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Retarded chylomicron apolipoprotein-B catabolism in Type 2 (non-insulin-dependent) diabetic subjects with lipaemia

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Summary. To define the kinetics of chylomicron apolipoprotein-B catabolism in diabetic subjects with lipaemia, autologous chylomicrons (S_f 400) harvested from plasma following an oral fat load were radioiodinated and re-injected. The radioactivity in the tetramethylurea-insoluble, non-lipid $S_f > 400$ lipoprotein fraction was followed in serial samples over 60–72 h on a fat-free, isocaloric diet in: (1) five normal subjects; (2) four hypertriglyceridaemic, non-diabetic subjects; and (3) five diabetic patients (one subject, No. 3, was studied twice). The plasma apolipoprotein-B decay curve for the S_f 400 fraction disclosed biphasic disappearance: a rapid first phase (residence time 0.8–1.9 h) accounting for the large majority of removal (60%–95%) and a slower second phase (residence time 3.6–47.6 h), accounting for the remainder. Total chylomicron apolipoprotein-B residence times were similar in normolipidaemic (1.8–7.3 h) and hypertriglyceridaemic (2.3–10.3 h) non-diabetic subjects and the mildly hypertriglyceridaemic diabetic patients (5.6 and 5.8 h). In the untreated lipaemic diabetic subjects (Nos. 1 and 2), only a single, much slower phase was observed (total chylomicron apolipoprotein-B residence time 38.5–58 h). Adipose tissue biopsy in one of these subjects (No. 1) disclosed profoundly low lipoprotein lipase activity. The lipaemic diabetic subject (No. 3) studied early during treatment showed an intermediate pattern. These studies suggest a key role for insulin-dependent, lipoprotein lipase-mediated triglyceride hydrolysis in the removal of chylomicrons from plasma.

Key words: Chylomicrons, diabetes, apolipoprotein B, lipoproteins, hyperlipidaemia, lipoprotein turnover, chylomicron catabolism.

Studies of the pathophysiology of 'diabetic lipaemia' [1, 2] have demonstrated deficiencies in adipose tissue lipoprotein lipase [3] and in the hydrolysis of endogenous plasma triglyceride in the very low density lipoproteins (VLDL) [4]. It has been postulated that a similar abnormality exists in the removal of triglyceride in chylomicrons, and their accumulation results from competition between chylomicron and VLDL triglyceride in a common, saturable lipolytic system mediated by lipoprotein lipase [5], an insulin-dependent enzyme [6].

The kinetics of the catabolism of the major structural apolipoprotein (apo) in chylomicrons, apo-B, have heretofore remained almost totally unexplored in man, normal or otherwise [7]. Since chylomicron apo-B (B-48) is not present in any other plasma lipoproteins, this apolipoprotein is presumably retained on the chylomicron particles until its removal in toto from the plasma, unlike the smaller, tetramethylurea (TMU) -soluble apolipoproteins (e.g., C-I–III), which shuttle between chylomicrons and high density lipoproteins (HDL) with the flow and ebb of alimentary lipaemia [8]. This property, together with its insolubility in TMU, allowed the present assessment of the catabolism of chylomicron apo-B in subjects with lipaemia. For comparison, similar studies were also performed in diabetic subjects with only modest hypertriglyceridaemia (and no chylomicrons after an overnight fast), non-diabetic subjects with endogenous hypertriglyceridaemia (including subject No. 6 with fasting chylomicronaemia and hypertriglyceridaemia of a degree comparable to the lipaemic diabetic subjects), and non-diabetic subjects with normal triglyceride levels.

Subjects and methods

Subjects

Five normolipidaemic, non-diabetic subjects volunteered for these studies (Table 1). All enjoyed good health and a stable, varied diet, and none was taking medications known to affect carbohydrate or lipid metabolism.

Four hyperlipidaemic, non-diabetic subjects were selected from subjects referred to the Northwest Lipid Research Clinic. None had a

Table 1. Clinical and chylomicron apo-B kinetic characteristics of study subjects

Subject	Sex	Age (years)	Weight/ height (kg/m^2)	Fasting plasma glucose (mmol/l)	Haemo-globin A_{1c} (%)	Fasting chylo-microns	Fasting plasma triglyc-eride (mmol/l)	Fasting cholesterol levels			Catabolic parameters of chylomicron apoprotein-B				
								Total (mmol/l)	LDL (mmol/l)	HDL (mmol/l)	First phase (delipation chain)		Second phase (slow removal)		Total residence time in the plasma (h)
											Fraction	Residence[a] time (h)	Fraction	Residence time (h)	
Diabetic															
1	M[b]	28	35.6	17.0		Yes	32.88	8.40	0.65	0.31	1.00	38.5	0	–	38.5
2	F	31	33.7	13.0		No	14.24	5.92	1.29	0.59	1.00	58.0	0	–	58.0
3a	F[e]	33	37.4	10.5	14.2	No	18.58	10.28	4.03	0.80	0.78	11.0	0.22	31.3	15.5
3b	F[d]	33	39.9	4.4	7.8	No	4.63	6.51	3.41	1.03	0.86	1.5	0.14	65.0	10.5
4	M	63	29.6	17.1	11.9	No	3.53	4.75	3.00	0.72	0.98	1.3	0.02	18.9	5.8
5	M[e]	76	32.2	6.9	10.4	No	3.10	6.18	3.77	0.98	0.62	1.6	0.38	12.2	5.6
Non-diabetic															
6	F	74	22.1	5.2		Yes	21.88	7.70	0.80	0.44	0.86	0.8	0.14	21.7	3.6
7	M	45	32.7	5.6		No	8.93	7.13	3.07	0.88	0.95	1.0	0.05	40.0	3.0
8	M	22	36.0	4.7		No	3.80	3.31	1.83	0.52	0.94	1.8	0.06	18.9	2.3
9	M	53	27.2	5.7		No	2.62	5.97	4.13	0.85	0.72	1.3	0.29	32.3	10.3
Normal															
10	M	57	28.0	4.8		No	2.05	6.41	4.68	0.93	0.70	1.4	0.30	13.3	5.0
11	M	55	25.4	5.4		No	1.89	6.20	4.52	0.98	0.60	1.2	0.40	13.9	6.3
12	M	25	19.9	4.3		No	0.95	4.32	2.82	1.03	0.67	1.3	0.23	27.8	7.3
13	M	24	22.4	5.7		No	0.90	3.46	2.14	0.90	0.84	1.5	0.16	3.6	1.8
14	F	24	19.7	4.1		No	0.79	3.62	2.12	1.11	0.96	1.9	0.04	35.7	3.2

[a] Half-life ($t_{½}$) values can be calculated as 0.693 × residence time; [b] eruptive xanthomata; [c] recent pancreatitis; [d] insulin therapy; [e] abnormal glucose tolerence test according to WHO criteria [9]

history of atherosclerotic disease. Subject 9, with familial combined hyperlipidaemia [10], was taking nicotinic acid (1.5 g/day) at the time of study. Subject 6 had a past history of chylomicronaemic pancreatitis but had been asymptomatic in that regard (despite continuing severe hypertriglyceridaemia) for over one year; she remained on her long-standing low dose corticosteroids (hydrocortisone (20 mg/day) and beclomethasone by inhalation three times daily) for chronic lung disease at the time of study. Subject 3 was studied twice, first when she had massive chylomicronaemia and later when she was treated for her diabetes with insulin.

Five diabetic subjects were selected from among participants in the placebo phase of a hypoglycaemic drug study (Nos. 2, 4, and 5) and those presenting with complications of their diabetes and/or hyperlipaemia (Nos. 1 and 3). No study was conducted while the subjects were acutely ill. Informed consent was obtained from all. All patients in our study had Type 2 (non-insulin-dependent) diabetes. None of the patients had ever had an episode of diabetic ketoacidosis. None of the diabetic subjects, except patient 3, was taking pharmacological therapy for their diabetes at the time of their study. The patient 3 was on insulin therapy during the second study.

Chylomicron apo-B catabolism study protocol

Circulating chylomicronaemia was induced by the ingestion of 255 ml whipping cream (395 mmol/l triglycerides) after an overnight (14–16 h) fast (reduced to 113 ml in subjects 1–3). The blood specimens (20–25 ml) were drawn 4–5 h later. Chylomicrons were harvested by preparative ultracentrifugation of plasma for 30 min in an SW41 Ti rotor (Beckman, Palo Alto, California) at 36,000 rev/min (10^6 g) and aspirating the upper 1.0 ml. Chylomicrons were washed once with saline solution (density, 1.006 g/ml, 150 mmol/l) under similar conditions, and radiolabelled with ^{131}I by the technique of McFarlane [11] as modified by Bilheimer et al. [12] and as extensively practiced previously in this laboratory [13, 14]. The labelled autologous chylomicrons were re-isolated, passed through a 0.45 μm millipore filter, pyrogen tested in rabbits and then re-injected. Chylomicrons were diluted four- to fivefold with saline (150 mmol/l) before passing through the millipore filter, which helped their passage through the filter. Fat loaded chylomicrons were characterized for their apoprotein composition (see Results).

The study subjects, except for hypertriglyceridaemic subjects (Nos. 1–3 and 6), were admitted to the Clinical Research Center the evening before the metabolic study. Subjects Nos. 1–3 and 6 were admitted to hospital for prolonged study and diabetic control. Labelled chylomicrons (20–40 μCi, spec. act. 15–20 μCi/mg protein) were injected after a 12–14 h fast. Blood sampling for lipoprotein determinations was carried out by standardized Lipid Research Clinics protocols [15]. According to these protocols, chylomicronaemia is defined by the visual detection of a creamy layer on the surface of plasma refrigerated overnight. They were started and remained on an isocaloric, fat-free liquid formula diet (15% protein, 85% carbohydrate). Study subjects consumed this diet in five equal feedings daily for the total duration of the study. A total of 18 blood samples (10 ml), was collected during the study, the first at 5–10 min and thereafter at ½, 1, 2 and 4 h, and at progressively increasing intervals for the duration of the study (60–72 h). The patients were in a steady-state condition as indicated by their triglyceride and cholesterol values.

Radioactivity in chylomicron apo-B in each sample (4–5 ml of plasma) was determined by isolating the S_f 400 fraction, as described above, using the SW55 Ti rotor (Beckman, Palo Alto, California) and slicing the upper 0.5 ml layer (but without the saline wash), precipitating the apo-B (and lipids) with TMU, as described by Kane [16], and counting the soluble fraction in a gamma counter (Nuclear-Chicago, Des Planes, Illinois). The radioactivity in lipids was determined by extracting chylomicrons (100 μl) with chloroform:methanol (2:1) and counting the lipid extract after evaporating the chloroform layer to dryness [13, 14]. Apo-B radioactivity was estimated as the difference between the total in the lipoprotein fraction and the lipid plus TMU soluble radioactivity [16]. The TMU insoluble radioactivity did not contain the radioactivity of apo-E or apo-C, because the TMU soluble radioactivity corresponded to that recovered in non-apo-B bands in urea gels.

Analysis of chylomicron apo-B catabolism

The plasma radioactivity decay curves for chylomicron apo-B were plotted as the fraction of the 10-min counts. In the initial decay of radioactivity, a concave downward slope was noticed in many individuals; this was most pronounced in the severely lipaemic diabetic patients. The characteristics of these decay curves are reminiscent of

those observed for the decay of radioiodinated VLDL apo-B which led originally to the postulation of a step-wise delipidation chain [17]. Phair et al. [17] proposed a compartmental model to describe VLDL apo-B metabolism consisting of a four-pool delipidation chain which accounted for the first phase of the plasma decay curve, and a slowly turning over pool which described the tail portion of the curve; Phair et al. [17] also proposed that a portion of the material degraded in the delipidation chain gave rise to this slowly turning over compartment. Because of the similarity between the chylomicron apo-B plasma decay curves and those observed for VLDL apo-B, a modified version of the Phair model [17] was used to estimate the residence time of chylomicron apo-B in plasma. A schematic of this model is shown in Figure 1. The difference between this model and the model for VLDL apo-B is the lack of a pathway describing conversion from the delipidation chain to the slow component. This model is used to estimate three parameters (Fig. 1): LD is the rate constant for delipidation in the stepwise delipidation chain; LS is the rate constant describing the turnover of the slow component, and S is the fraction of initial radioactivity in the slow component. Total apo-B residence time is calculated by the equation $S(1/LS)+(1\text{-}S)\,4/L_D$. The data analysis was carried out using SAAM computer program [18].

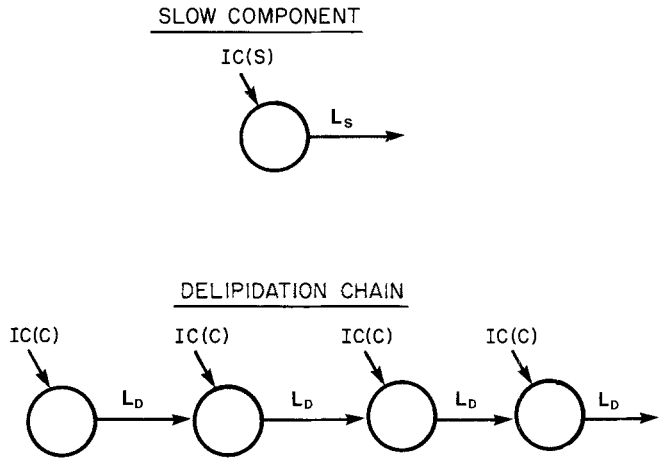

Fig. 1. Kinetic model used to estimate chylomicron apo-B residence time. This consists of a four compartment delipidation chain and a slowly turning over compartment; total chylomicron apo-B radioactivity is a sum of the radioactivity in all five compartments. The rate constant LD (h^{-1}) describes the rate of decay of each compartment in the delipidation chain and the rate constant LS (h^{-1}) describes the turnover of the slow component. The half times of each compartment may be obtained by calculating 0.693/LD and 0.693/LS respectively for compartment in the delipidation chain and the slow component. The initial condition of radioactivity in the slow component, IC(S), is S; the initial conditions in each component in the delipidation chain, IC(C), is equal to (1–S)/4

Adipose tissue lipoprotein lipase activity

This was determined in the heparin-elutable fraction of buttock adipose tissue aspiration biopsies in selected subjects (Nos. 1, 2 and 12; courtesy of Dr. J. D. Brunzell) [3].

Determination of glucose and haemoglobin A_{1c}

Plasma glucose was determined by the standard glucose oxidation method using a Beckman glucose analyzer (Beckman, Palo Alto, California). Haemoglobin A_{1c} was measured by the colorimetric procedure of Pecoraro et al. [19].

Results

Characterization of chylomicron apoproteins

To determine whither iodinated chylomicrons injected into study subjects were of intestinal origin or were contaminated with VLDL of hepatic origin, three normal subjects, a hyperlipidaemic non-diabetic and a hyperlipidaemic diabetic subject were fat loaded with whipping cream. Chylomicrons were isolated and the apoproteins were separated on 3.5% polyacrylamide containing 0.1% sodium dodecyl sulphate [20]. Figure 2 shows the patterns of apoproteins in these subjects. Fat loaded chylomicrons are rich in apoproteins that have molecular weights below 80,000 daltons (C band Fig. 2). The fasting diabetic subject had two higher molecular weight apoproteins (gel 1 Fig. 2). The A band corresponds to apoprotein B-100 and the B band corresponds to B-48 apolipoprotein [21]. All fat loaded chylomicrons have only one apo-B corresponding to B-48. There was no detectable amount of apo-B corresponding to B-100 on the gels. Thus, these particles are of intestinal origin at least with respect to their apo-B composition. Injected chylomicrons had $32.7 \pm 8.6\%$ radioactivity in apo-B protein as measured by the TMU precipitation method [16]. The remaining radioactivity was in smaller (non-apo-B) proteins.

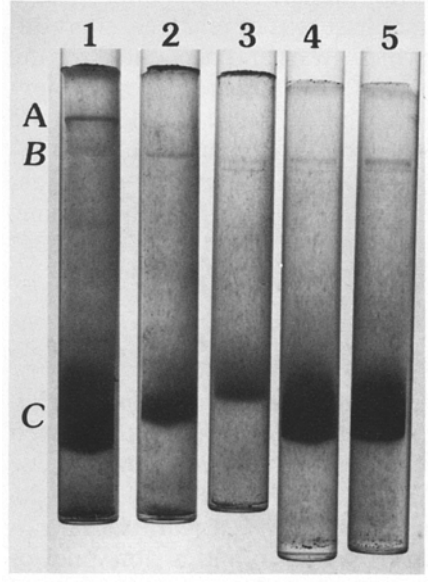

Fig. 2. Electrophoretic patterns of chylomicron (S_f 400) apolipoproteins on 3.5% polyacrylamide gels containing 0.2% sodium dodecyl sulphate: (1) from a fasting hyperlipidaemic diabetic subject; (2) from the same subject after a fat load of whipping cream; (3–5) from normal subjects after a similar fat load. Apolipoprotein band A corresponds to hepatic apo-B (B-100); band B corresponds to intestinal apo-B (B-48); and band C corresponds to apo-C and E with other minor peptides. Protein samples (40–60 μg) were incubated with β-mercaptoethanol before application to the gels

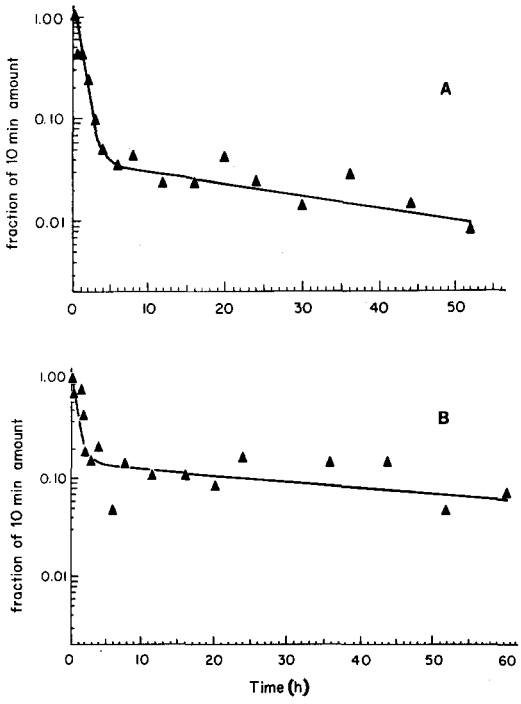

Fig. 3 A and B. Chylomicron ($S_f > 400$) ^{131}I-apo-B disappearance in **A** normolipidaemic, non-diabetic subject (no. 14) and **B** severely lipaemic subject (no. 3 b) after 6 months of treatment

Chylomicron apo-B catabolism in normolipidaemic subjects (Table 1)

In normolipidaemic subjects chylomicron apo-B disappeared from the plasma compartment in biphasic fashion (Fig. 3 A). The first phase was of relatively short duration (residence time 1.2–1.9 h) and accounted for the majority (60%–92%) of total chylomicron apo-B removal. The second phase had a longer residence time (3.6–35.7 h). The proportion of chylomicron apo-B removal accounted for by this phase was the major determinant of total chylomicron apo-B residence time, which varied from 1.8 to 7.3 h.

Chylomicron apo-B catabolism in hypertriglyceridaemic, non-diabetic subjects (Table 1)

The kinetics of chylomicron apo-B metabolism in hypertriglyceridaemic, non-diabetic subjects conformed closely to the model in normolipidaemic, non-diabetic subjects [18] (Fig. 4 A). The morphology of the curve describing the first phase was consistent with a chain of stepwise delipidation (first phase) and a slow pool of long residence time reflecting decay by the second phase. Accordingly, the fractions of total apo-B removal via the delipidation chain (from 72% to 95%) and the slow pool (5%–29%), the residence times in the chain phase (0.8–1.8 h) and the slow, second phase (19.9–40.0 h), and total chylomicron apo-B residence time (2.3–10.3 h) closely resembled comparable figures in the normolipidaemic, non-diabetic subjects. Hence

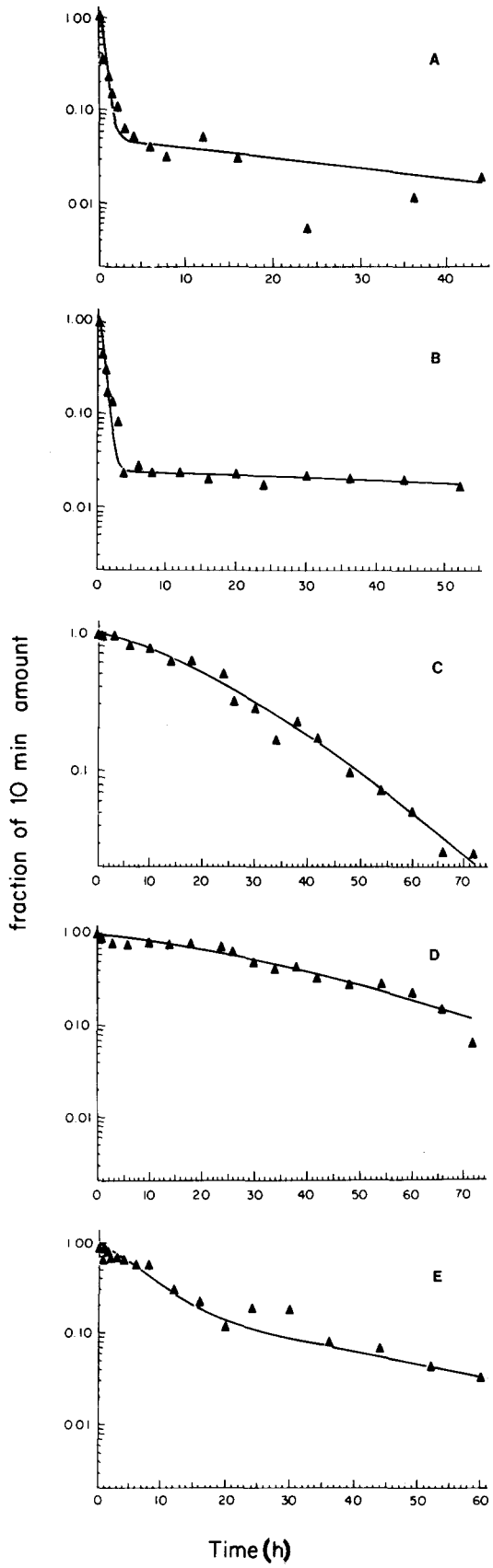

Fig. 4 A–E. Chylomicron ($S_f > 400$) ^{131}I-apo-B disappearance in **A** severely lipaemic, non-diabetic subject (no. 6); **B** modestly hypertriglyceridaemic, diabetic subject (no. 4); **C** severely lipaemic, diabetic subject (no. 1); **D** in severely lipaemic, diabetic subject (no. 2); and **E** in severely lipaemic, diabetic subject (no. 3 a) early during treatment

over a wide range of plasma triglyceride concentrations, these studies disclosed no evidence of saturation kinetics in chylomicron apo-B removal.

Chylomicron apo-B catabolism in hypertriglyceridaemic diabetic subjects (Table 1)

Triglyceride concentrations in the diabetic subjects varied widely, as did their clinical status and degree of hyperglycaemia and glycosuria. At one end of this spectrum (subject 5) hyperglycaemia was minimal, glycosuria absent, and hypertriglyceridaemia marginal (3.10 mmol/l). Not surprisingly, chylomicron apo-B kinetics in this subject could not be distinguished from those in non-diabetic subjects, whether normolipidaemic or hypertriglyceridaemic, with a total chylomicron apo-B residence time of 5.6 h (Fig. 4 B). Subject 4 also displayed normal chylomicron apo-B kinetics despite substantial fasting hyperglycaemia (17.09 mmol/l), glycosuria, and mild hypertriglyceridaemia (3.53 mmol/l). The inordinate residence time of his slow pool most likely represents a measurement error attributable to the small proportion (2%) of removal via this phase and the minimal radioactivity, just above background, in his chylomicron apo-B fraction after the first few hours of the study; accordingly, this altered the estimated residence time of his chylomicron apo-B only slightly.

At the other end of the spectrum were the three diabetic patients symptomatic from their hyperglycaemia and/or chylomicronaemia. Subject 1, with manifest polyuria, polyphagia, glycosuria (10–15 g/day), weight loss, and eruptive xanthomata, had been symptomatic and untreated for several months before hospitalization for these studies (and subsequent initiation of anti-diabetic treatment). His chylomicron apo-B kinetics contrasted markedly with the normal pattern: only the first phase of decay was evident, but his residence time (38.5 h) was in the range of that for the slow pool in the non-diabetic subjects (Fig. 4 C). However, that this decay curve represented a retarded first phase rather than a single second phase, was strongly suggested since a concave downward configuration of that curve is more consistent with a pattern of slow delipidation. Also consistent with this interpretation was the measured activity of his adipose tissue lipoprotein lipase (0.3 nmol fatty acid released/min per 10^6 cells) well below the normal range (0.8–4), especially considering his degree of obesity; a comparable value in normolipidaemic subject 12 was 3.0 nmol/min per 10^6 cells. A similar concave downward curve was seen in subject 2 (Fig. 4 D), who likewise had been progressively symptomatic from her untreated diabetes. Her estimated chylomicron apo-B residence time was 58 h.

Particularly informative were the paired studies performed in subject 3 at two different stages in diabetic treatment. Study 1 was initiated 21 days after she had presented with acute pancreatitis attributable to massive chylomicronaemia (triglyceride 77.3 mmol/l). Treatment of her diabetes with insulin had commenced concurrently with therapy for pancreatitis, such that she was pain free, her blood glucose was in the range of 10.49 mmol/l, and her triglyceride 19.07 mmol/l at the time of the first study of her chylomicron apo-B catabolism. This disclosed a pattern (Fig. 4 E) intermediate between that of the severely lipaemic subjects 1 and 2 and the non-lipaemic diabetic and non-diabetic subjects: two distinct phases were evident, the first of relatively long residence time (11.0 h) and suggestively convex upward accounting for 78% of total removal, the second of relatively normal residence time (31.3 h) accounting for 22% of total removal, resulting in a moderately long total residence time of 15.5 h.

Aggressive treatment of her diabetes with insulin (up to 300 U/day) over the ensuing 6 months was associated with a reduction in triglyceride levels (to 4.52–6.78 mmol/l) in spite of moderate weight gain (6 kg). A repeat chylomicron apo-B catabolism study when fasting glucose levels were normal (but glycosylated haemoglobin still moderately elevated at 10%) disclosed improved kinetics (Fig. 3 B): 86% via a first phase with residence time 1.5 h; 14% via a second phase with residence time 65.0 h; total chylomicron apo-B residence time 10.5 h.

Discussion

Characterization of chylomicron apo-B metabolism in man has proven elusive, especially by contrast with VLDL or low density lipoproteins (LDL). Thus, apart from a single study of a non-diabetic woman with a chylous pleural effusion [7], these studies are the first to address the kinetics of chylomicron apo-B metabolism in man (and the first in diabetic subjects). Even studies of chylomicron triglyceride metabolism have been few and beset with problems [22, 23]. The present studies were designed to circumvent certain problems in the study of chylomicron metabolism by capitalizing on the retention of apo-B on its particle of origin (in contrast to triglyceride and the TMU-soluble peptides). The apo-B radioactivity was measured by the TMU-method [16]. This technique, however, has a major drawback in that it measures both chylomicron apo-B (B-48) and B-100, and therefore any contamination of chylomicron apo-B with B-100 would give the decay of intestinal and hepatic lipoproteins. Since fat loaded chylomicrons in both normal subjects and hyperlipidaemic (diabetic and non-diabetic) subjects had only apo-B of intestinal origin (B-48), therefore, these studies are revealing regarding the regulation of intestinal lipoprotein metabolism in man and the role of lipoprotein lipase therein.

First, they suggest that chylomicron apo-B removal is biphasic, even in normolipidaemic subjects. Second, the morphology of the first phase curve suggests that, as in VLDL, a chain of delipidation steps is an essential feature of this more rapid phase. Third, the slow second

phase clearly existed in all subjects (except the most lipaemic diabetic subjects [see below]). There are two possible explanations for the existence of the slower second phase. First, the TMU-method [16] employed to measure the apo-B radioactivity may not give accurate values at low levels of radioactivity remaining in chylomicrons (after > 90% of total radioactivity has decayed in chylomicrons). Alternatively a small (variable) fraction of chylomicron apo-B circulates for an extraordinary duration, presumably as chylomicron remnants analogous to VLDL remnants (previously designated as 'compartment 21'). However, since the chylomicrons injected had no detectable apo-B-100, the radioactivity decaying by slower phase does not appear to be due to the apo-B-100.

Finally, these studies suggest a critical, permissive role of lipoprotein lipase (and chylomicron triglyceride hydrolysis) in the clearance of chylomicron apo-B from the circulation. Analysis of the monoexponential decay curves in lipaemic diabetic patients deficient in insulin clearly indicates that delipidation may be taking place, although at a retarded rate. Thus, the first-phase, and not the second-phase, of removal is rate-limiting to overall chylomicron apo-B removal. The slower first phase may, therefore, vastly prolong chylomicron apo-B circulation time in these subjects. That lipoprotein lipase is deficient (and triglyceride removal retarded [4]) in subjects like these has been convincingly demonstrated; this was directly determined in subject 1 when his diabetes was symptomatic and untreated. This observation provides a rationale for the severe and protracted lipaemia in undertreated diabetic subjects. However, since these studies were conducted using a limited number of subjects and the lipoprotein lipase was measured in only three subjects (two diabetic and one normal subject), these observations are preliminary and need to be confirmed in a larger group of patients.

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