Synthesis of plasma triglycerides in endogenous hypertriglyceridemia

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ABSTRACT Radioisotopic kinetic studies of triglyceride fatty acid synthesis from serum free fatty acids have been performed in 20 studies of normal and lipemic subjects. The lipemic subjects were characterized as having carbohydrate-responsive endogenous lipemia, and were classified as having either Type III or Type IV prebetalipoproteinemia. In the untreated state, triglyceride production was reduced relative to concentration of triglyceride when compared with the normal control population. In response to carbohydrate restriction an absolute reduction in triglyceride synthesis from free fatty acids was demonstrated. These data indicate that overproduction cannot be importantly implicated as the etiology of this form of endogenous lipemia. The patients thus represent a pathophysiological entity which is distinct from the normal physiological lipemia induced by carbohydrate feeding in which overproduction is reported to be the initiating event.

SUPPLEMENTARY KEY WORDS kinetic study · pre-β-lipoprotein

The importance of plasma FFA during fasting as the predominant precursors of plasma TGFA has been demonstrated in both normotriglyceridemic and hypertriglyceridemic subjects (1–3). However, the contribution of this synthetic pathway of production to the pathophysiology of abnormal endogenous lipemia has been the subject of some controversy. In 1965 Reaven, Hill, Gross, and Farquhar observed subjects with arteriosclerotic cardiovascular disease fed high carbohydrate diets, and they reported a close correlation between the subsequent rising TGFA production and rising $S_f > 20$ TGFA concentration (4). At the same time, Ryan and Schwartz studied a group of subjects with hyperlipemia of diverse etiology and concluded that plasma TGFA "clearance" is a more important determinant of plasma TGFA concentration than is production (5). A year later Sailer, Sandhofer, and Braunsteiner (6) reported that "influx" of triglyceride into the plasma is highly correlated with the log of TGFA concentration in normal and diabetic subjects, but not in patients with essential hyperlipemia. This led them to conclude that mechanisms of utilization were more important than production in determining lipemia in these patients. In each study, differences in patient population may have been partially responsible for the different conclusions as to the role of production in the physiology of endogenous hyperlipemia. For this reason, we have elected to examine the role of TGFA production in pathological lipemia by studying lipid metabolism in a selected population with carbohydrate-responsive endogenous lipemia, classed as Fredrickson Type III and IV.

The analysis has been performed utilizing a multicompartmental model which simulates the kinetic behavior of plasma FFA and TGFA in man under conditions of both normal and abnormal lipid metabolism (2). This model allows the evaluation of TGFA production from serum FFA precursors using data obtained from a single injection of tracer FFA. In the present investigation we have examined normal subjects, pathologically hyperlipemic subjects, and lipemic subjects both before and after dietary carbohydrate restriction. These studies suggest that TGFA production is linearly related to TGFA concentration in both control and lipemic populations. However, production appears inappropriately low in these hyperlipemic patients relative to triglyceride concentration as predicted from the study of control subjects. In response to carbohydrate restriction a significant reduction in TGFA production is uniformly observed.

Abbreviations: FFA, free fatty acids; TGFA, triglyceride fatty acids.

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METHODS

20 studies were carried out in 17 fasting subjects. Seven subjects were normal volunteers with no evidence of organic disease and with normal lipid levels (Tables 1 and 2). 10 subjects were classified as having pathologic carbohydrate-responsive endogenous hyperlipemia as shown in Table 1. All patients demonstrated an elevation in fasting plasma TGFA of at least three times that seen in controls, and all lipemic patients demonstrated a significant fall in serum TGFA concentration ranging from 84% to 30% of initial levels following carbohydrate restriction. One subject, J.W., could be classified as strictly Type III, based upon the demonstration of floating β-lipoprotein in the serum supernatant following ultracentrifugation at d 1.006. Four subjects, J.F., E.B., M.S., and M.J., could not be confirmed as Type III by ultracentrifugation, but the presence of xanthoma tuberosum or eruptum argued against a clear designation as Type IV, in spite of the Type IV pattern on paper electrophoresis. The last five subjects had no xanthoma, no floating β-lipoprotein, and distinct Type IV patterns as determined by paper electrophoresis of their serum lipoproteins. The group ranged in age from 30 to 50 yr, and was made up of six male and four female subjects. All patients were found to have a normal protein-bound iodine level, none were taking oral contraceptive therapy, and alcoholism was not apparent. The serum cholesterol ranged from 150 to 485 mg per 100 ml in the group with simple Type IV lipemia; from 320 to 800 mg per 100 ml in the group of indefinite

TABLE 1 General Description of Patients Studied

Patient	Age	Sex	Weight	Lipoprotein Lipase	Percentage fall in TGFA†	
	yr		*kg*	*μmoles/min/ml*		
Normal controls						
R.R.*	18	M	84.9			
L.F.*	19	M	79.0			
S.A.*	20	F	58.0			
L.B.*	20	F	67.9			
J.W.	64	F	67.0	0.18		
P.C.	57	F	55.0	0.21		
E.R.	48	F	76.6	0.16		
Hyperlipemic subjects					*μmoles/ml*	
J.W.	44	M	70.5	ND‡	63	Xanthoma tuberosum, floating beta lipoprotein at d 1.006; mother, Type IV; serum lipoprotein pattern, Type III; serum cholesterol 775 mg/100 ml untreated.
J.F.	38	M	86.5	0.13	80	Xanthoma tuberosum, no floating beta, serum cholesterol 400 mg/100 ml; daughter, Type IV; serum lipoprotein pattern, Type IV (?III).
E.B.	33	F	67.0	0.21	69	Xanthoma eruptum, no floating beta, serum cholesterol 700 mg/100 ml; serum lipoprotein pattern, Type IV; no chylomicrons in standing serum (?Type III).
M.S.	44	F	60.5	0.16	74	Xanthoma tuberosum, no floating beta, serum cholesterol 320 mg/100 ml; serum lipoprotein pattern, Type IV (?Type III).
M.J.	50	F	60.6	ND	85	Xanthoma tuberosum, no floating beta, serum cholesterol 800 mg/100 ml; serum lipoprotein pattern, Type IV (?Type III).
M.W.	44	F	74.5	0.24	79	No xanthoma, no floating beta, serum cholesterol 309 mg/100 ml; serum lipoprotein pattern, Type IV.
N.S.	30	M	78.0	ND	30	No xanthoma, no floating beta, serum cholesterol 261 mg/100 ml; serum lipoprotein pattern, Type IV.
A.B.	38	M	61.0	0.18	82	No xanthoma, no floating beta, serum cholesterol 485 mg/100 ml; serum lipoprotein pattern, Type IV; daughter, Type IV; brother, Type IV.
N.B.	34	M	86.0	ND	57	No xanthoma, no floating beta, serum cholesterol 213 mg/100 ml; serum lipoprotein pattern, Type IV.
J.J.	26	M	79.0	ND	68	No xanthoma, no floating beta, serum cholesterol 150 mg/100 ml; serum lipoprotein pattern, Type IV.

* Previously reported, Eaton, Berman, and Steinberg (2).
† Maximum change in serum TGFA concentration from pretreatment levels to postcarbohydrate restriction levels (see text).
‡ ND, not determined.

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TABLE 2 Relationship between Plasma FFA and TGFA Concentration, and TGFA Production from FFA Precursors in All Subjects without Treatment

Patient	Plasma FFA Concn	FFA Pool Size*	Plasma TGFA Concn	Production Rate of TGFA	Total Production from FFA of TGFA*
	μeq/ml	*μeq*	*μeq/ml*	*min⁻¹*	*μeq/min*
Normal controls					
R.R.†	0.70	2020	4.1	0.029	59
L.F.†	0.70	2020	3.8	0.014	28
L.B.†	0.60	1730	5.0	0.023	41
S.A.†	0.50	1160	3.4	0.014	16
J.W.	0.75	2180	5.4	0.028	61
P.C.	0.73	2120	4.8	0.029	61
J.R.	0.55	1590	3.3	0.019	30
Normal mean ± SD				0.022 ± 0.006	42 ± 17
Hyperlipemia, untreated					
J.W.(III)	0.75	2180	27.0	0.018	39
J.F. (III–IV)	0.65	1880	41.0	0.036	67
E.B. (III–IV)	0.78	2260	55.0	0.035	80
M.S. (III–IV)	0.93	2700	27.0	0.022	59
M.J. (III–IV)	0.75	2180	14.0	0.013	28
M.W. (IV)	0.62	1792	20.0	0.018	32
N.S. (IV)	0.50	1445	30.0	0.029	42
A.B. (IV)	0.70	2020	36.0	0.024	48
N.B. (IV)	0.49	1417	21.0	0.029	41
J.J. (IV)	0.40	1160	19.0	0.025	29
Lipemic group mean ± SD				0.024 ± 0.007	46 ± 16

* Calculated from estimated total plasma volume (41.3 ml/kg) and normalized to a body weight of 70 kg.
† Previously reported (2).

designation as Type III–IV; and was 775 mg per 100 ml in the pure Type III patient. No patient was found to have fasting chylomicrons by either lipoprotein electrophoresis or observation of the standing serum at 5°C. Lipoprotein lipase activity was normal in those patients tested and none were overtly diabetic in the sense of having glycosuria, polyuria, polydipsia, or requiring treatment for hyperglycemia. The glucose and insulin interrelationships in this population are the subject of a separate report.

All subjects were maintained for at least 2 wk before tests on an isocaloric standard clinical research unit diet with a caloric intake of 38% from carbohydrate, 45% from fat, and 17% from protein. At the end of this dietary period, diagnostic procedures were performed including postheparin serum lipoprotein lipase assayed with Lipomul as substrate (7), serum lipoprotein fractionation by ultracentrifugation and paper electrophoresis (8), and quantitative serum TGFA, cholesterol, and FFA analysis (2). Carbohydrate restriction was attained by feeding isocaloric low carbohydrate diets, with a caloric intake of 15% from carbohydrates, 53% from fat, and 42% from protein, for a period of 4 to 6 wk prior to tests.

The subjects were permitted no food after the evening meal, i.e., for about 16 hr before the injection of labeled substrate. Two intravenous catheters were inserted, one in each antecubital vein, after which the subjects rested comfortably in bed for at least 40 min before the administration of radioisotope. After three control blood samples had been drawn from one catheter, the appropriately labeled fatty acid albumin solution (palmitic-^{14}C or oleic acid-^{14}C) was rapidly injected through the other catheter (within 10 sec). The dose administered varied from 6 to 10 μCi of ^{14}C and represented a true tracer amount (between 0.005 and 0.01 meq of fatty acid). After rapid injection of FFA-^{14}C, blood samples were taken at 2-min intervals for 20 min, and then at 10-min intervals for the duration of the first hour. Thereafter, samples were taken at 15-min intervals for 3–4 hr. Blood samples were collected in dry syringes, transferred to tubes containing heparin, mixed thoroughly, and immediately placed in ice until analytical procedures could begin.

Measurements of plasma FFA and TGFA specific radioactivity were performed as previously described (2). The data were analyzed and fitted to the multicompartmental model previously reported by Eaton, Berman, and Steinberg in detail (2). As illustrated schematically in Fig. 1a, this model describes the synthesis of triglyceride from plasma FFA precursors (TGFA production). The actual computer model is shown in Fig. 1b, where the plasma pools of FFA and

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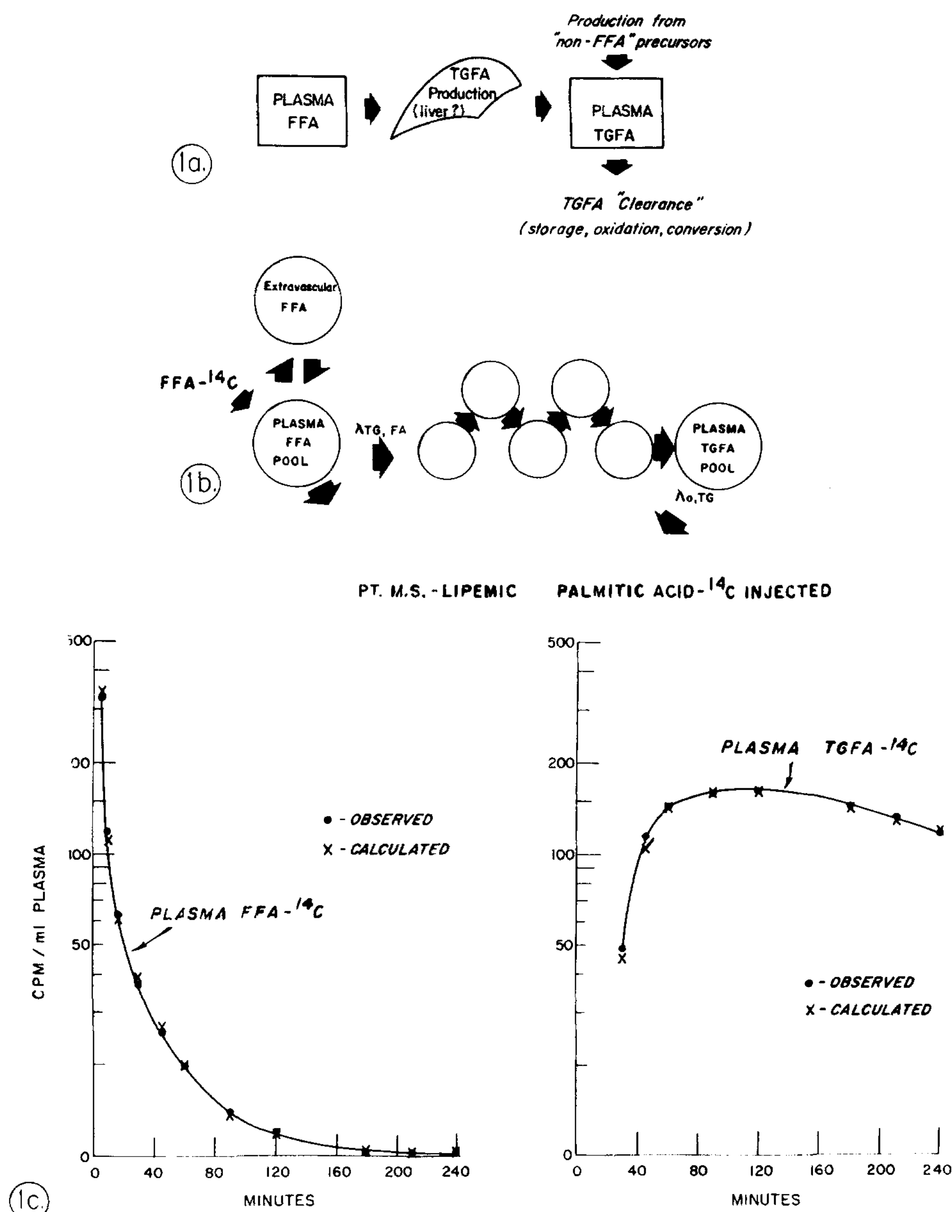

FIG. 1a. Schematic representation of plasma TGFA synthesis from plasma FFA precursors and other precursors, and of plasma TGFA clearance for various pathways of utilization.

1b. Compartmental model describing plasma FFA and TGFA metabolism in man as previously described in detail by Eaton et al. (2), derived by computer analysis.

1c. Plasma TGFA and FFA radioactivity as a function of time after the intravenous injection of FFA-^{14}C in a lipemic subject (M.S.). The closed circles represent the experimental values; the crosses represent the data generated from computer analysis using the multicompartmental model shown in Fig. 1b.

TGFA are calculated from the calculated plasma volume and the chemically determined lipid concentrations. The fraction of the plasma FFA pool flowing into TGFA synthesis is described by the rate constant λTG, FA, which is derived following the simultaneous fitting of both actual and theoretical data for precursor (FFA) and product (TGFA) specific activity. A least squares method using the NIH-SAAM program in the IBM 360 digital computer is utilized (2). In Fig. 1c is shown an example of the change in specific activity of plasma FFA with time, and of the buildup and decay of plasma TGFA activity in hyperlipemic subject M.W. It can readily be appreciated that the model-generated data (X) and the actual data (•) are superimposable, indicating the appropriate nature of the model and the resolved rate constants.

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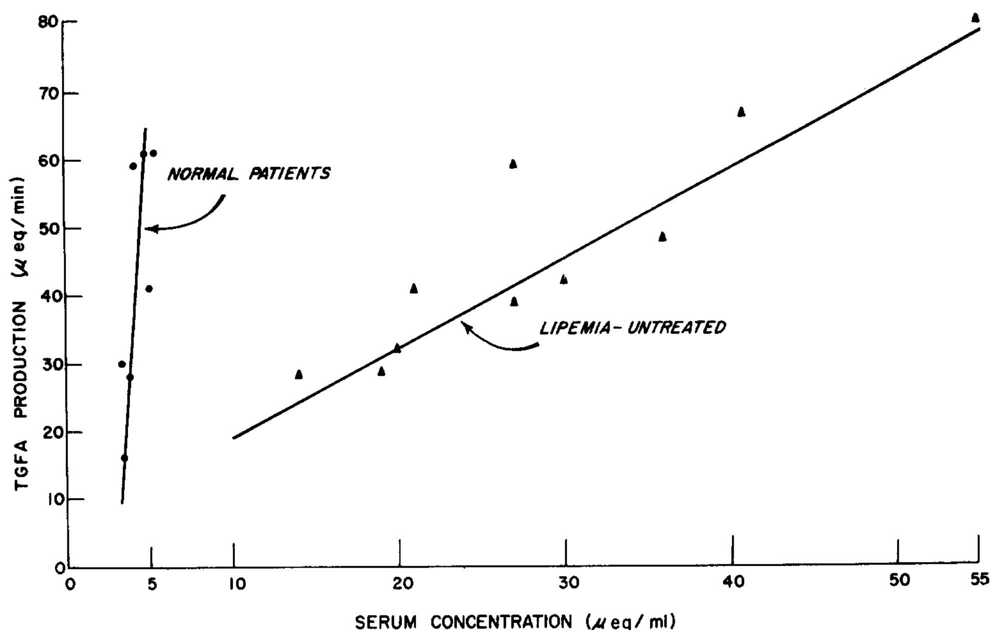

FIG. 2. Relationship between TGFA production and concentration in normal subjects and in untreated patients with endogenous lipemia.

RESULTS

Normal Subjects

The relationship in normal subjects between plasma triglyceride concentration and production from FFA precursors is tabulated in Table 2. The flow of FFA into TGFA production averaged 2.2 ± 0.6% of the plasma FFA pool per min (range 1.4–2.9%), resulting in a mean TGFA production of 42 ± 17 μeq/min (range 16–61 μeq/min).

The relationship between TGFA production and TGFA concentration is graphically depicted in Fig. 2. Within the narrow range of triglyceride concentration observed in normal subjects, with increasing triglyceride concentration there is a linear increase in triglyceride production ($r = 0.760$, $P < 0.05$). This observation is consistent with earlier data of Reaven et al. (4), who demonstrated in normal subjects a linear rise in triglyceride turnover rate up to concentrations of approximately 100 mg per 100 ml (i.e., 3 μeq/TGFA per ml), as well as with the data of Sailer et al. (6). However, the triglyceride concentrations in our studies range only from 3.3 to 5.4 μeq/ml, which prevents any generalizations relating production to concentration at higher triglyceride levels in normal subjects.

Hyperlipemic Subjects, Untreated

The triglyceride concentration in the overnight-fasted hyperlipemic subjects on a regular diet ranged from 14 to 55 μeq of TGFA per ml (Table 2). Neither the fractional conversion of FFA to TGFA (2.4 ± 0.7%) nor the absolute production from FFA precursors (46 ± 16 μeq/min) is significantly different from the observations noted in normal subjects.

As indicated in Fig. 2, the relationship between TGFA production and plasma TGFA concentration is abnormal relative to the control population. A tendency for increasing production relative to increasing concentration of TGFA is suggested ($r = 0.918$, $P < 0.001$), but at all concentrations of TGFA the production is inappropriately low. This observation in patients with pathological lipemia on a normal diet is at variance with the data in subjects with physiologic lipemia on a high carbohydrate diet reported by Reaven et al. (4). The latter group demonstrated a continuing extension of the normal rise in triglyceride turnover as higher triglyceride concentrations were obtained with carbohydrate induction. The limited number of subjects investigated in both studies requires confirmation of these observations by future investigations.

Response to Carbohydrate Restriction (*Table 3*)

Three hyperlipemic patients were studied both in the untreated state and again after a period of carbohydrate restriction (see Methods). Two of the patients had attained a maximum reduction in serum TGFA at the time of the second study, while the third (M.W.) was studied prior to the maximum response to therapy. In spite of the use of an isocaloric diet, all three patients demonstrated some reduction in body weight: 1.2%, 9.5%, and 3.7% for J.F., E.B., and M.W., respectively. In each case, the rate of production of TGFA from FFA

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TABLE 3 Plasma TGFA Production from FFA Precursors before and after Carbohydrate Restriction

Patient	Weight	Plasma FFA Pool	Plasma TGFA Concn	Production Rate of TGFA	Total Production from FFA
	kg	*μeq*	*μeq/ml*	*min⁻¹*	*μeq/min*
J.F. (III–IV)					
Before	87.1	1880	41	0.036	67
After	86.0	2590	6	0.013	33
E.B. (III–IV)					
Before	67.4	2260	55	0.035	80
After	61.0	2159	17	0.028	60
M.W. (IV)					
Before	76.3	1756	20	0.018	32
After†	73.5	1792	16	0.012	20

* Calculated from estimated total plasma volume (41.3 ml/kg) and normalized to a body weight of 70 kg.

† Studied prior to maximum reduction in TGFA.

was reduced following carbohydrate restriction relative to the untreated control state. The absolute change in total TGFA production from FFA was 34, 20, and 12 μeq/min, respectively, which corresponds with the observed fall in plasma TGFA concentration of 35, 38, and 4 μeq/ml.

DISCUSSION

The use of compartmental analysis in the evaluation of the physiology of plasma TGFA provides significant additional information to the understanding of endogenous lipemia by allowing the independent determination of TGFA production from FFA. The total TGFA production of 42 ± 17 μeq/min observed in this study is comparable to the data of Friedberg, Klein, Trout, Bogdanoff, and Estes, which indicated a range of 20 to 65 μeq/min in their normal subjects (9), and to the studies of Havel, Kane, Balasse, Segel, and Basso, in which net production of radioactive very low density TGFA in seven normal subjects averaged 37 ± 3.2 μmoles/min per 1.75 m^2 (10).

The mechanism involved in the normal physiological phenomenon of lipemia induced by carbohydrate has been studied by several investigators. Waterhouse, Kemperman, and Stormant reported that diets high in carbohydrate will increase the transfer of FFA into plasma triglycerides in normal subjects (11). Nestel and Hirsch studied three normal young men and observed that both increased production and decreased utilization contribute to the induced lipemia (12). Reaven et al. (4) examined an older population in which "arteriosclerotic cardiovascular disease" was the predominant diagnosis. These observations suggested that TGFA concentration and turnover are highly correlated, and that increased production of TGFA represents the basis for the rising plasma $S_f > 20$ TGFA concentration following carbohydrate feeding in this population (4).

The pathophysiology of lipema in the untreated hyperlipemic subjects studied in this investigation appears to be different from the overproduction state observed during physiological carbohydrate-induced lipemia. Instead of being increased, production is not significantly different from that seen in normal subjects; it is in fact distinctly low in relation to the plasma triglyceride concentration as predicted from the study of normal subjects. It seems likely that a clearance defect is the primary abnormality and that the inappropriately low triglyceride production relative to TGFA concentration represents a physiological attempt, albeit inadequate, to compensate for the inability to clear the excessive circulating TGFA. This conclusion is supported by the earlier studies of Ryan and Schwartz (5) and of Sailer et al. (6), also involving patients with essential endogenous lipemia. Utilizing a constant infusion of labeled fatty acid, both groups of investigators concluded that triglyceride production cannot account for the lipemia, and that removal of triglyceride from the blood is impaired. More recently, Havel et al. (10) and Quarfordt, Frank, Shames, Berman, and Steinberg (13) have independently concluded from isotopic data that efficiency of mechanisms for extrahepatic removal must be a major determinant of the concentration of triglycerides in the blood of both normolipemic and hyperlipemic subjects.

The mechanism of TGFA clearance is not defined by these isotopic kinetic studies. Since lipoprotein lipase activity was normal in those hyperlipemic subjects tested, it would appear that other changes in physiology not mediated by this mechanism must be of more quantitative importance for endogenous lipids. Fredrick-

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son, Ono, and Davis (7) have also reported that the plasma lipoprotein lipase activity is quantitatively normal in patients with carbohydrate-responsive pathologic lipemia.

The fall in serum TGFA concentration in response to therapy confirms many observations in the literature that carbohydrate restriction is of considerable clinical utility to this patient population. In spite of the isocaloric diet, each patient sustained a fall in body weight which, in subject E.B., was almost 10% of the control weight. The contribution of weight reduction to the effects of carbohydrate restriction cannot be separated in this study, but it is well known that weight reduction will reduce serum lipids in many patients. Our data suggest that the fall in serum triglyceride concentration is mediated largely by an absolute reduction in TGFA production from FFA. Nestel and Hirsch have reported a similar fall in TGFA production from FFA in normal subjects on reduced carbohydrate intake (12). This observation suggests that the reduction in serum lipid may not be the result of preferential sequestering of TGFA in vessel walls and other body tissues, but actually represents a normal physiological regulating mechanism focused at the site of TGFA production.

It is of interest that the physiological behavior of serum TGFA in this group of patients appears homogeneous, yet the classification based upon serum lipoprotein typing indicates two disease entities, Type III and Type IV. In terms of response to carbohydrate restriction, and in terms of TGFA production relative to concentration, it would appear that we are dealing with several populations which have in common this disturbance in pre-β-lipoprotein physiology. Further studies are needed to clarify this point.

This investigation describes only the contribution to TGFA production from FFA precursors and offers no information relative to a contribution from non-FFA precursors. However, extensive studies both in man and in animals have failed to implicate importantly any precursor in the fasting state other than the free fatty acid pool (1–3, 14, 15).

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