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Inter-Relationship between Insulin Secretion and Plasma Free Fatty Acid and Triglyceride Transport Kinetics in Maturity Onset Diabetes and the Effect of Phenethylbiguanide (Phenformin)

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Summary. The plasma free fatty acid and triglyceride transport kinetics in 16 non-obese and 7 obese maturity onset diabetics with hypertriglyceridaemia have been compared with results obtained in 27 control subjects. Changes in glucose and insulin responses were evaluated in relation to the lipid parameters. All the diabetics showed elevated plasma FFA levels and turnover rates. In the non-obese diabetics the plasma triglyceride turnover rate was within the normal range and their hypertriglyceridaemia was due to impaired triglceride clearance. In the obese diabetics the plasma triglyceride turnover rate was increased and they also had some impairment of triglyceride clearance, so that in them a double mechanism was observed to account for their hypertriglyceridaemia. The insulin levels in the diabetics were similar to, or greater than, those found in the controls. Our results suggested that the enhanced lipolysis and impaired triglyceride clearance observed in the diabetic patients were a manifestation of insulin unresponsiveness in adipose tissue and that the changes in insulin and glucose relationship could be secondary to elevated FFA and triglyceride levels. Further confirmation was obtained by the finding of an exaggerated insulin response to a glucose challenge in normal subjects infused with Intralipid. Treatment with phenethylbiguanide (Phenformin) significantly lowered the plasma FFA and triglyceride concentration in both diabetic groups. This was associated with normalisation of both plasma FFA turnover and triglyceride clearance. It also reduced the triglyceride turnover rate to the normal range in the obese diabetics. These changes were associated with a fall of plasma glucose and insulin levels to within the normal range. These results suggested an effect of Phenformin in reducing the rate of lipolysis leading to improved glucose tolerance.

Key words: Maturity onset diabetes, kinetic measurements, free fatty acid (FFA), triglyceride (TG), insulin, post-heparin lipolytic activity (PHLA), hypertriglyceridaemia, turnover rate, influx rate clearance, oral glucose tolerance test (O.G.T.T.).

The structural changes of atherosclerosis are, for practical purposes, probably irreversible. The most important therapeutic goal therefore is to prevent their development. Diabetics with vascular complications often have elevated levels of plasma lipids, including triglycerides [2]. Hypertriglyceridaemia has received increasing attention recently as a causative factor in atherogenesis in man [9, 25, 10].

Cross-sectional prevalence studies, on the other hand, have shown a correlation between the development of atherosclerosis in diabetics and the degree of insulinaemia observed in these patients [49, 28]. The association of these two risk factors has been stressed in several studies [15, 19, 20]. However, the relationship between hypertriglyceridaemia and hyperinsulinaemia in diabetic subjects remains to be clarified. The current view is that these patients have difficulty in peripheral glucose disposal. In an attempt to compensate for this difficulty insulin secretion is stimulated to maintain adequate glucose uptake at tissue level. The increased availability of glucose and insulin stimulates hepatic triglyceride synthesis and produces hypertriglyceridaemia. This view is supported by the finding of a positive correlation between plasma triglycerides and immuno-reactive insulin levels [19, 20]. This concept differs from that of Sailer *et al.* [43], who favour the view that the hyperinsulinaemia might be a consequence rather than the cause of hypertriglyceridaemia in these patients.

Unfortunately, the mechanisms involved in the control of plasma triglyceride levels in diabetics have not been clearly delineated. Knowledge of such mechanisms is of obvious importance in determining the appropriate therapy.

The aim of this work was to study the kinetics of plasma free fatty acid and triglyceride transport in patients with maturity onset diabetes and to determine the inter-relationship between insulin secretion and triglyceride levels. In addition, the effects of Phenformin (phenethylbiguanide) therapy on plasma free fatty acid and triglyceride transport kinetics have been evaluated in these patients.

Subjects

50 subjects were studied. The control group of 27 healthy subjects comprised 15 pre-menopausal female and 12 male volunteers, with normal fasting serum lipid levels and glucose tolerance. The mean age of this group was 35 (range 17–62) and all these subjects were non-obese (less than 115% of their ideal body weight obtained from data from Documenta Geigy). None were taking any drugs known to affect lipid or carbohydrate metabolism.

23 maturity onset diabetic patients of both sexes with fasting hypertriglyceridaemia were selected. 16 were non-obese, with relative body weights less than 115% of their ideal body weight, and 7 were obese with relative body weights in excess of 115% of their ideal body weight. These patients were selected from newly diagnosed, non-ketotic diabetics referred to our clinic. Oral glucose tolerance tests were performed on the patients and those who had an abnormal response and fasting hypertriglyceridaemia were stabilized on an isocaloric diet on an outpatient basis. The isocaloric diet provided 35 cals/kg ideal body weight, of which 38% was in the form of carbohydrate, 45% as fat and 17% protein. Two weeks later they were admitted to the Metabolic Ward for a further period of two weeks. During this period the fasting blood sugar, plasma lipids, urinary ketones (acetest), and body weights were recorded. At the end of this period the oral glucose tolerance test was repeated. Only those patients in whom glucose intolerance was substantially unchanged, plasma lipids and body weight stabilized, and who remained non-ketotic were selected for the study. Abnormal glucose tolerance was diagnosed when two or more of the following criteria were present: fasting plasma glucose >100 mg/100 ml, 60′ plasma glucose >180 mg/100 ml, and 120′ plasma glucose level of >140 mg/100 ml. Details of the ages and relative body weights of the subjects studied are shown in Tables 1a and b.

The control subjects were similarly stabilized on an isocaloric diet before testing. Kinetic measurements were then performed on both groups. Having completed these investigations the diabetic patients were treated with Phenformin 50—100 mgm b.d. for a period of 3—6 months. During this time the isocaloric diet was continued and the weights of the patients remained substantially unchanged. At the end of the treatment period the kinetic measurements were repeated.

Methods

A. Measurement of Kinetics of Plasma FFA and Triglyceride Transport

Plasma free fatty acid and triglyceride turnover rates were determined using a continuous infusion of ^{14}C labelled palmitate. The plasma free fatty acid flux was calculated from the rate of infusion of the label and plasma free fatty acid specific radioactivity at equilibrium. The plasma triglyceride turnover rate was estimated from the slope of the specific radioactivity disappearance curve of endogenously labelled triglycerides after stopping the ^{14}C-palmitate infusion. The technical details and validation of this method have been presented elsewhere [31, 32]. The Appendix shows three decay curves and a comparison between the turnover values calculated using this technique, and that obtained from studies in which pre-labelled lipoprotein triglycerides were re-injected into the same subject.

I. FFA turnover. After an overnight fast an indwelling catheter was inserted into a vein in both antecubital fossae and the patient was then rested for half an hour. 25 μci of ^{14}C palmitic acid was infused at a rate of 0.2 μci per minute, using a constant delivery syringe, for 2 h, blood samples being taken from the contralateral arm. Plasma free fatty acid turnover rate was then calculated as follows:

$$\text{Turnover } (\mu\text{eq/min}) = \frac{\text{Infused dose (dpm per min)}}{\text{FFA specific activity at equilibrium (dpm per } \mu\text{eq)}}$$

II. Triglyceride turnover. After stopping the labelled palmitate infusion, samples of blood were collected from the contralateral arm catheter at 30 min intervals for a period of 12 h. The plasma was separated and the triglyceride specific radioactivity was determined. The specific activity time curves were plotted semi-logarithmically and the fractional turnover rate of plasma triglyceride (Ka) was calculated, using the method of least squares. Triglyceride turnover rate (V) in μmol/kg/h was calculated from the formula:

$$V = S \times 50 \times Ka$$

where S represents the serum triglyceride concentration in μmol/ml. In this calculation the plasma volume was considered to represent the distribution space of triglycerides and the co-efficient 50 assumes that the plasma volume is 50 ml/kg body weight.

III. Clearance of endogenous triglyceride. The efficiency of endogenous triglyceride clearance was determined from the level of serum triglyceride and its turnover rate using the kinetic approach of Reaven *et al.* [41]. In this technique the transport of serum triglyceride was considered to be similar to a saturable enzyme system.

The results of the investigation performed in the normal subjects and in patients with experimental hypertriglyceridaemia [32] have added further confirmation for the use of this technique in determining endogenous triglyceride clearance. When serum triglyceride concentration (S) was plotted against turnover rate (V) a hyperbolic curve was obtained. At lower levels of triglyceride concentration, removal rates appeared to be proportional to the triglyceride concentration and the relationship was linear. As the maximum turnover rate (V-max) was reached the rate of removal appeared to be independent of triglyceride concentration and the reaction approached zero order kinetics. The relationship between serum triglyceride concentration and turnover in the normal group was constructed and the results from the diabetic patients were examined in this context. Using this approach the removal efficiency was characterised by means of the Michaelis constant, Km, which is the triglyceride concentration at which the triglyceride turnover rate is at half maximal velocity. To calculate the maximum turnover rate and Km more accurately a linear trans-

formation of the Michaelis-Menten formulation of [S] against [V] was constructed. As V-max equals the reciprocal of the slope of the regression line obtained, V-max was calculated for each individual group of subjects in the studies performed and individual Km values were calculated from the formula:

$$\text{Km} = \text{V-max} - \text{V} \times \frac{[\text{S}]}{\text{V}}$$

IV. Clearance of exogenous triglyceride. The efficiency of clearance of exogenous triglyceride was determined using the intravenous fat tolerance test described by Boberg *et al.* [8]. Intralipid (10% W/V) was given intravenously in a dose of 1 ml/kg body weight and the fractional turnover rate K_2 was calculated from the half-life time of turbidity determined nephelometrically.

V. Post-prandial plasma post-heparin lipolytic activity. Heparin 10 I.U./kg body weight was injected intravenously 90 min after the intake of an oral glucose load of 50 g. Blood was taken 10 min later and the plasma lipolytic activity was measured using the method of Boberg and Carlson [7].

B. Experimental Hypertriglyceridaemia

Experimental hypertriglyceridaemia was induced in 4 normal volunteers using a continuous infusion of Intralipid®. Immediately before the start of the infusion a priming dose of 0.1—0.3 mmol of triglyceride emulsion per kg body weight was given intravenously within a period of 2 min. The rate of the infusion was then adjusted to deliver between 20 and 30 μmol of triglyceride per kg per hour. This rate was found to be suitable to obtain a constant plasma triglyceride level during the infusion. 3 h later a glucose infusion at a rate of 0.4—0.6 g per kg per hour was commenced in addition to the Intralipid infusion. The combined infusion was continued for another 2 h. Blood samples were taken from a cannula in a vein in the contralateral arm throughout the 5 h study and the plasma was separated for triglyceride, free fatty acid, glucose and insulin measurements. At the end of the infusions 10 I.U. per kg of heparin were given intravenously and a blood sample was taken 10 min later for measurement of plasma lipolytic activity. This protocol was repeated with the same subject a week later using intravenous saline infusion instead of Intralipid.

C. Other Methods

Oral glucose tolerance tests were performed using 50 g of glucose. Samples of blood were taken at 0, 60 and 120 min after the glucose load. Measurements of plasma glucose and insulin were performed using the methods of Cramp [12] and Albano *et al.* [1] respectively. Serum triglyceride levels were measured using a semi-automated fluorimetric method [13]. Standard statistical methods were employed using student's "t" test.

Results

A. Plasma Free Fatty Acid and Triglyceride Transport Kinetics in Control and Diabetic Subjects

Table 2 summarises the results of our investigations in the control subjects and in the diabetic patients before and after Phenformin therapy.

I. Free fatty acid turnover rate. The mean plasma free fatty acid concentration and flux in the controls were 680 ± 172 μeq/l and 274 ± 45 μeq/min respectively. In both the obese and non-obese diabetic groups (Tables 1a and b) significant elevation of plasma free fatty acid concentration was associated with increased free fatty acid flux into the plasma. There was no significant difference in plasma free fatty acid concentration or flux between the two groups of diabetic patients. The mean plasma free fatty acid concentration was significantly reduced in the diabetic patients during Phenformin therapy. This reduction in free fatty acid concentration was associated with a simultaneous reduction in plasma free fatty acid turnover (Fig. 1).

II. Triglyceride turnover rate. The mean fasting triglyceride concentration in the control group was 1.008 ± 0.337 mmol/l. In both diabetic groups the mean triglyceride concentration was significantly higher, the greatest increase being in the non-obese patients. Fig. 2 shows the relationship between plasma triglyceride concentration and turnover rates in the control group and diabetics. The mean triglyceride turnover rate in the non-obese diabetics was similar to that of the control subjects so that the individual points of the former lay below the saturation curve of the controls, indicating that in these diabetic patients the hypertriglyceridaemia was due to a defect in triglyceride clearance. The mean triglyceride turnover rate in the obese diabetics was significantly increased so that their individual points lay above the saturation curve of the controls, indicating that in these diabetics hypertriglyceridaemia was in part due to increased triglyceride influx into the plasma. In the entire group of diabetic patients triglyceride turnover rate correlated significantly with the relative body weights of the patients ($r = 0.716$, $p < 0.001$).

In the non-obese diabetics there was no significant correlation between the plasma free fatty acid turnover and plasma triglyceride turnover rates. A significant correlation was, however, observed between these measurements in the obese diabetics ($r = 0.747$, $p < 0.05$).

With Phenformin therapy the plasma triglyceride concentration was markedly reduced in all the diabetics (Fig. 1 and Table 2). Triglyceride turnover rates were reduced during Phenformin treatment only in the obese diabetics, in whom the turnover values were originally elevated (Fig. 1, Table 2). No significant changes were observed in the non-obese diabetics. When the triglyceride concentration values were

Table 1a. *Somatic data, plasma FFA and triglyceride kinetic parameters in non obese adult onset diabetic subjects*

No.	Sex	Age yrs.	Wt. % ideal	O.G.T.T. Plasma glucose mg% 0′	60′	120′	Plasma insulin μu/ml 0′	60′	120′	Plasma FFA Conc. μeq/l	Flux μeq/min	Serum triglycerides Conc. mmol/l	K^a (h^{-1})	Turn-over rate μmol/kg/h	Km mmol/l	Intralipid Clearance K_2 %/m	PHLA FFA released μeq/l/h
1	F	39	106	105	198	225	8	65	26	3605	892	4.600	0.078	18	3.584	1.20	38
2	F	61	98	215	310	312	6	26	18	2650	640	3.200	0.094	15	3.621	1.60	49
3	M	66	108	93	195	186	11	72	36	4200	1200	6.400	0.066	21	3.355	0.64	26
4	M	42	114	110	220	180	16	140	49	4006	960	4.860	0.079	19	3.328	0.86	101
5	M	38	105	119	260	210	6	76	26	4870	1110	6.800	0.066	21	3.410	0.42	47
6	M	40	112	205	380	260	4	38	18	2650	762	4.200	0.091	19	2.873	0.82	32
7	F	46	106	100	218	310	5	12	18	3800	940	7.200	0.058	21	3.773	0.46	64
8	F	42	109	89	225	280	5	52	36	2112	460	3.560	0.079	14	4.572	1.60	48
9	M	54	112	130	250	267	12	60	40	4250	968	4.250	0.080	17	3.750	1.40	98
10	F	34	106	210	320	280	13	110	28	3800	872	3.800	0.089	17	3.360	2.40	73
11	M	47	109	200	310	260	12	16	6	3405	826	5.400	0.079	20	3.240	0.72	86
12	F	62	113	86	186	150	8	14	12	2350	412	2.350	0.111	13	3.439	0.96	48
13	M	37	102	105	215	176	6	44	65	4160	624	4.150	0.082	17	3.660	0.32	67
14	M	46	112	110	280	189	7	62	48	1120	410	1.900	0.126	12	3.160	3.80	39
15	F	38	114	107	260	220	6	86	52	1680	565	2.100	0.114	12	3.500	2.86	34
16	M	51	107	130	272	186	12	135	46	2950	915	4.600	0.078	18	3.580	1.02	69
Mean ±		46.5	108	132.1	259.3	230.7	8.6	63	32.8	3225.5	784.8	4.335	0.085	17.1	3.512	1.32	57.4
I.S.D.			4.5	46.7	52.8	51.1	3.6	39.9	16.5	1051.6	242.9	1.6	0.01	3.1	0.36	0.96	23.3
Controls Mean ±		33.5	103	79	140	90	7	40	19	680	274	1.008	0.312	16	0.849	4.1	290
I.S.D.			9	10	10	6	3	13	6	172	45	0.337	0.063	3	0.216	0.8	28
P				<0.001	<0.001	<0.001	NS	NS	NS	<0.001	<0.001	<0.01	<0.001	NS	<0.001	<0.001	<0.001

P = the significance between the diabetic patients and control subjects. NS = $p > 0.05$

Table 1b. *Somatic data, plasma FFA and triglyceride kinetic parameters in obese adult onset diabetic subjects*

No.	Sex	Age yrs.	Wt. % ideal	O.G.T.T. Plasma glucose mg% 0′	60′	120′	Plasma insulin μu/ml 0′	60′	120′	Plasma FFA Conc. μeq/l	Flux μeq/min	Serum triglycerides Conc. mmol/l	K^a (h^{-1})	Turn-over rate μmol/kg/h	Km mmol/l	Intralipid clearance K_2 %/m	PHLA FFA released μeq/l/h
17	M	35	124	250	530	560	4	65	26	2010	485	2.650	0.196	26	1.856	2.80	92
18	M	41	131	88	220	190	7	76	49	2430	560	3.250	0.203	33	1.109	1.460	58
19	F	30	125	96	185	155	14	168	128	1962	462	2.860	0.210	30	1.349	3.20	101
20	M	43	120	210	420	290	8	98	65	2362	620	3.650	0.181	33	1.243	1.60	76
21	F	56	130	86	210	186	5	40	25	2600	650	2.600	0.238	31	1.109	2.30	188
22	F	42	116	100	315	296	6	52	16	1950	312	1.950	0.308	30	0.923	2.84	112
23	M	52	132	98	280	188	8	105	82	2300	460	2.300	0.226	26	1.620	3.20	100
Mean ±		42.7	125.4	132.6	308.6	266.4	7.4	86.3	55.9	2230.6	507	2.751	0.223	29.9	1.315	2.49	103.9
I.S.D.			5.0	67.7	125.9	140.5	3.3	42.9	39.7	257.5	114.6	0.569	0.031	2.9	0.322	0.72	41.2
Controls Mean ±		33.5	103	79	140	90	7	40	19	680	274	1.008	0.312	16	0.849	4.1	290
I.S.D.			9	10	10	6	3	13	6	172	45	0.337	0.063	3	0.216	0.8	28
P				<0.01	<0.001	<0.001	NS	<0.05	NS	<0.001	<0.001	<0.001	<0.05	<0.001	<0.05	<0.001	<0.001

P = The significance between the diabetic patients and control subjects. NS = $p > 0.05$.

plotted against the triglyceride turnover rates the direction of change indicated that Phenformin treatment restored the turnover kinetics to the normal pattern in the majority of patients studied (Fig. 2). These results suggest that in the obese diabetics the improvement in serum triglyceride concentration was predominantly due to a decrease in plasma triglyceride turnover, whereas in the non-obese diabetics the reduction in serum triglyceride was due to the marked improvement in plasma triglyceride clearance.

plasma lipolytic activity was reduced in both groups of patients, the changes being more marked in the non-obese diabetics (Tables 1a and b).

During treatment with Phenformin the Km value was markedly reduced in all the patients; the values during treatment, with one exception, were within the normal range. These results suggest that Phenformin enhances the clearance of endogenous plasma triglyceride (Fig. 1 and 3). The clearance of exogenous triglyceride, as determined by the fractional transport

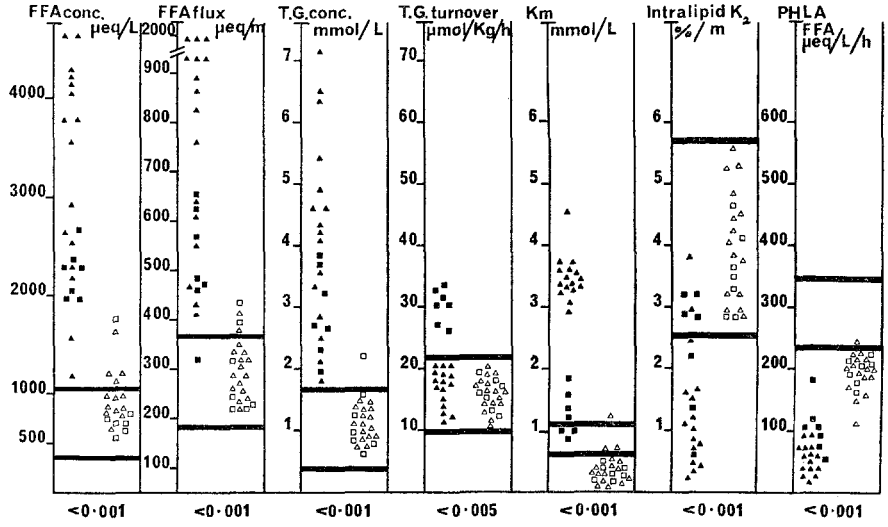

Fig. 1. Individual data of the kinetics of plasma FFA and triglyceride transport in non-obese and obese maturity onset diabetics. The two horizontal thick lines represent the means of the control group ± 2 S.D. The triangles (▲ △) represent the non-obese diabetics, the squares (■ □) represent the obese diabetics. The closed symbols indicate the results before therapy and the open symbols represent the values during treatment with Phenformin

III. Triglyceride clearance. The plot of [S] against $\frac{[S]}{V}$ (Fig. 3) showed that the kinetics of triglyceride transport were very different in the non-obese and obese diabetic patients. In the non-obese diabetics the V-max was similar to that of the control subjects (about 30 μmol/kg/h), while the V-max of the obese diabetics was greatly increased (44 μmol/kg/h). The Km of endogenous triglyceride clearance in the non-obese diabetics was significantly higher than that of the control subjects indicating that clearance of endogenous plasma triglyceride was severely impaired and that, in the presence of a normal triglyceride turnover rate, this accounted for the severe degree of hypertriglyceridaemia observed in these patients. On the other hand, the obese diabetics had only slight impairment of clearance of endogenous plasma triglyceride, as in this group elevation of the Km was small, thus indicating that enhanced triglyceride influx into the plasma was the predominant cause of their hypertriglyceridaemia.

Clearance of exogenous triglyceride was impaired in both groups of diabetic patients, as shown by low intralipid K_2 values (Table 1a and b). Post heparin

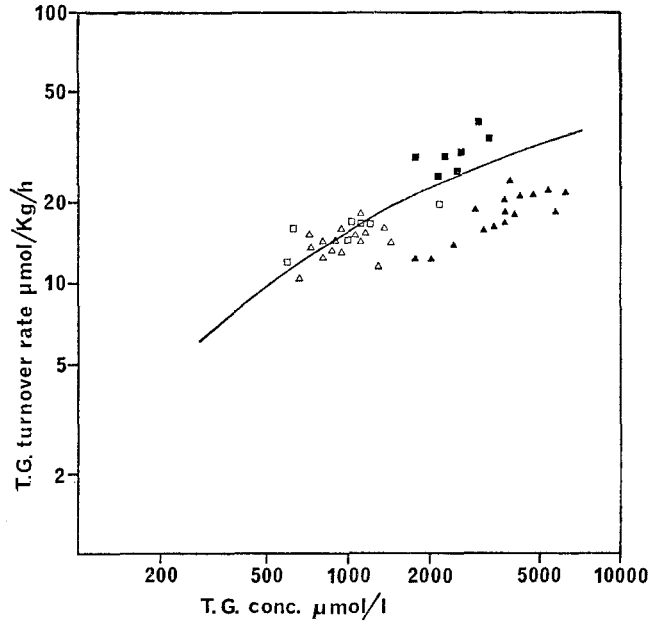

Fig. 2. Relationship between serum triglyceride concentration and turnover rate in controls and maturity onset diabetics before and after Phenformin treatment. The curved line represents the saturation curve for the control group. Symbols as in Fig. 1

rate of Intralipid, was also improved during Phenformin therapy (Fig. 1 and Table 2). The improvement in plasma triglyceride clearance induced by Phenformin was also associated with enhanced release of lipolytic activity into the plasma in response to heparin (Fig. 1 and Table 2).

B. Glucose Insulin Relationships in the Controls and Diabetics, and the Effects of Experimental Hypertriglyceridaemia

In both groups of diabetics plasma glucose levels fasting and during the oral glucose tolerance test were similar, and greatly exceeded the values observed in the control subjects (Tables 1a and b). Fasting plasma insulin levels were similar to those observed in the controls. Plasma insulin responses following oral glucose were not statistically different in the diabetic groups, though the levels in the obese diabetics tended to be higher than those in the non-obese. In the former patients the plasma insulin levels at 60 and 120 min were significantly higher ($p<0.01$, $p<0.05$ respectively) than those in the control subjects. In both diabetic groups there was no correlation between plasma glucose or insulin and triglyceride concentration and turnover rate.

During treatment with Phenformin there was a marked improvement in glucose tolerance and the mean values of fasting, 60 and 120 min plasma glucose and insulin fell within the range of the control subjects (Table 2). Fig. 4 shows the relationship between the insulin response and glucose levels at 60 min in the control subjects and in the diabetics, before and during Phenformin therapy. Before treatment, though the insulin responses in most of the diabetics were within or above the range observed in the controls, the plasma glucose levels were markedly elevated. During Phenformin therapy, the plasma insulin responses and glucose levels were reduced, so that the majority of individual points fell within the range observed in the control subjects.

In the healthy volunteers plasma glucose and insulin levels were not altered by the infusion of Intralipid, during which time plasma free fatty acid and triglyceride concentrations were elevated. During the glucose infusions, in the latter part of the experiment the resultant plasma glucose profiles were identical with and without the simultaneous infusion of Intralipid. However, the plasma insulin responses to glucose were significantly higher when Intralipid was being infused than during the saline infusion (Fig. 5, Table 3). Post-heparin, plasma lipolytic activity measured at the end of the experiments was significantly reduced by the Intralipid infusion, but not by saline.

Discussion

Despite several studies of the inter-relationship between diabetes and hyperlipidaemia it is difficult to

Table 2. *Effects of phenformin treatment on plasma glucose and insulin levels, and on the kinetic parameters of FFA and triglyceride kinetics in hypertriglyceridaemic maturity onset diabetic subjects*

Groups studied	O.G.T.T. Plasma glucose mg% 0′	60′	120′	Plasma insulin μu/ml 0′	60′	120′	Plasma FFA Conc. μeq/l	Flux μeq/min	Serum triglycerides Conc. mmol/l	Turnover rate μmol/kg/h	Km mmol/l	Intralipid clearance K_2 %	PHLA FFA released μeq/l/h
Normal controls (27)	79	140	90	7	40	19	680	274	1.008	16	0.842	4.1	290
	±10	±10	±6	±3	±13	±6	±172	±45	±0.337	±2.9	±0.220	±0.8	±28
Non-obese diabetics (16)													
Before treatment	132.1	259.3	230.7	8.6	63	32.8	3225.5	784.8	4.335	17.1	3.512	1.32	57.4
	±46.7	±52.8	±51.1	±3.6	±39.9	±16.5	±1051.6	±242.9	±1.60	±3.1	±0.36	±0.96	±23.3
After treatment	86	156	128	8	38	28	1210	296	1.158	15.6	0.413	3.8	191
	±12	±16	±9	±3	±13	±12	±864	±63	±0.474	±2.7	±0.282	±0.88	±28
P	NS	<0.01	<0.01	NS	NS	NS	<0.001	<0.001	<0.001	NS	<0.001	<0.001	<0.001
Obese diabetics (7)													
Before treatment	132.6	308.6	266	7.4	86.3	55.9	2230.6	507	2.751	29.9	1.315	2.49	103.9
	±67.7	±125.9	±140.5	±3.3	±42.9	±39.7	±257.5	±114.6	±0.569	±2.9	±0.322	±0.72	±41.2
After treatment	94	162	125	10	36	24	795	290	1.220	15.5	0.324	3.75	172.3
	±8	±11	±8	±2	±11	±12	±154	±52	±0.460	±1.6	±0.141	±0.76	±32
P	NS	<0.01	<0.01	NS	NS	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

P compares the values before and after Phenformin therapy. NS = P > 0.05 using the students "t" test. Values are means ± S.D. The values between parenthesis represent the number of subjects studied in each group.

draw conclusions concerning the plasma lipid pattern in patients with diabetes. The plasma levels of triglyceride and cholesterol may vary from day to day and are dependent on the dietary habit and degree of obesity. Furthermore, the type of diabetes and the degree of diabetic control may also influence the plasma triglyceride levels. Khachadurian and Uthman [29] and Avogaro *et al.* [3] reported a significant elevation of serum triglycerides in a large, heterogeneous group of diabetics, as compared to non-diabetic subjects. Hayes [27] studied 106 untreated diabetics and found an incidence of 68% of hyperlipidaemia in these patients. On the other hand, Östman [39] did not find increased triglyceride levels in untreated diabetics, compared to a non-diabetic group matched for age and obesity.

The mechanism of hypertriglyceridaemia associated with juvenile diabetes has been thoroughly investigated in man and experimental animals. In man, deficiency of lipoprotein lipase and impaired clearance of triglyceride has been reported [4, 24].

The contribution of hepatic production of triglycerides in juvenile diabetes remains unclear. In alloxan diabetic rats the *in vitro* synthesis of hepatic triglycerides was markedly increased [11]. However,

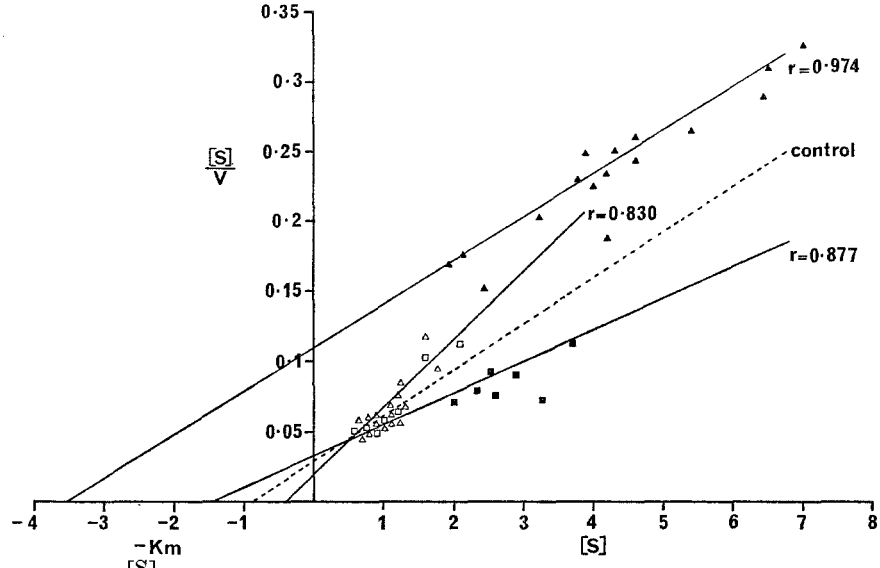

Fig. 3. The plot [S] against $\frac{[S]}{V}$ in the control group (---) and in obese and non-obese maturity onset diabetics before and after Phenformin therapy. Symbols as in Fig. 1. (S = serum triglyceride concentration in mmoles/l. V = serum triglyceride turnover rate in μmoles/kg/h)

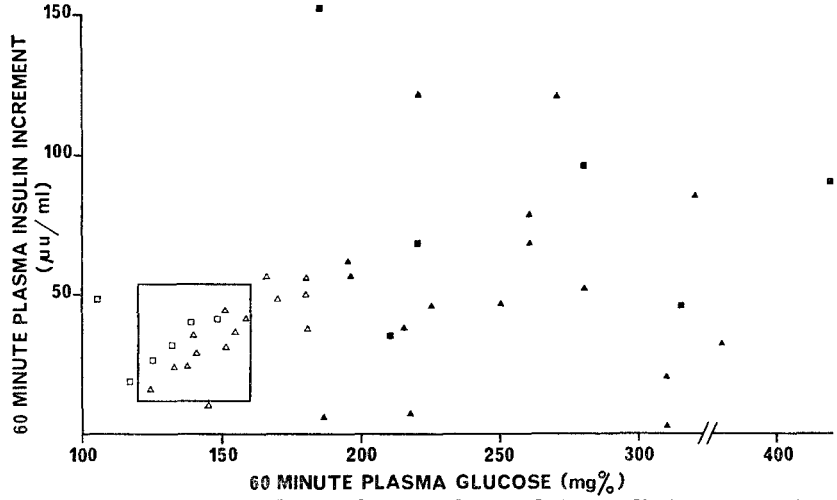

Fig. 4. The relationship between the 60 min plasma glucose values and the insulin increment above the fasting values in the control subjects and diabetic patients before and after Phenformin administration. The rectangle represents the mean values ± 2 S.D. in the control subjects. Symbols as in Fig. 1

in diabetic dogs the incorporation of plasma free fatty acid into plasma VLDL triglycerides was decreased [5]. In human juvenile diabetes early reports suggested that the hepatic production of triglyceride was normal [14, 42]. However, a recent investigation [38] has shown that hepatic triglyceride production was markedly raised and accounted for the hypertriglyceridaemia associated with this type of diabetes.

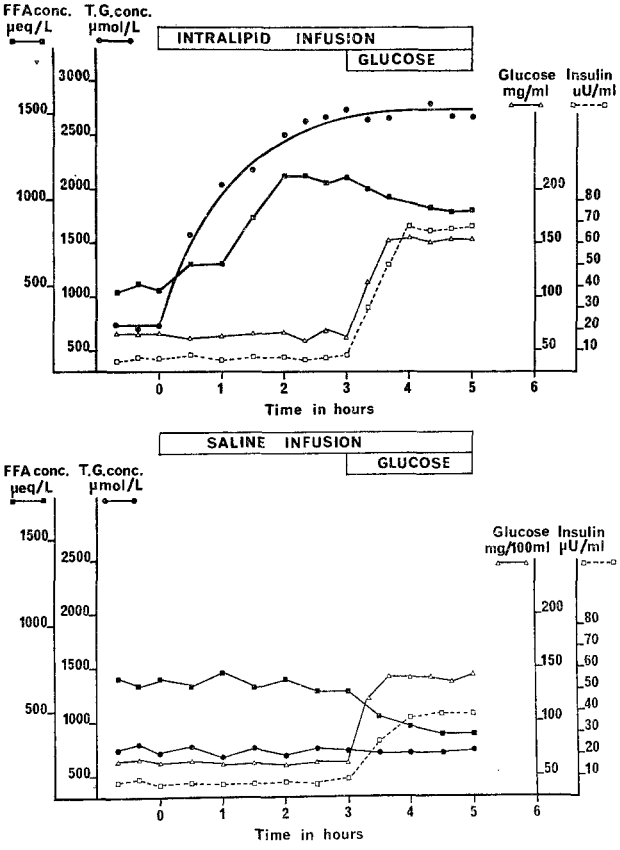

Fig. 5. Effect of experimental hypertriglyceridaemia induced by Intralipid infusion on plasma FFA and insulin response to glucose infusion in a normal subject. The lower graph shows the same results when saline was infused instead of Intralipid.

The dynamics of triglyceride transport in maturity onset diabetics have not been extensively studied and therefore it has not been possible to determine the relative importance of any one factor in the pathogenesis of the hypertriglyceridaemia associated with this disease. Elkeles *et al.* [17], in a preliminary survey, reported elevated triglyceride levels in 25% of maturity onset diabetics. It was further observed by the same authors that patients with moderate or high insulin response to oral glucose had higher triglyceride levels than those with poor insulin response. Farquhar *et al.* [19] studied the effects of high carbohydrate feeding on serum triglycerides and concluded that patients with high insulin responses were those who developed hypertriglyceridaemia. These results suggested that the hypertriglyceridaemia in this situation might be secondary to the high levels of circulating insulin stimulating hepatic triglyceride synthesis. In the present study an attempt has been made to evaluate the various parameters of free fatty acid and triglyceride transport kinetics in a group of 23 maturity onset hypertriglyceridaemic diabetics.

In the non-obese diabetic patients elevated plasma triglyceride concentrations were associated with normal triglyceride turnover rates. These results suggested that decreased efficiency of the triglyceride clearing mechanisms was the basis of the hypertriglyceridaemia observed in these subjects. This conclusion was also substantiated by the finding of a marked increase in the apparent Km of endogenous triglyceride clearance. Furthermore, the individual plasma triglyceride concentrations were not related to their production rate, which indicates that the degree of increase in triglyceride levels was determined by the deficiency of the removal system in this group of diabetics. The impairment of fractional transport rate of exogenous triglyceride in the form of Intralipid, added further evidence for this hypothesis.

The obese diabetics, on the other hand, showed higher values of triglyceride turnover rate as compared to the control group, suggesting that elevated triglyceride secretion into the plasma was in part responsible for their state of hypertriglyceridaemia. However, some degree of impairment of both endogenous and exogenous triglyceride clearance was observed in these patients. No definite conclusion, however, can be drawn from this study, since the number of patients studied in this group was small and our control group did not contain obese subjects. It is of importance to recognise that obesity is associated with increased plasma triglyceride turnover [34]; in fact there was a significant correlation between the degree of obesity and triglyceride turnover in this limited group of diabetics.

In the present study the plasma free fatty acid turnover in the diabetics was markedly accelerated, suggesting that fatty acid mobilisation was increased. Previous reports [39] have shown that adipose tissue from diabetics exhibits higher rates of lipolysis than non-diabetic tissue, when incubated *in vitro*. The association of enhanced lipolysis and impaired triglyceride clearance observed in these diabetic patients is not surprising since the enzymes, triglyceride lipase and lipoprotein lipase, regulating these two processes are known to change in a reciprocal direction in response to a number of physiological and pathological conditions. Thus Shafrir and Biale [47] have observed a decrease in adipose tissue lipoprotein lipase during stimulation of lipolysis, and Nikkilä and Pykälistö [35] found a negative correlation between the rates of mobilisation of fatty acids and the lipoprotein lipase activity in rat adipose tissue. In the present study we have also observed impaired release of lipoprotein lipase into the plasma, in response to heparin, associated with en-

hanced lipolysis. The activity of the lipoprotein lipase enzyme determines the overall disappearance of plasma triglyceride [44].

Wing and Robinson [51] reported evidence suggesting that the triglyceride lipase and lipoprotein lipase enzymes are controlled by a common mediator, 3′—5′ c-AMP. Elevation in tissue levels of this compound activates triglyceride lipase and inhibits lipoprotein lipase, resulting in enhanced lipolysis and impaired triglyceride clearance. Nikkilä and Pykälistö [35], on the other hand, suggested that the changes in lipoprotein lipase activity in this situation were secondary to the increased intracellular concentration of fatty acids, associated with enhanced lipolysis, which might exert regulatory control over the synthesis of the lipoprotein lipase.

be secondary to inhibition of lipolysis, resulting in reduced supply of fatty acids for plasma triglyceride synthesis. In support of this is the observation that reduction of plasma FFA by nicotinic acid is associated with lowering of the serum triglyceride levels in patients with maturity onset diabetes [3].

It is difficult to reconcile the association of enhanced lipolysis and the normal or moderately elevated insulin levels observed in the diabetic patients, since insulin exerts marked anti-lipolytic effects *in vivo* and *in vitro* [16, 50, 40]. It is possible, however, that the insulin sensitivity of the fat cells of these patients is reduced, resulting in less inhibition of fatty acid release. The observed insulin levels could therefore be secondary to the high concentration of circulating fatty acids and triglyceride substances, which have been reported to

Table 3. *Effects of experimental hypertriglyceridaemia on plasma triglycerides, F.F.A. and insulin response to glucose infusion*

Exp. No.	Type of study	Steady state concentrations						PHLA FFA released
		Before glucose infusion				During glucose infusion		
		Triglyc. conc. mmol/l	FFA conc. μeq/l	Glucose conc. mg%	Insulin conc. μu/ml	Glucose conc. mg%	Insulin conc. μu/ml	μeq/l/h
1.	Control	0.720	620	72	7	145	38	285
	Intralipid	2.650	908	68	9	156	68	190
2.	Control	1.210	910	96	11	140	42	320
	Intralipid	3.860	1260	110	8	149	82	118
3.	Control	1.016	725	66	4	162	28	226
	Intralipid	4.860	1286	74	3	148	113	174
4.	Control	0.650	680	96	8	138	29	318
	Intralipid	6.800	1220	82	6	146	126	126
P		< 0.001	< 0.01	NS	NS	NS	< 0.001	< 0.001

P compares the paired data from the infusion studies with the control values. Levels of significance were determined using the students "t" test.
NS = $P > 0.05$.

It is therefore possible that, in the present study, the impairment in plasma triglyceride clearance observed in the diabetic patients was secondary to enhanced lipolysis. The changes observed during Phenformin therapy add further support to this hypothesis. Phenformin has been shown to exert anti-lipolytic effects in rat adipose tissue [6, 18]. Similar effects have also been observed in human adipocytes [Kissebah unpublished]. The results of the present investigation also show that this drug lowers the plasma FFA concentration and flux in diabetic subjects. The reduction in fatty acid mobilisation was associated with marked improvement in plasma triglyceride clearance. Thus the Km of endogenous triglyceride clearance and the K_2 of Intralipid removal were markedly improved during Phenformin therapy. The post heparin plasma lipolytic activity was also increased during treatment. In the obese diabetics, however, the reduction in serum triglyceride concentration was, at least in part, due to inhibition of triglyceride release into the plasma. This effect might also increase insulin secretion both *in vivo* and *in vitro* [23, 36, 33]. In the present study normal subjects were infused with Intralipid and the plasma insulin response to a glucose infusion was determined. It was observed that the elevation in plasma free fatty acid and triglycerides during the Intralipid infusion augmented the insulin response of these subjects without alteration in the plasma glucose profile. Furthermore, in the diabetic patients the reduction in plasma FFA flux during Phenformin therapy was associated with a decrease in the plasma insulin response following a glucose load.

It is also possible that the hyperglycaemia observed in the diabetic patients was in part due to the enhanced lipolysis, since FFA compete with glucose for oxidation by skeletal muscle [40]. Elevations of plasma FFA by infusion of fat emulsions [45, 46, 22] resulted in impaired glucose utilisation. In the present study the reduction in plasma FFA flux induced by Phenformin was associated with improvement in glucose tolerance.

These results, however, do not exclude the possibility of direct effects by Phenformin upon the glucose insulin relationships.

As regards the nature of the initial lesion responsible for the insulin insensitivity observed in these patients, our previous studies in human adipose tissue biopsies suggested that the responsiveness of the glycerides were labelled using two different techniques. In the first technique the plasma VLDL triglycerides were labelled endogenously during a continuous infusion of ^{14}C palmitate, described under the method section in this paper. In the second technique plasma VLDL triglycerides were labelled endogenously with ^{14}C palmitate and withdrawn to be re-injected into the

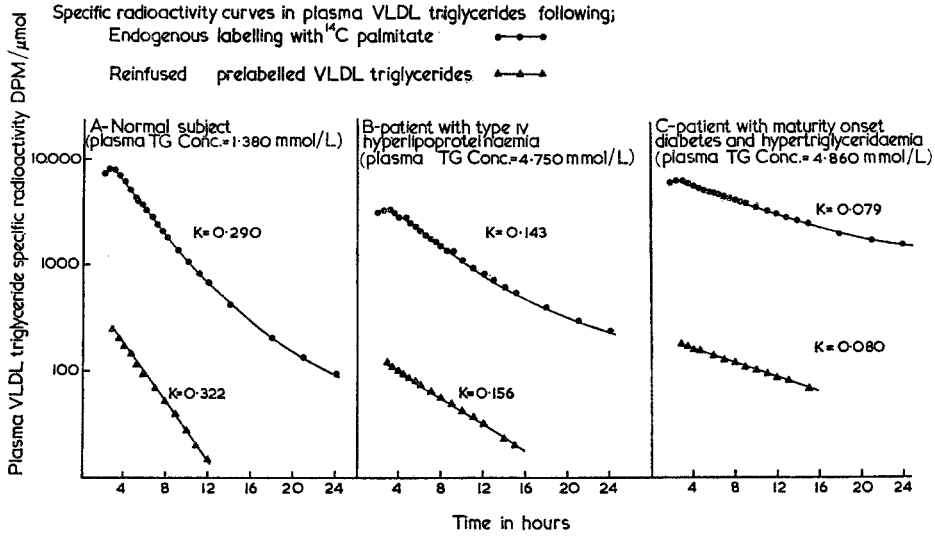

Fig. 6 (Appendix). Plasma VLDL triglyceride specific radioactivity following endogenous labelling with ^{14}C palmitate, and re-injected prelabelled VLDL triglyceride. $\left(K = \frac{0.693}{t\frac{1}{2}}\right)$

Table 4. *Mean (with ranges) measurements of plasma VLDL triglyceride transport in man obtained using two techniques (see text)*

Group studied	Plasma triglyceride Conc. mmol/l	Plasma VLDL triglyceride turnover rates		
		Endogenous labelling with 14C palmitate μmole/kg/h	Reinfusion of 14C prelabelled VLDL triglyceride	
			Total transport μmole/kg/h	% of total triglyceride transport converted to plasma FFA
Normal subjects N = 8	0.891 (0.500—1.380)	15.4 (11—20)	17.1 (14—22)	16.9 (12.7—26)
Type IV hypertriglyceridaemic patients N = 4	3.012 (2.10—4.75)	30 (26—34)	33.5 (26—36)	15.3 (10—18.5)
Diabetic patients N = 4	2.862 (2.10—3.80)	14.3 (12—17)	15 (12—18)	18.1 (14.4—21.3)

N = Number of subjects studied

enzymes regulating the c-AMP levels in this tissue was diminished [30]. The resultant, elevated intracellular c-AMP levels stimulate the release of fatty acids in spite of the normal levels of circulating insulin. How much of this derangement is due to a primary cellular abnormality or due to humoral factors remains to be identified.

Appendix

Fig. 6 shows the disappearance curves of VLDL triglycerides in three subjects in whom the plasma triglycerides were labelled using two different techniques. In the first technique the plasma VLDL triglycerides were labelled endogenously during a continuous infusion of ^{14}C palmitate, described under the method section in this paper. In the second technique plasma VLDL triglycerides were labelled endogenously with ^{14}C palmitate and withdrawn to be re-injected into the same patient two weeks later. These curves are part of a study performed in 8 normal subjects and 8 patients with hypertriglyceridaemia to assess the validity of the techniques employed to measure plasma triglyceride turnover [32].

The parallelism between the curves (Fig. 6) and the close agreement between the turnover values determined by the two techniques (Table 4) indicated that direct recycling of fatty acids from plasma triglycerides into the plasma FFA pool was minimal and did not affect the triglyceride radioactivity curves. In

order to quantitate this pathway more precisely, the plasma FFA radioactivity curves were analysed after injection of ^{14}C prelabelled VLDL triglycerides and ^{3}H labelled FFA [48]. The results shown in Table 4 indicate that less than 20% of the total plasma triglyceride transport can be converted immediately to plasma FFA. When one considers the fact that the plasma FFA flux is about 4—5 fold higher than that of triglycerides, this recycling can account for less than 5% of the plasma FFA flux. As evident from this study, as well as others [26], less than 20% of the plasma FFA flux is converted to triglycerides. It becomes apparent, therefore, that recycling can produce negligible effects on the turnover measurement both in normal and hypertriglyceridaemic subjects.

Using the technique described the rate constant for plasma triglyceride transport in normal subjects ranged between 0.211—0.492. These values are comparable to those of Friedberg *et al.* [21] who used an integration method and those reported by Reaven *et al.* [41], and Nikkilä and Kekki [37] who used the ^{3}H glycerol labelling technique. These results encouraged us to use the endogenous labelling technique for its simplicity and convenience.

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