closely correlated (13, 14), suggesting a precursor–product relationship. Under conditions of increased bile acid synthesis, induced by treatment with cholestyramine, there is a selective increase in the plasma levels of 7α-hydroxy-3-oxo-4-cholestenoic acid whereas levels of cholestenoic acid and 3β,7α-dihydroxy-5-cholestenoic acid are unaffected (14). This is compatible with a hepatic origin of part of the circulating 7α-hydroxy-3-oxo-cholest-4-stenoic acid under some specific conditions.

In a previous work we showed that the total flux of 27-hydroxycholesterol, cholestenoic acid, and the cholestenoic acid metabolites from extrahepatic sources is about 25 mg/24 h in healthy volunteers (4). If it is assumed that most of these metabolites are further oxidized into bile acids in the liver, this mechanism is responsible for about 4% of the total formation of bile acids.

Theoretically, circulating 27-hydroxycholesterol may be taken up by extrahepatic cells and further oxidized into cholestenoic acid by the sterol 27-hydroxylase system or by a yet undefined dehydrogenase system. No evidence for such a conversion has been presented, however, and the results of the present study do not favor this possibility. The lack of correlation between circulating levels of cholestenoic acid and cholesterol supports the contention that cholestenoic acid and its metabolites are produced by a mechanism utilizing a pool of cholesterol that is different from the bulk of cholesterol in the body.

Quantitative importance of the lung for formation of cholestenoic acid and other 27-hydroxylated products

The exceptionally high capacity of human alveolar macrophages to produce cholestenoic acid (**Fig. 6**), the reported high levels of sterol 27-hydroxylase mRNA in the lung (7), as well as the high content of sterol 27-hydroxylase in the lung (Table 1) led us to investigate the quantitative importance of this specific organ to produce 27-oxygenated products of cholesterol.

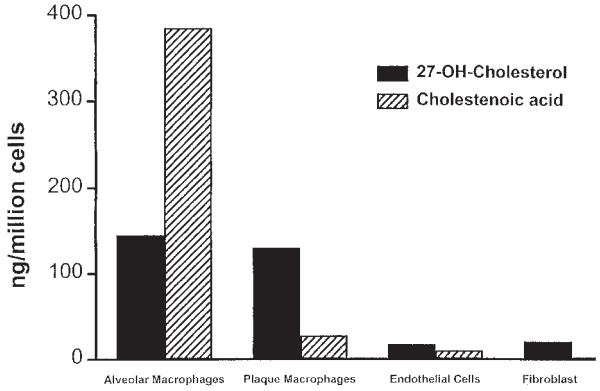

Fig. 6. Production of 27-OH-cholesterol and cholestenoic acid by some different cell types (data from ref. 5). Human alveolar macrophages were isolated from three different lung cancer patients; human monocyte-derived macrophages were from three different healthy volunteers. Endothelial cells were isolated from three different umbilical cords. Human fibroblasts were isolated from one healthy volunteer. All experiments were performed under the same standard conditions in the presence of 10% fetal calf serum in 3 mL of minimum essential medium for 24 h at 37°C as described in reference 5.

Several lines of evidence are presented here to support the contention that most of the cholestenoic acid in the circulation is of pulmonary origin. First, in spite of the very small arteriovenous difference in the concentration of cholestenoic acid in the pulmonary artery and vein, the calculated production of cholestenoic acid by the lung in vivo (14 mg/24 h) was similar to the previously reported elimination of cholestenoic acid and its metabolites by the liver (18 mg/24 h) (4). Second, pulmectomy caused about 50% reduction in the level of both circulating cholestenoic acid and its metabolites. Third, bypassing of the lung for 60 min led to a reduction of circulating cholestenoic acid (about 30%) that fits with a pulmonary origin when taking into account the half-life of cholestenoic acid. Fourth, patients with lung diseases associated with decreased active lung tissue and function had decreased plasma levels of cholestenoic acid.

Some of the above results require comments. We cannot exclude the possibility that the experiments performed under operation may have been disturbed by the anesthetic drugs which may have an effect on the turnover of cholestenoic acid. As both the lung and the heart were bypassed under these operations, part of the reduction in the levels of cholestenoic acid may theoretically be due to the exclusion of the heart. However, the possibility of a significant cardiac production is ruled out by the quantitative study carried out in these patients during the bypass operation. The most clear demonstration of the role of the lung in the production of cholestenoic acid is the results of the pulmectomy. It should be pointed out that

the blood samples obtained after the pulmectomy were collected with the patient in a steady-state condition 2–3 weeks after the operation.

As there is little or no urinary excretion of 27-oxygenated steroids under normal conditions (6), the liver must be the major eliminator of these compounds. **Figure 7** illustrates the importance of the lung in the flux of 27-oxygenated steroids to the liver under normal conditions. In a previous work we showed that the liver takes up about 8 mg 27-hydroxycholesterol and 4 mg of cholestenoic acid per 24 h in healthy volunteers (4). The corresponding figure for the cholestenoic acid metabolites was 14 mg per 24 h (4). In view of the close correlation between cholestenoic acid and its metabolites demonstrated previously (13, 14), most of the cholestenoic acid metabolites are likely to be formed from cholestenoic acid that is formed primarily in the lung. The present result that pulmectomy led to 18% reduction of levels of 27-hydroxycholesterol is consistent with the possibility that up to 30–40% of plasma 27-hydroxycholesterol originates from the lung. In view of the relatively great variations in these results and the low degree of significance ($P = 0.05$), no firm conclusion can be drawn, however. In any case the lung seems to be responsible for at least half of the total flux of 27-oxygenated cholesterol metabolites to the liver.

It is important to emphasize that in spite of our findings that the major part of cholestenoic acid in the circulation originates from the lung, production of this acid from cholesterol may be very important also in other organs and tissues.

We have also demonstrated high contents of sterol 27-hydroxylase in macrophages from other sources than the lung and in atheromas (5, 27). Even if the production of cholestenoic acid from these sources is low from a quantitative point of view, it may be of importance as a defense mechanism against accumulation of cholesterol. The production of cholestenoic acid by the sterol 27-hydroxylase is likely to be more dependent upon the ratio between the amount of enzyme and the amount of cholesterol substrate available than the absolute amount of enzyme. It is evident that such a high ratio between the enzyme and cholesterol must exist in the alveolar macrophages (4).

The high content of 27-hydroxycholesterol in the intestine is in accordance with the high levels of sterol 27-hydroxylase mRNA previously reported in this organ (7). However, whether or not elimination of cholesterol in the form of 27-hydroxycholesterol is of importance in the intestine is not known.

The high content of 27-hydroxycholesterol present in tendons may indicate that the sterol 27-hydroxylase mech-

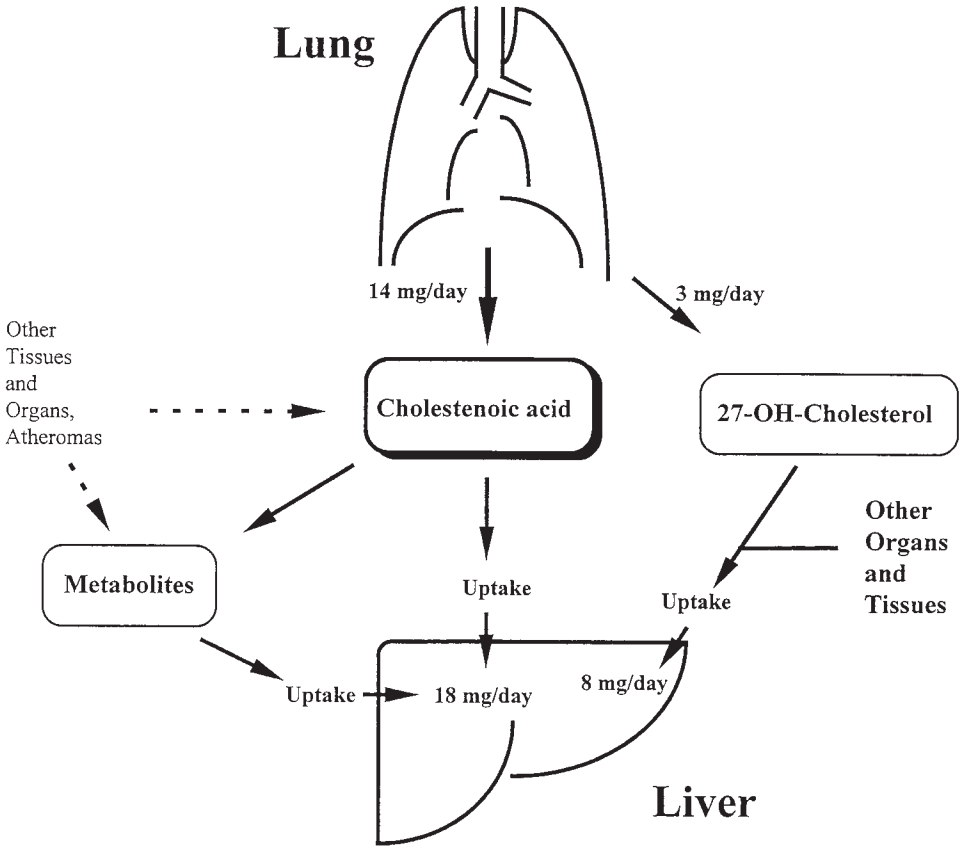

Fig. 7. Importance of the lung for production of 27-oxygenated cholesterol metabolites. The figure illustrates the importance of the lung as the major site for the production of 27-oxygenated steroids in the body and the role of the liver as the major eliminator of these products under normal conditions. The figures for the uptake of 27-oxygenated steroids were obtained from ref. 4.

anism may be important for transport of cholesterol from tendons, and it should be borne in mind that the deficiency of this enzyme in patients with cerebrotendinous xanthomatosis (CTX) leads to development of tendon xanthomas (28).

Cholesterol turnover in the lung and in the surfactant factor: possible role of the sterol 27-hydroxylase

Synthesis of cholesterol in the lung is relatively low and it can be assumed that a considerable part of the cholesterol in the lung originates from the circulation. The high flux of 27-oxygenated steroids from the lung suggests that sterol 27-hydroxylase may be of importance for the overall cholesterol homeostasis in this organ.

Cholesterol is a substantial (approximately 8%) but not an essential component of pulmonary surfactant. The cholesterol in this surfactant is continuously taken up by the alveolar macrophages (29). The very high content of sterol 27-hydroxylase in these cells is compatible with a role for this enzyme in the clearance of cholesterol from the surfactant.

It has clearly been shown that cholesterol modifies the properties of the surfactant factor and results have been presented suggesting that cholesterol has a negative effect on the surfactant surface properties (30, 31). The negative effects occurred at physiological concentrations of cholesterol in the surfactant and these effects could in part be counteracted by increasing the protein concentration. It is well documented that the concentration of cholesterol and the ratio between cholesterol and other lipids in the surfactant vary under different conditions (32–36). The physiological importance of this is, however, difficult to evaluate at the present level of knowledge.

If the activity of sterol 27-hydroxylase is of critical importance for the content of cholesterol in the surfactant factor, and if this content is important for the function of the lung, a lack of this enzyme would be expected to be associated with a respiratory insufficiency. Respiratory problems have been documented in only a minority of patients with cerebrotendinous xanthomatosis, lacking the sterol 27-hydroxylase (for a review see ref. 28). In a few cases of this disease, granoulomatous lesions have been observed in the lung at autopsy. Because patients with CTX have an accelerated degradation of cholesterol, this effect may, however, to some extent compensate for the lack of the sterol 27-hydroxylase and prevent accumulation of cholesterol in the lung surfactant. Further clinical studies on patients with CTX are required to evaluate whether the role of sterol 27-hydroxylase is of some importance for the function of the lung.

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