2,3-Diphosphoglycerate, Nucleotide Phosphate, and Organic and Inorganic Phosphate Levels During the Early Phases of Diabetic Ketoacidosis

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SUMMARY

The relation between serum and red blood cell (RBC) inorganic phosphate levels, RBC 2,3-diphosphoglycerate (2,3-DPG) levels, RBC nucleotide phosphate (P_n), and RBC total phosphate (P_t) levels were studied during the early phases of treatment and recovery from diabetic ketoacidosis (DKA).

A steady drop in serum inorganic phosphate was found during the first 24 hours of insulin treatment and was most profound at 24 hours. No statistically significant changes ($P>0.05$) were found in red cell inorganic phosphate or nucleotide phosphate levels during the 24-hour study period. The levels of total red cell phosphate were lower in this group of patients than in nonacidotic diabetic subjects and decreased slightly after 24 hours of treatment. The red cell 2,3-DPG levels were low at the initiation of therapy and remained low during the 24-hour study period. Glucose, bicarbonate, lactate, and ketone levels fell in linear patterns with treatment.

In view of the current evidence for the effects of low 2,3-DPG on oxygen delivery and the relation of low serum phosphate levels to RBC glycolysis and 2,3-DPG formation, this study reëmphasizes the need for phosphate replacement during the early phases of treatment of DKA. DIABETES *26*:429-33, May, 1977.

Since the early work of Guest and Rapaport,[1] low levels of red blood cell 2,3-diphosphoglycerate (RBC 2,3-DPG) have been reported in diabetic ketoacidosis (DKA).[2-6] It has been postulated that these low levels might lead to an impairment in oxygen delivery and thus to tissue anoxia. A marked and rapid drop in serum phosphate levels has been described in diabetic patients during treatment of DKA that might lead to RBC hypophosphatemia, interfering in turn with recovery of the 2,3-DPG levels within the red blood cell.[7,8]

We measured the serum and red blood cell (RBC) inorganic phosphate and RBC total phosphate, 2,3-diphosphoglycerate (DPG), and nucleotide phosphate levels during the early phases of treatment of DKA in order to study the relations between inorganic and organic phosphate levels during the early phases of treatment and recovery from ketoacidosis.

METHODS

Six patients in severe diabetic ketoacidosis who had been admitted to the Diabetes Service at the Los Angeles County-University of Southern California Medical Center were studied. The patients were included only if they met the following criteria: (1) serum glucose in excess of 400 mg./100 ml., (2) serum bicarbonate (HCO_3) less than 10 mEq./L., (3) total blood ketone levels more than 10 mEq./L. All patients were treated by the same protocol and received intravenous and subcutaneous insulin, hydration by intravenous fluids, bicarbonate (as NaHCO), and potassium (as KCl) according to protocol. Treatment of the underlying and/or precipitating cause of the ketoacidosis was also undertaken. Patients were monitored clinically and chemically and were started on oral food intake as soon as their condition was stable and they could tolerate food. No patient received oral feeding earlier than eight hours after treatment. For comparison of data with values obtained in nonketotic diabetic subjects, values obtained from a control group consisting of 30 ambulatory, well-controlled diabetic patients from the Diabetes Service at Rancho Los Amigos Hospital (RLAH) were used.

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Antecubital venous blood was drawn without stasis and treated according to the method previously described.[9,10] Phosphate determinations were performed with the Bessman phosphate analyzer.[11]

We determined the levels of serum inorganic phosphate (P_{is}), red blood cell inorganic phosphate (P_{ic}), red blood cell total phosphate (P_t), red blood cell total nucleotide phosphate (P_n), and red cell 2,3-diphosphoglycerate (2,3-DPG). Lactate levels were obtained from whole blood samples according to the method of Barker and Summerson;[12] total ketone and acetoacetate levels were determined by the Bessman-and-Anderson modified method.[13] Determinations of the serum glucose and bicarbonate were performed by the hospital laboratory using the SMA-12 Technicon Multiple-Channel AutoAnalyzer.

Student *t* test was used for the statistical analysis, and the data are presented as means ($\bar{X}$) ± standard error of the mean (S.E.M.).

RESULTS

Table 1 details the levels of serum inorganic phosphate (P_{is}), red blood cell inorganic phosphate (P_{ic}), red blood cell total phosphate (P_t), total nucleotide phosphate (P_n), and 2,3-DPG in patients with diabetic ketoacidosis during the first 24 hours of treatment.

The serum inorganic phosphate levels (P_{is}) dropped continuously with treatment. The 24-hour level reached the nadir of 0.49 ± 0.04 mEq./L. The drop of the serum inorganic phosphate levels was significant at the six-, eight-, and 24-hour intervals compared with the level at zero hour.

In contrast to the sharp drop in P_{is} levels, there seemed to be no significant difference ($P>0.05$) between the levels of the red cell inorganic phosphates (P_{ic}) obtained during the 24-hour study period and the levels obtained in control diabetics. A slight decrease in the P_{ic} levels was observed at the four-hour interval, with the lowest level obtained at the six-hour sample. These changes were not statistically significant.

At the initiation of treatment the RBC P_t levels were lower than those obtained in the control diabetics. These levels remained low in the early hours of treatment as well as after 24 hours of treatment. No statistically significant change in levels at the end of 24 hours was noted. The levels after eight and 24 hours showed no statistical difference ($P>0.05$) from the levels at the initiation of treatment (zero hour).

The total nucleotide phosphate (P_n) levels (which represent primarily the ATP and ADP levels within the RBC) were similar to the levels obtained in the controls. There were no significant changes during the course of treatment.

The 2,3-DPG levels at the initiation of therapy (time 0′) were significantly lower than the levels obtained in the diabetic control group. The DPG levels remained low and without significant change during

TABLE 1

Phosphate compound levels

Time	0 Hours	2 Hours	4 Hours	6 Hours	8 Hours	24 Hours	Controls*
P_t	15. 51§ ±1.97	15.20§ ±2.10	13.92§ ±1.62	14.32§ ±1.80	14.15§ ±1.74	12.74§ ±1.00	18.31 ±0.31
P_{is}	2.11 ±0.35	1.50 ±0.34	0.84‡ ±0.15	0.61† ±0.08	0.58† ±0.07	0.49† ±0.04	1.82 ±0.33
P_{ic}	0.87 ±0.12	0.87 ±0.12	0.65 ±0.12	0.58 ±0.15	0.65 ±0.13	0.67 ±0.15	0.88 ±0.21
P_n//	6.09 ±0.50	6.35 ±0.50	5.93 ±0.73	6.25 ±0.67	6.25 ±0.56	5.20 ±0.46	5.7 ±0.2
2,3-DPG	3.18¶ ±0.7	3.15¶ ±0.70	3.17¶ ±0.60	3.29¶ ±0.60	3.31¶ ±0.64	3.68§ ±0.60	4.89 ±0.11

Data give as mean ± standard error of the mean (S.E.M.).

P_t = Total phosphate (mM/L.RBC); P_{is} = serum inorganic phosphate (mEq./L. serum); P_{ic} = RBC inorganic phosphate (mM/L.RBC); P_n = total nucleotide phosphate (mM/L.RBC) (mM nucleotide P/L.RBC—see text); 2,3-DPG = mM DPG/L.RBC.

*Controls (for description, see METHODS).

†Significantly lower from 0′ at level of $P<0.005$

‡Significantly lower from 0′ at level of $P<0.01$

§Significantly lower than controls at level of $P<0.01$

¶Significantly lower than controls at level of $P<0.005$

//The nucleotide phosphate is approximately 90 per cent ATP and 7-8 per cent ADP

the 24-hour treatment study period.

Table 2 details the levels of glucose, lactate, total ketones, and acetoacetate measured every two hours from the initiation of treatment to eight hours and at 24 hours after initiation. The initially elevated glucose levels decreased with treatment to levels that were significantly lower than the zero-hour glucose level. The initially elevated lactate levels dropped consistently with treatment, and significantly lower levels ($P<0.05$) were found as early as six hours. The level at 24 hours was within the control group's level. The initially elevated total ketone levels dropped rapidly within the early hours of treatment, with a statistically significant decrease found as early as four hours from the start of therapy. Decrease of the initially elevated acetoacetate levels became statistically significant after eight hours.

The changes and relation between the levels of Pis, Pn, 2,3-DPG, and glucose during the early phases of treatment of DKA (zero to eight hours) are presented in figure 1.

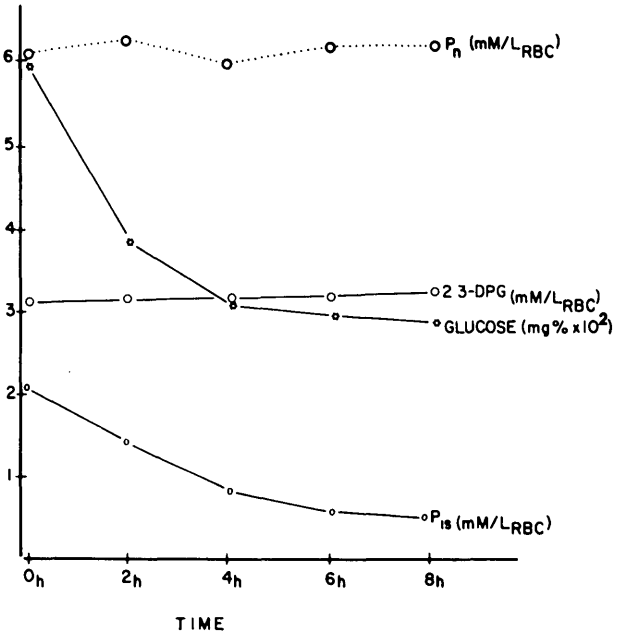

FIG. 1. Levels of nucleotide phosphate, 2,3-DPG, glucose, and inorganic phosphate during the first eight hours of treatment of ketoacidosis.

DISCUSSION

It is generally believed that insulin therapy causes a shift of phosphate and potassium into the cell. The profound decrease in extracellular (serum) phosphate documented in the current study is a reflection of this effect. However, in this study no significant changes in red blood cell inorganic phosphate levels and red blood cell nucleotide phosphate levels occurred. The absence of changes in these levels following insulin treatment of diabetic ketoacidosis confirms the consensus of the lack of direct effect of insulin on red blood cell metabolism. On the other hand the fall in total red blood cell phosphate levels at the end of 24 hours of treatment is possibly an indirect reflection of the effect of insulin on phosphate metabolism in other cells.

Since the discovery in 1967 by Chautin and Cornish[14] and simultaneously by Benesch and Benesch[15] that 2,3-DPG and, to a lesser extent, the nucleotide adenosine triphosphate (ATP) influence the affinity of hemoglobin for oxygen and facilitate oxygen release at the tissue levels, attention has been drawn to various conditions in which 2,3-DPG-level changes occur in the red blood cell. Low levels of 2,3-DPG have been reported in diabetic ketoacidosis[1,5,8] and in hypophosphatemic conditions.[16-19] 2,3-DPG, formed in the RBC by the Embden-Meyerhoff pathway, constitutes up to 65 per cent of all organic phosphates of the red cell. In other tissues, 2,3-DPG is about 0.01 or less of the RBC concentration. The fall in pH in diabetic ketoacidosis

TABLE 2

Glucose, lactate, total ketone, and acetoacetate levels

	0 Hours	2 Hours	4 Hours	6 Hours	8 Hours	24 Hours	Controls
Glucose (mg/100 ml.)	597±45	384±50*	308±37*	296±35*	287±19*	167±4†	91.7±2.2
Lactate (mEq./L.)	3.58±0.72	2.58±0.4	2.04±0.36	1.64±0.2*	1.39±0.26*	0.84±0.15*	0.97±0.08
Total ketones (mEq./L.)	17.2±1.53	15.27±1.5	12.68±1.3*	11.13±2.25*	8.39±2.3*	2.95±1.9†	0.24±0.02
Acetoacetate (mEq./L.)‡	8.53±0.88	8.15±0.91	7.97±0.96	5.94±1.24	4.66±1.31*	1.40±0.63†	0.13±0.02

Data given as mean ± standard error of the mean (S.E.M.).
*Significantly lower than zero hour at level of $P<0.05$.
†Significantly lower than eight hours at level of $P<0.01$.
‡Method includes acetone levels.

tends to shift the oxyhemoglobin dissociation curve to the right, facilitating oxygen release and counteracting the effect of the low 2,3-DPG levels. However, although the pH is returned to normal in a short period of time by treatment with insulin and intravenous fluids, the 2,3-DPG remains low and returns to normal levels slowly over three to five days.[6-8] Thus, the oxyhemoglobin dissociation curve may again be shifted to the left and oxygen delivery to tissues impaired.

In our patients, glucose levels returned to satisfactory levels within 24 hours and the ketone and lactate levels fell in a linear pattern. The bicarbonate levels also returned to near-normal over the initial 24-hour treatment period. However, 2,3-DPG levels remained low, with no significant return toward normal levels. Previous studies have shown that the nonketotic, nonacidotic chronic diabetic patient with evidence of complications maintains higher DPG levels (5.80 mM/L. RBC) than the chronic diabetic patient without complications (4.89 mM/L. RBC).[9] The latter group was used for comparison with the patients in the current study. Thus, low 2,3-DPG levels in the chronic diabetic patients with superimposed ketoacidosis might have an exaggerated effect on decreasing oxygen delivery.

One of the reasons for the slow recovery of 2,3-DPG levels might be the profound decrease in serum inorganic phosphate in the early phases of treatment of DKA. In 1948, Franks et al.[20] reported the occurrence of serum inorganic phosphate levels as low as 0.6 mEq./L. during the course of therapy of diabetic ketoacidosis, and they indicated a particular need for phosphates in the treatment regimen. In the patients in the current study, the Pis fell over the entire treatment period, at 24 hours reaching the low point of 0.49 mEq./L.

Although the need for phosphate replacement in the management of diabetic keotacidosis was recognized in the past,[20-22] supplementing phosphate in administered intravenous fluids is not a common procedure. Addition of phosphate up to a total of 65 mEq./L. of phosphorus in the first 24 hours of treatment of diabetic ketoacidosis has been recommended.[22] Anderson and Ditzel[3] showed that treatment of DKA with intravenous phosphate solution resulted in normalization of the 2,3-DPG content within hours rather than in days when no intravenous phosphate was given. In the face of the current evidence for the effect of low 2,3-DPG level on oxygen delivery and the relation between phosphate levels and 2,3-DPG formation, it seems important to reëmphasize that the regimen for treatment of DKA should include phosphate replacement in sufficient amounts to allow regeneration of 2,3-DPG and thus enhance oxygen delivery of tissue levels as early as possible in the ketoacidotic diabetic. The recommendations of the distant and recent past deserve renewed emphasis.

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