Transient triglyceridemia in healthy normolipidemic men increases cellular processing of large very low density lipoproteins by fibroblasts in vitro

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Abstract Exaggerated and prolonged postprandial triglyceridemia is a characteristic of patients with precocious coronary heart disease. Although large very low density lipoprotein (VLDL) particles accumulate during alimentary lipemia, the biological properties of the postprandial VLDL remain unknown. In the present study, an intravenous infusion of a chylomicron-like emulsion was given to healthy normolipidemic men to examine the effects of transient triglyceridemia in vivo on compositional and cell biological characteristics of VLDL. The postinfusion large(Svedberg flotation rate (S_f) (60–400) VLDL was found to have increased capacity to inhibit low density lipoprotein (LDL) binding to the LDL-receptor and a greater ability to suppress the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity of cultured fibroblasts compared to VLDL isolated from fasting plasma. These alterations in cellular interactions were accompanied by increases in the number of apolipoprotein (apo) E, C-I, and C-III molecules per large VLDL particle and loss of apoC-II, compositional changes similar to those observed after an oral fat load. The increase in number of apoE molecules per large VLDL particle correlated positively and significantly with the increase in the capacity of large VLDL to inhibit LDL binding to the LDL receptor ($r = 0.76$, $P = 0.01$, $n = 10$). In contrast, the composition of the small (S_f 20–60) VLDL particles did not change significantly, nor was the LDL receptor-mediated processing of these particles altered consistently. These observations indicate that large VLDL particles that accumulate during alimentary lipemia undergo compositional changes that render them more prone to cellular binding and uptake.—**Björkegren, J., F. Karpe, S. Vitols, P. Tornvall, and A. Hamsten.** Transient triglyceridemia in healthy normolipidemic men increases cellular processing of large very low density lipoproteins by fibroblasts in vitro. *J. Lipid Res.* 1998. **38:** 423–436.

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Very low density lipoproteins (VLDL) are a heterogeneous population of lipoprotein particles that vary in structure and metabolism. VLDL are made in the liver and contain apolipoprotein (apo) B-100 and endogenous lipids (1). Upon their entry into the bloodstream, VLDL achieve their normal particle composition after transfer of apos C and E from circulating plasma lipoproteins, primarily high density lipoproteins (HDL) in normolipidemic individuals (2) and also from VLDL particles in hypertriglyceridemic subjects (3). The apoB-100 molecule remains an integral part of the VLDL particle during its metabolism, whereas the triglyceride content is readily hydrolyzed by lipoprotein lipase (LPL), resulting in formation of smaller remnant particles. ApoC-II contained in the VLDL particle is required as an activator of LPL (4). ApoC-III, on the other hand, which has both non-glycosylated (apoC-III_0) and glycosylated isoforms containing either one (apoC-III_1) or two (apoC-III_2) moles of sialic acid, has been considered both to counteract the lipolysis-activating function of apo-C-II (5, 6) and to inhibit the receptor-mediated uptake of triglyceride-rich lipoproteins (7–9). Accordingly, overexpression of apoC-III in transgenic mice was shown to lead to an accumulation of VLDL (10) or a decrease in the clearance rate of chylomicron remnants (11). Conversely, an increased clearance of triglyceride-rich lipoproteins has been demonstrated in subjects with apoC-III deficiency (12). Furthermore, a mutation in the apoC-III gene locus re-

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; LPL, lipoprotein lipase; LPDS, lipoprotein-depleted serum; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; S_f, Svedberg flotation rate.

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cently described in two human subjects was found to be associated with low triglyceride and high HDL cholesterol concentrations (13). A similar opposing effect on the receptor-mediated uptake of remnant lipoprotein particles has also been shown for apoC-I in vitro (14, 15) and in vivo (16).

A substantial proportion of normal large (Svedberg flotation rate (S_f) 60–400) VLDL particles are lipolyzed to form smaller S_f 20–60 VLDL remnants which are eventually converted into low density lipoprotein (LDL). In contrast, large VLDL particles of hypertriglyceridemic individuals are to a large extent removed from the plasma before reaching the S_f 20–60 stage and without prior conversion to LDL (17). Whereas apoE-mediated binding and uptake by the LDL receptor has been consistently shown for hypertriglyceridemic large VLDL, small VLDL and IDL (18–20), a prevailing point of view has been that most native VLDL species from normolipidemic humans are incapable of undergoing LDL receptor-mediated catabolism (18, 21, 22). However, cellular processing through the LDL receptor has now been demonstrated for all subfractions of normolipidemic VLDL, although the capacity for both binding and catabolism decreases with increasing particle size (23).

Both the intestinal and the liver-derived lipoproteins contribute to the triglyceridemia seen after fat intake (24). However, the postprandial increase in plasma triglyceride-rich lipoprotein particle number is mainly accounted for by VLDL (25–27), particularly the large VLDL species (25). The postprandial triglyceridemia, on the other hand, is accounted for by chylomicron remnants containing apoB-48 as the structural protein, whereas around 90% of the increase in cholesterol during alimentary lipemia is associated with VLDL (27). However, the effect of the postprandial increase in VLDL on the plasma cholesterol concentration is quantitatively limited. Recent studies performed in our laboratory, both in rats (28), and in humans (29), have shown that reduced lipolysis of VLDL secondary to competition from chylomicrons is the major cause of the postprandial VLDL particle accumulation, a physiological phenomenon originally suggested by Brunzell et al. (30).

Multiple exchanges of lipids and apolipoproteins are likely to occur amongst the various species of triglyceride-rich lipoproteins, LDL and HDL during the postprandial state which could influence the metabolism of VLDL, and their involvement in atherogenesis. Indeed, we have recently shown that VLDL isolated during alimentary lipemia is enriched with apo-E, apoC-I, and cholesterol but depleted of apoC-II, compared to VLDL isolated from fasting plasma (31). These compositional perturbations could be of importance for the implication of large VLDL in thrombosis (32), and for the relation between small cholesteryl ester-enriched VLDL and precocious coronary atherosclerosis (33, 34).

Although large VLDL particles that accumulate during the postprandial state undergo significant compositional alterations, the biological properties of the postprandial VLDL remain unknown. In the present study, an intravenous infusion of a chylomicron-like emulsion was a given to healthy normolipidemic men to examine the effects of transient triglyceridemia in vivo on compositional and cell biological characteristics of VLDL. The infusion produced a stable 3-fold increase in plasma triglycerides and was used as a standardized model to study VLDL composition and cellular metabolism during alimentary lipemia. Because the metabolic fate of VLDL in vivo varies according to particle size and apolipoprotein and lipid composition, LDL-receptor-mediated processing by fibroblasts in culture was studied for large (S_f 60–400) and small (S_f 20–60) VLDL isolated from postabsorptive plasma and from plasma drawn at the end of the infusion of the chylomicron-like emulsion.

METHODS

Human subjects

A total of 18 healthy normolipidemic men aged 23 to 47 (29 ± 6 (mean ± SD)) years with apoE3/3 or apoE3/4 genotype as determined by restriction isotyping (35) were studied. All subjects gave oral, informed consent to the study, which was approved by the ethics committee of the Karolinska Hospital. Participants were nonobese (body mass index 23.6 ± 1.6 kg/m^2). Fasting plasma lipoprotein lipid concentrations are shown in **Table 1**. The major fasting plasma lipoproteins were determined by a combination of preparative ultracentrifugation and precipitation of apoB-containing lipoproteins followed by lipid analysis (36).

Intravenous infusion of a triglyceride emulsion

Subjects were admitted in the early morning to the Clinical Research Unit for an intravenous infusion of a triglyceride emulsion (Intralipid 100 mg/l, Pharmacia, Stockholm, Sweden). They had been fasting for 12 h and were asked to refrain from smoking during the fasting period and from alcohol during the preceding 3 days. The protocol has been described in detail (29). Briefly, venous blood was drawn immediately before a bolus dose of 0.15 g/kg body weight of Intralipid was given. The bolus dose was injected within 60 sec through an indwelling intravenous catheter inserted into the antecubital vein of one arm and was immedi-

TABLE 1. Fasting plasma and lipoprotein lipid concentrations of the study group

	Plasma	VLDL	LDL	HDL
		mmol/l		
Cholesterol	5.01 (4.47–5.56)	0.39 (0.28–0.50)	2.98 (2.57–3.39)	1.17 (1.00–1.33)
Triglycerides	1.21 (1.02–1.41)	0.77 (0.61–0.93)	0.27 (0.22–0.33)	0.12 (0.10–0.14)

Value are mean and 95% confidence limits, n = 18. VLDL, d < 1.006 kg/l lipoproteins; LDL, d 1.006–1.063 kg/l lipoproteins; HDL, d > 1.063 kg/l lipoproteins, as determined by preparative ultracentrifugation and precipitation of apoB-containing lipoproteins followed by lipid analysis (36).

ately followed by an infusion of Intralipid (0.15 g/kg/h) (infusion pump: IMED 922 H, Medical Market AB, Stockholm, Sweden). A second blood sample was subsequently drawn after 60 min of infusion. All blood samples were drawn into precooled sterile tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) containing Na_2EDTA (1.4 mg/ml), which were immediately put on ice.

VLDL subfractionation

Plasma was recovered within 30 min by low-speed centrifugation (1750 *g*, 1°C). VLDL was then immediately subfractionated by cumulative flotation in a density gradient (25). Ultracentrifugation was performed in SW40 Ti swinging bucket rotors at 40,000 rpm and 15°C (XL-70 Ultracentrifuge, Beekman Instrument, Palo Alto, CA). The gradients were first run for 32 min to float $S_f > 400$ lipoproteins (a fraction essentially containing Intralipid triglycerides). Then, two consecutive runs calculated to float S_f 60–400 (3 h 28 min) and S_f 20–60 (16 h) lipoprotein particles were made. The top 0.5 ml from each density gradient containing the respective lipoprotein subclasses was aspirated and immediately put on ice. NaCl solution (d 1.006 kg/l) was used to refill the tubes before the next run. All salt solutions used to prepare the density gradients were adjusted to pH 7.4 and contained 0.02% sodium azide and 0.01% EDTA. Densities were verified to the fourth decimal place.

Immunoaffinity chromatography to isolate VLDL particles in subfractions of triglyceride-rich lipoproteins from fasting and postinfusion plasma

Immunoaffinity chromatography was performed essentially according to McConathy et al. (37), Briefly, antiserum against human LDL was immunoaffinity purified against CNBr-Sepharose (Pharmacia, Uppsala, Sweden) immobilized human LDL to isolate a polyclonal antibody directed against apoB. The coupling procedure was according to the manufacturer's description. This antibody was then coupled to CNBr-activated Sepharose to form an immunoaffinity column to which 6 ml of either S_f 60–400 or S_f 20–60 fractions isolated from fasting and postinfusion plasma (1.0–2.0 mg of apoB-100) were applied and allowed to bind overnight at 4°C. Eluted fractions from the immunosorber were collected with use of an UV detector (UV-1, Pharmacia) and a fraction collector (Frac-200, Pharmacia). The unretained fractions, only containing Intralipid particles when postinfusion fractions were applied as verified by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (38), and the retained fractions, containing essentially VLDL, were eluted with phosphate-buffered saline (PBS) and 3 m NaSCN, respectively. The retained fraction was immediately passed through a PD-10 column (Sephadex G-25M, Pharmacia) in PBS and then concentrated under nitrogen pressure (<50 mPa) from 20–30 ml to 4–6 ml (Stired Cell, Model 52; Diaflo membrane IM-100, Amicon, Beverly, MA). The total binding capacity of the column was approximately 0.4 mg apoB-100 per ml suspended Sepharose 4B gel and the actual amount of apoB-100 applied on the anti-apo B column did not exceed 50% of the maximum capacity. The recovery of large VLDL apoB-100 after immunoaffinity chromatography, buffer exchange, and concentration procedures was 69% (n = 18). The corresponding recovery of apoE was 63% (n = 7).

Cell culture

All cell culture studies were performed on a human fibroblast cell line (GM08333, NIGMS, Coriell Institute for Medical Research, Camden, NJ) between the 6th to 13th passages. The cells (25×10^3) were grown in a humidified incubator at 8% CO_2 at a temperature of 37°C on 60 × 15 mm dishes (for the 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)reductase inhibition assay) or on 6-hole plates (for the ^{125}I-labeled LDL degradation analysis) (3060 and 3506, Tissue Culture Treated, Cambridge, MA) in 5 ml, respectively, 3 ml of 50% DMEM and 50% Ham's F12 medium, both supplemented with 10% fetal calf serum, 50 U penicillin and 50 μl streptomycin/ml (GIBCO BRL, Glasgow, Scotland), The cell medium was changed every third day. After 5 days of culture the cells were washed twice with 5 ml, respectively, 3 ml of 37°C PBS, and the medium was changed to medium containing 5% lipoprotein-depleted serum (LPDS) for 48 h. Lipoproteins were mixed with fresh LPDS medium before incubation with the cells. For the

HMG-CoA reductase inhibition assay, the cultured cells were incubated with VLDL subfractions (S_f 20–60 and S_f 60–400) and LDL in duplicate at three different concentrations for 9 h whereas for the ^{125}I-labeled LDL degradation assay, lipoproteins were incubated for 4 h. Control experiments were also performed with fibroblasts lacking the LDL receptor (GM 00486C, NIGMS, Coriell Institute for Medical Research, Camden, NY).

HMG-CoA reductase activity assay

The HMG-CoA reductase assay was essentially performed according to Brown, Dana, and Goldstein (39) with some modifications (21, 40). The assay was performed the day after lipoprotein incubation. The cell cultures were first washed 3 times with PBS 37°C before they were scraped off with a rubber policeman into 1 ml of PBS. The cells were then centrifuged for 5 min at 1,500 rpm; the supernatants were removed and the pellets were dissolved in 60 μl of PBS. A total of 20 μl of the dissolved pellets was added to 180 μl 0.28 mm NaOH for subsequent protein determination (41). Another 20 μl of the dissolved cell pellets was mixed with 7.5 μl of concentrated extraction buffer to a final concentration of 0.05 m $NaPO_4$ buffer, pH 7.4 (Merck, Darmstadt, Germany), 5 mm dithiothreitol (Sigma, St. Louis, MO), 1 mm EDTA, pH 7.4 (Merck), 0.15 m KCl (Merck) and 0.25% Zwittergent 3–14 (Calbiochem, La Jolla, CA). Samples were subsequently incubated at 37°C in a waterbath for 30 min. After the incubation samples were centrifuged at 6,000 rpm for 1 min, 20 μl of the supernatants was then added to a 10 μl cocktail containing 0.05 m Tris buffer, pH 7.4, 20 mm dithiothreitol, 80 mm glucose-6-phosphate (Sigma), 30 μg NADP (Sigma), and 1.2 U/l of glucose-6-phosphatedehydrogenase (Sigma). The HMG-CoA reductase inhibition assay was started by adding 1.7 nmol [^{14}C]HMG-CoA (47 mCi/mmol, NEN products, Boston, MA) and 1 nmol of unlabeled HMG-CoA (Sigma). The final volume of each HMG-CoA reductase inhibition assay was 35 μl. After a 1-h incubation at 37°C in a waterbath, the reaction was stopped by adding 10 μl of 60 mm mevalonate (Sigma) in 2.5 m HCl. Twenty μl of the incubation mixtures was then applied to 0.25 mm silica thin-layer chromatograms (Merck) without fluorescent indicator. The chromatograms were developed in acetone–benzene 1:1. The mevalonolactone formed was visualized by I_2 vapor, scraped off into 3 ml of toluene scintillation liquid (Packard, Groningen, Netherlands) after evaporation, and counted in a Beekman LS 1801 scintillator (Fullerton, CA). The maximum HMG-CoA reductase activity was 77.2 ± 38 pmol produced mevalonate/mg cell protein per h (mean ± SD, n = 8). HMG-CoA reductase inhibition was calculated as % of maximum reductase activity. LDL (d 1.019–1.063 kg/l), isolated from healthy men (n = 12) by density gradient ultracentrifugation as described for VLDL and cryopreserved (70°C) in 10% sucrose (42), was used to estimate between-experiment variation and run in duplicates at three different concentrations. Five μg of the LDL standard suppressed HMG-CoA reductase activity by 91.1 ± 5.3% (mean ± SD, n = 7).

^{125}I-labeled LDL degradation assay

LDL (d 1.019–1.063 kg/l) was isolated by ultracentrifugation in a density gradient and labeled with ^{125}I-labeled using the iodine monochloride method (43). The specific activity of the LDL varied from 170 to 390 cpm/ng of protein. More than 97% of the radioactivity was TCA-precipitated and <3% extractable with diethylether. The labeled LDL was sterilized by passage through two 0.45-μm filters (Millipor, Bedford, MA). The ^{125}I-labeled LDL degradation assay was essentially performed according to Goldstein and Brown (44). Briefly, after washing fibroblasts three times with 2 ml of PBS, 5 μg of ^{125}I-labeled LDL/ml medium together with three different concentrations of postabsorptive and postinfusion VLDL, respectively, or standard LDL dissolved in 2 ml F12/DMEM-medium containing 5% LPDS were incubated for 4 h at 37°C. Cellular protein was determined on every well. After the incubation, the medium was removed and centrifuged for 5 min (1,500 rpm, 4°C). A total of 0.8 ml of the supernatants was removed and mixed with 0.8 ml of 20% TCA to precipitate the remaining proteins, and subsequently centrifuged at 3,000 rpm for 15 min at 4°C. A total of 1 ml of the supernatants was then transferred to glass tubes and carefully mixed with 10 μl of 40% KI, 50 μl 30% H_2O_2, and 2 ml of chloroform at room temperature and subsequently centrifuged for 5 min at 2,000 rpm. One-half ml of the supernatants was transferred to 2-ml plastic tubes for counting in a gamma counter (Cobra/Autogamma, Packard Instruments, Menden, CT).

The percentage inhibition of the cellular ^{125}I-labeled LDL degradation rate in the presence of unlabeled VLDL was used as an indirect estimate of the capacity of VLDL to inhibit the binding of LDL particles to the LDL receptor (45). The relevance of this estimate was evaluated by incubating three concentrations of VLDL (S_f 20–60) in triplicate with 5 μg of ^{125}I-labeled LDL at 4°C to directly measure effects of VLDL on LDL at binding to the LDL receptor. Briefly, after up-regulating LDL receptors on fibroblasts as described above, 5 μg of ^{125}I-labeled LDL/ml medium and unlabeled VLDL dissolved in F12/DMEM-medium containing 5% LPDS were incubated for 1 h at 4°C. After discarding the medium, the fibroblasts were washed three times with ice-cold PBS; thereafter cells were incubated with 0.5 ml of a heparin solution (1 ml heparin (10,000

units/ml) to 5 ml of 50 mm NaCl/10 mm HEPES buffer, pH 7.4) for 20 min. Twenty percent of the retained heparin solution was subsequently transferred to tubes for counting in a gamma counter as described above. This control experiment showed similar curves of inhibition for ^{125}I-labeled LDL binding at 4°C and for ^{125}I-labeled LDL degradation at 37°C (data not shown). The corollary is that inhibition of ^{125}I-labeled LDL degradation at 37°C results from blockage of the LDL receptor by unlabeled VLDL, either through direct binding of VLDL to receptors or indirectly through steric hindrance by VLDL binding to the cellular matrix in the vicinity of receptors.

Unlabeled cryopreserved LDL was used to estimate variation between experiments and run in duplicate at three different concentrations together with 5 μg of ^{125}I-labeled LDL. The maximum cellular ^{125}I-labeled LDL degradation rate was 212 ± 98 ng LDL/mg cell protein per h (mean ± SD, n = 10). Five μg unlabeled LDL/ml medium inhibited the degradation rate of 5 μg ^{125}I-labeled LDL by 46 ± 8% (mean ± SD, n = 8). Background degradation of 5 μg ^{125}I-labeled LDL in the absence of cells was <7% of total degradation and was subtracted from the total degradation.

Determination of VLDL lipids and apolipoproteins

Triglycerides and phospholipids in the retained fractions were determined enzymatically (450032, Boehringer Mannheim Corporation, Indianapolis, IN; 990–54009, Wake Chemicals GmbH, Nueuss, Germany). Cholesterol was either determined enzymatically (14350, Merck, Darmstadt, Germany) or by a chemical method (46), the latter if very low concentrations were expected. ApoB and E were quantified by SDS-PAGE (38), whereas apoCs were determined by urea gel electrophoresis essentially according to Kane (47). Briefly, two portions of 100–400 μl (30–100 μg of total protein) of the S_f 60–400 and S_f 20–60 lipoprotein fractions were delipidated with methanol-diethylether. To determine apoB and E, the protein pellet was solubilized in 100 μl of sample buffer (2% SDS, 0.15 m sodium phosphate, 5% mercaptoethanol, 12.5% glycerol, and bromphenolblue (0.025 mg/ml) at pH 6.8) and denatured in a waterbath at 80°C for 10 min. ApoB-100 from LDL was used as a reference protein and for standard curve dilutions (38).

To determine apoCs, another pellet was solubilized in 100 μl of a urea containing loading buffer (6 m urea, 42 mm Tris, 46 mm glycine, and bromphenolblue (0.025 mg/ml), pH 9.8) and thereafter directly applied to a 10% polyacrylamide gel together with insulin (Actrapid, Novo Nordisk A/S, Gentofte, Denmark) which was used as a protein standard on the gel. One part of insulin was mixed with two parts of the urea containing buffer to a final insulin concentration of 1 μg/μl. Subsequently, 1, 2, 5, 7.5, 10, 12, and 15 μl of the insulin standard were run on a separate urea gel, first at 60 V for 10 min and then at 100 V for 1 h.

After electrophoresis all gels were fixed in a glass Petri dish using 12% trichloroacetic acid for 30 min, then stained with 0.2% Coomassie G-250 (Merck) in methanol–water–acetic acid 5:5:1 overnight and finally destained in ethano–water–acetic acid 12:81:7 for 12 h with 3–4 changes of destainer. Gels with standard curves were stained and destained in the same Petri disk as sample gels. A laser densitometer (Ultroscan XL, LKB Pharmacia, Bromma, Sweden) was used to scan the gels, and the contents of apoCs, apoE, and apoB-100 were subsequently calculated (Gelscan XL 2400 Software).

To determine the relative chromogenicity of insulin in comparison to that of apoC-I, C-II, and C-III, the dye uptake of the respective protein was compared with a protein determination made by amino acid analysis as described (31). The chromogenicity of apoE (1/2.6 compared with reference apoB-100 prepared from LDL, 1.030–1.040 kg/l) was determined according to the procedure described for determining the chromogenicities of apoB-100 and apoB-48 in subfractions of triglyceride-rich lipoproteins (38).

Calculations and statistical methods

Conventional methods were used for calculating means and standard deviations (mean ± SD) or means and 95% confidence intervals (mean(95% confidence interval)). The statistical significance of differences was tested by paired two-tailed *t*-test. Associations between lipoprotein parameters and between lipoprotein parameters and results of cell culture experiments were determined by calculation of Pearson correlation coefficients. The individual values of skewed parameters were log transformed prior to statistical computations. HMG-CoA reductase inhibition (y) at different VLDL apoB-100 concentrations (x) was expressed by the model $y = 100 + 10^{-kx}$. Using three different concentrations of VLDL apoB-100 in the incubation ($x_{1–3}$) and measuring the corresponding inhibition in % of maximal HMG-CoA reductase inhibition ($y_{1–3}$), a k-value could be calculated for each individual in this model. In the binding study, the relation between % of maximal inhibition of uptake of labeled LDL (y) and log-normalized concentration of VLDL apoB-100 (log(1 + x)) was shown to be linear, and a linear regression analysis was used to calculate the k-value of the slope of each individual. Concentration–response data for both HMG-CoA reductase inhibition and inhibition of ^{125}I-labeled LDL degradation were also analyzed as the effective

concentration for 50% inhibition (EC_{50}) of the response in the absence of VLDL.

RESULTS

Changes of plasma concentrations of VLDL subfraction constituents after intravenous infusion of a triglyceride emulsion

The plasma triglyceride concentration in the $S_f > 20$ fractions increased five-fold from 0.8 ± 0.6 mmol/l to 4.3 ± 1.8 mmol/l in response to the Intralipid bolus dose of 0.15 g/kg and the 60 min infusion at a rate of 0.15 g/kg per h. The $S_f > 400$ fraction was not applied an the affinity column, as it only contained minor amounts of apoB-100 as determined by SDS-PAGE, and the apoB-100 concentration in this fraction, i.e., the number of VLDL particles, did not increase during the infusion (data not shown). The plasma concentration of apoB-100 in the S_f 60–400 lipoprotein fraction, reflecting the plasma level of large VLDL, nearly doubled during the 60-min infusion (**Fig. 1**. left panel), whereas the S_f 20–60 apoB-100 concentration displayed a heterogeneous response to the infusion (Fig. 1, right panel).

Compositional changes of large and small VLDL particles after intravenous infusion of a triglyceride emulsion

Compositional analysis was performed on the S_f 60–400 and S_f 20–60 VLDL fractions isolated by immunoaffinity chromatography (**Table 2**). The large (S_f 60–400) VLDL particles contained significantly more molecules of apoE, C-I, and C-III_1 and significantly fewer molecules of apoC-II after the Intralipid infusion. In contrast, the lipid and apoC-III_2 content of the large VLDL particles did not change in response to the Intralipid infusion. The constituents of small (S_f 20–60) VLDL unaffected by the Intralipid infusion.

Effects of the triglyceride emulsion on VLDL processing by fibroblasts in vitro

HMG-CoA reductase inhibition in cultured fibroblasts incubated with VLDL is the result of uptake and degradation of lipoprotein particles with subsequent inhibition of the enzyme by cholesterol from degraded VLDL particles. The responses to 9–12 h incubations with different concentrations of VLDL particles are shown in **Fig. 2** and summarized in **Table 3**. Each experiment (n = 8) with three different concentrations of VLDL apoB-100 was best described by a model with a logarithmic fit, $y = 100 + 10^{-kx}$, where y is the percent-

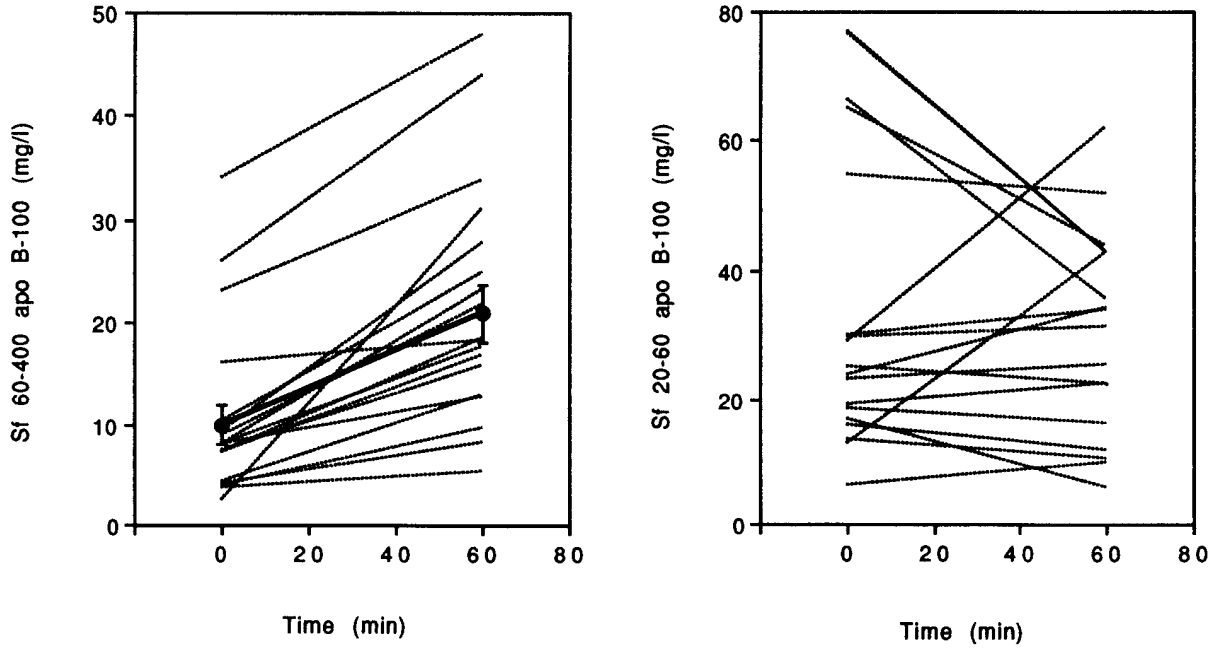

Fig. 1. Responses of plasma VLDL apoB-100 to infusion of a triglyceride emulsion in healthy human volunteers. Venous blood was drawn immediately before a bolus dose of 0.15 g/kg body weight of a triglyceride emulsion (Intralipid 100 mg/l, Pharmacia, Stockholm, Sweden) was given to 18 healthy normolipidemic men aged 23 to 47 (29 ± 6 (mean ± SD)) years with an apoE3/3 or apoE3/4 genotype. The bolus dose was immediately followed by an infusion of Intralipid (0. 15 g/kg/h), and a second blood sample was drawn after 60 min of infusion. VLDL was subfractionated by cumulative flotation in a density gradient (25). ApoB-100 in VLDL subfractions was quantified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (38). The left panel shows the mean (–) and individual (···) responses of VLDL S_f 60–400 apoB-100, and the right panel shows individual (···) responses of VLDL S_f 20–60 apoB-100.

TABLE 2. Composition of VLDL subfractions (no. lipid and apolipoprotein molecules per particle) is isolated from fasting plasma and after 60 min intravenous infusion of a triglyceride lipid emulsion

	Chol	TG	PhL	ApoC-I	ApoC-II	Apo-CIII$_1$	ApoC-III$_2$	ApoE
S_F 60–400 VLDL								
Fasting	3,600 (2,400–4,700)	19,000 (15,000–23,000)	4,700 (3,600–5,800)	19 (14–24)	27 (22–32)	22 (15–28)	21 (14–28)	1.5 (1.0–2.0)
Postinfusion	3,600 (2,800–4,500)	20,000 (16,000–24,000)	5,500 (4,300–6,600)	36 (27–44)	18 (13–23)	27 (19–35)	19 (13–25)	2.4 (1.3–2.3)
	NS	NS	NS	$P < 0.0001$	$P < 0.01$	$P < 0.05$	NS	$P < 0.0001$
S_f 20–60 VLDL								
Fasting	2,400 (1,800–3,000)	6,600 (4,900–8,200)	2,600 (2,100–3,100)	15 (10–21)(n = 17)	6.6 (4.8–8.6)(n = 17)	9.1 (6.4–12)(n = 17)	6.0 (4.1–7.8)(n = 17)	1.9 (1.3–2.3)
Postinfusion	2,600 (1,800–3,300)	7,200 (5,400–9,000)	2,900 (2,300–3,600)	19 (14–24)(n = 17)	6.7 (4.8–8.6)(n = 17)	9.0 (7.0–11)(n = 17)	7.6 (5.3–9.9)(n = 17)	2.0 (1.5–2.5)
	NS	NS	NS	NS	NS	NS	NS	NS

Data were pooled from experiments on 18 healthy human volunteers who had a bolus dose (0.15 g/kg body weight) followed by an intravenous infusion (0.15 g/kg/h) of Intralipid (Pharmacia). Values are means and 95% confidence limits. The statistical significance of differences between fasting and postinfusion plasma for each VLDL constituent was calculated by Student's paired *t* test. *P* values are given below for postinfusion value; NS, not significant.

age of maximum HMG-CoA reductase activity and x is the concentration of VLDL apoB-100 in the cell culture medium. Using this model, the HMG-CoA reductase inhibition by large VLDL isolated from fasting plasma did not differ significantly from the inhibition mediated by small VLDL isolated from fasting plasma. The ability of large VLDL to suppress HMG-CoA reductase activity had increased significantly at the end of the 60-min infusion of Intralipid (change in k-value from 0.05(0.03–0.07 to 0.12(0.05–0.18), $P < 0.05$). In contrast, the ability of small VLDL to suppress HMG-CoA reductase activity, which was more heterogeneous in the fasting state, had not changed significantly at the end of the infusion (k-values of 0.09(0.04–0.15) vs. 0.16(0.07–0.24), NS)(Fig. 2 and Table 3). These alterations were paralleled by changes in the corresponding EC_{50} values (Table 3).

Although there is evidence that VLDL processing by fibroblasts is LDL receptor-dependent (23), an experiment was performed to examine the extent of LDL receptor dependent VLDL uptake by fibroblasts. Briefly, triplicate samples of three different concentrations of large VLDL (5.0, 15, and 30 μg apoB-100/ml medium) and small VLDL (2.5, 5.0, and 15 μg apoB-100/ml medium) isolated by density gradient ultracentrifugation from plasma obtained from a normolipidemic individual who had fasted for 12 h, and duplicate samples of three different concentrations of control LDL (1.0. 3.0, and 10 μg apoB-100/ml medium) were incubated with fibroblasts lacking the LDL receptor. HMG-CoA reductase activity was thereafter measured according to the principle described above. There were no concentration–effect relationships between medium concentrations of VLDL or LDL and HMG-CoA reductase activities in that control experiment (data not shown). Nor were any of the VLDL species or the control LDL capable of suppressing HMG-CoA reductase activity to any substantial degree (<10%), suggesting that VLDL uptake by fibroblasts to a large extent is LDL receptor-dependent.

Effects of the triglyceride emulsion on the capacity of VLDL to inhibit LDL binding to the LDL receptor on fibroblasts in vitro

The result of 4-h incubations with three different concentrations of VLDL particles and 5 μg ^{125}I-labeled LDL/ml medium of cultured human fibroblasts are shown in **Fig. 3** and summarized in Table 3. Each experiment (n = 10) was best described by a model with a logarithmic fit, $y = 100 - k^{*}\log x$, where y is percentage of maximum ^{125}I-labeled LDL degradation rate and x is the concentration of VLDL apoB-100 in the cell culture medium. Large and small VLDL isolated from fasting plasma had a similar capacity to inhibit LDL binding to the LDL receptor. The inhibition of ^{125}I-labeled LDL binding to the LDL receptor by large VLDL increased significantly after 60 min infusion of Intralipid (in-

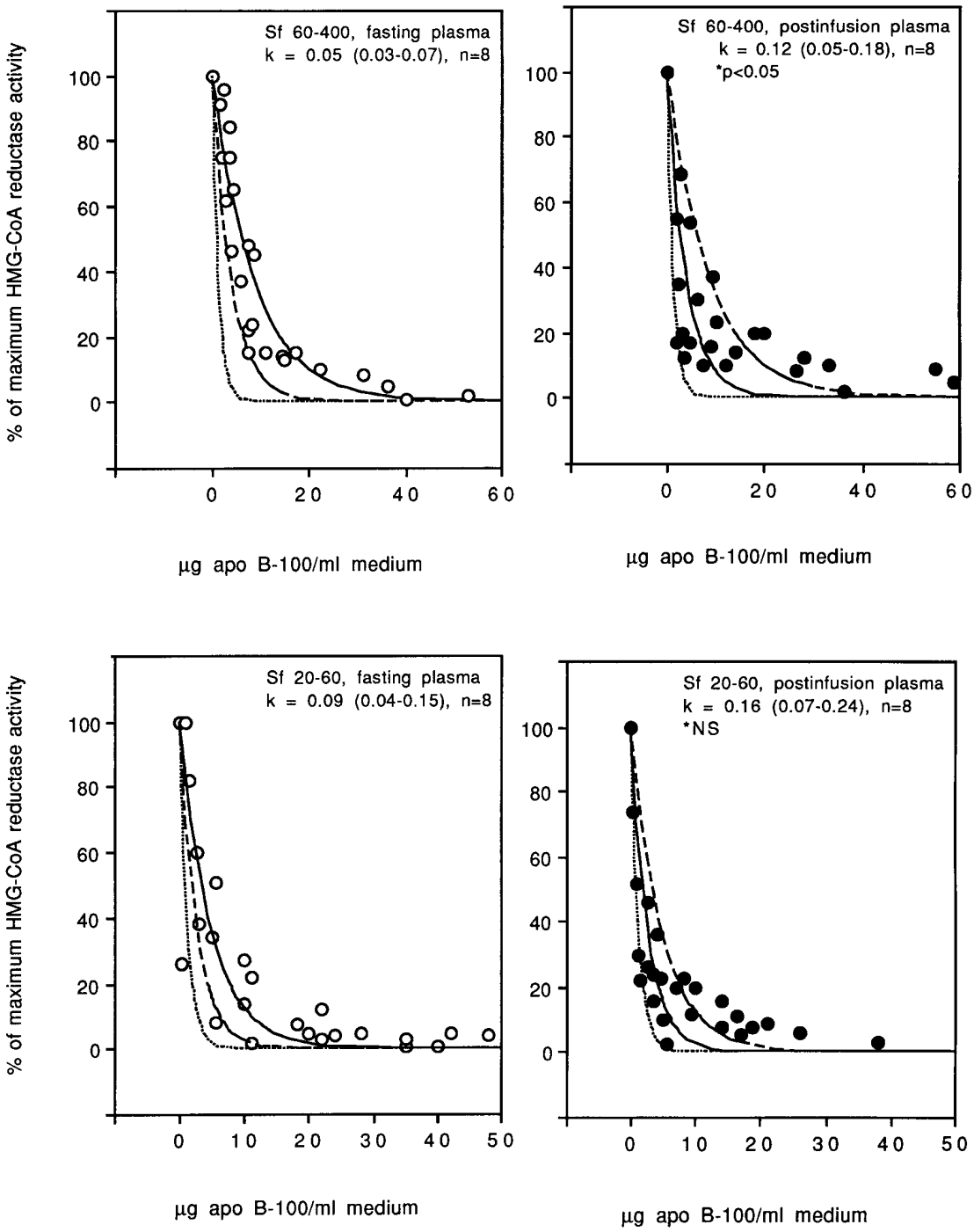

Fig. 2. Effect of transient triglyceridemia on VLDL processing by fibroblasts in vitro. Plasma samples were drawn immediately before and 60 min after a bolus dose (0.15 g/kg of body weight) followed by an intravenous infusion (0.15 g/kg/h) of a triglyceride-rich emulsion (Intralipid 100 mg/l) was administered to eight healthy human volunteers. S_f 60–400 and S_f 20–60 VLDL subfractions were isolated by cumulative flotation in a density gradient and subsequent immunoaffinity chromatography using polyclonal antibodies against apoB-100, as described under Experimental Procedures. The VLDL subfractions were then incubated for 9 h with human fibroblasts (GM08333 cell line) in duplicate at three different concentrations, whereafter inhibition of HMG-CoA reductase activity was assayed essentially according to Brown et al. (39). HMG-CoA-reductase inhibition was calculated as % of maximum reductase activity. LDL (d 1.019–1.063 kg/l), isolated from healthy men (n = 12) by density gradient ultracentrifugation as described for VLDL and cryopreserved (−70°C) in 10% sucrose, was used to estimate between-experiment variation and run in duplicate at three different concentrations. Each data point is the mean of duplicate HMG-CoA reductase activity determinations. Experiments were best described by a model with a logarithmic fit, $y = 100 + 10^{-kx}$, where y is percentage of maximum HMG-CoA reductase activity and x is the concentration of VLDL apoB-

TABLE 3. Processing of VLDL subfractions by fibroblasts in vitro

	HMG-CoA Reductase Inhibition Assay		^{125}I-Labeled LDL Degradation Assay	
	K Value	EC_{50} (μg apoB-100/ml medium)	K Value	EC_{50} (μg apoB-100 ml medium)
S_f 60–400 VLDL				
Fasting	0.05 (0.03)–0.07)	7.3 (4.1–10.5)	31 (25–38)	52 (24–110)
Postinfusion	0.12 (0.05–0.18)	3.9 (1.9–5.9)	41 (35–46)	19 (12–28)
	$P < 0.05$	$P = 0.06$	$P < 0.005$	$P < 0.01$
S_f 20–60 VLDL				
Fasting	0.09 (0.04–0.15)	4.6 (2.3–7.0)	33 (23–42)	48 (21–103)
Postinfusion	0.16 (0.07–0.24)	2.6 (1.2–4.1)	38 (32–43)	24 (15–33)
	$P = 0.16$	$P = 0.11$	$P = 0.18$	$P < 0.05$
LDL	0.30 (0.21–0.39)	1.1 (0.8–1.2)	59 (54–64)	7.2 (6.1–8.4)

Uptake and degradation of VLDL particles was measured as % inhibition of maximum HMG-CoA reductase activity, whereas the affinity of VLDL particles for the LDL receptor was estimated by the % inhibition of the maximum cellular ^{125}I-labeled LDL degradation rate in the presence of unlabeled VLDL or LDL. Values are means and 95% confidence limits. The statistical significance of differences between VLDL isolated from fasting and postinfusion plasma was calculated by Student's paired *t* test. *P* values are given below the postinfusion value. The results of the HMG-CoA reductase inhibition assay were best described by a model with a logarithmic fit, $y = 100 + 10^{-kx}$, where y is percentage of maximum HMG-CoA reductase activity and x is the concentration of VLDL or LDL apoB-100 in the cell culture medium. Similarly, the results of the ^{125}I-labeled LDL degradation assay were best described by a logarithmic fit, $y = 100 - k * \log x$, where y is percentage of maximum ^{125}I-labeled LDL degradation rate and x is the concentration of VLDL or unlabeled LDL apoB-100 in the cell culture medium. K values represent the average slope of the logarithmic fit. Dose–response data for both HMG-CoA reductase inhibition and inhibition of ^{125}I-labeled LDL degradation were also analyzed as the effective concentration (μg apoB-100/ml medium) for 50% inhibition (EC_{50}) of the corresponding maximum rates in the absence of VLDL or unlabeled LDL.

crease in k-value from 31(25–38) to 41(35–46), $P < 0.005$). The inhibitory capacity of small VLDL was less affected by the infusion. It appeared that large, and to some extent even small, VLDL isolated from fasting plasma were unable to inhibit labeled LDL binding to the LDL receptor by more than 50% (Fig. 3). This was also illustrated by the high EC_{50} values of large and small VLDL isolated from fasting plasma (52(24–110) and 48(21–103) μg apoB-100/ml medium). This pattern was not seen for large or for small VLDL isolated after Intralipid infusion (EC_{50} values (of 19(12–28) and 24(15–33) μg apoB-100/ml medium, respectively). The increased capacity of the post-infusion large VLDL to inhibit LDL binding to the LDL receptor, illustrated by the significant increase in k-value, was also evident when the post- and pre-infusion EC_{50} values were compared (19(12–28) vs. 52(24–110) μg apoB/ml medium, $P < 0.01$). The corresponding EC_{50} values of small VLDL were also significantly changed ($P < 0.05$).

Control experiments were performed to determine whether the immunoaffinity procedure materially influenced the ability of VLDL to interact with the LDL receptor. Inhibition of degradation of labeled LDL by fasting large VLDL isolated by density gradient ultracentrifugation was compared with inhibition of degradation of labeled LDL by VLDL isolated by density gradient ultracentrifugation followed by immunoaffinity chromatography as described. The mean k-value for the percentage inhibition of the cellular ^{125}I-labeled LDL degradation rate by large VLDL that had not undergone immunoaffinity chromatography was 22, i.e., slightly lower than the k-value of the corresponding VLDL fraction after immunoaffinity chromatography. The corollary of this control experiment is that *i*) large VLDL that has not undergone immunoaffinity has the capacity to interact with the LDL receptor, and *ii*) immunoaffinity chromatography, including buffer exchange and sample concentration, slightly increases the capacity of large VLDL to interact with fibroblasts in vitro.

100 in the cell culture medium. The solid line is the average logarithmic fit for all experiments (n = 8). K values given in the figure, with 95% intervals, represent the average slope of the logarithmic fit. The broken line in each panel represents the corresponding average logarithmic fit for either fasting or postinfusion VLDL, whereas the dotted line shows LDL for comparison. The statistical significance of differences in fibroblast uptake of VLDL subfractions isolated from fasting and postinfusion plasma samples was determined by testing for differences in k-values using Student's *t*-test. *P* values are indicated in the right panels showing the postinfusion data.

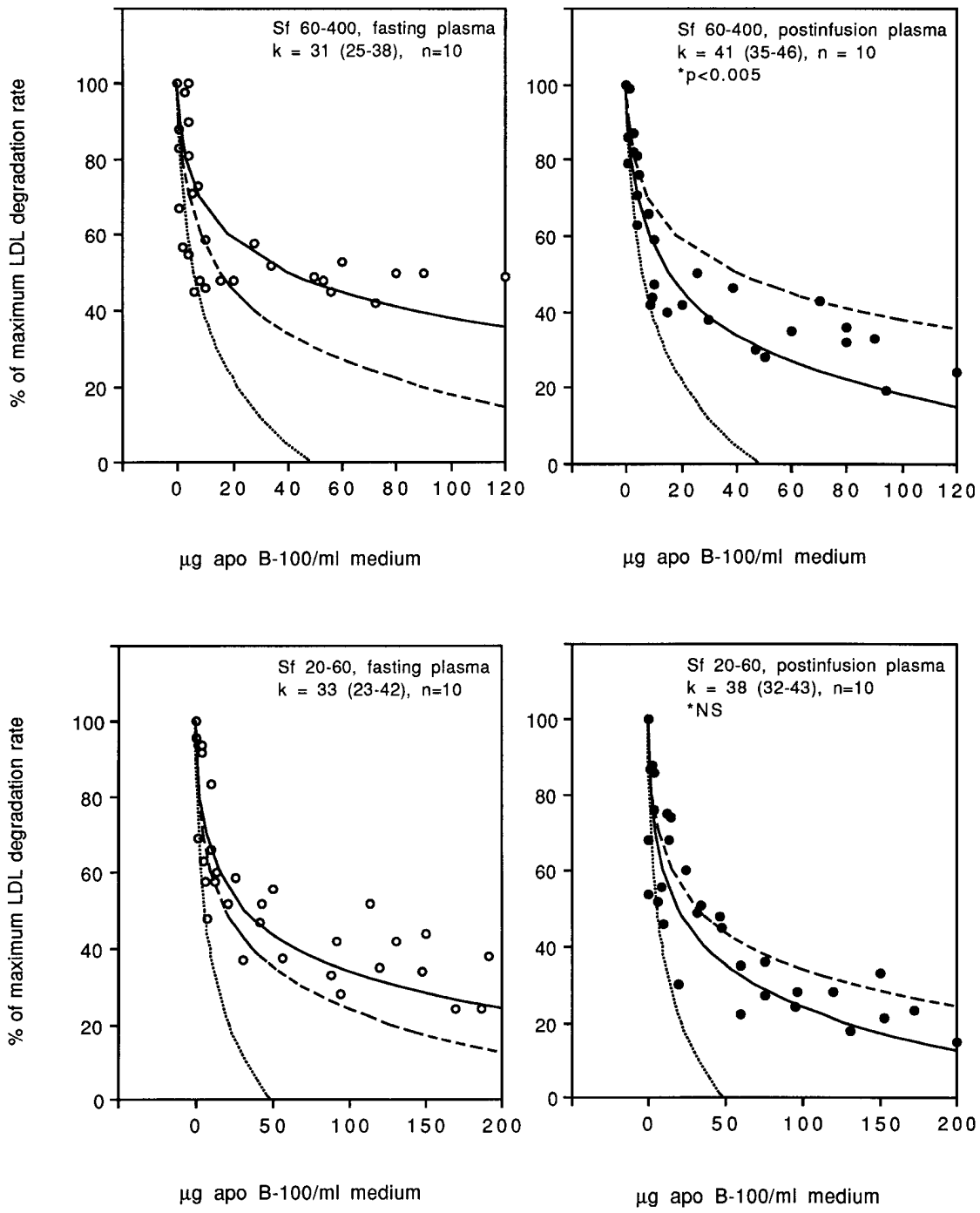

Fig. 3. Effect of transient triglyceridemia on the capacity of VLDL to inhibit the LDL metabolism by the LDL receptor in fibroblasts in vitro. S_f 60–400 and S_f 20–60 VLDL subfractions were isolated from postabsorptive and postinfusion plasma samples as described in the legend to Fig. 2 and under Experimental Procedures. VLDL subfractions at three different concentrations and 5 µg ^{125}I-labeled LDL / ml culture medium were then incubated in duplicate for 4 h with human fibroblasts (GM08333 cell line); hereafter the percentage inhibition of the cellular ^{125}I-labeled LDL degradation rate in the presence of unlabeled VLDL or LDL was measured. The same LDL standard that was used for the HMG-CoA reductase activity assay was used to estimate between-experiment variation and run in duplicate at three different concentrations. Each data point is the mean of duplicate measurements of cellular ^{125}I-labeled LDL degradation rate. Experiments were best described by a model with a logarithmic fit, $y = 100 - k*\log x$, where y is percentage of maximum ^{125}I-labeled LDL degradation rate and x is the concentration of VLDL apoB-100 in the cell culture medium. The solid line is the average logarithmic fit for all experiments (n = 10). K values given in the figure, with 95% intervals, represent the average slope of the logarithmic fit. The broken line

Correlations between changes in VLDL apolipoprotein composition and changes in cellular processing of VLDL

As the apolipoprotein content is likely to influence the cellular processing of the VLDL particles and also at least partly account for the altered properties of the VLDL particles isolated from plasma after Intralipid infusion, changes in compositional characteristics as a response to the infusion were correlated to the corresponding data from cell culture experiments (change of k-values for HMG-CoA reductase inhibition and ^{125}I-labeled LDL degradation experiments as a response to the infusion).

The extent to which the cellular uptake and degradation of large VLDL increased in response to the Intralipid infusion, as measured by the HMG-CoA reductase inhibition assay, did not correlate significantly with the concomitant changes in apolipoprotein content of the large VLDL particles. However, it is notable that the increase in the apoC-I content of large VLDL tended to be inversely related to degree of HMG-CoA reductase inhibition ($r = -0.65$, $P = 0.08$, n = 8).

The increase in the number of apoE molecules per large VLDL particle correlated positively and significantly with the increase in the capacity of large VLDL to inhibit LDL binding to the LDL receptor, as measured by the ^{125}I-labeled LDL degradation assay ($r = 0.76$, $P = 0.01$, n = 10). In contrast, there were no firm associations between the changes of apoC content of large VLDL and altered ability of VLDL to inhibit LDL binding to the LDL receptor (r-values <0.25, NS).

DISCUSSION

The present study examined effects of in vivo modulation of the apolipoprotein content of VLDL during transient triglyceridemia on VLDL processing by fibroblasts in vitro. The experimental design, with intravenous infusion of a chylomicron-like lipid emulsion producing a stable 3-fold increase in plasma triglyceride along with accumulation of large VLDL, was chosen to imitate in a standardized manner the alimentary lipemia occurring in the postprandial state. Large VLDL isolated from plasma after administration of the triglyceride emulsion was found to have increased capacity to inhibit LDL binding to the LDL receptor and a greater ability to suppress the HMG-CoA reductase activity of cultured fibroblasts, predominantly by LDL receptor-mediated pathways. These alterations in cellular interactions were accompanied by increases in the number of apoE, C-I, and C-III molecules per large VLDL particle and loss of apoC-II. The increase in the number of apoE molecules per large VLDL particle correlated closely with the increased capacity of large VLDL to inhibit LDL binding to the LDL-receptor. In contrast, the composition of the small VLDL particles did not change significantly, nor was the ability of these particles to suppress HMG-CoA reductase activity or to inhibit LDL binding to the LDL receptor altered significantly.

More than a decade ago, Gianturco et al. (18) and Bradley and colleagues (19) suggested that apoE is involved in the LDL receptor-mediated uptake of large VLDL that suppresses HMG-CoA reductase activity and later demonstrated that apoE is, in fact, necessary and sufficient for the binding of large triglyceride-rich lipoproteins to the LDL receptor (48). That these findings had a physiological significance in vivo was first indicated by Yamada et al. (49, 50) who showed in a rabbit model that the presence of apoE on VLDL particles influenced their elimination and conversion to LDL. More recently, the apoE content of apoB-containing lipoproteins purified by immunoaffinity chromatography was shown to increase their affinity for the LDL receptor (51), and the metabolism of normolipidemic VLDL through interaction with the LDL receptor was shown to be stimulated by exogenous apoE-3 (52). The present data extend the results of previous cell biological studies on the role of apoE in large VLDL, as the apoE content was modulated under physiological circumstances in vivo in humans during transient triglyceridemia.

The apoC-II depletion during transient triglyceridemia may also contribute to the increase in LDL receptor-mediated processing of large VLDL, as apoC-II has been proposed to counteract apoE-mediated receptor clearance of VLDL (53). The concomitant increases in the contents of apoC-I and apoC-III$_1$ did not appear to have a major influence on the changes in cellular processing that were displayed by large VLDL during triglyceridemia. Sehayek and Eisenberg (15) have previously shown by adding exogenous apoC and apoE to VLDL in vitro that apoE-dependent uptake of VLDL is inhibited by all apoCs, with apoC-I having the strongest inhibitory effect. However, it should be emphasized that the apoC content of large VLDL was modulated in

in each panel represents the corresponding average logarithmic fit for either fasting or postinfusion VLDL, whereas the dotted line shows LDL for comparison. The statistical significance of differences in suppressing degradation of ^{125}I-labeled LDL by the fibroblasts between VLDL subfractions isolated from fasting and postinfusion plasma samples was determined by testing for differences in k values using Student's paired t test. P values are indicated in the right panels showing the postinfusion data.

vivo in the present study, whereas previous studies pointing to a major role of the family of C apolipoproteins for processing of human VLDL and IDL have been based on addition of apoC in vitro. Addition of apoC to lipoproteins in vitro may result in a different function of the apoC molecules compared with endogenous apoC, which may inflate their role in the interaction with apoE and the LDL receptor.

The relevance of the experimental design in which a short-term intravenous infusion of a triglyceride emulsion provided the source of chylomicron-like triglyceride-rich particles could be disputed. Not least based on studies in which human plasma was incubated with Intralipid (3), it could be argued that Intralipid particles may deplete the large VLDL of functional apolipoproteins, particularly apoC-II, in a nonphysiological manner and thus perturb the interactions of these VLDL species with the LDL receptor. It is notable in this context that the number of apoC-II molecules per large VLDL particle decreased significantly during the Intralipid infusion. These restrictions notwithstanding, the post-infusion large VLDL had an increased content of apoE, C-1, and C-III and its processing by the LDL receptor was facilitated rather than impaired. Furthermore, the changes in VLDL composition induced by the Intralipid infusion were almost identical to the postprandial alterations in VLDL composition occurring 2 h after intake of a mixed meal (31). This indicates that intravenous infusion of a chylomicron-like emulsion such as Intralipid is a valid model for studying the effects of transient triglyceridemia on compositional and cell biological properties of VLDL under standardized conditions.

To exclude the possibility that the immunoaffinity chromatography procedure perturbed the apolipoprotein composition and conformation to an extent that it caused the enhanced ability of large VLDL to bind to the LDL receptor, large VLDL that had or had not undergone immunoaffinity chromatography were compared with respect to capacity to inhibit degradation of labeled LDL by fibroblasts. This control experiment showed that large VLDL particles that have not undergone immunoaffinity chromatography have the ability to interact with the LDL receptor. Secondly, the immunoaffinity chromatography procedure was shown to slightly increase the capacity of large VLDL to interact with fibroblasts in vitro. The influence of immunoaffinity chromatography is likely to lead to underestimation of the difference in capacity of postabsorptive and postinfusion large VLDL to interact with the LDL receptor on cultured fibroblasts.

The question arises of whether the compositional alterations acquired during transient triglyceridemia influence the atherogenic and/or thrombotic potential of VLDL. The large VLDL particles accumulating during triglyceridemia inhibited LDL binding to the LDL receptor more efficiently than the postabsorptive large VLDL. This can be due either to increased binding of VLDL to the LDL receptor or to increased binding of VLDL to heparan sulfate proteoglycans, resulting in blockage of LDL receptor sites. The greater change in the capacity of large postinfusion VLDL to inhibit LDL binding compared with the corresponding increase in the cellular uptake of large postinfusion VLDL, as measured by HMG-CoA reductase inhibition, suggests that VLDL effects other than direct binding to the LDL receptor, i.e., increased adhesiveness to matrix components, may play a role. VLDL binding to heparan sulfate proteoglycans is stimulated by apoE-3 (54) and followed by increased internalization and degradation through receptor-dependent catabolic routes. Because of their apoE enrichment it could be speculated that large VLDL particles are more prone to bind to matrix proteoglycans during triglyceridemia. This would promote retention, oxidative modification, and further processing of these VLDL particles in the arterial wall by receptor- and non-receptor-mediated mechanisms. Some triglyceride-rich lipoproteins are, indeed, deposited in the atherosclerotic plaque (55). These lipoproteins resemble the triglyceride-rich lipoproteins encountered in plasma, except for a slightly larger particle size and enrichment in apoE. Thus, triglyceride-rich lipoproteins deposited in the atherosclerotic plaque have striking compositional similarities with the large postinfusion VLDL particles investigated in the present study. A prothrombotic action of large VLDL particles interacting more avidly with the LDL receptor is also likely, as the LDL receptor is implicated in the VLDL-induced secretion of plasminogen activator inhibitor-l from cultured endothelial cells (56).

Exaggerated and prolonged postprandial triglyceridemia is a characteristic of patients with premature coronary heart disease (57), and accumulation of large VLDL is a predominant feature of the postprandial state (25–27). In summary, the present data suggest that large VLDL particles that accumulate during alimentary lipemia undergo compositional changes which render them more adhesive to cellular surfaces and thereby more prone to cellular uptake. These postprandial perturbations are likely to have both atherogenic and thrombotic consequences.

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REFERENCES

1. Gotto, A. M., Jr., H. J. Pownall, and R. J. Havel. 1986. Introduction to the plasma lipoproteins. *Methods Enzymol.* **128:** 3–41.
2. Havel, R. J., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32–38.
3. Tomoci, L., C. A. Scheraldi, X. Li, H. Ide, I. J. Goldberg, and N. A. Le. 1993. Abnormal activation of lipoprotein lipase by non-equilibrating apoC-II, further evidence for the presence of non-equilibrating pools of apoC-II and apoC-III in plasma lipoproteins. *J. Lipid Res.* **34:** 1793–1803.
4. LaRosa, J. C., R. I. Levy, P. Herbert, S. E. Lux, and D. S. Fredrickson. 1970. A specific apoprotein activator for lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **41:** 57–62.
5. Brown, M. V., and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem. Biophys. Res. Commun.* **46:** 375–382.
6. Wang, C-S., W. J. McConathy, H. U. Kloer, and P. Alaupovic. 1985. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J. Clin. Invest.* **75:** 384–390.
7. Shelburne, F., J. Hanks, W. Meyers, and S. Quarfordt. 1980. Effects of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J. Clin. Invest.* **65:** 652–658.
8. Windler, E., Y. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. Opposing effects of homologous apolipoprotein E and individual C apoproteins. *J. Biol. Chem.* **255:** 8303–8307.
9. Windler, E., and R. J. Havel. 1985. Inhibitory effect of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver. *J. Lipid Res.* **26:** 556–565.
10. Aalto-Setälä K., E. A. Fisher, X. Chen, T. Chajek-Shaul, T. Hayek, R. Zechner, A. Walsh, R. Ramakrishnan, H. N. Ginsberg, and J. L. Breslow. 1992. Mechanism of hypertriglyceridemia in human apolipoprotein (apo) C-III transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apoC-III and reduced apoE on the particles. *J. Clin. Invest.* **90:** 1889–1900.
11. deSilva, H. V., S. J. Lauer, J. Wang, W. S. Simonet, K. H. Weisgraber, R. W. Mahley, and J. M. Taylor. 1994. Overexpression of human apolipoprotein C-III in transgenic mice results in a accumulation of apolipoprotein B-48 remnants that is corrected by excess apolipoprotein E. *J. Biol. Chem.* **269:** 2324–2335.
12. Ginsberg, H. N., N-A. Le, I. J. Goldberg, J. C. Gibson, A. Rubinstein, P. Wang-Iverson, R. Norum, and W. V. Brown. 1986. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins C-III and A-I. *J. Clin. Invest.* **78:** 1287–1295.
13. von Eckardstein, A., H. Holz, M. Sandkamp, W. Weng, H. Funke, and G. Assmann. 1991. Apolipoprotein C-III (Lys-Glu). Identification of an apolipoprotein C-III variant in a family with hyperalphalipoproteinemia. *J. Clin. Invest.* **87:** 1724–1731.
14. Weisgraber, K. H., R. W. Mahley, R. C. Kowal, J. Herz, J. L. Goldstein, and M. S. Brown. 1990. Apolipoprotein C-I modulates the interaction of apolipoprotein E with β-migrating very low density lipoproteins (β-VLDL) and inhibits binding of β-VLDL to low density lipoprotein receptor-related protein. *J. Biol. Chem.* **265:** 22453–22459.
15. Sehayek, E., and S. Eisenberg. 1991. Mechanisms of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway. *J. Biol. Chem.* **266:** 18259–18267.
16. Shachter, N. S., T. Ebara, R. Ramakrishnan, G. Steiner, J. L. Breslow, H. N. Ginsberg, and J. D. Smith. 1996. Combined hyperlipidemia in transgenic mice overexpressing human apolipoprotein C-I. *J. Clin. Invest.* **98:** 846–855.
17. Packard. C. J., A. Munro, A. R. Lorimer, A. M. Gotto, Jr., and J. Shepherd. 1984. Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2178–2192.
18. Gianturco, S. H., F. B. Brown, A. M. Gotto, Jr., and W. A. Bradley. 1982. Receptor-mediated uptake of hypertriglyceridemic very low density lipoproteins by normal human fibroblasts. *J. Lipid Res.* **23:** 984–993.
19. Bradley, W. A., S-L. H. Hwang, J. B. Karlin, A. H. Y. Lin, S. C. Prasad, A. M. Gotto, Jr., and S. H. Gianturco. 1984. Low-density lipoprotein binding determinants switch from apolipoprotein E to apolipoprotein B during conversion of hypertriglyceridemic very low density lipoprotein to low density lipoproteins. *J. Biol. Chem.* **259:** 14728–14735.
20. Friedman, G., D. Gavish, T. Vogel, and S. Eisenberg. 1990. Cellular metabolism of human plasma intermediate density lipoprotein. *Biochim. Biophys. Acta.* **1044:** 118–126.
21. Gianturco, S. H., A. M. Gotto, Jr., R. L. Jackson, J. R. Patsch, H. D. Sybers, O. D. Taunton, D. L. Yeshurun, and L. C. Smith. 1978. Control of 3-hydroxy-3-methyl-glutaryl CoA reductase activity in cultured human fibroblasts by very low density lipoproteins of subjects with hypertriglyceridemia. *J. Clin. Invest.* **61:** 320–328.
22. Gianturco, S. H., C. J. Packard, J. Shepherd, L. C. Smith, A. L. Catapano, H. D. Sybers, and A. M. Gotto, Jr. 1980. Abnormal supppression of 3-hydroxy-3-methyl-glutaryl-CoA reductase activity in cultured human fibroblasts by hypertriglyceridemic very low density lipoprotein subclasses. *Lipids.* **15:** 456–463.
23. Chappell, D. A., G. L. Fry, M. A. Waknitz, L. E. Muhonen, and M. W. Pladet. 1993. Low density lipoprotein receptors bind and mediate cellular catabolism of normal very low density lipoproteins in vitro. *J. Biol. Chem.* **268:** 25487–25493.
24. Cohn, J. S., J. R. M. Namara, S. D. Cohn, J. M. Ordovas, and E. J. Schaefer. 1988. Plasma apolipoprotein changes in the triglyceride-rich lipoprotein fraction of human subjects fed a fat-rich meal. *J. Lipid Res.* **29:** 925–936.
25. Karpe, F., G. Steiner, T. Olivercrona, L. A. Carlson, and A. Hamsten. 1993. Metabolism of triglyceride-rich lipoproteins during alimentary lipemia. *J. Clin. Invest.* **91:** 748–759.
26. Schneeman, B. O., L. Kotite, K. M. Todd, and R. J. Havel. 1993. Relationships between the responses of triglyceride-rich lipoproteins in blood plasma containing apolipoproteins B-48 and B-100 to a fat-containing meal in normolipidemic humans. *Proc. Natl. Acad. Sci. USA.* **90:** 2069–2073.
27. Cohn, J. S., E. J. Johnson, J. S. Millar, S. D. Cohn, R. W. Milne, Y. L. Marcel, R. M. Russell, and E. J. Schaefer.

1993. Contribution of apoB-48 and apoB-100 triglyceride-rich lipoproteins (TRL) to postprandial increases in the plasma concentration of TRL triglycerides and retinyl esters. *J. Lipid Res.* **34:** 2033–2040.
28. Karpe, F., and M. Hultin. 1995. Endogenous triglyceride-rich lipoproteins accumulate in rat plasma when competing with a chylomicron-like triglyceride emulsion for a common lipolytic pathway. *J. Lipid Res.* **36:** 1557–1566.
29. Björkegren, J., C. J. Packard, A. Hamsten, D. Bedford, M. Caslake, J. Shepherd, L. Foster, P. Stewart, and F. Karpe. 1996. Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *J. Lipid Res.* **37:** 76–86.
30. Brunzell, J. D., W. R. Hazzard, D. J. Porte, and E. L. Bierman. 1973. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low lipoproteins in man. *J. Clin. Invest.* **52:** 1578–1585.
31. Björkegren, J., A. Hamsten, R. W. Milne, and F. Karpe. 1997. Alterations of VLDL composition during alimentary lipemia. *J. Lipid Res.* **38:** 301–314.
32. Hamsten, A., P. Eriksson, F. Karpe, and A. Silveira. 1994. Relationships of thrombosis and fibrinolysis to atherosclerosis. *Curr. Opin. Lipidol.* **5:** 382–389.
33. Tornvall, P., P. Båvenholm, C. Landou, U. deFaire, and A. Hamsten. 1993. Relation of plasma levels and composition of apolipoprotein B-containing lipoproteins to angiographically defined coronary artery disease in young patients with myocardial infarction. *Circulation.* **88:** 2180–2189.
34. Phillips, N. R., D. Waters, and R. J. Havel. 1993. Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events. *Circulation.* **88:** 2762–2770.
35. Hixson, J. E., A. McMahan, H. C. J. McGill, and J. P. Strong, 1992. Apolipoprotein B insertion / deletion polymorphism is associated with atherosclerosis in young black but not young white males. *Arterioscler. Thromb.* **12:** 1023–1029.
36. Carlson, K. 1973. Lipoprotein fractionation. *J. Clin. Pathol.* **26:** 32–37.
37. McConathy, W. J., E. Koren, H. Wieland, E. M. Campos, D. M. Lee, H. U. Kloer, and P. Alaupovic. 1985. Evaluation of immunoaffinity chromotography for isolating human lipoproteins containing apolipoprotein B. *J. Chromatogr.* **342:** 47–66.
38. Karpe, F., and A. Hamsten. 1994. Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. *J. Lipid Res.* **35:** 1311–1317.
39. Brown, M. S., S. E. Dana, and J. L. Goldstein. 1973. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in human fibroblasts by lipoproteins. *Proc. Nat. Acad. Sci. USA.* **70:** 2162–2166.
40. Gene, C. N., L. C. Pendelton, and A. S. Pendelton. 1987. Loss of NADPH during assays of HMG-CoA reductase: implication and approaches to minimize errors. *Lipids.* **22:** 409–412.
41. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
42. Rumsey, S. C., N. F. Galeano, Y. Arad, and R. J. Deckelbaum. 1992. Cryopreservation with sucrose maintains normal physical and biological properties of human plasma low density lipoproteins. *J. Lipid Res.* **33:** 1551–1561.
43. Bilheimer, D. W., S. Eisenberg, and R. J. Levy. 1972. The metabolism of very low density lipoprotein proteins I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **62:** 212–221.
44. Goldstein, J. L., and M. S. Brown. 1974. Binding and degradation of low density lipoprotein by cultured fibroblasts: comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J. Biol. Chem.* **249:** 5153–5162.
45. Eriksson, M., L. Berglund, M. Rudling, P. Henriksson, and B. Angelin. 1989. Effects of oestrogen on low density lipoprotein metabolism in males: short-term and long-term studies during hormonal treatment of prostatic carcinoma. *J. Clin. Invest.* **84:** 802–810.
46. Zlatkis, A., B. Zak, and A. Boyle. 1953. A new method for direct determination of serum cholesterol. *J. Lab. Clin. Med.* **41:** 486–492.
47. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
48. Bradley, W. A., and S. H. Gianturco. 1986. ApoE is necessary and sufficient for the binding of large very low density lipoproteins to the LDL receptor; apoB is unnecessary. *J. Lipid Res.* **27:** 40–48.
49. Yamada, N., D. M. Shames, J. B. Stoudemire, and R. J. Havel. 1986. Metabolism of lipoprotein B-100 in blood plasma of rabbits: heterogeneity related to the presence of apolipoprotein E. *Proc. Natl. Acad. Sci. USA.* **83:** 3479–3483.
50. Yamada, N., D. M. Shames, K. Takahashi, and R. J. Havel. 1988. Metabolism of apolipoprotein B-100 in large very low density lipoproteins of blood plasma. Kinetic studies in normal and Watanabe heritable hyperlipidemic rabbits. *J. Clin. Invest.* **82:** 2106–2113.
51. Agnani, G., J. M. Bard, L. Candelier, S. Delattre, J. C. Fruchart, and V. Clavey. 1991. Interaction of LpB, LpB:E, LpB:C-III and LpB:C-III:E lipoproteins with the low density lipoprotein receptor of HeLa cells. *Arterioscler. Thromb.* **11:** 1021–1029.
52. Sehayek, E., and S. Eisenberg. 1990. Abnormal composition of hypertriglyceridemic very low density lipoprotein determines abnormal metabolism. *Arteriosclerosis.* **10:** 1088–1096.
53. Shachter, N. S., T. Hayek, T. Leff, J. D. Smith, D. W. Rosenberg, A. Walsh, R. Kamakrishnan, I. J. Goldberg, H. N. Ginsberg, and J. L. Breslow, 1994. Overexpression of apolipoprotein C-II causes hypertriglyceridemia in transgenic mice. *J. Clin. Invest.* **93:** 1683–1690.
54. Ji, Z. S., S. Fazio, Y. L. Lee, and R. W. Mahley. 1994. Secretion-capture role for apolipoprotein E in remnant lipoprotein metabolism involving cell surface heperan sulfate proteoglycans. *J. Biol. Chem.* **269:** 2764–2772.
55. Rapp, J. H., A. Lespine, R. L. Hamilton, N. Colyvas, A. H. Chaumeton, J. Tweedie-Hardman, L. Kotite, S. T. Kunitake, R. J. Havel, and J. P. Kane. 1994. Triglyceride-rich lipoproteins isolated by selected-affinity anti-apolipoprotein B immunosorbtion from human atherosclerotic plaque. *Arterioscler. Thromb.* **14:** 1767–1774.
56. Stiko-Rahm, A., B. Wiman, A. Hamsten, and J. Nilsson. 1990. Secretion of plasminogen activator inhibitor-1 from cultured endothelial cells is induced by very low density lipoprotein. *Arteriosclerosis.* **10:** 1067–1073.
57. Patsch, J. R., G. Miesenböck, T. Hopferwieser, V. Mühlberger, E. Knapp, J. K. Dunn, A. M. Gotto, and W. Patsch. 1992. Relation of triglyceride metabolism and coronary artery disease. *Arterioscler. Thromb.* **12:** 1336–1345.