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Human Apo B Metabolism Investigated by Isotope Ratio Mass Spectrometry

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Introduction

Until recently human metabolism of apolipoprotein B-containing lipoproteins has been studied mainly by analysis of tracer kinetics following the injection of radio-iodine labeled VLDL (1-3). With the advent of new gas chromatography mass spectrometry techniques an alternative approach for investigation of apolipoprotein metabolism has become feasible. By intravenous administration of a stable isotope substituted amino acid lipoproteins are endogenously labeled in the liver and their metabolic fate can be followed by measurements of specific isotopic enrichment. In a previously published study a large dose of amino acid "tracer" had to be used due to limited sensitivity of the quadruple mass spectrometer applied (4). In this paper we report a new method for the investigation of human apo B metabolism using gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). This analytical principle is much more sensitive than conventional gas chromatography-mass spectrometry (GC-MS) and thus allowed for a considerable reduction of the dose of injected stable isotope labeled amino acid (5). It was the purpose of this study first to establish the feasibility of GCC-IRMS for analysis of the VLDL to LDL conversion in humans and secondly to test whether or not the results of metabolic studies were independent of the amount of tracer amino acid injected.

Subject and Methods

The subject investigated in this study was a 30 years old normolipidemic male who participated in three apo B turnover studies at intervals of several weeks in between. Carbon 13-labeled leucine (^{13}C-Leu; Cambridge Isotope Lab., Woburn, MA, USA) was given as an intravenous bolus injection at different doses in each of the three studies: The dosage was 6.0 mg/kg body weight in study I, 3.0 mg/kg in study II, and 0.6 mg/kg in study III. ^{13}C-Leu was injected at 8 a.m. after an overnight fast and plasma samples were collected at frequent intervals over the 24 h thereafter and then daily in the fasting state for 8 days. $VLDL_2$ (S_f 60-400), $VLDL_2$ (S_f 20-60), IDL (S_f 12-20), and LDL (S_f 0-12) were prepared from 2 ml of plasma by cumulative gradient ultracentrifugation as described elsewhere (6). Apo B was precipitated with isopropanol (7), delipidated with ethanol : ether (3 : 1) and ether, and subsequently hydrolyzed in 6N HCl at 110 °C for

22 h. HCl was evaporated and amino acids were derivatized to form N-acetyl-propyl-esters (8). Free amino acids were prepared from 1 ml of plasma by cation exchange chromatography (Dowex AG-50W-X8; Bio-Rad, Richmond, CA, USA) and finally derivatized as described above (8). These volatile, fully combustible compounds were analyzed for leucine $^{13}C/^{12}C$ isotope ratios using a VG-Optima gas chromatography-combustion-isotope ratio mass spectrometer (Fissons Instr. Ltd, Manchester, UK). Isotope ratios were transformed into tracer/tracee ratios and tracer mass data (9) were analyzed by multicompartmental modeling using the SAAM30 program (10).

Results

Plasma lipids and lipoprotein concentrations were measured several times in the same subject at the occasion of each of the three apo B turnover studies (Table 1). The time course for leucine tracer/tracee ratios from the apo B-containing lipoproteins $VLDL_1$, $VLDL_2$, IDL and LDL were determined as given in figure 1. It was found that the shape of curves and their relative position towards each other was similar in these studies which differed only by the amount of ^{13}C-Leu injected for endogenous apo B labeling. From this observation alone it could be concluded that the different dosage schemes had no qualitative impact on the observed tracer kinetics. Metabolic parameters calculated from the observed data by kinetic modeling are summarized in table 2. They are also in good agreement as could be expected from the similarity of observed data on which simulation calculations were based.

Discussion

Due to limited analytical sensitivity, in particular for the detection of low levels of isotopic enrichment in the LDL-apo B fraction, large doses of stable isotope labeled amino acids (approximately 5 mg/kg) had to be used in previous studies of apo B metabolism (4). It has been shown that still higher doses of leucine (up to 50 mg/kg), injected as a so-called flooding dose in studies of human muscle protein synthesis (11), can markedly stimulate protein synthesis and thus interfere with the parameters which are to be measured (12). This may be due to an increased insulin secretion in response to the raise of the plasma leucine concentration (13).

GC-C-IRMS is by at least two orders of magnitude more sensitive than conventional quadruple GC-MS. We used this method to test whether the amount of stable isotope labeled leucine which has to be used in GC-MS studies has an impact on apo B kinetics and metabolic parameters. ^{13}C-Leu injections in the range of 0.6 to 6.0 mg/kg resulted in similar curve patterns when tracer/tracee ratios were plotted against time. Metabolic parameters which are derived from observed data by multicompartmental modeling were also in good agreement. Therefore we conclude that leucine injected as a bolus of up to 6 mg/kg does not perturb apo B metabolism and that the dosage requirements for GC-MS measurements are compatible with valid results.

Table 1. Plasma lipids and lipoprotein concentrations of the subject investigated as measured during the three apo B metabolic studies.

Tracer dose	Trig	Chol	VLDL	LDL	HDL	Lp(a)
(mg/kg b.w.)			*(mg/dl)*			
I (6.0 mg/kg)	90	175	15	116	44	8
II (3.0 mg/kg)	89	168	21	105	42	9
III (0.6 mg/kg)	86	166	26	97	43	8

Table 2. Metabolic parameters for apo B-containing lipoproteins as calculated from tracer/tracee ratios by multicompartmental modeling.

Study	Direct Synth.	Flux from precursors	Plasma Pool	Direct Catab.	Transfer into metab. products
	mg/d		*mg*	*pools/d*	
$VLDL_1$ (S_f 60-400)					
I	396	-	34	0.33	11.3
II	416	-	36	0.36	11.2
III	339	-	26	0.19	12.8
$VLDL_2$ (S_f 20-60)					
I	300	385	86	0.95	7.01
II	340	403	92	3.10	4.90
III	197	334	51	2.43	8.14
IDL (S_f 12-20)					
I	229	603	248	1.56	1.79
II	216	454	168	1.67	2.32
III	90	415	169	0.43	2.56
LDL1 (S_f 0-12)					
I	10	445	1339	0.34	-
II	12	389	1180	0.34	-
III	52	433	1276	0.38	-

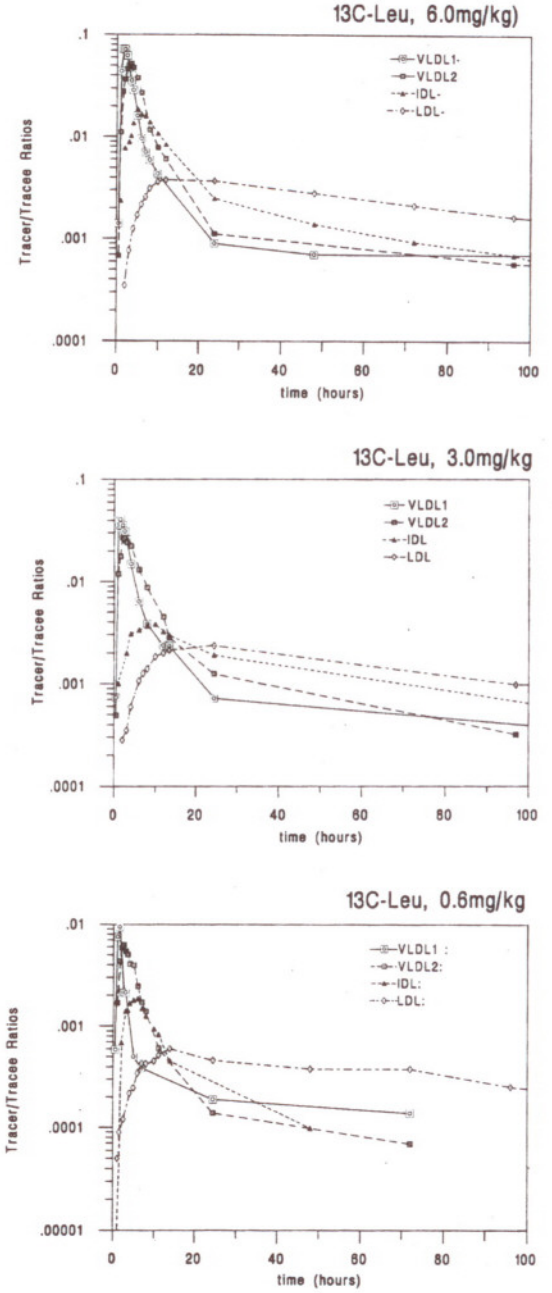

Fig. 1. Tracer/tracee ratios for apolipoprotein B-containing lipoproteins as measured after bolus injections of different amounts of ^{13}C-Leucine.

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