Sensitive Mass Spectrometry Techniques for Measuring Metabolism of Human Apolipoprotein B In Vivo, *Thomas Demant,[1,4] Christopher J. Packard,[2] Philip Stewart,[2] Andrea Bedynek,[1] A. Graham Calder,[3] James Shepherd,[2] and Dietrich Seidel[1]* ([1] Inst. für Klin. Chem., Klinikum Grosshadern, Marchioninistr. 15, 81366 München, Germany; [2] Inst. of Biochem., Royal Infirmary, Glasgow, UK; [3] Rowett Research Inst., Aberdeen, UK; [4] author for correspondence, fax Int + 49-89-7095 8888)

Until recently, in vivo studies of human apolipoprotein metabolism were based mainly on kinetic analyses of decay curves defined by exogenously radiolabeled li-

poprotein tracers. Distinct lipoprotein fractions such as large and small very-low-density lipoprotein ($VLDL_1$ and $VLDL_2$) or native and chemically modified low-density lipoprotein (LDL) were prepared from plasma, differentially labeled with either ^{131}I or ^{125}I, and reinjected into the donor subject. Subsequently, various lipoprotein fractions—the tracer fractions and, where appropriate, their metabolic products—were isolated from multiple plasma specimens collected as long as 12 days after tracer injection, and decay curves were constructed for radioactivity associated with specific apolipoproteins. These curves represent the database from which, by multicompartmental modeling, metabolic variables for apolipoprotein (apo) B-containing lipoproteins were calculated (*1, 2*).

With the advent of new, comparatively easy to use mass spectrometers, an alternative approach has become feasible: Stable-isotope-substituted amino acids are administered intravenously and used for endogenous labeling of apo B. Apo B-containing lipoproteins—$VLDL_1$, $VLDL_2$, intermediate-density lipoprotein (IDL), and LDL—are subsequently isolated from plasma by the same methods as used for the radioiodine tracer studies. Specific enrichment of the tracer amino acid, measured as atom percent excess (APE), is determined in plasma and in apo B hydrolysates by gas chromatography–mass spectrometry (GC-MS).

One of the main problems encountered in the initial studies with this approach was lack of a sufficiently accurate method for determining specific enrichment in a range from >10% APE for free amino acids in plasma to the <0.2% APE typically found in the LDL fraction (*3, 4*). We here describe a method for GC-MS analysis that reliably fulfills these requirements.

In our studies with eight normolipidemic subjects we used [$^2H_3$]leucine as a tracer compound, administering it as an intravenous bolus of 6 mg/kg body weight. All study participants gave informed consent and the study protocol met the requirements of the Ethical Committees of the respective hospitals. $VLDL_1$, $VLDL_2$, IDL, and LDL were isolated from plasma by cumulative flotation gradient ultracentrifugation (*5*). Apo B was precipitated with isopropanol and subsequently hydrolyzed in 6 mol/L HCl for 12 h at 115°C (*6*). Amino acids were dried under reduced pressure in a concentrator centrifuge and transformed into *tert*-butyldimethylsilyl derivatives (*7*).

APE values were determined by measurements with a quadrupole GC-MS (Trio 1000; Fisons, Manchester, UK). To achieve maximal sensitivity at low levels of tracer enrichment, we recorded the following *m/z* values and quantified them in the selected ion recording (SIR) mode: 277, 276, and 274. *m/z* 277 and 274 represent the $[M-85]^+$ fragments of the labeled (tracer) and the unlabeled (tracee) molecules, respectively, whereas *m/z* 276 is an isotope peak related to *m/z* 274. *m/z* 276 and 274 are in constant quantitative relation; i.e., *m/z* 276:*m/z* 274 equals 0.099±0.001 (n = 15). Since *m/z* 277 and 276 are of similar size, their ratio can be determined with greater precision than *m/z* 277:*m/z* 274 for which, particularly for low amounts of specific tracer enrichment, *m/z* 274 is orders of magnitude greater than *m/z* 277. The ratio *m/z* 277:*m/z* 276 is transformed into values for specific isotopic enrichment *E* by multiplication with a constant factor (i.e., *m/z* 276:*m/z* 274 as given above) and using the formula $E = (R - R_0)/[(1 + R) + (1 + R_0)]$, where R is *m/z* 277:*m/z* 274 of a given sample and R_0 is *m/z* 277:*m/z* 274 of naturally occurring leucine. From specific enrichment *E*, the, tracer/tracee ratios, *Z*, are derived by the formula $Z = E/(E_t - E)$, which takes into account the specific enrichment of the [$^2H_3$]leucine tracer, $E_t = 0.998$ (*8*).

Table 1 shows the *m/z* 277:*m/z* 276 ratios determined in aqueous solutions of naturally occurring leucine and [$^2H_3$]leucine in appropriate mixtures. The CV is <2.0% for calibrators with up to 1.00% APE and <4.0% for calibrators up to 10.0% APE. As shown in Fig. 1, the calibration curve is linear from 0% to 10% APE, and at least 0.1% APE can be distinguished from 0% reliably. Fig. 2 shows the leucine tracer/tracee ratios for the apo B-containing lipoproteins, which were determined by duplicate measurements with the same precision as the aqueous calibration solutions (CV <2.5%).

Crucial for this method is the use of multiply labeled tracer amino acids. Analogous results were obtained with [$^2H_5$]phenylalanine and measurement of *m/z* 313, 311, and 308.

From the curves for the four lipoproteins (labeled/unlabeled) shown in Fig. 2, metabolic variables, e.g., rates for synthesis, transfer, and catabolism, could be calculated by multicompartmental modeling with the SAAM 30 kinetics program (*9*). A comparison of these determinations of

Table 1. Ratios *m/z* 277:*m/z* 276 determined in 120 leucine calibrator solutions at various APE values.

APE, %	*m/z* 277:*m/z* 276 Mean	SEM	CV, %
0.00	0.1697	0.0023	1.4
0.25	0.1909	0.0024	1.3
0.50	0.2112	0.0028	1.3
1.00	0.2551	0.0051	2.0
2.50	0.3927	0.0066	1.7
5.00	0.6326	0.0135	2.1
10.00	1.1266	0.0371	3.3

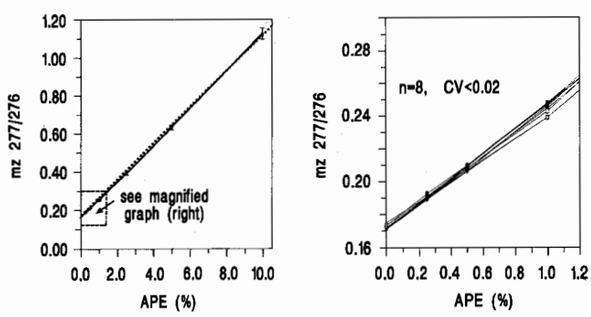

Fig. 1. Calibration curves for *m/z* 277:*m/z* 276 ratios in aqueous leucine solutions with [$^2H_3$]leucine content (APE) ranging from 0% to 10% (*left*) or from 0% to 1.2% (*right*).

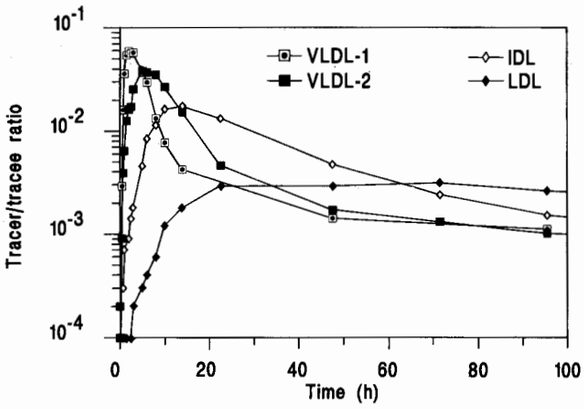

Fig. 2. Leucine tracer/tracee ratios for apo B-containing lipoproteins prepared at various times after an intravenous bolus injection of [$^2H_3$]leucine, 6 mg/kg body wt.

variables with those derived from our previous VLDL double-turnover studies showed good agreement.

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