Effects of insulin on plasma lipoproteins in diabetic ketoacidosis: evidence for a change in high density lipoprotein composition during treatment

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Abstract To determine the acute effects of insulin on lipoprotein metabolism, we have followed the plasma lipoprotein lipid and apolipoprotein levels during insulin therapy for the first 24 hr in 13 patients with diabetic ketoacidosis. Corrections were made for plasma volume changes during treatment. Before insulin treatment, mean plasma triglyceride and cholesterol levels were 574 mg/dl (range 53–2355) and 212 mg/dl (range 118–416), respectively. Insulin therapy resulted in rapid decreases in triglyceride-rich lipoproteins, chylomicrons, and very low density lipoproteins (VLDL), with most patients achieving plasma triglyceride levels below 150 mg/dl at 24 hr. Mean basal levels of intermediate density lipoproteins (IDL) and low density lipoproteins (LDL)-cholesterol were low (9.9 and 72 mg/dl, respectively) and were statistically invariant with therapy. Mean basal levels of high density lipoprotein (HDL) cholesterol were also low (26 mg/dl, range 5–48) and were invariant during the first 12 hr and increased significantly to 29 mg/dl by the 24th hr. Plasma apoprotein (apo) B levels were in the upper normal range (101 mg/dl) before treatment and decreased with therapy due to significant decreases in VLDL, but not IDL or LDL apoB. VLDL appeared to have a normal apoprotein composition which did not change with treatment. Mean apoA-I levels which were near normal in plasma and HDL before therapy, decreased significantly (16%) by 12 hr and subsequently increased towards basal levels between 12 and 24 hr. The ratio of apoA-I to cholesterol in HDL also fell significantly during the entire 24 hr. Density gradient ultracentrifugal analysis of the d > 1.006 g/ml fractions indicated a selective decrease in "lighter" density fractions of HDL-apoA-I during treatment. These results provide evidence that insulin may decrease the secretion of apoA-I into plasma or increase catabolism.—**Weidman, S. W., J. B. Ragland, J. N. Fisher, Jr., A. E. Kitabchi, and S. M. Sabesin.** Effects of insulin on plasma lipoproteins in diabetic ketoacidosis: evidence for a change in high density lipoprotein composition composition during treatment. *J. Lipid Res.* 1982. **23:** 171–182.

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Diabetics and others with abnormal glucose tolerance have a higher prevalence of vascular disease of all kinds than do non-diabetics (1–6). There is also considerable evidence that hypertriglyceridemia and hypercholesterolemia are among the risk factors for atherosclerosis in these patients (1, 7–9). Several mechanisms have been suggested whereby the insulin deficiency of the Type I diabetic (10) may induce derangements in lipoprotein metabolism: *a*) decreased activity of lipoprotein lipase impairs the clearance of TG-rich lipoproteins (CM and VLDL) and *b*) increased lipolysis in the adipocyte increases the supply of free fatty acids to the liver, enhancing both ketone body production and hepatic TG synthesis with resultant fatty liver and hypertriglyceridemia. On the other hand, the hyperinsulinemia of Type II diabetes may stimulate hepatic synthesis and secretion of TG and CH-containing lipoproteins. These effects of insulin deficiency, or excess, provide an explanation for the frequent observations of elevated levels of VLDL and the associated low levels of HDL in untreated or poorly-controlled diabetes (7, 11–20).

Diabetic ketoacidosis is a condition of severe insulin deficiency which provides an opportunity to study alterations in plasma lipoprotein composition and metabolism in response to insulin therapy. Although studies of lipoprotein composition in diabetes have been reported, little is known of the short-term effects of insulin per se on lipoprotein metabolism and lipoprotein composition. We have, therefore, undertaken a detailed analytical study of the plasma lipoproteins in 14 patients with di-

Abbreviations: apo, apolipoprotein; CH, cholesterol; TG, triglyceride; CM, chylomicrons; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; HDL_2, high density lipoprotein isolated in the density range d 1.063–1.125 g/ml; HDL_3, high density lipoprotein isolated in the density range d 1.125–1.21 g/ml; DKA, diabetic ketoacidosis; DTNB, dithiobisnitrobenzoic acid; LCAT, lecithin:cholesterol acyltransferase (EC 2.3.1.43); EDTA, ethylenediaminetetraacetic acid; SEM, standard error of the mean; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; LPL, lipoprotein lipase; i.v., intravenous; i.m., intramuscular.

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abetic ketoacidosis treated with a low-dose insulin protocol for 24 hr. Before insulin and re-hydration therapy, most of the patients were hypertriglyceridemic. Insulin therapy resulted in marked decreases in the TG-rich lipoproteins, CM and VLDL. We also observed significant decreases in plasma and HDL-apoA-I during the first 24 hr of insulin treatment. These observations provide evidence for a previously unrecognized role of insulin in the short-term regulation of lipoprotein metabolism of the diabetic.

METHODS

Patients and protocols

Fourteen Type I diabetic patients with DKA (eight female, six male, ages 16–50 yr) were studied in the Clinical Research Center of the University of Tennessee Center for the Health Sciences. **Table 1** contains the clinical laboratory data obtained on admission and the total volume of i.v. fluids administered during the study. DKA was defined by *a*) arterial pH < 7.30, *b*) serum bicarbonate < 15 mEq/l, *c*) plasma glucose > 300 mg/dl, and *d*) serum acetone-positive at greater than 1:2 dilution (21). Criteria for acceptance in the study were: the above-mentioned clinical profile and informed consent by the patient. There was no attempt to select patients on the basis of their plasma lipid parameters.

The 14 subjects were studied in detail at complete bedrest and with no food intake by mouth during the first 24 hr following initiation of insulin therapy. The patients were treated with the low dose insulin protocol described by Sacks et al. (22). The initial dose of insulin was 0.44 U/kg body weight, half by i.v. bolus and half by i.m. injection, into the deltoid muscle. When plasma glucose had decreased to 250 mg/dl, 5% dextrose was added to the saline infusion. At this time, if the ketoacidosis was still uncontrolled (control being defined as an arterial pH equal to or greater than 7.30, bicarbonate concentration greater than 15 mEq/L, and plasma acetone-negative at 1:2 dilution), regular insulin was given i.m. every 2 hr according to a "sliding scale" based on the degree of hyperglycemia. The following schedule was employed: plasma glucose greater than 300 mg/dl, 12 units: 250–300 mg/dl, 8 units; 201–250 mg/dl, 6 units; 150–200 mg/dl, 4 units (23, 24). no insulin was given if plasma glucose was less than 150 mg/dl even in the presence of glycosuria. As soon as DKA was brought under control and the patient could take food by mouth, but *not* before 24 hr after admission, maintenance insulin therapy was started.

Collection of samples and sample handling

Thirteen of the patients were sampled at 0, 6, 12, and 24 hr. Blood samples for lipids and apoprotein analyses were not obtained for patient No. 2 at 6 and 12 hr; therefore, this patient was not included in the analyses of changes in lipids and apoproteins that occur over the first 24 hr of insulin therapy. The patient was included in the group of patients studied at 24, 48, and 72 hr after initiation of the study protocol.

All blood samples for lipoprotein studies were collected in EDTA (1.5 mg/ml) tubes to which DTNB (1 mM) was added to inhibit LCAT activity. The plasma lipoproteins were separated at 17°C as follows: *a*) CM was isolated and washed by ultracentrifugation in the Beckman SW 50.1 rotor for 10^6 g-min at d 1.006 g/ml; *b*) VLDL was recovered from a 17-hr spin in the SW 50.1 rotor (2×10^8 g-min) at d 1.006 g/ml; and *c*) the d > 1.006 g/ml fraction (IDL + LDL + HDL) was separated in a 10-ml exponential salt plus sucrose gradient

TABLE 1. Clinical profiles of DKA patients at admission

Patient No.	Age	Sex	Duration of Diabetes	Previous Diabetic Treatment	Serum Glucose	HCO_3	pH	Plasma Triglycerides	Plasma Cholesterol	Total 24 hr I.V. fluids	Weight
					mg/dl	*meq/l*		*mg/dl*	*mg/dl*	*liters*	*Kg*
1	49	F	New	None	349	11.7	7.26	2800	495	6.77	87.7
2	50	F	New	None	840	9	7.22	308	238	9.65	73.4
3	35	M	4 yr	NPH-4OU; Reg-2OU	346	3	7.06	1921	255	6.12	73.3
4	45	M	16 yr	NPH-7OU	630	1.2	7.00	287	216	7.61	69.7
5	31	M	New	None	980	11.7	7.28	1542	452	11.10	108.6
6	26	M	10 yr	NPH-55U	334	6.6	7.2	63	166	4.18	73.9
7	29	F	New	None	338	11	7.28	139	237	2.72	87.4
8	35	F	3 yr	NPH-35U; Reg-4OU	325	8	7.21	369	245	4.06	68.6
9	16	M	1 yr	NPH-5OU; Reg-3OU	306	8.6	7.21	781	296	8.96	67.7
10	18	F	New	None	369	9.7	7.24	257	186	7.10	37.6
11	18	F	1 yr	NPH-25U; Reg-1OU	334	10.2	7.11	194	232	4.42	49.6
12	23	F	19 yr	NPH-3OU; Reg-2OU	590	2.5	6.97	210	232	5.12	50.4
13	23	M	15 yr	NPH-3OU	475	2.6	6.97	611	304	7.50	47.3
14	25	F	12 yr	NPH-4OU	545	5.7	7.05	309	195	5.78	46.2

extending from d 1.01 to d 1.21 g/ml. Two ml of the plasma remaining after VLDL isolation was adjusted to density 1.2406 g/ml with 762 mg of sucrose and 391 mg of NaBr and overlaid with the gradient using an Isco model 570 gradient former and a Buchler Densiflow. The solutions mixed to form the gradient were: heavy solution—2.133 m NaBr plus 32.38 g/100 ml sucrose (final density = 1.2295 g/ml) and 0.1 g (per liter) EDTA-Na_2; and light solution—0.195 m NaCl plus 0.1 g/liter EDTA-Na_2. After spinning at 40,000 rpm for 24 hr in a Beckman SW 41 rotor, the tube was punctured and 20 separate 0.6-ml fractions were collected from the top by pumping 7.95 m NaBr (d 1.49 g/ml) in the bottom of the tube using an Isco Model 640 gradient fractionator. Plasma proteins were located in the fraction 15–20 of the gradient (d 1.169–1.403 g/ml). Blank gradient fraction densities were measured with a Newtec (Newtec Inc., Birmingham, AL) high precision digital densimeter. TG, CH (total and unesterified), apoB, and apoA-I were determined in whole plasma, CM, VLDL, and the d > 1.006 g/ml lipoprotein fraction. All density gradient fractions were analyzed for total and unesterified CH. Fractions No. 1–9 (top of the gradient) were analyzed for apoB and fractions No. 10–20 were analyzed for apoA-I.

HDL-CH was determined by heparin-Mn^{2+} precipitation of apoB-containing lipoproteins from plasma (25). A turbid supernate from a hypertriglyceridemic sample was cleared by centrifugation at 12,000 *g* for 10 min.

Analytical techniques

CH and CH esters were determined by gas–liquid chromatographic analysis of extracts of saponified and non-saponified samples containing a known amount of β-sitosterol as an internal reference (26). TG was determined by the colorimetric method of Biggs, Erickson, and Morrehead (27). Phospholipid phosphorus was determined in the fractions obtained from the density gradient fractionation of the d > 1.006 g/ml lipoproteins from a normolipidemic subject (**Fig. 1**). The samples were extracted by sequential addition of 50 volumes of methanol and 50 volumes of chloroform. Extracts were dried under nitrogen, digested with 70% perchloric acid and phosphorus determined by the method of Rouser, Fleischer, and Yamamoto (28). Phosphorus values were converted to phospholipid values using a factor of 25.

Apolipoprotein A-I and B were determined in undelipidated samples by the electroimmunoassay (rocket) method of Laurell (29) using antibodies prepared in rabbits (30, 31). The apoB assay was linear over the range of 1/200 to 1/50 dilution of the reference plasma (standardized with LDL) in 0.024 M barbital buffer (pH 8.6, Bio Rad Laboratories, Richmond, CA) or 28–280 ng

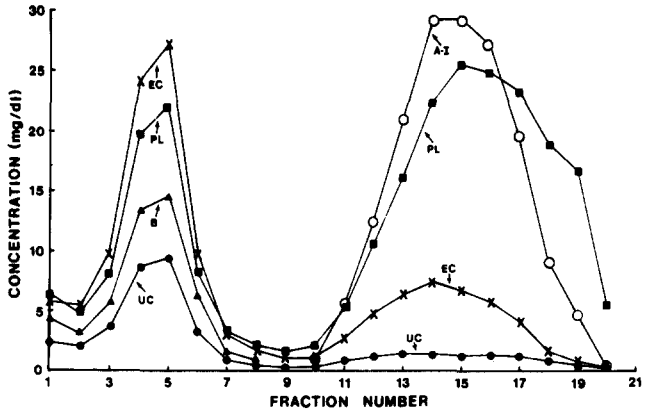

Fig. 1. Distribution of esterified CH (EC), phospholipids (PL), apoB, unesterified CH (UC), and apoA-I in the d > 1.006 g/ml fraction from a normolipidemic subject. Concentrations are corrected to that in plasma by volume measurements. Concentrations of EC, PL, apoB, UC, and apoA-I in the d > 1.006 g/ml fraction (corrected to that in plasma) were 134, 325, 75, 46, and 170 mg/dl, respectively.

applied to the plate, while the apoA-I assay was linear over the range of 1/200 to 1/30 dilution of the reference plasma (standardized with pure apoA-I) in the same buffer or 32–325 ng applied. The range of apoA-I and apoB concentrations obtained for normolipidemic subjects in our laboratory are 130 to 190 mg/dl and 75 to 110 mg/dl, respectively. Samples of whole plasma and sequential ultracentrifugal lipoprotein fractions from all times for a given patient were always run on the same immunoassay plate.

We assayed apoA-I in the plasma CM fraction, VLDL in the d > 1.006 g/ml fraction, and density gradient fractions numbers 9–20 of six additional diabetic patients with plasma TG ranging from 118–429 mg/dl by the method described above and by the method of Miller et al. (32) which employs dilution of the samples in 9 M urea. These authors have shown that dilution in 9 M urea yields apoA-I values equivalent to those obtained by delipidation with tetramethylurea. Although the mean values of apoA-I were somewhat higher for the method using dilution in 9 M urea, we found no statistical difference either by the unpaired or paired *t* test between the two methods. There was also no difference between the total amount of apoA-I recovered in the gradient as determined by two methods.

The recoveries of total CH, TG, apoB, and apoA-I from the ultracentrigfugal separation of CM, VLDL, and the d > 1.006 g/ml fraction were 93, 93, 98, and 90%, respectively. Recoveries of total CH, apoB and apoA-I from the density gradient were 89, 64, and 94%, respectively. The reason for the lower recovery of apoB from the gradient samples is not presently known. Sequential ultracentrifugal preparation of IDL (d 1.006 to 1.019 g/ml), LDL (d 1.019 to 1.063 g/ml), HDL (d 1.063 to 1.21 g/ml), HDL_2 (d 1.063 to 1.125 g/ml),

and HDL_3 (d 1.125 to 1.21 g/ml), and subsequent density gradient fractionation revealed that these lipoproteins floated in fractions No. 1, 2–8, 9–19, 9–13, and 14–20, respectively. However, since HDL_3/HDL_2 ratios are frequently > 5:1, and the technique does not resolve a distinct shoulder or peak in the HDL_2 density region except in subjects with high HDL_2 levels, fractions 9–13 in the gradient would be contaminated with HDL_3 (33). Therefore, we have denoted the summation of total CH or apoA-I in fractions 9–13 and 14–20 as "light" and "heavy" HDL, respectively. The summation of total CH in fractions 9–19 in the gradient was highly correlated with HDL-CH by the heparin-Mn^{2+} precipitation technique ($r = 0.929$, $P < .001$, n = 62), and the average agreement between HDL-CH as determined by the two methods was 101%.

A unique feature of the density gradient employed for separation of lipoproteins in the d > 1.006 g/ml fraction is that it includes a large amount of sucrose mixed with NaBr in order to decrease ionic strength and hopefully decrease dissociation of apolipoproteins during centrifugation. The density gradient profile of the d > 1.006 g/ml fraction from a normolipidemic subject is shown in Fig. 1, where the concentration (corrected to plasma) of unesterified and esterified CH, phospholipid, apoB, and apoA-I are plotted for each gradient fraction. Fractions 2–8 are seen to contain apoB and lipids, and represent the distribution of LDL in the density gradient, while fractions 9–20 contain apoA-I and lipids and represent HDL.

IDL and LDL-CH were calculated from the gradient analyses as follows: *1*) the ratio of IDL-CH/(IDL + LDL)-CH was calculated using analyses from the appropriate gradient fractions; *2*) this ratio was then multiplied by the d > 1.006 g/ml plasma fraction-CH minus HDL-CH (heparin-Mn^{2+}) value, yielding IDL-CH; and *3*) LDL-CH was obtained by subtracting IDL-CH from the d > 1.006 g/ml plasma fraction minus HDL-CH (heparin-Mn^{2+}). A similar procedure was used for IDL-apoB and LDL-apoB calculation. This procedure normalizes the results to the d > 1.006 g/ml value minus HDL-CH and assumes slight losses or over-recoveries are equal for all fractions in the density gradient.

"Light" and "heavy" HDL-CH were determined similarly: *1*) the ratio of "light" HDL/total HDL was calculated using the analyses of the appropriate density gradient fractions; *2*) this ratio was then multiplied by HDL-CH as determined by the heparin-Mn^{2+} technique, yielding "light" HDL-CH; and *3*) "heavy" HDL-CH was obtained by difference. "Light" and "heavy" HDL-apoA-I were determined similarly.

Plasma glucose was measured by the glucose oxidase technique and the Beckman Glucose Autoanalyzer (Beckman Instruments, Inc., Palo Alto, CA).

Proteins were determined by the technique of Markwell et al. (34) using crystalline bovine albumin standards. VLDL apoB was determined by the isopropanol precipitation technique of Holmquist, Carlson, and Carlson (35). Other VLDL apoproteins were analyzed by two electrophoretic techniques: *1*) SDS-PAGE by the method of Laemmli (36), employing 10–20% gradient gels stained with 0.025% Coomassie Brilliant Blue R-250 and destained in the solutions of Weber and Osborn (37), and *2*) isoelectric focusing on polyacrylamide gels by the method of Catapano et al. (38), except that gel staining was with Coomassie Brilliant Blue G-250 (39). All gels were scanned with an Isco Model 1310 gel scanner attachment to the model UA-5 absorbance monitor. The first method allowed calculation of the apo E/apo C dye uptake ratio and the latter method allowed quantitation of relative dye uptake of apoC-II and apoC-III subspecies.

Plasma volume change correlations

Table 1 lists the total volume of i.v. fluids administered to each patient during the study. The average volume given was 6.5 liters. This treatment aong with blood sampling necessitated a correction of the measured plasma lipid and apoprotein parameters to compensate for plasma expansion during the study. These corrections were made on the basis of hemoglobin determinations at 0, 6, 12, and 24 hr. Hemoglobin was determined as part of a procedure developed by Trivelli (40) for hemoglobin $A1_c$ determination. The reference time was chosen as the 24-hr time point, since the patients should have been almost fully hydrated at this point. Thus, each plasma lipid or apoprotein measurement was divided by the factor: hemoglobin concentration at time "t" divided by the hemoglobin concentration at 24 hr. In this manner, data at each time point were corrected to the more fully hydrated state at 24 hr. The average factors were 1.30, 1.17, and 1.10 at 0, 6, and 12 hr, respectively.

Statistical Methods

Tests for overall differences over time (0, 6, 12, 24 hr) were made by analysis of variance. When such analysis indicated significant variation across time, differences between means were analyzed using the paired Student's *t* test (41).

RESULTS

Effects of insulin therapy on lipoprotein lipid levels

Before insulin treatment, plasma TG levels in DKA ranged from 63–2800 mg/dl (mean ± SEM = 729 ± 233,

uncorrected for plasma volume change). When these values were corrected to the 24-hr state of plasma dilution the mean was 574 ± 188 (range 53–2355 mg/dl). (In the following sections all lipid and apoprotein values given will be corrected for plasma volume changes). Two of the patients (Nos. 1 and 3) had severe chylomicronemia (>700 mg/dl CM-TG) along with elevations of VLDL-TG (>400 mg/dl). The remainder of the patients had low levels of CM-TG and normal to high levels of VLDL-TG (20–1020 mg/dl).

All patients (except No. 6) demonstrated dramatic decreases in plasma TG levels by the 24th hr or insulin therapy (**Fig. 2**). Chylomicron TG levels generally decreased with insulin treatment, reaching negligible levels by 24 hr in all subjects except Nos. 1 and 3 (**Fig. 3A**). The mean CM-TG increased after 6 hr of treatment due to increases in CM levels in three patients (No. 3, 10, and 12). That the TG-rich particles were mostly CM and not $S_f > 400$ VLDL was indicated by their low CH/TG ratio (<0.15) and the observation that they contained apoA-I (Table 2).

Significant decreases in mean VLDL-TG were obtained at 6, 12, and 24 hr. The mean TG concentration in the $d > 1.006$ g/ml plasma fraction was invariant during insulin therapy. Since TG levels were not determined in density gradient fractions, data for TG in IDL and LDL were not available.

Mean plasma CH levels were in the normal range before therapy (**Fig. 4**) with only patients No. 1, 5, 9, 10, 11, having CH levels > 200 mg/dl. During therapy mean plasma CH levels fell significantly at 12 and 24 hr due to decreases in CM- and VLDL-CH levels (Fig. 3B). There were no significant changes in either IDL or LDL-CH levels with therapy (Fig. 3C), although the mean LDL-CH level at 24 hr was elevated from that of 0-hr (72 to 80 mg/dl, from 0 to 24 hr). Basal mean HDL-CH levels were low (26 ± 3.8 mg/dl; range 5–48 mg/dl) and did not change significantly after 6 and 12 hr of treatment even though analysis of variance indicated a significant ($P < 0.001$) variation across time. However, there was a small but significant increase in HDL-CH after 24 hr of treatment (26 to 29 mg/dl, from 0 to 24 hr, $P < 0.05$).

Combining the VLDL-CH and TG data allowed calculation of VLDL CH/TG ratios. The mean ratio before treatment was 0.28 ± 0.15 and did not change significantly during insulin treatment (0.28, 0.31, 0.31, and 0.24 at 0, 6, 12, and 24 hr, respectively).

ApoA-I levels during insulin treatment

Mean plasma and HDL-apoA-I levels decreased progressively throughout the first 12 hr of the study (**Table 2**). The changes in apoA-I levels were small (16% decrease at 12 hr) but were statistically significant: apoA-I levels were lowest for the more hypertriglyceridemic subjects. Between the 12th and 24th hr of therapy the mean levels of plasma and HDL-apo-A-I rose slightly, but the 24-hr means were still lower than the mean basal values. ApoA-I was detected in the CM fraction, but the levels were low (mean CM-apoA-I < 1.1 mg/dl) and did not change significantly during the 24-hr period. No VLDL-apoA-I could be detected by electroimmunoassay.

When these data are combined with the CH data, it was revealed that the ratio of HDL-apoA-I to HDL-CH (determined by heparin-Mn^{2+} precipitation technique) also decreased with therapy (Table 2). The ratios were highest for the hypertriglyceridemic patients. The mean ratio decreased progressively throughout the 24-hr

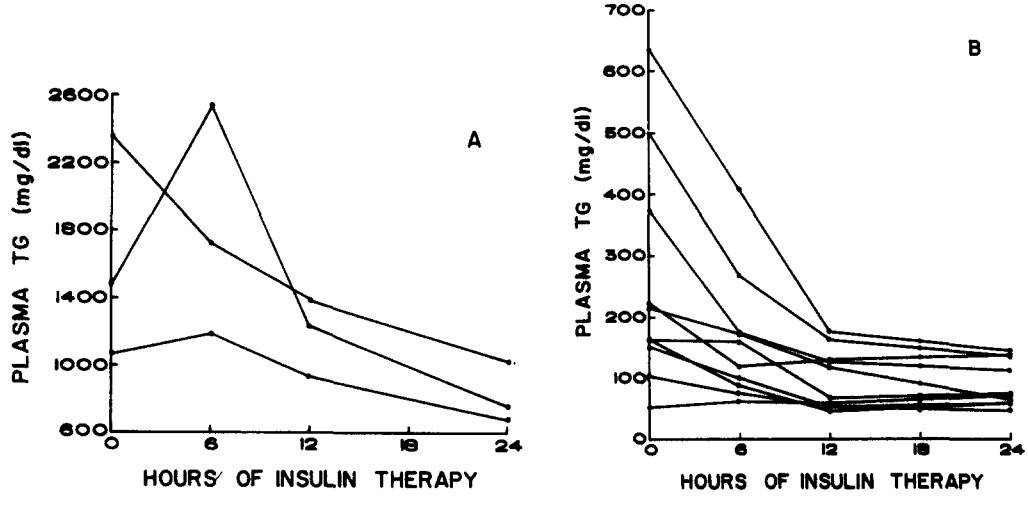

Fig. 2. Changes in plasma TG for each patient with hours of insulin therapy. A, the three most triglyceridemic patients (Nos. 1, 3 and 5); B, remainder of the patients.

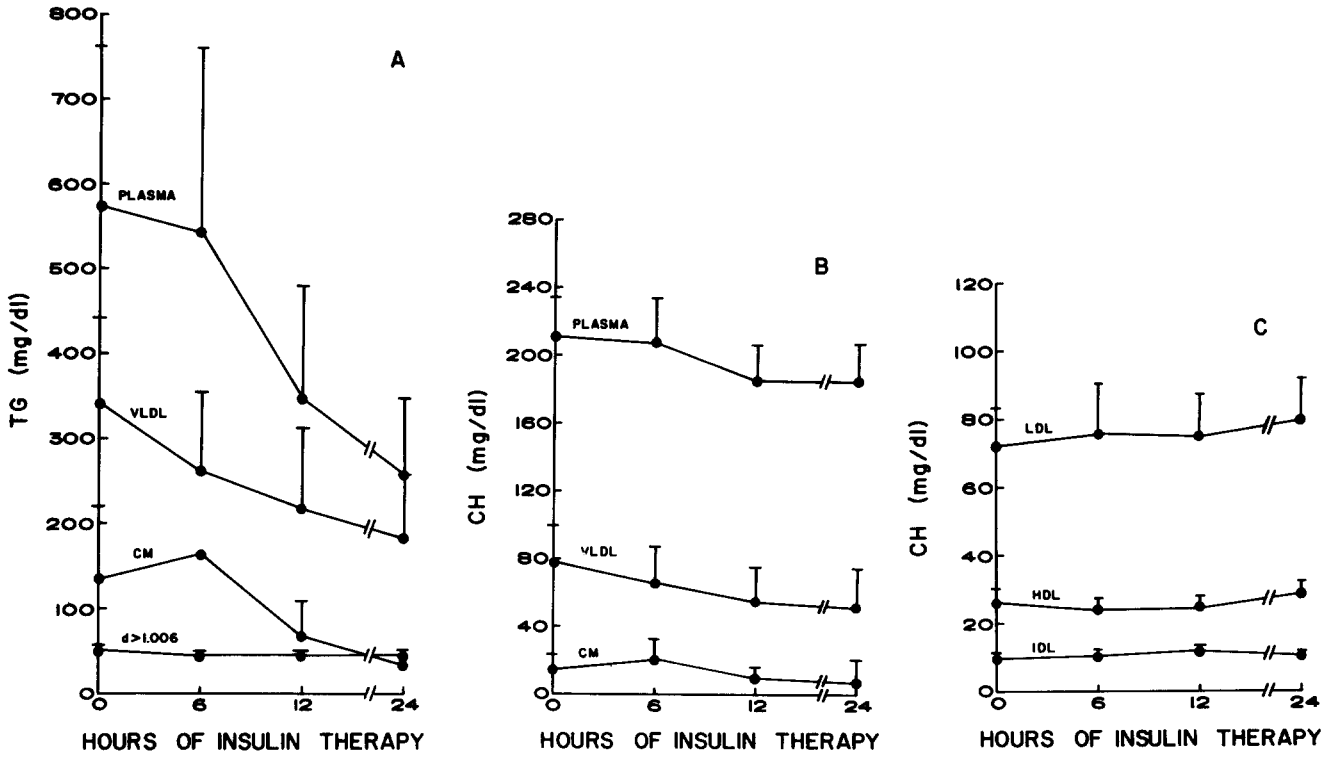

Fig. 3 Mean plasma and lipoprotein TG or CH levels as a function of hours of insulin therapy: A, triglyceride (TG); B, cholesterol (CH); C, cholesterol. Bars indicate 1 SEM. Bars were left off at two points (6-hr CM-SEM = 115 and 24-hr CM-SEM = 18) due to overlap with the curve above. In part C-IDL, the SEM is contained in the circle diameters at 0 and 24 hr. Analysis of variance indicated significant ($P < 0.01$) effects across time for plasma and VLDL-TG and plasma, VLDL, and HDL-CH. The following were statistically different from 0 hr: A) 12- and 24-hr plasma TG ($P < 0.01$) and 6-, 12-, and 24-hr VLDL-TG ($P < 0.01$); B), 12- and 24-hr plasma and VLDL-CH ($P < 0.01$) and 6-hr VLDL-CH ($P < 0.02$); C), 24-hr HDL-CH ($P < 0.05$).

period and was significantly different from basal at 12 and 24 hr.

HDL subspecies during insulin therapy

Summation of appropriate fractions obtained from the density gradient ultracentrifugal separation of IDL, LDL, and HDL (see Experimental Section for details) allowed calculation of "light" and "heavy" HDL-CH and apoA-I at each time point (**Table 3**). Mean "light" HDL-CH and apoA-I decreased by 16 and 32%, respectively, during the first half of the study followed by increases towards basal levels at 24 hr. The ratio of "light" HDL-apoA-I to CH did not change significantly. Mean "heavy" HDL-CH levels did not change significantly during treatment. Although the mean "heavy" HDL-apoA-I level decreased progressively during treatment, analysis of variance did not indicate a significant time trend. The "heavy" HDL-apoA-I to CH ratio decreased progressively during the study, reaching statistical significance by the 24th hr. More CH and apoA-I were found in "heavy" than in "light" HDL in all patients. The data support a selective decrease in the "light" HDL fraction during insulin treatment.

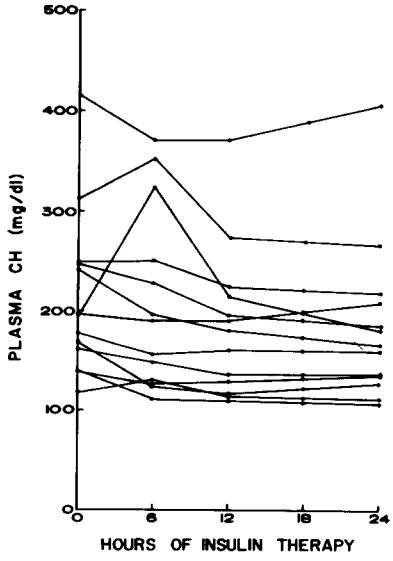

Fig. 4. Changes in plasma CH for each patient with hours of insulin therapy.

Changes in apoB levels in plasma and lipoprotein fractions

The mean basal plasma apoB concentration was in the upper range of normal for our laboratory and decreased significantly by 16% during the first 12 hr of treatment and did not change thereafter (**Table 4**). The apoB carried in the VLDL decreased significantly by 44%.

TABLE 2. Changes in plasma and lipoprotein apoA-I during insulin therapy of DKA patients

Time	ApoA-I Plasma[a,c]	ApoA-I CM	ApoA-I HDL[b,c]	HDL ApoA-I/CH
hr		*mg/dl*		
0	119 ± 11	1.1 ± 0.3	103 ± 12	4.96 ± 0.82
6	106 ± 12**	1.4 ± 0.4	92 ± 11	4.86 ± 0.77
12	100 ± 10****	0.9 ± 0.2	87 ± 9***	4.26 ± 0.62*
24	104 ± 8**	1.4 ± 0.3	91 ± 8	4.04 ± 0.65***

[a] Mean ± SEM, n = 13.
[b] ApoA-I concentration of the d > 1.006 g/ml fraction. Since IDL and LDL contain no apoA-I, this measurement can be taken as equivalent to the HDL-apoA-I concentration.
[c] Analysis of variance indicated a significant ($P < 0.01$) variation across time.
*, $P < 0.05$ vs 0-hr; **, $P < 0.02$ vs 0-hr; ***, $P < 0.01$ vs 0-hr; ****, $P < 0.001$ vs 0-hr.

The CM fraction represented a minor amount of the total apoB and did not change. The IDL- and LDL-apoB fractions also did not change. No significant changes for the apoB/CH ratio for either IDL or LDL as a function of treatment time were found (data not shown). The ratio of VLDL CH/apoB increased with treatment in two patients (Nos. 1 and 3) with severe chylomicronemia at 0 time (mean—4.58, 4.83, 5.27, and 6.11 at 0, 6, 12, and 24 hr, respectively). However, in the remaining patients, the mean VLDL CH/apoB ratio decreased with therapy (3.11, 2.56, 2.24, and 2.36 at 0, 6, 12, and 24 hr, respectively; N.S.). Normal VLDL in our laboratory has a CH/apo B ratio of 2.29 ± 0.12.

VLDL apoprotein composition during insulin therapy

Since it is possible that insulin-mediated catabolism of VLDL might involve a direct effect of insulin on VLDL apoproteins, we examined the apoproteins in VLDL by SDS electrophoresis and isoelectric focusing. The percent apoB in VLDL apoproteins was also determined. The mean value was 53 ± 3% at the start of treatment and did not change significantly during treatment, indicating a constant ratio of apoB and soluble apoprotein. Quantitation of the soluble apoprotein in VLDL by SDS electrophoresis revealed a mean apoE/apoC ratio of 0.40 ± 0.07 at 0-time which did not change significantly with insulin treatment. Similarly, determination of the C-II and C-III apoproteins by isoelectric focusing gave a mean ratio of apoC-II/apoCII + apoC-III at 0-time of 0.23 ± 0.04 which did not change significantly during therapy. ApoE isoforms were also examined in a few patients using a different pH gradient; there were no apparent changes in E isoform distribution during treatment. Thus, the dramatic changes in lipoprotein levels found are not associated with any apparent abnormalities in VLDL apoprotein composition.

Follow-up studies

Five of the subjects (Nos. 2, 5, 7, 8, 10) were also studied at 48 and 72 hr after the initiation of the study protocol (**Table 5**). It was assumed that the plasma volume of the patients was constant during this period. Mean plasma CH, TG, and LDL-apoB levels decreased during this period; but HDL-CH and apoA-I and LDL-CH increased slightly. None of the changes were statis-

TABLE 3. Changes in "light" and "heavy" HDL-CH, apoA-I and apoA-I/CH ratio with insulin therapy

Time	"Light" HDL[c] CH[a,b]	"Light" HDL[c] ApoA-I[a]	"Light" HDL[c] ApoA-I/CH	"Heavy" HDL[d] CH	"Heavy" HDL[d] ApoA-I	"Heavy" HDL[d] ApoA-I[a]/CH
hr	*mg/dl*	*mg/dl*		*mg/dl*	*mg/dl*	
0	13.2 ± 2.2	29.2 ± 5.4	2.45 ± 0.47	14.6 ± 2.3	75.0 ± 9.1	6.86 ± 1.48
6	11.1 ± 1.9***	21.4 ± 3.4*	2.42 ± 0.43	14.4 ± 2.0	72.3 ± 9.9	5.85 ± 0.88
12	11.4 ± 2.0***	19.9 ± 2.9	2.34 ± 0.54	14.4 ± 1.8	69.0 ± 7.8	5.38 ± 0.68
24	13.5 ± 2.4	24.6 ± 3.5	2.15 ± 0.33	16.3 ± 2.2	66.8 ± 6.9	4.97 ± 0.86**

[a] Analysis of variance indicated a significant ($P < 0.05$) variation across time. Differences between means were analyzed using the paired Student's *t* test.
[b] Mean ± SEM; n = 13.
[c] Fractions 9–13 (d 1.063–1.125 g/ml) in the density gradient analysis of d > 1.006 g/ml lipoproteins.
[d] Fractions 14–20 in the density gradient analysis of d > 1.006 g/ml lipoproteins.
*, $P < 0.05$ vs 0-hr; **, $P < 0.02$ vs 0-hr; ***, $P < 0.01$ vs 0-hr.

TABLE 4. Changes in plasma and lipoprotein apoB during insulin therapy of DKA patients

Time	ApoB				
	Plasma[a,b]	CM	VLDL[b]	IDL	LDL
hr			*mg/dl*		
0	101 ± 10	1.2 ± 0.4	28 ± 7	7.6 ± 0.9	56 ± 7
6	92 ± 11	1.6 ± 0.6	22 ± 6**	8.0 ± 1.0	53 ± 7
12	85 ± 7*	1.0 ± 0.3	17 ± 6**	8.7 ± 1.1	54 ± 7
24	84 ± 6*	1.5 ± 0.4	16 ± 6**	8.2 ± 1.2	58 ± 8

[a] Mean ± SEM; n = 13.
[b] Analysis of variance indicated a significant ($P < 0.05$) variation across time.
*, $P < 0.02$ vs 0-hr; **, $P < 0.01$ vs 0-hr.

tically significant. Thus, mean HDL-apoA-I levels which decreased significantly during the first 24 hr of insulin therapy, do not appear to increase rapidly during maintenance therapy.

DISCUSSION

To our knowledge this study is the first detailed characterization of plasma and lipoprotein lipids and apolipoproteins in DKA and of the sequential changes in these parameters during the first 24 hr of insulin therapy. Also, we believe this is the only such study where corrections have been made for plasma volume changes. Tuller et al. (42) in 1954 investigated plasma, VLDL subfractions, and LDL cholesterol levels before and during insulin treatment in 25 cases of DKA and reported that treatment led to correction of the plasma lipids toward normal levels. In order to assess the role of insulin availability on TG removal, Bagdade, Porte, and Bierman (43) investigated plasma TG levels and post-heparin lipolytic activity (PHLA) in insulin-dependent diabetics before and 48 hr after insulin withdrawal. Two of the seven subjects developed ketoacidosis during this period. TG was elevated and PHLA diminished after 48 hr of insulin withdrawal, suggesting impaired TG removal. The study did not include investigation of the effects of reinstitution of insulin. Other studies of lipid derangement parameters in diabetes have dealt only with the non-ketotic state (44–48).

The presenting lipid pattern of DKA probably reflects the rapidity of the onset of ketoacidosis and the recent food intake of the patient. The predominant lipid abnormality in the patients of the present study was that of hypertriglyceridemia due to increased levels of VLDL-TG with minor levels of chylomicron-TG. There was a wide range in basal plasma TG from normal to 2800 mg/dl (uncorrected). Severely hypertriglyceridemic patients (except patient No. 5) had massive concentrations of both chylomicrons and VLDL, resembling the classic "diabetic lipemia" pattern (49).

Insulin therapy resulted in rapid decreases in TG-rich lipoprotein levels in all patients with the exception of the normolipidemic subjects. We have observed similar rapid decreases in plasma lipids in 75 other unselected DKA patients who were not studied in detail.[2] Since it has previously been demonstrated that insulin regulates LPL activity, these results were not unexpected (50). However, they had not been previously demonstrated in DKA. It should be pointed out that correction of abnormalities in lipid metabolism, of DKA patients by in-

[2] Weidman, S. W., J. B. Ragland, and S. M. Sabesin. Unpublished results.

TABLE 5. Plasma and lipoprotein parameters in DKA patients after 24 hours

Time	Plasma TG[a]	Plasma CH	LDL CH	LDL ApoB	HDL[b] CH	HDL ApoA-1
hr			*mg/dl*			
24	208 ± 117	186 ± 26	96 ± 21	69 ± 14	27 ± 6	79 ± 10
48	239 ± 131	174 ± 27	89 ± 22	65 ± 13	27 ± 6	82 ± 12
72	169 ± 75	166 ± 22	100 ± 11	65 ± 12	29 ± 4	88 ± 9

[a] Mean ± SEM; n = 5.
[b] Determined by the heparin-Mn^{2+} precipitation technique.

sulin therapy may not be solely related to insulin treatment, since correction of the variables (hyperglucagonemia, hypercatecholaminemia, initial hypocaloric state) could also be involved in the changes seen (51).

Three patients (Nos. 3, 9, and 11) had an unusual response to insulin therapy. Their CM-TG levels increased during the first 6 hr of treatment. The explanation for this response is not apparent at this time. After 24 hr, all patients except Nos. 1, 3, and 5 had decreased plasma TG levels to <200 mg/dl. Triglycerides of patients Nos. 1 and 5 were still elevated after 72 hr of therapy (587 mg/dl, and 463 mg/dl, respectively). The slow response to therapy of these patients probably reflects their high basal TG levels, since patients No. 1 and 5 were not severely acidotic. It is also possible that patients Nos. 1, 3, and 5 had a genetic form of hyperlipidemia, accounting for their higher basal TG levels (52).

It has been postulated that the CH/apoB ratio decreases during the conversion of VLDL to LDL (53). We examined this ratio and found that it increased in the two patients with severe chylomicronemia, but decreased in all but one of the remaining patients. The increase in VLDL/CH apoB in the two patients with severe chylomicronemia may have been caused by contamination of VLDL with CM remnants. Thus the VLDL to LDL metabolic pathway "turned-on" by insulin treatment appeared to be normal in the patients and is consistent with our inability to demonstrate gross abnormalities in apoprotein composition of VLDL, i.e., apoB content, apoE/apoC ratio, and apoC-II/apoC-II + apoC-III ratio were all within normal limits.

The clearance of VLDL from the plasma might be expected to lead to an increase in IDL, since it has been shown that IDL is an intermediate in the conversion of VLDL to LDL in humans (54). However, IDL did not appear to change during treatment. Since the IDL density region might also be expected to contain CM remnants, the lack of change in IDL may be caused by offsetting changes, an increase due to VLDL remnants (IDL) and a decrease due to CM remnant (IDL) removal by the liver. An alternative pathway for VLDL catabolism, not involving IDL and LDL, cannot be ruled out. Evidence for binding and degradation of VLDL by peripheral cells involving a specific cell receptor for either the apoB or apoE moieties has been reported (55). This pathway involves the same receptor-specific pathway for LDL catabolism reported by Brown and Goldstein (56). Furthermore, Chait, Bierman, and Albers (57) have found that insulin, at physiological levels, stimulates degradation of VLDL and IDL by cultured human skin fibroblasts. Thus, it is conceivable that a considerable fraction of VLDL in the plasma of DKA patients during insulin therapy could be catabolized via this pathway and would not involve nor generate IDL. Another possibility is that a decrease in plasma free-fatty acids secondary to insulin therapy would result in decreased synthesis and secretion of VLDL.

There were statistically insignificant increases in both LDL-CH and apoB after 24 hr of insulin treatment. Therefore, the above mentioned arguments for IDL may apply to LDL as well. An additional effect of insulin in enhancing LDL receptor number, as demonstrated by Chait, Bierman, and Albers (58) for cultured fibroblasts, may have increased LDL catabolism during therapy as well.

It has been shown that, during VLDL lipolysis, surplus surface lipids and apolipoproteins are removed from the particle. Deckelbaum et al. (53) found that these surface materials could be isolated, at least in part, in HDL density range (1.063–1.21 g/ml). These authors speculated that complexes of phospholipid, cholesterol, and apolipoprotein originating from the surface of lipolyzed triglyceride-rich lipoproteins were HDL precursors, thus providing an explanation for the observed relationships of post-heparin lipoprotein lipase activity and HDL-CH in man (46). Tall (59) has demonstrated that phospholipid can combine with HDL_3 in human plasma, converting HDL_3 to HDL_2. The insulin treatment of DKA patients leads to clearance (lipolysis) or plasma triglyceride-rich lipoproteins and hence, might be expected to produce excess VLDL and CM surface material in the HDL density region. However, we observed very little change in HDL-CH during the first 12 hr of treatment and only a slight increase over basal at 24 hr. Furthermore, plasma and HDL-apoA-I decreased during the first 12 hr and the data indicated a selective decrease in "light" HDL-apoA-I. It is possible that "excess surface material" from VLDL and CM was catabolized rapidly; therefore, no accumulation could be observed. Other factors possibly involved could be a decrease in VLDL input by the liver and hypocaloric state of the patients.

The observed decrease in the ratio of HDL apoA-I to HDL-CH could have resulted from an impairment in the transfer of apoA-I from CM to HDL in the hyperlipidemic state (14). A selective transfer of CH in excess VLDL and CM surface material to HDL would cause the above ratio to decrease during insulin treatment.

The HDL-cholesterol in normals and Type I diabetics has been shown to be strongly and positively correlated with LPL activity of adipose tissue (60, 61). Type I diabetics in reasonable control actually have normal or slightly elevated HDL-cholesterol levels and adipose tis-

sue LPL activity (45). Previous workers (62, 63) have examined HDL-CH and adipose tissue LPL levels in insulin deficient (but non-acidotic) subjects and the response of these parameters to insulin therapy. Before therapy, these parameters were lower than control values. After insulin treatment, LPL activity and HDL-cholesterol increased significantly but were still subnormal after 2 weeks even though VLDL levels had returned to normal. Thus, the elevation to normal HDL and LPL levels following insulin treatment is a slower process than the decrease to normal VLDL levels. Our results with DKA patients followed for 72 hr are consistent with these findings.

The mechanisms whereby insulin could acutely lower HDL apoA-I in plasma are not clear at this time. Insulin could lower plasma HDL by decreasing secretion of HDL precursors or by enhancing HDL catabolism. The decreases observed in plasma and HDL-apoA-I during insulin treatment of DKA patients were small, but statistically significant. These results suggest that further research would be fruitful, particularly in the newly diagnosed Type I diabetic, where plasma volume changes and insulin resistance would be minimized, and in DKA patients where the effects or re-hydration on plasma lipoproteins could be investigated before initiation of insulin.

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