# Overproduction of small very low density lipoproteins ($S_f$ 20–60) in moderate hypercholesterolemia: relationships between apolipoprotein B kinetics and plasma lipoproteins

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**Abstract** An analysis of apolipoprotein (apo) B turnovers conducted in subjects with moderate hypercholesterolemia was performed to discover relationships that may exist between apoB kinetic parameters and plasma lipid and lipoprotein levels. A group of 21 subjects with plasma cholesterol in the range 250–300 mg/dl and triglyceride < 265 mg/dl were injected with tracers of $^{131}$I-labeled very low density lipoprotein 1 ($VLDL_1$, $S_f$ 60–400) and $^{125}$I-labeled $VLDL_2$ ($S_f$ 20–60) prepared by cumulative flotation ultracentrifugation. The metabolism of apoB in these fractions was followed through intermediate density (IDL, $S_f$ 12–20) to low density (LDL, $S_f$ 0–12) lipoprotein. The most consistent feature giving rise to the higher apoB levels that occurred in $VLDL_2$, IDL, and LDL in the hypercholesterolemic group was increased input of $VLDL_2$ (787 ± 607 (SD) mg/day vs. 349 ± 213 in normals, $P < 0.01$). $VLDL_1$ apoB input was variably affected and not significantly different from normal. However, the plasma residence time of this subfraction was increased (0.15 ± 0.07 days vs. 0.08 ± 0.03 days in normals, $P < 0.001$) due to a decreased fractional rate of direct catabolism. Fractional transfer rates (FTR) down the delipidation cascade and other fractional rates of direct catabolism were, overall, not significantly different from normal. The plasma residence time of $VLDL_2$ apoB and LDL apoB was similar in hypercholesterolemic and normal subjects, while that of IDL apoB was slightly increased. Variation in LDL apoB mass within the hypercholesterolemic group correlated with $VLDL_1$ apoB input ($r = 0.58$, $P = 0.006$), the fractional rate of transfer from IDL to LDL ($r = 0.61$, $P = 0.003$), and direct LDL input ($r = 0.64$, $P = 0.002$). The proportion of LDL apoB mass derived by direct, i.e., VLDL-independent input, varied from 5 to 50% and was inversely correlated with plasma triglyceride ($r = -0.53$, $P = 0.014$) and positively with $HDL_2$ ($r = 0.66$, $P = 0.002$). In addition, the amount of direct LDL input was related to the amount of $VLDL_1$ removed by direct catabolism ($r = 0.53$, $P = 0.013$). The analysis indicated that moderate hypercholesterolemia arose principally from overproduction of small VLDL, while variation in $VLDL_1$ input and the IDL to LDL conversion rate (presumably hepatic lipase-mediated) modulated the extent of the elevation in LDL apoB.—**Gaw, A., C. J. Packard, G. M. Lindsay, B. A. Griffin, M. J. Caslake, A. R. Lorimer, and J. Shepherd.** Overproduction of small very low density lipoproteins ($S_f$ 20–60) in moderate hypercholesterolemia: relationships between apolipoprotein B kinetics and plasma lipoproteins. *J. Lipid Res.* 1995. **36:** 158–171.

**Supplementary key words** kinetics • SAAM • multicompartmental model • direct LDL production

Many individuals who succumb to early coronary heart disease have moderate hypercholesterolemia often accompanied by an elevation in plasma triglyceride levels (1). These lipid abnormalities arise from disturbances in the metabolism of apolipoprotein B (apoB), the major protein associated with very low and low density lipoprotein (VLDL, LDL). Current evidence suggests that apoB-100 is secreted by the liver in the form of triglyceride-rich VLDL which undergoes intravascular lipolysis via intermediate particles (intermediate density lipoprotein, IDL) to LDL, a cholesterol-rich end product that is cleared from the circulation by a receptor pathway and alternative scavenger mechanisms. Endothelium-bound lipases in conjunction with receptor-mediated processes control the rates of lipoprotein delipidation and catabolism and their failure leads to hyperlipidemia of varying degree. Severe disorders such as familial hypercholesterolemia (FH) are well characterized in terms of the underlying genetic mutation, phenotypic expression, and impact on apoB metabolism. However, they are only minor contributors to the burden of coronary disease in a community. In the

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; SAAM, Simulation Analysis and Modeling computer program; TMU, tetramethylurea; FCR, fractional catabolic rate; FTR, fractional transfer rate; U(I), input into compartment I; L(I,J), rate constant for transfer from J to I; R(I,J), flux or transport in mg/time from J to I; IC(I), initial conditions of I.

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bulk of individuals, more modest changes give rise to elevated plasma lipids which, in conjunction with other risk factors, promote atherosclerosis.

The present study was designed to investigate apoB metabolism in a large group of subjects with plasma cholesterol levels in the range of 250–300 mg/dl, i.e., moderate hypercholesterolemia. Using a dual-tracer technique that had previously provided insight into the metabolic aberrations present in subjects with genetic disorders such as FH (2) and lipoprotein lipase deficiency (3), we determined kinetic parameters describing VLDL, IDL, and LDL apoB turnover and related these to plasma lipid levels, Lp[a] concentration, and the HDL and LDL subfraction distribution. Several issues were addressed in our analysis *i)* What causes elevated LDL levels in moderate hypercholesterolemia? This question has been examined in previous turnover studies (4–7) that have provided evidence that moderate hypercholesterolemia can arise for a number of reasons including an increased cholesterol content in LDL (i.e., elevated cholesterol/apoB ratio) (6), over-production of LDL apoB (4–6), and defective clearance of LDL apoB (6, 7). Excessive input of apoB appeared to be the most frequent abnormality and we sought to expand current knowledge by testing the hypothesis that the increased input occurred within a specific subfraction in the VLDL density interval. *ii)* What relationships are present between plasma lipid levels, HDL and LDL subfraction distributions, Lp[a] levels, and apoB kinetics? *iii)* Can the controversial phenomenon of "direct," (i.e., VLDL-independent) LDL input be characterized in greater detail?

## METHODS

### Subjects

A group of 21 subjects (15 males, 6 females) were recruited from the Risk Factor and Cardiology Clinics at Glasgow Royal Infirmary. On presentation, each subject had a plasma cholesterol level in excess of 250 mg/dl despite adherence to a standard lipid-lowering diet (European Atherosclerosis Society dietary recommendations (8)) and a plasma triglyceride of less than 265 mg/dl. Patients were screened by routine clinical and biochemical analyses and excluded from further study if they showed signs of hepatic, renal, hematological, or endocrine dysfunction. Subjects with evidence of FH (9) were also excluded as were pre-menopausal females, those homozygous for the apoE2 phenotype, and those taking lipid-lowering drugs. A number of individuals were receiving medication for angina or hypertension; one subject was on hydrochlorothiazide and another on propranolol which may have had a moderate impact on plasma lipid levels. Inclusion of the latter two patients did not unduly influence the statistical relationships observed in the study.

### Turnover protocol

Patients were recruited to three concurrent studies of the mechanism of action of lipid-lowering drugs that were conducted according to a standard protocol. The present report focuses on the metabolism of apolipoprotein B in 21 participants during the baseline dietary phase that was common to each study. One complete investigation comparing baseline and drug treatment phases in six of the present group of patients has already been published (10).

The methods used to prepare the $VLDL_1$ ($S_f$ 60–400) and $VLDL_2$ ($S_f$ 20–60) tracers have been described in detail elsewhere (10). Briefly, subjects admitted at 8:00 AM after an overnight fast received injections of autologous $^{131}$I-labeled $VLDL_1$ and $^{125}$I-labeled $VLDL_2$. ApoB-containing lipoproteins ($VLDL_1$[$S_f$ 60–400], $VLDL_2$[$S_f$ 20–60], IDL[$S_f$ 12–20], and LDL [$S_f$ 0–12]) were isolated from blood samples obtained at frequent intervals after tracer administration by a modification (2) of the cumulative gradient ultracentrifugation procedure of Lindgren, Jensen, and Hatch (11). ApoB was precipitated by the addition of an equal volume of redistilled 1,1,3,3-tetramethylurea (TMU) at 37°C to each lipoprotein fraction (12) and its specific activity was determined by radioactivity counting and protein assay (10, 13). The concentration of apoB in $VLDL_1$, $VLDL_2$, IDL, and LDL was determined by replicate analysis at four times throughout the study. Correction for centrifugal losses was made by comparing the recovered $VLDL_1$ + $VLDL_2$ + IDL + LDL cholesterol to the "non-HDL" cholesterol in plasma (10). Pool sizes for apoB in the four lipoprotein fractions were calculated as the product of plasma volume (taken as 4% of body weight) and the plasma concentration of apoB in each fraction.

The composition of each lipoprotein fraction was determined by assaying total and esterified cholesterol, triglycerides, phospholipids and protein (10). The method of Lowry et al. (13) was used for protein analysis while commercial kits were used to quantify the lipids.

Thyroidal uptake of radioiodide was blocked by the oral administration of mono-potassium iodate (170 mg twice daily). This regimen was commenced 3 days prior to injection and continued for the next 28 days. Turnovers were conducted on an out-patient basis and all subjects were instructed to adhere strictly to their established lipid-lowering diet and lifestyle. The study was approved by the Ethical Committee of Glasgow Royal Infirmary and written informed consent was obtained from each participant.

### Lipid and lipoprotein analysis

The plasma lipid and lipoprotein levels were assessed on three occasions throughout the 13-day turnover period according to the Lipid Research Clinic's Protocol (14). HDL subfraction masses were measured by analytical ultracentrifugation (15) and plasma LDL subfraction profiles were determined by non-equilibrium density gra-

dient centrifugation as described by Griffin et al. (16). This separation technique generated LDL profiles in which it was possible to resolve three subfractions that corresponded in size and density to those described by Krauss (17), namely LDL-I, LDL-II, and LDL-III. The individual subfraction areas beneath the LDL profile were quantified, adjusted by specific extinction coefficients determined previously for LDL-I, -II, and -III, and expressed as percentages. The total mass (cholesteryl ester plus triglyceride, phospholipid, cholesterol, and protein) of LDL prepared at density limits of 1.019–1.063 g/ml (11) was proportioned between the subfractions to give concentrations for each in milligrams of lipoprotein per 100 ml of plasma. Lp[a] was measured using a commercial ELISA kit, Innotest Lp[a] (Innogenetics SA, Bruges, Belgium).

### Modeling of apolipoprotein B kinetics

Kinetic constants and compartmental masses were calculated from the decay curves and pool sizes by multicompartmental modeling using the SAAM30 program (18). The modeling strategy is detailed in a companion paper (19). Derived kinetic constants and transport rates were considered acceptable when the calculated curves fit observed data for both $^{131}$I-labeled $VLDL_1$ and $^{125}$I-labeled $VLDL_2$ tracers without systematic error and the calculated masses for $VLDL_1$, $VLDL_2$, IDL, and LDL apoB were within 15% of measured values. Individual calculated rate constants and compartmental masses are given for 15 patients in the Appendix. The remaining subjects were those studied by Gaw et al. (10) with the exception of G. W. who was hypertriglyceridemic.

Summary kinetic parameters for $VLDL_1$, $VLDL_2$, IDL, and LDL apoB transport (in mg/d), the fractional rates of transfer from less dense to denser fractions, the fractional rates of direct catabolism (i.e., apoB lost by degradation), and residence times (the reciprocal of the overall fractional catabolic rate – direct catabolism plus transfer) were derived from the individual rate constants and compartmental masses as described in the companion article (19). It should be noted that while the kinetics of $VLDL_1$ and $VLDL_2$ were determined directly using the above tracers, the metabolic behavior of IDL and LDL was implied from the labeled apoB entering the density interval from VLDL delipidation. The values obtained for IDL and LDL kinetics are, therefore, subject to limitations described by Packard et al. (19).

### Statistical analysis

Relationships between plasma lipids, lipoprotein levels, and apoB kinetics were sought by determining univariate correlation coefficients with the exception of Lp[a] whose correlation with metabolic parameters was assessed using appropriate non-parametric tests. Multiple regression in an analysis of variance general linear model was used to determine the independent predictors of LDL direct input, while the Student's *t*-test was used in the comparison of data from normal and hypercholesterolemic subjects. Statistical calculations were performed using the Minitab PC Program (Minitab Version 8.0, State College, Pennsylvania, PA).

## RESULTS

Plasma lipid and lipoprotein levels for the 21 hypercholesterolemic and 7 normal subjects (taken from references 2 and 20) are given in **Table 1** and apoB pool sizes and kinetics are given in Tables 2A and B. In order to discover potential determinants of LDL apoB circulating mass in the hypercholesterolemic group, univariate correlations were calculated between this parameter and plasma lipid levels, lipoprotein concentrations, and apoB kinetic variables. These subjects were also divided into quartiles on the basis of their LDL apoB mass to reveal the changing apoB kinetic patterns that accompanied increases in circulating LDL. Predictably, plasma and LDL cholesterol were higher in the patients than the normals (Table 1) but no difference was seen in plasma triglyceride, or VLDL and HDL cholesterol. Likewise, although there was a nonsignificant tendency for plasma and LDL cholesterol to rise with increasing LDL apoB mass, there was no trend in plasma triglyceride, or VLDL and HDL cholesterol across the quartiles in Table 1. Lipoprotein subfraction concentrations in LDL and HDL also showed no association with LDL apoB mass.

When apoB pool sizes in $VLDL_1$, $VLDL_2$, IDL, and LDL were examined in the hypercholesterolemic group and its quartiles (**Table 2A, B**) it was observed that all four fractions were higher than normal. However, the increase in $VLDL_1$ apoB was variable and only obvious in quartiles 3 and 4. $VLDL_2$ and IDL apoB pools, on the other hand, were uniformly elevated with mean rises for the whole group of 152% and 218%, respectively. LDL apoB mass rose from just above the normal mean in quartile 1 to more than three times this value in quartile 4 (Table 2B). In the group of hypercholesterolemics, $VLDL_1$ apoB input was not significantly different from normal whereas the mean $VLDL_2$ input was increased 125% (Table 2A). On the other hand, the residence time for $VLDL_1$ apoB was 87% higher ($P < 0.001$) while that of $VLDL_2$ apoB was the same as that seen in normal subjects. The fractional rates of apoB transfer from $VLDL_1$ to $VLDL_2$ and from $VLDL_2$ to IDL did not differ from normal nor did they vary across the quartiles of, or correlate with, LDL apoB mass (Tables 2A, B). The mean fractional rate of $VLDL_1$ apoB direct catabolism was reduced compared to normal but this was of borderline significance. Other fractional rates of direct catabolism for $VLDL_2$ and IDL and, importantly, the LDL FCR

TABLE 1. Plasma lipid and lipoprotein levels in 21 hypercholesterolemic subjects divided into quartiles of LDL apoB mass

| | Plasma Concentration of | | Cholesterol in | | | Total Lipoprotein Mass in | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Triglyceride | Cholesterol | VLDL | LDL | HDL | $HDL_2$ | $HDL_3$ | LDL-I | LDL-II | LDL-III | Lp[a] |
| | *mg/dl* | | *mg/dl* | | | *mg/dl* | | | | | |
| Normal (n = 7)[a] | 126 ± 66 | 194 ± 38 | 24 ± 12 | 118 ± 38 | 55 ± 12 | | | | | | |
| Whole group (n = 21) | 164 ± 42 | 278 ± 27[b] | 32 ± 10 | 198 ± 27[b] | 46 ± 7 | 47 ± 23 | 235 ± 35 | 63 ± 28 | 222 ± 80 | 121 ± 104 | 25 ± 31 |
| Quartile 1 (n = 5) | 177 ± 68 | 268 ± 27 | 35 ± 15 | 187 ± 28 | 41 ± 7 | 49 ± 30 | 226 ± 43 | 52 ± 38 | 201 ± 81 | 128 ± 130 | 18.4 ± 21.7 |
| Quartile 2 (n = 5) | 163 ± 31 | 268 ± 25 | 29 ± 7 | 193 ± 18 | 46 ± 7 | 46 ± 13 | 238 ± 15 | 68 ± 27 | 203 ± 47 | 111 ± 74 | 46.2 ± 47.3 |
| Quartile 3 (n = 5) | 165 ± 45 | 278 ± 24 | 33 ± 12 | 198 ± 27 | 48 ± 9 | 46 ± 23 | 240 ± 56 | 62 ± 29 | 196 ± 72 | 140 ± 154 | 8.4 ± 8.4 |
| Quartile 4 (n = 6) | 152 ± 27 | 294 ± 30 | 31 ± 9 | 212 ± 31 | 48 ± 7 | 46 ± 27 | 234 ± 21 | 66 ± 22 | 276 ± 96 | 106 ± 72 | 28.8 ± 28.4 |
| Correlation vs. LDL apoB mass for whole group (n = 21) | −0.09[c] | 0.29 | 0.0 | 0.26 | 0.18 | 0.0 | 0.04 | 0.17 | 0.30 | 0.07 | −0.06 |

Values are given as mean ± SD.
[a] Normal values are taken from the five E3/E3 homozygous individuals published in reference 20 and subjects N2 and N3 in reference 2.
[b] Two sample *t*-test comparison of normals versus hypercholesteromics, $P < 0.001$.
[c] Univariate correlation coefficient determined within hypercholesterolemic group; no variable exhibited a significant correlation with LDL apoB observed mass.

were not abnormal or associated with change in LDL apoB mass. Thus, in the hypercholesterolemic group as a whole, the increment above normal in $VLDL_2$, IDL, and LDL apoB pools was the result of increased $VLDL_2$ production primarily from de novo input but also in a few subjects from enhanced $VLDL_1$ to $VLDL_2$ apoB transport due to the low fractional rate of $VLDL_1$ apoB direct catabolism. Variation in LDL apoB, on the other hand, was associated with alterations in $VLDL_1$ apoB input, the fractional rate of IDL to LDL transfer, and direct LDL apoB input (Tables 2A, B, **Fig. 1**).

### Influence of plasma triglyceride on apolipoprotein B kinetics in moderate hypercholesterolemia

Plasma triglyceride levels in the hypercholesterolemic group ranged from 90 to 250 mg/dl and varied inversely with HDL cholesterol (**Table 3**) but not with LDL cholesterol or total LDL apoB mass (Table 1). To explore further the impact of variation in plasma triglyceride on lipoprotein levels and apoB kinetics, the 21 hypercholesterolemic patients were again divided into quartiles, this time on the basis of their plasma triglyceride concentration (Table 3 and **Table 4**). The only lipoprotein subfraction concentration that showed a significant association with plasma triglyceride was LDL-III (Table 3) although, when the LDL subfractions were expressed in relative abundance (i.e., percentage of total LDL mass), both %LDL-I and %LDL-III correlated with triglyceride, $r = -0.44$, $P = 0.045$ and $r = 0.56$, $P = 0.008$, respectively (data not shown). The concentration of plasma triglyceride was determined by the content of the lipid in $VLDL_1$ and $VLDL_2$ (data not shown) but the mass of apoB in these VLDL subfractions showed no clear relationship to plasma triglyceride (Table 4A). The residence time of $VLDL_1$ apoB but not $VLDL_2$ apoB was positively associated with the plasma triglyceride level (Table 4A); it rose from normal levels in quartile 1 to approximately twice normal in quartile 4. There was no relationship between the plasma concentration of the lipid and $VLDL_1$ or $VLDL_2$ apoB input rates. $VLDL_1$ to $VLDL_2$ apoB and $VLDL_2$ to IDL/LDL apoB transfer rates were also unrelated to plasma triglyceride level. However, the fractional rate of $VLDL_2$ apoB direct catabolism did exhibit an inverse correlation ($r = -0.46$, $P = 0.034$) (Table 4A). LDL kinetics, on the other hand, appeared to be strongly influenced by plasma triglyceride concentration (Table 4B, **Fig. 2**). The LDL apoB FCR was inversely related to plasma triglyceride levels ($r = -0.52$, $P = 0.015$; Fig. 2) as was the percentage of LDL apoB derived by direct input (**Fig. 3A**). The former association was concordant with that seen previously (21) in normolipemic subjects examined in an LDL turnover study (Fig. 2).

TABLE 2A. Apolipoprotein B kinetics in 21 hypercholesterolemic subjects divided into quartiles of LDL apoB mass

| | $VLDL_1$ ApoB | | | | | $VLDL_2$ ApoB | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Fractional Rate[b] | | | | | Fractional Rate[b] | |
| | Input | Pool | Residence Time[b] | Direct Catabolism | Transfer to $VLDL_2$ | Input | Pool | Residence Time[b] | Direct Catabolism | Transfer to IDL/LDL |
| | *mg/day* | *mg* | *days* | *pools/day* | | *mg/day* | *mg* | *days* | *pools/day* | |
| Normals[a] | 700 ± 399 | 57 ± 36 | 0.08 ± 0.03 | 8.58 ± 6.10 | 6.16 ± 4.85 | 349 ± 213 | 149 ± 70 | 0.25 ± 0.05 | 1.11 ± 0.77 | 3.00 ± 0.97 |
| Whole group | 889 ± 553 | 121 ± 70[d] | 0.15 ± 0.07[f] | 2.65 ± 2.59[e] | 5.45 ± 3.26 | 787 ± 607[d] | 376 ± 130[f] | 0.31 ± 0.11 | 1.10 ± 1.18 | 2.60 ± 0.93 |
| Quartile 1 | 503 ± 125 | 82 ± 27 | 0.18 ± 0.08 | 2.31 ± 1.80 | 4.25 ± 1.96 | 603 ± 328 | 298 ± 28 | 0.37 ± 0.14 | 0.75 ± 0.97 | 2.34 ± 0.56 |
| Quartile 2 | 547 ± 144 | 77 ± 36 | 0.14 ± 0.06 | 0.97 ± 1.73 | 7.11 ± 3.80 | 733 ± 255 | 352 ± 130 | 0.28 ± 0.06 | 1.10 ± 0.41 | 2.64 ± 1.26 |
| Quartile 3 | 1245 ± 547 | 156 ± 78 | 0.14 ± 0.08 | 3.02 ± 3.08 | 5.87 ± 4.39 | 626 ± 402 | 417 ± 176 | 0.30 ± 0.15 | 1.08 ± 0.75 | 2.87 ± 1.36 |
| Quartile 4 | 1198 ± 81 | 158 ± 81 | 0.15 ± 0.09 | 4.04 ± 2.97 | 4.70 ± 2.71 | 1119 ± 1004 | 428 ± 129 | 0.28 ± 0.09 | 1.41 ± 2.00 | 2.58 ± 0.58 |
| Correlation vs LDL apoB mass for whole group[g] | 0.58 $P = 0.006$ | 0.53 $P = 0.013$ | −0.10 | 0.33 | −0.04 | 0.16 | 0.36 | −0.15 | 0.07 | 0.13 |

Values are given as mean ± SD.

[a] Normal subjects were E3/E3 group in reference 20 plus subjects N2 and N3 in reference 2.

[b] The overall fractional catabolic rate (FCR) of $VLDL_1$ is the sum of losses by direct catabolism and transfer to $VLDL_2$, and for $VLDL_2$ is the sum of losses by direct catabolism and transfer to IDL/LDL. It is presented as its reciprocal, the plasma lipoprotein apoB residence time (1/FCR).

[c] Two sample t-test of normals versus hypercholesterolemics, $P < 0.05$; [d] $P < 0.01$; [e] $P < 0.001$; [f] $P < 0.0001$.

[g] Univariate correlation coefficient versus LDL apoB observed mass in all 21 hypercholesterolemic subjects.

TABLE 2B. Apolipoprotein B kinetics in 21 hypercholesterolemic subjects divided into quartiles of LDL apoB mass

| | IDL ApoB | | | | LDL ApoB | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Fractional Rate[b] | | | | | | | |
| | Pool | Residence Time[b] | Direct Catabolism | Transfer to LDL | Input | Mass derived from VLDL | Observed Mass | Residence Time[b] | FCR | Total ApoB Input |
| | *mg* | *days* | *pools/day* | | *mg/day* | *mg* | *mg* | *days* | *per day* | *mg/day* |
| Normals[a] | 262 ± 113[b] | 0.70 ± 0.25 | 0.36 ± 0.19 | 1.33 ± 0.77 | 83 ± 103 | 1131 ± 571 | 1444 ± 682 | 3.38 ± 0.77 | 0.31 ± 0.08 | 1132 ± 534 |
| Whole group | 833 ± 194[f] | 0.97 ± 0.24[f] | 0.40 ± 0.20 | 0.71 ± 0.27 | 241 ± 137[d] | 2353 ± 688[e] | 3272 ± 971[f] | 4.09 ± 0.88 | 0.26 ± 0.06 | 1758 ± 601[f] |
| Quartile 1 | 724 ± 178 | 1.06 ± 0.14 | 0.40 ± 0.17 | 0.56 ± 0.18 | 166 ± 114 | 1482 ± 336 | 2078 ± 227 | 3.94 ± 0.97 | 0.27 ± 0.06 | 1274 ± 364 |
| Quartile 2 | 865 ± 232 | 1.11 ± 0.26 | 0.37 ± 0.17 | 0.58 ± 0.24 | 180 ± 106 | 2974 ± 360 | 2778 ± 233 | 4.17 ± 1.05 | 0.25 ± 0.06 | 1460 ± 261 |
| Quartile 3 | 955 ± 218 | 0.97 ± 0.28 | 0.44 ± 0.23 | 0.66 ± 0.22 | 219 ± 114 | 2634 ± 391 | 3530 ± 256 | 4.25 ± 0.48 | 0.24 ± 0.03 | 2217 ± 607 |
| Quartile 4 | 795 ± 124 | 0.79 ± 0.14 | 0.31 ± 0.13 | 1.00 ± 0.20 | 371 ± 123 | 3076 ± 177 | 4461 ± 445 | 4.00 ± 1.11 | 0.27 ± 0.07 | 2689 ± 1581 |
| Correlation vs. LDL apoB mass for whole group[g] | 0.14 | −0.47 $P = 0.03$ | 0.15 | 0.61 $P = 0.003$ | 0.64 $P = 0.002$ | 0.91 $P < 0.0001$ | | 0.0 | −0.01 | 0.48 $P = 0.026$ |

Values are given as mean ± SD.

[a] Normal subjects were E3/E3 group in reference 20 plus subjects N2 and N3 in reference 2.

[b] The overall fractional catabolic rate (FCR) for IDL is the sum of losses by direct catabolism and transfer to LDL. It is presented as its reciprocal, the plasma lipoprotein apoB residence time (1/FCR). The residence time for LDL is, similarly, the reciprocal of its FCR.

[c] Two sample *t*-test of normals versus hypercholesterolemics, $P < 0.05$; [d] $P < 0.01$; [e] $P < 0.001$; [f] $P < 0.0001$.

[g] Univariate correlation coefficient versus LDL apoB observed mass in all 21 hypercholesterolemic subjects.

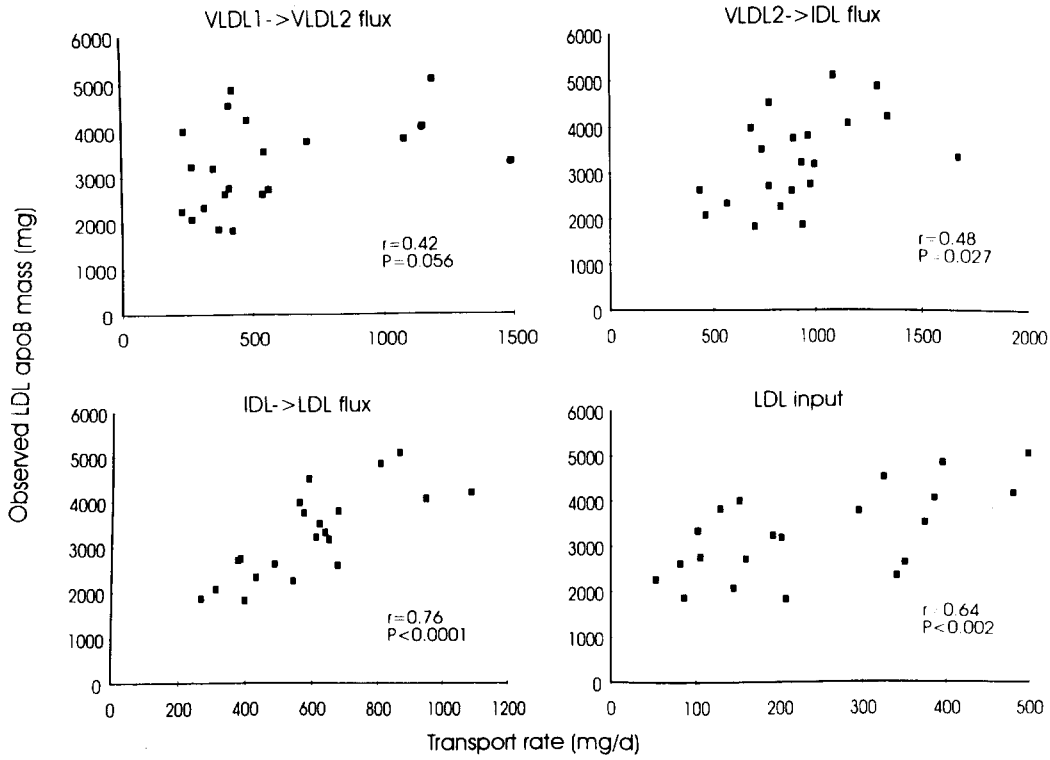


**Fig. 1.** Relationship between apolipoprotein B transport and LDL apoB circulating mass. The scatter diagrams demonstrate the correlations between plasma LDL apoB mass and the transport of apoB from one density class to the next in the delipidation sequence. The lower right hand panel is the de novo input of apoB into LDL. Correlation coefficients were obtained by linear regression.

### Direct LDL apoB input

In all subjects there was a discrepancy between the "VLDL-derived" and observed LDL apoB mass of 5–50% (Fig. 3). As described in the companion paper (19) "VLDL-independent" or "direct" input of LDL apoB was invoked to make up the shortfall. To gain insight into the regulation of this pathway, associations were sought between the percentage of LDL derived by direct input or the estimated amount of direct LDL apoB input (in mg/day) and plasma lipid and lipoprotein concentrations and other, apparently independent apoB kinetic constants. As the overall observed LDL FCR was assumed to apply to LDL apoB not derived from VLDL delipidation then the "%LDL derived by direct input" could equally be termed the "%LDL mass unaccounted for." The percentage of LDL apoB derived by direct input was negatively related to the plasma triglyceride level and the fractional rate of $VLDL_2$ apoB transfer to IDL/LDL (**Table 5A**) and positively related to $HDL_2$ concentration (Fig. 3B, Table 5A). It also positively correlated with the fractional rate of $VLDL_1$ apoB direct catabolism although this did not achieve significance. In addition, the amount of LDL direct input was related to the amount of $VLDL_1$ apoB direct catabolism ($r$ = 0.53, $P$ = 0.013, **Fig. 4**). The fractional rate of $VLDL_1$ apoB direct catabolism was also related to the $HDL_2$ concentration ($r$ = 0.52, $P$ = 0.02).

Multivariate analysis was used to explore the independence of the predictors identified by simple regression. The parameters, which in the 21 hypercholesterolemic subjects exhibited significant univariate correlation coefficients of $r > 0.4$ with "%LDL derived by direct input" (Table 5A), were entered into a general linear model. Plasma triglyceride and $HDL_2$ remained predictors of direct LDL apoB input although they fell either side of the nominal 0.05 significance level (Table 5B). The fractional rates of $VLDL_2$ apoB transfer to IDL/LDL and $VLDL_1$ apoB direct catabolism in addition to the VLDL cholesterol did not add to the prediction.

### LDL subfractions, Lp[a] and apoB kinetics

Associations were present between the LDL subfraction profile and apoB kinetic parameters. The most significant were negative relationships between the $VLDL_1$ apoB residence time and plasma LDL-I ($r$ = −0.58, $P$ = 0.006) and LDL-II ($r$ = −0.54, $P$ = 0.012) concentrations. There was an inverse correlation of borderline significance between LDL-I concentration and $VLDL_1$ apoB circulating mass ($r$ = −0.44, $P$ = 0.045).

TABLE 3. Plasma lipid and lipoprotein levels in 21 hypercholesterolemic subjects divided into quartiles of plasma triglyceride level

| | Plasma | | Cholesterol in | | | Total Lipoprotein Mass in | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Triglyceride | Cholesterol | VLDL | LDL | HDL | $HDL_2$ | $HDL_3$ | LDL-I | LDL-II | LDL-III | Lp[a] |
| | *mg/dl* | | | | | *mg/dl* | | | | | |
| Quartile 1 (n = 5) | 112 ± 17 | 260 ± 34 | 19 ± 6 | 183 ± 31 | 51 ± 10 | 64 ± 26 | 251 ± 51 | 73 ± 20 | 262 ± 73 | 70 ± 64 | 4.4 ± 1.5 |
| Quartile 2 (n = 5) | 149 ± 5 | 294 ± 27 | 30 ± 3 | 215 ± 29 | 49 ± 3 | 47 ± 28 | 223 ± 20 | 75 ± 26 | 245 ± 83 | 47 ± 38 | 51.4 ± 38.0 |
| Quartile 3 (n = 5) | 169 ± 7 | 286 ± 20 | 35 ± 7 | 209 ± 17 | 42 ± 2 | 39 ± 15 | 242 ± 34 | 55 ± 35 | 203 ± 84 | 165 ± 137 | 18.2 ± 23.1 |
| Quartile 4 (n = 6) | 214 ± 30 | 272 ± 21 | 42 ± 8 | 188 ± 21 | 42 ± 7 | 37 ± 16 | 221 ± 28 | 49 ± 26 | 183 ± 76 | 186 ± 92 | 28.0 ± 32.5 |
| Correlation vs. plasma triglyceride for whole group (n = 21)[a] | | 0.11 | 0.90 | 0.0 | - 0.61 | - 0.37 | - 0.36 | - 0.40 | - 0.37 | 0.50 | 0.07 |
| | | | $P = < 0.0001$ | | $P = 0.003$ | | | | | $P = 0.021$ | |

Values are given as mean ± SD.
[a]Univariate correlation coefficient versus plasma triglyceride concentration.

The concentration of Lp[a] varied widely in the subjects. No significant associations were found between the plasma Lp[a] level and any kinetic parameter of VLDL or LDL apoB turnover. In particular, the hypothesis that the Lp[a] level was related to the activity of the LDL direct input pathway was unsupported (Lp[a] versus percentage of direct LDL apoB input, $r = 0.22$, $P = 0.328$).

## DISCUSSION

A number of VLDL and LDL apoB turnover studies conducted over the last two decades (4–7) have examined the nature of the defects that give rise to elevated LDL cholesterol levels in primary, moderate hypercholesterolemia. Increased input of apoB into the LDL density range is the usual but not universal finding. Janus et al. (4) and Kesaniemi and Grundy (5) studied small groups of non-FH hypercholesterolemics and found that in contrast to FH the elevation in apoLDL was due to increased input while the FCR of apoLDL was normal. Simons, Balasubramaniam, and Holland (7), on the other hand, reported in 20 moderate hypercholesterolemics a 15% mean reduction in FCR with no significant rise in input rate for apoLDL. In their comprehensive examination of 134 middle-aged men with primary hypercholesterolemia, Vega, Denke, and Grundy (6) identified the presence of three abnormalities: cholesterol enrichment of LDL, defective catabolism, and overproduction of apoLDL. The last was the most prevalent finding occurring in about half of the subjects and our findings, presented in Table 2 and Fig. 1, also lead to the conclusion that overproduction rather than under-catabolism of apoB-containing lipoproteins was the primary kinetic feature that predisposed to increased LDL apoB pool size. The elevated plasma cholesterol levels in the individuals whom we examined were due to consistent and substantial increases in $VLDL_2$ and IDL as well as LDL, a finding that suggested metabolic abnormalities were present at early stages in the VLDL-LDL delipidation cascade. Because dual tracers of $VLDL_1$ and $VLDL_2$ were injected, we were able to define more precisely the source of the increased apoB input. This occurred mainly at the level of $VLDL_2$ apoB whose mean input was increased above normal in all quartiles in Table 2A (but not, it should be noted, in every patient (10, Appendix)). $VLDL_1$ apoB input, although it influenced LDL apoB mass, was not abnormal in the hypercholesterolemic group as a whole. In fact, the range (352–2280 mg/d) as well as the mean (Table 2A) of $VLDL_1$ apoB input rates in the patients was similar to those seen in normals (range 237–1288 mg/d, (2, 20)). These observations suggest that while $VLDL_2$ overproduction, whether from increased de novo input or from enhanced $VLDL_1$ to $VLDL_2$ apoB transport, was at the root of the elevated plasma cholesterol and apoB level,

TABLE 4A. Apolipoprotein B kinetics in 21 hypercholesterolemic subjects divided into quartiles of plasma triglyceride

| | $VLDL_1$ ApoB | | | | | $VLDL_2$ ApoB | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Fractional Rate[a] | | | | | Fractional Rate[a] | |
| | Input | Pool | Residence Time[a] | Direct Catabolism | Transfer to $VLDL_2$ | Input | Pool | Residence Time[a] | Direct Catabolism | Transfer to IDL/LDL |
| | *mg/day* | *mg* | *days* | *pools/day* | | *mg/day* | *mg* | *days* | *pools/day* | |
| Quartile 1 | 1099 ± 782 | 110 ± 63 | 0.11 ± 0.02 | 4.09 ± 2.75 | 5.22 ± 0.99 | 1150 ± 1113 | 391 ± 123 | 0.31 ± 0.16 | 2.17 ± 2.02 | 1.85 ± 0.32 |
| Quartile 2 | 868 ± 390 | 116 ± 85 | 0.14 ± 0.09 | 2.86 ± 3.85 | 6.81 ± 4.57 | 782 ± 441 | 395 ± 143 | 0.27 ± 0.06 | 0.84 ± 0.71 | 2.98 ± 1.05 |
| Quartile 3 | 775 ± 510 | 118 ± 82 | 0.16 ± 0.08 | 1.93 ± 1.99 | 5.30 ± 2.50 | 625 ± 202 | 388 ± 170 | 0.32 ± 0.14 | 0.85 ± 0.18 | 2.73 ± 1.09 |
| Quartile 4 | 825 ± 584 | 135 ± 69 | 0.19 ± 0.08 | 1.89 ± 1.55 | 4.61 ± 4.14 | 623 ± 319 | 338 ± 116 | 0.32 ± 0.10 | 0.63 ± 0.58 | 2.81 ± 0.88 |
| Correlation vs. plasma triglyceride for whole group (n = 21)[b] | −0.19 | 0.18 | 0.50<br>$P = 0.021$ | −0.26 | −0.29 | −0.39 | −0.19 | 0.26 | −0.46<br>$P = 0.034$ | 0.16 |

Values are given as mean ± SD.
[a]The overall fractional catabolic rate (FCR) is the sum of direct catabolism and transfer to denser lipoproteins. The residence time is the reciprocal of the overall FCR.
[b]Univariate correlation versus plasma triglyceride.

TABLE 4B. Apolipoprotein B kinetics in 21 hypercholesterolemic subjects divided into quartiles of plasma triglyceride

| | IDL ApoB | | | | LDL ApoB | | |
|---|---|---|---|---|---|---|---|
| | | | Fractional Rate[a] | | | | |
| | Pool | Residence Time[a] | Direct Catabolism | Transfer to LDL | Mass Derived from VLDL | Observed Mass | FCR |
| | *mg* | *days* | *pools/day* | | *mg* | *mg* | *per day* |
| Quartile 1 | 737 ± 168 | 1.11 ± 0.31 | 0.25 ± 0.12 | 0.73 ± 0.34 | 1830 ± 718 | 2881 ± 907 | 0.32 ± 0.02 |
| Quartile 2 | 966 ± 211 | 0.94 ± 0.27 | 0.43 ± 0.23 | 0.70 ± 0.30 | 2616 ± 440 | 3603 ± 746 | 0.25 ± 0.07 |
| Quartile 3 | 924 ± 150 | 1.02 ± 0.11 | 0.36 ± 0.18 | 0.63 ± 0.25 | 2545 ± 736 | 3579 ± 1181 | 0.23 ± 0.04 |
| Quartile 4 | 726 ± 161 | 0.84 ± 0.19 | 0.45 ± 0.11 | 0.78 ± 0.25 | 2408 ± 712 | 3063 ± 1066 | 0.24 ± 0.04 |
| Correlation vs. plasma triglyceride for whole group (n = 21)[b] | −0.18 | −0.19 | 0.21 | 0.0 | 0.12 | −0.09 | −0.52<br>$P = 0.015$ |

Values are given as mean ± SD.
[a]The overall fractional catabolic rate (FCR) is the sum of direct catabolism and transfer to denser lipoproteins. The residence time is the reciprocal of the FCR.
[b]Univariate correlation versus plasma triglyceride.

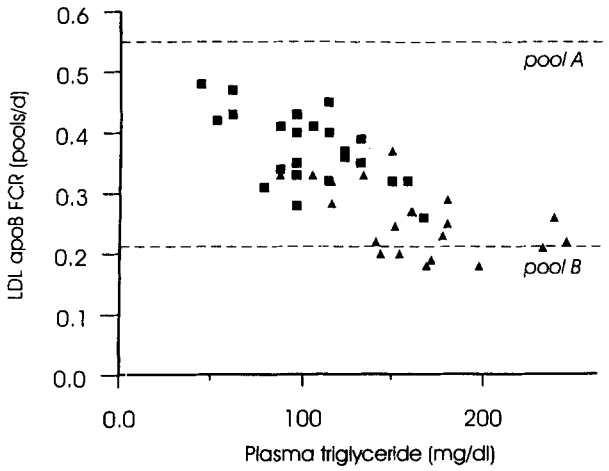


**Fig. 2.** Influence of plasma triglyceride levels on LDL apoB FCR. The diagram shows the relationship between LDL apoB FCR in the present group of 21 hypercholesterolemic subjects (triangles) and 25 normals (squares) studied previously (21). In the latter study FCRs were measured using a tracer of $^{125}$I-labeled LDL. The correlation coefficient for LDL FCR versus plasma triglyceride for the hypercholesterolemics was $r = -0.52$, $P = 0.015$ and for the normals was $r = -0.54$, $P < 0.01$ (21).

the condition was aggravated or ameliorated by variation in the rate of $VLDL_1$ apoB input (Table 2A). A minor contribution from direct LDL apoB input was also present (Table 2B).

Fractional rates of transfer down the delipidation cascade from VLDL to IDL, which appear from previous studies to be controlled by lipoprotein lipase and to some extent hepatic lipase (3, 22, 23), were within the normal range and not affected by the level of hypercholesterolemia in the patient group. IDL to LDL conversion on the other hand, although not different from normal for the group as a whole (Table 2B), was a further significant modulator of LDL apoB mass; individuals with low fractional rates of IDL to LDL transfer had less circulating LDL apoB, presumably due to lower hepatic lipase activity as this is the principal enzyme governing this metabolic step (22, 23). All four apoB-containing lipoproteins were subject to direct catabolism from the plasma which for $VLDL_2$, IDL and, importantly, LDL apoB occurred at near normal rates. The fractional rate of $VLDL_1$ apoB direct catabolism was reduced compared to normal but this parameter was highly variable in both normal and hypercholesterolemic subjects.

As a large number of turnovers were conducted according to a standard protocol, we were also able to explore other regulatory phenomena in an attempt to explain the marked inter-individual variation that is observed in all investigations of apoB metabolism. Fasting plasma triglyceride levels are, of course, largely a reflection of VLDL triglyceride metabolism. VLDL apoB kinetic parameters were related to the plasma triglyceride level (Table 4A). There was a near doubling of $VLDL^1$ apoB residence time as plasma triglyceride levels rose twofold (Tables 3 and 4A). There was also a negative correlation with the fractional rate of $VLDL_2$ apoB direct catabolism. Upon ranking subjects according to plasma triglyceride concentration, we also observed associated changes in LDL kinetics. As plasma triglyceride levels rose the FCR for LDL apoB fell as did the percentage of LDL derived by direct input. In a previous investigation of a large group of normolipemic subjects (21) we also observed a negative relationship between the FCR of LDL apoprotein (apoLDL) and plasma triglyceride level. Figure 2 shows that both data sets are in surprising agreement given the different methodological approaches. Our interpretation of the plasma triglyceride-associated variation in apoLDL kinetics in the earlier study of normal subjects was that LDL comprised at least two metabolically distinct species, a rapidly metabolized pool "A" with

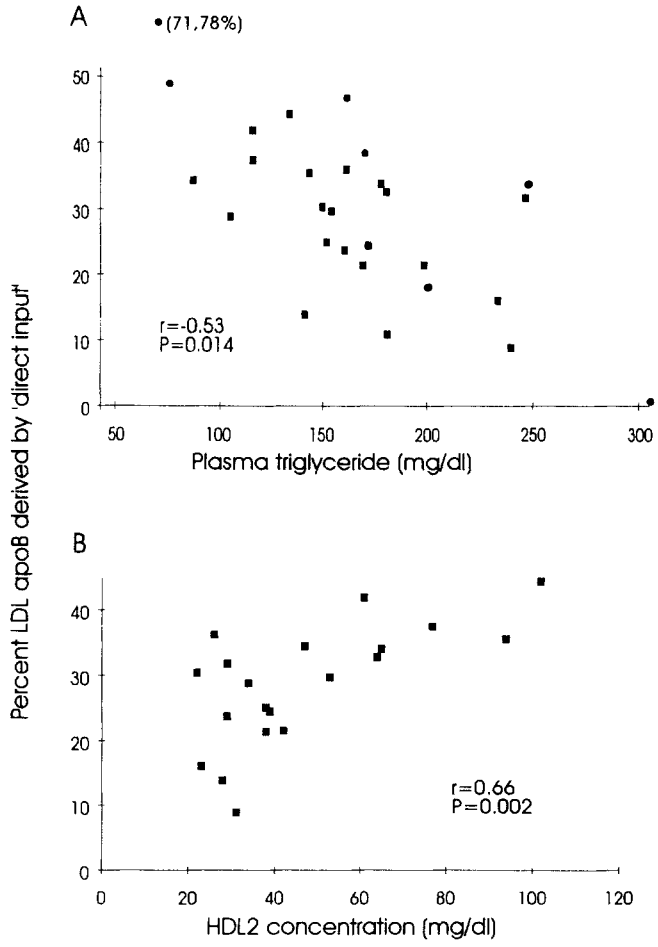


**Fig. 3.** Relationship between the percent of LDL apoB derived by direct input and (A) plasma triglyceride and (B) $HDL_2$ concentration. Correlation coefficients were obtained by univariate linear regression. In (A) the squares refer to the hypercholesterolemic subjects in the present study and the circles to the seven FH homozygotes described in reference (2). The outlying point is denoted by its coordinates (71, 78%) in order not to compress the scales.

TABLE 5. Relationship between plasma lipoprotein levels, VLDL apoB kinetics, and direct LDL input

A. Univariate correlates ($r \geq 0.4$) of % LDL apoB derived by direct input

| Variable | $r$ | $P$ |
|---|---|---|
| Plasma triglyceride | −0.53 | 0.014 |
| VLDL cholesterol | 0.40 | 0.075 |
| $HDL_2$ concentration | 0.66 | 0.002 |
| Fractional rate of $VLDL_1$ apoB direct catabolism | 0.40 | 0.07 |
| Fractional rate of $VLDL_2$ apoB transfer to IDL/LDL | −0.58 | 0.006 |

B. Multiple regression analysis of predictors of percentage of direct LDL apoB input

| Variable | $r^2$ | $P$ |
|---|---|---|
| Plasma triglyceride | 10.1% | 0.060 |
| $HDL_2$ concentration | 12.1% | 0.042 |
| Fractional rate of $VLDL_2$ apoB transfer to IDL/LDL | 7.0% | 0.112 |

a removal rate of approximately 0.55 pools/day and a slower metabolized pool "B" with a removal rate of about 0.2 pools/day. These, we speculated, corresponded, respectively, to LDL with high and low affinity for the receptor. A similar explanation may be invoked to account for the observations in the present hypercholesterolemic group. That is, as plasma triglyceride levels rose the proportion of LDL in pool "A" fell (Fig. 2) and thus the overall FCR was reduced to a limiting value of about 0.2 pools/day (the removal rate of pool "B" material). Previously published studies by other workers (e.g., references 5 and 7) did not detect an overall relationship between plasma triglyceride concentration and apoLDL FCR. Indeed in the study of Vega et al. (6) a higher FCR was observed in a sub-group of patients with increased plasma triglyceride. This discrepancy is most likely due to patient selection but may also arise from the different methods used to isolate LDL and to derive its FCR (19). On the basis of the present and other studies (21, 24, 25) we speculate that the FCR of apoLDL within the plasma triglyceride range 45–200 mg/dl is a function of both the nature of the LDL ligand, whose apoB conformation has been shown to vary (26, 27), and LDL receptor activity in both normal and moderately hypercholesterolemic individuals.

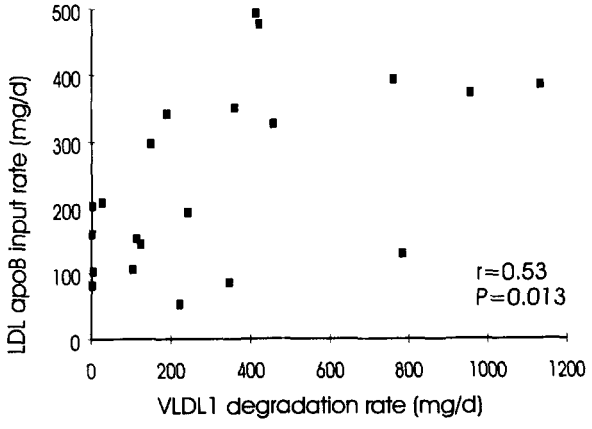


**Fig. 4.** Relationship between amount of LDL derived by direct input and $VLDL_1$ apoB degradation (i.e., direct catabolism) rate. Correlation coefficient was obtained by univariate linear regression.

The mechanism responsible for "direct" or "VLDL-independent" LDL input has been the subject of controversy. Two possible explanations have been proposed. In the first, particles the size and density of LDL are released from the liver (2, 28–30); cell culture experiments with HepG2 cells indicate that this can occur (31). In the second (32–34), it is suggested that within VLDL (either in plasma or in a pre-plasma hepatic compartment) a rapidly turning over, but poorly labeled fraction, generates LDL outside the normal delipidation cascade. There is indirect kinetic evidence to support this hypothesis (34) but recent stable isotope-based tracer studies that overcome the problems associated with interpretation of radio-iodination experiments also invoke direct input of LDL (35). The approach taken in the present study was to identify variables that exhibited a statistical association with the activity of this LDL production pathway in the hope of gaining insight into its origins. We found that the percentage or amount of apoB input directly into the LDL density interval was linked primarily to the plasma triglyceride concentration (Fig. 3A), the $HDL_2$ level (Fig. 3B), and the amount of $VLDL_1$ apoB removed by direct catabolism (Fig. 4, Table 5). The inverse relationship between plasma triglyceride and direct LDL input was observed not only in the present study but also in our previous turnovers in homozygous FH patients (Fig. 3A and ref. 2) and in a report by Ginsberg, Le, and Gibson (36) where it was noted that in hypertriglyceridemic subjects undergoing weight reduction, the magnitude of direct LDL input appeared to be related inversely to the VLDL triglyceride secretion rate. Recent cell culture studies (31) have shown that apoB-100 synthesis occurs continuously in hepatocytes but the rate of apoB-100 secretion, and the nature of the particle released, depends on the availability

of lipid within the cells. As the plasma triglyceride concentration in normotriglyceridemic subjects is largely a function of the synthetic rate of the lipid (37, 38), we postulate that the latter is a major determinant of direct LDL production. In our hypercholesterolemic subjects, total apoB input rates are high (Table 2B) and if there is insufficient hepatic triglyceride available to form VLDL particles then smaller LDL-like species are released. Conversely, in subjects where the liver has ample triglyceride available, then $VLDL_1$ and $VLDL_2$ are the major secretory products.

The connection between $HDL_2$ and the percentage of direct LDL apoB input was the most significant relationship observed (Table 5, Fig. 3B). It may simply be a reflection of the inverse relationship between fasting plasma triglyceride and $HDL_2$ concentrations but we found that the association persisted when the latter were adjusted for variation in plasma triglyceride levels. It is unlikely that there is a direct metabolic link between direct LDL input and $HDL_2$ and we hypothesize that the $HDL_2$ concentration in our subjects is a reflection of the efficiency of chylomicron triglyceride clearance mechanisms (39). Thus, those with high $HDL_2$ levels have active lipolysis in muscle and adipose tissue and disperse most of their dietary triglyceride to the periphery, leaving little to be delivered in remnants to the liver with the above-noted consequences for the form in which apoB is secreted. The contribution of chylomicron triglyceride to VLDL synthesis has not been extensively examined in vivo and so this suggestion is tentative. However, the potential for chylomicron remnant-mediated delivery of dietary lipid to influence VLDL secretion rates has been well documented in cultured cells (40).

An obvious explanation for the positive correlation between the amount of $VLDL_1$ apoB degradation and the amount of LDL apoB direct input (Fig. 4) was that $VLDL_1$ apoB removed at the top of the delipidation chain contributed directly to LDL production. However, when this link was tested by inclusion of a side route from compartment 1 to 11 in the model (Fig. 10 in ref. 19) the amount of apoB transport possible was limited by the very slow rise in $^{131}I$-labeled apoB in the LDL density range. Thus the maneuver did not improve the fit or account for the shortfall in LDL apoB mass. Remaining possibilities are that the relationship was fortuitous or that enhanced $VLDL_1$ direct catabolism was a further feature of subjects with efficient lipolysis mechanisms as $HDL_2$ levels correlated with the fractional rate of $VLDL_1$ apoB direct catabolism. Some support for the latter suggestion comes from a study of lipoprotein lipase-deficient patients (3) in whom fractional rates of $VLDL_1$ apoB direct catabolism were reduced 90% while those of $VLDL_2$, IDL, and LDL were normal or elevated. Thus, $VLDL_1$ metabolism was linked more closely to that of chylomicrons than $VLDL_2$ and may also depend on lipoprotein lipase for hepatic uptake (41).

Alternative metabolic schemes accounting for the direct LDL input-plasma triglyceride link can be postulated. For example, if VLDL is released and directly converted into LDL in hepatic lymph (34, 42), then this pathway would be expected to be more active in subjects with lower VLDL secretion rates and plasma triglyceride levels. However, the enzyme responsible for the rapid lipolysis is likely to be hepatic lipase whose action is known to lower $HDL_2$ levels. Also, the percentage of direct input was related inversely to the $VLDL_2$ delipidation rate (Table 5); a positive association would have been expected if small VLDL formed LDL rapidly (42).

## APPENDIX

TABLE 6. Plasma lipid values

| Subject | Age | Sex | Plasma Trig | Plasma Chol | VLDL Chol | LDL Chol | HDL Chol |
|---|---|---|---|---|---|---|---|
| | *yr* | | | | *mg/dl* | | |
| 1 | 50 | M | 117[a] | 225 | 20 | 164 | 42 |
| 2 | 45 | M | 235 | 249 | 43 | 163 | 42 |
| 3 | 64 | M | 181 | 270 | 40 | 190 | 41 |
| 4 | 44 | M | 161 | 281 | 27 | 213 | 41 |
| 5 | 53 | M | 152 | 256 | 31 | 177 | 48 |
| 6 | 51 | M | 162 | 268 | 32 | 194 | 42 |
| 7 | 55 | M | 135 | 233 | 28 | 143 | 40 |
| 8 | 45 | M | 181 | 252 | 38 | 170 | 45 |
| 9 | 36 | M | 179 | 290 | 45 | 207 | 39 |
| 10 | 59 | M | 117 | 302 | 17 | 225 | 61 |
| 11 | 41 | F | 144 | 302 | 36 | 221 | 46 |
| 12 | 47 | F | 241 | 306 | 48 | 222 | 37 |
| 13 | 49 | M | 150 | 333 | 28 | 255 | 47 |
| 14 | 52 | F | 248 | 279 | 51 | 192 | 35 |
| 15 | 60 | F | 170 | 317 | 38 | 235 | 46 |

TABLE 7. Computed rate constants

| Subject | L(0,1) | L(2,1) | L(0,12) | L(1,13) | L(12,13) | L(4,2) | L(6,2) | L(0,6) | L(0,4) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *pools/day* | | | | |
| 1 | 7.2 | 8.0 | 0.32 | 20.4 | 0.03 | 7.3 | 0.04 | 0.47 | 0.98 |
| 2 | 6.6 | 9.2 | 1.30 | 15.6 | 0.05 | 24.0 | 0.12 | 0.62 | 2.78 |
| 3 | 0.0 | 33.8 | 0.59 | 21.5 | 0.02 | 24.0 | 0.10 | 0.67 | 0.00 |
| 4 | 0.0 | 14.1 | 0.28 | 25.1 | 0.04 | 24.0 | 0.18 | 0.41 | 0.0 |
| 5 | 7.0 | 7.9 | 0.53 | 14.8 | 0.02 | 24.0 | 0.40 | 0.60 | 1.94 |
| 6 | 7.0 | 20.4 | 1.8 | 15.4 | 0.07 | 8.2 | 0.05 | 0.75 | 1.65 |
| 7 | 3.9 | 6.7 | 19.4 | 33.3 | 0.45 | 9.7 | 0.45 | 0.71 | 0.0 |
| 8 | 9.0 | 4.9 | 1.7 | 14.8 | 0.24 | 5.4 | 0.17 | 0.96 | 0.70 |
| 9 | 1.2 | 6.0 | 0.64 | 7.4 | 0.02 | 8.3 | 0.11 | 0.50 | 0.72 |
| 10 | 16.2 | 9.3 | 0.73 | 24.4 | 0.02 | 7.5 | 0.13 | 0.73 | 1.31 |
| 11 | 18.2 | 16.6 | 0.75 | 35.8 | 0.02 | 24.0 | 0.34 | 0.76 | 0.0 |
| 12 | 3.3 | 3.4 | 0.41 | 8.0 | 0.02 | 4.4 | 0.09 | 0.35 | 0.47 |
| 13 | 3.9 | 4.8 | 0.96 | 6.7 | 0.23 | 6.2 | 0.07 | 0.60 | 1.05 |
| 14 | 2.9 | 6.4 | 0.58 | 7.8 | 0.02 | 6.6 | 0.20 | 0.57 | 0.62 |
| 15 | 2.9 | 6.3 | 0.99 | 9.7 | 0.05 | 9.5 | 0.16 | 0.32 | 0.21 |

TABLE 8. Computed rate constants (continued)

| Subject | L(8,4) | L(11,4) | L(9,4) | L(0,5) | L(7,5) | L(10,5) | L(0,8) | L(11,8) | L(0,9) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *pools/day* | | | | |
| 1 | 3.28 | 0.30 | 0.0 | 2.35 | 1.06 | 0.0 | 0.13 | 0.43 | 0.07 |
| 2 | 3.39 | 0.12 | 0.88 | 0.0 | 5.65 | 0.0 | 0.02 | 1.61 | 0.49 |
| 3 | 4.25 | 0.59 | 0.72 | 0.98 | 3.59 | 0.0 | 0.26 | 1.15 | 0.47 |
| 4 | 1.76 | 0.24 | 1.42 | 0.0 | 5.08 | 0.0 | 0.24 | 0.34 | 0.57 |
| 5 | 1.02 | 0.15 | 0.14 | 1.72 | 5.14 | 0.0 | 0.0 | 0.49 | 0.16 |
| 6 | 6.16 | 0.24 | 0.17 | 1.50 | 3.12 | 0.74 | 0.24 | 0.89 | 0.36 |
| 7 | 1.47 | 0.04 | 0.26 | 0.0 | 2.81 | 0.0 | 0.30 | 0.50 | 0.31 |
| 8 | 2.16 | 0.02 | 0.22 | 0.0 | 3.54 | 0.0 | 0.09 | 0.62 | 0.31 |
| 9 | 0.58 | 0.0 | 0.40 | 0.81 | 2.88 | 0.01 | 0.20 | 0.91 | 0.34 |
| 10 | 1.57 | 0.01 | 0.27 | 2.15 | 1.81 | 0.0 | 0.08 | 0.50 | 0.14 |
| 11 | 1.32 | 0.15 | 1.42 | 1.23 | 4.77 | 0.0 | 0.02 | 2.0 | 0.50 |
| 12 | 2.04 | 0.0 | 0.26 | 0.0 | 3.60 | 0.0 | 0.41 | 0.55 | 0.23 |
| 13 | 1.24 | 0.0 | 0.25 | 0.45 | 3.72 | 0.0 | 0.13 | 1.09 | 0.30 |
| 14 | 0.73 | 0.02 | 0.86 | 0.0 | 3.36 | 0.0 | 0.0 | 0.89 | 0.48 |
| 15 | 0.48 | 0.02 | 0.74 | 1.61 | 3.12 | 0.0 | 0.0 | 1.05 | 0.43 |

TABLE 9. Computed rate constants (continued)

| Subject | L(0,7) | L(10,7) | L(0,11) | L(0,10) |
|---|---|---|---|---|
| | | *pools/day* | | |
| 1 | 0.0 | 2.24 | 0.39 | 0.23 |
| 2 | 2.22 | 2.28 | 0.19 | 0.35 |
| 3 | 0.53 | 1.30 | 0.27 | 0.31 |
| 4 | 0.29 | 1.07 | 0.19 | 0.30 |
| 5 | 0.52 | 0.80 | 0.21 | 0.25 |
| 6 | 1.54 | 2.16 | 0.25 | 0.42 |
| 7 | 0.0 | 1.12 | 0.26 | 0.39 |
| 8 | 1.25 | 1.68 | 0.22 | 0.26 |
| 9 | 0.38 | 1.37 | 0.22 | 0.24 |
| 10 | 0.58 | 1.19 | 0.20 | 0.37 |
| 11 | 0.0 | 1.97 | 0.18 | 0.21 |
| 12 | 0.42 | 0.99 | 0.18 | 0.29 |
| 13 | 0.27 | 1.43 | 0.20 | 0.46 |
| 14 | 0.25 | 1.03 | 0.17 | 0.24 |
| 15 | 0.0 | 1.50 | 0.15 | 0.19 |

TABLE 10. Computed compartmental masses

| Subject | M(1) | M(2) | M(4) | M(5) | M(6) | M(7) | M(8) | M(9) | M(10) | M(11) | M(12) | M(13) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *mg/plasma pool* | | | | | | |
| 1 | 50 | 54 | 87 | 142 | 4 | 67 | 501 | 5 | 658 | 874 | 3 | 37 |
| 2 | 117 | 45 | 151 | 55 | 8 | 70 | 313 | 268 | 456 | 2736 | 5 | 120 |
| 3 | 16 | 22 | 96 | 113 | 3 | 221 | 289 | 146 | 911 | 1421 | 1 | 25 |
| 4 | 25 | 15 | 103 | 130 | 6 | 487 | 313 | 257 | 1737 | 694 | 2 | 14 |
| 5 | 34 | 11 | 82 | 162 | 7 | 634 | 171 | 72 | 2010 | 433 | 2 | 35 |
| 6 | 58 | 145 | 144 | 54 | 10 | 45 | 786 | 69 | 329 | 2942 | 4 | 103 |
| 7 | 47 | 31 | 173 | 95 | 20 | 238 | 316 | 147 | 679 | 627 | 0 | 15 |
| 8 | 87 | 77 | 134 | 272 | 14 | 329 | 408 | 96 | 2096 | 1188 | 12 | 82 |
| 9 | 119 | 84 | 413 | 169 | 18 | 277 | 213 | 488 | 1570 | 899 | 4 | 116 |
| 10 | 59 | 72 | 169 | 234 | 12 | 239 | 457 | 327 | 1062 | 1142 | 2 | 61 |
| 11 | 25 | 17 | 138 | 82 | 8 | 197 | 91 | 391 | 1814 | 1107 | 1 | 24 |
| 12 | 67 | 51 | 81 | 178 | 13 | 453 | 173 | 90 | 1543 | 519 | 3 | 56 |
| 13 | 101 | 78 | 190 | 282 | 9 | 616 | 192 | 161 | 1909 | 1025 | 32 | 132 |
| 14 | 42 | 39 | 116 | 83 | 14 | 217 | 95 | 207 | 913 | 509 | 2 | 50 |
| 15 | 38 | 25 | 164 | 154 | 12 | 321 | 75 | 280 | 2600 | 539 | 2 | 37 |

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