ACID-BASE STATUS OF ARTERIAL AND FEMORAL-VENOUS BLOOD DURING AND AFTER INTENSE CYCLE EXERCISE

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ABSTRACT

Intense exercise depends on energy from both aerobic and anaerobic processes. These processes produce CO_2 and lactate, respectively, and both metabolites affect blood's acid-base status. To examine how the acid-base status of arterial and femoral-venous blood is affected and regulated, seven healthy young men cycled for 2 min at constant power to exhaustion. Blood samples were drawn from indwelling catheters in the femoral artery and vein during exercise and for 1 h after, and the samples were analysed for lactate (La^-), acid-base parameters, and plasma electrolytes (Na^+, K^+, Cl^-, La^-, HCO_3^-). The chloride concentration in red blood cells (cCl_{RBC}) was also determined to quantify the chloride shift. Arterial (femoral-venous, fv, mean values) blood lactate concentration rose to 13.8 mmol L^{-1} (fv 15.7), pH fell to 7.18 (fv 7.00), pCO_2 changed to 41 hPa (fv 114), and blood bicarbonate concentration was more than halved after exercise. cCl_{RBC} rose by 5 (a) and 8 mmol L^{-1} blood (fv) during exercise. pCO_2 and pH fell linearly by the lactate concentration. Consequently, blood bicarbonate concentration fell by 81% of the increase in blood lactate concentration, while blood base deficit rose 30% more than lactate did. Bicarbonate thus neutralised 62% of the total acid load. cCl_{RBC} rose in proportion to the amount of hydrogen ions buffered by haemoglobin, and chloride shift amounted to 31% of the total acid load. pH was lower and pCO_2 and bicarbonate concentration were higher in femoral-venous than in arterial blood with the same lactate

concentrations. The relationship between base deficit and blood lactate concentration did not differ between arterial and femoral-venous blood. In conclusion, after intense exercise pH falls more in femoral-venous than in arterial blood because of a lack of respiratory compensation of the metabolic acidosis.

Key words: acid-base balance, anion gap, arterio-venous difference, blood, buffering, chloride, chloride shift, electrolytes, exercise, lactate, pH, plasma.

INTRODUCTION

Intense exercise depends on anaerobic energy release, and breakdown of glycogen to lactate in the working muscles is quantitatively the most important anaerobic process [29]. Part of the lactic acid produced is released to the blood, thus reducing blood pH and altering its acid-base status [4, 24, 27, 33, 36, 43]. If exercise lasts for more than a few seconds, there is in addition a large aerobic energy release that leads to production of CO_2 that again may lead to a respiratory acidosis of venous blood. The blood may also receive hydrogen ions by a process independent of release of lactate or CO_2 from muscle to blood [2], possibly by a Na^+–H^+ exchange [24]. Each process contributes to acidification of the blood and thus to a drop in blood pH.

The relationship between the amount of lactic acid added to arterial blood and its acid-base status has been examined in several studies [4, 27, 33, 36]. It is thus well established that not only pH but also the CO_2-pressure (pCO_2) of arterial blood falls by the lactate concentration, meaning that there is a respiratory compensation for the metabolic acidosis introduced by lactate production. Venous blood may act as a closed compartment unable to reduce its pCO_2 and thereby buffer acids by bicarbonate. A raised pCO_2 will expectedly reduce the pH of venous blood considerably below that of arterial blood [16]; femoral-venous pCO_2 may double during strenuous exercise, reducing pH further by 0.2 below that of arterial blood [37].Further differences in the acid-base status between venous and arterial blood have apparently not been studied.

Acids added to the blood can be neutralised by bicarbonate as well be buffered as by several nonbicarbonate buffers where haemoglobin and plasma proteins are quantitatively the most important ones. The

contribution from each component in relation to intense, short-lasting, anaerobic types of exercise is not well known. Moreover, red blood cells can exchange bicarbonate with plasma chloride by the so called chloride shift mechanism [42]. The process is very fast, taking less than one second for equilibration [19]. However, the extent of this process in relation to high-intensity, anaerobic types of exercise has only been studied in arterialised blood [3], and possible differences between arterial and venous blood is therefore not known.

As pH falls, the net negative charge on plasma proteins is reduced, and anion gap of plasma would expectedly fall too. However, it has been suggested that anion gap may increase after intense exercise [27]. If so, other, unmeasured ions may perhaps appear in plasma, but this possibility has not been examined. On the other hand, our former study did not distinguished between concentrations of lactate and bicarbonate in plasma and in whole blood [27]. These ions are not evenly distributed in plasma and red blood cells, and that may have influenced conclusions drawn.

To examine changes in the acid-base status of blood further, healthy young men cycle for 2 min to exhaustion since this duration leads to a maximum anaerobic energy release [26, 29]. Blood samples were drawn from catheters in the femoral artery and vein during the exercise and at intervals for 1 h after. The samples have been analysed for lactate, acid-base parameters, and plasma electrolytes and the acid-base status of blood as well as red blood cell chloride concentration have been calculated.

MATERIALS AND METHODS

Subjects

Seven healthy male students at the Norwegian Police Academy volunteered to serve as subjects in this study after being given oral and written information about the experimental procedures and possible risks. The subjects were 25±2 yr old (mean ± SD), 1.85±0.04 m tall, weighed 81±4 kg, and their maximal O_2 uptake was 39±2 μmol kg^{-1} s^{-1} (53±3 ml_{STPD} min^{-1} kg^{-1}). The experiments were approved by The Ethics Committee of Health Region 1 in Norway.

Procedures

All exercise was carried out at a Krogh-type cycle ergometer [20] at a constant pedalling frequency of 1.5 Hz. The maximal O_2 uptake and the highest cycle power that could be kept for 2 min were determined for each subject during pretests the last weeks before the experiments.

Each subject arrived at the laboratory in the morning after an overnight fast. Catheters were inserted into the femoral artery and vein. Further details on the catherisation procedures and the experiments have been given in more detail elsewhere [24], see their Figure 1). In short, the subject warmed up for 15 min at a power corresponding to ≈50% of his maximal O_2 uptake. After a 10 min rest he cycled at a constant power established during the pretests for ≈2 min to exhaustion. Blood samples from the femoral artery and vein were drawn in parallel in 5 ml syringes before the exercise, after 30, 60 and 90 s of exercise, and at 30 s, 1, 3, 6, 10, 15, 20, 30, 45, and 60 min after the exhausting bout. The blood samples were handled as described elsewhere [24] to allow measurement of haematocrit (Hct), blood lactate (La) and haemoglobin (Hb) concentrations, blood acid-base parameters (pH, pCO_2, pO_2, sO_2), concentration of plasma electrolytes (Na^+, K^+, Cl^-, La^-, HCO_3^-), and plasma albumin, and proteins.

Analyses

Maximal O_2 uptake was established by the levelling-off criterion of Taylor et al. [40] using a discontinuous protocol of stepwise increases of cycle power, measuring the O_2 uptake during the last 30 s of a 3 min exercise bout at constant power. The expired volume was measured in a wet Tissot-type spirometer [41], and fractions of CO_2 and O_2 in the expired air was measured by analysers from Applied Electrochemistry Instruments (Pittsburgh, PA, USA).

Blood and plasma parameters were measured as described elsewhere [24, 25]. In short, the lactate concentration in plasma and whole blood was measured by enzymatic photofluorometry according to Passoneau and Lowry [34]. Blood pH, pCO_2, and pO_2 were measured on an IL 1312 blood gas manager (Instrumentation laboratory, Milan, Italy), while blood oxygen saturation (sO_2) was measured on an OSM 2 hemoximeter (Radiometer, Copenhagen, Denmark). Blood haemoglobin concentration (cHb) was measured by a hemiglobin-cyanide method of Baxter Dade AG (Düdingen, Switzerland). The

values are reported as concentrations in mmol L^{-1} of blood using a molecular mass of haemoglobin of 16.114 kDa. Blood haematocrit (Hct) was measured on blood samples in heparinised capillary test tubes centrifuged for more than 3 min at more than 15 000 g ($>1.5 \cdot 10^5$ m s^{-2}) on a Cellokrit 2 centrifuge (AB Lars Ljungberg, Stockholm, Sweden), thus giving a fraction of trapped plasma of ≈0.02 between the red blood cells [10, 11].

Plasma sodium, potassium, and chloride concentrations were measured on a Microlyte ion selective analyser (Kone corporation, Espoo, Finland). Plasma albumin concentration was measured by procedure 631 of Sigma Diagnostics (St. Louis, MO, USA) where albumin binds to bromcreosol green. The concentration of the product formed was measured in a spectrophotometer at 477 THz (628 nm) and expressed in SI-units using a molecular mass of albumin of 66.5 kDa [31]. The protein concentration was measured by the DC Protein assay method of Biorad laboratories (Hercules, CA, USA) using the two-step alkaline copper tartrate and folin reaction. The concentration of the product formed was measured in a spectrophotometer at 400 THz (750 nm).

The concentration of nine elements (Ca, Cu, Fe, K, Mg, Na, P, S, and Zn) in selected plasma samples were measured on an Optima 3000 inductive coupled plasma (ICP) emission spectroscopy analyser (Perkin Elmer, Norwalk, CT, USA). It appeared that the values of *c*Zn were 32 ± 5 μmol L^{-1}, which is 2–4 times the normal value. It is well known that in particular caps of test tubes like those used in this study may add zinc to plasma samples (Yngvar Thomassen, personal communication). Data on *c*Zn are therefore not given further consideration.

Calculations

Blood acid-base parameters were calculated along the principles of Siggaard-Andersen [39] as modified for use on modern computers [24]. The bicarbonate concentration is given as the "total" or titratable bicarbonate concentration that includes carbonate and carbamino compounds, both for plasma and for whole blood.

The (net) acid load on the blood was taken as the algebraic sum of the measured changes in the blood bicarbonate ($\Delta cHCO_3^-{}_B$) and blood base deficit (ΔcBD_B) concentrations from the normal values of

19.5 mmol L^{-1} ($cHCO_3^-{}_B$) and 0 mmol L^{-1} (cBD_B, see Ref. [24] for further details):

[1] $$c\text{Acid load} = \Delta cHCO_3^-{}_B + \Delta cBD_B$$

After exercise the bicarbonate concentration fell, and the net acid load is thus less than base deficit and reflects the part of base deficit load buffered by other means than bicarbonate.

Plasma anion gap concentration was taken as

[2] $$cAG_P = cNa^+{}_P + cK^+{}_P - cCl^-{}_P - cLa^-{}_P - cHCO_3^-{}_P$$

Here La^- denotes lactate ions, and the index $_P$ means that all entities refer to concentrations in plasma.

Red blood cell chloride concentration was calculated as

[3] $$cCl^-{}_C = ([0.658 - 0.350\ \Delta pH_P] \cdot cCl^-{}_P) \cdot \phi_{w,C} / \phi_{w,P}$$

where cCl^- is the chloride concentration, the indices $_C$ and $_P$ refer to red blood cells and plasma, respectively, and $\phi_{w,C}$ and $\phi_{w,P}$ refer to the water fraction in the red blood cells and plasma, respectively. A separate analysis showed that the relative error of each estimated value was ≈ 2 mmol L^{-1} cell or 3% (not shown). The expression inside the curly brackets was taken from Funder and Wieth [8]. They expressed their concentrations on a molal basis (per kg of red blood cell or plasma water), while we report our values per litre of red blood cell or plasma volume. Conversions between mol L^{-1} and mol kg^{-1} water were done using water fractions of $\phi_{w,C} = 0.73$ and $\phi_{w,P} = 0.94$ for red blood cells and plasma, respectively, (p. 79 in Ref. [39]**,** taken from Ref. [9] assuming a red cell density of 1.1 kg L^{-1}). It could be argued that if water leaves or enters the vascular bed or the red blood cells, the calculated values will be biased. However, if 10% of the plasma water was removed, water would still comprise 93.4% of the total plasma volume. Moreover, if 10% of the red cell water left the cell, water would still account for 70.9% of the red blood cell volume, showing that possible water fluxes have limited effect on the calculated intracellular concentration. Funder and Wieth [8] established the relationship above from measurements on fully oxygenated blood covering a large span of CO_2 pressures and pH-values, and only minimal deviations from the relationship were found [8]. Thus, the

equation was assumed to hold for our blood samples too. Possible deviations for venous blood with reduced O_2-saturation, are addressed in the discussion. Böning and co-workers [3] used a similar approach calculating $c\mathrm{Cl}^-_C$ from a relationship proposed by Dell and Winters [6]. Their approach reported identical values of that proposed above when correcting for different water fractions ($\phi_{w,C}$ / $\phi_{w,P}$).

The chloride concentration in red blood cells has been expressed per litre of whole blood by weighting (prescript w) for haematocrit (Hct), that is, the concentrations were multiplied by haematocrit:

$$[4] \qquad w c\mathrm{Cl}^-_C = c\mathrm{Cl}^-_C \cdot \mathrm{Hct}$$

The effect of a reduced CO_2-pressure on acid-base status of the blood was calculated as follows: When pCO_2 is reduced, bicarbonate reacts with hydrogen ions, forming carbonic acid and CO_2. The hydrogen ions absorbed are taken from nonbicarbonate buffer bases (here symbolised as X' along with Ref. [39]. Thus, $dc\mathrm{HCO}_{3,B}/d\mathrm{pH}_P = -dcX'_B/d\mathrm{pH}_P = \beta X'_{B,P}$ (p. 45 in Ref.)[39]. The latter expression denotes here the buffer capacity of nonbicarbonate buffers in whole blood expressed for a unit change in plasma pH. It was calculated as explained elsewhere [24], and it appeared that $\beta X'_{B,P} \approx 29$ mmol L^{-1} pH^{-1} for the present data. Thus, an increase $\Delta cX'_B$ in cX'_B (and a corresponding decrease in the blood bicarbonate concentration) results in an increase in plasma pH of $\Delta\mathrm{pH}_P = \Delta cX'_B/\beta X'_{B,P}$. The bicarbonate concentration of whole blood can be taken from the concentration in plasma and vice versa (see p. 46 in Ref. [39] or Ref. [24] for details), and consequently the bicarbonate concentration in plasma can be calculated from the new, lower bicarbonate concentration in whole blood. The new CO_2-pressure can now be calculated from the new pH_P and $c\mathrm{HCO}_3^-{}_P$ by solving the Henderson-Hasselbalch equation for pCO_2. This procedure was iterated in steps of $\Delta cX'_B = 1$ mmol L^{-1} or $\Delta c\mathrm{HCO}_3^- = -1$ mmol L^{-1} to a pCO_2 of 53 hPa was found; a separate examination showed that steps much larger than 1 mmol L^{-1} did not introduce nonlinear effects (not shown). The amount of acid neutralised by reducing pCO_2 was taken as the difference between the initial and final bicarbonate concentrations of whole blood. The effect of increasing pCO_2 was calculated correspondingly. The numerical approach used is an Euler method [18] and was carried out in a spread sheet.

The *Bohr-Haldane effect* was taken as the reduction in O_2 saturation (sO_2) times blood haemoglobin concentration, using the Haldane coefficient $k_H = -0.31$ (p. 79 in Ref. [39]):

[5] $$\beta_{BH} = -k_H \cdot (1 - sO_2) \cdot c\mathrm{Hb}_B$$

Statistics

The data are given as mean ± SEM unless otherwise stated. Tests of statistical significance were carried out using *t*-tests. Linear regression was calculated as the geometric mean, thus taking into account that both parameters were subject to error [5, 35].

RESULTS

The subjects cycled at 5.48±0.17 W kg^{-1} for 122±7 s (mean ± SEM) to exhaustion.

Time course of blood lactate and acid-base parameters

The arterial blood lactate concentration rose during the exercise and peaked at 14 mmol L^{-1} in the interval 1–6 min after the exercise before declining (Figure 1a). The lactate concentration in femoral-venous blood was higher than that in arterial blood during the exercise and for the first 20 min of the recovery period. The lactate concentration in plasma was ≈60% higher than that in whole blood.

The bicarbonate concentration of both arterial and femoral-venous whole blood fell during the exercise and was around half the pre-exercise value in the early recovery before returning to the pre-exercise level in the late recovery (Figure 1c). The bicarbonate concentration in plasma was 20–25% higher than that in whole blood throughout the study. Thus for both lactate and bicarbonate the concentration in plasma was considerably higher than that in whole blood. Blood base deficit concentration rose during the exercise and further in the early recovery before returning to the pre-exercise value within 1 h of recovery (Figure 1e). In arterial blood standard base deficit was always within 1.0 mmol L^{-1} of the reported (actual) base deficit, and the changes in standard base deficit were in average 95% of those of (actual) base deficit (not shown further).

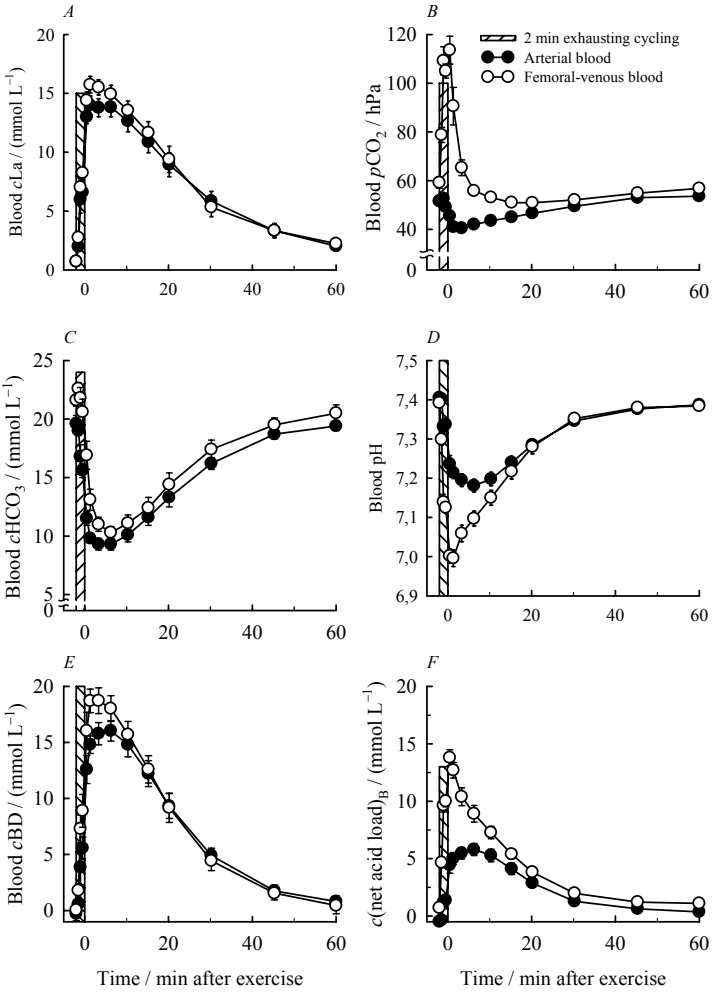

Figure 1. Blood lactate concentration and related acid-base parameters of arterial (●) and femoral-venous whole blood (○) during and after the exercise. *A*, blood lactate concentration (*c*La); *B*, blood CO_2 pressure (pCO_2); *C*, whole blood bicarbonate concentration ($cHCO_3^-{}_B$); *D*, pH; *E*, blood base deficit (*c*BD); *F*, net acid load on whole blood [$c(\text{net acid load})_B$]. The data are mean ± SEM of seven subjects during 2 min cycling at constant power to exhaustion and for 1 h after the exercise. Error bars not shown are hidden by the symbols.

The CO_2-pressure (pCO_2) of arterial blood fell during the exercise and further in the early recovery, while pCO_2 of femoral-venous blood doubled during exercise and dropped to below the pre-exercise level in the early recovery (Figure 1b). pCO_2 was back to the pre-exercise level within 45 min after the exercise.

pH of arterial blood fell during the exercise and further in the early recovery (Figure 1d). pH of femoral-venous blood fell twice as much, reaching a lowest value of 7.00±0.02 seen during the first minute of the recovery period. The arterial pH of 7.387±0.004 seen 1 h after exercise was still below the pre-exercise value (P=0.01).

The net acid load of the arterial blood rose during the exercise and further to a peak 6 min into the recovery period. The net acid load of femoral-venous blood rose more than twice as much (Figure 1f). One hour after exercise the net acid load of both arterial and femoral-venous blood was still above the pre-exercise level.

Table 1. Summary of the main blood values.

Parameter	Unit of	Before 2 min cycling		Just after 2 min cycling	
	measurement	Artery	Vein	Artery	Vein
cLa_B	mmol L^{-1}	0.7±0.2	0.7±0.2	13.0±0.7	14.2±0.8
pH		7.40±0.01	7.39±0.01	7.24±0.02	7.00±0.02
pCO_2	hPa	51.6±1.8	59.2±2.3	45.5±0.9	113.6±5.7
$cHCO_{3,B}$	mmol L^{-1}	19.6±0.7	21.6±0.5	11.5±0.6	16.9±1.2
cBD_B	mmol L^{-1}	–0.3±0.6	0.0±0.6	12.4±1.3	16.4±1.7
*c*Acid load	mmol L^{-1}	–0.5±0.2	0.7±0.4	4.4±0.7	13.8±0.7
cHb_B	mmol L^{-1}	9.64±0.20	9.58±0.15	10.39±0.21	10.50±0.19
Hct		0.452±0.006	0.458±0.008	0.473±0.008	0.491±0.006
pO_2	hPa	137±5	57±5	149±3	43±10*
sO_2		0.963±0.006	0.670±0.056	0.965±0.007	0.291±0.102*
$cO_{2,B}$	mmol L^{-1}	9.3±0.2	6.4±0.6	10.0±0.2	3.1±1.1*
$cAlb_P$	mmol L^{-1}	0.640±0.013	0.652±0.024	0.714±0.020	0.791±0.032
$wcAlb_P$	mmol L^{-1}	0.351±0.008	0.353±0.012	0.377±0.012	0.396±0.012
ρPr_P	g L^{-1}	67.5±1.6	67.0±1.8	73.1±2.2	77.3±1.9

The data are blood lactate concentration (cLa_B), pH, CO_2-pressure (pCO_2), blood bicarbonate ($cHCO_{3,B}$), base deficit (cBD_B), and haemoglobin concentrations (cHb_B), blood haematocrit (Hct), O_2-pressure (pO_2), O_2-saturation (sO_2) and blood O_2-concentration ($cO_{2,B}$). All data refer to the values in whole blood. Further follows plasma albumin concentration ($cAlb_P$), plasma albumin concentration weighted for haematocrit ($wcAlb_P$) and plasma protein mass concentration (ρPr_P). *The blood samples just after 2 min cycling were drawn 20–30 s after the end of exercise. pO_2, sO_2 and cO_2 of femoral-venous blood near the end of exercise were ≈30 hPa (pO_2), <0.2 (sO_2) and <2 mmol L^{-1} ($cO_{2,B}$). The data are mean ± SEM of seven subjects.

Blood haemoglobin concentration rose by 8% during the exercise, thus reflecting haemoconcentration (Table 1). The concentration fell in the recovery period, was back to the pre-exercise level within 15 min and below that level from 30 min after the exercise. Haematocrit of arterial blood rose during the exercise and peaked 7% above the pre-exercise value 3 min into the recovery. Haematocrit of femoral-venous blood was 9% above the pre-exercise level at exhaustion. Haematocrit fell to the pre-exercise level within 20 min and was below that level from 30 min into the recovery period.

The ratio between plasma albumin concentration weighed for haematocrit and blood haemoglobin concentration was 0.0367±0.0014 (mol Alb/mol Hb) before the exercise. There was at no time point any statistically significant arterial-femoral-venous difference for this ratio, nor did this ratio change during the exercise or in the recovery period. These data thus suggest that the vascular contents of albumin and haemoglobin were constant or changed in parallel during the study.

Scatterplots of acid-base parameters

pCO_2 and pH of the arterial blood fell by the blood lactate concentration (Figure 2A, C), and consequently the bicarbonate concentration of arterial blood fell by 0.8 mmol L^{-1} for a 1 mmol L^{-1} increase in the blood lactate concentration (Figure 2E). Arterial base deficit concentration rose 30% more than blood lactate concentration did (Figure 2G). These data thus show that in average bicarbonate and other, nonbicarbonate buffers each buffered 63% (HCO_3^-) and 37% (nonbicarbonate buffers), respectively, of the total acid load on arterial blood.

pCO_2 of femoral-venous blood ($pCO_{2,fv}$) doubled during the last minute of the exercise and stayed high for the first minute of the recovery period (Figure 1B). These values with a $pCO_{2,fv} > 80$ hPa are therefore marked separately in Figure 2 and are not included in the regression analyses. $pCO_{2,fv}$ was systematically higher than that of arterial blood with the same lactate concentration, and $pCO_{2,fv}$ showed no simple relationship to the lactate concentration (Figure 2B). pH of femoral-venous blood fell by the lactate concentration (Fig. 2D). Even when disregarding samples with $pCO_2 > 80$ hPa, pH in femoral-venous blood was 0.03–0.06 less than that in arterial blood with the same lactate concentration, and the relationship was slightly curved. The bicarbonate concentration of femoral-venous blood fell by the blood lactate concentration. When excluding samples with $pCO_2 > 80$ hPa, there was a relationship almost parallel to that of arterial blood but displaced to 1.7 mmol L^{-1} higher bicarbonate concentrations for the same lactate concentration (Figure 2F). The base deficit concentration of femoral-venous blood rose showed almost the same relationship to lactate concentration as seen in arterial blood, and there was no difference between samples with high and low pCO_2 (Figure 2H). When excluding samples with $pCO_{2,fv} > 80$ hPa, bicarbonate buffered 61% of all acid added to the femoral-venous blood, a value very close to that of arterial blood.

The dashed lines in *E* and *G* are lines of identity, that is, relationships assuming that each lactate ion added to the blood replaced one bicarbonate ion (*E*), and that each lactate ion added to the blood was accompanied by one and only one hydrogen ion (*G*). The difference between the solid and the dashed line in *G* thus shows the excess hydrogen ions added to the arterial blood. The dotted line in *G* is taken from the regression line in *E*, that is, by assuming that the amount of hydrogen ions added to the blood equalled the amount of bicarbonate ions disappearing. The difference between the solid and dotted line thus shows the amount of hydrogen ions buffered by nonbicarbonate buffers. The data are from seven subjects who cycled at constant power for 2 min to exhaustion and were followed for 1 h after.

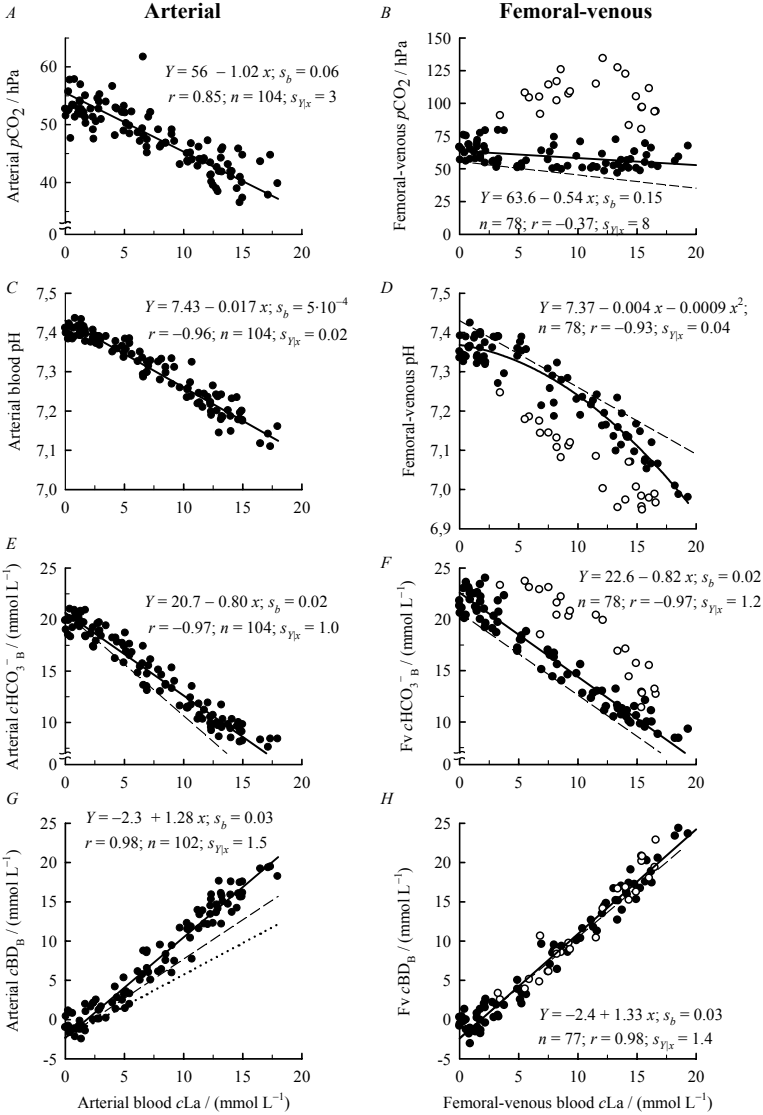

Figure 2. Arterial (left panels) and femoral-venous acid-base parameters (right panels) versus blood lactate concentration. Top row, blood pCO_2; second row, blood pH; third row, whole blood bicarbonate concentration; bottom row, blood base deficit. The solid lines are the regression lines

with the relevant regression parameters given in each panel. For femoral-venous plots (right) the corresponding regression line of arterial data is shown as a dashed line, and the difference between each pair of lines thus shows the difference between arterial and femoral-venous blood for the same lactate concentration. For femoral-venous blood ○ refers to samples taken during the last minute of the exercise and for the first minute after, that is samples with $pCO_2 > 80$ hPa ; these values are not included in the calculated regressions. ● refers to all other blood samples and on which values the regressions are based.

Plasma electrolyte concentrations

The plasma sodium concentration rose by 10 mmol L^{-1} during the 2 min exhausting exercise, mainly as a consequence of haemo-concentration during exercise, while the chloride concentration rose somewhat less (Table 2). The plasma potassium concentrations measured ≈30 s after the exercise was 2.3 mmol L^{-1} higher than before the ride. The plasma lactate concentration rose by 20 mmol L^{-1}, while the plasma bicarbonate concentration fell by around half this value (Figure 3A). Therefore, the sum of these two ion concentrations rose during the exercise, and more so for femoral-venous than for arterial plasma. Consequently, anion gap fell during the exercise, and more so for femoral-venous than for arterial plasma (Figure 3B). Anion gap rose linearly by pH, whether expressed in absolute terms (Figure 3C) or as anion gap weighted for changes in haematocrit [$wcAG_P$ / (mmol L^{-1}) = 6.8+13.3 (pH–7.4); $s_b = 0.8$; $s_{Y|x} = 1.1$; $r = 0.75$]. There was no difference between arterial and femoral-venous samples. One hour after the exercise all plasma electrolyte concentrations except those of lactate and bicarbonate were back to the pre-exercise values, and the anion gap was restored.

Table 2. Summary of plasma electrolytes and derived quantities.

Parameter	Unit of measurement	Before 2 min cycling		Just after 2 min cycling	
		Artery	Vein	Artery	Vein
cNa^+_P	mmol L^{-1}	134.5±1.2	135.3±1.1	143.9±1.9	147.0±1.9
cK^+_P	mmol L^{-1}	3.84±0.06	3.83±0.09	6.11±0.27*	6.21±0.39*
cCl^-_P	mmol L^{-1}	101.8±0.6	100.5±0.4	107.9±1.3	105.3±1.3
cLa^-_P	mmol L^{-1}	1.4±0.3	1.6±0.3	20.0±1.4	23.1±1.6
$cHCO^-_{3,P}$	mmol L^{-1}	24.0±0.6	26.6±0.8	14.1±0.8	20.2±1.4
cAG_P	mmol L^{-1}	11.1±0.7	10.3±0.7	8.1±1.1	4.7±0.8
cCl^-_C	mmol L^{-1}	57.8±0.3	59.0±0.2	66.1±0.9	70.4±0.8
$wcCl^-_C$	mmol L^{-1}	23.5±0.4	24.3±0.4	28.5±0.4	32.7±0.9

The data are plasma electrolytes (Na^+, K^+, Cl^-, lactate and bicarbonate), and anion gap (cAG_P). Finally, calculated concentration of chloride in red blood cells (cCl^-_C) and weighted for haematocrit ($wcCl^-_C$). * The blood samples just after 2 min cycling were drawn 20–30 s after the end of the exercise. cK^+_P near the end of exercise was ≈7 mmol L^{-1}. The data are mean ± SEM of seven subjects.

The ratio between the plasma sodium concentration weighted for haematocrit and blood haemoglobin concentration [cNa^+_P (1 – Hct) / cHb_B], was 7.7 ± 0.2 (mmol Na^+ / mmol Hb) for both arterial and femoral-venous blood before the exercise. The ratio fell to 7.3±0.2 (aP, –5%) and 7.0±0.2 (fvP, –9%) at exhaustion and stayed at those levels for the following 6 min. During this period there was a significant a–fv difference, compatible with an uptake of sodium in the leg (see Ref. [24] for further details and interpretation). Since we in the recovery period flushed the catheters with a heparinised isotonic NaCl-solution, changes later in the recovery are difficult to interpret physiologically, and further data are therefore not given.

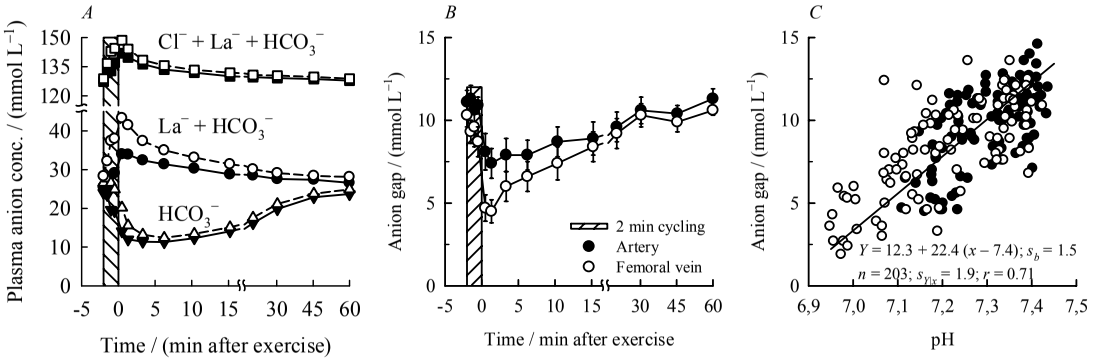

Figure 3. *A*, concentration of bicarbonate (HCO_3^-), lactate plus bicarbonate ($La^- + HCO_3^-$), and of chloride plus lactate plus bicarbonate ($CL^- + La^- + HCO_3^-$, sum of all measured anions) in arterial (filled symbols) and femoral-venous plasma (open symbols). *B*, plasma anion gap concentration, taken as $cAG_P = (cNa^+{}_P + cK^+{}_P - cCl^-{}_P - cLa^-{}_P - cHCO_3^-{}_P)$. *C*, plasma anion gap versus pH in arterial (●) and femoral venous (○) plasma. The data are from seven subjects cycling at constant power for 2 min to exhaustion and for 1 h after the exercise. In *B* the values are mean ± SEM; error bars not shown are hidden by the symbols (SEM ≈1 mmol L^{-1}).

In arterial blood the relationship between plasma bicarbonate and lactate concentrations was $c\mathrm{HCO}_{3,\mathrm{aP}} = 25\ \mathrm{mmol\ L^{-1}} - 0.65\ c\mathrm{La}_{\mathrm{aP}}$ ($s_b = 0.02$, $s_{Y|x} = 1.7\ \mathrm{mmol\ L^{-1}}$, $n = 105$, $r = -0.95$). The corresponding relationship for plasma from femoral-venous blood was $c\mathrm{HCO}_{3,\mathrm{fvP}} = 27\ \mathrm{mmol\ L^{-1}} - 0.63\ c\mathrm{La}_{\mathrm{aP}}$ ($s_b = 0.02$, $s_{Y|x} = 1.8\ \mathrm{mmol\ L^{-1}}$, $n = 74$, $r = -0.95$) when samples with a $p\mathrm{CO}_2 > 80$ hPa were excluded. The slopes of the two relationships do not differ. Thus, in average a 1 mmol $\mathrm{L^{-1}}$ increase in the plasma lactate concentration resulted in a 0.64 mmol $\mathrm{L^{-1}}$ drop in the plasma bicarbonate concentration.

Concentrations of eight elements in plasma. The concentrations of eight elements in plasma (Ca, Cu, Fe, K, Mg, Na, P, and Sn) were measured before the exercise and 1 min after the exercise. There was no major change during the exercise nor any major a–fv differences, and the moderate changes and differences seen are compatible with haemoconcentration and the fluid shift that took place.

Red blood cell chloride concentration

The calculated chloride concentration rose by 8 (+15%) and 12 mmol $\mathrm{L^{-1}}$ (+23%) for arterial and femoral-venous red blood cells, respectively, during the exercise. To correct for fluid shifts between plasma and red blood cells, the concentration were expressed per litre of whole blood by weighing the concentrations expressed per litre of cell volume for haematocrit. The weighted chloride concentration rose by 21% (a) and by 34% (fv) during the exercise (Figure 4A, Table 2). The chloride concentrations of red blood cells fell in the recovery. From 10 min into the recovery period the weighed chloride concentration of femoral-venous red blood cells did not differ from that of arterial blood.

The weighted chloride concentration of red blood cells rose linearly by the net acid load on blood and by reduced pH (Figure 4 B, C). The weighed chloride concentrations of the red blood cells was in average 0.6 mmol $\mathrm{L^{-1}}$ less in femoral-venous than in arterial blood for the same blood pH or acid load. The calculated chloride uptake of the red blood cells amounted to 82% of the net acid load on the blood (Figure 4B).

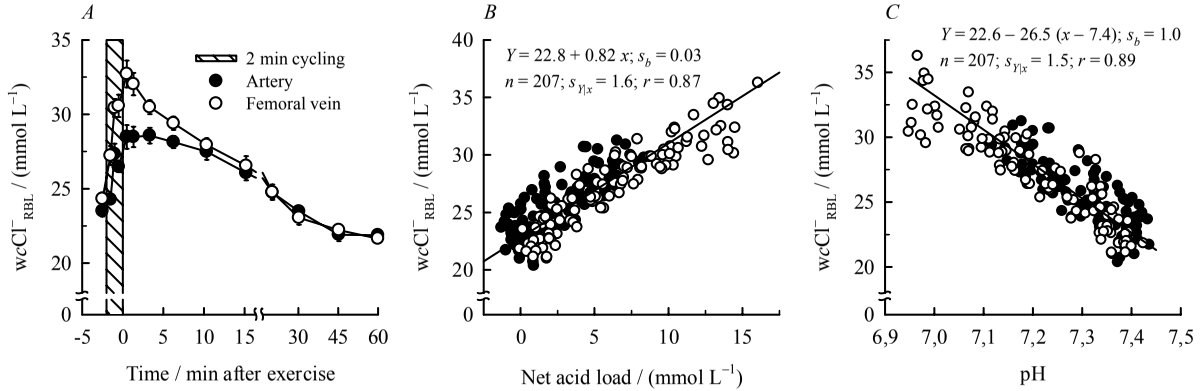

Figure 4. *A*, weighted red blood cell chloride concentration for seven subjects during 2 min cycling to exhaustion and for 1 h after. The data are mean ± SEM, and error bars not shown are hidden by the symbols. *B*, weighted red blood cell chloride concentration versus net acid load on the blood. *C*, weighted red blood cell chloride concentration versus pH. The chloride concentration of red blood cells was calculated as explained in the method section, and weighted values given are expressed per volume of whole blood by multiplying the red blood cell concentrations by haematocrit. The data are from seven subjects who cycled at constant power for 2 min to exhaustion.

DISCUSSION

Blood pH fell by increasing blood lactate concentrations, and more so in femoral-venous blood with either an additional respiratory acidosis or only limited respiratory compensation. Blood base deficit concentration rose 30% more than the blood lactate concentration did, and the relationship did not differ between arterial and femoral-venous blood with different pCO_2. Bicarbonate buffered in average 62% of the hydrogen ions added to the blood. Chloride left the plasma space and entered red blood cells in proportion to the net acid load or change in pH and amounted to >30% of the total acid load. Plasma anion gap fell linearly by a falling pH or increasing acid load.

Comparison with former studies

Blood lactate concentration rose during the exercise and continued to rise for a few minutes into the recovery period and fell thereafter. This pattern and the values seen are in line with several former studies [3, 4, 24, 27, 33, 36, 43]. Blood and plasma bicarbonate concentration fell linearly by the lactate concentration, which is in line with data from former studies [4, 27, 33]. In arterial blood pCO_2 fell linearly by the blood lactate concentration or the degree of acidosis, as has also been shown earlier [27]. Thus, our data on arterial blood are in line with former studies. During exercise there was no respiratory compensation in femoral-venous blood but in fact a doubling of the pCO_2 near the end of the exercise and in the early recovery, thus adding a respiratory acidosis to the metabolic one. That observation is in line with that of Sahlin and coworkers [37]. Finally, the observed changes in plasma chloride concentration are in line with a recent study [3]. Thus, when comparisons are possible, our data are in line with former studies and thus appear typical for very intense exercise. Consequently, it is therefore conceivable that further analyses and calculations on our data are not atypical.

Acid-base status of arterial and femoral-venous blood

For arterial blood pH, pCO_2, and the bicarbonate concentration all fell linearly by increasing lactate concentrations, while base deficit rose linearly by the lactate concentration. For femoral-venous blood there were corresponding relationships between pH and bicarbonate concentration versus blood lactate concentration when samples with particularly high pCO_2 were excluded. The relationship between blood

base deficit and blood lactate concentrations did not differ systematically between arterial and femoral-venous blood, nor did base deficit differ between samples with high and low pCO_2. The latter observation suggests that respiratory acidosis or alkalosis had no influence on the calculated base deficit concentration, which was to be expected since base deficit measures the metabolic or nonrespiratory component of the acidosis in blood. The finding that base deficit did not differ between arterial and femoral-venous blood with the same lactate concentration even when pH differed, suggests that the differences in pH and acid-base status between arterial and femoral-venous blood was due to different degrees of respiratory acidosis or compensation.

Just after exercise pH differed by as much as 0.2 between arterial and femoral-venous blood, and the main reason was different pCO_2, as has been shown earlier [36]. These results thus show the importance of pCO_2 for blood pH, as has been addressed in a recent review [16].

Base deficit and excess hydrogen ions

Blood base deficit changed ≈30% more than the blood lactate concentration did. This is compatible with the idea that also hydrogen ions of nonlactic and noncarbonic origin appeared in the blood. It was first suggested that lactate and hydrogen ions appeared in equal amounts in the blood [43]. Later studies found that base deficit exceeded the blood lactate concentration after intense exercise. One interpretation was that the excess seen meant that base deficit overestimated the amounts of hydrogen ions added to blood [4, 33]. It was later shown that there was an excess of hydrogen ions added to blood in the early recovery period after intense exercise [2, 27, 36], at the same time as muscle pH recovers much faster than muscle lactate concentration does [13, 14, 38], compatible with the idea that muscle removed hydrogen ions to the blood independently of lactate ions in the early recovery after intense exercise. It is now known that muscle can release hydrogen ions independently of lactate [2, 17, 21]. We have more recently shown that in the leg sodium ions left plasma in equal amounts to the excess hydrogen ions appearing in the blood after intense exercise [24]. Those data are compatible with an activation of a Na^+,H^+-exchange in the early recovery, which is well established to take place for the amiloride-sensitive Na^+,H^+-exchanger when muscle pH is reduced [21]. Thus, we now know that base deficit is an accurate measure of the metabolic acidosis in blood and that the

excess hydrogen ions appearing in the blood after exercise is a physiologic phenomenon reflecting processes with well established mechanisms.

The view of excess hydrogen ions released to blood has recently been questioned by Böning and co-workers who found that changes in standard base deficit, assumed to measure metabolic acidosis averaged over the whole extracellular compartment, equalled the calculated extracellular lactate concentration [3]. Their approach assumes sufficiently fast equilibration of lactic acid and bicarbonate in all extracellular space, which may not be the case [30]. A further discussion of possible excess release of hydrogen ions is beyond the scope of the present paper.

Uptake of chloride in red blood cells

Chloride left plasma and entered the red blood cells in proportion to the change in pH and to the net acid load. Chloride shift and its relation to pCO_2 and blood acid-base status has been known for more than hundred years [12, 32]. The phenomenon was later shown to be due to a property of red blood cells and not of plasma [7], to be a chloride-bicarbonate-exchange across the red cell membrane [15], and to be a very fast process, reaching equilibrium within 1 s [19, 42]. Bicarbonate neutralised most of the hydrogen ions added to the blood, but of the remaining hydrogen ions added, our data suggest that 82% could be accounted for by processes related to chloride uptake in red blood cells. Thus, the calculated chloride shift amounted in average to 31% of the total acid load on the blood.

At each time point the chloride concentration in red blood cells was higher in femoral-venous than in arterial blood. That observation is compatible with an uptake of chloride by the red blood cells in exchanged with bicarbonate in the peripheral capillaries (and a reversal in the lungs). Our data show in addition that the chloride concentration in red blood cells rose by a falling pH or an increasing net acid load. Since plasma bicarbonate concentration fell during these conditions, this means that the cells took up chloride during acidosis while the bicarbonate concentration in plasma fell. It is likely that excess hydrogen ions in plasma have reacted with bicarbonate, thus forming CO_2 that have diffused into the red blood cells, formed bicarbonate that again have been exchanged with chloride in plasma, a process known as the Jacobs-Steward cycle [15]. The process may

take place even without carbonic anhydrase available in plasma, but then requiring 1 min or more for approaching equilibrium.

The chloride concentration in red blood cells was in this study not measured but calculated from the measured pH and plasma chloride concentration using equation [3] taken from [8]. Funder and Wieth established their relationship from measurements covering a large span of pCO_2- and pH-values, but only on fully oxygenated blood. They found no systematic deviations from the relationship they established, and a separate analysis on their data showed that the error in each estimate was ≈2 mmol L^{-1} or 3%. Dell and Winters [6] developed the same relationships using others data. Thus, two independent sets of data support our calculated chloride concentrations. Others have calculated the chloride concentration of red blood cells from the measured amounts of chloride in haemolysed whole blood and plasma [22, 23]. That approach that is less precise than direct measurements but would expectedly not introduce systematic errors. The chloride concentration of red blood cells in two other studies rose almost as much as in our study [3, 23]. Thus, these studies give further support to our data on the red blood cell chloride concentration calculated from equation [3]. However, as discussed below, our calculations may have underestimated the uptake in red blood cells of deoxygenated femoral-venous blood. Thus, in future studies red blood cell chloride concentration should be measured directly, at least in deoxygenated venous blood.

Plasma electrolyte balance

The pooled concentration of cations and anions in plasma rose during the exercise, and the plasma volume fell, as shown by the increased albumin concentration. Haemoconcentration is one major reason for the increases in plasma electrolyte concentrations. However, several factors modified this picture. Most important is that during the exercise and for the first minutes after lactate replaced bicarbonate in plasma at a ratio of ≈1 : 0.64 (mot including femoral-venous samples with pCO_2 > 80 hPa). In addition, both sodium and chloride left the plasma space. While sodium was probably taken up by acidotic muscle cells in exchange with H^+ [24] chloride was most likely taken up by the red blood cells. Finally, the plasma potassium concentration rose during the exercise and fell in the early recovery period, as studied in much more detail elsewhere [28].

The concentration of anions raised more than the concentration of cations did, mainly because of the smaller loss of bicarbonate than accumulation of lactate. Consequently, plasma anion gap concentration fell linearly by a falling pH or by an increasing net acid load, that is, by an increasing lactate concentration. That finding is in apparent conflict with our former study where we reported an apparent increase in the calculated anion gap after the exercise [27]. However, in that study we compared the lactate concentration in whole blood with the concentration of bicarbonate and other electrolytes in plasma. In the present study the plasma lactate concentration was as much as 10 mmol L^{-1} higher than the concentration in whole blood, and this difference may explain the whole difference between the two studies.

Anion gap reflects the net negative electric charge on electrolytes that have not been measured, preferentially on plasma proteins. It could be that plasma exchanged other ions. That possibility was examined by measuring the concentrations of eight elements in plasma samples taken before the exercise and 1 min into the recovery. These analyses showed only moderate changes in the concentration of each of the measured elements, compatible with haemoconcentration and fluid shift and thus suggest that there was no detectable exchange between muscle, plasma, and red blood cells of the measured elements or ions other than sodium, potassium, chloride, lactate, and bicarbonate. Moreover, the production and release of pyruvate from muscle is minimal [4, 36]. However, anion gap fell by 22.4 mmol L^{-1} for a unit drop in plasma pH. If that value did reflect buffering by plasma proteins, the slope would correspond to a buffer capacity of >0.3 mmol pH^{-1} g^{-1} protein, which is three times the value found by others by titration of plasma (p. 42 in Ref. [39]). A correction for changes in the protein mass concentrations during the study has no influence on this conclusion (not shown). Thus, it could also be that the concentration of other ions in plasma, perhaps some organic ions, was changed after the exercise. The results of a change in anion gap in excess of changed charge of plasma proteins is in line with data of Adrogue and co-workers [1] who also proposed changes in unmeasured organic anions as a likely explanation.

The ratio between blood concentrations of albumin and haemoglobin was constant throughout the study, meaning that the vascular content of both species was probably constant, apart from loss due to blood sampling. The mass concentration of plasma proteins rose relatively less than the albumin concentration. That observation is

compatible with loss of plasma proteins other than albumin during exercise and in the early recovery, as also suggested by another study [22].

Neutralisation of acids added to the blood

In femoral-venous blood base deficit rose by 20 mmol L^{-1} at the extreme. Blood pH fell from 7.4 to 7.0, which is an increase in the activity of hydrogen ions from 40 to 100 nmol L^{-1}. Using an activity coefficient of 0.84 (p. 28 in Ref. [39], this corresponds to an increase in the concentration of free hydrogen ions of ≈70 nmol L^{-1}. This means that for each million of hydrogen ions added to the blood, less than four remained free at any time while the others were neutralised. A corresponding calculation for the data on arterial blood shows that less than three out of one million hydrogen ions released to the blood were free. Blood has several means of neutralising added acids. Bicarbonate may react with hydrogen ions, form carbonic acid that again is split to water and CO_2 that may be lost through the lungs. It appeared that ≈62% of the total acid load, taken as the base deficit, was neutralised by bicarbonate in both arterial and femoral-venous blood (disregarding samples with $pCO_2 > 80$ hPa). Thus, bicarbonate was quantitatively the most important means of neutralising acids added to the blood.

Our data suggest that of the portion of hydrogen ions added to the blood but not neutralised by bicarbonate, 82% entered the red blood cells by a chloride shift and were buffered intracellularly. That amounts in average to 31% of the total acid load (base deficit). Thus, other means accounted for only 7% of the total acid load.

The magnitude of base deficit, its components and how this acid load was accounted for, is shown in Figure 5. In arterial samples reduction of blood bicarbonate concentration neutralised in both cases 61% of the acid load, taken as the base deficit concentration. The main component was due to the effect of a reduced bicarbonate concentration as pH fell, keeping the CO_2-pressure constant. As a consequence of hypervention pCO_2 of arterial blood fell to 41–45 hPa, which reduced the bicarbonate concentration further by ≈1 mmol L^{-1} and thus neutralised the same amount of hydrogen ions. In femoral-venous blood pCO_2 was increased, and that led to an extra acid load of to 5.3 mmol L^{-1} (0 min, fv) and 0.4 mmol L^{-1} (6 min, fv).

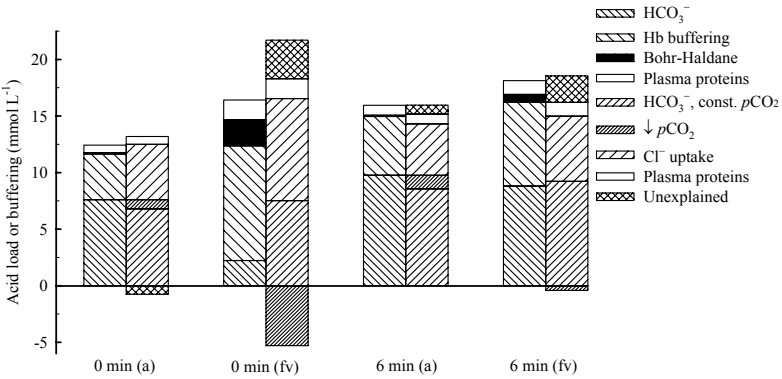

Figure 5. *Left column* in each pair, components of base deficit taken as (bottom to top) a reduced bicarbonate concentration in whole blood (HCO_3^-), buffering on haemoglobin without the Bohr-Haldane effect (Hb buffering), the Bohr-Haldane effect (Bohr-Haldane), and buffering by plasma proteins (Plasma proteins). *Right column* in each pair, to what extent the base deficit was reflected in a reduced bicarbonate concentration at a constant pCO_2 (HCO_3, const. pCO_2), by a reduced pCO_2 ($\downarrow pCO_2$), by the calculated uptake of chloride in the red blood cells (Cl^- uptake), by buffering by plasma proteins (Plasma proteins), and finally the difference between the base deficit and the sum of the components above (Unexplained). The data are mean values of seven subjects who cycled at constant power for 2 min to exhaustion. The values are for arterial (a) and femoral-venous (fv) blood just after the cycling (0 min) and 6 min after the exercise (6 min). The chloride concentration of the red blood cells and the effect of a reduced or increased CO_2-pressure were calculated as explained in the method section; for femoral-venous blood with a raised CO_2-pressure this component came out negative. Base deficit and its components were calculated as explained elsewhere [24].

In arterial blood haemoglobin neutralised 33% of the hydrogen ions, but the Bohr-Haldane effect was minimal. In femoral-venous blood haemoglobin neutralised 76% (0 min fv) and 45% of the acid load (6 min fv). Those percentages include contributions by the Bohr-Haldane effect of 14% (2.3 mmol L^{-1}, 0 min fv) and 4% (0.7 mmol L^{-1}, 6 min fv) of the total neutralisation . Thus, for femoral-

venous blood just after the exercise the Bohr-Haldane effect neutralised more hydrogen ions that bicarbonate did.

For arterial blood the calculated chloride uptake in red blood cells was similar to the amount of hydrogen ions neutralised by haemoglobin; the small mismatch seen are well within the analytical errors. For femoral-venous blood there was an apparently unexplained component of 3.4 mmol L^{-1} (0 min fv) and 2.3 mmol L^{-1} (6 min fv). The chloride concentration of the red blood cells was in this study calculated, using equation [3] worked out for fully oxygenated blood and thus disregarding the Bohr-Haldane effect. If hydrogen ions neutralised by the Bohr-Haldane effect is included, the apparent mismatch is largely within the error of analysis and estimation.

Neutralisation by plasma proteins accounted for 10% of the base deficit in femoral-venous blood just after the exercise and 5–6% in the other samples shown.

CONCLUSION

Bicarbonate neutralised most of the acids added to the blood, and haemoglobin neutralised most of the remaining part. Base deficit did not differ between arterial and femoral-venous blood with the same lactate concentration. The acid-base status of femoral-venous and arterial blood differed because of different degrees of respiratory acidosis or compensation. The chloride concentration of red blood cells rose in proportion to the amount of hydrogen ions buffered by haemoglobin.

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