The Hyperlipemic Effect of a Low-fat, High-carbohydrate Diet in Diabetic Subjects

Edwin L. Bierman, M.D., and James T. Hamlin, III, M.D., New York

Low-fat, high-carbohydrate diets, until recently avoided in the treatment of diabetic patients, have been shown to lower serum cholesterol levels and reduce the severity of vascular complications, while improving glucose tolerance and lowering insulin requirements.[1-5]

However, increases in the concentration of plasma triglyceride (hyperlipemia) have been noted in nondiabetic subjects during the administration of low-fat, high-carbohydrate diets. Many of the patients with essential hypertension who had been maintained on rice diets (85 to 95 per cent carbohydrate) for several months developed a "neutral fat" lipemia.[6,7] Increased plasma tritriglyceride levels have been observed in normal subjects[8] and patients with coronary artery disease[9] during the feeding of low-fat, liquid formula diets. Increases in plasma low density lipoproteins (S_f 100-400) were also noted in normal subjects on low-fat diets.[10] A major proportion of patients with essential hyperlipemia became more markedly lipemic when carbohydrate was isocalorically substituted for fat in the diet.[11]

The lipemic effect of these low-fat, high-carbohydrate diets may, however, be only temporary. In a recent study of the long-term effects of reduction of dietary fat calories in South African white and Bantu prisoners, it was observed that serum triglyceride levels returned to normal after several months.[12] Elevated levels in patients with essential hyperlipemia, maintained on rice diets, also returned toward control values after three months.[13]

Previous observations in diabetic subjects appeared to indicate that hyperlipemia was not the result of increased dietary fat levels;[14] indeed, amelioration of lipemia during treatment with high-fat diets had been reported.[15,16] The more recent evidence linking hyperlipemia with the administration of low-fat, high-carbohydrate diets raised the question of whether insulin-dependent diabetics would respond similarly.

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From the Hospital of The Rockefeller Institute, New York, New York.

METHODS

Patients with severe adult or juvenile diabetes, without renal or hepatic disease, served as subjects. Six studies were performed in five patients on a metabolic ward. They received diets kept at constant caloric levels designed to maintain body weight. Insulin dosages were also kept constant whenever possible. Two diets were compared: (1) a basal formula, containing 15 per cent protein calories, 40 per cent fat calories as corn oil, and 45 per cent carbohydrate calories, fed as a liquid diet; (2) a low-fat, high-carbohydrate diet, containing 15 per cent protein calories, 85 per cent carbohydrate calories, and virtually no fat, fed as a liquid diet with rice and matzoh (unleavened bread) supplements, for periods ranging from two to ten weeks. The subjects received supplementary multivitamins, iodized salt, and ferrous gluconate.

Samples of venous blood were obtained after an overnight fast prior to the administration of insulin. Glucose was measured in filtrates of whole blood[17] using the enzymatic reagent Glucostat (glucose oxidase, horseradish peroxidases, phosphate buffer, and o-dianisidine; Worthington Biochemical Corp., Freehold, New Jersey), as described by Saifer and Gerstenfeld.[18] Plasma nonesterified fatty acids were determined by the single extraction method of Dole.[19] Measurements of total cholesterol[20] and lipid phosphorus[21] were made on aliquots of serum.

Triglyceride analyses were performed by a modification of a procedure previously described.[22] A 1 ml. aliquot of plasma is extracted in 20 ml. chloroform-methanol (2:1 V:V), filtered into a glass-stoppered, narrow-necked bottle graduated at 25 ml., and made up to volume. Five ml. water is added, and the two phases allowed to separate by standing overnight at 4° C. The upper aqueous phase is removed by gentle suction and the chloroform phase brought to a final volume of 25 ml. with additional chloroform-methanol (2:1 V:V). Aliquots of this extract are dried and then redissolved in petroleum ether (BP 30°-60°).

Lipids in the petroleum ether extract are fractionated

by a modification of the procedure described by Hirsch and Ahrens[23] using batch elution.[24]

An aliquot of the petroleum ether extract is shaken several minutes with 0.5 gm. activated silicic acid (Bio Rad Laboratories, Berkeley, California). The petroleum ether is then removed and discarded by suction through a glass-wool-plugged glass adaptor placed into the silicic acid slurry. The silicic acid is next eluted with anhydrous ethyl ether (approximately 3 ml. shaken with silicic acid followed by three ether washes). This fraction contains glycerides, free of phospholipids and interfering substances. After evaporation to dryness it is saponified and extracted by the Carlson method,[25] modified as follows: 1 ml. of freshly prepared 0.4 per cent of KOH in 95 per cent ethanol is added to the samples, to a tripalmitin standard (approximately 0.5 μM), and to a blank tube. The glycerides are hydrolyzed at 65° C. for thirty minutes. Following cooling, 2.0 ml. water, 0.1 ml. 10 N H_2SO_4, and 4 ml. of ethyl ether are added to each tube. The phases are mixed well, allowed to separate, and the upper phase carefully removed and discarded. One ml. aliquots of lower phase are transferred into flasks for determination of glycerol;[26,27] 0.5 ml. .05 M sodium metaperiodate is added, and the reaction allowed to proceed ten minutes at room temperature. Then 0.5 ml. 0.5 M sodium arsenite and 3.0 ml. water are added and the flasks thoroughly mixed. Duplicate 1.0 ml. aliquots are transferred into 100 x 13 mm. tubes, and 10 ml. of 0.2 per cent chromotropic acid reagent is added. The tubes are capped loosely, mixed thoroughly and heated in a boiling water bath away from direct light for thirty minutes. After cooling, optical densities are read at 570 mμ.

Total urine collections were made, preserved under toluene and refrigerated. Aliquots were analyzed for reducing substances by titration with copper sulfate,[28] and for total organic acids by the method of Van Slyke and Palmer[29] modified for use with a *p*H meter.

Paper electrophoresis was performed with RSCO Model E-800-2 apparatus (Research Specialties Co., Richmond, Calif.) on Whatman No. 3 MM paper in Veronal buffer, *p*H 8.6, ionic strength 0.06. Aliquots of plasma (approximately 0.05 ml.) were applied to a free hanging filter paper strip (3.5 x 54 cm.) and a constant voltage of 200 volts applied for sixteen hours at room temperature. The strips were dried in air and stained for lipoproteins with Fat Red 7 B (Ciba) according to the method of Straus.[30] Densitometric measurements of the lipid stained strips were made on a Photovolt recording densitometer using a 525 mμ filter.

The removal rate of particulate fat from plasma was measured in one subject during each of the two dietary periods. A trace quantity of C^{14}-labeled tripalmitin (7-11 μc) was complexed with the patient's plasma in vitro, generating labeled "chylomicrons" (particle size <800 mμ), and given intravenously as a single rapid injection. Blood samples were obtained from an indwelling needle at one-minute intervals for the first ten minutes, and at two- to ten-minute intervals thereafter. Plasma samples were extracted in chloroform-methanol (2:1 V:V), and, after filtration, drying, and re-extraction in 5 per cent ethanol-95 per cent ethyl ether, passed slowly through columns of freshly charged Amberlite IRA-400 ion exchange resin to remove fatty acids.[31] Eluates were assayed for radioactivity with a Packard TriCarb automatic scintillation counter using 2,5-diphenyloxazole and p-bis 2-(5-phenyloxazolyl)-benzene as phosphors.

RESULTS

On the high-carbohydrate, low-fat diet, plasma triglyceride concentrations increased two- to fourfold (p <0.01) in five of the six studies (figure 1) (table 1). This increment became evident during the first week and reached maximum levels in one to four weeks. (The patient who did not show this response was a seventeen-year-old male juvenile diabetic who was also atypical in that he gained weight (0.1/kg./day) and demonstrated increased glycosuria throughout the six weeks of high carbohydrate feeding). In two patients (OD; JW) following a rapid initial response, a tendency to return toward control levels was noted (figure 2). This tendency was defined more clearly in a study on one patient (FF II) who was maintained on the test diet for ten

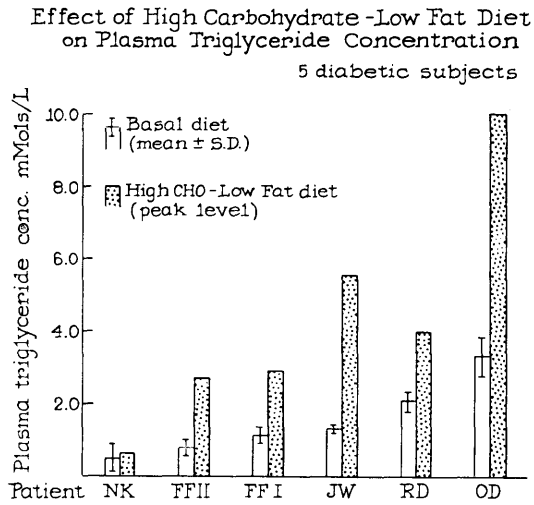

FIGURE 1

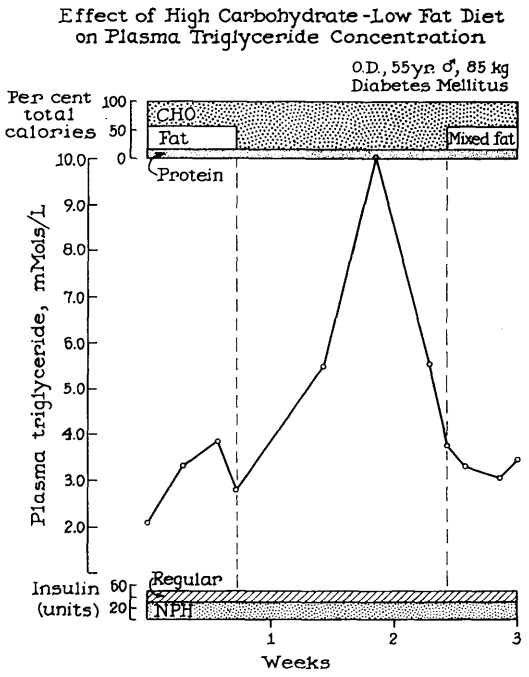

FIGURE 2

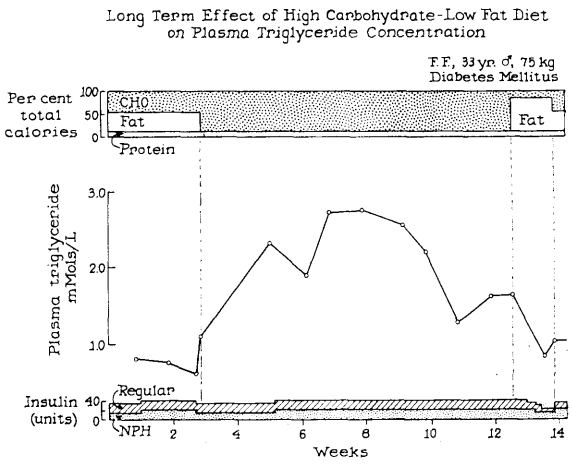

FIGURE 3

weeks (figure 3). After seven weeks, triglycerides were significantly reduced from previous levels ($p<0.02$), but remained higher than control values ($p<0.05$). In all studies, return to the basal diet promptly restored baseline triglyceride concentrations.

Small increases in phospholipid levels were observed ($p<0.05$; paired comparisons) paralleling the elevations in triglyceride. Serum cholesterol levels remained unchanged ($p>0.10$) (table 1).

Plasma turbidity increased during the high-carbohydrate, low-fat period. Filter paper electrophoresis of the plasma showed a rise in the proportion of lipoprotein remaining at the origin ($p<0.02$) (figure 4), including the patient (NK) in whom increased plasma triglyceride concentration could not be demonstrated (table 2). One previously untreated diabetic (RD), who had a marked hyperlipemia (triglycerides, 92 mM/L.; normal range, 0.6-1.7 mM/L.)[22] that responded to insulin therapy prior to the study, maintained a high proportion of origin lipid on the basal diet, although plasma triglyceride concentrations were near normal. In this case, no shift in lipoprotein distribution was apparent.

Despite the increased load of dietary carbohydrate, insulin requirements were not raised, as reflected by unchanged fasting blood glucose and plasma fatty acid levels, urinary sugar and organic acid excretion.

On the low-fat, high-carbohydrate diet, the removal rate of a trace dose of C^{14}-labeled tripalmitin during the first ten minutes after rapid intravenous administra-

TABLE 1

Subject	Age	Sex	Wt. (kg.)	Daily insulin dose (units)	TRIGLYCERIDES Basal Mean* mM/L.±S.D.	(n)	High carbohydrate Max. mM/L.	Level %↑	p†	PHOSPHOLIPIDS Basal Mean mg%±S.D.	(n)	High carbohydrate Mean mg%±S.D.	(n)	p	CHOLESTEROL Basal Mean mg%±S.D.	(n)	High carbohydrate Mean mg%±S.D.	(n)	p
FF I	33	M	75	50	1.18±.20	(5)	2.96	151	<.002	170±0	(3)	213±9	(3)	<.01	157±9	(3)	164±17	(4)	N.S.
FF II	33	M	75	40	.81±.21	(4)	2.75	240	<.01	133±15	(2)	225±40	(7)	<.05	107±6	(2)	169±24	(7)	<.02
JW	47	F	56	60	1.34±.08	(4)	5.53	313	<.001	219±26	(2)	291±2	(2)	N.S.	150±11	(2)	157±7	(2)	N.S.
OD	55	M	85	50	3.30±.53	(3)	9.96	202	<.02	297±5	(2)	344±54	(2)	N.S.	205±11	(2)	219±24	(2)	N.S.
RD	21	M	65	50	2.07±.26	(3)	4.00	93	<.05	183±3	(2)	204±17	(3)	N.S.	140±23	(2)	125±12	(3)	N.S.
NK	17	M	46	45	.54±.40	(3)	.83	54	N.S.	154±37	(2)	143±9	(7)	N.S.	95±3	(3)	111±6	(7)	<.01

*Standard deviation
†Probability that differences between values for basal and test periods are significant (N.S. = $p>.05$).

TABLE 2

Effect of high-carbohydrate, low-fat diet on lipoprotein distribution (filter paper electrophoresis)

Patient	Basal diet Per cent of total			High carbohydrate Per cent of total		
	"O"	β	α	"O"	β	α
FF I	16	62	22	53	37	11
FF II	23	66	11	57	37	6
JW	26	54	20	51	32	17
OD	15	70	15	44	45	11
RD	45	47	8	44	49	7
NK	18	43	39	28	50	22

"O" = lipoprotein remaining at origin.
β = Beta lipoprotein.
α = Alpha lipoprotein.

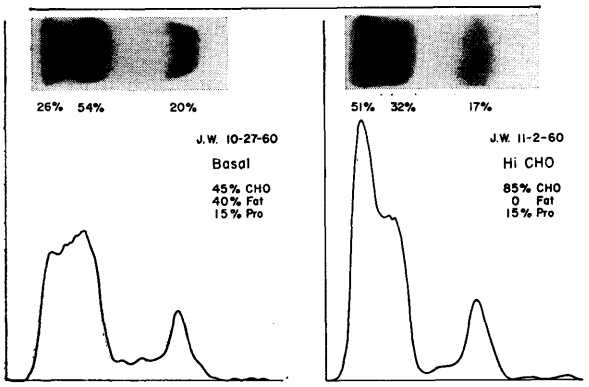

FIGURE 4

tion ($T_{1/2} = 4.1 \pm 0.3$ min.) was similar to that observed on the basal diet ($T_{1/2} = 4.5 \pm 0.2$ min.) when the identical test was performed (figure 5).

DISCUSSION

These results indicate that the insulin treated diabetic is capable of exhibiting a hyperlipemic response to a low-fat, high-carbohydrate diet, similar to that observed in normal, hyperlipemic, and hypercholesterolemic patients. Thus, plasma triglyceride levels in postabsorptive diabetic patients are clearly dependent on antecedent dietary fat and carbohydrate levels.

The relation of this dietary effect to the hyperlipemia with concomitant lipemia retinalis and eruptive xanthoma, occasionally observed in the uncontrolled diabetic, has not been defined in this study. The latter type of lipemia is characteristically more marked, and clears rapidly with insulin therapy. When the one patient who exhibited a massive hyperlipemia that responded

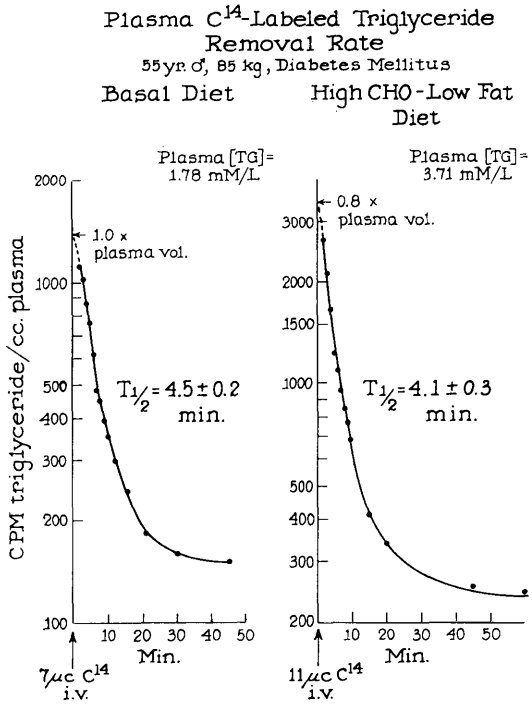

FIGURE 5

to insulin was tested, fasting plasma triglyceride levels doubled in two weeks on the low-fat, high-carbohydrate diet. However, when insulin was omitted while the patient was on this diet, the plasma became milky with a tenfold increase in triglyceride concentration in forty-eight hours.[32]

The possibility that the hyperlipemic response to low-fat, high-carbohydrate feeding may be only transient is suggested by the present data. The earliest complete reversal of the increment in plasma triglyceride concentration observed by Antonis and Bersohn was three months.[12] In this study, three patients exhibited a peak triglyceride level with a subsequent decline in less than two months. More long-term studies are needed, however, further to define this apparent adaptation.

The ability of the diabetic patient to tolerate an extreme increase in the proportion of carbohydrate calories in the diet without raising insulin requirements or showing other untoward effects is confirmed. No reduction of serum cholesterol level was observed in the present study, in contrast to the findings with hypercholesterolemic diabetics.[2,3,5] This discrepancy could be related to the fact that the serum cholesterol had already been lowered by a basal diet in which the relatively unsaturated fat, corn oil, served as the sole source of dietary fat.

The alteration in circulating lipoproteins associated with the additional plasma triglyceride is of interest.

The increase in stainable lipid remaining at the origin on filter paper electrophoresis suggests a rise in the concentration of chylomicrons and very low density lipoproteins.[33] The increments in plasma phospholipids and triglycerides had a molar ratio of about 1:5; this would be compatible with an increase in a lipoprotein of density < 1.006 ($S_f > 20$).[34] These results are in accord with the ultracentrifugal data reported by Nichols et al.[10] for normal subjects on similar diets.

The mechanism by which a low-fat, high-carbohydrate diet induces hyperlipemia remains obscure. Presumably the effect is not due merely to lack of dietary fat, since low-fat, low-calorie diets reduce the level of low-density lipoprotein.[10] Increasing the proportion of dietary carbohydrate, then, must in some manner either limit the removal or increase the entry of triglyceride-rich, low-density lipoproteins into plasma. The limited data available do not support the first possibility. The C^{14}-labeled tripalmitin "chylomicrons" were cleared at the same rate during basal and high-carbohydrate feeding periods. If native very low-density lipoproteins are also removed at an unchanged rate when the plasma triglyceride pool is increased during high-carbohydrate feeding (figure 5), then the total triglyceride removed from plasma during this period may be increased. Supporting this interpretation, carbohydrate fed rats clear intravenously injected homologous chylomicrons at the same rate as fasted rats.[35,36]

Alternatively, increasing the proportion of dietary carbohydrate may enhance synthesis and esterification of fatty acids, and accelerate their release into plasma as very low-density lipoproteins. Some experimental support for this possibility is derived from studies of lipogenesis in liver and adipose tissue. Carbohydrate feeding enhances the synthesis of fatty acid from glucose,[37-39] and incorporation of fatty acids into glycerides in liver,[40] muscle,[41] and adipose tissue.[42] Insulin treatment of the diabetic may preserve this pathway of glucose utilization, while at the same time rendering him subject to hyperlipemia.

SUMMARY

Insulin treated diabetics exhibited a hyperlipemic response to isocaloric substitution of carbohydrate for fat in the diet similar to that previously observed in normal, hyperlipemic, and hypercholesterolemic subjects. Increases in plasma turbidity, alterations in plasma lipoprotein patterns (filter paper electrophoresis) and small elevations in serum phospholipid levels, suggest that the additional triglyceride in plasma circulates as very low density lipoproteins.

The marked increase in dietary carbohydrate did not raise insulin requirements, as reflected by unchanged fasting blood glucose and fatty acid levels, urinary sugar and organic acid excretion.

High carbohydrate feeding did not appear to alter the clearance of C^{14}-labeled triglyceride from plasma.

SUMMARIO IN INTERLINGUA

Le Effecto Hyperlipemic de un Dieta Povre in Grassia e Ric in Hydratos de Carbon in Subjectos Diabetic

Diabeticos tractate con insulina exhibiva un responsa hyperlipemic al substitution isocaloric de hydrato de carbon pro grassia, simile al responsa previemente observate in subjectos normal, hyperlipemic, e hypercholesterolemic. Augmentos in le turbiditate de plasma, alterationes in le configuration del componentes lipoproteinic in le plasma (studiate per electrophorese a papiro-filtro), e leve augmentos del concentration seral de phospholipido suggere que le triglycerido additional in le plasma circula como lipoproteinas de bassissime densitate.

Le marcate augmento de hydrato de carbon in le dieta non augmentava le requirimentos de insulina, a judicar per le non-alterate valores pro glucosa e acido grasse del sanguine in stato jejun e pro le excretion urinari de sucro e acido organic.

Le dieta ric in hydrato de carbon non pareva alterar le clearance de triglycerido marcate con C^{14} ab le plasma.

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