A Study of the Metabolism of Apolipoprotein B100 in Relation to Insulin Resistance in African American Males (44418)

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Abstract. **The purpose of this study was to determine the relationship between insulin resistance and apoB100 metabolism in African American males. Fifteen subjects, 33 ± 7.6 years old, were divided into two groups, insulin-resistant (IR) or insulin-sensitive (IS), based on the sum of the plasma insulin concentrations during an oral glucose tolerance test. The IR group ($n = 8$) differed significantly from the IS group ($n = 7$) with respect to body mass index (BMI) (30.1 vs 23.1 kg/m^2; $P = 0.0003$), fasting triglycerides, (118 vs 54 mg/dl, $P = 0.013$), and total plasma apolipoprotein B100 (80 vs 59 mg/dl, $P = 0.014$). Significantly elevated apoB100 levels in the IR group were seen in very low density lipoprotein (VLDL) (5.1 vs 3.4 mg/dl, $P = 0.045$) and intermediate density lipoprotein (IDL) (18 vs 12 mg/dl, $P = 0.017$) but not in low density lipoprotein (LDL) (57 vs 46 mg/dl, $P = 0.19$). Total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), apolipoprotein A-I, and blood pressure were not significantly different between the two groups. There was a high correlation between the sum of insulins during the oral glucose tolerance test and the BMI ($\rho = 0.88$, $P = 0.0001$).**

In five IR and five IS subjects, apoB100 kinetics were determined in the fasting state using a bolus dose of deuteroleucine and multicompartmental modeling. IR subjects had significantly lower fractional catabolic rates (FCR) in the larger $VLDL_1$ (–70%), the smaller $VLDL_2$ (–71%), and the IDL (–53%) fractions. No significant differences in production rates were observed for any lipoprotein class. There was a significant correlation between the sum of insulins and the FCR of the apoB100 of $VLDL_1$ ($\rho = -0.65$, $P = 0.05$) and of IDL ($\rho = -0.85$, $P = 0.004$). The correlation coefficient of the sum of insulins and the FCR of $VLDL_2$ was –0.61 with $P = 0.067$. We conclude that in this population of African American males, IR is correlated with a decreased FCR of apoB100 in VLDL and IDL and elevated plasma levels of apoB and triglycerides (TG). These changes might be explained by decreased clearance of the TG-rich lipoproteins. We postulate that this may reflect decreased lipoprotein and/or hepatic lipase activity related to insulin resistance and its association with obesity.

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Insulin resistance is recognized as an important risk factor for the development of atherosclerosis and coronary heart disease (1). IR has been correlated with obesity as well as with type 2 diabetes mellitus (2). In previous work from our laboratories (3), insulin resistance has also been correlated with hypertension in African Americans, a population at greater risk than the general population in the United States for the complications of atherosclerosis (4).

Insulin resistance and obesity are associated with one another and with elevated fasting triglycerides (5). Though there have been several studies of steady-state levels of

VLDL and triglycerides in relation to insulin resistance (6), there have been fewer studies of VLDL-triglyceride kinetics and no studies of VLDL apoB100 kinetics. Packard and Shepherd (7) have demonstrated the metabolic heterogeneity of VLDL. They have shown that the VLDL fractions isolated by ultracentrifugation at $d = 1.006$ are composed of at least two metabolically distinct subfractions: $VLDL_1$, large particles with flotation rates in the S_f 60–400 range and smaller $VLDL_2$ particles of S_f 20–60. The present experiments were undertaken to explore the hypothesis that IR in African Americans is related to increased plasma levels of apoB100 in $VLDL_1$ or $VLDL_2$. If plasma levels of apoB100 are increased, the study was designed to determine if the increase was due to overproduction or to decreased catabolism. All 15 subjects had fasting plasma levels of apoB100 measured in all of the lipoprotein density classes. Insulin sensitivity was assessed by determining insulin levels during an oral glucose tolerance test. Ten of the subjects underwent stable isotope kinetic studies of apoB100 metabolism.

Materials and Methods

Population Parameters. The group of 15 male African American subjects averaged 33 ± 7.6 years old. The population was clinically well and fully ambulatory. The subjects were recruited from the hypertension clinic of the Philadelphia Veterans Affairs Medical Center and from a cohort that has been under study since adolescence in ongoing investigations of blood pressure regulation (3). Two subjects were taking one of two types of antihypertensives, angiotensin converting enzyme inhibitors or calcium channel blockers, which are not known to affect plasma lipid levels. The protocol was approved by the Institutional Review Boards of the Philadelphia Veterans Affairs Medical Center and the Allegheny University of the Health Sciences (now the MCP-Hahnemann University). Each subject gave his informed consent prior to participation.

All of the subjects came to the clinical research unit after a 12-hr overnight fast. An enrollment assessment and an oral glucose tolerance test were performed. The enrollment assessment consisted of a medical, social, and dietary history, a physical examination, anthropometric measurements (height, weight, and skinfold thicknesses), and blood pressure determinations. The subjects then had a standard 75-g oral glucose tolerance test (Glucola, Ames Laboratories, Elkhart, IN). Fasting blood samples were obtained for glucose, insulin, and lipid and apolipoprotein levels. Glucose and insulin levels were also obtained at 30, 60, and 120 min. Plasma glucose concentrations were determined by the glucose oxidase method (Glucostat, Yellow Springs Instrument Co, Yellow Springs, OH). Plasma insulin concentrations were determined by a solid phase radioimmunoassay (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA). The fasting lipid profile and the concentrations of plasma apoA-I and apoB were measured by the Lipid Disorders Center of the former Allegheny University of the Health Sciences, a CDC standardized laboratory. Total cholesterol and TG were analyzed using enzymatic methods and an automated analyzer (Hitachi 704). HDL-C was isolated by the method of Bachorik *et al.* (8), and LDL-C was calculated by the Friedewald equation. Apolipoproteins A-I and B were assayed turbidimetrically using commercial antibodies (Boehringer-Mannheim) according to the general procedures for apoB (9).

Kinetic Study Design. Ten of the 15 subjects were randomly selected to participate in the kinetic study, which was performed 2–4 weeks after the oral glucose tolerance test. After a 12-hr fast on the day of the study, a blood sample was drawn for the measurement of initial lipid parameters. Eight of the subjects were then given an intravenous dose of 60 μmoles/kg body weight of deuteroleucine ($^2H_3$-leucine, 99% enriched, CDN Isotopes, Pointe-Claire, Quebec). Subjects 1 and 2 drank the same dose of deuteroleucine dissolved in 100 ml of water. Blood samples were collected in Vacutainer tubes containing EDTA at 5-min intervals for the first hour, at 15-min intervals for the second hour, at 30-min intervals for the third and fourth hours, and at hourly or two-hourly intervals thereafter until the 12th hour. After the 12-hr sample was collected, the subjects were allowed to eat a meal and return home. They returned the next morning, following a 12-hr overnight fast, for a blood sample. Subsequent fasting blood samples were drawn at 2, 4, 7, and 12 days. For several subjects, samples were obtained on Day 5 instead of Day 4 and Day 8 instead of Day 7. Plasma was isolated from the collected blood samples by centrifugation in the cold for 30 min. Then 3 ml of plasma were used to isolate lipoproteins by sequential density ultracentrifugation as described below. Additional aliquots of the plasma were treated with 10% trichloroacetic acid for the isolation of plasma amino acids as previously described (10).

Lipoprotein Isolation and Measurement of the Steady State Concentration of ApoB100 in Each Lipoprotein Class. $VLDL_1$ lipoproteins were isolated essentially as described by Evans *et al.* (11). With a thin spinal needle, the plasma was layered under 5.5 ml of 0.15 *M* NaCl- 2 m*M* EDTA containing 0.1 m*M* 4-(2-aminoethyl) benzene sulfonyl fluoride (AEBSF) and 0.02% sodium azide in a polycarbonate ultracentrifuge tube. The samples were then centrifuged at 12°C in a Beckman 50Ti rotor for 2 hr at 41,000 rpm (152,000*g* max). The top milliliter was carefully removed as the $VLDL_1$ fraction. Then 1.0 ml of the 0.15 *M* NaCl solution containing the inhibitors was added to the tube with as little mixing as possible, and the tubes were then centrifuged at 40,000 rpm (145,000*g*) for 16 hr at 5°C. The top milliliter was removed as the $VLDL_2$ fraction, and the salt density of the remaining solution was adjusted to 1.02 with 70% NaBr in 0.01 *M* phosphate buffer pH 7.4. After thorough mixing, the solution was brought to a final volume of 8.5 ml and centrifuged for 18 hr at 42,000 rpm (160,000*g*) at 5°C to isolate the IDL fraction in the top milliliter. Following the removal of the IDL fraction, the

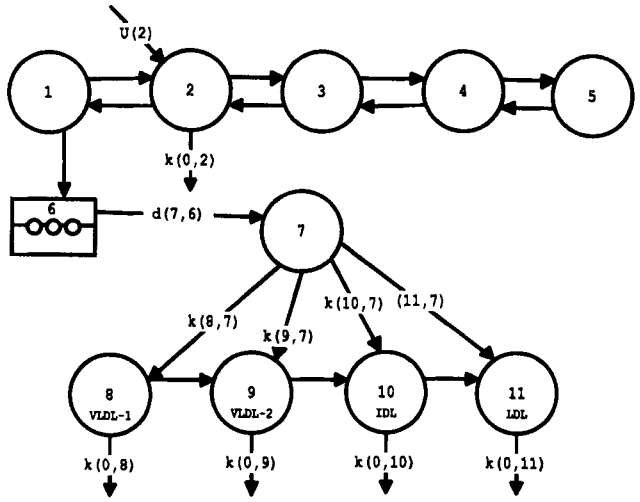

Figure 1. Multicompartmental model employed in the determination of the kinetic constants for apoB100. In this model, compartment 1 represents the hepatic leucine pool, compartment 2, the plasma pool with the tracer entering it by the intravenous route as shown by the symbol U(2). Compartments 3–5 represent other tissue leucine pools that equilibrate with the plasma. Compartment 6 represents the delay required for leucine to be incorporated into apoB100 and subsequently secreted by the liver into a precursor pool, compartment 7. Compartments 8–11 represent apoB100 in $VLDL_1$, $VLDL_2$, IDL, and LDL, respectively.

salt density of the remaining solution was increased to 1.063, and it was centrifuged for 24 hr at 47,000 rpm (200,000*g*) at 5°C to isolate LDL in the top milliliter. The isolated LDL fraction was dialyzed against 0.02 *M* phosphate buffer pH 7.4-2 m*M* EDTA containing 20 μ*M* AEBSF and 0.02% sodium azide.

Aliquots of the isolated lipoprotein fractions were removed for the analysis of total protein by the Morton modification (12) of the bicinchoninic acid method. The remaining $VLDL_1$, $VLDL_2$, and IDL fractions were treated with 0.25 volume of a solution of 12% SDS in 0.05 *M* Tris buffer pH 7.0 containing 30% glycerol, 0.016% bromphenol blue, and 12% mercaptoethanol and stored at −20°C until used for SDS-PAGE isolation of apoB100. The LDL solutions were treated with 2 volumes of a similar buffered solution of 3% SDS containing 10% glycerol, 3% mercaptoethanol, and 0.004% bromphenol blue. We have found that the separation of the apoproteins remains excellent even after storage under these conditions for 6 years, though the maximum time until analysis in this study did not exceed 1 year. Electrophoresis of the lipoprotein fractions was carried out in a 3.5% polyacrylamide gel containing glycerol (13). The gels were stained with Coomassie Blue R250, destained, and preserved in 5% acetic acid until scanned with a laser densitometer. The percentage of stained protein on the gel corresponding to apoB100, (which also included any apoB95 present) as judged by a rat plasma VLDL standard, was calculated and multiplied by the measured total protein to give the value for apoB100. No corrections were made for differences in dye-binding between the various apoproteins. These measurements of apoB100 were applied only to $VLDL_1$, $VLDL_2$, and IDL. The sum of the apoB100 found in these fractions was subtracted from the total apoB measured in the clinical laboratory to give the concentration of LDL apoB100.

Measurement of the Isotopic Enrichment of Plasma Leucine and ApoB100 Leucine. ApoB100 bands were cut from the gel, lyophilized, and hydrolyzed in 12 *N* HCl under nitrogen for 24 hr at 110°C. Amino acids were isolated after ion-exchange chromatography and derivatized as tertiary butyl N-trimethysilyl compounds as previously described (10). Plasma leucine concentrations were determined at each time point by the isotope dilution method using a 99% D_3-leucine standard as the diluent. GC-MS was carried out with a 25-m Hewlett-Packard Ultra2 column and a temperature program with a Hewlett-Packard 5988A mass detector operated in SIM mode and monitoring at m/z of 200 and 203 after electron impact ionization. The 203/(200 + 203) ratio was determined from the area under the peak, and the isotopic enrichment was determined from a standard curve obtained by mixing unenriched leucine with known amounts of deuteroleucine. The tracer/tracee ratio was calculated from the observed mole percent enrichment (14).

Multicompartmental Modeling of Lipoprotein ApoB100 Kinetics. The compartmental model developed in these studies is similar to that previously described by Welty *et al.* (15) and is shown in Figure 1. The SAAMII program (SAAM Institute, Seattle, WA) was used for model development and analysis of the tracer data (16). Compartments 1–5 were used to describe the leucine kinetics. Compartment 2 represented plasma leucine. In eight of these studies, deuterated leucine was administered intravenously (i.e., directly into compartment 2 of the model). In subjects 1 and 2, who received an oral dose of leucine, the model was modified to include a compartment 13 that described the oral input—a fast and a slow pathway (with a delay) for the absorption and appearance of deuteroleucine in plasma. The leucine section of the model was fit to the leucine kinetic data initially and independently of the apoB kinetic data. The leucine rate constants were subsequently fixed when the model was fit to the apoB kinetic data. The model assumed that apoB leucine was derived from compartment 1 after passing through delay compartment 6 that represents the time required for the synthesis of apoB. Compartment 7 represented a nonplasma compartment, from which plasma apoB was derived. Within the plasma there are four pools of apoB: $VLDL_1$, $VLDL_2$, IDL, and LDL, as described by compartments 8–11, respectively. In addition to pathways that show the conversion to a higher density fraction and direct removal of the apoB-containing particles, the model permitted the direct secretion of apoB into all density fractions. This model was fit to the apoB data for all fractions simultaneously.

The plasma leucine mass in compartment 2 for each tracer experiment was calculated as the concentration at

Table I. Subject Parameters

Subject	Age (yrs)	Insulin sum	BMI	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	ApoB100 (mg/dl)	Apo A-1 (mg/dl)	Syst. BP (mmHg)
IR										
1	59	268	26.5	224	200	37	147	96	122	121
2	30	274	29.7	184	103	35	128	68	105	117
6	25	240	26.5	207	228	44	117	90	152	125
8	32	305	33.8	173	78	34	123	67	125	125
9	31	355	33.2	162	124	54	83	74	115	108
12	31	371	32.6	222	108	47	153	95	154	147
13	32	340	31.1	154	56	40	105	82	103	130
14	35	409	27.2	136	54	48	79	65	124	109
Mean	34.4	320	30.1	183	118	42.4	117	79.6	123	125
SD	10.3	58.1	3.05	32.4	65.6	7.07	27	12.9	19.1	12.5
IS										
3	32	227	26.2	158	54	46	101	54	116	142
5	25	139	21.1	152	10	48	96	47	127	139
7	34	152	23.9	143	44	52	82	57	152	131
10	31	101	24.6	124	52	36	65	42	157	99
11	35	169	25.2	163	—	63	78	70	180	110
16	32	108	18.6	203	77	66	122	66	180	137
17	28	168	22.3	155	84	40	98	74	109	131
Mean	31	152	23.1	157	53.5	50.1	91.7	58.6	146	127
SD	3.46	42.6	2.64	24.0	26.3	11.1	18.5	11.9	29.2	16.2
P, IR vs IS	0.96	0.0003	0.0003	0.12	0.013	0.19	0.054	0.014	0.12	0.36
Correlation with insulin										
ρ			0.88	0.25	0.44	−0.14	0.34	0.54	−0.45	−0.16
P			0.0001	0.36	0.11	0.62	0.21	0.04	0.09	0.57

each time point averaged for all of the time points in which plasma was obtained, multiplied by the plasma volume which was assumed to be 4.5% of the body weight. For the apoB100 leucine mass in the various lipoprotein fractions, the pool size was measured as the mass of apoB100 multiplied by 0.121, the leucine fraction of the amino acids of apoB100. The delay time for the synthesis of apoB100 in the liver was initially set at 0.5 hr but was allowed to adjust in each subject during the curve-fitting procedure. Fractional catabolic rates (FCR) and production rates were determined from the parameters that gave the best model fit.

Statistical Methods. The Spearman nonparametric method was employed using the GraphPad Instat2 program (GraphPad, San Diego, CA) to determine correlation coefficients. Student's *t* test was employed to determine *P* values for the differences in population means, with $P < 0.05$ taken as statistically significant.

Results

Population Parameters. The subjects were divided into two groups, IR or IS, based on a median split of the sum of the insulin values for all 15 subjects. The body mass indices (BMI) of the men, their systolic blood pressure, and their fasting plasma lipid and apolipoprotein concentrations are shown in Table I. Compared to the IS group, the IR group had a significantly higher BMI (+30%). For all subjects there was a highly significant correlation between the sum of the insulins and the BMI ($\rho = 0.88$, $P = 0.0001$), and a significant correlation of the insulins with the apoB100 ($\rho = 0.54$, $P = 0.04$). The IR subjects, compared

Table II. ApoB100 Levels in IR and IS Subjects (mg/l)

Subject	$VLDL_1$	$VLDL_2$	VLDL	IDL	LDL
IR					
1	9.5	11.4	20.9	234	705
2	16.3	41.2	57.5	145	478
6	5.2	48.5	53.7	168	679
8	16.3	61.3	77.6	238	355
9	21.3	45.3	66.6	238	436
12	12.1	42.2	54.3	130	766
13	5.8	37.9	43.7	130	645
14	5.1	30.6	35.7	141	473
Mean	11.5	39.8	51.3	178	567
SD	6.09	14.5	17.7	50	149
IS					
3	9.3	16.7	26.0	80.9	433
5	4.6	28.8	33.4	104.0	333
7	3.2	7.5	10.7	83.6	476
10	4.6	31.1	35.7	135.0	249
11	19.5	24.1	43.6	146.0	693
16	9.5	32.3	41.8	150.0	468
17	8.5	35.4	43.9	130.0	566
Mean	8.5	25.1	33.6	119	460
SD	5.5	9.9	12.0	28.9	146
P, IR/IS	0.338	0.042	0.045	0.017	0.185

to the IS group, had significantly higher TG levels (+122%, $P = 0.013$). There were no significant differences between the IR and IS groups in the levels of LDL-C, HDL-C, apoA-I, or systolic blood pressure.

Table II shows that in the IR subjects, the plasma concentration of apoB100 was significantly increased in total VLDL and IDL but not in LDL. The increase was 26% for $VLDL_1$ ($P = 0.34$), 37% for $VLDL_2$, ($P = 0.042$), 35% for total VLDL ($P = 0.045$) and 33% for IDL ($P = 0.017$). No significant differences were found between the two groups in the apoprotein composition of any lipoprotein class (data not shown).

ApoB100 Kinetic Parameters Figure 2 shows the fit of the data to the curve calculated from the model (Fig. 1) for deuteroleucine given IV (Fig. 2A) or orally (Fig. 2B). Figure 3 shows the fit of the data for apoB in a representative IS subject (Fig. 3A) or IR subject (Fig. 3B).

Table III shows the kinetic parameters for apoB100 in the four density classes in the IR and IS groups. No significant differences in production rate were observed in any of the lipoprotein density classes, and there were no significant correlations with the insulin sum in any instance. The FCRs were decreased in the IR subjects in $VLDL_1$, $VLDL_2$, and IDL by 70% ($P = 0.03$), 71% ($P = 0.015$), and 53% ($P = 0.004$), respectively. There was no significant change in the FCR of apoB100 in LDL. However, when the individual values for the FCR of apoB100 were correlated with the sum of insulins, significant negative correlations of −0.65 and −0.85 were obtained for $VLDL_1$ and IDL; and for $VLDL_2$, the ρ value was −0.61 ($P = 0.067$).

Discussion

Large studies have demonstrated the strong correlation between obesity, insulin resistance, and hyperlipidemia (5, 17, 18). The present study shows that for African American men, even when the sample size was small ($n = 15$), the association between obesity, IR, and hyperlipidemia was highly significant. As measured by the sum of insulin concentrations during an oral glucose tolerance test, IR subjects showed a very strong correlation with the BMI (Spearman correlation coefficient $\rho = 0.88$, $P = 0.0001$, Table I). All of the lipid parameters except HDL-C were higher in the IR group though the difference was significant only for TG which was elevated more than two-fold. ApoB100 levels were also significantly increased in $VLDL_2$, total VLDL, and IDL but not in $VLDL_1$ or LDL (Table II).

Several studies have shown a correlation between hyperinsulinism and VLDL-triglyceride production rates in

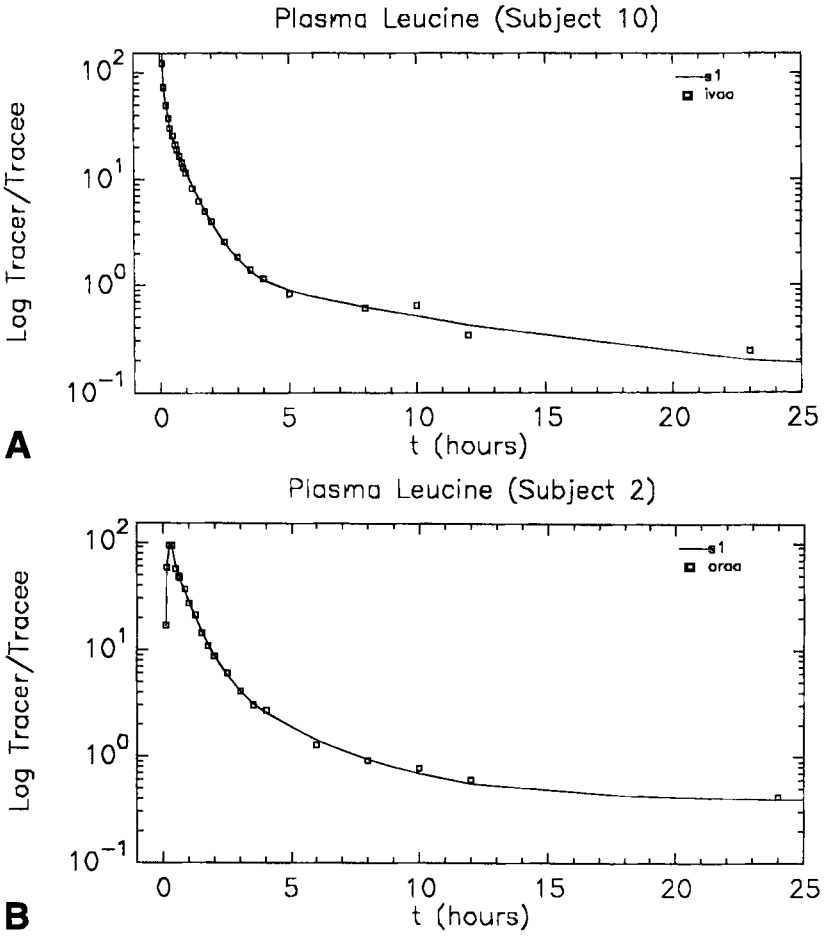

Figure 2. Leucine kinetics. An example of the time course of the isotopic enrichment of plasma leucine in a subject given the isotope (A) intravenously or (B) orally. In the upper right-hand corner of the figure, the calculated curve is represented by a solid line, followed by an s number (s1). The *s* number represents the parameter (in this case, plasma leucine) being sampled by the SAAMII program and the symbol for the data points (in this case, an open square) is shown below the s number, followed by a shorthand notation for the parameter (in this case, ivaa or oraa).

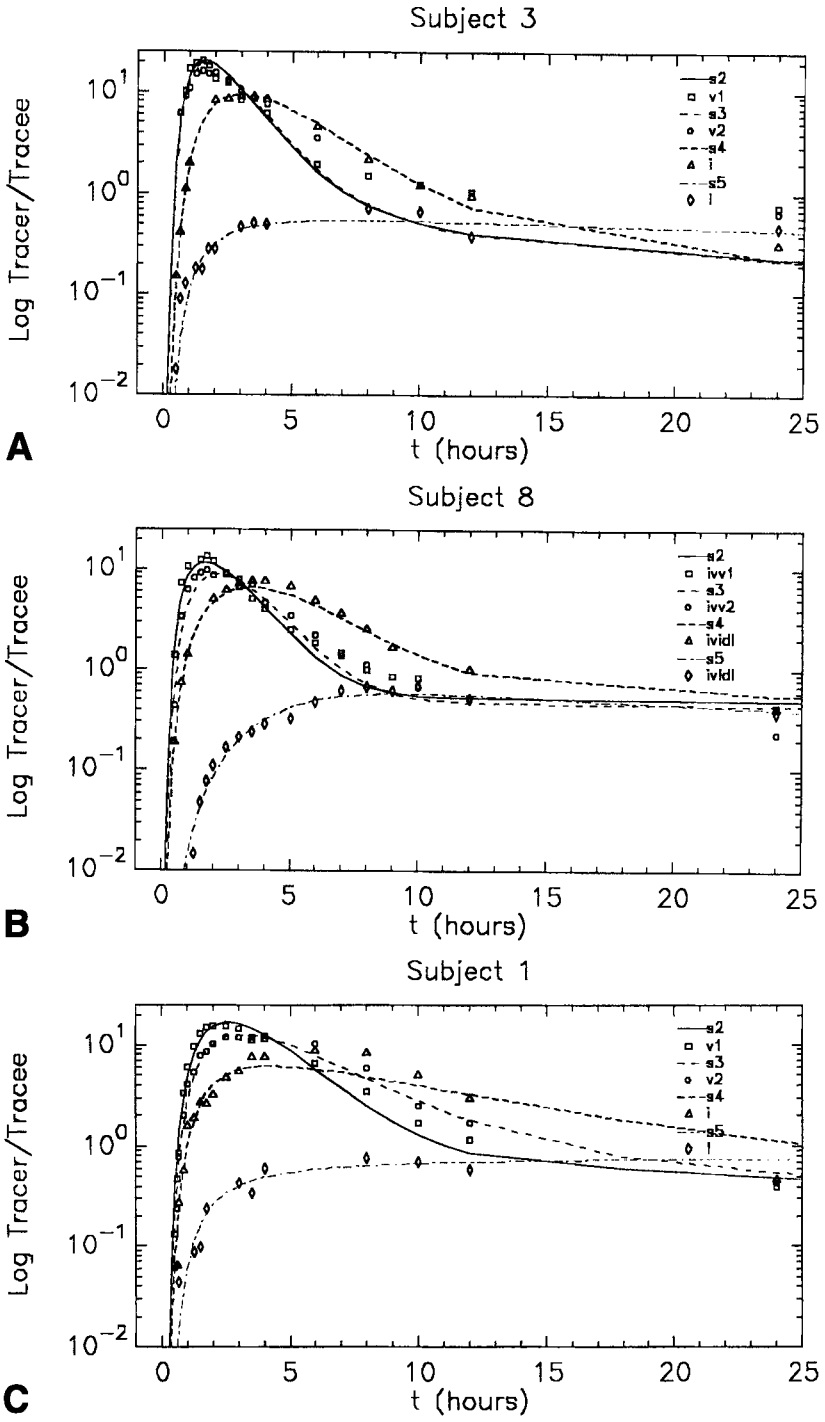

Figure 3. ApoB100 kinetics. (A) ApoB100 kinetics in subject 3 representing the IS group and (B) in subject 8 representing the IR group with an intravenous input and (C) in subject 1 of the IR group with an oral input of deuteroleucine. The small squares denote the apoB100 for $VLDL_1$; the circles denote $VLDL_2$; the triangles, IDL; and the diamonds, LDL. The *s* numbers in the upper right-hand corner denote the density class in which apoB has been sampled by the SAAMII program, as indicated in the legend to Figure 2. The average coefficient of variation (defined as the mean divided by the SD) for the rate constants used for the calculation of the FCRs (for example, *k* (0,8) and *k* (9,8) for compartment 8, as shown in Figure 1) was not significantly different between the IR and IS groups. For leucine leaving compartment 8 the coefficient of variation averaged 1.1 ± 0.09; for compartment 9, 0.80 ± 0.50; for compartment 10, 0.86 ± 0.52; and for compartment 11, 0.51 ± 0.69. It should be noted that although isotopic enrichment values were obtained at 2, 4, 7, and 12 days, they are not depicted in the figure(s) to avoid compression of the early portions of the curves. In the SAAMII program, each tracer/tracee ratio value was weighted according to the extent of enrichment. This was done from statistical data obtained from many determinations of the standard curves included in every run on the mass spectrometer. Consequently, the low values at the longer time points did not exert an undue influence on the calculated FCRs of apoB for the lipoprotein fractions with relatively short residence times.

humans (19, 20, 21, 22). Although apoB is required for VLDL assembly, an increased output of VLDL-TG does not necessarily result in an increased apoB100 output since larger particles may be formed. Insulin is known to decrease production of apoB100 in acute experiments with hepatocytes (23). Lewis *et al.* (24) have shown that acute hyperinsulinemia inhibits VLDL apoB and triglyceride production in humans. It might be expected that in IR subjects, there would be greater release of fatty acids from adipose triglyceride stores and that this would result in greater production of VLDL apoB. However, we were unable to demonstrate an effect of IR on apoB100 PR, which is consistent with studies performed in primary human hepatocytes (25) in which addition of oleic acid did not increase apoB production. In our IR subjects, there was a significantly decreased FCR of apoB100 in all triglyceride-rich lipoprotein fractions and good correlations with the sum of insulins (Table III).

How can the decreased FCR of apoB100 in the triglyceride-rich lipoproteins be explained? First, receptors such as the LDL or VLDL receptors may be decreased, but there is no evidence at present to support this possibility. Second,

Table III. ApoB100 Kinetic Parameters

Subject	Body Wt. (kg)	V1 PR (mg/kg/day)	V2 PR (mg/kg/day)	IDL PR (mg/kg/day)	LDL PR (mg/kg/day)	V1 FCR (Pools/day)	V2 FCR (Pools/day)	IDL FCR (Pools/day)	LDL FCR (Pools/day)
IR									
1	91.8	6.8	4.3	33.5	10.2	15.9	8.3	3.2	0.322
2	99.6	7.4	17.1	29.3	12.4	10.1	9.2	4.5	0.578
6	87.5	5.2	57.8	67.3	17.0	22.0	26.5	8.9	0.557
8	102.6	32.9	77.6	91.1	17.2	44.8	28.1	8.5	1.080
9	96.0	10.0	21.0	65.3	17.2	10.4	10.3	6.1	0.516
Mean	95.5	12.5	35.6	57.3	14.8	20.6	16.5	6.2	0.611
SD	6.02	11.6	30.8	25.8	3.29	14.4	9.92	2.47	0.281
IS									
3	91.5	13.4	37.9	40.1	13.4	31.9	50.4	11.0	0.689
5	66.7	5.2	32.6	61.7	8.4	24.9	25.2	13.2	0.559
7	81.5	11.2	29.0	38.4	13.4	77.5	85.8	10.2	0.626
10	73.8	21.3	118.0	109.0	16.5	103.0	84.5	18.0	0.842
11	70.0	69.5	71.9	86.6	26.6	107.0	38.3	13.2	0.854
Mean	76.7	24.1	57.9	67.2	15.7	68.9	56.8	13.1	0.714
SD	9.54	26.0	37.7	30.5	6.77	38.7	27.3	3.04	0.131
P, IR/IS	0.006	0.39	0.34	0.59	0.8	0.031	0.015	0.004	0.479
Correlation with insulins									
ρ	0.89	−0.07	−0.39	−0.2	0.28	−0.65	−0.61	−0.85	−0.27
P	0.001	0.87	0.26	0.58	0.43	0.049	0.067	0.004	0.45

the apoE content of the TG-rich lipoproteins may be low, resulting in less hepatic receptor binding, but we did not find significant differences in the lipoprotein apoE content in the IR compared to the IS subjects (data not shown). Third, it might be related to decreased lipase activity. We favor this hypothesis because Knudsen *et al.* (26) in a study of nondiabetic first degree relatives of individuals with Type 2 diabetes, found that the insulin-resistant subjects had less postheparin plasma lipoprotein lipase activity. Recently, Vega *et al.* (27) reported that African American males aged 20–40 years had 39% less postheparin hepatic lipase activity than a comparable white population. Thus, the stage may be set in African American males for decreased lipase activity associated with insulin resistance.

It is not possible at present to determine whether obesity or insulin resistance itself was responsible for the alterations of apoB100 metabolism we observed. Cummings *et al.* (28), using a stable isotope technique, compared VLDL apoB100 production in six obese subjects (three males and three females) with six controls and found significant increases in PR, expressed per kg of fat-free mass, in the obese subjects, studied under fasting conditions. However, they also found a 50% decrease in the FCR with $P = 0.06$. No significant correlations of either FCR or PR with BMI emerge from their data. The trend, however, was clearly in the direction of an association of obesity with both a decreased FCR and an increased PR. In the present study, we observed a significant decrease in FCR but no increase in PR in our insulin-resistant subjects who were also obese. In comparing our results with those of the British investigators (28), it should be noted that their obese subjects were not shown to be insulin-resistant, and they had an average BMI of 37.6, higher than the value of 30 for the five IR subjects in the present study. In addition, the population studied differed markedly from that of the present study, since half of the subjects were female, and the racial composition of the participants was not stated. Further investigation appears warranted to determine whether or not increased VLDL apoB100 production in insulin-resistant subjects, both obese and nonobese, is demonstrable in a Caucasian population. The present study suggests, however, that decreased clearance is more likely to account for elevated plasma levels of apoB100 in VLDL and IDL in the fasting state in African American males.

Packard and Shepherd (7) have shown that $VLDL_2$, rather than the larger $VLDL_1$ fraction, is a more significant precursor of the apoB of LDL and that the two VLDL fractions may be regulated independently of one another. In the present kinetic study, on average most of the $VLDL_1$ apoB was transferred to $VLDL_2$ but the individual variations were too great to permit conclusions to be drawn.

In assessing the validity of our kinetic results, it should be noted that subjects 1 and 2 received the deuteroleucine tracer by the oral route. We believe the inclusion of these subjects can be justified since, as explained in the footnote,[3] no overall trend or significant difference in kinetic parameters was observed in a separate experiment with five subjects in which labeled leucine was administered simultaneously by both oral and intravenous routes. Also, when the results for subjects 1 and 2 are excluded from the analysis, there is an even higher negative correlation between the sum of insulins and the FCR for IDL ($\rho = -0.91$). Because of the small number of subjects studied, and also because the antecedent diet was not controlled (though the studies were carried out in the fasting state), caution is warranted in extrapolating these results to the general population of African American males.

In summary, we have observed that insulin resistance in a group of African American males is associated with elevated levels of apoB100 and TG-rich lipoproteins. These elevations were mainly due to a decrease in FCR in the fasting state. Further studies are needed to determine lipoprotein and hepatic lipase activity to clarify the effect of these enzymes on the lipolysis of nascent lipoproteins. The relationship between the FCR of apoB100 and obesity, especially under nonfasting conditions, needs further investigation in the African American population. There is also a need to determine whether IR African Americans differ in response when compared to a Caucasian population.

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[3] In a separate series of experiments, we attempted to determine whether or not the oral route of isotope administration would yield the same kinetics for apoB100 as the intravenous route. We administered a 60 mmole/kg dose of D_3-deuteroleucine intravenously and the same dose of $^{15}N^{13}C$-leucine orally at the same time in five subjects. The electron impact fragmentation pattern of the tertiary butyl dimethysilyl (TBDMS) derivative of leucine enabled us to determine the isotopic enrichment of each species of leucine in the presence of the other. We then compared the results for VLDL, IDL, and LDL apoB. In two of five subjects studied in this manner, there was good agreement of the calculated FCRs, and in three subjects the agreement was poor. However, in 33 total determinations using two different multicompartmental models, the average difference in FCRs in all lipoprotein fractions between the two modes of isotope administration was $-6.4\% \pm 43\%$, and no consistent positive or negative trend was discernible. The large error indicates that with the above experimental design, we have been unable to prove or disprove the equivalence of the two methods.

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