Mutation in Apolipoprotein B Associated with Hypobetalipoproteinemia Despite Decreased Binding to the Low Density Lipoprotein Receptor*

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Received for publication, December 9, 2004, and in revised form, March 4, 2005
Published, JBC Papers in Press, March 28, 2005, DOI 10.1074/jbc.M413877200

Mutations in apolipoprotein B (APOB) may reduce binding of low density lipoprotein (LDL) to the LDL receptor and cause hypercholesterolemia. We showed that heterozygotes for a new mutation in APOB have hypobetalipoproteinemia, despite a reduced binding of LDL to the LDL receptor. APOB R3480P heterozygotes were identified among 9,255 individuals from the general population and had reduced levels of apoB-containing lipoproteins. Most surprisingly, R3480P LDL bound with lower affinity to the LDL receptor than non-carrier LDL in vitro, and these results were confirmed by turnover studies of LDL in vivo. In very low density lipoprotein (VLDL) turnover studies, the amount of VLDL converted to LDL in R3480P heterozygotes was substantially reduced, suggesting that this was the explanation for the hypobetalipoproteinemia observed in these individuals. Our findings emphasized the importance of combining in vitro studies with both human in vivo and population-based studies, as in vitro studies often have focused on very limited aspects of complex mechanisms taken out of their natural context.

Low density lipoprotein (LDL)1 particles are cleared from plasma mainly by binding to high affinity LDL receptors and subsequent internalization and degradation in the liver. Affinity of LDL to the receptor is dependent on intact structural and functional domains in both the receptor and the ligand, apoB-100 (1). ApoB-100, a 513-kDa glycoprotein composed of 4536 amino acid residues, is the predominant protein component of VLDL, IDL, and LDL (2).

Studies of the three-dimensional structure of apoB-100 by immunoelectron microscopy have suggested that apoB-100 enwraps VLDL, IDL, and LDL particles like a belt, completing the encirclement by about amino acid residue 4050, and that the carboxyl-terminal end forms a bow that crosses backwards over the chain between residues 3000 and 3500 (3). Chatterton et al. (3) speculated that the carboxyl-terminal sequence of apoB-100 could act as a negative regulator of LDL receptor binding, and they proposed that in VLDL particles the bow would inhibit apoB-100 binding to the LDL receptor. In contrast, after lipolysis had transformed VLDL into IDL and finally LDL, the carboxyl-terminal bow moved sufficiently to allow interaction of apoB-100 with the LDL receptor. Furthermore, mutagenesis studies in mice by Borén et al. (4) suggested that the region where the carboxyl-terminal bow of apoB-100 crosses the main chain of apoB-100 is located around residue 3500 in LDL.

In humans, three point mutations have been reported around this site in the APOB gene. All three mutations result in the loss of arginines and in varying degrees of decreased receptor affinity in vitro, reflected in the varying effects on lipid and lipoprotein levels in vivo; R3500Q and R3500W are associated with moderate to severe hypercholesterolemia, whereas R3531C has no effect or a marginal effect on lipid levels in the general population in vivo (5–8). Mutations resulting in truncations of apoB-100 are known to abolish LDL receptor binding when located amino-terminal to the putative binding site at amino acids 3359–3369 (site B), indicating that this site is crucial for receptor interaction. In contrast, apoB truncations enhance LDL receptor binding when located carboxyl-terminal to the binding site, indicating that the negative regulatory effect of the bow of apoB-100 can be abolished by removal of the carboxyl-terminal tail (9–11).

A mutation in APOB, R3480P, located in the region where the carboxyl-terminal bow crosses over the main apoB-100 chain, and briefly mentioned in a previous paper (12), led us to the following hypothesis. Because this mutation causes a substitution of the basic side chain of arginine with the non-polar of proline in an α-helical region, the R3480P mutation might have an impact on both the secondary and tertiary structure of apoB-100, and thus on the affinity of apoB-100 for the LDL receptor via a change in the negative regulatory effect of the carboxyl-terminal bow. This then could affect LDL levels, catabolism, and production in R3480P heterozygotes.

To study the effect of the R3480P mutation in apoB-100 on the levels of lipids, lipoproteins, and apolipoproteins and to characterize the effect of the mutation on LDL metabolism, we screened 13,427 Danish individuals (9,255 individuals from the general population (The Copenhagen City Heart Study) and 4,172 patients with ischemic cardiovascular disease or hyper-
lipidemia) for the presence of this mutation. In R3480P heterozygotes, we determined lipids, lipoproteins, and apolipoproteins, and we compared these with the corresponding values in non-carriers in the general population of the same age, sex, and APOE genotype. The relative LDL receptor affinity of R3480P LDL and non-carrier LDL was determined in vitro in a competitive receptor-binding assay in normal human fibroblasts. In vivo turnover studies of iodinated LDL, we compared fractional catabolic rate (FCR) and production rate (PR) of LDL from R3480P, R3480W, R3500Q, and R3531C heterozygotes with simultaneously injected iodinated LDL from non-carriers. Finally, in vivo turnover studies of iodinated VLDL, we estimated the amount of VLDL from R3480P heterozygotes converted to LDL compared with the conversion of non-carrier VLDL. This design with simultaneous injection of differently labeled R3480P LDL (or VLDL) and non-carrier LDL (or VLDL) excludes all variation because of differences between recipients, because the two types of lipoprotein are metabolized by the exact same pathways in the same recipient. Thus, even small differences in metabolism because of differences in LDL (or VLDL) particles can be detected accurately.

MATERIALS AND METHODS

Subjects—The Copenhagen City Heart Study (1991–1994) comprised an almost equal number of women (55%) and men stratified into 10-year age groups from 20 to 80 years and above, drawn randomly from the Copenhagen Central Population Register, with the aim of drawing a sample representative of the adult Danish general population (13, 14); 9,255 individuals were genotyped for the present study. A second population comprised 4,172 patients with a primary diagnosis of ischemic heart disease (n = 3,479), ischemic cerebrovascular disease (n = 452), or hyperlipidemia (n = 241). In total, 13,427 individuals (n = 9,255 + 4,172) were genotyped for the present study, and 99% of these were white and of Danish descent.

The studies were approved by institutional review boards and Danish ethical committees as follows: 100.2039/91, KF 01-375/94, KF 01-372/94, Copenhagen and Frederiksberg committee, and KA 93125 and KA 92273. Copenhagen County committee and was conducted according to the Declaration of Helsinki. Participants gave written informed consent. Rabbit studies were approved by the Danish Animal Experiment Board.

Detection of Mutations—The R3480P mutation was caused by the substitution of guanine for asparagine at position 10,648 in complementary DNA, in exon 26 of the APOB gene on chromosome 2p24 (15, 16). DNA from each of the 13,427 individuals was subjected to PCR (forward primer, 5′-GGGCAATCTCATCCTTTCTTGGAAAGATG-3′; and reverse primer, 5′-CTTGGTACCACTCCTCTTGATGCTG-3′) with an annealing temperature of 65°C followed by digestion with MspI. The mismatched C introduced 3′ in the reverse primer introduced an internal control site for MspI. The R3500Q and R3531C mutations were identified as described previously (8); a single carrier with R3480W was identified accidentally during sequencing of this apoB-100 region in 60 years.

Plasma Lipids, Lipoproteins, and Apolipoproteins—Colorimetric and turbidimetric assays were used to measure plasma levels of total cholesterol, HDL cholesterol, triglycerides, and apoB (8). The sizes of LDL and apoB were determined as described previously (18–20). From plasma samples of three mutation carriers (proband number 1, proband number 4, and his 39-year-old son) and 30 non-carriers, matched for age in 10-year age groups, sex, and APOE genotype, lipoprotein fractions were isolated using ultracentrifugation in a fixed angle rotor (Beckman 50.4 Ti rotor and Beckman L8–70 ultracentrifuge, Beckman Instruments, Inc., Palo Alto, CA) at 50,000 × g using density adjustment with NaCl/NaBr solution. Cholesterol and triglycerides were measured in VLDL (d < 1.006 g/ml), IDL (1.006 g/ml < d < 1.019 g/ml), LDL (1.019 g/ml < d < 1.063 g/ml), and HDL cholesterol (d > 1.063 g/ml). In vitro Competitive Receptor Binding Assay of LDL—Competitive receptor binding assays were performed as described by Arnold et al. (21). In brief, human wild type fibroblasts were cultured in Dulbecco's modified Eagle's medium (with Glutamax I and Hepes 25 mM) containing 10% fetal bovine serum. To up-regulate LDL receptors, cells were transferred to Dulbecco's modified Eagle's medium with 10% lipopo-

**Table 1**

| Characteristics of subjects heterozygous for R3480P and R3480W compared with non-carriers in the general population | n | N | n-1 |
|---|---|---|---|
| | | | 1 |
| | | | Probands 1 2 3 4 5 |
| Age (years) | 51 | 69 | 70 | 71 | 85 |
| Cholesterol (mmol/liter) | 3.9 (1%) | 6.1 (28%) | 5.2 (19%) | 6.0 (45%) | 5.4 (1%) |
| HDL cholesterol (mmol/liter) | 1.22 (5.1%–2.48) | 1.25 (6.0%–2.60) | 1.24 (5.9%–2.33) | 1.25 (5.5%–2.33) | 1.25 (5.6%–2.35) |
| LDL cholesterol (mmol/liter) | 3.0 (1.5%–5.6) | 4.9 (2.3%–6.9) | 3.7 (1.3%–5.8) | 4.4 (2.2%–5.9) | 3.9 (1.8%–5.5) |
| ApoA1 (mg/dl) | 121 (35%) | 168 (71%) | 141 (64%) | 164 (86%) | 135 (94–194) |
| ApoB (mg/dl) | 2.0 (0.6–4.4) | 2.0 (0.8–11.9) | 1.8 (0.7–2.9) | 2.0 (0.6–4.4) | 2.0 (0.8–11.9) |
| Lp(a) (mg/dl) | 48.5 (80%) | 34.1 (67%) | 13.9 (4%) | 0.4 (7%) | 22.9 (0–82.8) |
| Other lipids and lipoproteins | | | | | |
tein-deficient serum 2 days before each experiment. Non-carrier human 125I-labeled LDL cholesterol (5 μg/ml), along with increasing concentrations of unlabeled R3480P or non-carrier LDL, was added to the cells in phosphate-buffered saline and 10% human lipoprotein-deficient serum. After 2 h of incubation at 4 °C, the surface-bound radioactivity was determined. Counts per min in each well were adjusted for differences in cell growth by measuring the protein content in lysates and calculating the number of counts/mg of cell protein. The number of counts/mg of cell protein was corrected for unspecific binding. The amount of apolipoprotein B was precipitated in lipoprotein fractions with isopropyl alcohol (19), instead of total plasma protein precipitation with trichloroacetic acid as in the LDL study (22).

Fractional catabolic rate (FCR) of LDL, defined as the fraction of the intravascular pool of LDL catabolized per day, was calculated according to the method of Matthews (25), where $F = \lambda/[\lambda - \rho] C_{b0}$, using the SAAM II software (26). Production rate was calculated by multiplying the pool size of LDL (mmol/kg) with the FCR (1/day) of LDL and was expressed as mmol/kg body weight/day. Pool size of LDL was the product of plasma LDL (mmol/liter) and plasma volume (liter/kg), estimated by isotope dilution at 10 min after injection of labeled LDL. Because steady state plasma LDL cholesterol levels were significantly lower in R3480P heterozygotes than in non-carriers, we used the individual LDL cholesterol levels of R3480P heterozygotes and of non-carriers to calculate the estimated pool sizes of LDL.

The amount of VLDL converted to LDL (mmol/kg/day) was calculated by multiplying the pool size of VLDL (mmol/kg) with the FCR of VLDL (pool/day). FCR of VLDL was estimated directly from human in vivo VLDL turnover studies using a four-compartment model in the SAAM II software (26); Dr. P. Hugh Barrett helped us optimize the model that best described the VLDL kinetic data. Fractional catabolic rate of VLDL cholesterol $k_{3,1}$, defined as the fraction of the intravascular pool of
VLDL catabolized to LDL per day, was determined with the SAAM II software using a four-compartment model. The model consisted of two VLDL compartments (q1 and q2) and two LDL compartments (q3 and q4). Dose was injected into q1 and q2, and samples were drawn from q1 + q2 and q3. Fluxes were estimated for the following: k(0,1), the direct removal of VLDL from compartment q1; k(0,2), the direct removal of VLDL from compartment q2; k(0,3), the direct removal of LDL from compartment q3; k(2,1), the transfer from compartment q1 to q3, i.e., the fractional catabolic rate of VLDL or the fraction of VLDL converted to LDL in pools per day; k(3,4) and k(4,3) the transfer between compartments q3 and q4. Pool sizes (mmol/kg) for the two lipoprotein fractions were taken as the product of plasma volume (liter/kg) estimated by isotope dilution at 10 min after injection of the labeled VLDL and the plasma concentration at steady state (mmol/liter). Production of LDL from VLDL (mmol/kg/day) was calculated by multiplying the pool size of VLDL (mmol/liter) with the fractional catabolic rate of VLDL (pools/day).

Statistical Analysis—Because the four R3480P heterozygotes identified in the general population were all homozygous for the APOE e33 genotype, all comparisons were restricted to individuals with the e33 genotype throughout the study. Data were analyzed with SPSS and Minitab computer programs (27, 28).

To examine the effect of R3480P on phenotype in heterozygotes identified in the general population, we converted values for continuous variables for heterozygotes to their respective percentiles within each 10-year age group and by sex, and we compared them with the values of e33 homozygotes in the general population as a whole, using z-scores, as described previously (8, 29). The absolute increase or decrease in a given variable in R3480P heterozygotes was calculated by subtracting the 50th percentile value for non-carriers in the general population matched for age (in 10-year age groups), sex, and APOE genotype (e33) from the value of the individual R3480P heterozygote; the mean increases or decreases for a given variable were compared with the normal distribution with μ = 0 by a 1-sample test. All p values are two-sided, and a p value less than or equal to 0.05 is considered to indicate statistical significance.

RESULTS

Frequency of the APOB R3480P Mutation—Four of 9,255 subjects in the general population (carrier frequency, 0.04%; 95% confidence interval, 0.01–0.11%) and 1 of 4,172 patients with ischemic cardiovascular disease or hyperlipidemia (0.02% and 95% confidence interval, 0.00–0.15%) were heterozygous for the R3480P mutation in apoB (χ², p = 0.59).

Phenotypic Characteristics of APOB R3480P Heterozygotes in the General Population—Compared with non-carriers in the general population of the same age, sex, and APOE genotype (e33), R3480P and R3480W heterozygosity tended to be associated with lower than average levels of plasma cholesterol, LDL cholesterol (including IDL cholesterol), apoB, triglycerides, VLDL cholesterol, and cholesterol/HDL cholesterol ratio, and with higher than average HDL cholesterol and HDL cholesterol/apoA1 ratio (Table I). None of the R3480P heterozygotes had a family history of hypercholesterolemia or of premature ischemic heart disease, although one subject, a 71-year-old male with type 2 diabetes and hypertension, had a non-fatal myocardial infarction at age 68 (Table I).

Because all continuous characteristics were measured simultaneously in all participants of the Copenhagen City Heart Study, it was possible to assign each proband to a percentile in the relevant 10-year age group by sex and APOE genotype (Table I; Fig. 1), thus controlling for well known effects of age, sex, and APOE genotype on lipid and lipoprotein levels. Heterozygosity for R3480P was associated with significantly lower total cholesterol (mean percentile, 23rd; 95% confidence interval, 0–52nd percentile, p = 0.05), LDL cholesterol (16th percentile; 0–35th percentile; p = 0.03), apoB (14th percentile; 0–27th percentile, p = 0.02), and cholesterol/HDL cholesterol (9th percentile; 4–14th percentile, p = 0.005), compared with...
non-carriers in the general population of the same age, sex, and APOE genotype (Fig. 1). R3480P heterozygosity was associated with increased HDL cholesterol (80th percentile; 53–100th percentile; \( p = 0.06 \)) and HDL cholesterol/apoAI (89th percentile; 75–100th percentile; \( p = 0.008 \)) compared with non-carriers in the general population. A single R3480W heterozygote had percentile values for these variables similar to those for R3480P heterozygotes (Table I). Triglycerides, VLDL cholesterol, and age (Fig. 1), as well as lipoprotein(a), fibrinogen, glucose, body-mass index, and ratio of waist to hip circumfer-

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**FIG. 4.** Plasma decay curves for different ligand-defective mutations in APOB. Plasma decay curves of radiolabeled R3500Q LDL (○) (top panel, left and right), R3531C LDL (○) (2nd panel, left and right), non-carrier LDL (○) (3rd panel, left and right), R3480P LDL (○) (4th panel, left and right), R3480W LDL (○) (bottom panel, left and right) and non-carrier LDL (■) determined in human non-carriers after simultaneous intravenous injection.
ence did not differ between R3480P heterozygotes and non-carriers in the general population (data not shown). Sex distribution, smoking habits, frequencies of ischemic heart disease, hypertension, peripheral arterial disease, ischemic stroke, xanthelasma, arcur corneae, and diabetes mellitus also did not differ between R3480P heterozygotes and non-carriers in the general population (data not shown).

The R3480P mutation was associated with average decreases in levels of LDL cholesterol, apoB, triglycerides, VLDL cholesterol, and ratio of cholesterol/HDL cholesterol of 1.1 mmol/liter, 24 mg/dl, 0.4 mmol/liter, 0.2 mmol/liter, and 1.6, respectively, and with average increases in levels of HDL cholesterol, and the ratio of (HDL cholesterol/apoAI) × 10, of 0.5 mmol/liter and 0.9 (Table II), when compared with individuals in the general population of the same age (in 10-year age groups, sex, and APOE genotype (e33)).

**Lipoprotein Fractions Separated by Ultracentrifugation**—R3480P heterozygosity (n = 3) was associated with decreased cholesterol content in VLDL and LDL fractions when compared with non-carriers (n = 30) of the same age (in 10-year age groups, sex, and APOE genotype (e33)) (median VLDL cholesterol 0.1 mmol/liter (range, 0.1–0.2) and IDL 0.1 mmol/liter (0.0–0.1) versus LDL 0.3 mmol/liter (0.1–1.0) and IDL 0.3 mmol/liter (0.1–0.5); p = 0.03 and p = 0.01, respectively), whereas there was a trend toward an association with lower LDL cholesterol levels (p = 0.12). R3480P heterozygosity was not associated with differences in HDL cholesterol levels between the two groups (p = 0.51). R3480P heterozygosity was associated with decreases in triglycerides in plasma compared with non-carriers (0.7 mmol/liter (0.6–0.9) versus 1.3 mmol/liter (0.6–2.6); p = 0.008) as well as with decreases in triglycerides in VLDL, LDL, and HDL fractions (p = 0.004 to p = 0.05). Finally, R3480P heterozygosity was associated with decreases in plasma apoB and phospholipids compared with non-carriers (apoB, 77 mg/dl (71–93) versus 113 mg/dl (69–174), and phospholipids, 1.7 mmol/liter (1.6–2.5) versus 3.0 mmol/liter (2.5–4.0); p = 0.004 and p = 0.001, respectively).

**LDL Particle Size and Size of ApoB**—Particle diameters of the isolated LDL were similar in R3480P heterozygotes and non-carriers (median 24.5 nm (n = 3; range, 19.4–29.6) versus 24.2 nm (n = 44; range, 24.1–24.3), p = 0.61). Separation of delipidated apoB (from the LDL, IDL, and VLDL fractions obtained by ultracentrifugation) on a 7% acrylamide SDS-polyacrylamide gel showed no differences between R3480P heterozygotes and non-carrier apoB, indicating that the R3480P mutation was not associated with truncated forms of apoB.

**LDL Competitive Receptor Binding Assay**—The ability of R3480P LDL to compete with 125I-labeled non-carrier LDL for binding to normal human fibroblasts was impaired compared with non-carrier LDL (Fig. 2). A 50% displacement of 125I-labeled non-carrier LDL from human fibroblasts required 7.9 μg of R3480P LDL protein per ml compared with 4.6 μg of non-carrier LDL protein/ml.

**LDL Turnover Studies**—Plasma decay curves of R3480P LDL (n = 4) and non-carrier LDL (n = 4) (Figs. 3 and 4) determined simultaneously in humans (n = 4) or in rabbits (n = 3) demonstrated that R3480P LDL is metabolized more slowly than non-carrier LDL in both humans with low LDL receptor activity and in rabbits with high receptor activity (Fig. 3). In accordance with this, fractional catabolic rate of R3480P LDL was on average decreased by 23% compared with non-carrier LDL in humans (Table III). Furthermore, because plasma LDL levels were reduced in R3480P heterozygotes compared with non-carriers in the general population (Table I, Fig. 1, and Table II), LDL production rate was reduced by 29% in R3480P heterozygotes when compared with non-carriers (Table III).

For comparison with other known ligand-defective mutations, in vivo turnover studies were performed in R3500Q LDL, R3531C LDL, non-carrier LDL, R3480P LDL, and R3480W LDL versus non-carrier LDL simultaneously injected in the same recipients (Fig. 4). The reductions in FCR compared with non-carriers were as follows: 33% for R3500Q (Fig. 4, top panel, left and right), 12% for R3531C LDL (Fig. 4, 2nd panel, left and right), no difference for non-carrier LDL (Fig. 4, 3rd panel, left and right), 26% for R3480P LDL (Fig. 4, 4th panel, left and right), and 21% for R3480W LDL (Fig. 4, bottom panel, left and right).

Fig. 5 compares LDL cholesterol percentiles (top), LDL fractional catabolic rate ratios (middle), and LDL production rate ratios (bottom) between heterozygotes for R3500Q, R3531C, R3480P, and R3480W LDL.
non-carriers, R3480P, and R3480W all identified in The Copenhagen City Heart Study. LDL cholesterol percentiles were increased in R3500Q heterozygotes, unaltered in R3531C heterozygotes, and decreased in R3480P heterozygotes compared with non-carriers in the general population. LDL fractional catabolic rate ratio was decreased for all four mutations studied compared with non-carriers; the decrease was largest for R3500Q heterozygotes, intermediate for R3480P and R3480W heterozygotes, and smallest for R3531C heterozygotes. By contrast, LDL production rate ratio was unaltered in R3500Q and R3531C heterozygotes compared with non-carriers, although it was decreased in R3480P and R3480W heterozygotes.

**DISCUSSION**

In this study, we report the characteristics of a new ligand-defective mutation in *APOB*, R3480P, located in a region of apoB-100 critical for modulation of binding of VLDL, IDL, and LDL to the LDL receptor (4, 7, 12). Our data indicate that *APOB* R3480P heterozygosity occurs at a frequency of 0.04% (in 2,000) in a general population of whites and is associated with hypobetalipoproteinemia, despite an impaired ability to interact with the LDL receptor. The explanation for this apparent contradiction appears to be a considerable reduction in the amount of VLDL converted to LDL in these individuals *in vivo*.
Because we identified four R3480P heterozygotes among 9,255 randomly selected individuals from the general population and only 1 among 4,172 patients with ischemic cardiovascular disease or hyperlipidemia, our results indicate that R3480P is not associated with these diseases. In support of this, probands identified in the general population had on average lower levels of LDL cholesterol (by ~1 mmol/liter), apolipoprotein B, triglycerides, and VLDL cholesterol, and HDL levels tended to be higher when compared with individuals in the general population matched for age, sex, and APOE genotype. Thus, R3480P is the first ligand-defective mutation in APOB associated with a moderate hypobetalipoproteinemia phenotype.

These results were further supported by ultracentrifugation of fasting plasma samples from three R3480P heterozygotes and 30 non-carriers from the general population matched for age, sex, and APOE genotype and were examined 10 years after the initial measurements. Taken together, these findings suggested a decreased production and/or an increased catabolism of apoB-100 containing lipoproteins in R3480P heterozygotes.

Most surprisingly, R3480P LDL showed a considerable reduction in LDL receptor affinity in vitro competitive receptor binding studies, suggesting a hypercholesterolemia phenotype. The reduction in receptor affinity was confirmed in vivo turnover studies of LDL, where fractional catabolic rate of R3480P LDL was on average reduced by 23% compared with non-carrier LDL in both humans and rabbits. Despite a reduced affinity of LDL for the LDL receptor in vivo as shown in the turnover studies, steady state levels of all apoB-100 containing lipoproteins in plasma were lower than average when compared with non-carriers, suggesting a large reduction in production rate of LDL. This was exactly what we observed, as the LDL production rate in R3480P heterozygotes was reduced by ~29% compared with non-carriers. In support of this, direct VLDL turnover studies showed that the amount of VLDL converted to LDL was reduced by ~70% in R3480P heterozygotes compared with non-carriers. Furthermore, an accidentally identified R3480W heterozygote had hypobetalipoproteinemia as well as both reduced production and catabolism of LDL, supporting our findings for R3480P heterozygotes. Finally, R3480W has been shown by others to have reduced affinity for the LDL receptor in vivo (7).

It would appear that there is a discrepancy between the conversion rate of VLDL to LDL obtained in VLDL turnover studies (70%) and the production rate of LDL obtained in LDL turnover studies (30%). This apparent discrepancy is because of the different levels of LDL and VLDL in the subjects used for turnover studies and not because of excessive (40%) in vivo “escape” of VLDL. The method used in our turnover studies is very precise when comparing the fractional catabolic rate ratios of R3480P heterozygous LDL (or VLDL) with non-carrier LDL (or VLDL) between the individuals, because the method eliminates the inter-individual variation, but is less accurate when the fractional catabolic rates are multiplied with lipoprotein levels in the individual participants to estimate production rate of LDL and VLDL converted to LDL. However, although the absolute levels of LDL production and of VLDL converted to LDL in R3480P heterozygotes versus non-carriers have relatively wide confidence intervals, the fractional catabolic rate ratios as well as the lipoprotein levels in the general population are both very accurately determined. Taken together, the combined data suggest that the observed hypobetalipoproteinemia in R3480P heterozygotes is explained by a reduction in the conversion of VLDL to LDL.

Immunoelectron microscopy studies have shown that 89% of apoB-100 enwraps the LDL particle like a belt and that the carboxyl-terminal 11% constitutes a bow that crosses over this belt, bringing the carboxyl-terminal portion of apoB-100 close to amino acid 3500 (3). The carboxyl terminus normally functions to inhibit the interaction of apoB-100 VLDL with the LDL receptor, but after conversion of triglyceride-rich VLDL to smaller cholesterol-rich LDL, arginine 3500 interacts with the carboxyl terminus, permitting normal interaction between LDL and its receptor (4). The loss of arginine at this site as in familial defective apolipoprotein B-100 (R3500Q/R3500W) destabilizes this interaction, resulting in receptor-binding defective LDL. Thus, mutations distal to the receptor-binding region, residues 3,359–3,369 (site B), may modulate the affinity of apoB-100 containing lipoproteins to the LDL receptor. Recently, a model has been developed of how the carboxyl-terminal bow interacts with the backbone of apoB-100, which enwraps the LDL particle (7). This model predicts that arginine 3500 interacts with tryptophan 4369 and that this interaction is essential for correct conformation of the carboxyl-terminal tail of apoB-100. The model further implies that tryptophan 4369 interacts with other arginines in addition to arginine 3500 in apoB-100 during conversion of VLDL to LDL. Only five naturally occurring mutations in apoB-100 have been unequivocally linked to varying degrees of defective receptor binding and all are located distal to the receptor-binding region: R3500Q (5), R3500W (24), R3531C (6), R3480W (7), and R3480P, the novel mutation characterized in this study. All

**TABLE IV**

| recipient | R3480P VLDL | Non-carrier VLDL | R3480P VLDL/ non-carrier VLDL |
|-----------|-------------|-----------------|-----------------------------|
| Recipient 5 | | | |
| VLDL cholesterol (mmol/liter) | 0.21 | 0.84 | |
| VLDL fractional catabolic rate (pools/day) | 5.0 | 5.2 | 0.96 |
| VLDL converted to LDL (mmol/kg/day) | 0.024 | 0.102 | 0.24 |
| Recipient 6 | | | |
| VLDL cholesterol (mmol/liter) | 0.21 | 0.84 | |
| VLDL fractional catabolic rate (pools/day) | 9.2 | 8.4 | 1.10 |
| VLDL converted to LDL (mmol/kg/day) | 0.043 | 0.161 | 0.27 |
| Recipient 7 | | | |
| VLDL cholesterol (mmol/liter) | 0.31 | 0.96 | |
| VLDL fractional catabolic rate (pools/day) | 9.2 | 9.7 | 0.95 |
| VLDL converted to LDL (mmol/kg/day) | 0.083 | 0.273 | 0.30 |
| Average in 3 recipients | | | |
| VLDL cholesterol (mmol/liter) | 0.24 | 0.88 | |
| VLDL fractional catabolic rate (pools/day) | 7.8 | 7.8 | 1.0 |
| VLDL converted to LDL (mmol/kg/day) | 0.050 | 0.179 | 0.28 |
these mutations result in the loss of an arginine and presumably interfere with correct conformation of the carboxyl-terminal tail of apoB-100. By using the Copenhagen City Heart Study, we have shown previously that although probands with R3500Q identified in the general population had moderately to severely increased levels of total cholesterol, LDL cholesterol, and apoB, probands identified in the same population carrying R3531C had total cholesterol, LDL cholesterol, and apoB levels that did not differ from those of non-carriers (8). In accordance with this, R3500Q was associated with a 7-fold increase in risk of ischemic heart disease, whereas risk was not increased in R3531C heterozygotes. In the present study we demonstrate that R3480P is associated with a reduction in apoB-100 containing lipoproteins, a reduced affinity of mutant LDL for the LDL receptor both in vivo and in vitro, and with a considerably reduced production of LDL cholesterol from VLDL cholesterol. In accordance with this, R3480P heterozygotes are not at an increased risk of ischemic cardiovascular disease. In summary, we have identified the first ligand-defective mutation in apoB associated with hypobetalipoproteinemia, and we have shown that the phenotype in apoB mutation carriers is the net result of several processes involving production rate of VLDL, conversion of VLDL to LDL, and removal of LDL via the LDL receptor. Thus, the effects of a reduced affinity for the LDL receptor can be counteracted by other mechanisms and result in a hypobetalipoproteinemia phenotype. Our findings emphasize the importance of combining in vitro studies with both human in vivo and population-based studies, as in vitro studies often focus on very limited parts of complex mechanisms taken out of their natural context.

Acknowledgments—We thank Mette Refstrup, Kurt Svarre Jensen, Hanne Damm, and Jesper Schou for expert technical assistance and Professor P. Hugh Barrett (Perth, Western Australia) for creating the compartment model used in analyzing the VLDL turnover studies.

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