We have devised a combined in vivo, ex vivo, and in vitro approach to elucidate the mechanism(s) responsible for the hypoalphalipoproteinemia in heterozygous carriers of a naturally occurring apolipoprotein A-I (apoA-I) variant (Leu159 to Arg) known as apoA-I Finland (apoA-IFIN). Adenovirus-mediated expression of apoA-IFIN decreased apoA-I and high density lipoprotein cholesterol concentrations in both wild-type C57BL/6J mice and in apoA-I-deficient mice expressing native human apoA-I (hapoA-I). Interestingly, apoA-IFIN was degraded in the plasma, and the extent of proteolysis correlated with the most significant reductions in murine apoA-I concentrations. ApoA-IFIN had impaired activation of lecithin:cholesterol acyltransferase in vitro compared with hapoA-I, but in a mixed lipoprotein preparation consisting of both hapoA-I and apoA-IFIN there was only a moderate reduction in the activation of this enzyme. Importantly, secretion of apoA-I was also decreased from primary apoA-I-deficient hepatocytes when hapoA-I was co-expressed with apoA-IFIN, following infection with recombinant adenoviruses, a condition that mimics secretion in heterozygotes. Thus, this is the first demonstration of an apoA-I point mutation that decreases LCAT activation, impairs hepatocyte secretion of apoA-I, and makes apoA-I susceptible to proteolysis leading to dominantly inherited hypoalphalipoproteinemia.

Plasma concentrations of high density lipoprotein (HDL)1

This work was supported in part by a group grant from the Medical Research Council of Canada. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Supported by a postgraduate scholarship from the Heart and Stroke Foundation of Canada.

‡ Supported by a scholarship from the National Sciences and Engineering Research Council of Canada and an Ontario Graduate Scholarship.

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The abbreviations used are: HDL, high density lipoproteins; HDL-C, high density lipoprotein cholesterol; apoA-I, apolipoprotein A-I; CE, cholesteryl ester; TRL, triglyceride-rich lipoproteins; FC, free cholesterol; LCAT, lecithin:cholesterol acyltransferase; ABCA1, ATP-binding cassette transporter protein A1; PLTP, phospholipid transfer protein; FHA, familial hypoalphalipoproteinemia; apoA-IFIN, apoA-I Finland; aa, amino acid; hapoA-I, native human apoA-I; Ad5, recombinant adenovirus(es); apoA-IFIN, Ad5 carrying the apoA-IFIN cDNA; hapoA-I, Ad5, Ad5 carrying the hapoA-I cDNA; PAGE, polycrylamide gel electrophoresis; luc, Ad5, Ad5 carrying the firefly luciferase cDNA; cholesterol (HDL-C) are inversely correlated with the risk of developing coronary heart disease (1). However, the complex and often poorly understood etiology for variations in HDL-C concentrations within the general population has made the therapeutic control of HDL levels an elusive target to date. This is attributed to the intricate nature of HDL metabolism that involves many components including the major HDL structural protein apolipoprotein A-I (apoA-I) and multiple factors required for cholesteryl ester (CE) formation, lipolysis, lipid transfer, cellular lipid efflux, and cell surface interactions (reviewed in Refs. 2–4).

Nascent HDLs that are derived from the liver and intestine are poorly lipidated (2, 5) and must acquire additional lipids for their maturation into the more stable α-migrating HDLs in plasma. Defective clearance of triglyceride-rich lipoproteins (TRL) is recognized as a major determinant of HDL-C concentrations. Recessive mutations in lipoprotein lipase and its major activator protein apolipoprotein C-II, which result in impaired hydrolysis of TRL, also contribute to low HDL-C concentrations. Also the efficient conversion of free cholesterol (FC) to CE on HDL by lecithin:cholesterol acyltransferase (LCAT) is necessary for HDL maturation and depends on apoA-I as its physiological activator (reviewed in Refs. 2–4). Recent work has also highlighted the importance of both the ATP-binding cassette transporter A1 (ABCA1) protein and phospholipid transfer protein (PLTP) in maintaining normal HDL-C concentrations. PLTP-deficient mice have HDL-C levels that are reduced by 60–70% (6) as a result of enhanced catabolism of HDL apparently due to defective transfer of lipids from TRL to nascent HDL (7). Mutations in the ABCA1 cause Tangier disease (8–10) in which affected individuals have less than 5% of the normal HDL-C concentrations due to impaired cellular lipid efflux to nascent HDL.

Isolated familial hypoalphalipoproteinemia (FHA) is more common than Tangier disease, and heterozygous mutations in ABCA1 can give rise to FHA (8, 11, 12). However, mutations in apoA-I also contribute significantly to FHA in the general population. A recent study has shown that in a group of 1264 Japanese school children, 6% of FHA cases defined as HDL-C 21292

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21292

pfu, plaque-forming units; TC, total cholesterol; PAGGE, polycrylamide gradient gel electrophoresis; mapoA-I, murine apoA-I; FBS, fetal bovine serum; m.o.i., multiplicity of infection; DMEM, Dulbecco’s modified minimal medium; rec.hapoA-I, His-tagged purified recombinant hapoA-I; rec.mapoA-I, His-tagged purified recombinant mapoA-I; Lp2A-I, recombinant lipoproteins containing two molecules of apoA-I; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; BSA, bovine serum albumin; Lp2A1-IFIN, Lp2A-I with two molecules of apoA-I; Lp2A1-IFIN, Lp2A-I with two molecules of apoA-IFIN; Lp2A1-IFIN, Lp2A-I containing two apoA-I prepared with an equimolar amount of hapoA-I and apoA-IFIN; pCPT-cAMP, cAMP analog 8-(4-chlorophenylthio)-cAMP; apoA-II, apolipoprotein A-II.
concentrations below the 5th percentile matched for age and sex were related to apoA-I mutations (13). This would represent an incidence of 0.3% for apoA-I mutations in the general population, which is significant and warrants mechanistic studies on the relationships between these mutations and hyperalphalipoproteinemia. Many apoA-I mutations have been identified (reviewed in Refs. 3, 14), but particularly interesting among them are those associated with a dominant FH phenotype, such as apoA-I Finland (apoA-I FIN). This mutation was originally identified in a Finnish kindred and results from a Leu to Arg substitution at amino acid (aa) 159 (15). These individuals are heterozygous carriers of this mutation and yet have HDL-C and apoA-I concentrations that are 20 and 25% of the normal plasma concentrations, respectively (15). The cause for this dominant negative effect on HDL-C concentrations remains largely unknown. ApoA-I FIN was shown to have reduced LCAT activity compared with native human apoA-I (hapoA-I) (16), but it has not been established whether this mutant can inhibit LCAT activity by hapoA-I and confer its dominant negative phenotype in this manner. We present the results of a combined in vivo, ex vivo, and in vitro study of the mechanisms responsible for the dominant negative effects on HDL-C brought about by apoA-I FIN, and we demonstrate that the mutation exerts its effects at multiple levels.

**EXPERIMENTAL PROCEDURES**

**Mutagenesis of ApoA-I cDNA**—The apoA-I FIN cDNA was generated by the QuickChange™ mutagenesis protocol from Stratagene (La Jolla, CA) with sense 5′GATGCCGTCGCCGACGATCTGACCGT3′ and antisense 5′GATGCCGTCGCCGACGATCTGACCGT3′ primers (Life Technologies, Inc.). The underlined sequence indicates the mutagenic bases required for the Leu to Arg conversion at aa 159 within apoA-I. To generate the purified recombinant His-tagged apoA-I FIN (see below), mutagenesis was carried out on plasmid pXL2116. This plasmid is a modified pET3a vector (Strategene) that contains the apoA-I cDNA (minus the region coding for the prepro sequence) and an upstream region coding for an 11-aa N-terminal extension containing a His tag as described previously (17). For generation of the recombinant adenoivirus (Ad5) carrying the apoA-I FIN cDNA (apoA-I FIN, see below), the mutagenesis was carried out on plasmid pcAI13 (Microbis Biosystems Inc., Toronto, Canada) harboring the hapoA-I cDNA. For both sets of mutagenesis reactions, positive clones were determined by the loss of the HpaI restriction site that occurs as a result of a T to G substitution for this mutation as documented previously (15). The full-length apoA-I FIN cDNAs were sequenced prior to generation of the recombinant apoA-I FIN protein and the apoA-I FIN-Ad5.

**Production and Screening of First Generation ApoA-I Recombinant Adenoviruses**—The apoA-I FIN-Ad5 was generated and purified in the same manner as the hapoA-I adenoivirus (hapoA-LAd5) recently described by our laboratory (18). Prior to the studies with the primary hepatocytes and mice (see below), we confirmed that apoA-I FIN was produced and secreted from COS-7 cells following infection with the recombinant adenoivirus. Medium was analyzed 2–3 days after infection by 12% SDS-polyacrylamide gel electrophoresis (PAGE) performed under reducing conditions, which was then subjected to Western blot analysis following transfer to nitrocellulose membrane. The membrane was probed with anti-apoA-I monoclonal antibodies 4H1 and 5F6, and the apoA-I FIN protein in the medium was detected by chemiluminescence (WestPico SuperSignal Substrate, Pierce) following treatment with horseradish peroxidase-conjugated anti-mouse IgG (Amersham Pharmacia Biotech).

**Animals**—ApoA-I-deficient (apoA-I<sup>−/−</sup>) and wild-type c57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME) and Charles River Laboratories (Wilmington, MA), respectively. Mice were maintained on a 12-h light/12-h dark schedule and were fed either a normal chow (Charles River rodent diet no. 5075, 18% protein and 4.5% fat) or high fat Western (Harlan Teklad TD 88137, 19.5% protein, 42% fat, 0.15% cholesterol) diet as indicated. All experiments were performed in accordance with protocols approved by the University of Ottawa Animal Care Committee. Mice used for these studies were 3–8-month-old females, except where indicated.

**In Vivo Metabolism of HapoA-I and ApoA-I FIN**—Wild-type and apoA-I-deficient c57BL/6J mice were injected via the tail vein with either the hapoA-LAd5, the apoA-I FIN-Ad5, or the control luciferase adenoivirus (luc-Ad5) at a dose of 2 × 10⁸ plaque-forming units (pfu) (~3.7 × 10⁹ adenoviruses). ApoA-I-deficient mice were also co-injected with 1 × 10⁸ pfu of each of the hapoA-LAd5 and the apoA-I FIN-Ad5. Blood was collected in K<sup>2</sup>-EDTA tubes at various times following the injections, and plasma samples isolated were immediately placed on ice and incubated with a protease inhibitor mixture (complete EDTA-free® (Roche Molecular Biochemicals)). The plasma and HDL total cholesterol (TC), FC, and phospholipid concentrations were measured with enzymatic kits as described (18), and human apoA-I concentrations (hapoA-I and apoA-I FIN) were measured by a solid-phase immunoassay (19). HDL fractions were isolated from the plasma by discontinuous gradient density ultracentrifugation (18). ApoA-I present in α- and β-precipitating HDL was determined by agarose gel electrophoresis (Beckman Bilipod) and Western blot analysis. Western blots for apoA-I were also performed on plasma after it was subjected to 12% SDS-PAGE under reducing conditions or 4–20% polyacrylamide gradient gel electrophoresis (PAGE) under native conditions. Anti-human apoA-I Western blots, monoclonal antibodies 4H1 and 5F6 were biotinylated with Sulfo-NHS-Biotin (Pierce), and chemiluminescence was performed following treatment with horseradish peroxidase-conjugated streptavidin (Amersham Pharma- bia Biotech). Murine apoA-I (mapoA-I) was detected with an anti-mouse apoA-I antibody (BIODESIGN International, Kennebunk, ME), and chemiluminescence was carried out as above following treatment with anti-rabbit horseradish peroxidase-conjugated IgG (Amersham Pharmacia Biotech).

**Secretion of HapoA-I and ApoA-I FIN from Primary Mouse Hepato- cytes**—Primary hepatocytes were prepared from apoA-I-deficient mice according to an established protocol (21, 22). Briefly, the cells were seeded in fibronectin-coated (25 μg/ml) 6-well plates at an initial density of 1–2 × 10<sup>6</sup> cells per well in William’s medium containing penicillin (100 units/ml), streptomycin sulfate (100 units/ml), Fungizone® (250 ng/ml) (Life Technologies, Inc.), and 10% fetal bovine serum (FBS) (Sigma). Cells were infected the following day (24 h) with either the hapoA-LAd5 or the apoA-I FIN-Ad5 at a multiplicity of infection (m.o.i.) of 75:1 (pfu/cell) or with a mixture of hapoA-LAd5 and apoA-I FIN-Ad5 each at an m.o.i. of 37.5:1 (total m.o.i. ~ 75:1). 24 h after the initial infection, the William’s medium was removed, and the hepatocytes were incubated in Dulbecco’s minimal essential medium (DMEM) without methionine and cysteine containing 10% FBS (pre-labeling medium) for 1 h. Following this, the cells were pulse-labeled for 1 h with labeling medium (pre-labeling medium plus 150 μCi/well [<sup>35</sup>S]Methionine). The labeling medium was then removed, and growth medium (5% FBS) was added. Cells were reinfected as described previously and collected in K<sub>3</sub>-EDTA tubes at various times following the injections, and plasma samples isolated were immediately placed on ice and incubated with a protease inhibitor mixture (complete EDTA-free®). Cholesterol, phospholipids (PL), and glycine were measured by a solid-phase immunoassay (18). Treatment of the LDL fractions with heparin and dextran sulfate resulted in 80% removal of LDL ApoB-100 and 100% removal of HDL ApoA-I and α-2-macroglobulin (α-2M). The remaining material was dialyzed against sodium bicarbonate (5 mM, pH 8) containing EDTA (1 mM) and azide (0.02%), lyophilized, and stored at −20 °C. Prior to the in vitro studies, the lyophilized recombinant proteins were solubilized in 6 M guanidinium hydrochloride and dialeyzed extensively against phosphate-buffered saline or Tris-buffered saline as required.

**Preparation of Reconstituted Lipoproteins (Lp2A-I)**—Discoidal reconstituted lipoproteins containing two apoA-I molecules per particle (Lp2A-I) were prepared by the cholate dispersion/Bio-Beads removal technique using a starting 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC/FC/apoA-I ratio of 80:10:1 as published by Sparks et al. (24)). The homogeneity and hydrodynamic diameters of the Lp2A-I were determined by non-denaturing PAGE as described previously (17).

Proteolysis and Impaired Hepatocyte Secretion of ApoA1-FIN 21293

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The reconstituted lipoproteins were shown to contain two molecules of recombinant apoA-I by cross-linking with dimethyl suberimidate according to an established protocol (25). The Lp2A-I phospholipid compositions were determined with an enzymatic kit (Wako Chemicals, Neuss, Germany) as was FC (Roche Molecular Biochemicals). The apoA-I concentrations were measured by the Markwell Lowry method (26) using bovine serum albumin (BSA) as the standard.

Stability of Association on Lp2A-I—The stability of association of rec.hapoA-I and rec.apoA-I\textsubscript{FIN} on the Lp2A-I was measured by CD spectroscopy using a Jasco J41A spectropolarimeter. The change in molar ellipticity at 222 nm in the presence of increasing amounts of guanidine hydrochloride was used to calculate the standard free energy of denaturation (ΔG°) as described previously (24).

Lecithin:Cholesterol Acyltransferase Assay—LCAT was purified according to Albers et al. (27) and the cholesterol esterification studies were performed as outlined previously (28). The Lp2A-I used in these studies (initial POPC/FC/apoA-I ratio of 80:10:1) were labeled with [1\textsuperscript{4}C]-cholesterol (PerkinElmer Life Sciences) and contain either rec.hapoA-I alone (Lp2A-I\textsubscript{WT}), rec.apoA-I\textsubscript{FIN} alone (Lp2A-I\textsubscript{FIN}), or an equimolar mixture of the two (Lp2A-I\textsubscript{WT/FIN}). The Lp2A-I\textsubscript{WT/FIN} are hybrid populations that were prepared to resemble most closely the nascent lipoproteins formed in heterozygous carriers of the apoA-I\textsubscript{FIN} mutation. Since rec.hapoA-I and rec.apoA-I\textsubscript{FIN} have equal lipid binding capabilities and given that all proteins were incorporated into the particles, the Lp2A-I\textsubscript{WT/FIN} represent a heterogeneous lipoprotein population that might form in vivo. Approximately 50\% of the Lp2A-I contain one molecule of each, 25\% two molecules of rec.hapoA-I, and 25\% two molecules of rec.apoA-I\textsubscript{FIN}. Two different types of experiments were performed. The initial rate constants apparent K\textsubscript{m} (appK\textsubscript{m}) and V\textsubscript{max} were calculated by incubating the Lp2A-I at the concentrations indicated (given as μg of apoA-I) with the enzyme for 10 min at 37 °C and terminating the reaction with the addition of 2 ml of ethanol. Under these conditions, there is minimal substrate conversion (29). In the second experiment, the time course of CE formation was followed over 5 h by incubating Lp2A-I particles at a final apoA-I concentration of 2.0 μg/ml with 3.5 units of LCAT. For both sets of experiments, the values are the mean (± S.E.) of triplicate measurements and represent the average of two independent experiments. The analysis was performed using the Student's t-test.

One unit of LCAT is defined as the amount required to convert 1 nmol of FC per h using a standard Lp2A-I particle (prepared as described above) at a final apoA-I concentration of 2.0 μg/ml.

Cholesterol Efflux Studies—Efflux of cholesterol from mouse J774 macrophages (ATCC TIB-67) to rec.hapoA-I or rec.apoA-I\textsubscript{FIN} with or without stimulation by cAMP was performed as outlined previously (29). Briefly, J774 macrophages in 12-well plates were loaded with [1\textsuperscript{4}C]-cholesterol (10 μCi/well) using acetylated low density lipoproteins (75 μg/ml) in DMEM containing 10% FBS, penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml). After 36 h, the loading medium was removed and replaced with serum-free DMEM containing 0.2% (w/v) BSA (DMEM/BSA) with or without the membrane-permeable cAMP analog 8-(4-chlorophenylthio)-cAMP (pCPT-cAMP) (0.15 mM final) (Sigma). Cholesterol was allowed to equilibrate with the cells for 10–12 h at which time the medium was removed and replaced with DMEM/BSA with or without pCPT-cAMP containing rec.hapoA-I or rec.apoA-I\textsubscript{FIN} as lipid-free proteins or as Lp2A-I (1.7 μg final apoA-I concentration). Efflux was measured over 3 h after subtracting the basal 1\textsuperscript{4}C-FC efflux to medium of cells without apoA-I (DMEM/BSA control) which was less than 10\% of the apoA-1-specific efflux.

RESULTS

Since apoA-I\textsubscript{FIN} causes hypoalphalipoproteinemia in carriers of this mutation, we first sought to reproduce this phenotype in a mouse model by de novo expression of apoA-I\textsubscript{FIN}. This was accomplished by two different approaches. First, wild-type C57BL/6J mice were injected with either the apoA-I\textsubscript{FIN}-Ad5 or the hapoA-I Ad5, and their effects on mupen-I (Fig. 1) and HDL-C concentrations were compared. The concentrations of mapoA-I were decreased significantly by apoA-I\textsubscript{FIN} (Fig. 1B, lower right panel, lanes 1–4) but not by hapoA-I (Fig. 1A, lower right panel, lanes 1–4). Interestingly, evidence of apoA-I\textsubscript{FIN} proteolysis was seen in the plasma (Fig. 1B, upper right panel), and the greatest decreases in mapoA-I concentrations correlated with plasma samples that had the most significant amounts of apoA-I\textsubscript{FIN} degradation (Fig. 1B, lower right panel, lanes 3 and 4). Overall, low concentrations of circulating apoA-I\textsubscript{FIN} (50 ± 4.6 mg/dl) caused a statistically significant decrease (p < 0.03) in mapoA-I concentrations (to 32 ± 22% of pre-injected values) that was not found for higher and more physiological concentrations (141 ± 27 mg/dl) of hapoA-I (p = 0.14) (Fig. 1C). Furthermore, apoA-I\textsubscript{FIN} decreased the HDL-C concentrations and caused a remodeling of HDL in these mice converting the large HDL\textsubscript{L} to smaller HDL\textsubscript{S} (not shown). This is similar to what was observed when hapoA-I and apoA-I\textsubscript{FIN} were co-expressed in apoA-I-deficient mice (below).

To confirm these results and more closely mimic the human heterozygous state, apoA-I-deficient mice were co-injected with the hapoA-I Ad5 and the apoA-I\textsubscript{FIN}-Ad5, and the circulating apoA-I and total cholesterol concentrations were compared with mice injected with either the hapoA-I Ad5 or the apoA-I\textsubscript{FIN}-Ad5 alone (Fig. 2). Mice injected with the hapoA-I Ad5 had high circulating concentrations of hapoA-I that reached physiological concentrations by 4 days and peaked at high levels between 7 and 9 days before returning to lower concentrations at 12 days (Fig. 2A, ○). In contrast, co-expression of hapoA-I and apoA-I\textsubscript{FIN} (Fig. 2A, ▲) resulted in only moderate concentrations of circulating apoA-I (50–70 mg/dl) that were 3–8-fold lower in these mice throughout the time course of expression compared with hapoA-I. In fact, the apoA-I concentrations following co-expression of the two proteins were similar to mice expressing only apoA-I\textsubscript{FIN} (Fig. 2A, ■), except that apoA-I levels were sustained longer in the plasma when both proteins were present. The increases in total cholesterol concentrations were confined to the HDL pool (p > 1.6 g/ml) and paralleled the expression of the human apoA-I proteins in these mice (Fig. 2B). Native hapoA-I increased the HDL-TC concentrations reaching a maximum between 7 and 9 days (Fig. 2B, ○), whereas co-expression of hapoA-I and apoA-I\textsubscript{FIN} had only a moderate effect on the HDL-TC concentrations (2–3-fold increase) (Fig. 2B, ▲) throughout the time course (Table I). ApoA-I\textsubscript{FIN} (Fig. 2B, ▼) produced a similar effect, but the return to base-line plasma cholesterol concentrations occurred more rapidly (by 6–8 days) (Table I) and followed the more transient expression of this protein in apoA-I-deficient mice.

A more detailed analysis of the effects of the different adenovirus injections on plasma lipid concentrations in these mice is provided in Table I. Shown are the changes for 3 different days (2, 4, and 8 days) and are representative of early, intermediate, and late time points following injections of the adenoviruses. Statistically significant increases (p < 0.05) in all pre-injected plasma lipid concentrations occur following expression of hapoA-I by 4 days post-injection and are maintained at day 8 and beyond. Expression of apoA-I\textsubscript{FIN} or co-expression of hapoA-I and apoA-I\textsubscript{FIN}, on the other hand, did not produce these large changes in plasma lipid concentrations. Therefore, by 4 days post-injections there were significant differences in plasma lipid concentrations between mice expressing apoA-I\textsubscript{FIN} alone or co-expressing hapoA-I and apoA-I\textsubscript{FIN} and mice expressing hapoA-I (p < 0.05). Co-expressing hapoA-I and apoA-I\textsubscript{FIN} produced significant changes by 8 days post-injections compared with mice expressing apoA-I\textsubscript{FIN} alone (p < 0.05) due to the return to base-line plasma lipid concentrations in mice expressing only the mutant protein.

HDL size and charge were also monitored 4 days following injections of the different adenoviruses (Fig. 3). Interestingly, small HDL only were formed (8–9 nm) in apoA-I-deficient mice injected with the hapoA-I Ad5 and the apoA-I\textsubscript{FIN}-Ad5 (Fig. 3A, lanes 3 and 5) similar in size although somewhat larger than in mice injected with the apoA-I\textsubscript{FIN}-Ad5 (lane 6). The absence of large HDL in mice expressing both proteins is consistent with that found in heterozygous carriers of the apoA-I\textsubscript{FIN} mutation
Proteolysis of apoA-I<sub>F</sub> is correlated with reductions in murine apoA-I concentrations in C57BL/6J mice expressing sub-physiological concentrations of this human apoA-I mutant. Plasma (1.5 μl) was isolated from mice and subjected to 12% SDS-PAGE and then Western blot analysis following transfer to nitrocellulose. The blots were probed with either a mixture of biotinylated anti-human apoA-I monoclonal antibodies (a-human) (upper panels) or a polyclonal anti-mouse (a-mouse) apoA-I antibody (lower panels) as described under “Experimental Procedures.” A, plasma was isolated from 4 mice prior to (left panels) or 4 days after injections of 2 × 10<sup>9</sup> pfu of the hapoA-I<sub>Ad5</sub> (right panels). B, plasma was isolated from 4 mice prior to (left panels) or 4 days after injections of 2 × 10<sup>9</sup> pfu of the apoA-I<sub>F</sub>-Ad5 (right panels). C, densitometric scanning of the apoA-I bands before (white bars) or following injections of the hapoA-I<sub>Ad5</sub> (gray bar) or the apoA-I<sub>F</sub>-Ad5 (black bar). There is a statistically significant reduction (p < 0.03) in mapoA-I concentrations (33 ± 22% of normal values) following expression of apoA-I<sub>F</sub> (50 ± 4.6 mg/dl) but not with hapoA-I at higher and more physiological concentrations (141 ± 27 mg/dl). The greatest reductions in mapoA-I concentrations correlate with the most extensive proteolysis of apoA-I<sub>F</sub>.

Proteolysis of apoA-I<sub>F</sub> was also also evident following adenovirus-mediated expression in apoA-I-deficient mice (Fig. 4), similar to that observed in C57BL/6J mice (Fig. 1). For these experiments, female apoA-I-deficient mice between 4 and 6 months of age were maintained on a Western diet 3 weeks prior to injection of the recombinant adenoviruses. Under these dietary conditions, apoA-I<sub>F</sub> reached significantly higher levels (>100 mg/dl) allowing direct detection of the degradation products in the plasma by both Ponceau staining (not shown) and Western blot analysis (Fig. 4A). The monoclonal antibodies used for Western blot analysis recognize epitopes N-terminal to the Leu<sup>159</sup> → Arg mutation (4H1 and 5F6 epitopes map to residues 1–8 and 118–148 of human apoA-I, respectively). Therefore, the major 18.5-kDa fragment represents an N-terminal degradation product and corresponds to the size expected if cleavage occurred at or near the site of the mutation. HDL fractions were isolated by centrifugation of pooled plasma collected from fasted (9–11 h) apoA-I-deficient mice on the Western diet 4 days after injection with either the hapoA-I or the apoA-I<sub>F</sub> recombinant adenoviruses (Fig. 4B). The apoA-I<sub>F</sub> degradation products were confined to the smaller HDL and lipid-poor fractions where the majority of this mutant was located (p > 1.13 g/ml). In contrast, no degradation products were found for hapoA-I which was found predominantly as buoyant HDL<sub>2</sub> (p = 1.07 g/ml).

The delayed appearance of apoA-I in the plasma of apoA-I-
deficient mice either co-expressing the two proteins or expressing apoA-I FIN alone (Fig. 2) suggested that the apoA-I FIN mutation might also impair hepatocyte secretion of apoA-I. To address this, apoA-I secretion was monitored in primary apoA-I-deficient murine hepatocytes infected with either the hapoA-I.Ad5 or apoA-I FIN.Ad5 (m.o.i. = 75:1 pfu/cell). As well, in order to simulate hepatic secretion of this mutant in heterozygous individuals, cells were co-infected with equal amounts of both recombinant adenoviruses at a half-dose (m.o.i. = 37.5) in which the combined titer was equivalent to that used to study secretion independently (Fig. 5). These results are representative of three separate experiments, and significant differences in the amounts of secreted and cell-associated 35S-apoA-I were detected under the different conditions during the initial chase.

![Image](http://www.jbc.org/Downloaded from)

**Fig. 2.** ApoA-I-deficient mice co-expressing hapoA-I and apoA-I FIN or apoA-I FIN alone have greatly reduced apoA-I and HDL total cholesterol concentrations compared with mice expressing only hapoA-I over the time course of expression. ApoA-I-deficient mice were pre-bled and then injected with either 2 × 10⁹ pfu of the hapoA-I.Ad5 alone (3 males), 1 × 10⁹ pfu of each of the hapoA-I.Ad5 and the apoA-I FIN.Ad5 (2 males and 1 female), and for comparison 2 × 10⁹ pfu of the apoA-I FIN.Ad5 alone (3 females) as described under "Experimental Procedures." ApoA-I concentrations in mouse plasma samples were measured with a standard radioimmunoassay as described previously (19, 20). In mice expressing hapoA-I alone (●), the apoA-I concentrations reach physiological concentrations 4 days following injections of the adenoviruses and peaks between days 7 and 9 before returning to physiological concentrations once again by day 12. In contrast, mice co-expressing hapoA-I and apoA-I FIN (▲) have a delayed appearance of apoA-I in the plasma, and the apoA-I concentrations attained are much lower. The apoA-I concentrations are more similar to mice expressing only apoA-I FIN (■), except the duration of expression is sustained longer in mice expressing both proteins. B, The plasma total cholesterol concentrations were determined as described under "Experimental Procedures," and the increases following apoA-I expression are confined to the HDL pool. Mice expressing hapoA-I alone (●) have large increases in HDL total cholesterol that parallel the apoA-I concentrations over the time course of expression. Mice co-expressing hapoA-I and apoA-I FIN (▲) or apoA-I FIN alone (■) have only moderate increases in HDL total cholesterol concentrations, which demonstrate the dominant negative effect of this human mutation in these mice. The transient expression of apoA-I FIN is paralleled by a return to normal plasma cholesterol concentrations in these mice 6 days post-injection (■).

**Table 1.** The changes in apoA-I-deficient plasma lipid concentrations as a function of time of expression of either hapoA-I or apoA-I FIN alone or co-expression of hapoA-I and apoA-I FIN.

| Lipid | Day 2 | Day 4 | Day 8 | Day 12 |
|-------|-------|-------|-------|--------|
|        | apoA-I-expressed | HapoA-I (n = 3) | HapoA-I (n = 3) | HapoA-I (n = 3) |
|        | Total cholesteral | 138 ± 20 | 157 ± 4^a | 153 ± 4^b | 134 ± 4 |
|        | Cholesterol (mg/dl) | 62 ± 8^a | 62 ± 8 | 62 ± 8 | 62 ± 8 |
|        | Phospholipid (mg/dl) | 40 ± 6 | 40 ± 6 | 40 ± 6 | 40 ± 6 |
|        | Lipid (mg/dl) | 59 ± 5 | 59 ± 5 | 59 ± 5 | 59 ± 5 |
|        | Free cholesterol (mg/dl) | 35 ± 4 | 35 ± 4 | 35 ± 4 | 35 ± 4 |
|        | Cholesteryl ester (mg/dl) | 64 ± 6 | 64 ± 6 | 64 ± 6 | 64 ± 6 |

Statistically significant lipid concentrations (p < 0.05) from pre-injected mice.

- Statistically significant lipid concentrations (p < 0.05) for apoA-I FIN mice compared with apoA-I FIN mice.

- Statistically significant lipid concentrations (p < 0.05) for hapoA-I Ad5 mice compared with apoA-I FIN mice.

- Statistically significant lipid concentrations (p < 0.05) for hapoA-I Ad5 mice compared with apoA-I FIN mice.
period \((t = 1 \text{ h}) (p < 0.05)\). Less apoA-I was secreted in cells co-infected with the two adenoviruses (Fig. 5A, \(\Delta\)) compared with hepatocytes infected with the hapoA-I.Ad5 alone (Fig. 5A, \(\bullet\)). The apoA-I secreted was decreased further when apoA-IFIN was expressed alone (Fig. 5A, \(\oplus\)). Interestingly, there was also less \(^{35}\text{S}\)-apoA-I associated with the hepatocytes expressing both apoA-I proteins (Fig. 5B, \(\Delta\)) at 1 h compared to hepatocytes expressing only hapoA-I (Fig. 5B, \(\Box\)), and between 20 and 30\% of the \(^{35}\text{S}\)-apoA-I is unaccounted for in these cells during the chase. Likewise, there was also cell-associated \(^{35}\text{S}\)-apoA-I than expected in apoA-IFIN expressing hepatocytes throughout the chase even though this mutant accumulated in the hepatocytes (Fig. 5B, \(\square\)) as anticipated from its poor secretion.

The physicochemical properties of rec.hapoA-I and rec.apoA-I FIN purified from \(E.\) coli were also compared and found to be very similar. The two proteins have identical kinetics of association with dimyristoylphosphatidylcholine and behaved similarly to apoA-I purified from human plasma in this assay (not shown). When recombined with lipids \textit{in vitro}, there were also no significant differences in the final molar compositions of \(\text{Lp2A-I}_{\text{WT}}, \text{Lp2A-I}_{\text{FIN}}, \) or \(\text{Lp2A-I}_{\text{WT/FIN}}\) (Table II). All reconstituted lipoproteins contained two molecules of apoA-I as demonstrated by cross-linking with dimethyl suberimidate and were homogeneous in size (single band between 10.0 and 10.6 nm in diameter), and all proteins were incorporated into the lipoproteins as assessed by native 8–25\% PAGGE (not shown). In addition, the stability of association \((\Delta G_D^0)\) of rec.apoA-I FIN with lipids was similar to rec.hapoA-I (Table II).

The effect of the apoA-IFIN mutation on LCAT activation was assessed (Fig. 6). As mentioned (see “Experimental Procedures”), the \(\text{Lp2A-I}_{\text{WT/FIN}}\) were prepared to represent the nascent lipoprotein population that would most likely form in heterozygotes for this mutation. These lipoproteins were expected to contain one molecule of each recombinant protein (\(\approx 50\%\) of total), two molecules of rec.hapoA-I (\(\approx 25\%\) of total), or two molecules of rec.apoA-I FIN (\(\approx 25\%\) of total). The Michaelis-Menten con-
Proteolysis of apoA-I FIN is confined to HDL₃ and lipid-poor species in the plasma of apoA-I-deficient mice expressing this human apoA-I mutant. ApoA-I-deficient mice were maintained on the Teklad Western diet for 3 weeks prior to injection of either the hapoA-I and the apoA-IFIN recombinant adenoviruses (2 × 10⁹ pfu). A, plasma isolated from apoA-I-deficient mice at the given days following injections with either the hapoA-I Ad5 or the apoA-IFIN Ad5 was subjected to 12% SDS-PAGE under reducing conditions followed by Western blot analysis. Proteolysis of apoA-IFIN is detected in the plasma, and the size of the major fragment (→) was found to be 18.5 kDa. This is the size predicted for an N-terminal fragment if cleavage occurs at or very close to the site of the apoA-IFIN mutation (antibodies used recognize N-terminal epitopes on apoA-I). B, selected HDL fractions were isolated from the plasma of fasted (9–11 h) apoA-I-deficient mice 4 days following injections of the recombinant adenoviruses and were subjected to 12% SDS-PAGE and Western blot analysis as described under "Experimental Procedures." Degradation products for apoA-IFIN are confined to the smaller HDL (HDL₃) and lipid-poor species, and none are detected for hapoA-I.

Fig. 5. The apoA-I FIN mutation impairs apoA-I secretion from hepatocytes and enhances its intracellular clearance. Primary hepatocytes from apoA-I-deficient mice were prepared as described previously (21, 22) and infected with the recombinant adenoviruses as described under "Experimental Procedures." 24 h after the initial infection, the cells were labeled with [³⁵S]methionine for 1 h and chased for up to 4 h. A, the accumulation of [³⁵S]apoA-I (given as the percentage of initial cell-associated apoA-I) in the medium is reported as a function of chase time following expression of hapoA-I (○), apoA-I FIN (□), or both (▲). The initial rates of apoA-I secretion at the 1-h time point during the chase (before the plateaus are reached) show that hepatocytes co-expressing hapoA-I and apoA-I FIN (▲) have impaired apoA-I secretion compared with hepatocytes expressing hapoA-I (○) (p < 0.05, Student's t test). ApoA-I secretion is reduced further in hepatocytes expressing apoA-I FIN (□) (p < 0.05 at 1 h). B, the amount of [³⁵S]apoA-I remaining in the cells (as a percentage of initial cell-associated apoA-I) is reported as a function of chase time following expression of hapoA-I (○), apoA-I FIN (□), or both (▲). Interestingly, at the 1-h time point less cell-associated apoA-I is found in hepatocytes co-expressing hapoA-I and apoA-I FIN (□) compared with hepatocytes expressing hapoA-I (○) (p < 0.05, Student's t test). The apoA-I FIN mutation not only interferes with hepatocyte secretion but makes apoA-I susceptible to intracellular clearance as well. This finding is supported by the observation that less than the expected amount of apoA-I FIN accumulates in the cells (□). Statistically significant differences in medium and cell-associated apoA-I between hepatocytes co-expressing the two proteins or expressing apoA-I FIN alone and hepatocytes expressing hapoA-I are shown (▲, p < 0.05, or ★★, p < 0.10).

The apoA-I FIN mutation does not negatively affect cholesterol efflux from cells in culture (16).

DISCUSSION

This study offers new insights into the multiple and complex effects of apoA-I FIN on HDL metabolism. The cause for the dominant hypoalphalipoproteinemia induced by this mutation in heterozygous carriers has remained poorly understood since it was first identified by Miettinen et al. (15). The mutation was found to impair the ability of apoA-I to activate LCAT, but it was suggested that this could not account for the initial observations that the apoA-I FIN mutation does not negatively affect cholesterol efflux from cells in culture (16).
The apoA-I FIN mutation (Leu 159 to Arg) occurs on the hydrophobic face of helix 6 (aa 143–165), a region of the protein that has been shown by both in vivo studies (30–38) and more recently by in vitro proteolysis and impaired hepatocyte secretion of apoA-I FIN to be important for LCAT activation. This helix is highly conserved among species (41), and therefore non-conserved amino acid substitutions in this region might be expected to interfere with the activity of this enzyme by native human apoA-I. The three sets of reconstituted lipoproteins (Lp2A-I) were prepared as described under “Experimental Procedures.”

A, the esterification of [3H]cholesterol in three different reconstituted Lp2A-I particles by LCAT is shown. The Lp2A-I prepared with rec.hapoA-I alone (Lp2A-IWT) (◊), rec.apoA-IFIN alone (Lp2A-I FIN) (●), or a 1:1 molar ratio of two (Lp2A-IWT/FIN) (△) were incubated with purified LCAT as described under “Experimental Procedures.” Values are the mean (± S.E.) of triplicate measurements at each data point. B, the 4–5-fold reduction in HDL-C and apoA-I concentrations (70–80% of which contain at least one molecule of apoA-I FIN) activates LCAT almost as efficiently as the Lp2A-I WT.

HDL-β carriers of apoA-I FIN, it is clear that other mechanisms may contribute to the dominant hypoalphalipoproteinemia in these individuals (16). Therefore, we have expanded on the initial in vivo LCAT studies and designed informative in vitro experiments using recombinant adenoviruses to study the effect of the apoA-I FIN mutation on the metabolism of HDL. Our results show that while impaired LCAT activation may contribute to the dominant hypoalphalipoproteinemia in carriers of apoA-I FIN, it is clear that other mechanisms are also responsible for conferring this dominant negative phenotype. Furthermore, this study illustrates the advantages of using recombinant adenoviruses in expression of apoA-I variants in mice and primary hepatocytes when in vitro studies alone are not sufficiently informative for the study of HDL metabolism.

The apoA-I FIN mutation (Leu159 to Arg) occurs on the hydrophobic face of helix 6 (aa 143–165), a region of the protein that has been shown by both in vivo studies (30–38) and more recently by in vitro studies (18, 39, 40) to be important for LCAT activation. This helix is highly conserved among species (41), and therefore non-conserved amino acid substitutions in this region might be expected to interfere with LCAT activation by apoA-I. This was demonstrated for apoA-I FIN (16) and more recently in a study where three conserved Arg residues in this region were mutated (38). However, it
remains unclear how lipoproteins containing both apoA-I\textsubscript{FIN} and hapoA-I activate LCAT compared with lipoproteins that contain only hapoA-I (see below). Also, the effect of apoA-I\textsubscript{FIN} on LCAT activity has not been analyzed \textit{in vivo} in the homozygous state. In this study apoA-I\textsubscript{FIN} generates HDL with predominantly pre-\(\beta\) migration in apoA-I-deficient mice (Fig. 3\textbf{B}, \textit{lane} 6), and analysis of plasma lipoproteins from these mice by negative staining EM demonstrates that this mutant but not hapoA-I forms discoidal HDL (not shown). As well, even during peak expression of this mutant (2–3 days) the CE/TC ratio in the plasma samples averaged only 0.39 and is even lower than pre-injected values (derived from Table I). Therefore, these \textit{in vivo} findings support previous and current (see below) \textit{in vitro} experiments that apoA-I\textsubscript{FIN} has impaired LCAT activation in the absence of hapoA-I.

Detailed \textit{in vitro} LCAT experiments were next performed to address the possibility that the apoA-I\textsubscript{FIN} mutation might inhibit LCAT activation in lipoproteins containing both apoA-I\textsubscript{FIN} and hapoA-I and contribute to its dominant negative effect on HDL-C and apoA-I concentrations in this manner (Fig. 6). Lp2A-I of similar size, lipid composition, and stability are formed with rec.hapoA-I (Lp2A-I\textsubscript{FIN}), rec.apoA-I\textsubscript{FIN} (Lp2A-I\textsubscript{WT}), or both (Lp2A-I\textsubscript{WT/FIN}) (Table II). This ensures that we are studying an effect of the mutation on LCAT activation and not secondary effects due to differences in lipid composition that can independently alter the activity of this enzyme (28, 42). The Lp2A-I\textsubscript{WT/FIN} are important because they most closely represent the nascent HDL that would form in apoA-I\textsubscript{FIN} heterozygotes. This heterogeneous lipoprotein population consists of Lp2A-I with either one molecule each of rec.hapoA-I and rec.apoA-I\textsubscript{FIN} or Lp2A-I containing two molecules of rec.hapoA-I or two molecules of rec.apoA-I\textsubscript{FIN}. As such, the majority (70–80%) of the Lp2A-I would contain at least one molecule of rec.hapoA-I and one molecule of rec.apoA-I\textsubscript{FIN}. Therefore, if apoA-I\textsubscript{FIN} is dominant over hapoA-I with respect to LCAT activation, the Lp2A-I\textsubscript{WT/FIN} should behave similarly to the Lp2A-I\textsubscript{FIN} in this assay. If not, the LCAT activation of the Lp2A-I\textsubscript{WT/FIN} should more closely resemble that of the Lp2A-I\textsubscript{WT}. The latter is observed here. We find that the Lp2A-I\textsubscript{FIN} (Fig. 6A, \(\square\), and Table II) does have a marked reduction in the affinity for the enzyme (large increase in \(K_{m}\)) which is consistent with our \textit{in vivo} data (above), whereas the Lp2A-I\textsubscript{WT} (Fig. 6A, \(\Delta\)) have only slightly impaired LCAT activation compared with Lp2A-I\textsubscript{WT}. There is a small increase in the \(K_{m}\) and no difference in \(V_{\max}\) for the Lp2A-I\textsubscript{WT/FIN} compared with Lp2A-I\textsubscript{WT} (Table II). Furthermore, the Lp2A-I\textsubscript{WT/FIN} are more efficient at activating LCAT than is a 1:1 mixture of preformed Lp2A-I\textsubscript{WT} and Lp2A-I\textsubscript{FIN} (Fig. 6B, \(\circ\)). These data clearly demonstrate that the apoA-I\textsubscript{FIN} mutation does not negatively affect LCAT activation of hapoA-I, and hapoA-I even appears to overcome some of the inhibition of apoA-I\textsubscript{FIN}. Our \textit{in vivo} data support these \textit{in vitro} results. Both pre-\(\beta\) and mature \(\alpha\)-migrating HDL are present in apoA-I-deficient mice expressing both proteins (Fig. 3\textbf{B}, \textit{lanes} 4 and 5) but not in mice expressing only apoA-I\textsubscript{FIN} (Fig. 3\textbf{B}, \textit{lane} 6). Taken together, these data indicate that LCAT activation is only moderately impaired in the heterozygous state and can not fully account for the hypoalphalipoproteinemia in carriers of apoA-I\textsubscript{FIN}.

Since many prohormones are cleaved at paired basic amino acids (43), it was suggested that the presence of two consecutive arginines within apoA-I produced by the Leu\textsuperscript{150} \(\rightarrow\) Arg substitution may make the mutant protein susceptible to proteolytic cleavage (15). Of note, the pro-sequences of both human and mouse apolipoprotein A-II (apoA-II) are cleaved in the plasma at a paired basic sequences immediately downstream (−1,−2) of the first amino acid in the mature protein. Nonetheless, proteolysis of apoA-I\textsubscript{FIN} was not detected by Western blot analysis of plasma samples isolated from heterozygotes in the initial studies of Miettinen \textit{et al}. (15) and the possibility that apoA-I\textsubscript{FIN} was degraded was not explored further in these studies. However, it is clear in this study that even with precautions to reduce degradation artifacts that apoA-I\textsubscript{FIN} but not hapoA-I undergoes proteolysis in both wild-type (Fig. 1) and apoA-I-deficient mice (Fig. 4) following adenovirus-mediated expression. The higher concentrations of apoA-I\textsubscript{FIN} obtained following injections of the recombinant adenoviruses have made possible the detection of these proteolytic cleavage products of apoA-I\textsubscript{FIN}, which under normal circumstances are likely cleared rapidly from the circulation and escape detection. This proteolysis appears to play an important role in reducing HDL-C and apoA-I concentrations. In our \textit{in vivo} system, we show that there is a direct correlation between the extent of apoA-I\textsubscript{FIN} proteolysis and the decrease in mapoA-I concentrations (Fig. 1). In fact, mapoA-I is barely detectable in the plasma sample where there is greatest amount of detectable apoA-I\textsubscript{FIN} proteolysis (Fig. 1\textbf{B}, \textit{lane} 4, lower right panel). This reduction in mapoA-I levels occurs with only low to moderate concentrations of apoA-I\textsubscript{FIN} (50 ± 4.6 mg/dl) and is specific for the mutant protein. Higher and more physiological concentrations of hapoA-I (147 ± 26 mg/dl) do not have this statistically significant effect (Fig. 1). This finding appears different from that reported previously with human apoA-I transgenic mice, in which high expression of hapoA-I was shown to reduce mapoA-I concentrations (44). However, the two systems are not directly comparable. Two major differences between this study and the previous one is that in the prior study the human apoA-I transgenic mice were fasted overnight and had higher circulating concentrations of human apoA-I. In the present study, the C57BL/6J mice were not fasted and hapoA-I is found only to slightly reduce mapoA-I plasma concentrations without reaching statistical significance (\(p = 0.14\)). In contrast, sub-physiological concentrations of apoA-I\textsubscript{FIN} significantly reduce mapoA-I levels (\(p < 0.03\)) (Fig. 1C). Furthermore, we observe a similar and dominant effect of apoA-I\textsubscript{FIN} on apoA-I and HDL-C concentrations when this mutant is co-expressed with hapoA-I in apoA-I-deficient mice (Fig. 2), a system that more closely mimics the heterozygous state. ApoA-I concentrations are 3–8-fold lower in apoA-I-deficient mice expressing both proteins compared with mice expressing hapoA-I alone throughout the time course of expression (Fig. 2A). Consequently, the HDL-C (Fig. 2\textbf{B}) and plasma lipid (Table I) concentrations are greatly reduced and large HDL\(_{2}\) are absent (Fig. 3\textbf{A}, \textit{lanes} 3 and 5) in these mice, similar to findings reported previously (16) for heterozygous carriers of this mutation.

The dramatic reductions in apoA-I and HDL-C concentrations caused by apoA-I\textsubscript{FIN} proteolysis should not come as a surprise. Numerous studies have demonstrated this can have a major impact on HDL metabolism. ApoA-I degradation by elastase was shown to enhance the binding and intracellular clearance of HDL by macrophages (45). Limited proteolysis of apoA-I by matrix metalloproteinases has also been shown to decrease cholesterol efflux from cholesterol-loaded macrophages (46), and similarly, mild trypsinization of HDL effectively abolishes apolipoprotein-mediated cholesterol efflux from cholesterol-loaded fibroblasts (47). Therefore, even though the apoA-I\textsubscript{FIN} mutation does not affect cholesterol efflux from cells in culture as we (not shown) and Miettinen \textit{et al}. (16) have found, it is likely that proteolysis of apoA-I\textsubscript{FIN} \textit{in vivo} interferes with HDL-mediated efflux in mice expressing this mutant. In support of this, apoA-I\textsubscript{FIN} is selectively degraded on smaller
HDL and lipid-poor species (Fig. 4B), the most important acceptors of cell-derived phospholipids and cholesterol (reviewed in Ref. 48). Furthermore, another study has shown that proteolysis leads to dissociation of apoA-I from HDL, especially on the less buoyant HDL₄, and produces an unstable HDL population (49). We also find that apoA-I FIN is preferentially degraded on HDL₄ and lipid-poor species, and this is likely to promote a more rapid clearance of native apoA-I and other HDL apolipoproteins and in the process interfere with their maturation into larger and more buoyant HDL. This hypothesis is consistent with our findings that mice co-expressing apoA-I FIN and hapoA-I (Fig. 2B, lanes 3 and 5) and wild-type mice expressing this mutant are devoid of HDL₄. It also provides an explanation as to why other HDL apolipoproteins, such as apoA-II, are also found at lower than normal concentrations in heterozygotes for this mutation (15).

This is also the first demonstration that the apoA-I FIN mutation decreases the rate of apoA-I secretion from primary hepatocytes (Fig. 5). The effect is greatest under conditions that mimic apoA-I FIN homozygosity (infection with the apoA-I FIN adenovirus alone), but it is also observed in the heterozygous state (co-infection with the two adenoviruses). This is particularly true early on in the chase (t = 1 h) before the plateaus in the secretion time course are reached. In fact, hepatocytes expressing both apoA-I FIN and hapoA-I have statistically significant decreases (p < 0.05) in secreted ³⁵S-apoA-I (Fig. 5A, ▲) as well as cell-associated ³⁵S-apoA-I (Fig. 5B, ▲) at the 1 h-time point compared with hepatocytes expressing only hapoA-I (Fig. 5A, ●, and Fig. 5B, ○). This suggests that the apoA-I FIN mutation interferes with apoA-I secretion and causes apoA-I to be degraded intracellularly given that between 20 and 30% of the initial cell-associated apoA-I cannot be accounted for throughout the chase. These data are consistent with hepatocytes expressing apoA-I FIN alone. The decreased secretion of apoA-I FIN (Fig. 5A, ▼) into the medium is apparently accounted for by the accumulation of this mutant within the hepatocytes (Fig. 5B, ▼). However, some of the initial cell-associated apoA-I is lost and cannot be accounted for in the hepatocytes expressing this mutant. Therefore, these are the first data to suggest that heterozygous carriers of the apoA-I FIN mutation also have impaired apoA-I secretion in addition to enhanced apoA-I clearance from the plasma. Some studies have suggested that HDL-C levels are inversely correlated with the fractional catabolic rate of apoA-I and not with the apoA-I secretion rate (50). However, a more recent study utilizing endogenously labeled apoA-I has shown that both the fractional catabolic rate and secretion rate contribute to plasma HDL-C levels (51). Therefore, the reduced secretion rate of apoA-I in hepatocytes expressing both apoA-I FIN and hapoA-I most likely contributes to the low apoA-I and HDL-C concentrations in heterozygous carriers of this mutation. We also propose that there is an increased intracellular clearance of hapoA-I in the presence of apoA-I FIN, since a proportion of apoA-I secreted from these primary hepatocytes is in the form of lipid-associated apoA-I dimers. Proteinolysis of apoA-I FIN inside the hepatocytes would prevent efficient secretion of this nascent HDL population, similar to its effect on the clearance of this lipid-poor HDL pool in the plasma.

There are at least 7 other mutations identified within the central domain of apoA-I that are also associated with reduced plasma HDL-C and apoA-I concentrations (52, 52–60). We have shown in this study that apoA-I FIN has a greatly reduced ability to activate LCAT, but this alone cannot account for the hypoalphaproteinemia in heterozygous carriers of this mutation. In contrast, a recent in vivo study suggests that deletion of an 143–164 within apoA-I may negatively affect LCAT activation by native apoA-I (40). It was proposed that this might explain the dominant negative effect on HDL-C concentrations seen in heterozygotes for the apoA-I FIN mutation (deletion of an 146–160), although it should be noted that the two mutations are structurally different. In addition, no in vitro LCAT studies have been reported to confirm this hypothesis. Conversely, it has been suggested that the low HDL-C concentrations in heterozygotes for apoA-I mutations such as apoA-I Haplo and apoA-I FIN and apoA-I Zevafin (Leu¹⁰⁵ to Pro) result from hypercatabolism of apoA-I that is only partially due to or independent of a decrease in LCAT activation (55). This latter viewpoint is consistent with the results obtained from this study in which we show that proteolysis of apoA-I FIN and a reduced secretion rate of this mutant from hepatocytes are more likely to account for the low HDL-C and apoA-I concentrations than is dysfunctional LCAT activation.

In summary, this extensive metabolic study of a naturally occurring apoA-I variant known as apoA-I FIN should also prove valuable in the study of other apoA-I mutations contributing to hypoalphaproteinemia and further our understanding of the roles of apoA-I domains in the metabolism of HDL. We have shown that the apoA-I FIN mutation does not affect the ability of apoA-I to associate with lipids and form stable reconstituted lipoproteins. However, apoA-I FIN has a significantly reduced ability to activate LCAT (5-fold increase in ampK₄) compared with hapoA-I, but in a heterogeneous lipoprotein preparation with hapoA-I there is only a slight decrease in the affinity for the enzyme (1.4-fold decrease in ampK₄). Importantly, this mutation impairs apoA-I secretion from primary hepatocytes and leads to proteolysis of apoA-I in plasma. These effects appear to be primarily responsible for the remodeling of and decrease in the HDL pool size in both wild-type C57BL/6J mice expressing this mutant and in apoA-I-deficient mice co-expressing apoA-I FIN and hapoA-I. Therefore, we propose the combination of these defects account for the 4–5-fold reductions in HDL-C and apoA-I concentrations in heterozygous carriers of the apoA-I FIN mutation.

Acknowledgments—We thank Tracey Neville and Susha Zachariah for excellent technical assistance with the LCAT and primary hepatocyte experiments, respectively, and to the Animal Care Staff at the University of Ottawa Heart Institute for expert assistance. We also thank Drs. Ruth McPherson and Ross Milne for their suggestions and critical reading of this manuscript.

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Proteolytic Degradation and Impaired Secretion of an Apolipoprotein A-I Mutant Associated with Dominantly Inherited Hypoalphalipoproteinemia
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J. Biol. Chem. 2001, 276:21292-21302.
DOI: 10.1074/jbc.M100463200 originally published online April 5, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M100463200

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