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DIURNAL CHANGES OF SERUM TRIGLYCERIDE IN DIABETIC PATIENTS

FUMIO KUZUYA, MICHIRO KITAGAWA, AND NOBORU YOSHIMINE

3rd Department of Internal Medicine, Nagoya University Medical School
(Director: Prof. Kozo Yamada)

SUMMARY

The diurnal changes of serum triglycerides (T.G.) in diabetic patients were studied. The results obtained are as follows:

1) The value of T.G. was minimum at 7 : 00 a.m. and changed along a monophasic curve with its peak at 2 : 00 p.m.

2) A distinctly positive correlation was observed between the T.G. value at 7 : 00 a.m. and the maximal T.G. during a day.

3) There was no correlation at all between the changes of blood sugar and T.G.

4) Increase of T.G. was marked in obesity and slight in thinness.

5) Increase of T.G. in insulin-treated cases was marked, as compared with that in non-treated cases.

6) The changes of T.G. certainly mean not only the changes of exogenous chylomicron, but also those of endogenous T.G. itself.

From the above results the role of insulin in the genesis of hyper-T.G.-emia in diabetes was discussed.

INTRODUCTION

Many reports have already been made of a high level of serum lipids, especially of triglycerides (T.G.) in case of diabetes. An abnormally high level of T.G. is of frequent occurrence in case of diabetes with a high degree of metabolic disorders, while diabetics with vascular complications often reveal also an increased T.G.[1] Besides, non-diabetic patients showed also a close relation-ship between arteriosclerosis and abnormal T.G. values[2].

Study of the diurnal changes of T.G., however, has not yet been carried out thoroughly. We made, therefore, a study of the changes of T.G. in diabetic patients during 24 hours, in pursuit of correlations between the value of T.G., insulin therapy and changes of blood sugar value, and made some investigations on the role of insulin in the metabolism of T.G. in diabetes.

METHODS

1) Subjects

Our subjects were 14 patients including 6 males and 8 females who had

葛谷文男，北川道弘，吉峰　徳
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been treated in our diabetic clinic and again admitted for the purpose of control. The diet of these patients containing 90-100 g protein, 40-50 g fat and 200-350 g carbohydrates was 1,600-2,000 Cal. in total. Major drugs administered were insulin or oral hypoglycemics, coronary dilator drugs, stomachics etc. and no hypolipemic agents or hormones other than insulin were used. Except in one case of hemochromatosis the results of hepatic function test were within the normal limits, and the serum cholesterol level was always less than 250 mg/dl. The body weight of patients, except for 2 obese and 1 slim person, was always within the range of standard body weight ±10%.

2) Determination of T.G. and Blood Sugar

Blood was taken from the patients 7 times each at 7 : 00 a.m., 10 : 00 a.m., 11 : 30 a.m., 2 : 00 p.m., 4 : 00 p.m., 9 : 00 p.m. and 0 : 00 a.m., *i.e.* 29 times in total to determine blood sugar and T.G. The blood sugar was determined by Autoanalyser-Method and the T.G., by Triglyceride-Test-Wako.

3) Chylomicron-free T.G.

From additional 10 diabetic patients blood was taken in the same way and chylomicron-free T.G. was also determined[3]. The serum was filtered through a cellulose membrane filter of 0.1 micron pore size and the very low density lipoprotein contained in the filtrate was determined by SFL Micronepherometer. With the serum before and after filtration lipoprotein electrophoresis on cellulose acetate[4] was done, in order to ascertain whether or not chylomicron was only and specifically removed through this membrane filter.

RESULTS

1) Changes of Blood Sugar and T.G.

The mean values at each time are given in Table 1 and Fig. 1. The value of T.G. was minimum at 7 : 00 a.m. and showed a monophasic curve with its peak at 2 : 00 p.m. Against this the changes of blood sugar were shown as a polyphasic curve with peaks after each meal at 10 : 00 a.m., 2 : 00 p.m. and 7 : 00 p.m.

TABLE 1. Diurnal Changes of Serum Triglyceride and Blood Sugar in Diabetics (29 cases)

	A.M. 7 : 00	A.M. 10 : 00	A.M. 11 : 30	P.M. 2 : 00	P.M. 4 : 00	P.M. 7 : 00	A.M. 0 : 00
Triglyceride	127±59*	139±67	142± 71	167± 78	160± 90	159±106	139± 83
Blood sugar	171±80	222±90	194±111	203±116	184±114	223±109	190±104

* standar deviation

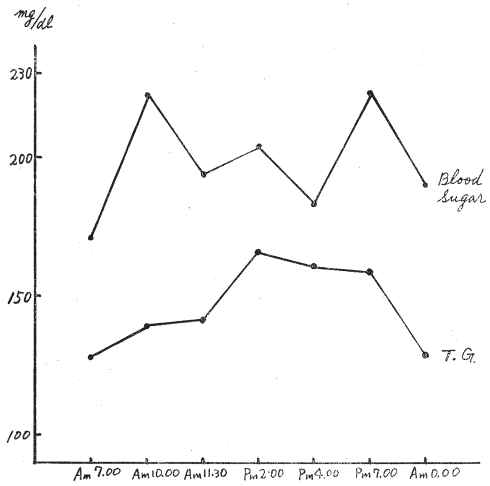

FIG. 1. Mean diurnal serum triglyceride and blood sugar levels in diabetics (29 cases).

2) T.G. at 7 : 00 a.m. in Relation to its Maximum

By indication of T.G. at 7 : 00 a.m. on the abscissa and its maximum during a day on the ordinate, the correlation between the two is given in Fig. 2. There can be observed a distinct positive correlation between them. The higher the T.G. level in the fasting state early in the morning is, the more

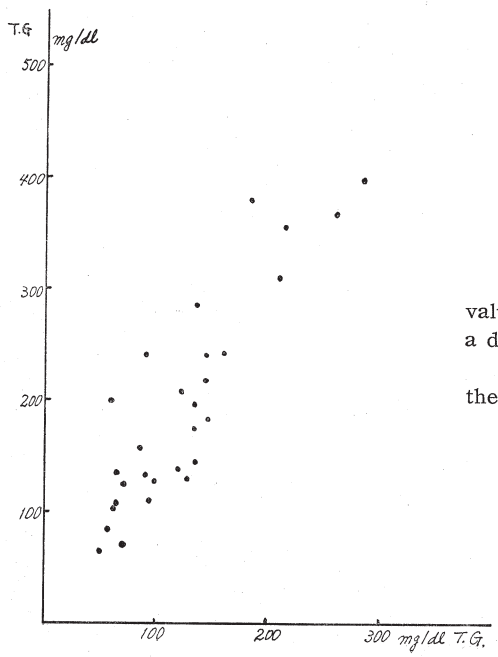

FIG. 2. Correlation of T.G. between the value at 7 : 00 a.m. and the maximum during a day.

Abscissa; T.G. at 7 : 00 a.m., Ordinate; the maximum T.G. during a day.

widely it changes in the daytime and the T.G. rises obviously to a higher level.

3) Correlation between Blood Sugar and T.G.

The correlation between the blood sugar and T.G. at 7 : 00 a.m. is given in Fig. 3; there was no correlation at all between them. Fig. 4 shows a comparison between blood sugar at 7 : 00 a.m. and the maximal T.G. in the daytime; also between them no particular correlation was found. It seems, therefore, that there is no correlation at all between daytime variation in T.G. and blood sugar value, which is a sign of metabolic abnormalities of carbohydrate in diabetes.

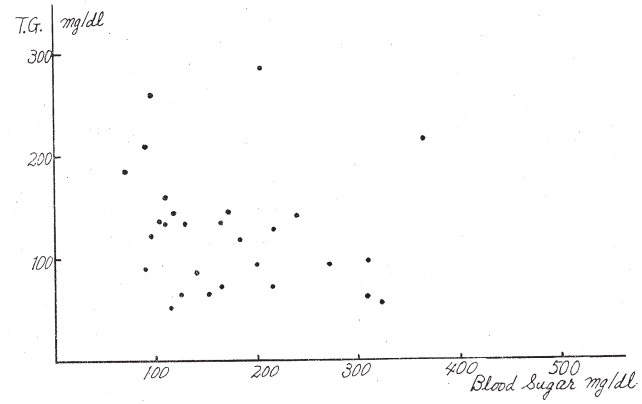

FIG. 3. Correlation between T.G. and blood sugar at 7 : 00 a.m.

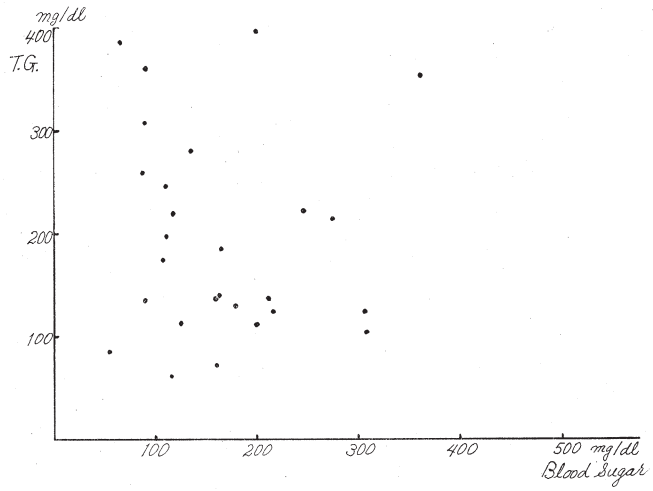

FIG. 4. Correlation between the maximal T.G. in the day (ordinate) and blood sugar (abscissa).

4) Obesity, Thinness and Variation of T.G.

It is said that the T.G. value of an obese person, depending upon the body weight, is generally high. In this study the number of both obese and thin patients were too few to be dealt with statistically. So a few cases will be described. To a 33 year-old thin male of −18% body weight in Fig. 5, 24 U insulin were administered. At 7 : 00 a.m. T.G. attained a rather high content of 134 mg/dl, which thereafter remained during a day almost unchanged. The T.G. in a 36 year-old patient of −22% body weight given 32 U insulin, as shown in Fig. 6, reached a rather low value of 71 mg/dl at 7 : 00 a.m. and, increased some what to a value of 125 mg/dl at 2 : 00 p.m., but otherwise very little increase was found. On the contrary a 43 year-old obese male of +27% body weight, as shown in Fig. 7, was treated with oral hypoglycemic. In this case T.G. attained a high content of 143 mg/dl at 7 : 00 a.m. and increased fairly largely to 219 mg/dl at 2 : 00 p.m., so that the values obtained at each time were always higher than the value in the fasting state early in the morning.

5) Application of Insulin and Changes of T.G.

The question was investigated, whether or not the changes of T.G. during

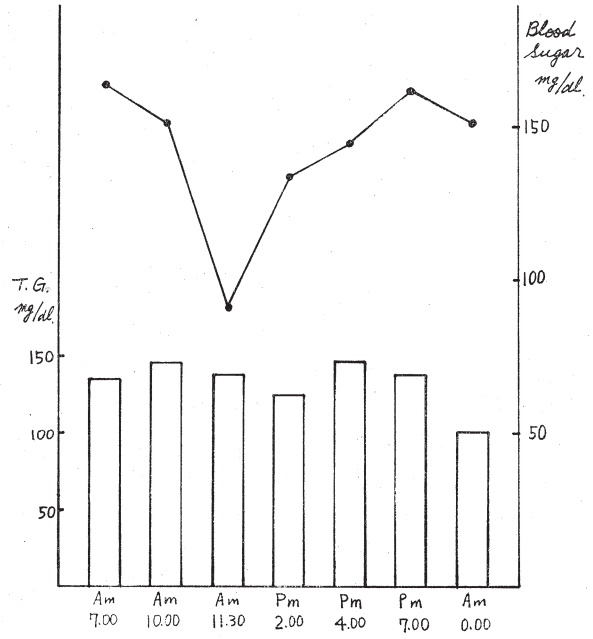

FIG. 5. Thin diabetic patient treated with insulin 24 unit, 33 years old male. Body weight −18%. Upper line represents blood sugar and column refer to T.G.

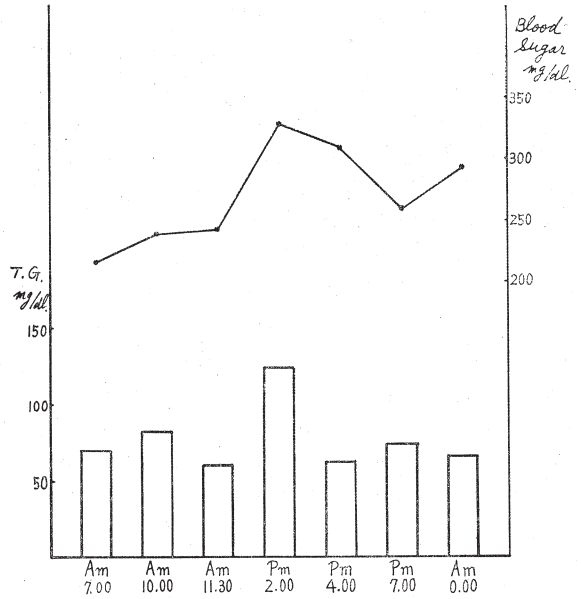

FIG. 6. Thin diabetic patient treated with insulin 32 units, 36 years old male. Body weight −23%. See legend of Fig. 5 for key.

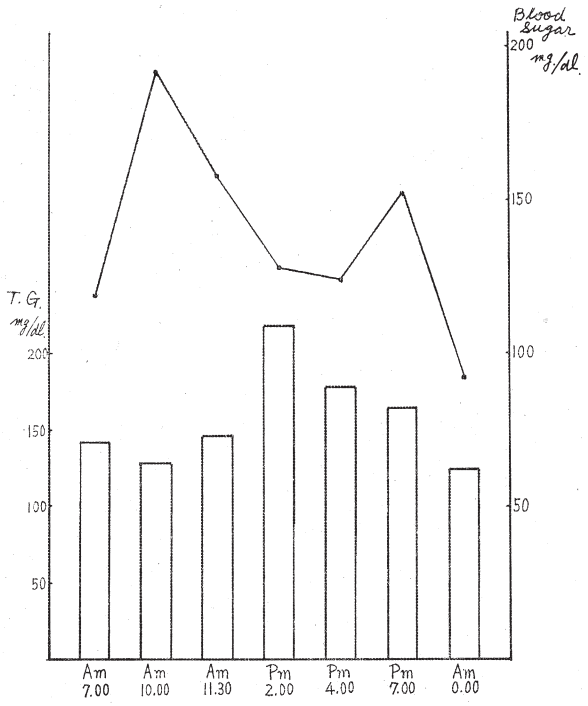

FIG. 7. Obese diabetic patient treated with oral hypoglycemic, 43 years old male. Body weight +27%. See legend of Fig. 5 for key.

twenty-four hours differ with insulin for control of diabetes and with exclusive oral hypoglycemic or dietetic therapy. Of 15 selected cases without factors influencing T.G. values from among our objects, except cases of hemochromatosis, obese or thin patients, 9 were treated with insulin and other 6 were untreated. The values obtained for each in these cases are shown in Table 2. In the cases treated with insulin, compared with the non-treated cases, changes of T.G. are marked and the value was particularly high at 2 : 00 p.m. (Fig. 8). In one case of a 45 year-old female, in which observation was made during the periods of therapy with and without insulin, as shown in Fig. 9, T.G. increased fairly much, as compared with the value at 7 : 00 a.m., from 10 : 00 a.m. to 2 : 00 p.m. After ceasing insulin therapy and replacement with an oral hypoglycemic, biguanide about a week later, the blood sugar level became slightly high, while T.G. remained almost unchanged all day. In a case of a 27 year-old male, as shown in Fig. 10, the increase of T.G. was marked during the periods of insulin therapy with 20 and 16 U, but was markedly reduced

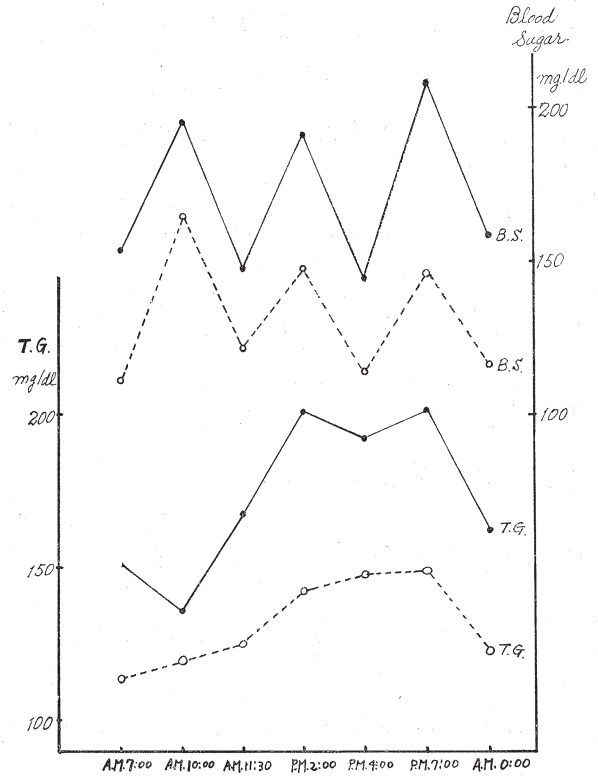

FIG. 8. Diurnal changes of T.G. and blood sugar in regard to insulin therapy. Solid line; the means of patients treated with insulin. Dotted line; the means of patients without insulin.

TABLE 2. Diurnal Changes of T.G. and Blood Sugar in Regard to Insulin Therapy

	No.	Triglyceride							Blood sugar						
		A.M. 7 : 00	A.M. 10 : 00	A.M. 11 : 30	P.M. 2 : 00	P.M. 4 : 00	P.M. 7 : 00	A.M. 0 : 00	A.M. 7 : 00	A.M. 10 : 00	A.M. 11 : 30	P.M. 2 : 00	P.M. 4 : 00	P.M. 7 : 00	A.M. 0 : 00
Insulin (+)	1	87	111	132	157	126	83	83	140	170	102	89	38	266	81
	2	259	312	365	322	298	302	306	96	179	167	249	205	172	135
	3	282	170	234	203	320	525	447	206	296	296	296	270	296	346
	4	185	189	230	329	376	378	170	71	129	155	145	124	222	235
	5	133	121	110	284	255	199	104	129	185	106	133	108	180	74
	6	159	102	103	240	138	147	104	110	135	90	169	82	117	93
	7	62	51	—	102	81	62	92	306	138	73	264	258	—	—
	8	130	80	66	88	87	86	88	213	336	237	220	166	318	213
	9	63	95	107	95	56	47	72	126	185	114	162	56	99	103
	Mean	151	137	168	202	193	203	163	155	195	149	192	145	209	160
	S.D.	75	74	94	91	113	156	122	69	69	70	65	80	76	90
Insulin (−)	1	209	220	239	249	282	308	216	91	174	102	126	95	143	147
	2	123	174	181	174	186	207	165	97	90	100	113	87	126	135
	3	136	131	129	175	171	160	138	108	108	66	108	—	82	94
	4	90	83	88	132	125	96	113	91	133	88	88	88	82	60
	5	51	53	48	64	56	48	42	114	263	160	239	126	220	81
	6	72	56	64	72	65	72	64	166	221	218	215	178	228	187
	Mean	114	120	125	144	148	149	123	111	165	122	148	115	147	117
	S.D.	51	62	67	64	77	89	59	26	62	51	57	35	59	43

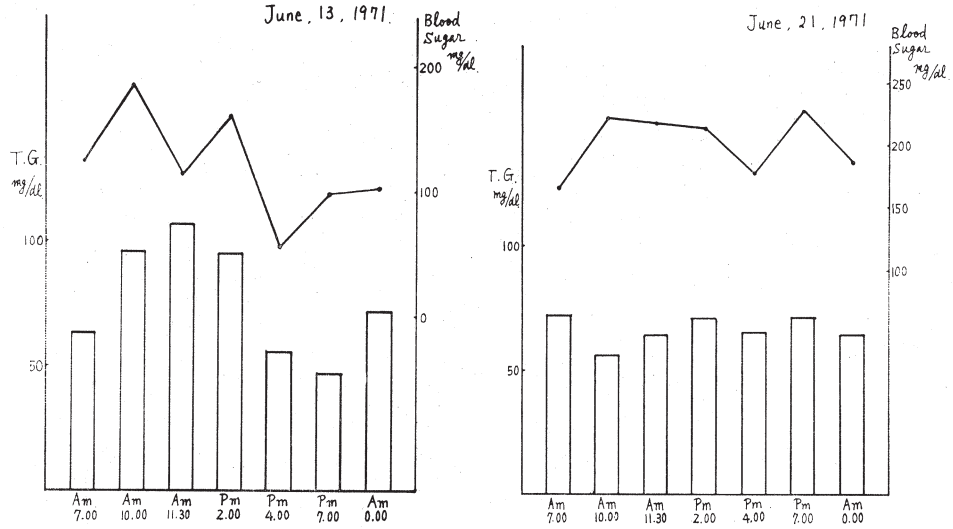

FIG. 9. Diurnal changes of T.G. and blood sugar in 45 years old female. On June, 13, 1971, the patient was treated with insulin 12 units. On June, 21, 1971, insulin was replaced with oral hypoglycemic, biguanide. See legend of Fig. 5 for key.

after replacement with oral hypoglycemics, whereas no signficant changes were found in the blood sugar level. This makes us suspect, that insulin plays a significant role in the diurnal changes of T.G., especially in its postprandial increase, while the blood sugar level itself has little to do with it.

6) Changes of Chylomicron-free T.G.

As the postprandial serum contains absorbed chylomicron, it seems probable that the above changes of T.G. are only reflected in the changes of absorbed chylomicron, and the endogenous T.G. synthesized and secreted in the liver undergoes by no means a change. Another approach to the study was taken in this respect. The high-T.G. serum (T.G.=260 mg/dl) three hours after a meal rich in fat was filtrated with a membrane filter of 0.1 micron pore size, and the determined T.G. content of the filtrate was 210 mg/dl. Next, this serum was fractionated by lipoprotein-electrophoresis into chylomicron, β, pre-β and α-lipoprotein, as given in Fig. 11. The postfiltrated serum was entirely free from chylomicron, while the other β-, pre-β- and α-lipoprotein, as in the original serum, definitely remained. By filtration, therefore, only chylomicron was separated specifically, and the endogenous T.G. can then be determined with the thus obtained sample.

In 10 cases of diabetes, selected in order to investigate the changes of endogenous T.G., T.G. (total), as shown in Table 3 and Fig. 12, began to increase from 7 : 00 a.m., reached the peak at 2 : 00 p.m. and decreased thereafter, while the endogenous T.G. decreased a little once from 7 : 00 a.m. to 10 : 00

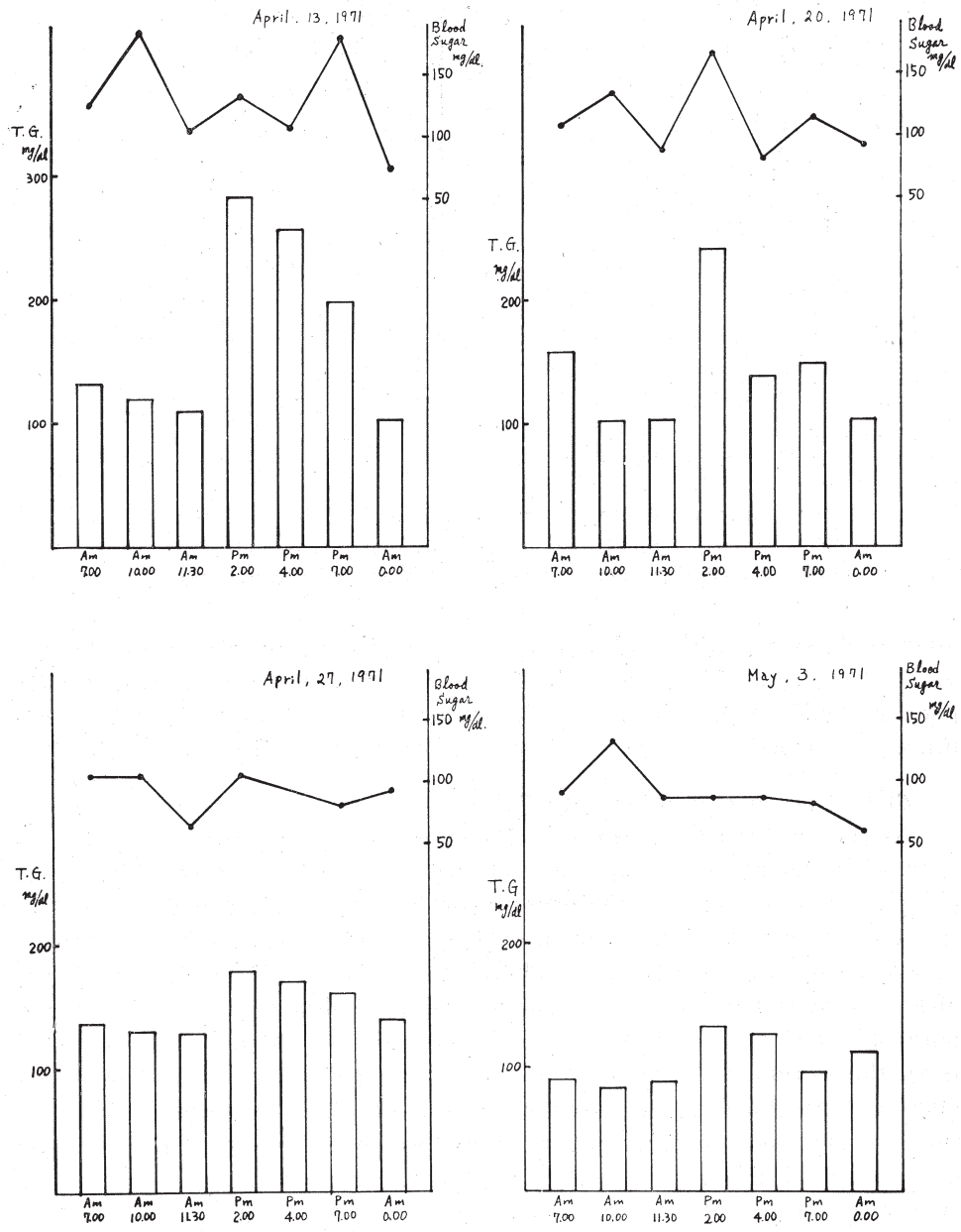

FIG. 10. Diurnal changes of T.G. and blood sugar in 27 years old male. On April, 13, 1971, and April, 20, 1971, the patient was treated with insulin 20 and 16 units respectively. On April, 27, 1971 and May, 3, 1971, he was treated with oral hypoglycemic, sulfonyl urea.

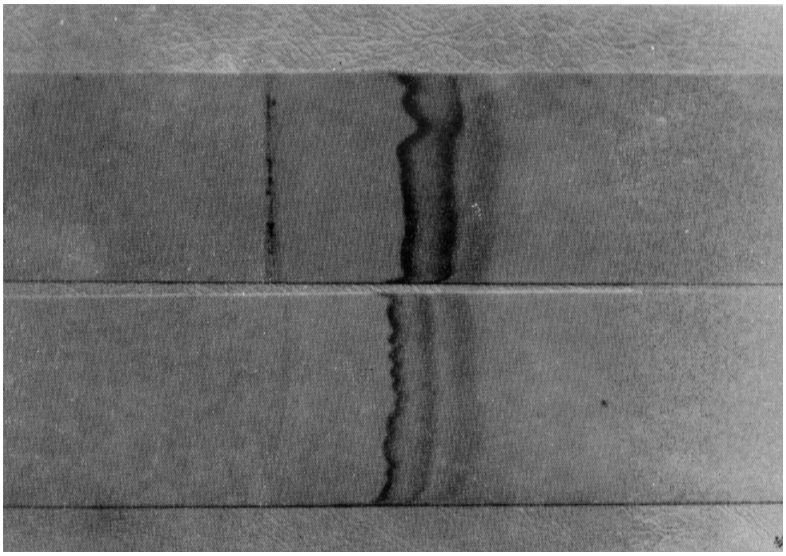

FIG. 11. Electrophoresis of hypertriglycemic serum (upper strip). From left to right; chylomicron, β, pre-β, and α-lipoprotein. Lower strip is the electrophoresis of the serum filtrated through membrane filter. Note the absence of chylomicron.

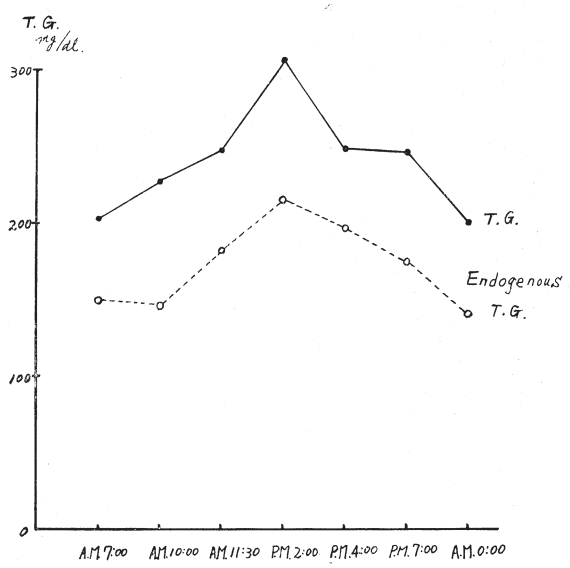

FIG. 12. Diurnal changes of endogenous T.G. in diabetes.

TABLE 3. Diurnal Changes of Endogenous T.G. in Diabetics

No.	T.G.							Endogenous T.G.						
	A.M. 7 : 00	A.M. 10 : 00	A.M. 11 : 30	P.M. 2 : 00	P.M. 4 : 00	P.M. 7 : 00	A.M. 0 : 00	A.M. 7 : 00	A.M. 10 : 00	A.M. 11 : 30	P.M. 2 : 00	P.M. 4 : 00	P.M. 7 : 00	A.M. 0 : 00
1	208	214	222	327	233	301	156	166	139	200	246	182	234	132
2	128	119	172	181	126	106	71	107	105	119	138	100	101	70
3	92	96	104	108	100	119	88	79	82	91	94	97	91	65
4	216	145	152	310	335	271	210	203	125	125	270	278	250	186
5	154	138	131	262	262	246	169	138	103	119	230	220	195	138
6	192	233	246	300	300	274	167	136	—	210	224	254	—	152
7	220	247	323	386	377	332	224	119	166	207	243	332	193	113
8	256	260	318	278	215	314	359	89	89	107	113	77	94	159
9	152	161	196	202	109	148	148	88	119	192	97	88	109	54
10	408	673	610	717	439	368	430	379	379	460	493	355	317	355
Mean	203	229	247	307	250	248	202	150	146	183	215	198	176	142
S.D.	83	158	139	156	110	88	107	84	86	102	113	100	77	82

a.m., if any, then increased and reached the peak at 2 : 00 p.m., so that the endogenous T.G. changed in parallel with T.G. (total). The diurnal changes of T.G. were not merely a reflex of the changes of exogenous chylomicron, but it means evidently the changes of the endogenous T.G. itself.

DISCUSSION

Many reports have been made on the fact that lipid metabolic disorders, especially hyper-T.G.-emia, are of common occurrence in diabetes. For instance Albrink *et al.*[1] reported in 1963, that an increase of T.G. was observed in diabetic patients with vascular complications, and New *et al.*[5] and Shrade *et al.*[6] also presented similar reports.

On the contrary in case of hyperlipemia of Fredrickson Type IV, in which serum T.G. increases, a positive glucose tolerance test[7] is commonly known to be given, and a hyper-T.G.-emia can be induced by massive carbohydrate loading. It is certain, therefore, that a close relationship will exist between disorders of carbohydrate metabolism and hyper-T.G.-emia.

As T.G. varies with meals and the postprandial serum with absorbed fat as chylomicron contains much T.G., an overnight fasting should strictly be kept at the time of T.G.-determination, while T.G. in the reported cases was determined only in the fasting state early in the morning and rarely in the postprandial state.

The results obtained by the authors indicate that the T.G. changed along a monophasic curve showing a maximum at 2 : 00 p.m. and a minimum between 0 : 00 a.m.—7 : 00 a.m., and the changes of T.G. were never related to those of blood sugar. It was found further that not only these changes reflect those of the dietary chylomicron, but also the endogenous T.G. itself changes in parallel therewith.

In the insulin-treated cases, as compared with the non-treated cases, the increase of T.G. was, and really in the same individual, remarkable, which was not related with the changes of blood sugar. The results should be of particular interest too, in order to study the genesis of hyper-T.G.-emia in diabetes.

Farquhar[8], Reaven[9] *et al.* attached importance to the fact that the insulin-induced increase of T.G.-synthesis in the liver causes hyper-T.G.-emia. They indicate that for the hyper-T.G.-emia following supplies of carbohydrate-rich diet is responsible the increase of blood sugar and insulin caused by this diet. Concerning further the carbohydrate induced lipemia they found hyper-T.G.-emia in 31 out of 33 cases supplied 3 weeks with the diet, of which 85% of the total calories was provided by carbohydrate. The hyper-T.G.-emia in these cases was related fairly well and only to the increase of insulin content, but not to the blood sugar, F.F.A. and obesity. In case of insulinoma, despite the

presence of hyperinsulinemia, hyper-T.G.-emia does not take place because of hypoglycemia affecting high carbohydrate diets and in case of maturity onset diabetes, despite high blood sugar level, T.G. does not increase in the presence of hypoinsulinemia. These facts make them suspect that for the outbreak of hyper T.G.-emia, normoglycemia or hyperglycemia of more than moderate degree and hyperinsulinemia must preexist as necessary conditions.

The results obtained by the authors indicate that under the same dietetic treatment the increase rate of postprandial T.G. differs significantly with drug therapy with and without insulin, so that the insulin probably accelerates intrahepatic synthesis or secretion of T.G. Goto *et al.*[10] determined I.R.I. and T.G. of diabetic patients after a load of 50 g glucose, and observed a significant positive correlation between T.G. and the sum total of insulin (total I.R.I.), and indicated the possible responsibility of insulin for the hyper-T.G.-emia of diabetes in hyper-insulinemic stage.

On the contrary, however, insulin can really dispose T.G. from serum and some investigators explain the hyper-T.G.-emia[11] in diabetes from the weakening of this disposal. Consequently, on the genesis of hyper-T.G.-emia in diabetes, there should be now taken into consideration at all times, balance of insulin-status in the first place, then intrahepatic increase of T.G.-synthesis and decrease of its disposal in peripheral tissues. On the strength of our results obtained, however, it should be indicated, that exogenous insulin will possibly be a trigger of increase in T.G. of diabetic patients.

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