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### Research Article

Tangier disease is a rare familial disorder characterized by extremely low levels of apolipoprotein A-I (apoA-I) and high density lipoproteins (HDL). In normal subjects, proapoA-I is secreted into plasma and converted to mature apoA-I by the cleavage of the amino-terminal six amino acids with the major isoprotein in plasma being mature apoA-I. In contrast, in Tangier disease there is a marked relative increase of proapoA-I as compared with mature apoA-I. ProapoA-I and mature apoA-I were isolated from normal and Tangier disease subjects, radio-labeled, and autologous apoA-I isoproteins injected into normal and Tangier subjects. The in vivo catabolism and conversion of proapoA-I and mature apoA-I in normal and Tangier disease subjects were quantitated. A comparison of the rate of catabolism of apoA-I isoproteins from plasma revealed a significantly faster rate of catabolism of both isoproteins of apoA-I in Tangier subjects when compared with normal subjects. The fractional conversion rate of proapoA-I to mature apoA-I in normal subjects and 3.6 d-1 in Tangier subjects. The results indicate that (a) apoA-I enters plasma as the pro isoprotein in both normal and Tangier subjects, (b) Tangier disease subjects have a normal fractional rate of conversion of proapoA-I to mature apoA-I, (c) proapoA-I is catabolized at the same rate as mature apoA-I in Tangier subjects, and (d) Tangier subjects catabolize both pro [...]



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## In Vivo Metabolism of Proapolipoprotein A-I in Tangier Disease

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#### Abstract

Tangier disease is a rare familial disorder characterized by extremely low levels of apolipoprotein A-I (apoA-I) and high density lipoproteins (HDL). In normal subjects, proapoA-I is secreted into plasma and converted to mature apoA-I by the cleavage of the amino-terminal six amino acids with the major isoprotein in plasma being mature apoA-I. In contrast, in Tangier disease there is a marked relative increase of proapoA-I as compared with mature apoA-I. ProapoA-I and mature apoA-I were isolated from normal and Tangier disease subjects, radiolabeled, and autologous apoA-I isoproteins injected into normal and Tangier subjects. The in vivo catabolism and conversion of proapoA-I and mature apoA-I in normal and Tangier disease subjects were quantitated. A comparison of the rate of catabolism of apoA-I isoproteins from plasma revealed a significantly faster rate of catabolism of both isoproteins of apoA-I in Tangier subjects when compared with normal subjects. The fractional conversion rate of proapoA-I to mature apoA-I was 3.9  $d^{-1}$  in normal subjects and 3.6  $d^{-1}$  in Tangier subjects. The results indicate that (a) apoA-I enters plasma as the pro isoprotein in both normal and Tangier subjects, (b) Tangier disease subjects have a normal fractional rate of conversion of proapoA-I to mature apoA-I, (c) proapoA-I is catabolized at the same rate as mature apoA-I in Tangier subjects, and (d) Tangier subjects catabolize both pro and mature apoA-I at a much greater rate than do normal subjects. Therefore, the relative increase in proapoA-I in Tangier disease is due to a marked decrease in mature apoA-I resulting from rapid catabolism of both pro- and mature apoA-I and not to defective conversion of proapoA-I to mature apoA-I.

#### Introduction

Tangier disease is a rare familial syndrome characterized by hypocholesterolemia, moderate hypertriglyceridemia, abnormal chylomicron remnants, and plasma concentrations of HDL-cholesterol as well as apoA-I and apoA-II of  $\sim 1-5\%$  of normal values (1). Clinically, these patients have enlarged orange tonsils, hepatosplenomegaly, lymphadenopathy, intermittent peripheral neuropathy, and there is a possible increased incidence of premature atherosclerotic vascular disease (2). Initial metabolic studies from our laboratory established that Tangier subjects were able to synthesize apoA-I at a normal rate and the deficiency of plasma apoA-I was due to accelerated catabolism (3, 4).

lational proteolytic cleavage to proapoA-I, which is secreted into human plasma or lymph (apoA-I+1,+2 isoforms) still containing the six amino acid propeptide attached to the aminoterminus of mature apoA-I (5, 6). In normal subjects proapoA-I is converted in plasma to mature apoA-I (apoA- $I_{0,-1,-2}$ isoforms) by a specific calcium requiring peptidase (7-9). ApoA-I<sub>+2</sub> and apoA-I<sub>o</sub> are the major pro and mature isoforms, respectively. In addition, there is a very slow loss of 1 or 2 positive changes from proapoA-I and mature apoA-I<sub>o</sub> without proteolysis to generate the respective minor isoforms, apoA-I+1 and apoA-I<sub>-1,-2</sub> (9). Human proapoA-I is a relatively minor isoprotein in plasma, comprising  $\sim 5\%$  of the total circulating apoA-I. Patients with Tangier disease have a marked increase in plasma of proapoA-I relative to mature apoA-I (10). This observation led to the speculation that the biochemical abnormality in Tangier disease is a defect in the conversion of proapoA-I to mature apoA-I, resulting in rapid catabolism of proapoA-I and low plasma apoA-I levels (5, 10, 11). We have subsequently demonstrated that Tangier plasma can convert proapoA-I to mature apoA-I at a normal rate in in vitro experiments (8); the reason, however, for the relative increase in proapoA-I in Tangier disease subjects remained unknown. Therefore, in order to elucidate the kinetic etiology of the altered apoA-I isoform pattern in Tangier disease, we have performed metabolic studies with purified proapoA-I and mature apoA-I in normal subjects and patients with Tangier disease. Methods Study subjects. The study subjects included two normal volunteers and two homozygous Tangier disease subjects including a member (subject 2) from the initial family described with this disease (12). The results for the normal subjects have been previously published (9). All of the

The precursor of human apoA-I, preproapoA-I, is a 267

amino acid protein containing 24 additional amino acids at-

tached to the amino-terminal end of the mature apoA-I. 18

amino acids are contained within the prepeptide followed by a

six amino acid propeptide. PreproapoA-I undergoes cotrans-

for the normal subjects have been previously published (9). All of the subjects had normal hepatic, renal, and endocrine function and were in good health at the time of the study. The study protocol was approved by the Institute Review Board of the National Heart, Lung, and Blood Institute and informed consent was obtained from each subject. The studies were performed while the subjects were inpatients on the metabolic ward of the Clinical Center of the National Institutes of Health. Starting 10 d before the study, the subjects began an iso-weight diet containing 16% protein, 42% fat, 42% fat (polyunsaturated/saturated fat ratio of 0.2), and 200 mg cholesterol/1,000 kcal. Subjects were given potassium iodide (0.9 g/d) in divided doses beginning 1 d before the injection of the radiolabeled apolipoproteins and continuing for the duration of the study to inhibit thyroid uptake of radioactivity.

Quantification and isolation of plasma lipids, lipoproteins, and apolipoproteins. Blood was collected after a 12-h fast in EDTA (0.1 mg/ml) and the plasma separated by centrifugation. Thoracic duct lymph was obtained from patients undergoing thoracic duct drainage and lymph chylomicrons were obtained by centrifugation at d = 1.006 g/ml for 0.5 h at 27,000 rpm in a 60-Ti rotor in an ultracentrifuge (both

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from Beckman Instruments, Inc., Palo Alto, CA). Lipoproteins from Tangier patients were obtained by ultracentrifugation of plasma (53,000 rpm, 4°C), at 1.21 g/ml in a 60 Ti rotor for 18 h. Normal HDL were isolated by ultracentrifugation of plasma at d 1.063-1.21 g/ml using KBr for density adjustment as previously described (13). The protein content was determined by the method of Lowry et al. (14). Plasma lipid and lipoprotein cholesterol values were quantitated as previously described (9). Apolipoproteins were quantitated by radial immunodiffusion (15). The apolipoprotein A-I isoproteins were isolated as previously described (9, 16, 17). Briefly normal proapoA-I (apoA-I+2 isoform) and mature apoA-I (apoA-I0 isoform) were isolated from lymph lipoproteins (d < 1.006 g/ml) and HDL, respectively, by preparative isoelectrofocusing. Total apoA-I<sub>Tangier</sub> was purified from delipidated lipoproteins (d < 1.21 g/ml) by gel permeation chromatography and proapoA-I<sub>Tangier</sub> as well as mature apoA-I<sub>Tangier</sub> isolated by preparative isoelectrofocusing (17).

Radioiodination of apolipoprotein A-I isoproteins. Purified normal and Tangier isoproteins (50–100  $\mu$ g) were dissolved in 25–50  $\mu$ l of 6 M guanidine-HCl, 1 M glycine (pH 8.50) and iodinated with <sup>125</sup>I or <sup>131</sup>I (New England Nuclear, Boston, MA) by the iodine monochloride method (18, 19). Less than 1 mol of iodine was incorporated per 2 mol of isoprotein with the efficiency of iodination being assessed by precipitation of protein with 20% trichloroacetic acid.

Recombination of radiolabeled apoA-I isoproteins with lipoproteins. Autologous radiolabeled apoA-I isoproteins were incubated with 35 ml of normal or Tangier plasma containing EDTA for 60 min at room temperature, the density adjusted to 1.21 g/ml using solid KBr, and the samples centrifuged. Tangier plasma containing radiolabeled Tangier apoA-I isoproteins was centrifuged in a 60 Ti rotor at 53,000 rpm, 4°C, for 18 h, and normal plasma containing radiolabeled apoA-I isoproteins was centrifuged in a 60 Ti rotor at 59,000 rpm, 4°C, for 24 h. The 1.21 g/ml supernate lipoproteins were isolated by tube slicing and the unbound iodide removed by dialysis against 150 mM NaCl, 0.01% EDTA, and 0.1 M Tris-HCl (pH 7.4). All preparations were filtered (0.22 micron filters; Millipore Corp., Bedford, MA) and tested for pyrogenicity, and sterility. Radiolabeled apoA-I reassociated with lipoproteins were analyzed by analytical isoelectrofocusing with unlabeled apoA-I standards to ascertain the electrophoretic position of the apoA-I isoforms (9).

Electrophoretic methods. Analytical two-dimensional gel electrophoresis was conducted as previously described (20). Isoelectrofocusing was performed at 250 V for 26 h with 3% ampholines (pH 5.0–6.0) followed by NaDodSO<sub>4</sub> slab gel electrophoresis (15% acrylamide, 0.085% bisacrylamide). The relative proportion of the different isoforms of apoA-I on the two-dimensional gel was quantitated as previously reported (9) by densitometric scanning of the gel.

Preparative isoelectrofocusing was conducted with 300–600  $\mu$ g of protein per well (5 well gels) on 3 × 100 mm slab gels, using either 1.21 g/ml supernate apolipoproteins (Tangier plasma), chylomicron apolipoproteins (lymph), or HDL apolipoproteins (normal plasma) (17, 21). Lyophilized apolipoprotein samples were dissolved in aqueous 10 M urea and fractionated by isoelectrofocusing on a dual slab cell (Bio-Rad 220; Bio-Rad, Richmond, CA) with a 5.0–6.0 pH gradient (Serva Ampholines; Serva AB, Heidelberg, FRG) using gels containing 7.5% acrylamide and 0.2% bisacrylamide. The upper tray buffer was 0.02 M NaOH and the lower buffer 0.01 M H<sub>3</sub>PO<sub>4</sub>. The gels were electrophoresed for 18 h at 250 V, the isoprotein bands visualized from a portion of the gel with Coomassie Blue G-25, and the isoprotein bands cut from the unstained gel, eluted, and concentrated as previously described (21).

Metabolic studies. Two normal subjects were injected simultaneously with 100  $\mu$ Ci of normal <sup>125</sup>I proapoA-I and 25  $\mu$ Ci of normal <sup>131</sup>I mature apoA-I. Similarly, 100  $\mu$ Ci of <sup>125</sup>I proapoA-I<sub>Tangier</sub> and 25  $\mu$ Ci of <sup>131</sup>I mature apoA-I<sub>Tangier</sub> were injected in two homozygous Tangier disease subjects. The studies were performed for 14 d in normal subjects, and for 2 and 3 d in the Tangier patients. In normal individuals, blood samples were obtained in 0.1% EDTA just before injection of radiolabeled apoA-I, and after injection at 10 min, 1 h, 3 h, 6 h, 12 h, 24 h, 36 h, d 2, d 3, d 5, d 7, d 9, d 11, and d 14. In Tangier patients blood samples were obtained prior to the injection, and at 10 min, 1 h, 2 h, 4 h, 6 h, 12 h, 18 h, 24 h, 36 h, 48 h, and 72 h after injection of radiolabeled lipoproteins. The radioactivities in plasma, normal HDL (d = 1.063 - 1.21 g/m), and the Tangier 1.21 g/ml supernate lipoproteins were quantified in an autogamma counter (Packard Model 560; Packard Instrument Co., Inc., Downers Grove, IL). Desalted aliquots of HDL or 1.21 g/ml supernate lipoproteins were lyophilized, delipidated with chloroform methanol (2:1), and the pellets dried under a stream of nitrogen. The protein was resolubilized in 10 M urea and ampholines, and the apoA-I isoforms separated by preparative isoelectrofocusing (9). The gel segments corresponding to the individual apoA-I isoforms (apoA- $I_{+2}$  to apoA- $I_{-2}$ ) were cut and the radioactivity quantitated to determine the relative distribution of radioactivity among the apoA-I isoproteins. The sum of the radioactivity recovered in the five apoA-I isoforms was 75-85% of the initial total counts at each time point. The plasma residence times of the apoA-I isoproteins were determined by computer assisted multiexponential curve fitting using the SAAM27 computer program. The apoA-I production rates were calculated from the following formula: production rate = (apoA-I concentration  $\times$  plasma volume)/(weight  $\times$  residence time).

#### Results

Characterization of plasma lipoproteins and apolipoproteins. Plasma lipid and lipoprotein cholesterol concentrations of all study subjects are shown in Table I. As has been previously noted, plasma total cholesterol, HDL cholesterol, and LDL cholesterol values were lower in Tangier homozygotes than in normal subjects while VLDL cholesterol as well as plasma triglyceride values were higher in Tangier homozygotes than in normal subjects.

The plasma apoA-I isoforms from a normal subject and Tangier patient are illustrated in Fig. 1. In normal subjects the predominant apoA-I isoforms in plasma are the mature apoA-I isoproteins (apoA- $I_{0,-1}$  isoforms). ProapoA-I migrates in the apoA- $I_{+1}$  and apoA- $I_{+2}$  isoform positions and is only a relatively minor isoprotein in normal plasma; however, it is markedly increased in thoracic duct lymph after fat feeding (22). The isoform pattern of apoA-I<sub>Tangier</sub> contains approximately equal quantities of pro and mature apoA-I<sub>Tangier</sub>. Purified normal, as well as Tangier, pro- and mature isoproteins migrated as single electrophoretic bands in NaDodSO<sub>4</sub> electrophoresis and polyacrylamide electrophoresis at pH 8.9 as previously described (17). In addition to the electrophoretic evaluation, the purity and type of apoA-I isoprotein was also established by Edman amino terminal analysis of the isolated isoproteins (17).

Recombination of radiolabeled apoA-I isoproteins with lipoproteins. Each radiolabeled apoA-I isoprotein was recombined with plasma lipoproteins before injection, and the free apoA-I separated from lipoprotein bound apoA-I by ultracentrifugation at 1.21 g/ml. For the normal proapoA-I and mature apoA-I, 82% and 84% recombined with plasma lipoproteins, respectively (mean of 2 values each), while 73% of proapoA-I<sub>Tangier</sub> and 83% of mature apoA-I<sub>Tangier</sub> recombined with plasma lipoproteins.

Metabolism of proapoA-I and mature apoA-I in normal and Tangier disease subjects. The plasma decay curves of radioiodinated normal proapoA-I and mature apoA-I in a normal subject are illustrated in Fig. 2. The figure depicts the whole plasma decay curves without fractionation into lipoproteins or apolipoprotein isoforms. From this it is apparent that the overall fractional catabolic rate of proapoA-I and its meta-

	Table I.	<b>Characteristics</b>	of the	Study	, Sub	jects
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Subjects				Weight	Plasma triglyceride	Cholesterol			
	Age	Sex	Height			Plasma	VLDL	LDL	HDL
	yr		ст	kg	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl
Normal									
1	23	F	167	59	59	182	8	88	86
2	20	Μ	180	71	69	232	10	146	76
Tangier disease									
1	62	Μ	178	76	143	59	16	39	4
2	30	F	175	76	384	55	25	28	2

bolic product, mature apoA-I, is the same as mature apoA-I in normal subjects. The plasma decay curves of proapoA-I and mature apoA-I are very similar because normal subjects rapidly convert virtually all of the proapoA-I to mature apoA-I, and the mature apoA-I derived from proapoA-I is catabolized at the same rate as the radiolabeled mature apoA-I.

In order to quantitate the catabolism of both proapoA-I<sub>Tangier</sub> and mature apoA-I<sub>Tangier</sub>, and the conversion of proapoA-I to mature apoA-I in Tangier disease subjects, the respective isoproteins of apoA-I were isolated from Tangier disease subjects, radioiodinated, reassociated with lipoproteins from the Tangier disease subjects, and injected into these subjects. Fig. 3 depicts the plasma decay curves of proapoA-I<sub>Tangier</sub> and mature apoA-I<sub>Tangier</sub> injected into a Tangier disease subject. The decay curves are very similar indicating that both proapoA-I<sub>Tangier</sub> and its metabolic product, mature apoA-I<sub>Tangier</sub>, are both catabolized from plasma at a similar fractional catabolic rate. If this was not the case, these two curves would not be virtually superimposable. Compared to apoA-I in normal subjects, both proapoA-I and mature apoA-I in Tangier disease subjects were catabolized at a much greater rate than the corresponding apoA-I isoproteins in normal subjects.

The kinetics of conversion of proapoA- $I_{Tangier}$  to mature apoA- $I_{Tangier}$  for one of the Tangier disease subjects studied is

illustrated in Fig. 4. ProapoA-I<sub>Tangier</sub> and mature apoA-I<sub>Tangier</sub> were injected into this subject with Tangier disease, the d < 1.21 g/ml lipoproteins isolated from plasma at various time intervals, the apoA-I isoproteins isolated by isoelectrofocusing gel electrophoresis, and the quantity of radioactivity in each isoform band quantitated in a gamma counter. The proapoA-I isoprotein was catabolized at an extremely rapid rate with both direct catabolism from plasma and conversion to mature apoA-I. Mature apoA-I<sub>Tangier</sub> derived from proapoA-I<sub>Tangier</sub> was catabolized at the same rate as the injected radiolabeled mature apoA-I<sub>Tangier</sub>. These results are qualitatively very similar to the results in normal subjects but quantitatively the fractional catabolic rates of both proapoA-I and mature apoA-I are much faster in the Tangier disease subjects.

The kinetics of apoA-I synthesis, conversion, and catabolism in normal and Tangier disease subjects were quantitated, and the results are summarized in Table II. ApoA-I is secreted into plasma as the proapoA-I isoprotein with little or no direct catabolism of proapoA-I in normal plasma, and it is converted to mature apoA-I with a  $t_{1/2}$  of conversion of  $\sim 4$  h. In the Tangier disease subjects, there is extremely rapid direct catabolism of both proapoA-I and mature apoA-I with a plasma residence time < 5% of apoA-I in normal subjects. In contrast, the  $t_{1/2}$  of conversion of proapoA-I to mature apoA-I in the



Figure 1. Two-dimensional plasma gel electrophoretogram of apoA-I. Panel (a), apoA-I from a normal subject; panel (b), apoA-I from a patient with Tangier disease. ProapoA-I is apoA-I isoforms +1 and +2 while mature apoA-I is isoforms 0, -1, and -2. Only the portion of the two-dimensional

plasma gel containing the apoA-I isoforms is shown in the gel. Panels (c) and (d) are two-dimensional electrophoretograms of purified proapoA-I and mature apoA-I, respectively, from the plasma of the patient with Tangier disease.



Figure 2. Plasma catabolic decay curves of radioiodinated normal proapoA-I ( $\bullet$ ) and mature apoA-I ( $\bullet$ ) injected into a normal subject. The curve depicts the total plasma decay curve without separation of the individual apoA-I isoforms.



Figure 3. Plasma catabolic decay curves of proapoA-I ( $\Box$ ) and mature apoA-I ( $\triangle$ ) isolated from a subject with Tangier disease, radioiodinated, and injected into a Tangier disease subject. The curves depict the total plasma decay curve without separation of the individual apoA-I isoforms.

Tangier disease subjects was the same as in normal subjects, and the synthesis rates of proapoA-I (i.e., newly secreted apoA-I) were similar to normal. Even though the fractional rate of conversion of proapoA-I to mature apoA-I was normal in these subjects, the absolute amount of proapoA-I converted was decreased. This was because  $\sim 50\%$  of the proapoA-I was directly catabolized from plasma before it was converted to mature apoA-I. Therefore, Tangier disease subjects had a normal apoA-I synthesis rate and normal fractional rate of conversion of proapoA-I to mature apoA-I. The decreased proapoA-I levels in these subjects are due to rapid catabolism of proapoA-I while the decreased levels of mature apoA-I are due to both a decreased conversion of proapoA-I to mature apoA-I in absolute terms and rapid catabolism of mature apoA-I.

Kinetic model of proapoA-I and mature apoA-I metabolism of Tangier disease subjects. Our current schematic model for apoA-I metabolism in normal and Tangier disease subjects is depicted in Fig. 5. The fractional conversion rates and the fractional catabolic rates of pro and mature apoA-I in both normal and Tangier disease subjects are obtained from the results of the above experiments. The width of the arrows is proportional to the absolute transport rates which were also obtained from the above experiments. The pool size values given at the bottom of the figure are calculated values from this



Figure 4. ApoA-I metabolic decay curves of proapoA-I and mature apoA-I in a Tangier disease subject. ProapoA-I and mature apoA-I were isolated from a Tangier disease subject, radioiodinated, and injected into a subject with Tangier disease. Timed plasma samples were collected following injection, plasma lipoproteins were isolated by ultracentrifugation, and the apoA-I isoforms separated by IEF gel electrophoresis. The curves for injected proapoA-I ( $\bullet$ ), mature apoA-I derived from the conversion of proapoA-I to mature apoA-I ( $\circ$ ), and injected mature apoA-I ( $\bullet$ ) are depicted on the figure.

model utilizing the given fraction rates of catabolism and conversion. All of the synthesis of apoA-I in both types of subjects is into the proapoA-I pool. In normal subjects, the fractional conversion rate is so much greater than the fractional catabolic or degradative rate for proapoA-I that virtually all of the proapoA-I is converted to mature apoA-I, and it is then slowly degraded. In Tangier disease subjects, the fractional catabolic rates of pro and mature apoA-I are markedly increased with the fractional catabolic rate now being approximately equal to the fractional conversion rate for proapoA-I. Therefore,  $\sim 50\%$  of the proapoA-I is directly degraded and 50% is converted to mature apoA-I followed by rapid degradation. The fractional catabolic and conversion rates that were utilized

Table II. Kinetics of	f Apolipoprotein A-I	Metabolism in Norma	l and Tangier .	Disease Subject:
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Subject	ProapoA-I	ProapoA-I			Mature ApoA-I		
	Concentration	RT*	PR	Concentration	RT	PR	Conversion rate
	mg/dl	d	mg/kg-d	mg/dl	d	mg/kg-d	d <sup>-1</sup>
Normal							
1	5.5	0.19	11.6	133	5.2	10.2	3.1
2	6.0	0.27	8.8	143	7.7	7.5	4.6
Tangier disease							
1	1.8	0.12	6.0	1.5	0.30	2.0	3.4
2	1.1	0.06	7.3	0.5	0.13	1.5	3.7

\* RT, residence time; PR, production rate; conversion rate, fractional rate of conversion of proapoA-I to mature A-I.



Figure 5. Scheme for the metabolism of apoA-I in normal subjects and Tangier disease subjects. The width of the arrows is proportional to the flux of material through that pathway, the fractional metabolic rates are derived from the above experimental results, and the pool size estimates at the bottom of the figure are calculated from the above model using the pathways and fractional metabolic rates given.

from the above studies predict very closely the independently determined densitometric gel scan distribution of plasma proapoA-I and mature apoA-I isoproteins in both normal and Tangier disease subjects.

#### Discussion

In normal subjects, apoA-I is secreted into plasma as proapoA-I, and while in the circulation is converted to mature apoA-I (5–9). This conversion is the result of cleavage of the amino terminal six amino acids of proapoA-I by a calcium requiring protease (7, 8). As we have previously demonstrated, the in vivo rate of conversion and the rate of catabolism of proapoA-I and mature apoA-I result in  $\sim 95\%$  of circulating apoA-I in normal subjects being the mature isoprotein while the remaining 5% is proapoA-I (9). In Tangier disease subjects, only 25 to 50% of the circulating apoA-I is the mature isoprotein while the remaining 50 to 75% is proapoA-I (10).

It has been proposed that apoA-I from subjects with Tangier disease is structurally abnormal (1), and that this structural abnormality results in rapid metabolism of apoA-I<sub>Tangier</sub> (4) and a block in the conversion of proapoA-I<sub>Tangier</sub> to mature apoA-I<sub>Tangier</sub> (5, 10, 11). In addition, it has been proposed that this structural abnormality results in an inability of proapoA-I<sub>Tangier</sub> to bind normally to lipoproteins (11). Our laboratory has recently determined the nucleotide, and derived amino acid sequence of preproapoA-I from a subject with Tangier disease (23). This subject had the characteristic Tangier disease distribution of apoA-I between the pro and mature isoproteins and rapid catabolism of apoA-I, and had an essentially normal preproapoA-I sequence. Therefore, we concluded that the abnormal apoA-I isoform distribution and rapid apoA-I catabolism in Tangier disease subjects is not secondary to a structural abnormality in the amino acid sequence of apoA-I.

It has also been proposed that the primary defect in Tangier disease may be an abnormality in the proapoA-I to mature apoA-I converting protease (5, 10, 11). This abnormal protease would then lead to an accumulation of proapoA-I, and the proapoA-I would be catabolized very rapidly resulting in a very low plasma level of apoA-I and HDL cholesterol. In our investigations of the metabolism of proapoA-I and mature apoA-I in normal subjects, proapoA-I was catabolized at the same rate as mature apoA-I (9). In addition, we and others have demonstrated with in vitro experiments, that normal and Tangier disease plasmas convert proapoA-I to mature apoA-I at the same rate (8, 24). Therefore, we concluded that the plasma proapoA-I to mature apoA-I converting protease activity is normal in Tangier disease subjects, that proapoA-I is not normally catabolized at a faster rate than mature apoA-I, and that the abnormal isoform distribution and rapid catabolism of apoA-I is not secondary to an abnormal converting protease activity in Tangier disease subjects (8, 9).

Our current investigations have elucidated the kinetic etiology of the abnormal plasma apoA-I isoform distribution in subjects with Tangier disease. This abnormal distribution is the result of a normal fractional rate of conversion of proapoA-I to mature apoA-I, and a rapid catabolism of both apoA-I isoproteins in subjects with Tangier disease. When proapoA-I is secreted into plasma, there are two possibilities for its catabolism. It may be directly catabolized from plasma or it may be converted to mature apoA-I and the mature apoA-I degraded. In normal subjects, the proapoA-I is rapidly converted to mature apoA-I with the slow catabolism of mature apoA-I resulting in a low plasma proapoA-I concentration compared to mature apoA-I. In subjects with Tangier disease, proapoA-I is secreted into plasma. The fractional rate of conversion to mature apoA-I is normal but the catabolic rate of the proapoA-I and mature apoA-I is increased 30-fold. Since the rate of direct catabolism of proapoA-I and its rate of conversion to mature apoA-I are now nearly equal in these subjects, they catabolize 50% of their proapoA-I directly from plasma and convert 50% on to mature apoA-I. This results in a moderately decreased (by  $\sim$  50%) proapoA-I concentrations in plasma from Tangier disease subjects as compared to normal plasma. It is not commonly appreciated that it is not the level of proapoA-I that is increased in Tangier disease but only the relative proportion of the proisoprotein of apoA-I compared to the mature apoA-I isoprotein.

In Tangier disease subjects, the plasma concentration of mature apoA-I is very low compared to normal plasma. In normal subjects > 95% of the proapoA-I is converted to mature apoA-I with slow catabolism of mature apoA-I; however, in Tangier disease only 50% of proapoA-I is converted to mature apoA-I. In addition to a decreased percentage of conversion of proapoA-I to mature apoA-I, the fractional catabolic rate of mature apoA-I is  $\sim 30$  times greater in Tangier disease subjects than in normals. From these results it is apparent that the extremely low plasma concentrations of mature apoA-I in subjects with Tangier disease is a result of the rapid catabolism of both proapoA-I and mature apoA-I. Therefore, the kinetic explanation for the relative increase in proapoA-I in Tangier disease subjects is not an increase in proapoA-I secondary to a decreased rate of conversion of the proisoprotein to mature apoA-I, but a marked decrease in the mature isoprotein secondary to extremely rapid catabolism of apoA-I. It is of interest that the rapid rate of catabolism of proapoA-I and mature apoA-I in Tangier disease does not result in any marked alteration in the apoA-I synthesis rate.

What is unanswered, though, at the present time is the question of the biochemical explanation for the rapid catabolism of both proapoA-I and mature apoA-I. From the studies of the apoA-I sequence in subjects with Tangier disease, it is apparent that they synthesize and secrete a structurally normal proapoA-I (23). From the above and previous studies we conclude that Tangier disease subjects secrete proapoA-I at a normal rate, and that there is no defect in the fractional rate of conversion of proapoA-I to mature apoA-I in these subjects (8). There is a recent report of abnormal catabolism of HDL and apoA-I by monocyte-macrophages obtained from subjects with Tangier disease (25), but there have been no additional reports to confirm this finding in other kindreds. Therefore, it is still unknown as to why Tangier disease subjects rapidly catabolize apoA-I.

From the present studies we now have a kinetic explanation for the increase in the relative proportion of proapoA-I compared to mature apoA-I in Tangier disease subjects. This increase is not due to an increase in the concentration of proapoA-I but is secondary to a moderate decrease in the proisoprotein concentration and a very marked decrease in the concentration of the mature apoA-I isoprotein; both of these decreases in plasma concentrations being secondary to the rapid catabolism of both proapoA-I and mature apoA-I in Tangier disease subjects with a normal fractional rate of conversion of the pro-isoprotein to mature apoA-I. Additional studies are in progress to determine the biochemical etiology for the rapid catabolism of proapoA-I and mature apoA-I in subjects with Tangier disease.

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