Original Article*

Classification of Disease Pattern of Diabetes Mellitus as Seen from the Function of Production and Disposal of Pyruvic Acid

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Pyruvic acid (to be abbreviated hereafter as *PA*) stands at the key point of the metabolic system common to glucide, lipid and protein. Since *PA* adapts itself according to various situations, and changes moment by moment, the occasional determination of the *PA* value in the blood will have little clinical significance.

However, when tracing the changes of the *PA* value in the blood continuously under the situation of being loaded with special conditions, certain patterns are believed to be obtained, according to the normal or sick conditions.

In the cases of diabetes mellitus, the *PA* value at the fasting varies, correlative with metabolic abnormalities, and it is generally believed that in mild cases of diabetes mellitus or those not involved with serious complications, there are no significant difference from healthy persons.¹⁻⁷⁾

If loaded with glucose (to be abbreviated hereafter as *G*) or fructose, however, the attitude of *PA* is very much different from that of healthy persons, i. e. according to Gitelson⁸⁾, if *G* is injected intravenously into diabetic patients, the increase of the blood *PA* value one hour afterward is more than healthy persons, and the decrease of the value two hours afterward is delaying.

On the contrary, Alslev⁹⁾, Miller¹⁰⁾ and Horwitt¹¹⁾ reported that the increase of the *PA* value after being loaded with *G* was delaying, furthermore, Amatuzio¹²⁾ and Schrifter¹³⁾ reported that the *PA* value rather went down thirty minutes after *G* was injected.

There are other reports^{1,5,7)} to the effect that the changes of the *PA* value of diabetic patients are similar to those of healthy persons. Thus, opinions of various authors do not agree each other.

Now, if the process of loading *G* in the vein and its becoming *PA* is called *A*, and the consequent disposal of *PA* is called *B*, the following four patterns theoreti-

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cally exist, according to where hindrances predominate. In other words, there are Pattern N where both Process A and Process B are not hindered, Pattern A where only Process A is hindered, Pattern B where Process B only is hindered, and Pattern AB where both Process A and Process B are hindered.

The authors considered these four patterns as follows: As the Fig. 1 shows, in Pattern N there is a certain balance being maintained between Process A and Process B, the blood sugar level immediately rises after the injection of G, and presently comes back to the level before the injection, and the PA value rises a little later than the rise of blood sugar, and presently comes back to the level before the injection.

Pattern A is the type where the blood sugar level, which rose after the injection of G, comes back to the former level in a longer time than Pattern N, and the PA value makes little change after the injection of G, then it gradually rises after some time, and presently returns to the level before the injection.

Pattern B is a type where the changes of the blood sugar level is similar to Pattern N, but the PA value reaches the maximum soon after the injection, and its returning to the former level is later than Pattern N.

In Pattern AB where the changes of the blood sugar level is similar to Pattern A, the PA value gradually rises after the injection, taking more time than Pattern N and B before reaching the maximum which is larger than Pattern A.

Reports of various other authors stated above^{1,5,7-13)} may belong either of these four patterns, and the authors consider that in diabetes mellitus the four patterns can exist, trying to classify this disease according to the predominance of hindrances of either Process A or B.

Fig. 1. Theoretical four patterns after glucose loading.

Subjects and Methods

1. Subjects

Thirty-one cases were chosen, consisting of 17 cases of healthy males and 14 cases of healthy females as healthy persons, who have no family record of diabetes.

mellitus, with no liver and endocrinological troubles, of ages ranging from 16 to 64.

One hundred cases were chosen, consisting of 62 cases of males and 38 cases of females, aged 13 to 69, who were diagnosed to be diabetics or suspected after careful examinations of glucosuria, hyperglycemia before and after meals, abnormal sugar tolerance and other clinical features of diabetes mellitus.

2. Methods

In early morning at the fasting after urination, let patients drink 180 cc. of water. One hour later blood was collected before loading with G. Immediately after blood collection G was injected intravenously in about three minutes as 50% solution with the ratio of 1 g. per 3 kg. of body weight. After G was loaded, blood was collected 6 times, i. e. at 15 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes and 150 minutes, respectively.

The blood sugar level and PA value were determined with each sample, besides, the excreted amount of urinary sugar in 150 minutes after G was loaded was determined, too. The blood sugar was determined by Somogyi's method. The blood pyruvic acid analysis was performed by Friedemann Haugen's method²¹, i. e. cubital venous blood collected was mingled at once into iced trichlor acetic acid solution.

From this supernatant fraction including various keto acids only pyruvic keto acid was extracted into xylol as pyruvic hydrazone after adding DNPH (dinitro phenyl hydrazine).

Then, pyruvic hydrazone was transferred to sodium carbonate solution, and reacting to sodium hydroxide, it showed violet-reddish tone which was estimated colorimetrically. As regards diabetic patients, we gave them the diet containing more than 250 g. hydrocarbon daily 3 days before the examination, and administered more than 15 mg. of thiamine daily several days before the test. For insulin-treated patients the administration was discontinued 24 hours before the examination, suspending the administration of oral antidiabetics, too, on the day before the examination.

The authors decided to call the examination as above PPDT (Pyruvate Production and Disposal Test) tentatively.

Results

1. Results of PPDT of Healthy Persons

Results were summarized in Table 1, and Fig. 2 shows the transitive curve obtained by connecting the blood sugar level and the PA value at each point of time. In the graph it can be observed that the blood sugar level at 15 minutes after being loaded with G was highest, and the difference of this highest level and former level was within 150 mg./dl., but the highest figure dropped to the previous level within 90 minutes after being loaded with G.

Table 1. Results of PPDT in Healthy Persons

Age	Sex	Before Loading		After Loading												Total Urinary glucose after Loading (g/dl)
		Blood Sugar	Pyruvic Acid	15 min		30 min		60 min		90 min		120 min		150 min		
				Blood Sugar	Pyruvic Acid	Blood Sugar	Pyruvic Acid	Blood Sugar	Pyruvic Acid	Blood Sugar	Pyruvic Acid	Blood Sugar	Pyruvic Acid	Blood Sugar	Pyruvic Acid	
19	♂	84	1.20	176	1.88	110	1.80	69	1.30	77	1.00	81	0.96	84	1.20	1.15
19	♂	89	1.30	177	1.68	100	1.86	70	1.56	73	1.12	77	1.00	84	1.04	0.48
20	♂	84	1.38	116	1.48	86	1.62	66.5	1.34	80.5	1.22	76	1.22	82	1.34	0.90
20	♂	108	0.76	175	0.82	93.5	0.82	90.5	0.80	105	0.50	105	0.66	110	0.54	1.29
20	♂	88	1.88	156	1.92	119	1.84	80	1.74	84	1.22	84	1.04	91	1.46	0.25
21	♂	92	1.46	197	1.48	143	1.62	85	1.24	75	0.78	83	0.72	92	0.64	0.37
23	♂	92	1.16	200	1.36	166	1.18	96	1.36	72	1.10	69	1.08	84	0.98	0.75
27	♂	80	0.58	230	0.88	193	0.80	17	0.74	50	0.56	83	0.50	60	0.42	1.76
29	♂	97	0.82	161	1.06	113	1.08	95	0.78	89.5	0.68	80	0.66	77	0.68	0.70
31	♂	76	1.74	172	1.92	88	1.68	68	1.46	66	1.22	77	1.32	75	1.76	1.05
39	♂	76	0.92	160	1.42	95	1.22	61	0.82	63	1.16	74	0.96	76	0.92	0.41
43	♂	87	1.50	190	2.26	152	1.90	99	1.22	77	1.18	69	1.36	74	0.96	0.55
49	♂	74	1.14	167	1.86	87	1.06	66	0.96	77	0.96	78	0.88	78	0.96	0.87
54	♂	85	0.90	222	1.04	236	1.22	94	0.90	71	0.96	70	0.90	76	0.68	0.26
55	♂	74	0.72	154	1.44	121	0.96	85	0.72	69	0.96	74	0.72	80	0.96	0.34
60	♂	78	1.20	197	1.70	142	1.48	75	1.16	78	0.98	83	0.96	83	0.96	0.26
64	♂	83	1.20	161	1.80	109	1.48	80	1.30	73	0.96	75	0.96	77	1.00	0.45
16	♀	91	1.42	195	1.92	132	1.36	77	1.34	81	1.30	87	1.22	80	1.22	0.55
17	♀	90	1.18	156	1.40	76	1.42	72	1.12	83	0.98	81	1.12	83	1.18	0.35
18	♀	89	0.92	172	1.42	101	1.42	54	1.12	75	0.92	85	0.58	89	0.70	0.60
23	♀	77	1.16	214	1.30	129	1.36	85	1.00	73	1.08	73	1.12	79	1.20	0.30
25	♀	90	1.12	148	1.38	80	1.16	74	1.24	84	1.00	87	0.96	85	1.14	1.50
30	♀	79	0.96	122	1.40	65	1.21	71	0.98	75	0.80	71	0.92	74	1.12	0.37
32	♀	76	1.42	179	2.08	115	1.34	62	1.22	62	1.42	58	1.24	68	1.10	0.59
39	♀	91	1.46	116	1.82	58	0.38	59	1.52	83	1.30	81	1.00	84	0.96	0.80
45	♀	75	0.92	158	1.18	78	1.96	49	0.72	76	0.68	62	0.78	80	0.76	0.78
46	♀	94	1.52	236	1.72	148	1.84	81	1.42	66	1.12	68	1.48	80	1.18	1.10
52	♀	94	1.44	232	1.44	162	1.20	83	1.14	66	1.06	74	1.06	80	1.18	0.95
55	♀	79	1.42	174	2.20	143	1.66	111	1.22	87	1.12	80	1.18	78	1.14	1.34
60	♀	103	1.06	223	1.78	169	1.12	107	0.96	81	0.88	83	1.16	91	1.16	1.01
60	♀	87	0.92	191	1.90	120	1.44	58	0.92	67	0.96	75	1.40	78	0.92	0.62
Mean Value		86	1.19	178	1.58	120	1.37	79	1.14	75	1.01	78	1.00	81	1.01	
S.D.		±8	±0.30	±31	±0.36	±39	±0.30	±16	±0.26	±10	±0.21	±8	±0.24	±8	±0.27	

The PA value reached highest in 30 minutes after being loaded, and the difference of the highest value and the previous value was within 0.8mg./dl. In 60 minutes after it reached highest, it returned to the former value. This development corresponds to Pattern N which the authors set forth, and therefore it was confirmed that PPDT of healthy persons would show Pattern N in all cases.

2. Classification of Disease Pattern of Diabetes Mellitus by PPDT

A. Standard of Classification

Results of Pattern N of healthy people were taken as the standard, and the standard value as shown in Table 2 was established in order to actually classify Patterns A, B and AB which we already set forth.

B. Results of PPDT of Diabetic Patients

Results of PPDT conducted 223 times in total with 100 cases of diabetic patients are classified as follows:

- 24 cases of Pattern N
- 59 cases of Pattern A
- 23 cases of Pattern B
- 34 cases of Pattern AB

Fig. 2. Results of PPDT in healthy persons.

Table 2. Standard of Classification of Disease Pattern in Diabetes Mellitus by PPDT

Type	Blood Sugar			Pyruvic Acid		
	When reached highest value after loading (min)	Difference of between highest level and previous level (mg/dl)	When dropped to previous level after loading (min)	When reached highest value after loading (min)	Difference of between highest level and previous level (mg/dl)	When dropped to previous level after loading (min)
N	15	150 >	30 ~ 90	15 ~ 30	0.8 >	30 ~ 90
A	15	150 <	90 <	60 ~ 150	0.8 >	90 <
B	15	150 >	30 ~ 90	15 ~ 30	0.8 <	120 <
AB	15	150 <	90 <	60 ~ 150	0.8 ≥	150 <

The transitive curve, which was obtained by connecting the mean blood sugar level and the mean PA value at the point of each time is shown in Figs. 3, 4, 5 and 6.

Fig. 3. Pattern N.

Examining the remaining 83 cases which did not belong to any of four patterns, 21 cases were found to show the blood sugar transition of Pattern A while the transition of PA was suggestive of Pattern N, and 30 cases showed the blood sugar transition of Pattern N, and PA, of Pattern A. These 51 cases were

Fig. 4. Pattern A.

regarded as the sub-pattern of Pattern A, because the production process of PA was hindered for reasons to be explained afterwards.

Fig. 5. Pattern B.

Fig. 6. Pattern AB.

Next, 21 cases among the rest were suggestive of Pattern N for the blood sugar and Pattern AB for PA, and 20 cases indicated Pattern A for blood sugar and Pattern B for PA. These 32 cases were regarded as the sub-pattern of Pattern AB, because both the production and disposal processes were hindered for reasons to be explained afterwards.

The above results were summarized and shown in Table 3, among which 63% showed our fundamental Patterns of N, A, B and AB, while in 37% the attitudes of blood sugar and PA did not come to an agreement, and we took them as sub-patterns of Pattern A or Pattern AB, and the final classification suggested that in diabetes mellitus Pattern A constituted about 50%, followed by 30% of Pattern AB and about 10% each of Pattern N and Pattern B.

Table 3. Summarized Data of PPDT in Diabetic Patents

	Blood Sugar	
Pyruvic Acid	N	A
N	[N] 24cases	(A) 21cases
A	(A) 30cases	[A] 59cases
B	[B] 23cases	(AB) 20cases
AB	(AB) 12cases	[AB] 34cases

[] Fundamental Pattern
() Sub Pattern

Discussion

As stated in the introductory chapter, reports on the changes of the blood PA value when diabetic patients are loaded with G, are not always in agreement with

each other, and their results only represent one of four Patterns of N, A, B and AB which the authors established. However, the authors thought that in diabetes mellitus all these four patterns existed which could be confirmed with this experiment.

Regarding the time for PA to reach the highest value after G was loaded, for Pattern N it requires 15 to 30 minutes, which is believed to correspond to the time required in ordinary cases from G invading into the cell, going through Channel A and being metabolized into PA.

In Pattern A it required at least 60 minutes for PA to reach the highest value after G was loaded, however, unlike the presumed sketch in Graph 1, the value at 15 minutes after loading actually made almost no change from the previous value, and sometimes it was observed to be lower than the previous value.

This fact was already pointed out by Amatuzio¹²⁾ and Schifter¹³⁾ as the initial drop, but the cause is not clear. According to Bueding¹⁴⁻¹⁶⁾, Root¹⁷⁾, Flock¹⁸⁾ and Moorhouse¹⁹⁾, insulin accelerates the production of PA out of G, but it does not influence on the disposal process of PA.

On the other hand, Stadie's²⁰⁾ experiment in vitro of muscle homogenate of a pigeon added by PA, proved the increase of O₂ consumption and the decrease of PA by the addition of insulin.

The authors considered that the initial drop of Pattern A was due to the fact that in this pattern the production of PA after G was loaded was delaying, and therefore insulin action by the loading of sugar appeared before PA was produced, so that the disposal process of PA was proceeding.

Thirty-seven per cent of diabetic patients did not correspond to the fundamental patterns in either of the blood sugar curve or PA curve.

First, taking the case of the blood sugar curve indicating Pattern N and the PA curve, Pattern A, the case was considered that there was no hindrance in the process of G's invasion into the cell, but that the PA production afterward was hindered, so that the case was classified as the sub-pattern of Pattern A.

In the other case of the blood sugar curve showing Pattern A and the PA curve, Pattern N, blood sugar, which remarkably increased instantaneously, was taken in the cell promptly up to a certain blood sugar level even in Pattern A, which was metabolized into PA, and therefore the PA curve indicated Pattern N, however, coming below this blood sugar level, the invasion into the cell was hindered and the blood sugar curve indicated Pattern A. This is why the authors made it the sub-pattern of Pattern A. In the similar way of thinking, the case of the blood sugar curve being Pattern A and the PA curve, Pattern B, and the case of the blood sugar curve being Pattern N and the PA curve, Pattern AB, these were considered to be similar with Pattern AB and made them sub-patterns of Pattern AB.

There have hitherto been many methods of classifying the disease patterns of diabetes mellitus, however, no satisfactory method has ever been found which enables clinicians to foresee the complications or prognosis of the disease and afford a clue

to the most appropriate therapy.

The classification of this disease by the plight of intermediary metabolism of glucide is believed to be one step nearer to the above objective, so that the authors adopted the above method of classifying it into four patterns according to the function of production and disposal of PA, and we wonder how far our classification will be helpful for clinicians treating diabetic patients.

The facts that there are many cases of diabetic retinopathy or nephrosis among Pattern A or AB, and that biguanide preparations are excellently effective for Pattern A, and sulfonylurea preparations, for Pattern B or AB, imply that our classification is clinically significant, and details thereof will be reported separately by Yoshino, one of the authors.

Summary

By the predominance of hindrances to either of the production process (A) and disposal process (B) of PA, we could classify diabetes mellitus into four patterns, i.e. Pattern A in which the production process of PA was hindered, Pattern B in which the disposal process of PA was hindered, Pattern AB in which both processes were hindered, and Pattern N in which both processes were not hindered.

Pattern A was most common and occupied about 50% of all, followed by Pattern AB which was about 30%, and Pattern B and Pattern N were about 10% respectively.

The authors believe that our classification is clinically significant.

References

- 1) Smith, M. J. H., and Taylor, K. W.: Blood Pyruvate and α -Ketoglutarate in Normal and Diabetic Subjects. *Brit. Med. J.*, 2: 1035 (1956).
- 2) Amatuzio, D. S. et al.: Blood Pyruvic Acid Response to Intravenous Glucose or Insulin in the Normal and in Patients with Liver Disease and with Diabetes Mellitus. *J. Clin. Invest.*, 31: 751 (1952)
- 3) Hills, O. W. et al.: Diabetes Mellitus and Cushing's Syndrome: Concentrations of Lactate and Pyruvate in Blood after Ingestion of Glucose. *Diabetes*, 1: 351 (1952)
- 4) Himwich, W. A. and H. B. Himwich: Pyruvic Acid in Exercising Depancreatized Dogs and Diabetic Patients. *J. Biol. Chem.*, 165: 513 (1956)
- 5) Anderson, J. and V. Marks: Pyruvate in Diabetes Mellitus. Concentrations in Urine and Blood. *Lancet*, 1: 1159 (1962)
- 6) Seligson, D. and B. Shapiro: Alpha-keto Acids in Blood and Urine Studied by Paper Chromatography. *Anal. Chem.*, 24: 754 (1952)
- 7) Kennedy, G. C. and Hill, L. E.: Some Effects of Sulphydryl Compounds on Keto-Acid Metabolism in Experimental Diabetes Mellitus. *J. Endocrin.*, 18: 409 (1959)
- 8) Gitelson, S.: The Blood Pyruvic Acid Level in Patients with Stress Hyperglycaemia and Diabetes Mellitus. *Acta Endocrin.*, 22: 87 (1956)
- 9) Alslev, J.: Vegieichende Untersuchungen über den Intermediären Stoffwechsel der Fructose bei Gesunden und Diabetikern. *Dtsch. Arch. Klin. Med.*, 202: 368 (1955)
- 10) Miller, M. et al.: Studies on Pyruvate and Citrate Metabolism in Man and Animal. *Proc. Am. Diab. A.*, 9: 83 (1949)

- 11) Horwitt, M.D. et al.: Lactic and Pyruvic Acids in the Blood after Glucose and Exercise in Diabetes Mellitus. *Am. J. Phys.*, **156**: 92 (1949)
- 12) Amatuzio, D.S. et al.: Blood Pyruvic Acid Response to Intravenous Glucose or Insulin in the Normal and in Patients with Liver Disease and with Diabetes Mellitus. *J. Clin. Invests.*, **31**: 751 (1952)
- 13) Schrifter, N. and Kritzer, M.: Carbohydrate Metabolism. II. Changes in the Serum Pyruvic Acid during Glucose-Tolerance Test in Normals, Diabetics, and Prediabetic Women. *Arch. Int. Med.*, **98**: 28 (1956)
- 14) Buedings, E. et al.: Blood Pyruvate Curves Following Glucose Ingestion in Normal and Thiamine Deficient Subyests. *J. Biolog. Chem.*, **140**: 697 (1941)
- 15) Bueding, E. et al.: Pyruvic Acid Metabolism in Diabetes Mellitus. *Am. J. M. Sc.*, **204**: 838 (1942)
- 16) Bueding, E. et al.: Effect of Insulin on Pyruvic Acid Formation in Depancreatized Dogs. *Science*, **95**: 282 (1942)
- 17) Root, H.F. et al.: The Respiratory Quotient and Blood Pyruvate and Lactate Responses after Oral Ingestion of Glucose and Fructose in Diabetes Melluitus with and without Insulin. *Am. J. M. Sc.*, **211**: 189 (1946)
- 18) Flock, E. et al.: The Utilization of Pyruvic Acid by the Dog. *J. Biol. Chem.*, **125**: 49 (1938)
- 19) Moorhouse, A.: Pyruvate-Tolerance Tests in Healthy and Diabetic Subjects. *Lancet*, **1**: 689 (1964)
- 20) Stadie, W.C. et al.: The Effect of Insulin upon Pyruvate Utilization by Pigeon Muscle. *J. Biol. Chem.*, **172**: 567 (1948)
- 21) Friedmann, T.E. and Haugen, G.E.: The Determination of Keto Acids in Blood and Urine. *J. Biol. Chem.*, **147**: 415 (1943)