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The effects of 2,3-diphosphoglycerate, adenosine triphosphate, and glycosylated hemoglobin on the hemoglobin-oxygen affinity of diabetic patients

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Abstract

The position of the oxygen dissociation curve (ODC) is modulated by 2,3-diphosphoglycerate (2,3-DPG). Decreases in 2,3-DPG concentration within the red cell shift the curve to the left, whereas increases in concentration cause a shift to the right of the ODC. Some earlier studies on diabetic patients have reported that insulin treatment may reduce the red cell concentrations of 2,3-DPG, causing a shift of the ODC to the left, but the reports are contradictory. Three groups were compared in the present study: 1) nondiabetic control individuals (N = 19); 2) insulin-dependent diabetes mellitus (IDDM) patients (on insulin treatment) (N = 19); 3) non-insulin-dependent diabetes mellitus (NIDDM) patients using oral hypoglycemic agents and no insulin treatment (N = 22). The overall position of the ODC was the same for the three groups despite an increase of the glycosylated hemoglobin fraction that was expected to shift the ODC to the left in both groups of diabetic patients (HbA_{1c}: control, 4.6%; IDDM, 10.5%; NIDDM, 9.0%). In IDDM patients, the effect of the glycosylated hemoglobin fraction on the position of the ODC appeared to be counterbalanced by small though statistically significant increases in 2,3-DPG concentration from 2.05 (control) to 2.45 µmol/ml blood (IDDM). Though not statistically significant, an increase of 2,3-DPG also occurred in NIDDM patients, while red cell ATP levels were the same for all groups. The positions of the ODC were the same for control subjects, IDDM and NIDDM patients. Thus, the PO_2 at 50% hemoglobin-oxygen saturation was 26.8, 28.2 and 28.5 mmHg for control, IDDM and NIDDM, respectively. In conclusion, our data question the idea of adverse side effects of insulin treatment on oxygen transport. In other words, the shift to the left reported by others to be caused by insulin treatment was not detected.

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Introduction

In a pioneering study, Benesch and Benesch (1) found that 2,3-diphosphoglycerate (2,3-DPG) keeps the oxygen dissociation curve (ODC) within its normal operational range. An increase of 2,3-DPG reduces hemoglobin-oxygen (HbO_2) affinity, i.e., causes a shift to the right of the ODC, and increases the cooperativity of the four hemoglobin chains. On the other hand, decreased levels of 2,3-DPG cause a shift to the left and reduce cooperativity.

Red cell 2,3-DPG levels may counteract the effects of blood acid-base status on the ODC (2,3). For example, acute high altitude hypoxia combined with hyperventilation-induced alkalosis shifts the ODC to the left. An increase of red cell 2,3-DPG may, however, oppose this effect and return the ODC to its normal position (4). Modulations of 2,3-DPG concentrations may also occur during lack of oxygen due to congenital cardiac problems, anemia and chronically reduced cardiac output. Under these circumstances, 2,3-DPG concentrations increase with a consequent shift to the right of the ODC (for a review, see Ref. 5). The concomitant intracellular alterations have been examined in a large number of studies. Within the red cell, the glycolytic pathway includes the transformation steps from glucose to lactate. An intermediate step consists of the transformation of fructose-6-phosphate to 1,3-DPG. As a specific feature, the red cell has a shunt in which 2,3-DPG is formed from 1,3-DPG, involving a diphosphoglycerate mutase. Acidosis acts on this step to reduce 2,3-DPG formation and, moreover, depresses the main pathway in an anterior phosphofructokinase-mediated transformation step (5). Ketoacidosis (pH <7.1) in insulin-dependent diabetes mellitus (IDDM) can be associated with considerable reductions of 2,3-DPG levels (2,6-10), which partly compensate for the acid-induced shift to the right of the curve. Data for diabetic patients without ketoacidosis are, however, inconsistent. Thus, it has been reported that insulin administration decreases plasma concentrations of inorganic phosphates, leading to a reduced production of 2,3-DPG (11). Reduced levels of 2,3-DPG increase HbO_2 affinity, which, predictably, reduces oxygen delivery to the tissues (12,13). Farber et al. (13) induced diabetes in rats and subsequently measured 2,3-DPG levels and quantified the extent of nerve tissue degeneration. They found degeneration only in insulin-treated rats with lowered combined levels of 2,3-DPG and ATP. However, in a clinical study, Bodnar and Pristupiuk (14) reported a significant increase in 2,3-DPG in diabetic patients when compared to nondiabetic subjects.

In diabetes mellitus an increased glycosylation of hemoglobin tends to shift the ODC to the left, which would reduce oxygen delivery to tissues (15,16). Concomitantly, elevated levels of 2,3-DPG could shift the ODC to the right, which would then recover its normal position (17-19). Story et al. (20) concluded, however, that the increases of erythrocyte 2,3-DPG concentrations were insufficient to compensate for the formation of glycosylated hemoglobin (HbA_{1c}) and to maintain normal HbO_2 affinity in IDDM patients. By contrast, studying streptozotocin-induced diabetic rats, Alder et al. (21) observed that HbA_{1c} formation was counterbalanced by elevated 2,3-DPG. As a further complication, Kihara et al. (22) reported that the position of the ODC and the concentration of red cell 2,3-DPG of insulin-treated rats did not suffer any physiologically significant changes of the ODC or levels of 2,3-DPG. On the other hand, insulin treatment reduced tissue perfusion and nutrient supply to nerve tissue.

Considering these contradictory data, we decided to re-examine a possible influence of diabetes on red cell concentrations of 2,3-DPG, ATP and HbA_{1c}. In addition, the position of the ODC was determined. These measurements were performed in nondia-

betic individuals and in non-insulin-dependent diabetes mellitus (NIDDM) and IDDM patients by applying standard selection criteria (6,23-26). The paper focuses on the possible specific consequences of insulin treatment on 2,3-DPG levels and on the position of the ODC. As a special precaution, we have used a firmly established method to construct the ODC (see Methods). In addition, the curves were constructed based on several saturation points for PO_2/O_2-saturation relationships. In short, we based our study on multiple curve points combined with high n-values for each point.

Material and Methods

A total of 60 individuals were studied, divided into three groups: 1) nondiabetic individuals (N = 19, 12 males and 7 females; mean age ± SEM: 34.7 ± 0.4 years; range: 23 to 52 years); 2) IDDM (N = 19, 10 males and 9 females; mean age ± SEM: 31.5 ± 1.0 years; range: 14 to 59 years); 3) NIDDM (N = 22, 8 males and 14 females; mean age ± SEM: 52 ± 0.6 years; range: 30 to 70 years). Individuals with renal disturbances, smokers, alcohol-dependent persons and also individuals using medication for any disease other than diabetes were excluded from the study. Patients were recruited at the Diabetes Outpatient Clinic, Division of Endocrinology and Metabolism, Department of Clinical Medicine, University Hospital, Faculty of Medicine of Ribeirão Preto, University of São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil. The study was approved by the Ethics Committee of the University Hospital, FMRP-USP, and all samples were obtained with informed consent from all patients.

Blood samples

In the early morning hours, heparinized venous blood (17 ml) was withdrawn from fasting and resting patients and control individuals. The samples were immediately analyzed for *in vivo* pH, PCO_2, PO_2 and HbO_2 saturation using a Corning model 178 blood gas analyzer (Corning Inc., New York, NY, USA). Hematocrit and hemoglobin concentrations were then determined with a model T-890 Coulter (Beckman Coulter International, Nyon, Switzerland). In order to exclude individuals with abnormal hemoglobin, electrophoresis was carried out according to the method of Marengo-Rowe (27). In addition, 50 µl of fresh blood was hemolyzed to assess HbA_{1c}. Part of the blood was stored on dry ice and analyzed for ATP and 2,3-DPG within 15 min after withdrawal. In addition, tonometry for ODC construction was initiated within 10 min after sampling. Blood for the determination of glucose levels was withdrawn into a vacuum tube containing EDTA (Vacutainer, Franklin Lakes, NJ, USA).

Construction of the oxygen dissociation curve

The ODC was constructed on the basis of the "blood mixing technique" (28,29). In this procedure, the blood sample is divided into two equal volumes, each placed inside a tonometer. One tonometer was equilibrated with a gas mixture of 30% O_2, 6% CO_2 and balance N_2, which assures maximal saturation. The other tonometer received a mixture of 0% O_2, 6% CO_2 and balance N_2 to deoxygenize the blood. In both tonometers CO_2 was kept constant at 6%, corresponding closely to 40 mmHg at the altitude of FMRP-USP. Equilibration of the blood with these gas mixtures was achieved within 30 min, after which a 1-ml Hamilton syringe was used to withdraw known fractions of saturated and desaturated blood to obtain several saturations (20, 30, 50, 70, and 80%). Mercury was used not only to clear the syringe dead space but also to mix the blood samples within the syringe. The mixed blood volume (1 ml total) was then analyzed for PO_2, PCO_2, pH and HbO_2 saturation, using a blood gas analyzer (Corning model 178).

The curves are reported using the classical Hill plot, which is a logarithmic approach that permits the best possible definition of the position of the curve, since linear regression analysis can be applied.

Table 1. Relationships between hemoglobin-oxygen (HbO_2) saturation and partial oxygen pressure (PO_2) for the three groups studied.

HbO_2 saturation (%)	PO_2 (mmHg)		
	Control (N = 19)	IDDM (N = 19)	NIDDM (N = 22)
20	16.2 ± 0.4	16.9 ± 0.4	16.9 ± 0.4
30	19.5 ± 0.4	20.3 ± 0.4	20.6 ± 0.3
50	26.8 ± 0.6	28.2 ± 0.5	28.5 ± 0.3
70	38.2 ± 0.5	38.8 ± 0.6	38.9 ± 0.4
80	46.8 ± 0.9	47.6 ± 3.6	47.9 ± 0.6

Data are reported as means ± SEM.
IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus.

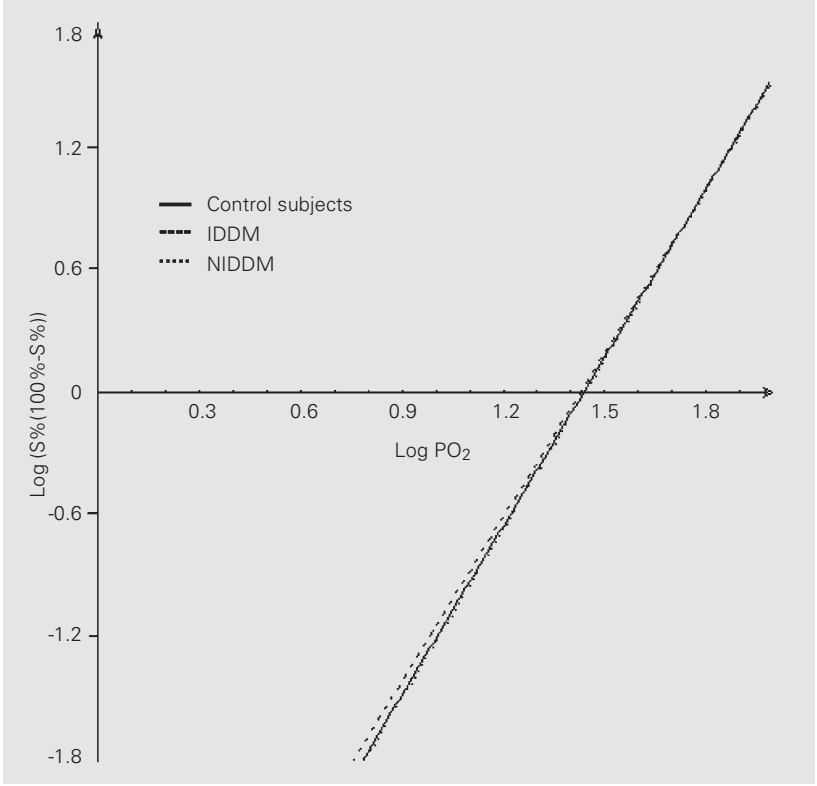

Figure 1. Hill plots for the oxygen dissociation curves for the three groups. See Table 2. IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus.

Analysis of glucose, 2,3-DPG, HbA_{1c} and ATP

Glucose levels were determined using a Sera-Pak diagnostic kit (Sera-Pak, Bayer Corp., New York, NY, USA) based on the method of Trinder (30). According to standard procedures for evaluation of diabetes mellitus, the HbA_{1c} fraction (%) was measured using a diagnostic kit (Glyco-Test II, Pierce, Rockford, IL, USA). Concentrations of 2,3-DPG were determined with a 35 UV kit (Sigma, St. Louis, MO, USA) according to the method of Lowry et al. (31), modified by Rose and Liebowitz (32). ATP concentrations were determined using a Sigma 366 UV kit (33,34).

Statistical analysis

Data are reported as means ± SEM (see n-values above). Analysis of variance (ANOVA) was performed and the Tukey or Student *t*-test was applied to determine the level of statistical difference between individual groups. Values of $P<0.05$ were considered to be significant.

The Pearson test for linear regression was used to analyze Hill plots in relation to correlation coefficient and 95% confidence intervals for the intercept of the regression line with the Y-axis.

Results

The PO_2-saturation relationships were identical for the three groups (see Table 1). The ODC are presented as Hill plots in Figure 1, that compares control individuals to IDDM and NIDDM patients. To perform a statistical analysis, the data of Table 1 were transformed into the form of Hill plots. The overlap of 95% confidence intervals for the groups (control, IDDM and NIDDM) implies that the overall curves were not statistically different. The constants and statistics for the Hill plots are presented in Table 2. The position of the ODC was not significant-

ly different between groups.

The acid-base status of venous blood samples was different for the three groups. Both groups of patients had a reduced pH relative to the control group, and the lowest *in vivo* values were measured in IDDM patients, while the reduction of the NIDDM group was not significantly different from control (Table 3). Consistently, venous PCO_2 was slightly elevated in both groups of diabetic patients, but was not statistically different from control (Table 3). During *in vitro* tonometry with 6% CO_2 (identical CO_2% and PCO_2) the blood pH values became statistically identical when equilibrated at the same PCO_2 (control 7.40, IDDM 7.41, NIDDM 7.41).

Plasma glucose and HbA_{1c} concentrations were in the same range in IDDM and NIDDM patients and, as expected, significantly higher than in the nondiabetic control group (Table 3). Hematocrit concentrations were identical for all groups (control individuals and IDDM and NIDDM patients).

2,3-DPG levels were significantly elevated in IDDM patients when compared to the control group, whereas increased levels in NIDDM patients were not statistically significant. ATP concentrations were identical for the three groups (control, IDDM and NIDDM) (Table 3).

Discussion

Our ODC data for the control individuals (PO_2 at 50% saturation = 26.8 mmHg; Table 1) closely agree with the extensive list of standard ODC reported for human blood (26.9 mmHg; Ref. 35). As mentioned above, Ditzel's group (9,11,12) suggested that insulin treatment would shift the ODC to the left, reduce oxygen delivery to the systemic tissues and potentially cause tissue necrosis in patients. They suggested that the ODC would undergo a shift to the left due to a combination of formation of glycosylated hemoglobin and a decrease of 2,3-DPG. Furthermore, they proposed that tissue damage would develop as a long-term consequence of impaired oxygen delivery. This idea received support from experiments on diabetic rats (13). Our results did not confirm these reports, since the ODC positions were not affected by diabetes mellitus or by insulin treatment. In both groups of diabetic patients, the elevated ($P<0.05$) HbA_{1c} fraction could shift the ODC to the left. This effect on the ODC, however, was masked by an elevated ($P<0.05$) level of red cell 2,3-DPG in the IDDM group. A tendency to an increase occurred in the NIDDM group but was not statistically significant. Consistently, Roberts et al. (36) reported increased red cell 2,3-DPG concentrations in IDDM patients with elevated HbA_{1c}. Moreover, these patients had no symptoms of hypoxic stress. Triolo et al. (37) reported similar data for patients without ketoacidosis. Furthermore,

Table 2. Parameters of the Hill plots and oxygen dissociation curves.

	Control	IDDM	NIDDM
Correlation coefficient	>0.99	>0.99	>0.99
Regression equation	y = -3.81 + 2.67x	y = -3.97 + 2.76x	y = -3.97 + 2.75x
95% CI for intercept	(-3.94 to -3.67)	(-4.09 to -3.85)	(-4.08 to -3.85)

Regression equations for the Hill plot. The slope of the curve is the Hill coefficient. P_{50} is the partial oxygen pressure that saturates 50% of the hemoglobin. The P_{50} values (see Table 1) and Hill coefficients were not statistically different, since the 95% confidence intervals (95% CI) of the group overlap (Pearson test). IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus.

Table 3. Comparison of blood variables of the studied groups.

Variable	Control	IDDM	NIDDM
Glycemia (mg/dl)	93.6 ± 1.7	151.2 ± 21.0*	160.5 ± 11.6*
HbA_{1c} (%)	4.6 ± 0.1	10.5 ± 0.7*	9.0 ± 0.4*
2,3-DPG (µmol/ml whole blood)	2.05 ± 0.08	2.45 ± 0.12*	2.21 ± 0.08
pH	7.40 ± 0.01	7.36 ± 0.01*	7.38 ± 0.01
ATP (µmol/g Hb)	4.08 ± 0.11	4.17 ± 0.15	4.25 ± 0.12
PCO_2 (mmHg)	42.3 ± 1.0	47.2 ± 2.4	44.1 ± 2.1

Data are reported as means ± SEM (n-values stated in the text). PCO_2 indicates the values prior to tonometry of the blood. HbA_{1c} = glycosylated hemoglobin; 2,3-DPG = 2,3-diphosphoglycerate; IDDM = insulin-dependent diabetes mellitus; NIDDM = non-insulin-dependent diabetes mellitus. *$P<0.05$ compared to the control group (ANOVA and Student *t*-test).

Rodriguez et al. (38) reported that diabetic patients with micro- or macroangiopathy had elevated levels of 2,3-DPG. On the other hand, Giardina et al. (39) measured 2,3-DPG concentration in diabetic patients and concluded that the values were equal to those of normal control individuals. A decreased level of red cell ATP plus 2,3-DPG was related to nerve tissue degeneration by Farber et al. (13), studying insulin-treated rats with induced diabetes mellitus. Neither group of patients in our study showed any change in red cell ATP levels due to diabetes mellitus.

The contradictions in the literature are difficult to explain. Results obtained with experimental animals may not apply to human physiology. Even within the framework of human physiology, conflicts may arise from the selection of specific groups for study. Using standard criteria for patient selection, we conclude that neither diabetes nor insulin treatment modified the position of the ODC when comparisons were performed at identical PCO_2/pH. This questions the idea of tissue damage due to an impaired pressure for oxygen delivery to tissues. In particular, it was not possible to confirm an effect of insulin treatment on the ODC.

Information on tissue damage in diabetes mellitus has increased in recent years. Importantly, clinical complications in patients have been related to alterations of taurine metabolism rather than to an altered position of the ODC (for a recent review, see Ref. 40). Therefore, several important tissue damages can be explained without implying any adverse shift to the left of the position of the ODC.

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