Preexercise metabolic alkalosis induced via bicarbonate ingestion accelerates \( \dot{V}_{O_2} \) kinetics at the onset of a high-power-output exercise in humans

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Zoladz, Jerzy A., Zbigniew Szkutnik, Krzysztof Duda, Joanna Majerczak, and Bernard Korzeniewski. Preexercise metabolic alkalosis induced via bicarbonate ingestion accelerates \( \dot{V}_{O_2} \) kinetics at the onset of a high-power-output exercise in humans. J Appl Physiol 98: 895–904, 2005. First published October 29, 2004; doi:10.1152/japplphysiol.01194.2003.—The present study investigated the effect of preexercise metabolic alkalosis on the primary component of oxygen uptake (\( \dot{V}_{O_2} \)) kinetics, characterized by \( \tau_1 \). Seven healthy physically active nonsmoking men, aged 22.4 ± 1.8 yr, maximum \( \dot{V}_{O_2} (\dot{V}_{O_2\max}) \) 50.4 ± 4 ml·min\(^{-1} \cdot kg\(^{-1} \), performed two bouts of cycling, corresponding to 40 and 87% of \( \dot{V}_{O_2\max} \), lasting 6 min each, separated by a 20-min pause, once as a control study and a few days later at ~90 min after ingestion of 3 mmol/kg body wt of NaHCO\(_3\). Blood samples for measurements of bicarbonate concentration and hydrogen ion concentration were taken from antecubital vein via catheter. Pulmonary \( \dot{V}_{O_2} \) was measured continuously breath by breath. The values of \( \tau_1 \) were calculated by using six various approaches published in the literature. Preexercise level of bicarbonate concentration after ingestion of NaHCO\(_3\) was significantly elevated (\( P < 0.01 \)) compared with the control study (28.96 ± 2.11 vs. 24.84 ± 1.18 mmol/l; \( P < 0.01 \)), and [H\(^+\)] was significantly (\( P < 0.01 \)) reduced (42.79 ± 3.38 mmol/l vs. 46.44 ± 3.51 mmol/l). This shift (\( P < 0.01 \)) was also present during both bouts of exercise. During cycling at 40% of \( \dot{V}_{O_2\max} \), no significant effect of the preexercise alkalosis on the magnitude of \( \tau_1 \) was found. However, during cycling at 87% of \( \dot{V}_{O_2\max} \), the \( \tau_1 \) calculated by all six approaches was significantly (\( P < 0.05 \)) reduced, compared with the control study. The \( \tau_1 \) calculated as in Borroni et al. (Borroni R, Candau R, Millet GY, Perrey S, Fuchsloscher J, and Rouillon JD. J Appl Physiol 90: 2212–2220, 2001) was reduced on average by 7.9 ± 2.6 s, which was significantly different from zero with both the Student’s \( t \)-test (\( P = 0.011 \)) and the Wilcoxon’s signed-ranks test (\( P = 0.014 \)).

Acid-base status; oxygen uptake; oxygen uptake kinetics; oxidative phosphorylation

The transition from rest to work requires rapid acceleration of the ATP production in the working muscle cells. At the onset of a moderate or heavy exercise, the ATP demands are met mainly by acceleration of the creatine kinase reaction and oxidative phosphorylation, with rather minor contribution of anaerobic glycolysis (see, e.g., Ref. 38).

It was established that the \( \tau_1 \), characterizing the primary component of oxygen uptake (\( \dot{V}_{O_2} \)) kinetics, varies between 20 and 65 s, being shorter in physically trained subjects and longer in untrained individuals (42) or in patients suffering from cardiopulmonary insufficiency (28, 44).

It has been also reported that acute respiratory alkalosis slows down the primary component of the \( \dot{V}_{O_2} \) kinetics at the onset of exercise (13, 39). Despite the fact that an ergogenic effect of preexercise alkalization on the power generating capabilities was reported (see, e.g., Ref. 8), little is known about the effect of acute preexercise metabolic alkalization on the \( \dot{V}_{O_2} \) kinetics in humans. Previously, we have shown (46) and others confirmed (32) that preexercise metabolic alkalization does not modulate the magnitude of the slow component of \( \dot{V}_{O_2} \) kinetics in humans, estimated as the difference in \( \dot{V}_{O_2} \) between the sixth and third minute of work (see Ref. 46). But, to our best knowledge, no study has been performed to investigate the effect of preexercise metabolic alkalization on the primary component of the \( \dot{V}_{O_2} \) kinetics. Previous theoretical studies using our model of oxidative phosphorylation (23) showed that initial alkalization of the muscle cells (22), increase in resting pH (21), and inhibition of anaerobic glycolysis by protons (20) may play an important role in the acceleration of oxidative phosphorylation in the mammalian muscles at the onset of exercise. This is why we hypothesize that preexercise alkalization can influence the primary component of \( \dot{V}_{O_2} \) kinetics in humans at the onset of exercise via an effect on intracellular pH. The main purpose of the present investigation was to examine the effect of preexercise metabolic alkalosis induced by ingestion of 3 mmol/kg body wt of NaHCO\(_3\) on the \( \tau_1 \) characterizing the primary component of \( \dot{V}_{O_2} \) kinetics, as described by Barstow et al. (3). Various approaches to modeling the \( \dot{V}_{O_2} \) kinetics have been proposed in the literature (see, e.g., Refs. 3, 4, 5, 43). The secondary aim of our study was thus to investigate the extent to which the choice of a particular model could influence the analysis of the behavior of \( \tau_1 \).

METHODS

Subjects

Seven healthy, physically active men (means ± SD: age 22.4 ± 1.8 yr, body weight 75.8 ± 4.3 kg, height 183.3 ± 7.8 cm, body fat 12.1 ± 1.8% of body wt) participated in this study. The maximum \( \dot{V}_{O_2} (\dot{V}_{O_2\max}) \), determined during incremental exercise test; see the next section) of the subjects was 50.4 ± 4.0 ml·kg\(^{-1} \cdot \text{min}^{-1} \). Concentrations of their basic blood variables such as hematocrit value, hemoglobin, erythrocyte, leukocyte, Na\(^+\), K\(^+\), and creatinine were in the range of physiological values (see Table 1). The subjects abstained

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from a fatiguing physical activity 1 day before and on the day of the experiments. All were experienced in laboratory exercise tests. Ethical approval (Cancer Institute Medical Ethics Committee) was obtained for this study. Subjects were aware of the aims of the study and gave informed, written consent.

Exercise Tests Procedures and Alkalization

The incremental exercise tests. All subjects performed incremental exercise test until exhaustion on a cycloergometer Ergoline 800 S, to determine the VO2 max. The test started with a 6-min rest seated on the ergometer, followed by a gradual increase of power output by 30 W every 3 min. During the test, gas-exchange variables were measured continuously by breath by breath via the Oxycon Champion Jaeger, starting from the fifth minute before the exercise until the test was stopped. Before and after each test was finished, gas analyzers were calibrated with certificated calibration gases, as previously described by Zoladz et al. (47). One minute before the start of the first bout of exercise and at the end of each step (the last 15 s), antecubital venous blood samples were taken. The samples were used only for measurements of plasma lactate concentration ([La]pl) and for the description of the effectiveness of the alkalization. The tests were stopped when the subjects could no longer maintain the required pedaling rate and power output. The tests were performed ~2 wk before the constant-power-output tests.

Constant-power-output tests. The subjects performed two constant-power-output exercise tests (each of 6-min duration), separated by a 20-min pause. The power output during the first bout of exercise was planned to correspond to 40% VO2 max and the second bout (the high-power-output exercise) was planned to be performed at power output corresponding to 80% VO2 max. One minute before the start of the first bout of exercise and at the end of each minute of work (the last 15 s), venous blood samples were taken for [La]pl and blood acid-base balance variables. The lactate threshold (for overview of this concept, see Ref. 40) in this study, as previously (see Refs. 45, 47), was identified as the highest power output, above which [La]pl showed a sustained increase of >0.5 mmol·l−1·step−1.

The calculation of the power outputs corresponding to 80% of VO2 max was based on the linear relationship of the VO2-power output data, obtained during incremental tests and taking into consideration only data below the stage of sustained increase in [La]pl (see Refs. 45, 47). For this calculation, values of VO2 reached during the third minute of each stage of incremental test were applied. Because of the nonlinear increase in VO2 occurring above the lactate threshold, during the second bout of exercise the measured oxygen uptake corresponded to 87% VO2 max, instead of 80% VO2 max, as planned before the test (see also Refs. 45, 47). The first series of the constant-power-output tests (two bouts of exercise each of 6 min duration separated by a 20-min pause, as described above) was considered as the control study. At ~1 wk after performing the control study, the subjects reported to the laboratory once again to perform the constant-power-output exercise bouts, but on this occasion after ingestion of NaHCO3.

Ingestion of NaHCO3. The preexercise alkalization was induced by ingestion (within ~30 min) of 250 mg/kg body wt, i.e., 3 mmol/kg body wt of NaHCO3, placed in capsules containing 420 mg (5 mmol) of NaHCO3 each. During ingestion of the capsules the subjects were allowed to drink up to 500 ml of water. At the beginning of ingestion and every 15 min during ingestion of NaHCO3, blood samples were taken to determine the changes in the blood acid-base balance. When a significant alkalinaemia had occurred, which normally happened ~1.5 h after ingestion of the planned dosage of the NaHCO3, the subjects performed the constant-power-output test exactly as in the control study. Care was taken that meals ingested up to 1 day before the control experiment were exactly the same as those ingested before exercise performed after alkalization.

Blood Sampling and Biochemical Analysis

Venous blood samples (1 ml each) were taken by using Abbot Int-Catheter (18-G/1.2 × 45 mm), inserted into the antecubital vein ~30 min before the onset of exercise. The catheter was connected with the extension set (a tube 10 cm in length), using a “T” adapter (SL Abbot). Immediately before taking a 1-ml blood sample for analysis, we took 1 ml of blood volume to eliminate the blood from the catheter and the T adapter. One part of each sample was taken for the detection of blood gases (PO2 and PCO2) and blood hydrogen ion concentration ([H+]b), using a heparinized 90-μl capillary. The second part (0.5 ml) of the blood sample was placed in 1.8-ml Eppendorf tubes, containing 1 mg of ammonium oxalate and 5 mg of sodium fluoride, and mixed for ~20 s. Subsequently, to separate plasma for performing lactate and ammonium measurements, the blood samples were centrifuged. Samples of blood plasma (0.2 ml) were stored for further analysis at a temperature of ~25°C. PO2 and PCO2, as well as [H+]b, were determined using a Ciba-Corning 238 analyzer. The blood bicarbonate concentration ([HCO3]b) and the base excess were calculated by that unit. [La]pl was measured with an automatic analyzer Ektachem XR 700, Kodak. Serum Na+ and K+ concentrations were determined by using a flame photometer, Ciba Corning model 480. Blood creatinine level was determined by the kinetics method, on the basis of reaction with picric acid, by use of an automatic analyzer, Express 3. Hemoglobin concentration, hematocrit value, erythrocyte count, and leukocyte count were determined by using an automatic hematological analyzer, Baker 9000.

Modeling and Estimation of VO2 Kinetics

To investigate the effect of various data analysis scenarios, and to choose the most suitable one for the present analysis, six different approaches to estimation of the VO2 kinetics were tried. Each approach was characterized by the kind of data used and the kind of model fitted. The data were either raw (breath by breath) or smoothed (10-s averages). The model was either a three-component exponential function [as proposed by Barstow et al. (3)] or one of its two modifications, with the first cardiodynamic component (and respective data) ignored. Ten-second-averaging was used in many studies to reduce the random noise (see, e.g., Refs. 5, 30). The aim of ignoring the cardiodynamic phase has been to reduce the complexity of the model and thus to enhance the estimation precision for the remaining parameters. In many studies (e.g., Refs. 3 and 33), the raw data were preprocessed by interpolating and averaging over multiple exercise transitions. In this study, each exercise was performed only once, because of unknown pharmacokinetic effect of the ingestion of NaHCO3.

### Table 1. Concentration values in antecubital venous blood samples, taken at rest

<table>
<thead>
<tr>
<th></th>
<th>Hct, vol. %</th>
<th>Hb, g %</th>
<th>E, ml/min</th>
<th>V̇O2, l/min</th>
<th>[Na+]b, mmol/l</th>
<th>[K+]b, mmol/l</th>
<th>[Cr], mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min.</td>
<td>40.4</td>
<td>13.9</td>
<td>4.65</td>
<td>5.6</td>
<td>142.5</td>
<td>3.62</td>
<td>76.0</td>
</tr>
<tr>
<td>Max.</td>
<td>50.2</td>
<td>16.9</td>
<td>5.58</td>
<td>8.2</td>
<td>147.0</td>
<td>4.47</td>
<td>110.9</td>
</tr>
<tr>
<td>Means ± SD</td>
<td>45.56±3.02</td>
<td>15.49±1.04</td>
<td>5.24±0.29</td>
<td>6.76±0.77</td>
<td>144.6±1.44</td>
<td>4.16±0.34</td>
<td>90.96±11.54</td>
</tr>
</tbody>
</table>

Minimal (Min.), maximal (Max.), and mean ± SD values for 7 subjects. Hct, hematocrit value; Hb, hemoglobin; E, erythrocyte concentration; [Na+]b, sodium concentration; [K+]b, potassium concentration; [Cr], creatinine concentration.
Approach A. Use raw data and fit the complete model, as e.g., in Barstow et al. (3) and Scheuermann et al. (33). The model is as follows:

\[
\dot{V}_O_2(t) = \dot{V}_O_2(0) + A_0 \times (1 - e^{-\min(t;TD)\tau_1}) + A_1 \times (1 - e^{-\min(t;TD)\tau_2}) \quad \text{Phase 1}
\]

\[
+ A_2 \times (1 - e^{-\min(t;TD)\tau_3}) \quad \text{Phase 2}
\]

\[
+ A_3 \times (1 - e^{-\min(t;TD)\tau_4}) \quad \text{Phase 3}
\]

\[
\dot{V}_O_2(0) \text{ is the resting baseline value, calculated as an average over the 3-min resting period (seated on the ergometer) before the onset of the exercise} \quad (t=0), \quad \text{where the first exponential term starts. The other terms start after independent time delays (TD). The phase I response is terminated at} \quad t=TD_1 \quad \text{and given the value at that time.}
\]

Apporach B. Use smoothed data and fit model given by Eq. 1, used in approach A.

Approach C. Use raw data, ignore data from the first 20 s of the exercise [cf. e.g., Brittain et al. (5)] and fit the model:

\[
\dot{V}_O_2(t) = \dot{V}_O_2(0) + A_0' \times (1 - e^{-t/20}) + A_1 \times (1 - e^{-t/20}) \quad \text{(2)}
\]

The first exponential term starts at \( t = 20 \) and the second one at \( t = \text{TD}_2 \). This corresponds to setting \( \text{TD}_1 = 20 \) and omitting phase I in the model given by Eq. 1. \( A_0' \) is then the increase of \( \dot{V}_O_2 \) during phase I, the shape of which is not modeled. This model is just another parameterization of the model used in approaches E and F below. It was suggested as “model 3” in Whipp et al. (43).

Approach D. Use smoothed data, ignore data from the first 20 s, and fit model given by Eq. 2, used in approach C.

Approach E. Use raw data, ignore data from the first 20 s of the exercise and fit the model:

\[
\dot{V}_O_2(t) = \dot{V}_O_2(0) + A_0 \times (1 - e^{-t/20}) + A_1 \times (1 - e^{-t/20}) \quad \text{(3)}
\]

The first exponential term starts at \( t = 20 \) and the second one at \( t = \text{TD}_2 \). The time delay \( \delta \) is similar to (but not identical with) \( \text{TD}_1 \) from the model given by Eq. 1. The same approach was used, e.g., by Borrami et al. (4).

Approach F. Use smoothed data, ignore data from the first 20 s, and fit model given by Eq. 3, used in approach E.

In all cases, the relevant models were fitted to the corresponding data by the least-squares method, using the Nonlinear Estimation module of Statistica, version 6. Several starting points for the iterative minimization procedure were tried, to find the best fit. Figure 1 illustrates approaches B, D, and F.

For high-power-output exercise, addition of the slow phase (Phase 3) considerably improved the fit and the estimation precision for \( \tau_1 \). This is illustrated in Fig. 2 for approach F and one of the subjects. In addition to the curve given by Eq. 3, a single exponential curve, obtained from Eq. 3 by dropping the slow phase component, was fitted to the control data. Dropping the slow phase component more than doubled the residual sum of squares (1,021,858 vs. 447,351) and almost doubled the standard deviation of the estimate of \( \tau_1 \). Moreover, the residuals clearly showed a lack of fit for the single exponential and a significantly better fit for the complete model. Note that, for the subject reported in Fig. 1, the slow phase started rather early (TD1 = 75). For other subjects, the slow phase started later (between \( t = 100 \) s and \( t = 250 \) s) but the effect of the incomplete model specification was similar and clearly visible as well.

Low-power-output exercise, there was no slow phase component, and reduced models, with Phase 3 dropped, were fitted.

 Statistics

The significance of the difference between \( \tau_1 \) (and other variables) measured in the control study and that after alkalinization was tested with the matched-pairs Wilcoxon’s signed-ranks test, which means that for each subject the change \( \Delta \tau_1 \) of \( \tau_1 \) was computed and the Wilcoxon’s test was used to check whether those differences are significantly nonzero. Exact (i.e., nonasymptotic), one-sided \( P \) values were computed with a method described in Ex. 3.7.3 in Hettmansperger (14).

The nonparametric test with the exact \( P \) values was used because of the small sample size (7 subjects), on which the comparisons of the control and alkaliotic \( \tau_1 \) were based. With seven data points, it is, of course, impossible to reliably verify the assumption of normality, needed for an application of standard parametric tests such as the Student’s \( t \)-test. For the nonparametric Wilcoxon’s signed-ranks test, one only needs to assume the symmetry of the distribution with respect to its median, which is much weaker and, in our opinion, acceptable for the present study. It should be stressed that this is a much more conservative approach to testing the significance of the differences than parametric testing. In other words, it is much easier, in small samples, to “prove the significance” of the difference by means of the \( t \)-test, but it is, at the same time, quite likely that such “significance” is mainly due to (unverifiable) normality assumption rather than due to the evidence contained in the data. One-sided \( P \) values were used because, as explained in the introduction, previous theoretical studies had suggested that, if there is at all a change in the primary component of the \( \dot{V}_O_2 \) kinetics because of the preexercise alkalinization, then it should be its acceleration.

RESULTS

Bicarbonate Concentration and pH at Rest in the Control Study and Before NaHCO3 Ingestion

\([\text{HCO}_3^-]_b\), measured at rest before the exercise in the control study, was not significantly different from its value determined before the onset of ingestion of \( \text{NaHCO}_3 \) (90 min before exercise), amounting to 24.84 ± 1.18 vs. 24.6 ± 0.70 mmol/l, respectively, in both studies. Similarly, no difference in hydrogen ion concentration ([H\(^+\)]) was found in the control study (46.44 ± 3.51 mmol/l) and before the onset of ingestion of \( \text{NaHCO}_3 \) (46.60 ± 3.60 mmol/l).

Effect of Ingestion of 3 mmol/kg of \( \text{NaHCO}_3 \) on the Acid-Base Balance and Plasma Lactate Concentration at Rest and During Cycling at 40 and 87% of \( \dot{V}_O_2\) max

Low-power-output cycling exercise, amounting to 107 ± 14 W, corresponding to 40% \( \dot{V}_O_2\) max. The preexercise \([\text{HCO}_3^-]_b\) after ingestion of 3 mmol/kg of \( \text{NaHCO}_3 \) was significantly higher \((P<0.01)\) than in the control study. \([\text{H}^+]\) measured at rest after preexercise alkalinization was significantly lower \((P<0.01)\) than in control conditions. No statistically significant effect of ingestion of \( \text{NaHCO}_3 \) was found on the preexercise level of \([\text{La}]_{pl}\) (see Table 2).

During exercise (between the 1st and 6th minutes of work), performed after preexercise alkalinization, the time-averaged \([\text{HCO}_3^-]_b\) was significantly higher \((P<0.01)\) than in the control study. Time-averaged \([\text{La}]_{pl}\) during exercise performed after preexercise alkalinization was slightly but not significantly higher \((P=0.14)\) than in the control study. Time-averaged \([\text{H}^+]\) during exercise performed after preexercise alkalinization was significantly lower \((P<0.01)\) than in the control study (see Table 2).

High-power-output cycling exercise amounting to 256 ± 24 W, corresponding to 87% \( \dot{V}_O_2\) max. At rest before the second bout of exercise, \([\text{HCO}_3^-]_b\), after ingestion of \( \text{NaHCO}_3 \) was significantly higher \((P<0.01)\) than in the control study. \([\text{H}^+]\) after ingestion of \( \text{NaHCO}_3 \) was significantly lower \((P<0.02)\) than in the control study. \([\text{La}]_{pl}\) measured also at rest before
Fig. 1. Models of oxygen uptake (\(\dot{V}O_2\)) kinetics fitted to subject 6 control (left) and alkalotic (right) data. Vertical dashed lines mark the beginning of the fit range. \(\tau_0\), \(\tau_1\), and \(\tau_2\), Transition times; TD1 and TD2, time delays.
performed after preexercise alkalinization was significantly lower ($P < 0.01$) than in the control study (see Table 3).

**Effect of Ingestion of 3 mmol/kg of NaHCO$_3$ on the V$_O$$_2$ Kinetics at the Onset of Exercise**

The quantitative conclusions were based on the values of $\tau_1$ estimated with **approach F**, which, as discussed in more detail in the next section, most adequately described the primary component of V$_O$$_2$ kinetics. Qualitatively, the conclusions obtained with all approaches ($A$–$F$) were thoroughly consistent.

Ingestion of 3 mmol/kg of NaHCO$_3$ had no significant effect on the preexercise V$_O$$_2$, both before cycling at $40\%$ and at $87\%$ of V$_O$$_2$$_{\max}$. The preexercise V$_O$$_2$, measured before cycling at $40\%$ of V$_O$$_2$$_{\max}$, has amounted to $391 \pm 57$ ml/min in the control experiment and to $440 \pm 40$ ml/min after ingestion of NaHCO$_3$. Before cycling at $87\%$ of V$_O$$_2$$_{\max}$, the preexercise V$_O$$_2$ in the control study amounted to $417 \pm 70$ ml/min and to $412 \pm 72$ ml/min after preexercise alkalinization.

The values of $\tau_1$, representing the primary component of V$_O$$_2$ kinetics in the control study and after preexercise alkalinization, are presented in Table 4. For four typical subjects, the models fitted to the control data and the models fitted to the alkalotic data are shown, superimposed, in Figs. 3 and 4 ($40\%$ V$_O$$_2$$_{\max}$) and Figs. 5 and 6 ($87\%$ V$_O$$_2$$_{\max}$), along with corresponding data and residuals.

During low-power-output exercise, preexercise alkalinization had no effect on $\tau_1$. However, the $\tau_1$ measured during high-power-output exercise (corresponding to $87\%$ V$_O$$_2$$_{\max}$), performed after preexercise alkalinization, was significantly reduced ($P = 0.014$) (see Table 4).

**DISCUSSION**

The main and original finding of the present study is that preexercise metabolic alkalinization of blood, induced by ingestion of 3 mmol/kg of NaHCO$_3$, did cause a significant acceleration of the primary component of the pulmonary V$_O$$_2$ kinetics (shortening of the $\tau_1$ by $\sim 25\%$: $7.9 \pm 2.6$ s, on average) at the onset of a high-power-output exercise, corresponding to $87\%$ of V$_O$$_2$$_{\max}$ (see Table 4). It should be noticed, however, that preexercise alkalinization had no effect on the $\tau_1$ during low-power-output exercise, corresponding to $40\%$ of V$_O$$_2$$_{\max}$.

In the present study, six different approaches to modeling the V$_O$$_2$ kinetics were used, not to increase the significance of

![Fig. 2. Model F with (slow) phase 3 (solid lines) and without the slow phase (dashed lines), fitted to subject 2 data [87% maximal V$_O$$_2$ (V$_O$$_2$$_{\max}$), control; top] and the corresponding residuals (bottom). Dropping the slow phase component results in a considerably worse fit, with the residual sum of squares more than twice as large as that for the model with the slow component included.](http://jap.physiology.org/)

**Table 2. Venous [HCO$_3^-$]$_b$, [H$^+$]$_b$, and [La]$_{pl}$ at rest and during each minute of the exercise corresponding to 40% V$_O$$_2$$_{\max}$, performed as a control and after preexercise alkalinization**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>[HCO$_3^-$]$_b$, mmol/l</th>
<th>[H$^+$]$_b$, mmol/l</th>
<th>[La]$_{pl}$, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Alkalization</td>
<td>Control</td>
</tr>
<tr>
<td>Rest</td>
<td>24.84±1.18</td>
<td>28.96±2.11</td>
<td>46.44±3.51</td>
</tr>
<tr>
<td>1</td>
<td>25.36±1.28</td>
<td>29.30±1.83</td>
<td>45.93±2.74</td>
</tr>
<tr>
<td>2</td>
<td>24.91±1.34</td>
<td>29.03±2.22</td>
<td>46.99±3.30</td>
</tr>
<tr>
<td>3</td>
<td>24.67±1.39</td>
<td>29.69±1.91</td>
<td>48.20±3.78</td>
</tr>
<tr>
<td>4</td>
<td>24.46±1.38</td>
<td>28.76±1.83</td>
<td>48.73±3.70</td>
</tr>
<tr>
<td>5</td>
<td>24.49±1.40</td>
<td>29.27±2.13</td>
<td>48.79±3.50</td>
</tr>
<tr>
<td>6</td>
<td>24.20±1.26</td>
<td>29.14±1.65</td>
<td>49.37±3.44</td>
</tr>
</tbody>
</table>

Values are means ± SD for 7 subjects. V$_O$$_2$$_{\max}$, maximal oxygen uptake; [HCO$_3^-$]$_b$, blood bicarbonate concentration; [H$^+$]$_b$, blood hydrogen ion concentration; [La]$_{pl}$, plasma lactate concentration.
the conclusions, but rather to check whether the choice of a particular approach influenced the results. Although the qualitative conclusions from all six approaches were consistent, we believe that approach F best served our purposes. Phase 1 parameters could not be well estimated from the data obtained in our experiment. This seems to be a general problem: to control (and reproduce in repeated experiments) very exactly the increase of the generated power output in the first seconds of exercise, before the target level is reached. In the complete model, used in approaches A and B, it has been observed that this uncertainty propagated to phase 2 parameters. Moreover, the estimates heavily depended on (even small) shifts of the starting point of the exercise. The unavoidable uncertainty in the latter was a very influential source of instability of the estimates. Approach F eliminated the two factors by ignoring data from the first 20 s and restricting the estimated model to phases 2 and 3 only. Moreover, the parameterization of the model used in approach F was superior to that used in approach D in two respects: the estimates were less sensitive to the choice of the starting point for the minimization procedure used in the least squares fitting, and the parameters were easier to interpret (approaches C and D sometimes produced negative values of the $A_0$ parameter). Thus, in our opinion, the values of $\tau_1$ estimated in approach F most adequately described the primary component of $V_{O2}$ kinetics, and they were used as the basis for the final conclusions.

The calculations of $\tau_1$ in our study are based only on one repetition of each exercise. We are aware of the fact that this is usually not enough to allow precise kinetics analysis with partitioning among different phases. Therefore, studying the impact of alkalization on the parameters of, e.g., the slow component of the $V_{O2}$ kinetics was not possible, because those

Table 3. Venous $[HCO_3^-]_{pl}$, $[H^+]_{pl}$, and $[La]_{pl}$ at rest and during each minute of the exercise corresponding to 87% $V_{O2 max}$, performed as a control and after alkalization.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>[HCO$<em>3^-$]$</em>{pl}$, mmol/l</th>
<th>[H$^+$]$_{pl}$, mmol/l</th>
<th>[La]$_{pl}$, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Alkalization</td>
<td>Control</td>
</tr>
<tr>
<td>Rest</td>
<td>24.80±0.74</td>
<td>30.24±1.09</td>
<td>44.56±2.87</td>
</tr>
<tr>
<td>1</td>
<td>25.24±1.32</td>
<td>30.01±1.20</td>
<td>45.11±3.13</td>
</tr>
<tr>
<td>2</td>
<td>23.83±1.48</td>
<td>28.31±1.57</td>
<td>49.00±4.25</td>
</tr>
<tr>
<td>3</td>
<td>22.91±1.16</td>
<td>27.17±1.05</td>
<td>53.87±4.68</td>
</tr>
<tr>
<td>4</td>
<td>22.13±1.23</td>
<td>25.63±1.06</td>
<td>56.53±5.04</td>
</tr>
<tr>
<td>5</td>
<td>21.51±1.20</td>
<td>24.41±1.33</td>
<td>58.19±5.32</td>
</tr>
<tr>
<td>6</td>
<td>20.64±1.18</td>
<td>23.63±1.31</td>
<td>58.61±5.33</td>
</tr>
</tbody>
</table>

Values are means ± SD for 7 subjects.

Table 4. Estimated values of the primary component of the oxygen uptake kinetics ($\tau_1$) during cycling at 40 and 87% $V_{O2 max}$ in the control study and after preexercise alkalization for all subjects and Approach F.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Cycling at 40% $V_{O2 max}$</th>
<th>Cycling at 87% $V_{O2 max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Alkalization</td>
</tr>
<tr>
<td>1</td>
<td>25.5±8.1</td>
<td>33.1±12.9</td>
</tr>
<tr>
<td>2</td>
<td>8.4±4.7</td>
<td>18.6±5.8</td>
</tr>
<tr>
<td>3</td>
<td>26.0±5.7</td>
<td>18.1±7.1</td>
</tr>
<tr>
<td>4</td>
<td>25.4±6.5</td>
<td>26.8±8.8</td>
</tr>
<tr>
<td>5</td>
<td>29.2±24.2</td>
<td>27.8±10.9</td>
</tr>
<tr>
<td>6</td>
<td>33.7±14.4</td>
<td>38.7±13.8</td>
</tr>
<tr>
<td>7</td>
<td>18.4±3.9</td>
<td>18.3±6.4</td>
</tr>
<tr>
<td>Means ± SD</td>
<td>23.8±8.2</td>
<td>25.9±8.1</td>
</tr>
</tbody>
</table>

Estimated values ± estimation SD. The matched-pairs Wilcoxon signed-ranks test did not detect any difference ($P > 0.50$) between control and alkalotic data for cycling at 40% $V_{O2 max}$. For cycling at 87% $V_{O2 max}$, the shortening of transition time ($\tau_1$) was statistically significant ($P = 0.014$). Also statistically significant ($P = 0.014$) was the shortening of $\tau_1$ at 87% $V_{O2 max}$ vs. 40% $V_{O2 max}$ in the control study. There was no such statistically significant shortening effect ($P = 0.47$) in the study with preexercise alkalization.

Fig. 3. Models of $V_{O2}$ kinetics (top) fitted to control (c, dashed line) and alkalotic (4, solid line) data and the corresponding residuals (bottom) for subject 3 at 40% of $V_{O2 max}$. There is no slow component at low-power-output exercise.
parameters could not be estimated with sufficient precision. However, the aim of the present study was not to estimate very accurately the values of all parameters of the model, but rather to investigate the effect of alkalinization on the primary component of \( \dot{V}O_2 \) kinetics, expressed by the change in \( \tau_1 \). If the effect is strong enough, it should be detectable, even if the estimations of \( \tau_1 \) cannot be very precise. What really matters is the ratio of the median of \( \Delta \tau_1 \) (“the signal”) to its variance (“the noise”), the latter being the sum of the intersubject variance and the independent estimation variance. For high-power-output exercise, the effect was statistically significant (Wilcoxon’s signed-ranks test) in the analysis based on seven independent “measurements” of \( \tau_1 \).

The values of \( \tau_1 \), which characterize the primary component of the \( \dot{V}O_2 \) kinetics, obtained in our study during cycling at 40% of \( \dot{V}O_2 \max \) and at 87% of \( \dot{V}O_2 \max \) (see Table 4), were close to the typical data reported in the literature (for review, see Ref. 42). It should be noted that, in the present study, we have found in the control experiment significantly (\( P < 0.02 \)) longer \( \tau_1 \) values during cycling at 87% \( \dot{V}O_2 \max \) compared with cycling at 40% of \( \dot{V}O_2 \max \). This effect was not seen during cycling after ingestion of NaHCO$_3$, because of the significant shortening of \( \tau_1 \) during cycling at 87% \( \dot{V}O_2 \max \), caused by ingestion of NaHCO$_3$. This additionally supports our main finding: the shortening of \( \tau_1 \) at high-power-output exercise after ingestion of NaHCO$_3$. Although the effect of exercise intensity on \( \tau_1 \) was examined in several experiments, some authors have reported no effect or only a minor effect of exercise intensity on the magnitude of \( \tau_1 \) (2, 41), whereas others have reported significant lengthening of \( \tau_1 \) during exercise of higher power outputs (see, e.g., Refs. 9, 15, 26). A possible cause of lengthening of \( \tau_1 \) could be inadequate oxygen delivery to the working muscle cells at the onset of a high-power-output exercise. This is, however, not in agreement with the growing body of evidence suggesting that not oxygen delivery to the working muscle but other factors operating in the muscle cells (kinetic properties of the bioenergetic system, especially oxidative phosphorylation) determine the transition time of \( \dot{V}O_2 \) between rest and work (1, 10, 11). We postulated recently (21) that the main parameter determining the \( \tau_1 \) at a given energy demand (ATP consumption rate) is the change in PCr concentration (\( \Delta \)PCr) between rest and work. Moreover, on the basis of our theoretical study (20, 21), we postulate that important role in the lengthening of \( \tau_1 \) at high power outputs may be played by the intensification of glycolytic ATP supply.

The most interesting finding of this study, as mentioned above, was a significant shortening of \( \tau_1 \) during cycling at 87%
The effect of respiratory alkalosis on the V\(_{\text{O}_2}\) kinetics at the working muscles. Interestingly, preexercise metabolic alkalinization, as presented in our study, was caused by respiratory alkalosis-induced delay of the kinetics of oxygen diffusion in the working muscles, as a result of the leftward shift of the oxygen dissociation curve of oxyhemoglobin, suggesting that the oxygen diffusion in muscle tissue is important for regulating the V\(_{\text{O}_2}\) response to exercise. The importance of acidosis as a facilitator of the oxygen delivery to the working muscle by shifting the oxyhemoglobin dissociation curve to the right was also postulated by Stringer et al. (36).

The shortening of the V\(_{\text{O}_2}\) on-kinetics is not the only effect of metabolic alkalinization on the functioning of skeletal muscle during exercise. Jones and coworkers (18) demonstrated that ingestion of a similar dose of NaHCO\(_3\) causes a significant lengthening of endurance time during cycling at 95% V\(_{\text{O}_2}\)\(_{\text{max}}\). This indicates that metabolic alkalinization may affect the muscle functioning both at the onset of exercise and during sustained high-power-output exercise. However, it is likely that the mechanisms by which alkalinization exerts its action are different in both experiments. In the study by Jones et al., alkalinization and blood-gas concentrations were determined in arterialized blood samples. We measured only blood pH and lactate in the blood samples taken from antecubital vein. In none of these studies was the muscle pH measured and, therefore, it is not clear whether the effect of metabolic alkalinization of blood on muscle pH was identical in both cases.

One may ask how far the ingestion of 3 mmol/kg body wt of NaHCO\(_3\), which significantly affects the blood acid-base status (see Table 3), could change the dynamics of the cytosolic [H\(^+\)] in myocytes at the onset of exercise. As mentioned above, in the present study, we did not measure the intracellular pH and, therefore, we have to review the literature data on the effect of a similar dose ingestion of NaHCO\(_3\) on the resting and exercise muscle pH in relation to control experiments. However, the data on this topic are not consistent. For example, Costill et al. (8) have shown that ingestion of 2.38 mmol/kg of NaHCO\(_3\) had no effect on resting muscle pH. Recently, Stephens et al. (35) reported that, after ingestion of 3.56 mmol/kg NaHCO\(_3\), intramuscular pH, measured with a pH microelectrode, was significantly higher compared with the control study, both at rest and during prolonged exercise. Similarly, Nielsen et al. (25) recently reported that intravenous administration of bicarbonate attenuated the decrease in intracellular muscle pH, measured in humans by means of the \(^{31}\)P-magnetic resonance spectroscopy, during a 5-min rhythmic handgrip exercise. The exercise-induced decrease in intracellular muscle pH, observed in the last minute of work, performed after administration of bicarbonate, was significantly (P < 0.05) attenuated compared with the control study (see Ref. 25). On the other hand, Hooi et al. (17), who studied the effect of ingestion of 4 mmol/kg NaHCO\(_3\) on intramuscular pH at rest and during exhausting exercise, found using NMR spectroscopy, despite systemic alkalosis, no effect on resting and end-exercise muscle pH, in relation to control study. In another study (16), in which the effect of ingestion of 3.56 mmol/kg NaHCO\(_3\) on the muscle metabolism during constant-power-output cycling exercise corresponding to 30, 60, and 75% V\(_{\text{O}_2}\)\(_{\text{max}}\) was examined, the intramuscular pH, calculated on the basis of lactate and pyruvate concentration [according to Sahlin et al. (31)], was not significantly different at rest from the control conditions (7.20 vs. 7.20), but, during cycling at 60 and 75% V\(_{\text{O}_2}\)\(_{\text{max}}\), muscle pH after alkalinization was significantly lower than in the control study (6.88 vs. 6.95 and 6.74 vs. 6.80, respectively).

Fig. 6. Models of V\(_{\text{O}_2}\) kinetics (top) fitted to control (○, dashed line) and alkalotic (△, solid line) data and the corresponding residuals (bottom) for subject 7 at 87% of V\(_{\text{O}_2}\)\(_{\text{max}}\). The slow component starts at TD\(_2\).
The above-presented literature data on the effect of ingestion of NaHCO₃ on the muscle pH remain controversial. In clinical conditions, this topic is still a matter of debate (see, e.g., Refs. 25, 29, 37). Further studies are needed to explain the effect of administration of NaHCO₃ on the dynamics of changes in the intracellular muscle pH at the onset of exercise and its role in the acceleration of VO₂ kinetics.

To sum up everything that has been said above, respiratory alkalosis lengths τ₁, whereas metabolic alkalosis decreases the value of this parameter. The main physiological difference between respiratory alkalosis and metabolic alkalosis is that the former decreases the concentration of CO₂ and HCO₃⁻ in blood, whereas the latter increases the concentration of CO₂ and HCO₃⁻. It is not clear how the blood concentration of CO₂ and/or HCO₃⁻ influences the intracellular pH at the onset of exercise. The experimental data do not provide an unequivocal solution of this problem. Therefore, we are forced to speculate.

In our previous theoretical studies, we analyzed several factors determining the VO₂ on-kinetics in skeletal muscle (20–22). We demonstrated that an increase in the total creatine pool [PCR + Cr] and in glycolytic ATP supply lengthen the transition time of VO₂, whereas increase in mitochondria content, in parallel activation of ATP supply and ATP usage, in oxygen concentration, in proton leak, in resting energy demand, in resting cytosolic pH, and in initial alkalinization diminish this parameter. In the context of the present study, the factors related to cytosolic pH seem to be most relevant. Because in most experiments no effect of NaHCO₃ on the resting cytosolic pH was observed, we focus below on the possible effect of initial alkalinization and of inhibition of anaerobic glycolysis by acidification.

The initial alkalinization of skeletal muscle cells that is usually observed at the onset of exercise (see, e.g., Refs. 7, 19, 24) is caused by consumption of protons by the reaction catalyzed by creatine kinase. This alkalinization shifts, in turn, the equilibrium of creatine kinase in the direction of ADP production. ADP is the main metabolite activating oxidative phosphorylation in mitochondria (6). In our previous theoretical studies, in which our computer model of oxidative phosphorylation in intact skeletal muscle was used (23), we demonstrated that the initial alkalinization accelerates the transition from resting oxygen consumption to working oxygen consumption (see Fig. 5 in Ref. 22). This is caused by a faster increase in ADP concentration and, therefore, a faster stimulation of oxidative phosphorylation. It should be stressed that the discussed effect does not result from the detailed properties of the computer model used for simulations but is a direct consequence of fundamental kinetic properties of the oxidative phosphorylation + creatine kinase system in skeletal muscle (for details, see Ref. 22). Therefore, any increase in the initial alkalinization should lead to a decrease in the transition time (τ₁) of the VO₂ kinetics.

On the other hand, if the ingestion of NaHCO₃ enhances intracellular acidosis in the working muscles, similarly as in the study by Hollidge-Horvat et al. (16), it is possible to explain the acceleration of VO₂ kinetics at the onset of exercise by an inhibition of ATP supply from anaerobic glycolysis. It is a well-known fact that H⁺ ions inhibit glycolysis (7). Computer-aided theoretical studies, using the model of oxidative phosphorylation developed previously (23), clearly suggest that an additional ATP supply from anaerobic glycolysis slows down the VO₂ kinetics at the onset of exercise, because it slows down the increase in ADP concentration and thus the activation of oxidative phosphorylation (20). It should be added that mostly anaerobic glycolysis and not aerobic glycolysis is inhibited by protons, because lactate dehydrogenase works near thermodynamic equilibrium and, therefore, it is much more sensitive to pyruvate concentration than oxidative phosphorylation. On the other hand, pH has essentially no effect on the rate of mitochondrial respiration in a broad range of its value (pH = 5.5–8.0) (27, 34). The hypothesis that the proton-induced inhibition of ATP supply from anaerobic glycolysis may be responsible for the shortening of τ₁ is supported by the fact that this effect is seen above the lactate threshold (87% VO₂ max), where glycolytic ATP supply is significant but not below the lactate threshold (40% VO₂ max).

It may seem paradoxical that alkalinization of blood increases the proton concentration in working muscle cells in relation to control, but there has been proposed a plausible mechanism that can be responsible for this effect (29). This mechanism consists in diffusion of CO₂ from blood to a muscle cell, transformation of CO₂ into HCO₃⁻, trapping of the latter within the cell, and increase in the cytosolic proton concentration.

If the above explanation of the observed acceleration of the VO₂ kinetics is correct, the factor that really matters is not alkalinization of blood per se, but the increase in the CO₂ and HCO₃⁻ concentrations.

In conclusion, preexercise metabolic alkalosis, induced by ingestion of 3 mmol/kg body wt of NaHCO₃, caused a significant acceleration of the primary component of VO₂ kinetics at the onset of a high-power-output exercise (87% VO₂ max), expressed by a significant shortening of the τ₁. This effect was detectable by all six approaches to calculation of the primary component of the VO₂ kinetics applied in this study. No effect of NaHCO₃ ingestion on the τ₁ values was found during cycling at 40% VO₂ max. This provides further support to the hypothesis that the factors regulating the pulmonary VO₂ kinetics are, at least in part, different at different exercise intensities.

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METABOLIC ALKALOSIS ACCELERATES THE VO₂ KINETICS


