

TANGIER DISEASE

Report of a Case and Studies of Lipid Metabolism

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Abstract To elucidate the deranged metabolism of Tangier disease, the turnover of esterified cholesterol, and the activities of lipoprotein lipase and of lecithin-cholesterol acyltransferase (LCAT) were studied in a patient with typical findings. The plasma concentration of high-density lipoproteins, assessed by electrophoretic, immunologic and ultracentrifugal means, was greatly reduced. Of a total plasma cholesterol of 59 mg per 100 ml (70 per cent esterified), only 7 mg was present in high-

density lipoprotein. In vivo turnover of plasma esterified cholesterol, measured after injection of radiolabeled mevalonic acid, was 48 mg per hour, similar to values obtained in normal subjects. Post-heparin plasma lipoprotein lipase activity was 0.071 μ moles of free fatty acids released per minute per milliliter of plasma, compared to a normal value of 0.218. LCAT activity was 2.5 μ g of cholesterol esterified per milliliter per hour of incubation, about half the normal value.

TANGIER disease was first described in two children by Fredrickson and his associates¹ in 1961. Ten other cases have since been described.²⁻⁸ The features of the disease are a very low content of high-density lipoprotein (HDL) in the circulating plasma, the accumulation of a large quantity of esterified cholesterol in the macrophages of many tissues,^{1,9} enlarged tonsils having a yellow-orange color, reflecting their high content of esterified cholesterol, frequently elevated plasma triglyceride concentrations and abnormal quantities of circulating chylomicrons in the fasting state. Both parents of affected persons have low plasma concentrations of HDL, indicating that the disorder is transmitted as an autosomal recessive trait.

In the additional case reported below, since disordered metabolism of esterified cholesterol is a feature of the disease, the turnover of this class of lipids in the plasma was measured. Furthermore, HDL may be critically connected with the activity of two enzymes, lecithin-cholesterol acyltransferase (LCAT) and lipoprotein lipase, which are involved respectively in the metabolism of esterified cholesterol and triglyceride in plasma, and these enzymes were therefore studied.

CASE REPORT

A girl, born on November 30, 1955, at full term and weighing 2.3 kg had no apparent physical abnormality at birth. She appeared healthy and fed well, and growth was normal. Walking and the development of speech were also normal. Growth of permanent teeth was irregular, this fact being attributed to the distorting influence of a high arched palate. Between the ages of 5 months and 2 years she had 3 convulsions, each associated with a raised temperature. At the age of 2½ years the tonsils were found to be greatly enlarged and were removed at operation. The surgeon experienced difficulty in securing hemostasis, and during the 24 hours after operation, extensive hemorrhage from the tonsillar beds occurred. Multiple bruises appeared spontaneously on the arms and legs within a few hours of the operation. The platelet count was 108,000, and bleeding and

clotting times were normal; an enlarged spleen was palpable. A blood transfusion was given. Later in childhood, 2 teeth were removed and unusual hemorrhage again occurred. At the age of 6½ years, persistent deafness, thought to be due to eustachian-tube blockage, developed, and adenoidal tissue and tonsillar remnants were removed. The excised tissue had an unusual yellow appearance, microscopy of which revealed increased numbers of large, pale, foamy macrophages scattered diffusely throughout the tissue.

After the 2d operation the patient remained well. Her scholastic achievements were above average. Menstruation had been normal since the menarche in 1968. Occasional spontaneous nosebleeds of small volume had occurred recently. There had been no symptoms of diarrhea or paresthesia.

Abbreviations Used

| | |
|-------|--------------------------------------|
| HDL: | high-density lipoprotein |
| LCAT: | lecithin-cholesterol acyltransferase |
| LDL: | low-density lipoprotein |
| VLDL: | very-low-density lipoprotein |

The patient was 162.5 cm tall and weighed 55.5 kg; nutrition seemed normal. The blood pressure was 105/70. Heart sounds were normal. There was no lymphadenopathy, and the liver and spleen were not palpable. The fundi were normal, as were the corneas on superficial examination. The hard palate was high arched and associated with displacement of the secondary teeth. Small, orange-yellow, solid lesions, 1 cm in diameter, were present on the posterior aspect of the pharynx. Examination of the nervous system revealed no abnormality. Urinalysis was negative for blood, protein, sugar and bile. The Hess test for capillary fragility was negative. A radiograph of the chest and a plain radiograph of the abdomen were normal. Hemoglobin, white-cell count, serum alkaline phosphatase, serum glutamic oxaloacetic transaminase, prothrombin time, thrombin clotting time and plasma fibrinogen were also normal. Thromboplastin generation was only 56 per cent of normal. The platelet count was 82,000. The low platelet count and reduced thromboplastin generation were not evaluated further.

The father, 47, and the mother, 38 years of age, were well. The mother's maternal grandmother and the patient's father shared the same surname, but no consanguinity between the 2 families was known. The families of both father and mother were of Scottish origin. General medical examination of the patient's father and mother was negative.

METHODS

Plasma Lipids and Lipoproteins

Blood was taken in the fasting state with diso-

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dium EDTA as anticoagulant, and the plasma lipoproteins HDL, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) were separated by the preparative method of Fredrickson and his co-workers¹⁰; p-chloromercuriphenylsulfonate was added to the plasma in final concentration of 2 mM to inhibit the activity of LCAT during handling of the plasma.¹¹ The content of cholesterol¹² and of triglyceride¹³ was measured in each lipoprotein fraction. Plasma was submitted to electrophoresis on 1 per cent agarose after prestaining of the lipoproteins with Sudan Black B.¹⁴ Plasma from the patient and both her parents was tested against antihuman HDL antiserum (Behringwerke) in 1 per cent agarose by the method of double diffusion.

Esterified Cholesterol Turnover in Vitro

Approximately 140 μ c of ³H-DL-mevalonic acid* (Radiochemical Centre, Amersham) was injected intravenously into the patient and into each of her parents, and the specific activity of unesterified and esterified cholesterol in whole plasma and in HDL, LDL and VLDL was measured at frequent intervals after injection for as long as 72 hours. Specific activity-time curves were constructed, and the turnover of esterified cholesterol was then calculated with the method of Nestel and Monger.¹⁵

Plasma LCAT Activity (Rate of Cholesterol Esterification In Vitro)

The method of Glomset and Wright was used.¹⁶ Plasma samples of the patient with Tangier disease and her parents and a normal person's plasma were heated at 56°C for 45 minutes to abolish enzyme activity and used as the source of substrate lipoproteins.

Post-Heparin Lipoprotein Lipase Activity

Two methods were used: that of Fredrickson et al.¹⁷ and that of Boberg and Carlson.¹⁸ Heparin was given intravenously to the patient, her father and a normal person in a dose of 0.1 mg per kilogram of body weight. Blood was taken 10 and 20 minutes after injection and immediately chilled, and the plasma frozen at -10°C until ready for use as the source of enzyme.

RESULTS

Plasma Lipids and Lipoproteins

The concentrations of cholesterol and triglyceride in whole plasma and in individual lipoproteins are shown in Table 1. The cholesterol content of the whole plasma of the patient was 59 mg per 100 ml (70 per cent esterified), and of this only 7 mg per 100 ml was in the HDL fraction, about 1/8 the normal value for females.¹⁹ The cholesterol content of the HDL in both parents was lower than normal.²

*D-mevalonic acid is the biologic isomer, and the radioactivity associated with the L-isomer is excreted in the urine.

The plasma triglyceride concentration for the patient was at the extreme upper limit of the normal range expected for a person of her age and sex.¹⁹ When plasma from the patient was subjected to electrophoresis on 1 per cent agarose, no alpha 1 (high-density) or prebeta (very-low-density) lipoprotein was seen. No precipitin line was seen when plasma from the patient was diffused against anti-HDL antiserum in agarose. Precipitin lines were seen when the parents' and a normal person's plasma were used.

Esterified Cholesterol Turnover in Vivo

Specific activity time curves for esterified and unesterified cholesterol in whole plasma are shown in Figure 1. Similar kinds of curves were obtained for LDL and VLDL. The curves for HDL obtained for each parent are shown in Figure 2. The HDL curves for the patient could not be obtained because of low levels of radioactivity in the HDL cholesterol.

The steepness of the rise of a given specific activity-time curve for esterified cholesterol is an esti-

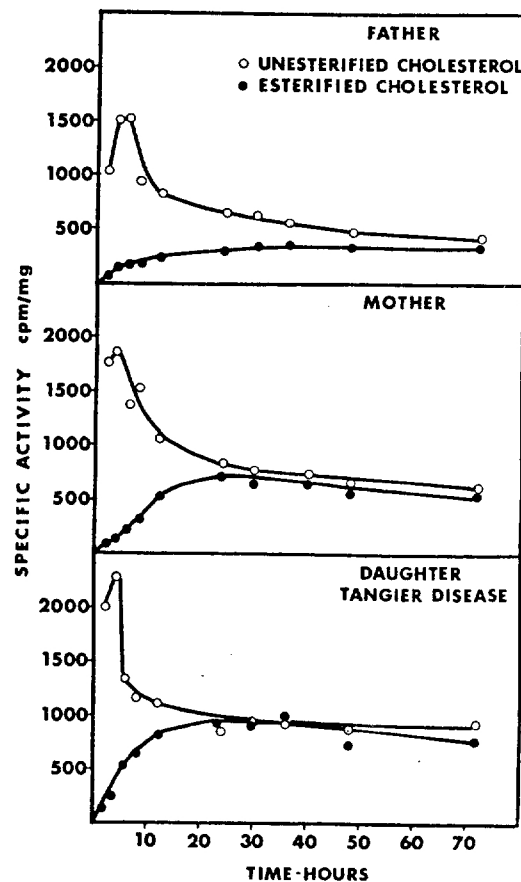


Figure 1. Specific Activity-Time Curves for Unesterified and Esterified Cholesterol in the Whole Plasma of the Patient and Her Parents after an Intravenous Injection of ³H-DL-Mevalonic Acid.

Table 1. Cholesterol and Triglyceride Content of Lipoprotein Fractions Derived from Whole Plasma.

| SUBJECT | AGE (Yr) | CHOLESTEROL CONTENT (MG/100 ML) | | | | TRIGLYCERIDE CONTENT (MG/100 ML) | | | |
|--------------------|----------|---------------------------------|-----|-----|------|----------------------------------|-----|-----|------|
| | | WHOLE PLASMA | HDL | LDL | VLDL | WHOLE PLASMA | HDL | LDL | VLDL |
| Father | 47 | 167 (75% esterified) | 31 | 113 | 23 | 86 | 14 | 30 | 42 |
| Mother | 38 | 200 (71% esterified) | 26 | 151 | 23 | 88 | 37 | 12 | 39 |
| Daughter (patient) | 15 | 59 (70% esterified) | 7 | 47 | 5 | 136 | 7 | 49 | 80 |
| Male control | 31 | 166 | 60 | 98 | 8 | 56 | 16 | 23 | 17 |

mate of the turnover rate* of the esterified cholesterol pool in question. A measure of the turnover rate and turnover of the plasma esterified cholesterol pool can be obtained by means of the method of calculation used by Nestel and Monger¹⁵ and Moutafis and Myant.²⁰ The values are shown in Table 2 for the whole-plasma esterified cholesterol pool and for individual plasma lipoprotein pools. Since a direct measure of the turnover rate of

low value of 1.7 μg was also obtained for the patient when plasma from a normal person was used as substrate, indicating that the additional lipoprotein substrate provided did not enhance the rate of esterification.

Post-Heparin Lipoprotein Lipase Activity

The results are shown in Table 4. Maximum values for lipoprotein lipase were seen in each case at

Table 2. Turnover of Esterified Cholesterol in Vivo.

| SUBJECT | ESTERIFIED CHOLESTEROL POOL (MG) | | | | WHOLE PLASMA | TURNOVER RATE (HR ⁻¹) | | | WHOLE PLASMA | TURNOVER (MG/HR) | | |
|---------|----------------------------------|------|------|-----|--------------|-----------------------------------|-------|--------|--------------|------------------|-----|-----|
| | WHOLE PLASMA | VLDL | LDL | HDL | | VLDL | LDL | HDL | | VLDL | LDL | HDL |
| Father | 2913 | 189 | 1872 | 558 | 0.015 | 0.022 | 0.026 | 0.024 | 44 | 4 | 49 | 13 |
| Mother | 4213 | 229 | 3218 | 465 | 0.022 | 0.021 | 0.029 | 0.022 | 93 | 5 | 93 | 10 |
| Patient | 639 | 90 | 537 | 12* | 0.075 | 0.039 | 0.043 | 1.750* | 48 | 4 | 23 | 21 |

*Estimate.

esterified cholesterol in HDL could not be obtained in the patient with Tangier disease, an estimate was made by subtracting the values for the turnover for LDL and VLDL from that of whole plasma.¹⁵ Turnover rate for HDL was then calculated by division of turnover by the pool size.

Table 2 shows that the turnover rates for esterified cholesterol in the patient's whole plasma and in the individual lipoproteins were higher than the corresponding values for her parents. The difference is particularly striking for the HDL, in which the turnover rate for the Tangier patient was estimated at 1.750 per hour. However, the turnover of esterified cholesterol in whole plasma, 48 mg per hour, was similar to values reported by Nestel²¹ in normal persons. The turnover for the patient's HDL esterified cholesterol appears higher than for her parents, but it should be emphasized that this value was calculated indirectly and that the true magnitude of difference between the parents and the patient is uncertain. On the other hand, the turnover in the daughter's LDL is considerably less than that in her parents.

LCAT Activity

The results are shown in Table 3. The value of 2.5 μg of cholesterol esterified per milliliter of active plasma per hour of incubation was obtained for the patient when her own plasma was used as substrate; this was much lower than the values obtained for her parents and for a normal person. A

low value of 1.7 μg was also obtained for the patient when plasma from a normal person was used as substrate, indicating that the additional lipoprotein substrate provided did not enhance the rate of esterification.

DISCUSSION

The case reported satisfies the requirements for

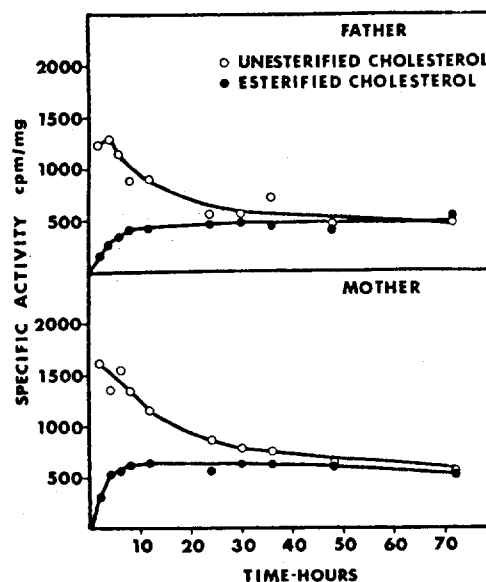


Figure 2. Specific Activity-Time Curves for Unesterified and Esterified Cholesterol in the HDL of the Parents of the Patient after an Intravenous Injection of ³H-Mevalonic Acid.

*Turnover rate is the fraction of the plasma pool replaced per hour.

Table 3. LCAT Activity (in Vitro Cholesterol Esterification Rate)

| ACTIVE PLASMA | SOURCE OF SUBSTRATE | ESTERIFICATION RATE* |
|---------------------------|---------------------|----------------------|
| A: | | |
| Normal person | Normal person | 4.5 ± 0.4 |
| Father | Father | 4.1 ± 0.1 |
| Mother | Mother | 7.0 ± 0.3 |
| Patient | Patient | 2.5 ± 0.2 |
| B: | | |
| Father | Normal person | 4.5 ± 0.1 |
| Mother | Normal person | 6.1 ± 0.3 |
| Patient (Tangier disease) | Normal person | 1.7 ± 0.3 |

* μg of cholesterol esterified/ml of fresh test plasma/hr of incubation (mean \pm SD).

the diagnosis of Tangier disease in the following respects: a low total plasma cholesterol in the patient with a normal proportion esterified; near absence of HDL from the plasma as assessed by ultracentrifugal, electrophoretic and immunologic techniques; a high plasma triglyceride concentration but an absence of pre-beta migrating VLDL on electrophoresis; and a low HDL cholesterol concentration in the plasma of both parents.

In the present case lymphoid tissue was not directly analyzed for esterified cholesterol content. However, tissue of a yellow-orange color observed in the pharynx of our patient was similar in appearance to lesions in the pharynx described in other tonsillectomized patients with Tangier disease.^{3,5} Also, microscopical examination of adenoidal tissue in our patient showed large numbers of pale macrophages with foamy cytoplasm assumed to contain lipid material but not proved to be esterified cholesterol. The patient had a low platelet count on two occasions, as previously described by Hoffman and Fredrickson³ and by Kummer et al.⁶ in this disease. The explanation for her episodes of unusual bleeding is unknown.

The turnover of esterified cholesterol in the whole plasma for the patient and her father was normal,²¹ and therefore the pathogenesis of the deposition of esterified cholesterol in macrophages cannot be linked with abnormal plasma turnover. The normal turnover in the patient was brought about by a high turnover rate in all lipoproteins, especially in the HDL. Furman et al.²² found that labeled human HDL disappeared more rapidly from the circulation when the HDL pool size was reduced.

Table 4. Plasma Lipoprotein Lipase Activity 10 and 20 Minutes after Heparin.

| SUBJECT | LIPOPROTEIN LIPASE ACTIVITY* | | | |
|---------------|------------------------------|-----------|-----------------------|-----------|
| | COCONUT OIL SUBSTRATE† | | INTRALIPID SUBSTRATE‡ | |
| | at 10 min | at 20 min | at 10 min | at 20 min |
| Normal person | 0.218 | 0.160 | 0.080 | 0.021 |
| Father | 0.234 | 0.201 | 0.111 | 0.061 |
| Patient | 0.071 | 0.040 | 0.033 | 0.009 |

* μmoles of free fatty acids released/ml of plasma/min of incubation.

†In the form of Ediol; method of Fredrickson et al.¹⁷

‡Method of Boberg & Carlson.¹⁹

One of the factors thought to regulate the turnover of esterified cholesterol in plasma is the enzyme LCAT, the activity of which has been associated with the presence of HDL in the plasma.^{23,24} Patients with genetic deficiency of LCAT also have low plasma HDL concentrations.^{25,26} The activity of LCAT has been positively correlated with the concentration of unesterified cholesterol in the plasma.²⁷ In our patient, low HDL concentration or low unesterified cholesterol concentration, or both, may have contributed to the low LCAT activity. However, the LCAT activity in the patient's plasma did not rise when additional substrate was provided by use of normal plasma, suggesting that the enzyme concentration itself was diminished. Because in vivo turnover of total plasma esterified cholesterol was normal, it seems likely that factors in addition to LCAT are responsible for the turnover of esterified cholesterol in the plasma.

The postheparin lipoprotein lipase activity of our patient's plasma was found to be much lower than that in her father and in a normal person. Low plasma postheparin lipase activity has previously been described in a patient with LCAT and HDL deficiency.²⁶ Lipoprotein lipase appears to require HDL, or at least one apoprotein common to HDL and VLDL as a cofactor, to achieve maximal activity,²⁸⁻³³ and the low enzyme activity in the plasma of our patient with Tangier disease is consistent with this view. The frequently observed increase in the plasma triglycerides in patients with Tangier disease may be related to diminished lipoprotein lipase activity.

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