

MATERIAL AND METHODS

Study cohort—Forty-six subjects were recruited at two study centers according to the following inclusion criteria: men or postmenopausal women, 35–65 years of age, BMI 25–40 kg/m², abdominal obesity according to the National Cholesterol Education Program/Adult Treatment Panel III (waist circumference above 88 cm for women and above 102 cm for men) and at least one lipid abnormality (plasma triglycerides 1.7–4.5 mmol/L and/or HDL cholesterol below 1.29 mmol/L for women and below 1.03 mmol/L for men). Exclusion criteria are described in.¹

Study design—The protocol included three study visits comprising a fasting kinetic study, determination of intra-abdominal fat depots, and a heparin test on separate dates. The ethics committee at each site approved the study design and each subject gave written informed consent before participation in the study (trial registered as NCT00408148).

Standardization between centers—Protocols and methods were described in joint SOPs, all analyses were harmonized between the centers, assays that were not standardized (as LPL activity, LPL mass, HL activity, adiponectin, resistin, leptin and RBP4) were performed in one laboratory. Modeling VLDL kinetics was performed by one person and the HDL kinetic modeling was performed by one person.

Kinetic protocol—The subjects were admitted at 7:30 am and baseline blood samples were taken for the kinetic study and biochemical analyses. At 8:00 am, a bolus injection of [1,1,2,3,3-²H₅]glycerol [500 mg] and [5,5,5-²H₃]leucine [7 mg/kg] was given and blood drawn as previously described.²

Isolation of apolipoproteins and glycerol: measurement of isotopic enrichment—VLDL₁ and VLDL₂ were isolated from plasma as reported.² ApoB isolated from VLDL₁ was hydrolyzed, derivatized and subjected to gas chromatography mass spectrometry (GC/MS) to measure tracer leucine enrichment.^{2,3} Triglycerides were isolated from VLDL₁ and the tracer glycerol enrichment determined as previously described.²

Modeling—Apolipoprotein enrichment data were modeled using SAAM-II (The Epsilon Group, Charlottesville, VA, USA).⁴ The injected amount of [²H₃]leucine and [²H₅]glycerol, the leucine and glycerol pool sizes (blood volume calculated as 4.5% of body weight) in VLDL₁ and the enrichment curves of plasma leucine and glycerol in VLDL₁ and leucine and glycerol were used for a multi-compartment model that allowed simultaneous modeling of apoB and triglyceride kinetics as described previously.² The model output included secretion rate (SR), fractional clearance rate (FCR), fractional direct catabolic rate (FDCR) and fractional transfer rate (FTR). The kinetics of VLDL₁-TG and VLDL₁-apoB were modelled using a combined model for apoB100 and TG metabolism in VLDL subfractions as previously described.²

We also modelled the kinetic parameters of VLDL₁ apoB and triglycerides independently of each other and compared with kinetic parameters derived from the combined model (**Supplementary Figure II**).

Determination of liver, subcutaneous and visceral fat—Liver fat was determined using proton magnetic resonance spectroscopy and subcutaneous abdominal and visceral fat were measured by magnetic resonance imaging.⁵

Biochemical analyses—Fasting plasma glucose, triglycerides, HDL-cholesterol, LDL-cholesterol and plasma liver enzymes were determined by standard procedures. Plasma apoAI, apoB, apoE, apoC-II, apoC-III, apoA5, adiponectin, resistin, leptin and RBP4 were measured by ELISA. Post-heparin LPL and HL activities and LPL mass were measured as described.⁶ LDL peak particle diameter was measured with 2-10% gradient polyacrylamide gel electrophoresis.⁷ Cholesteryl ester transfer protein (CETP) activity was determined as the capacity of a plasma sample to promote the transfer of radiolabeled cholesteryl esters ([³H]cholesteryl ester) from [³H]cholesteryl ester-HDL to apoB-containing lipoproteins.⁸ Plasma phospholipid transfer protein (PLTP) activity was determined as the capacity of a plasma sample to induce the transfer of radiolabeled [¹⁴C]dipalmitoyl phosphatidylcholine from [¹⁴C]dipalmitoyl phosphatidylcholine liposomes to an excess of isolated HDL.⁹ Plasma CETP and PLTP activity levels were related to the activity of a reference plasma analyzed in each run and are expressed in arbitrary units (AU).

Statistical analysis—Statistical calculations were performed using the SPSS software package

(Chicago, Illinois, USA). Data are reported as mean±SD for normally distributed variables or as median (min–max) for non–normally distributed variables. For continuous variables, a Kolmogorov–Smirnov analysis was performed to test for normality. The Pearson correlation coefficients (r) were determined for bivariate correlations. Multivariable analyses were performed by stepwise linear regression, including into the model all the variables that correlated in bivariate analysis with $P \leq 0.10$ and potential confounding factors such as gender and study center. For correlation– and multivariable analyses, data that were not normally distributed were log–transformed. A two–tailed probability level of 0.05 was accepted as statistically significant. The proportion of the variance of the dependent variable that is explained by the independent variable(s) is expressed by the r^2 (the square of the regression coefficient) for univariate analyses and by the adjusted r^2 in multivariable analyses.

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