Effect of Sustained Hand Grip Isometric Exercise on the Response of Erythrocyte 2,3-diphosphoglycerate in Untrained Men[1]

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ABSTRACT. **Experiments were designed to examine the role of erythrocyte 2,3-diphosphoglycerate (2,3-DPG) in a purely isometric exercise (hand-grip dynamometer) using the human arm as an *in vivo* model, since the literature contains very little information concerning the contribution of this phosphate in such exercise. 2,3-DPG provides a compensatory adjustment to facilitate oxygen delivery during hypoxic conditions. Its breakdown occurs under acidosis. Twelve untrained males, age ranging from 19 to 22 years, exercised using a hand-grip dynamometer at a sustained contraction of load greater than 50% of their individual maximal voluntary contractions (MVC) until exhaustion occurred between 1.8–3.2 min. There were no significant post-exercise changes in the mean levels of 2,3-DPG when measured as either $\mu mol \cdot ml^{-1}$ red blood cell (RBC) or $mol \cdot mol^{-1}$ hemoglobin (Hb) immediately after exercise and 50 min after exercise even though immediate post-exercise increase in mean lactate levels and decrease in mean pH levels were significant ($P < 0.001$). It is concluded that the duration of exercise in the present study was too brief for acidosis to affect 2,3-DPG metabolism.**

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INTRODUCTION

Erythrocyte 2,3-diphosphoglycerate (2,3-DPG, also known as 2,3-BPG) enhances oxygen unloading from hemoglobin to tissues as it causes the oxygen dissociation curve to shift to the right (Benesch and Benesch 1967, Brown and Keith 1993). In many forms of hypoxia, the levels of 2,3-DPG increase (Brown and Keith 1993, Quatrini et al. 1993, Mairbaurl 1994), but the literature on the role of 2,3-DPG following exercise is replete with anomalous results. Some workers have observed an increase in post-exercise levels of 2,3-DPG (Meen et al 1981, Lijnen et al. 1988) while others have noted a decrease (Ramsey and Pipoly 1979, Odje and Ramsey 1995). Still others have reported no changes in 2,3-DPG levels following exercise (Shappell et al. 1971, Katz et al. 1984, Weight et al. 1992, Engfred et al. 1994). Investigations in our laboratory also found no change in the level of 2,3-DPG when a second group of subjects exercised for a longer duration (Odje and Ramsey 1995). However, little or nothing is known concerning the response of 2,3-DPG levels following isometric exercise alone, a form of exercise which occurs in some occupational settings.

Metabolic acidosis and respiratory alkalosis which are known to affect 2,3-DPG metabolism (Rose 1970, Ramsey and Pipoly 1979) may occur in this form of exercise (Tesch and Karlsson 1977, Sadamoto et al. 1992). Thus, experiments were designed to examine the effect of isometric exercise on 2,3-DPG levels in untrained males following a one-time strenuous hand grip exercise at a workload greater than 50% of the maximum voluntary contraction (MVC). The levels of 2,3-DPG and other factors were measured before exercise, immediately after exercise, and following 50 min of rest because some studies suggest that there is a time lag in 2,3-DPG response following exercise (Meen et al. 1981, Lijnen et al. 1988, Odje and Ramsey 1995).

MATERIALS AND METHODS

Selection of Subjects

Twelve untrained and otherwise healthy Caucasian males ranging in age from 19 to 22 years volunteered for the present study. One of the volunteers was a member of the college rugby team and none was undergoing any sort of drug therapy. At the time of recruitment, each volunteer was briefed concerning the goals, benefits, and method of investigation, in accordance with the Helsinki convention. The University Committee on the use of human subjects for research approved the study. Each subject reported to the laboratory at 9:00 AM. At the conclusion of each experimental phase, the subjects were briefed on their performances. The subjects reported fasting (8 hr) before each of their appearances and remained fasted during the exercise and recovery periods.

Isometric Exercise

The volunteers reported two at a time to the laboratory where a total of 12 ml of blood was withdrawn from the antecubital vein of their dominant arms in order to establish the control levels of all parameters in question (at time 0.0 min). The blood samples were collected in heparinized evacuated glass tubes and stored in ice. The samples for the determination of 2,3-DPG were immediately deproteinized with the aid of ice-cold 8% trichloroacetic acid (w/v), while the samples for the determination of lactic acid were deproteinized using 8% perchloric acid. Determination of blood pH and PCO_2 occurred within one hour of blood collection. The subjects then exercised using a hand-grip dynamometer (Lafayette Instrument Co., Lafayette, IN).

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Each subject exercised with his dominant hand maintaining a grip force of 30 kg. This workload, which was determined a week or more before the exercise, was greater than 50% of the MVC of each of the 12 volunteers' muscle tension. When the subject could no longer sustain the workload on the hand grip, blood samples were obtained from the antecubital vein of the exercising arm and analyzed. After 50 min of rest, blood samples were again taken and analyzed. The venous blood samples were analyzed for the following parameters: 2,3-DPG level; hemoglobin concentration (Hb); hematocrit (Hct); blood pH; and blood PCO_2. Oxygen consumption was also monitored with the aid of a Warren E. Collins respirometer (closed-circuit technique).

Specific Assays

Levels of 2,3-DPG were determined by a modification of the method of Rose and Liebowitz (1970) and values were reported in two ways: as $\mu mol \cdot ml^{-1}$ red blood cell (RBC); and as $mol \cdot mol^{-1}$ hemoglobin (Hb). All reagents for 2,3-DPG determination were obtained from the Sigma Chemical Company (St. Louis, MO). Determination of Hb was by the cyanmethemoglobin method, while Hct was determined with the aid of an International Microcapillary Centrifuge, Model MB (International Equipment Co., Boston, MA). Whole blood pH and PCO_2 were determined with the aid of a blood gas analyzer (Model 813 Instrumentation Laboratory, Inc., Lexington, MA). Blood lactate levels were determined by an ultraviolet method with the aid of the Sigma Technical kit #826. VO_2 was measured as described earlier.

Statistical Analysis

The changes in the levels of 2,3-DPG and all of the parameters in question were compared to their pre-exercise levels with the aid of the Student *t*-test for two means, in addition to analysis of variance (ANOVA) for multiple means. The results are presented as means ± standard error of the means (SEM). For the determination of statistical significance, a 5% level of significance was selected. The product-moment correlation coefficients of selected-paired variables were determined to assess the relationships among these variables. All statistical analyses were conducted using the procedures described by Sokal and Rohlf (1995).

RESULTS

The duration of sustained hand grip by the subjects ranged from 1.8 min to 3.2 min. Mean levels of parameters examined before exercise, immediately after exercise, and following 50 min of rest have been summarized (Table 1). The Pearson product-moment correlation coefficients of selected paired variables and the levels of significance were also determined (Table 2). The levels of mean post-exercise 2,3-DPG were not significantly different from the pre-exercise means, when expressed as either $\mu mol \cdot ml^{-1}$ of RBC or $mol \cdot mol^{-1}$ Hb. There were no changes in the mean levels of either Hb or Hct immediately after the exercise. Mean PCO_2 increased significantly from 7.28 ± 0.41 Kpa at the start of the exercise to 7.57 ± 0.13 Kpa at exhaustion ($P < 0.05$). Increase in mean lactate level and decrease in mean pH immediately after exercise were significant at $P < 0.001$. Immediate post-exercise mean value for oxygen consumption (VO_2) increased significantly ($P < 0.05$). Changes in immediate post-exercise pH were negatively correlated with changes in immediate post-exercise lactate and PCO_2.

TABLE 1

Levels of hematological parameters and oxygen consumption (VO_2) in untrained males following exhaustive hand grip isometric exercise at workload greater than 50% of their MVCs.

Time (min)	0.0 Pre-exercise	1.8–3.2 Post-exercise	51.8–53.2 After 50 min rest
Parameters			
Hemoglobin ($g \cdot 100\ ml^{-1}$)	14.97 ± 0.19	15.50 ± 0.28	14.78 ± 0.28
Hematocrit (%)	45.45 ± 0.60	46.33 ± 0.70	44.42 ± 0.43
2,3-DPG ($\mu mol \cdot ml^{-1}$)	2.14 ± 0.10	2.31 ± 0.08	2.10 ± 0.06
2,3-DPG ($mol \cdot mol\ Hb^{-1}$)	0.93 ± 0.03	0.96 ± 0.03	0.91 ± 0.03
Oxygen consumption ($ml \cdot kg^{-1} \cdot min^{-1}$)	4.04 ± 0.22	6.34 ± 0.39*	4.54 ± 0.18
Venous PCO_2 (Kpa)	7.28 ± 0.41	7.57 ± 0.13*	7.30 ± 0.54
pH	7.324 ± 0.01	7.192 ± 0.01**	7.330 ± 0.01
Lactate ($mmol \cdot l^{-1}$)	1.73 ± 0.17	7.40 ± 0.33**	1.60 ± 0.23

Values are reported as means ± SEM.
*Significantly different from pre-exercise means at $P < 0.05$.
**Significantly different from pre-exercise means at $P < 0.001$.

DISCUSSION

The present study is the first in which the role of 2,3-DPG has been evaluated with respect to isometric exercise alone. The forearm provided an *in vivo* model for the assessment of the relationship between 2,3-DPG and its modulating variables. Lack of changes in 2,3-DPG has been reported following dynamic exercise (Ramsey and Pipoly 1979, Weight et al. 1992, Engfred et al. 1994, Odje and Ramsey 1995). However, the findings here are at variance with reports following dynamic exercise in which the levels of 2,3-DPG either increased (Meen et al. 1981, Lijnen et al. 1988, Cade et al. 1984, Alvarez et al. 1992) or decreased (Ramsey and Pipoly 1979, Hasibeder et al. 1987, Odje and Ramsey 1995).

The short duration of exercise, common in sustained isometric contraction, is a possible cause for the lack of

TABLE 2

Pearson Product-Moment Correlation Matrix of paired variables in 12 untrained males following exhaustive hand grip isometric exercise at workload greater than 50% of their MVCs.

Variables (X)	Variables (Y)	Correlation Coefficient	Significance
Changes in 2,3-DPG at 50 min ($\mu mol \cdot ml^{-1}$ RBC)	Changes in post-exercise lactatea ($mmol \cdot l^{-1}$)	-0.41	NSb
Changes in 2,3-DPG at 50 min ($mol \cdot mol^{-1}$ Hb)	Changes in post-exercise lactate ($mmol \cdot l^{-1}$)	-0.17	NS
Post-exercise lactate ($mmol \cdot l^{-1}$)	Post-exercise pH	-0.77	$P < 0.005$
Post-exercise VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)	Post-exercise lactate ($mmol \cdot l^{-1}$)	-0.38	NS
Post-exercise PCO_2 (Kpa)	Post-exercise pH	-0.88	$P < 0.001$

[a]Time ranged from 1.8 to 3.2 min.
[b]Not significant.

change in the levels of erythrocyte 2,3-DPG immediately after exercise in the present study. It is known that 2,3-DPG synthesis develops slowly (Meen et al. 1981, Brown and Keith 1993). The presence of acidosis brought about in this study by lactate and PCO2 might have led to a reduction of 2,3-DPG because 2,3-DPG phosphatase, the enzyme that degrades 2,3-DPG, has been shown to become active at acid pH *in vitro* (Rose 1970). The levels of 2,3-DPG were not changed following rest, even though there was an immediate post-exercise acidosis. Lactate or pH levels do not recover immediately following isometric exercise (Sadamoto et al. 1992). The duration of exercise in the present study ranged from 1.8–3.2 min. The observed decrease in blood pH following purely isometric exercise was not accompanied by a decrease in 2,3-DPG following 50 min of rest as was noted in the same subjects (Odje and Ramsey 1995) following a 10 min bout of dynamic exercise. This may be caused by the difference in the duration of time that pH remained depressed in the different studies, resulting in an insufficient time for enzyme (2,3-DPG phosphatase) induction to occur.

In the present study, changes in blood pH were probably more localized to the exercising arm since it has been shown that sustained isometric exercises are accompanied by significant reductions in blood flow in the exercising muscles (Sjogaard et al. 1988). These investigators derived muscle blood flow from forearm blood flow measurements; forearm blood flow was measured by venous occlusion plethysmography. Once normal blood circulation returned to the arm at the end of exercise, the buffering capacity of the blood reversed the pH changes that were recorded immediately following exercise, thus returning the pH to homeostasis before it could have significant effects on 2,3-DPG status.

Although the forearm has provided a physiological model for the *in vivo* evaluation of 2,3-DPG in isometric exercise, data from the present study suggest that 2,3-DPG does not provide a compensatory adjustment to facilitate oxygen delivery in a one-time exhaustive isometric (hand grip) exercise. Perhaps studies of the effect of repeated (work, relax) and long lasting isometric exercises at lower MVC in which acidosis may be present for longer periods of time, planned for the future, may help to clarify the response of 2,3-DPG during isometric exercise.

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