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National Research Centre, Dokki, Cairo (Egypt) and Kasr El-Aini Faculty of Medicine, Cairo University

Blood-reduced glutathione, pyruvic acid, citric acid, ceruloplasmin oxidase activity and certain mineral changes in diabetes mellitus before and after treatment

R. Awadallah, E. A. El-Dessoukey, H. Doss, and *K. Khalifa*

With 1 table

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Many authors reported ample informations that provide indirect evidence of impaired pyruvate utilization in diabetes, both in man and experimental animals (6, 10). *Guly* (10) reported a grave disturbance in tricarboxylic acid cycle. Also, an increased value of pyruvate was shown by *Mincu* et al. (25) when a simultaneous glucose and insulin load was given. They showed also that in diabetes seems to be affected by sulphydryl group donors.

Zinc administration seems to stimulate glucose uptake (28) as well as an increase in plasma-insulin-like activity and seems to enhance the hypoglycaemic action of insulin itself (12).

Buckowski et al. (4) stated that there was no correlation between serum zinc and glucose levels determined in fasting patients, but *Shustov* (31) found that zinc content in the plasma tended to be low in diabetic patients.

There is a normal level of serum copper in diabetes (36). *Anspaugh* (2) however reported that a highly significant inverse correlation exists between copper levels and age of onset of symptoms of diabetes.

Shustov (32) stated that the serum copper levels in diabetic cases but without ketosis were only slightly higher than normal, but that during ketosis considerably higher values of serum copper were found. On the other hand, *Kosenko* (19) found that the blood copper was lower in diabetes mellitus in comparison to values found in healthy individuals.

Heintzelmann (13) found that the serum iron does not change in diabetic patients, but according to *Gobarets* (9) it was found that injection insulin led to reduction in serum iron.

Josinski et al. (16) found that the total and ionized plasma calcium were decreased in diabetic patients.

Slightly increased values of serum magnesium have been reported in diabetes mellitus (5), but lower blood magnesium level was found by *Ruichi* et al. (30).

Water and electrolyte metabolism were studied in diabetes mellitus by many investigators (3, 17).

The present study was performed to study the metabolism of blood pyruvic acid, reduced glutathione, serum citric acid, ceruloplasmin oxidase activity and certain inorganic elements such as zinc, copper, iron, calcium, magnesium, potassium and sodium in maturity onset of diabetes before and after treatment with some hypoglycaemic drugs and insulin.

Materials and methods

The subjects in this were divided into two groups. The first group comprised twenty normal male and female individuals of different ages, mostly laboratory personnel, medical staff and medical students. The female subjects were not taking any type of oral contraceptive. Second group included 40 patients with diabetes mellitus, they were all in- or out-patients in Kasr El-Aini Hospital and were treated with different hypoglycaemic drugs. This group was divided into four subgroups, each containing patients, treated with Tolbutamide "Rastinon-Hoechst", Glibenclamide "Daonil-Hoechst", Metformin "Glucophage-Cid" and insulin respectively.

Blood samples were taken from the fasting patients both before treatment and thirty days after treatment with hypoglycaemic drugs.

A portion of blood was used for analysis of blood glucose, reduced glutathione and pyruvic acids. The remaining portion was allowed to clot for two hours in polyethylene tube before centrifugation and the serum was separated for the analysis of serum citric acid, ceruloplasmin and minerals.

The method of *Nelson's* modification of *Somogyi's* (34) was used for determination of blood sugar. The total amount of blood pyruvic acid was estimated by the method of *Friedman* and *Haugen* (8). Serum citric acid was assayed by the method of *Marrier* and *Boulet* (23). Reduced blood glutathione was determined by method of *Thompson* and *Watson* (35). Serum zinc and copper were determined by the method of *Sinaha* and *Gabrieli* (33). The ceruloplasmin oxidase activity was determined by the method of *Henry* et al. (14). Serum iron, potassium and sodium were estimated by the method published in *Beckman* Analytical method by Atomic Absorption Spectrophotometer. Serum calcium and magnesium were determined using the method of *Willis* (37).

Results and discussion

Table 1 illustrates the data obtained for blood pyruvic acid, serum citric acid and reduced blood glutathione in maturity onset diabetes. These data showed that there was a significant elevation in the levels of total blood pyruvic acid and serum citric acid while there was a significant decrease in the level of reduced blood glutathione as compared with normal adult's levels. Our results are in concordance with the previous work (18, 24).

Thirty days after initiating treatment of these diabetic patients with Rastinon, Daonil and insulin the blood pyruvic acid, serum citric acid and reduced blood glutathione had returned to the normal levels. When Glucophage was used instead, these diabetics showed a slightly higher than normal blood pyruvic acid, serum citric acids, while reduced blood glutathione had returned to the normal level.

Decreased pyruvate utilization in diabetic patients may be caused by defective enzymatic reaction or by accumulation in some metabolites partially derived from pyruvate such as acetate, citrate, fumarate, which may lead to inhibition of pyruvate utilization. *Moorhouse* and *Kark* (26) suggested the presence of impaired pyruvate disposal in diabetes, both in

Table 1. Fasting blood glucose, reduced glutathione, pyruvic acid, citric acid, cerulo after treatment with some hypoglycaemic

Item	Normal (20)*	Diabetic (40)	Rastinon (10) before	after
F. Blood glucose (mg%)	103.2 ± 11.3	312.2 ±101.5	283.2 ± 76.6	183.5 ± 52.7
P >	–	0.0005	–	0.01
Blood GSH (mg%)	38.8 ± 2.78	27.6 ± 2.01	28.6 ± 1.88	37.6 ± 2.18
P >	–	0.0005	–	0.025
Blood Pyruvic (mg%)	1.58 ±0.16	2.63 ±0.28	2.42 ±0.23	1.76 ±0.12
P >	–	0.0005	–	0.005
Serum Citric (mg%)	2.63 ±0.28	3.77 ±0.58	3.66 ±0.35	2.94 ±0.48
P >	–	0.0005	–	0.01
Serum Zn (µg%)	124.9 ± 14.2	80.2 ±16.6	74.4 ±24.1	101.2 ± 35.4
P >	–	0.0005	–	0.05
Serum Cu (µg%)	105.0 ± 11.7	104.1 ± 24.2	94.3 ±15.1	105.6 ± 27.8
P >	–	n.s.	–	n.s.
Serum Cerul. (units)	600.5 ± 87.2	582.4 ± 80.4	559.3 ± 70.4	570.6 ± 74.6
P >	–	n.s.**	–	n.s.
Serum Fe (µg%)	99.1 ±17.5	106.9 ± 16.5	109.1 ± 16.6	117.9 ± 23.0
P >	–	n.s.	–	n.s.
Serum Ca (mg%)	9.69 ±0.86	10.58 ± 0.90	10.69 ± 0.88	9.20 ±0.98
P >	–	0.05	–	0.05
Serum Mg (mg%)	3.98 ±1.11	3.77 ±0.79	4.12 ±1.20	4.53 ±0.80
P >	–	n.s.	–	n.s.
Serum K (mg%)	17.38 ± 1.58	15.88 ± 0.86	15.44 ± 1.17	17.80 ± 1.55
P >	–	0.025	–	0.05
Serum Na (mg%)	330.8 ± 32.58	281.9 ± 25.25	290.4 ± 32.82	334.8 ± 34.79
P >	–	0.025	–	0.01

* Figures between parentheses indicate number of patients
** n.s.: not significant

juvenile and adults. He found prolonged time for the disappearance of injected pyruvate in diabetes than in normals. On the other hand, the explanation of increased citrate in diabetic persons might be either due to increase of acetyl Co A (6) or to decreased utilization of citrate.

In diabetic cases, the level of blood-reduced glutathione was significantly decreased in the individual values when compared to normal adults. This phenomenon may be explained as a result of decrease

plasmino oxidase activity and minerals levels in maturity onset of diabetes before and drugs and insulin (mean ± S.D.).

Daonil (10)		Glucophage (10)		Insulin (10)	
before	after	before	after	before	after
324.5	257.8	272.1	249.0	369.1	285.8
± 99.4	± 87.6	± 40.3	±103.5	± 90.4	±107.8
–	0.05	–	n.s.	–	0.05
23.4	34.9	27.3	35.6	29.7	36.2
± 1.35	± 2.12	± 1.85	± 2.34	± 1.59	± 1.36
–	0.025	–	0.025	–	0.025
2.76	1.69	2.68	2.13	2.66	1.49
±0.16	±0.15	±0.24	±0.19	±0.29	±0.13
–	0.025	–	n.s.	–	0.005
3.26	2.98	4.35	3.22	3.82	2.90
±0.48	±0.36	±0.69	±0.47	±0.87	±0.26
–	0.005	–	n.s.	–	0.005
83.5	123.5	87.6	98.0	75.3	112.8
±10.7	± 20.8	±15.6	±15.6	±16.2	± 59.1
–	0.01	–	n.s.	–	0.05
110.1	129.1	108.2	132.4	103.8	119.9
± 31.2	± 29.1	± 22.7	± 21.2	± 21.2	± 26.8
–	n.s.	–	0.05	–	n.s.
590.6	610.2	600.6	640.8	580.7	595.8
± 60.4	± 80.0	± 75.6	± 77.0	± 70.2	± 75.4
–	n.s.	–	0.05	–	n.s.
107.3	120.5	97.6	90.7	133.7	110.9
± 18.6	± 24.9	±13.2	±19.6	± 17.4	± 17.7
–	n.s.	–	n.s.	–	n.s.
10.68	9.83	10.49	10.58	10.47	9.20
± 0.65	±1.62	± 1.19	± 1.43	± 0.90	±1.09
–	n.s.	–	n.s.	–	0.025
3.86	3.57	3.49	3.40	3.59	4.11
±0.71	±0.48	±0.71	±0.40	±0.54	±0.78
–	n.s.	–	n.s.	–	0.05
15.76	16.84	16.48	15.39	15.85	15.38
± 0.91	± 2.64	± 0.85	± 2.18	± 0.53	± 2.40
–	n.s.	–	n.s.	–	n.s.
273.8	321.7	289.8	328.5	273.7	309.8
± 23.70	± 27.10	± 23.03	± 28.09	± 20.46	± 44.48
–	0.01	–	0.05	–	0.05

synthesis of glutathione in the liver. This receives evidence from the work of *Krahl* (20), demonstrating diminished incorporation of labelled glycine into glutathione in the liver slices of severely diabetic rats.

The serum zinc was lower in untreated diabetics (80.2 ± 16.6 $\mu g\%$ as compared with normal subjects 124.9 ± 14.2 $\mu g\%$).

After thirty days of treatment with Rastinon, Daonil or insulin the serum zinc returned to a nearly normal level. When a glucophage was the

treating agent, however, the level of serum zinc remained low (table 1). These findings are in agreement with those of most of the workers in this field (32).

Zinc ions seem to be intimately involved in carbohydrate metabolism, the lower serum zinc in adult onset may be due to an excessive loss of zinc in the urine, in the polyuria of diabetes (31, 32). Another possibility may be that the reported inability of diabetics to fix zinc in tissues may lead to an excessive loss of zinc in their stools as well due to excessive loss in the normally zinc-rich pancreatic juice.

Further explanation that zinc helps in the metabolism of glucose, a drop in blood zinc would therefore help to make the impairment in glucose utilization worse, so would establish a metabolic vicious circle.

The serum copper in maturity onset of diabetes was within normal limits (104.1 ± 24.2 $\mu g\%$) against (105.0 ± 11.7 $\mu g\%$) in normal subjects. This is in agreement with the finding of *Vanghelovicia* et al. (36).

With regard to the serum ceruloplasmin level, we found it to be 585.4 ± 80.4 units, against 600.5 ± 78.2 units in normal control subjects.

Shustov (32) stated the serum copper level in maturity onset diabetes without ketosis is slightly higher than normal, but that during ketosis considerably higher values were found. The higher values may be related to the haemo-concentration which sometimes occurs as a result of the diuresis present in these cases.

Kosenko (19), however, found the total blood copper to be lower in adult diabetics. The lower copper was occasionally due to the excessive loss of copper in the urine.

With regard to the effect of hypoglycaemic drugs on the serum copper and ceruloplasmin levels, there is no statistically significant difference after thirty days of treatment with Rastinon, Daonil or insulin, while the serum copper and ceruloplasmin increases after thirty days of treatment with Glucophage.

Shustov (32) reported that insulin therapy was without any effect on serum copper.

The mean serum iron in our subjects with adult onset diabetes was 106.9 ± 16.5 $\mu g\%$, when compared with normal adults, there is no significant difference. These findings are supported by those of *Heintzelmann* (13) who found also no change in the serum iron in diabetes.

Treatment of our cases with hypoglycaemic drugs and insulin led to no change.

The mean calcium was significantly higher in our subjects with adult onset diabetes mellitus.

With regard to the effect of hypoglycaemic drugs on calcium after thirty days of treatment with Rastinon or insulin, the serum calcium returned to near the normal level, but after treatment with Daonil or Glucophage it was still high in our cases.

Calcium plays a role in the process of secretion of insulin from the beta cells and that drugs like ouabain which diminish the calcium flux inhibit the secretion of insulin (29). On the other hand, *Malaisse* et al. (22) stated that glucose-induced insulin release is probably associated with a concomitant release of calcium.

There was a normal level of serum magnesium in our subjects with maturity onset diabetes. Our findings are in agreement with those of *Poroa* (27) who found no significant difference in serum magnesium in compensated and non-compensated diabetes.

With regard to the effect of hypoglycaemic drugs on serum magnesium levels, no difference was noted after treatment for 30 days with Rastinon, Daonil or Glucophage, while the serum magnesium significantly increased from 3.59 ± 0.54 mg% to 4.11 ± 0.78 mg% after 30 days of treatment with insulin.

It is now an established fact that diuretics like frusemode impair carbohydrate tolerance especially in diabetics. Recently, it has been suggested that this is a result of their prompting magnesium as well as potassium loss in the urine.

In this work, the mean serum potassium in our subjects was 15.88 ± 0.86 mg%. This significantly lower than the normal. Our results are in agreement with those of *Alonso* (1).

The low serum potassium in most diabetes is probably the result of osmotic diuresis due to the loss of glucose in the urine. Once a lower serum potassium occurs, a metabolic vicious circle develops, since potassium is an important catalyst and co-factor in many enzyme process in carbohydrate metabolism.

Potassium therapy, it is now clear, increases the ability of the pancreas to secrete insulin in the presence of hyperglycaemia (15).

Thirty days after treatment with Rastinon, but not after treatment with Daonil, Glucophage or insulin, the level of serum potassium returned to its normal.

The mean serum sodium was significantly lower in our subjects. Our findings are in agreement with those of many investigators (7).

This is most probably due to the glucose-induced osmotic diuresis. Initially there are losses of water, sodium and chloride from the extracellular fluid, but if the glycosuria continues, loss also occurs from the intracellular compartment (7).

These losses of fluid and electrolytes may impair carbohydrate metabolism and responsiveness to insulin (7). *Hales* et al. (11) maintained that sodium pump plays a role in insulin secretion. Percontra, stimulation of insulin secretion occurred secondary to an increase in the intracellular sodium ion concentration (24). *Letarte* et al. (21) similarly reported that insulin-stimulated glucose transport into fat cells is dependent on sodium.

With regard to the effect of hypoglycaemic drugs on serum sodium, we found that with the normalization of the diabetic state and diminution in the polyuria, serum sodium returns to the normal level after treatment.

Summary

In maturity onset diabetes the blood levels of total blood keto acids in terms of pyruvic, serum citric, calcium are significantly higher than in normal adults, while there is a decrease in reduced-blood glutathione, serum zinc, potassium and sodium levels.

There were no significant differences between diabetes and normal adults in the serum levels of copper, ceruloplasmin oxidase activity, iron and magnesium.

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Authors' address:
Dr. *Raafat Awadallah*, Biochemistry Department, National Research Centre, Dokki, Cairo (Egypt)