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# PRE-EXERCISE ACIDIFICATION INDUCED BY INGESTION OF $NH_4Cl$ INCREASES THE MAGNITUDE OF THE SLOW COMPONENT OF $\dot{V}O_2$ KINETICS IN HUMANS

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Five healthy non-smoking men, aged 22.2±0.8 years (mean SD), $\dot{V}O_2$ max 50.2±6.2 ml·min$^{-1}$·kg$^{-1}$) performed two 6 minutes constant power output (PO) bouts of cycling at 70 rev·min$^{-1}$, separated by a 20 minutes of rest. The power output during the first bout of exercise corresponded to 40 % $\dot{V}O_2$max, while the second exercise corresponded to 75 %$\dot{V}O_2$max. The first bout of exercise was performed at a power output below the lactate threshold (LT) — determined during an incremental exercise test. In the second bout of exercise the subjects exercised above the LT. This experimental protocol was performed twice. Once as a control test (test C) and on a separate day, at about 90 minutes after ingestion of 3 mmol·kg$^{-1}$ BW of $NH_4Cl$ (test A). Ingestion of ammonium chloride developed a state of metabolic acidosis. Antecubital venous blood samples taken every one minute of cycling were analysed for pH, $HCO_3^-$, BE, $pO_2$, $pCO_2$ and plasma lactate concentration $[La]_{pl}$. Oxygen uptake was measured continuously using breath by breath system. The obtained acidotic shift of blood acid-base balance was also present throughout the 6 minutes bouts of exercise.

No significant difference in $\dot{V}O_2$ during the exercise corresponding to 40% $\dot{V}O_2$ max in the test C and the test A was observed. The total oxygen consumed throughout the 6 min cycling at the PO corresponding to 75 % of $\dot{V}O_2$ max was not significantly different in test C and test A (13.532 *vs.* 13.422 l $O_2$, respectively). The slow component of $\dot{V}O_2$ kinetics as expressed by the $\Delta\dot{V}O_2$ $^{6-3\,min}$ of exercise was significantly higher ($p = 0.03$) in test A than in test C (183±97 *vs.* 106±53 ml·min$^{-1}$ $O_2$, respectively). It should be noted that in each subject, the magnitude of the slow component of $\dot{V}O_2$ kinetics after pre-exercise acidification was consistently higher than in control experiment.

In conclusion: The original finding of our study is that pre-exercise acidification induced by ingestion of 3 mmol·kg$^{-1}$ B.W. of $NH_4Cl$ was accompanied by a significant increase in the magnitude of the slow component of $\dot{V}O_2$ kinetics. This is why we postulate that acidosis may play a significant role in the physiological mechanism responsible for the slow component of $\dot{V}O_2$ kinetics in humans.

Key words: *acid-base balance, ammonium chloride, metabolic acidosis, oxygen uptake kinetics, exercise*

## INTRODUCTION

It is well established that the oxygen uptake at the onset of low power output exercise follows a mono-exponential process reaching steady state

within approx. 3 minutes (1—3). However, during exercise at the power output above the anaerobic threshold a progressive increase in $\dot{V}O_2$ above the level reached in the 3rd minute of exercise occurs. This effect has been called the slow component of $\dot{V}O_2$ kinetics (2). The magnitude of the slow component of $\dot{V}O_2$ kinetics can be expressed as the difference between the $\dot{V}O_2$ reached in the 3rd minute of a constant power output exercise and the 6th minute of work (for review see Whipp (4, 5)).

The appearance of the slow component of $\dot{V}O_2$ kinetics illustrates the fact that performance of work above the „anaerobic threshold" is accompanied by a decrease of human mechanical efficiency. Moreover, as illustrated by the study by Poole *et. al.* (6) who have simultaneously measured the pulmonary and leg muscle $\dot{V}O_2$, 86% of the increase in the pulmonary $\dot{V}O_2$ observed between 3rd and 21st minute of a constant high power cycling exercise was attributable to the exercising muscle in the legs. This suggest the origins of the drop in locomotory efficiency takes place in the exercising muscle. Therefore, understanding the physiological mechanism responsible for the slow component of $\dot{V}O_2$ kinetics seams to be very important for improvement of exercise tolerance in humans in whom the anaerobic threshold occurs already at very exercise intensities. This seem to be specially the case for patients whose exercise tolerance is impaired by cardio-pulmonary insufficiency (for review see Poole *et al.* (7)).

A number of factors may be involved in the mechanism responsible for the slow component of $\dot{V}O_2$ kinetics, including increased blood lactate concentration (8, 9), rise in muscle hydrogen ions (10), increased muscle temperature (11—13), or the recruitment of less efficient type II muscle fibres (4, 14).

In the recent years most attention has been paid to the relationship between magnitude of blood lactate accumulation and the magnitude of the slow component of $\dot{V}O_2$ kinetics. Interestingly the onset of appearance of the slow component of $\dot{V}O_2$ kinetics is related to the lactate threshold, and the magnitude of the slow component of $\dot{V}O_2$ kinetics positively correlates with the level of blood lactate accumulation (8, 15, 16). Some authors (9, 10) postulated that blood lactate accumulation and accompanied acidosis may play the regulatory role in the mechanism responsible for the slow component of $\dot{V}O_2$ kinetics.

However, to our knowledge no data has been published showing the direct effect of pre-exercise pharmacologically induced metabolic acidosis on the magnitude of the slow component of $\dot{V}O_2$ kinetics.

This is why in the present study we have evaluated the effect of pre-exercise acidification induced by ingestion of $NH_4Cl$ on the magnitude of the slow component of $\dot{V}O_2$ kinetics in humans.

## METHODS

### *Subjects*

Five healthy, physically active non-smoking males (means ± SD.; age, 22.2 ± 0.8 years; body weight 68.2 ± 5.1 kg; height 175 ± 2 cm, body fat 9.2 ± 5.2 % of BW) experienced in laboratory exercise tests participated in this study. Maximal oxygen uptake of the studied subjects was 50.2 ± 6.2 ml · min$^{-1}$ · kg$^{-1}$. A medical history and physical examination were completed before the study. Basic blood tests for haemoglobin (Hb), hematocrit value (Ht), erythrocyte (E), leukocyte (L), sodium ($Na^+$), potassium ($K^+$) and creatinine (Cr) were performed (*Table 1*).

*Table 1.* Basic blood variables determined in the tested subjects.

| Subject | Ht (vol. %) | Hb (g%) | E (mln · mm$^{-3}$) | L (1000 · mm$^{-3}$) | $Na^+$ (mmol · l$^{-1}$) | $K^+$ (μmol · l$^{-1}$) | Cr |
|---|---|---|---|---|---|---|---|
| I | 44,2 | 16,7 | 5,59 | 5,9 | 148,5 | 4,49 | 104,9 |
| II | 44,2 | 15,4 | 5,23 | 5,2 | 144,5 | 4,12 | 67,7 |
| III | 46,9 | 15,6 | 5,18 | 5,5 | 145,0 | 4,10 | 89,6 |
| IV | 43,5 | 15,8 | 5,18 | 9,0 | 146,0 | 5,50 | 98,5 |
| V | 45,8 | 15,6 | 5,20 | 5,6 | 144,0 | 4,94 | 58,3 |

### *Incremental exercise tests*

In order to establish exercise intensities applied during the main part of this study, the subjects performed an incremental exercise test on a cycloergometer (Ergoline 800s the Netherlands). The exercise protocol started with a 5-minute resting period sitting on the cycloergometer. The initial power output during the incremental exercise test was 30 W, followed by a gradual increase by 30 W every 3 minutes. Subjects were encouraged to continue the test until exhaustion. The pedalling rate during all exercise tests was 70 rev · min$^{-1}$. The test was stopped when the subjects could not longer maintain the required pedalling rates. All exercise tests were performed on the same ergometer. The incremental exercise tests were performed about 2 weeks before the constant power output tests were applied.

### *The constant power output tests*

In this stage of the study the subjects performed two constant power output exercise tests (each of six minutes duration) separated by a 20 minute pause, on two occasions: once as a control experiment and about one week later after pre-exercise acidification. The power output during the first bout of exercise corresponded to 40 % $\dot{V}O_2$ max and the second bout was planned to be performed at a power output corresponding to 75 % $\dot{V}O_2$ max. The calculation of individual power output was based on the linear relationship of oxygen uptake *vs.* power output obtained during incremental tests, taking into consideration only data below the stage of sustained increase in $(La)_{pl}$ concentration, as previously described by Żołądź *et al.* (17). This calculation was based on the $\dot{V}O_2$ reached during the 3$^{rd}$ minute of each stage of incremental test.

## *Pre-exercise acidification*

The subjects ingested 3 mmol · $kg^{-1}$ BW of $NH_4Cl$, which was placed in a capsules containing 5 mmol of ammonium chloride each. Upon ingestion of the capsules the subjects were allowed to drink up to 500 ml of water. At the onset of ingestion and every 15 minutes during ingestion of the ammonium chloride, venous blood samples were taken to determine the changes in the blood acid-base status. *Fig. 1* illustrates the changes in antecubital blood $HCO_3^-$ and pH during and after the ingestion period. After reaching a significant level of acidosis, which normally occurred at about 90 minutes after ingestion of $NH_4Cl$, the subjects performed the constant power output tests. The meals ingested during the day preceding the control experiment were exactly repeated before the exercise performed after acidification.

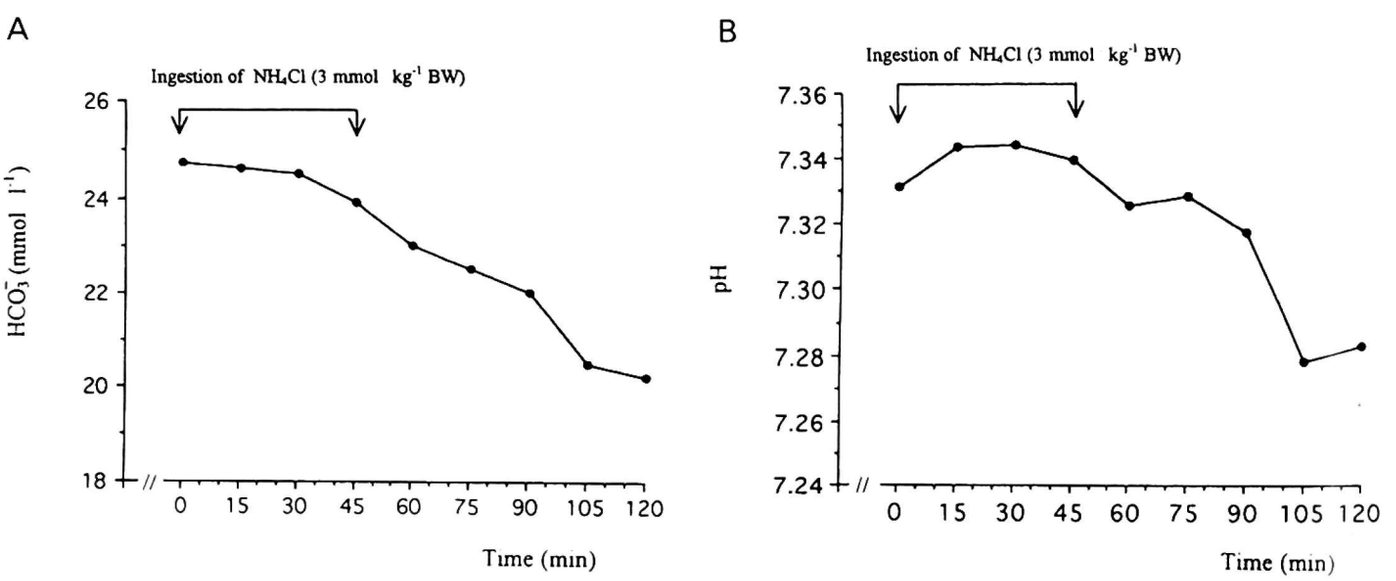


*Fig. 1.* Typical changes in blood $HCO_3^-$ (panel A) and blood pH (panel B) determined in antecubital venous blood samples taken before, during and after ingestion of ammonium chloride, for subject I.

## *Blood sampling and analysis*

Five minutes prior to the exercise, at the end of each stage of exercise (the last 15 seconds before increasing power output) and at the moment of ending the exercise protocol, antecubital blood samples (1 ml each) were taken using Abbot Int-Catheter Ireland (18G/1.2 × 45 mm) inserted into the antecubital vein about 30 minutes prior to the onset of exercise. The catheter was connected with the extension set using a „T" Adapter SL Abbot Ireland (a tube 10 cm in length). Immediately before taking 1 ml blood sample for analysis, 1 ml of blood volume was taken in order to eliminate the blood from the catheter and the T-set. One part of each sample was taken for the detection of blood gases ($PO_2$ and $PCO_2$) 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 ammonium oxalate and 5 mg sodium fluoride and mixed for about 20 seconds. Subsequently, in order to separate plasma for performing lactate and ammonium measurements, the blood samples were centrifugated. Samples of blood plasma (0.2 ml) were stored for further analysis at a temperature of minus 25°C. $PO_2$ and $PCO_2$, as well as $(H^+)_b$ were determined using a Ciba-Corning 248 analyser (England). The blood bicarbonate concentration ($[HCO_3^-]_b$) and the base excess (BE) were calculated by this unit. Plasma lactate concentration ($[La]_{pl}$) was measured using an automatic analyser (Ektachem XR 700, Kodak, USA). Serum sodium ($Na^+$) and potassium ($K^+$) concentration was determined using a flame photometer, Ciba

Corning Model 480 (England). Blood creatinine level was determined by the kinetics method based on reaction with picric acid using an automatic analyser, Express 550 CBI (England). Haemoglobin concentration (Hb), haematocrit value (Ht), erythrocyte count (E) and leukocyte count (L) were determined using and automatic haematological analyser, Baker 9000 USA. The percentage of body fat was assessed according to Hassager *et al.* (18).

The magnitude of the slow component of $\dot{V}O_2$ kinetics in this study was expressed as the difference in oxygen uptake reached in the 6$^{th}$ minute of exercise and in the 3$^{rd}$ minute of work ($\Delta\dot{V}O_2^{6-3\ min}$) (see Whipp and Wassermann (2)).

The lactate threshold in this study was defined as the highest power output above which $(La)_{pl}$ showed a sustained increase of $> 0.5\,mmol \cdot l^{-1} \cdot step^{-1}$ (17).

Gas exchange variables were measured continuously breath-by-breath using the Oxycon Champion Jaeger, Germany, starting from the 5$^{th}$ minute prior to the exercise until the test was stopped. Before and after each test was finished, gas analysers were calibrated with certificated calibration gases as previously described by Żołądź *et al.* (17).

### *Statistical analysis*

The presented results represents means ($\pm$SD). Statistical significance was tested using ANOVA for repeated measurements and paired t-test. The chosen level of significance was $*p<0.05$, $**p<0.02$, $***p<0.001$.

## RESULTS

### *Power output reached during the incremental test:*

The lactate threshold has occurred at the power output of $126\pm25$ W. The maximal power output reached at the $\dot{V}O_2$ max has amounted $258\pm11$ W.

### *Power output, acid base balance, plasma lactate concentration and oxygen uptake during the exercise corresponding to 40% $\dot{V}O_{2\,max}$.*

The power output during the first bout of exercise (40% $\dot{V}O_{2\,max}$) has amounted to $89.0\pm9.0$ W. The mean value of the applied power output was by $37\pm23$ W lower than the lactate threshold power output (LT). Four subject exercised below the lactate threshold, and one subject in this test cycled at the lactate threshold power output.

The $HCO_3^-$, pH and BE levels determined 1 minute prior to the first bout of exercise performed after ingestion of $NH_4Cl$ (test A) were significantly lower (paired t-test) than in the control condition (test C) (*Table 2*). The significant shift of acid base-balance into acidosis was also present throughout the 6-minutes period of exercise (see *Fig. 2* panel A, B and C).

Oxygen uptake during test C and test A was not significantly different (ANOVA) (see *Fig. 2* panel D). No slow component of $\dot{V}O_2$ kinetics was present during this exercise in neither of the experimental conditions (control and pre-exercise acidification) (*Fig. 2* panel D).

*Tabel 2.* Pre-exercie blood $HCO_3^-$, BE and pH, determined 1 minute before the exercise corresponding to 40 and 75% of $\dot{V}O_2$max, in the control study (C) and after pre-exercise acidification (A). Data are given as means ± SD for 5 subjects, where *p < 0.02, **p < 0.01 indicates significant difference in relation to the control values (Student's paired t-test).

| Exercie intensity | 40% $\dot{V}O_{2max}$ | | 75% $\dot{V}O_{2max}$ | |
|---|---|---|---|---|
| Variable | C | A | C | A |
| pH | 7.355 ± 0.019 | **7.302 ± 0.027 | 7.348 ± 0.028 | *7.285 ± 0.300 |
| $HCO_3^-$ (mmol · l$^{-1}$) | 25.8 ± 0.8 | **22.1 ± 1.2 | 25.1 ± 1.0 | *20.0 ± 1.4 |
| BE (mmol · l$^{-1}$) | 3.04 ± 1.33 | ** −1.30 ± 1.78 | 2.16 ± 1.70 | ** −4.26 ± 2.12 |

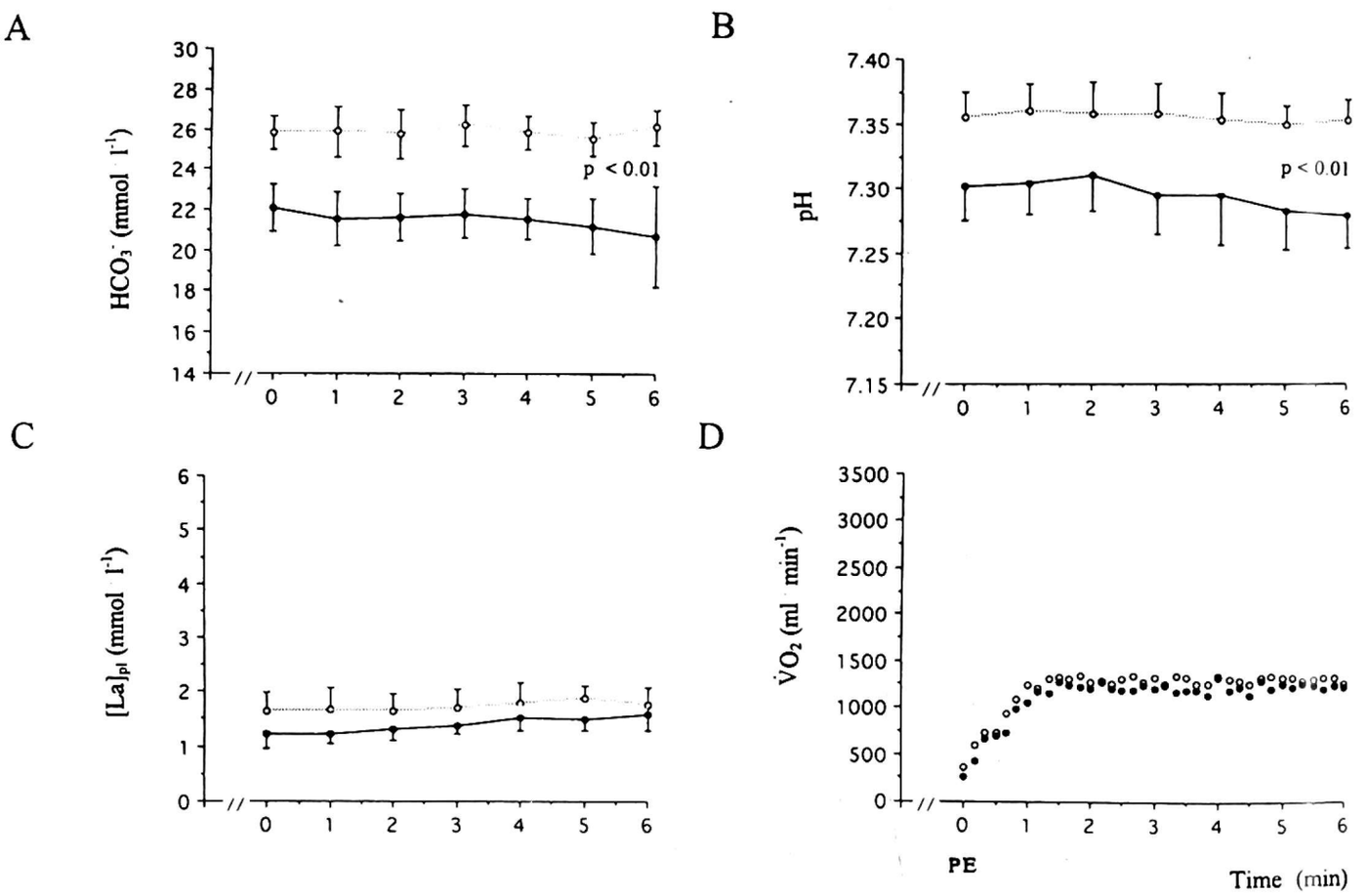


*Fig. 2.* Blood $HCO_3^-$ (panel A), pH (panel B) and plasma lactate concentration (panel C) and oxygen uptake (panel D) determined before and during cycling at 40% $\dot{V}O_2$ max performed in the control study (○) and after pre-exercise acidification (●). Values are means ± SD, n = 5. Data for $\dot{V}O_2$ (panel D) are given as mean values for 5 subjects, for 36 ten-second intervals.

*The power output during the exercise corresponding to 75% $\dot{V}O_{2\,max}$.*

The power output during the second bout of exercise (75% $\dot{V}O_{2\,max}$) has amounted to 196 ± 15 W. Each of the studied subjects exercised above the lactate threshold. The mean value of the applied exercise load was by 70 ± 18 W higher than the lactate threshold (LT).

Pre-exercise level of $(HCO_3^-)_b$ and pH determined 1 minute prior to the second bout of exercise performed after ingestion of $NH_4Cl$ was significantly

lower (paired t-test) than in the control condition (*Table II*). This shift of acid base-balance towards acidosis was also present throughout the 6-minutes period of exercise. (see *Fig. 3* panel A, B and C).

Plasma lactate concentration determined 1 minute prior exercise in test C and test A was not significantly different. There was a tendency towards lower values of $(La)_{pl}$ throughout the 6-minutes period of work after ingestion of $NH_4Cl$ in relation to the control experiment; however, this differences did not reach the level of significance (see *Fig. 3 C*).

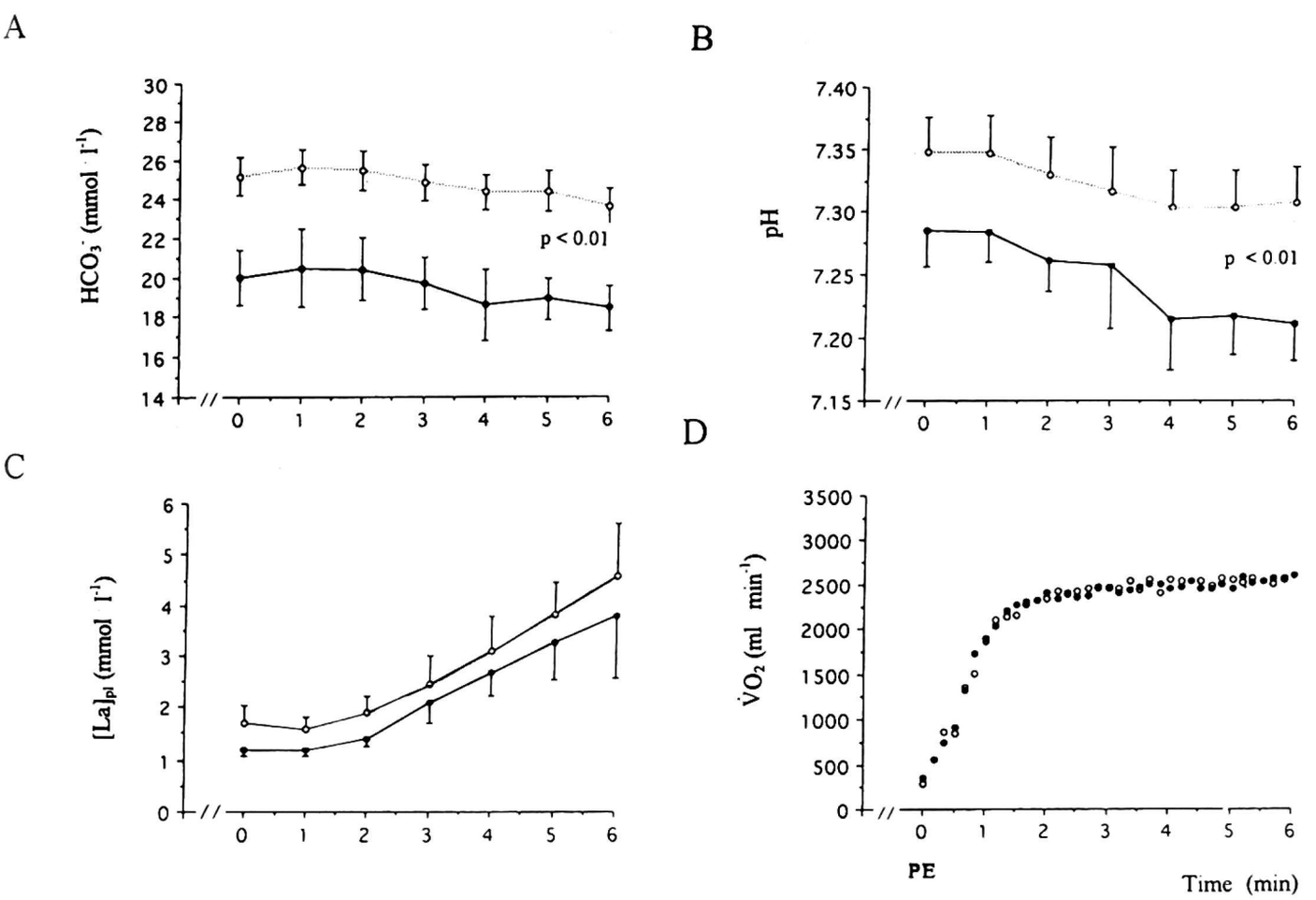


*Fig. 3.* Blood $HCO_3^-$ (panel A), pH (panel B) and plasma lactate concentration (panel C) and oxygen uptake (panel D) determined before and during cycling at 75% $\dot{V}O_{2max}$ performed in the control study (o) and after pre-exercise acidification (●). Values are means ± SD. n = 5. Data for $\dot{V}O_2$ (panel D) are given as mean values for 5 subjects, for 36 ten-second intervals.

The net $\dot{V}O_2$ / power output ratio established during the last 3 min of cycling was not significantly different (paired t-test) in both test (11.51 ± 0.47 *v.s* 11.37 ± 0.61 ml · $min^{-1}$ · $W^{-1}$). The total oxygen consumption thorough the 6 minutes cycling at the power output corresponding to 75% $\dot{V}O_{2max}$ was not significantly different (paired t-test) in the control study and after ingestion of ammonium chloride (13. 532 *vs.* 13. 422 l). However, the slow component of $\dot{V}O_2$ kinetics as expressed by the $\Delta\dot{V}O_2^{6-3\,min}$ was significantly higher (paired t-test) ($p = 0.03$) in test A than in test C. It amounted to 183 ± 97 *vs.* 106 ± 53 ml $O_2$ · $min^{-1}$, respectively for test A and test C (see *Fig. 4 A*). It should be noted

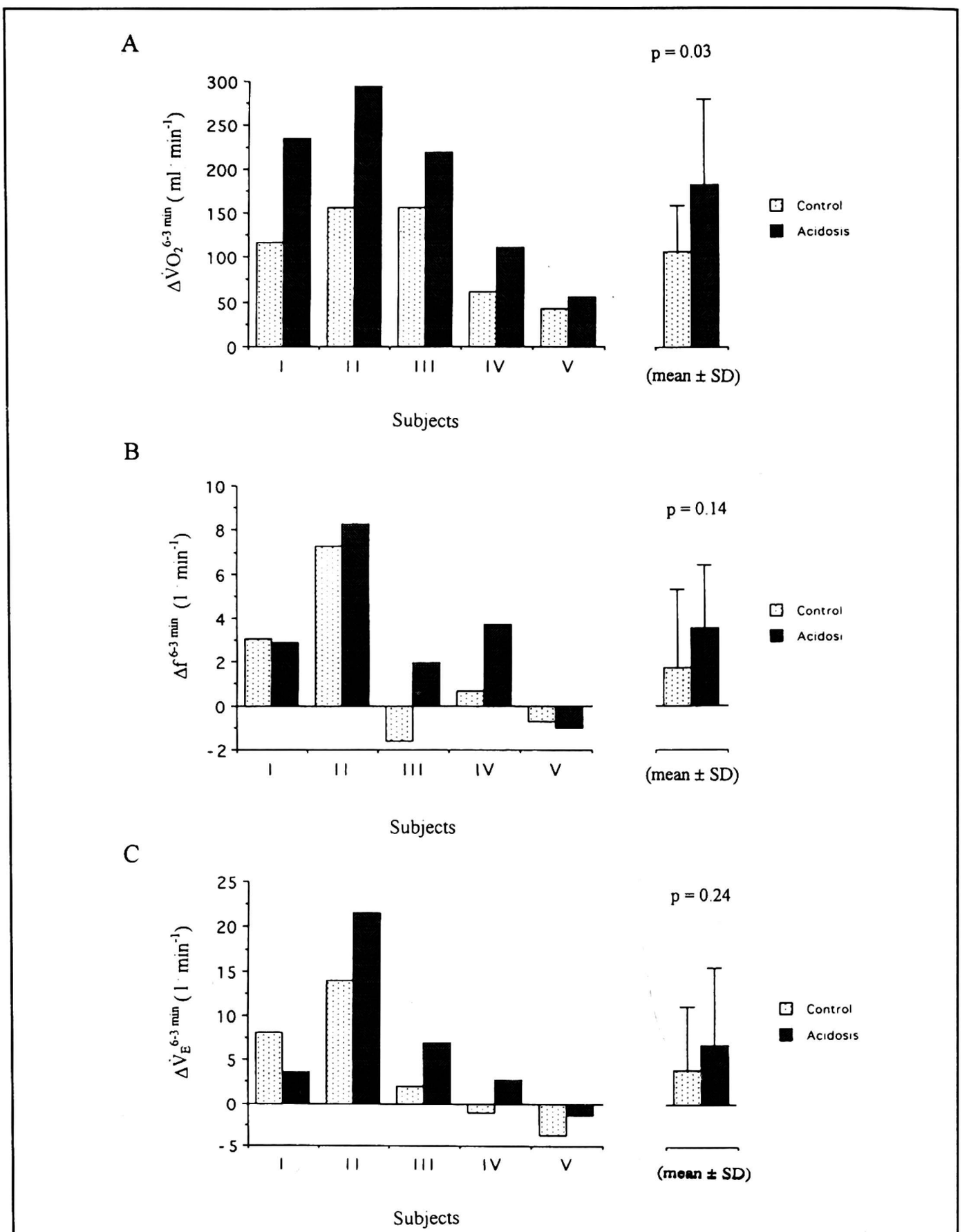


*Fig. 4.* Individual and mean (±SD) values of the slow component of $\dot{V}O_2$ kinetics ($\Delta\dot{V}O_2^{6-3\ min}$) (panel A), increase in breathing frequency between the 3rd and the 6th minute of exercise ($\Delta f^{6-3\ min}$) (panel B) and the increase in minute ventilation between the 3rd and the 6th minute of exercise ($\Delta\dot{V}_E^{6-3\ min}$) (panel C), determined during the exercise corresponding to 75 %$\dot{V}O_{2max}$, where: control -indicates the control study and acidosis — indicates experiment with pre-exercise acidification.

that in each subject the $\Delta\dot{V}O_2^{6-3\ min}$ was higher after pre-exercise acidification induced by ingestion of 3 mmol · kg $BW^{-1}$ of $NH_4Cl$ (*Fig. 4 A*).

The increase of breathing frequency between the 3rd and the 6th minute of exercise ($\Delta\ f^{6-3\ min}$) in test C was no significantly different form the value reached in test A (*Fig. 4 B*). Moreover, as illustrated at the *Fig. 4 C* the increase in $\dot{V}_E$ reached between the 3rd and the 6th minute of exercise ($\Delta\dot{V}_E^{6-3\ min}$) in test C was no significantly different from the level observed in test A.

## DISCUSSION

The original finding of our study is that pre-exercise acidification induced by ingestion of 3 mmol · $kg^{-1}$ B.W. of $NH_4Cl$ was accompanied by a significant increase in the magnitude of the slow component of $\dot{V}O_2$ kinetics. This was a systematic effect observed in each of the tested subject (*Fig. 4 A*). Our study illustrates that metabolic acidosis may play a significant role in the physiological mechanism responsible for the slow component of $\dot{V}O_2$ kinetics. There are several possible ways by which acidosis may increase the magnitude of the slow component of $\dot{V}O_2$ kinetics.

Perhaps the simplest possible explanation of the increase of the magnitude of the slow component of $\dot{V}O_2$ kinetics in acidotic conditions may be the increase of oxygen cost of breathing due to the increase in minute ventilation. This is why we have compared the differences in $\dot{V}_E$ between the 3rd and the 6th minute of exercise in both tests. The $\Delta\dot{V}_E^{6-3\ min}$ in test A was only by 2.8 l · $min^{-1}$ higher than in test C. This difference was not significant (*Fig. 4 C*). Using the data presented by Aaron *et al.* (19) we have assessed that a such intensification of $\dot{V}_E$ may account for ~5.6 ml $O_2$ · $min^{-1}$, whereas the magnitude of $\Delta\dot{V}O_2^{6-3\,min}$ in the acidotic state was by 77 ml $O_2$ · $min^{-1}$ higher than in the control test. This results supports the suggestion by Womack *et al.* (20) that „$\dot{V}_E$ contributes minimally, if at all, to the $\dot{V}O_2$ slow component" This is in accordance with the data by Poole *et al.* (6) showing that origin of the drop in locomotory efficiency expressed by the slow component of $\dot{V}O_2$ kinetics takes place in the exercising muscle. However, the mechanism by which the enhanced acidosis increases the magnitude of the slow component of $\dot{V}O_2$ kinetics is still not fully understood.

It has been postulated that acidosis may play a key role in the physiological mechanism responsible for the slow component of $\dot{V}O_2$ kinetics. Capelli *et al.* (10) has suggested that intramuscular $H^+$ accumulation may be responsible for the slow component of $\dot{V}O_2$ kinetics by increasing the free creatine concentration (see 21). The increase of free creatine may be caused by the shift in equilibrium of the creatine kinase reaction due to $H^+$ accumulation (22). However, so far there is no evidence from *in vivo* study to confirm this

explanation. Furthermore, the creatine production does not stimulate the mitochondrial respiration by itself, but eventually *via* phosphate production. However, the shift of the creatine kinase reaction towards creatine synthesis leads also to an increase in the ATP/ADP ratio, which can be expected to slow down oxygen consumption. For this reason, the relevance of the mechanism proposed by Capelli *et al.* (10) is no so obvious.

According to Stringer *et al.* (9) acidosis may contribute to the increase of the slow component of $\dot{V}O_2$ kinetics by a shift of the oxyhemoglobin dissociation curve to the right, increasing oxygen transport to the muscle and promoting aerobic metabolism during heavy exercise. Interestingly in a number of studies (8, 16, 23) the magnitude of the slow component of $\dot{V}O_2$ kinetics has been positively correlated with the magnitude of blood lactate accumulation. Moreover, recently, Zoladz *et al.* (17, 24, 25) have shown, that during an incremental exercise test the onset of blood lactate accumulation is accompanied by a pronounced non-linear increase in oxygen uptake. This result may suggest that exercise induced acidosis may be responsible for the decrease of muscle efficiency as expressed by the magnitude of the slow component of $\dot{V}O_2$ kinetics (2).

On the other hand lactate infusion to the working dog muscle (26) or epinephrine induced increase of blood lactate accumulation (27, 28) did not succeed in changing the magnitude of the slow component of $\dot{V}O_2$ kinetics. Moreover, our previous study (29), in which the effect of pre-exercise alkalisation induced by ingestion of 3 $mmol \cdot kg^{-1}$ BW of $NaHCO_3$, on the magnitude of the slow component of $\dot{V}O_2$ kinetics was examined, has shown, that significantly reduced blood $H^+$ concentration and significantly increased blood lactate accumulation did not change the magnitude of the slow component of $\dot{V}O_2$ kinetics in humans.

Interestingly, as illustrated by the present study, the significantly increased magnitude of the slow component of $\dot{V}O_2$ kinetics developed by pre-exercise acidification was accompanied by a reduction of blood lactate accumulation (*Fig. 3* panel C). This supports the early findings (26) that blood lactate accumulation *per se* is not responsible for the slow component of $\dot{V}O_2$ kinetics.

The interpretation of the effects of pharmacologically induced lactatemia (26, 27, 28), metabolic alkalosis (29) and metabolic acidosis (the present study) on the magnitude of the slow component of $\dot{V}O_2$ kinetics should, however, be done caution. The reason for this is that none of these interventions is physiological. The exercise induced acidosis is accompanied by enhanced lactatemia. This cannot be attained by ammonium chloride induced acidosis or intake of bicarbonate salt. The first increases acidosis with decreased lactatemia (see Jones *et al.* (30)), while the second enhances lactatemia with decreased $H^+$ concentration in relation to the physiological conditions (29—31). Moreover all three above mentioned interventions (pharmacologically induced lactatemia, metabolic alkalosis and metabolic acidosis)

affect primarily extracellular space with secondary effect on the intracellular space, whereas exercise induced acidosis has intracellular origin. This is why manipulations with the blood acid-base status may not directly reflect the effect of acidosis on the mechanism responsible for the slow component of $\dot{V}O_2$ kinetics, which may operate at the cellular level.

The increase of the magnitude of the slow component of $\dot{V}O_2$ kinetics induced by pre-exercise acidification observed in the present study demonstrates that pre-exercise acidification decreases muscle efficiency and accelerates oxygen uptake during exercise. In the light of earlier studies, the increase of muscle $H^+$ may affect its efficiency in several ways including decrease of force (32). It has been shown in animal studies that low muscle pH decreases maximal velocity of shortening (33, 34). If it were the case that decreased muscle pH affects the power output from the recruited muscle fibres population, then, in order to maintain the required power output, presumably more type II muscle fibres (less efficient) (14) would be recruited (for review see Sargeant (35)). This could be a potential cause of an increase in the magnitude of the slow component of $\dot{V}O_2$ kinetics.

Additionally it has been shown that enhanced muscle $H^+$ accumulation was accompanied by slowing of muscle relaxation (36, 37). This would also contribute to the decrease of muscle efficiency and enhancement of the slow component of $\dot{V}O_2$ kinetics. Another pH depended factor contributing to the increase of the slow component of $\dot{V}O_2$ kinetics could be the reduced free energy of ATP hydrolysis (38—41). On the other hand, recent data from Bangsbo *et al.* (42) have shown that a significant decrease of muscle pH (by about 0.2 units — from 6.82 to 6.65) did not affect the muscle efficiency expressed by the amount of ATP used per unit of work.

In conclusion: the present study demonstrates that pre-exercise acidification, induced by ingestion of 3 $mmol \cdot kg^{-1}$ BW of $NH_4Cl$, was accompanied by a significant increase of the magnitude of the slow component of $\dot{V}O_2$ kinetics. This was a systematic effect observed in each of the tested subjects. Therefore we have postulated that intensification of metabolic acidosis may play a role in the mechanism responsible for the increase in the slow component of $\dot{V}O_2$ kinetics.

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Local Ethical Committee approval was obtained for this study.

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