Factors influencing hydrogen ion concentration in muscle after intense exercise

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KOWALCHUK, JOHN M., GEORGE J. F. HEIGENHAUSER, MICHAEL I. LINDINGER, JOHN R. SUTTON, AND NORMAN L. JONES. *Factors influencing hydrogen ion concentration in muscle after intense exercise.* J. Appl. Physiol. 65(5): 2080–2089, 1988.—To assess the importance of factors influencing the resolution of exercise-associated acidosis, measurements of acid-base variables were made in nine healthy subjects after 30 s of maximal exercise on an isokinetic cycle ergometer. Quadriceps muscle biopsies ($n = 6$) were taken at rest, immediately after exercise, and at 3.5 and 9.5 min of recovery; arterial and femoral venous blood were sampled ($n = 3$) over the same time. Intracellular and plasma inorganic strong ions were measured by neutron activation and ion-selective electrodes, respectively; lactate concentration ([La^-]) was measured enzymatically, and plasma P_{CO_2} and pH were measured by electrodes. Immediately after exercise, intracellular [La^-] increased to 47 meq/l, almost fully accounting for a reduction in intracellular strong ion difference ([SID]) from 154 to 106 meq/l. At the same time, femoral venous P_{CO_2} increased to 100 Torr and plasma [La^-] to 9.7 meq/l; however, plasma [SID] did not change because of a concomitant increase in inorganic [SID] secondary to increases in [K^+], [Na^+], and [Ca^{2+}]. During recovery, muscle [La^-] fell to 26 meq/l by 9.5 min; [SID] remained low (101 and 114 meq/l at 3.5 and 9.5 min, respectively) due almost equally to the elevated [La^-] (30 and 26 meq/l) and reductions in [K^+] (from 142 meq/l at rest to 123 and 128 meq/l). Femoral venous P_{CO_2} rose to 106 Torr at 0.5 min postexercise and fell to resting values at 9.5 min. In contrast, femoral venous [La^-] rose progressively to 17.6 meq/l at 3.5 min postexercise and remained elevated at 14.2 meq/l after 9.5 min; femoral venous [SID] fell from 43 meq/l immediately after exercise to 30 meq/l at 9.5 min of recovery. In arterial plasma there were early and sustained falls in P_{CO_2} and [SID]; [La^-] rose to 13.9 meq/l at 2.5 min postexercise. The results of these studies demonstrated that several factors in addition to increases in [La^-] contribute to the changes in [H^+] and [HCO_3^-] after heavy exercise.

acid-base control; strong ion difference; lactate; muscle; electrolytes; femoral venous blood; arterial blood; carbon dioxide partial pressure; buffering

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THE PRESENT STUDY examined several factors that influence [H^+] in muscle during and after intense exercise of short duration. The importance of an increase in intramuscular lactic acid concentration ([La^-]) is well accepted, but recently other factors have been shown to exert important influences on [H^+]. Stewart (35, 36) has pointed out the importance of the physicochemical interactions between the independent and dependent variables contributing to [H^+] control, within the constraints imposed by the laws of electrical neutrality and conservation of mass. He showed that the contributions may be quantified by measuring the system independent variables and using them to solve a series of equations to obtain the dependent variables, including [H^+] and [HCO_3^-]. Considering the systems involved in plasma and intracellularly in muscle, the independent variables are the concentration of strong (fully dissociated) ions, of which the most important are [Na^+], [K^+], [Ca^{2+}], [Mg^{2+}], [Cl^-], and [La^-]; the total concentration of weak (partially dissociated) acids ([A_T]), mainly proteins and phosphates; and P_{CO_2}. The effect of strong ions may be expressed as the net difference between the total concentration of cations and anions, the strong ion difference ([SID]). In addition to these independent variables, the equations identified by Stewart (35) include values for the reaction equilibrium constants. The solution of the equations allows the important dependent variables, [H^+], [HCO_3^-], and dissociated weak anions ([A^-]), to be derived. Thus although increases in [La^-] during exercise play a very important role in changing [H^+] in plasma and muscle, changes in inorganic strong ions also may influence [SID], and changes in P_{CO_2} and [A_T] also are potentially important. Furthermore, when exchange of ions and CO_2 occurs between muscle and plasma, the effects may be different in extent and opposite in direction in the two sites. For example, intracellular [SID] is about four times the [SID] of plasma; a loss of K^+ from the cells into plasma will reduce intracellular [SID], but an accompanying increase in plasma [K^+] will increase [SID] in plasma, potentially to a larger relative extent. Increases in P_{CO_2} in cells will aid the transfer of CO_2 into plasma; such increases have relatively little effect on intracellular [H^+], but when transfer to plasma has occurred, a large increase in venous plasma [H^+] is to be expected. Finally, the 10-fold higher [A_T] in the cells compared with plasma means that this system is of greater importance in the cells than in plasma and that changes in [A^-] will be correspondingly greater. We need to know how the changes in all the independent variables interact to influence [H^+], if we are to understand how muscle homeostasis is achieved. Also, because the rate at which changes take place may vary among the systems, each may play a different role at different times during

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and after exercise. The relative importance of each system in the control of intracellular and extracellular [H^+] is unknown, and there is relatively little information on the rate at which changes take place in the independent variables. However, it is now possible to measure or estimate with reasonable precision the important variables both in plasma and in muscle (19).

For these reasons we conducted studies during and after 30 s of intense cycle ergometer exercise in which changes in independent variables were followed in muscle and in arterial and femoral venous plasma. Thus measurements or indirect estimates were made of the concentration of strong acid anions ([Cl^-] and [La^-]) and strong basic cations ([Na^+], [K^+], [Mg^{2+}], and [Ca^{2+}]), the P_{CO_2}, and [A_T]. From a knowledge of the dissociation constants of the equilibrium reactions involved, changes in these independent variables were used to quantify their relative importance in mediating changes in [H^+] in both plasma and muscle intracellular fluid (ICF).

METHODS

Subjects. Nine healthy male subjects (25 ± 6 yr of age, 181 ± 5 cm, 81 ± 5 kg body wt) participated in the study. After the experimental protocol and possible risks were outlined, informed consent was obtained from each subject. The study was approved by the institution's Ethics Committee.

General protocol. Two groups of studies were carried out, one ($n = 6$) in which muscle biopsies were taken and one ($n = 3$) in which blood was sampled from arterial and femoral venous catheters. The exercise protocol was the same in the two groups, and the studies were performed in the morning after a light breakfast. In studies in which needle muscle biopsies were taken, small incisions were made in the skin overlying the middle third of the vastus lateralis under local anesthesia and a resting biopsy was taken. In the studies in which blood was sampled, the subject rested on a laboratory bed and percutaneous Teflon catheters were placed under local anesthesia in the brachial or radial artery and the femoral vein. The femoral venous catheters were 10 cm long and inserted retrograde to flow.

After 30 min of rest, blood samples were drawn simultaneously from the artery and femoral vein. The subject was then seated on a constant-velocity cycle ergometer (23) at an optimal saddle height, and his feet were secured to the pedals. The motor was started, and the subject was instructed to catch up to the predetermined pedal speed of 100 rpm before exerting maximal force. Immediately on attaining the correct speed the subject began pedaling with maximal force for 30 s. Arterial and venous blood was drawn simultaneously, immediately after exercise, and at 0.5, 1.0, 1.5, 2.5, 3.5, 5.5, 7.5, and 9.5 min postexercise, while the subject remained seated on the ergometer. Muscle biopsies were taken immediately after and at 3.5 and 9.5 min after the completion of exercise.

Materials and methods. The design of the constant velocity cycle ergometer has been described in detail elsewhere (23). Briefly, a 3-horsepower direct-current (DC) electric motor, connected in series to a DC regenerative controller, maintained pedal speed constant despite maximal efforts by the subject. The torque generated on the pedal cranks was transmitted from strain gauges bonded to the pedal cranks to a laboratory computer (Digital Equipment PDP 11-03) via a brass slip-ring, Wheatstone bridge system. Calculations of peak power, average power, and total work for each leg during each pedal stroke were made by the computer. Data were smoothed by continuous averaging of three successive pedal revolutions.

Blood was drawn into heparinized glass syringes and immediately divided into two portions and stored on ice. One portion was used for measurements of plasma pH, P_{CO_2}, and P_{O_2} by electrodes (Corning 178 pH/blood gas analyzer). The mean differences between duplicate analyses were 0.004 ± 0.005 (SD) U for pH, 0.5 ± 0.04 Torr for P_{CO_2}, and 0.9 ± 1.3 Torr for P_{O_2}. Hemoglobin concentration and O_2 saturation were measured photometrically (Radiometer OSM2 hemoximeter). Plasma [HCO_3^-] was calculated (30). Plasma [Na^+], [K^+], and [Ca^{2+}] were measured by ion-selective electrodes (Radiometer KNA1, sodium-potassium analyzer; Radiometer ICA1 ionized calcium analyzer). Plasma [Cl^-] was measured by colorimetric titration (Buchler-Cotlove chloridometer, model 4-2008). All analyzers were calibrated immediately before and throughout the period of analysis. The second portion of the blood sample was added to tubes containing EDTA, stored on ice, and centrifuged within 30 min. The separated plasma was frozen for subsequent analysis of [La^-] by means of a fluorometric enzyme technique (21). The mean differences between duplicate sample measurements were (in meq/l) 0.6 ± 0.4 for Na^+, 0.02 ± 0.04 for K^+, 0.005 ± 0.005 for Ca^{2+}, 1.2 ± 1.0 for Cl^-, and 0.2 ± 0.2 for La^-.

Muscle biopsies were rapidly frozen in liquid N_2, wrapped in aluminum foil, and stored in liquid N_2 until analyzed for total tissue water (TTW) and strong ions. The frozen muscle was dissected free of connective tissue and blood and weighed. The sample was freeze-dried and TTW was determined. The sample was analyzed for total content (μeq/g dry wt) of Na^+, K^+, Mg^{2+}, Ca^{2+}, and Cl^- by means of instrumental neutron activation analysis (18). Tissue recoveries for this method were 111 ± 6% for Na^+, 104 ± 7% for K^+, 104 ± 12% for Cl^-, 108 ± 7% for Mg^{2+}, and 105 ± 4% for Ca^{2+}. The sample was also analyzed for [La^-] by means of enzymatic fluorometric techniques (21).

Calculations. The following equation (35) was applied to both the plasma and biopsy data to obtain values for the dependent variables [H^+], [HCO_3^-], and [A^-] from P_{CO_2}, [SID], and [A_T]

$$[H^+]^4 + (K_A + [SID])[H^+]^3 + \{K_A([SID] - [A_T]) - (K_c \times P_{CO_2} + K'_w)\}[H^+]^2 - \{K_A(K_c \times P_{CO_2} + K'_w) + (K_3 \times K_c \times P_{CO_2})\}[H^+] - (K_A \times K_3 \times K_c \times P_{CO_2}) = 0 \quad (1)$$

where K_A, K_c, K_3, and K'_w are the equilibrium constants for the dissociation of weak acids, carbonic acid, bicarbonate, and water, respectively (Table 1). Plasma [A_T]

TABLE 1. *Constants used in calculations of [H+]*

Equation	Site	Constant	Reference No.
$[H^+] \times [A^-] = K_A[HA]$	Plasma	$K_A = 3.0 \times 10^{-7}$	35, 38
	Muscle, rest	$K_A = 5.5 \times 10^{-7}$	18, 20
	Muscle, end of exercise	$K_A = 4.0 \times 10^{-7}$	18, 20
$[H^+] \times [HCO_3^-] = K_c Pco_2$	Plasma	$K_c = 2.46 \times 10^{-11}$	35
	Muscle	$K_c = 2.34 \times 10^{-11}$	31
$[H^+] \times [CO_3^=] = K_3[HCO_3^-]$	Plasma, muscle	$K_3 = 6.0 \times 10^{-11}$	5
$[H^+] \times [OH^-] = K'_w$	Plasma, muscle	$K'_w = 4.4 \times 10^{-14}$	6

K_A, K_c, K_3, and K'_w, equilibrium dissociation constants for weak acid, carbonic acid, bicarbonate, and water, respectively.

was assumed to be 17 meq/l (35), appropriate to a plasma protein concentration of 7 g/dl (26), and changes due to changes in plasma water content were calculated from changes in hemoglobin concentration and hematocrit (8). Plasma [SID] was calculated from $([Na^+] + [K^+] + [Ca^{2+}]) - ([Cl^-] + [La^-])$.

For muscle, intracellular ion contents were first corrected for the changes in the extracellular fluid volume (ECFV), established in previous studies of heavy exercise (34), which showed ECFV to increase relative to TTW from 12.5% (ECFV/TTW) at rest to 15% at 0.5 min postexercise, 14.5% at 3.5 min, and 13.5% at 9.5 min. ICF volume (ICFV) was calculated as the difference between TTW and ECFV (ml/g dry wt); ICFV (ml/g wet wt) was calculated by dividing ICFV (ml/g dry wt) by ratio of wet to dry weight. Intracellular ion concentrations were calculated by first converting the concentrations in dry tissue to wet tissue, by dividing by the wet-to-dry weight ratio, and expressing values in meq/l of ICFV. The corrected concentrations ($[K^+]$, $[Na^+]$, $[Mg^{2+}]$, $[Cl^-]$, and $[La^-]$) were expressed in meq/l ICF and used to derive intracellular [SID]. Intracellular $[A_T]$ was assumed to be 180 meq/l ICF at rest (20) and to change appropriately with changes in ICFV, as described above. Intracellular muscle Pco_2 was assumed equal to femoral venous Pco_2.

Statistical analysis. All values are reported as means ± SD. Recovery values were compared with preexercise values by a paired *t* test. Differences over time were compared by one-way analysis of variance. A paired *t* test was also used to compare means when a significant *F* ratio was obtained. Statistical significance was accepted at $P < 0.05$.

RESULTS

Maximum peak power, average power, and total work produced during 30 s of exercise in the blood-sampling studies were 1,330 ± 209 W, 845 ± 100 W, and 18.6 ± 1.2 kJ, respectively. In the subjects in whom muscle biopsies were obtained, comparable values were 1,799 ± 265, 1,143 ± 142, and 25.9 ± 1.9; only total work in 30 s was significantly different ($P < 0.05$) between the two groups of studies.

Muscle fluid and ion concentrations. Muscle TTW was 3.20 ± 0.09 ml/g dry wt at rest and increased by 6.5% during 9.5 min of recovery to 3.41 ± 0.08 ml/g dry wt (Table 2). Calculated ECFV was significantly greater than at rest at all points in recovery from exercise and accounted for 100, 43, and 29% of the changes in TTW at 0.5, 3.5, and 9.5 min, respectively (Table 2). There were also increases in ICFV during recovery, but these were not significant (Table 2).

Intracellular [SID] decreased from 154 to 106 meq/l ICF immediately after exercise and remained low for 9.5 min of recovery (Tables 2 and 3). The decrease initially was accounted for by an increase in $[La^-]$, which reached a peak value of 47 meq/l ICF at 0.5 min and gradually fell to one-half this value by 9.5 min postexercise. Although the increase in intracellular $[La^-]$ accounted for 87 ± 19% of the decrease in [SID] at 0.5 min postexercise, this proportion fell to 45 ± 13 and 52 ± 24% at 3.5 and 9.5 min of recovery, respectively (Table 3); at these times the reduced [SID] was due partly to changes in inorganic strong ions and partly to increases in $[La^-]$. Although changes in the intracellular concentrations of K^+, Na^+, Mg^{2+}, Ca^{2+}, and Cl^- were not statistically significant, some trends were apparent in the data. $[K^+]$ fell during exercise and remained low during recovery; $[Na^+]$ and $[Cl^-]$ increased during the initial 3.5 min and then returned to rest values (Table 2). No changes were apparent in $[Mg^{2+}]$ and $[Ca^{2+}]$.

Muscle $[H^+]$, calculated from *Eq. 1*, increased by 2.5-fold, from 132 to 328 neq/l at 0.5 min after the end of exercise, with a further increase to 417 neq/l at 3.5 min; at 9.5 min $[H^+]$ had returned to 345 neq/l, close to the value at 0.5 min (Table 3). Intracellular $[HCO_3^-]$ showed a delayed fall at 3.5 min of recovery to one-third of the resting value and remained low at 9.5 min (Table 3).

Changes in arterial and femoral venous plasma. Compared with the resting state, plasma volume (arterial and venous) decreased after exercise as reflected in increases in hemoglobin concentration (Table 4). In femoral venous blood the fall was maximal immediately after exercise and amounted to 13.5%; smaller increases were maintained for the remainder of the recovery period. In arterial blood an initial decrease of 9.8% did not change significantly in the succeeding 9 min.

The femoral venous plasma $[Na^+]$, $[K^+]$, and $[Ca^{2+}]$ were all increased in blood sampled immediately after exercise; although the absolute increase was greatest for $[Na^+]$, the largest relative increase was in $[K^+]$ (a 44% increase over rest). The increases were accounted for by the decreases in plasma volume for all ions except K^+, for which only 30% of the increase could be accounted

TABLE 2. *Muscle TTW, ECFV, ICFV, and intracellular strong ion concentrations at rest and after 30 s of maximal exercise*

	Rest	Recovery, min		
		0.5	3.5	9.5
TTW, ml/g dry wt	3.20±0.02	3.30±0.08	3.43±0.11*	3.41±0.08*
ECFV, ml/g dry wt	0.40±0.02	0.50±0.01*	0.50±0.02*	0.46±0.01*
ICFV, ml/g dry wt	2.78±0.14	2.80±0.07	2.93±0.10	2.96±0.07
[La$^-$], meq/l ICF	5.5±0.4	47.0±7.3*	29.9±5.4*	26.4±1.8*
[K$^+$], meq/l ICF	142±12	138±10	123±10	128±8
[Na$^+$], meq/l ICF	9.3±2.6	11.4±2.2	11.5±3.0	9.7±3.4
[Cl$^-$], meq/l ICF	8.8±1.3	9.1±1.6	13.0±5.6	9.6±1.6
[Mg^{2+}], meq/l ICF	21±2	24±1	19±1	22±1
[Ca^{2+}], meq/l ICF	2.5±0.3	2.6±0.3	2.3±0.3	2.7±0.3

Values are means ± SD. TTW, total tissue water; ECFV, extracellular fluid volume; ICFV, intracellular fluid volume; ICF, intracellular fluid. * Significantly different from rest value ($P < 0.05$).

TABLE 3. *Measured and calculated variables in plasma and muscle*

	Arterial Plasma	Muscle	Venous Plasma
Rest			
[K$^+$], meq/l	5	142	5
[La$^-$], meq/l	1	6	1
[SID], meq/l	37	154	42
P_{CO_2}, Torr	41	46†	46
[A$^-$],* meq/l	14	145	15
[HCO_3^-], meq/l	26	9*	27
[H$^+$], neq/l	38		41
[H$^+$],* neq/l	45	132	42
0.5 min postexercise			
[K$^+$], meq/l	7	138	8
[La$^-$], meq/l	10	47	13
[SID], meq/l	34	106	39
P_{CO_2}, Torr	37	106†	106
[A$^-$],* meq/l	16	98	13
[HCO_3^-], meq/l	19	7*	28
[H$^+$], neq/l	49		95
[H$^+$],* neq/l	51	328	99
3.5 min postexercise			
[K$^+$], meq/l	5	123	5
[La$^-$], meq/l	14	30	18
[SID], meq/l	29	101	29
P_{CO_2}, Torr	30	48†	48
[A$^-$],* meq/l	15	98	15
[HCO_3^-], meq/l	14	3*	15
[H$^+$], neq/l	54		76
[H$^+$],* neq/l	54	417	76
9.5 min postexercise			
[K$^+$], meq/l	5	128	5
[La$^-$], meq/l	12	26	15
[SID], meq/l	31	114	30
P_{CO_2}, Torr	32	40†	40
[A$^-$],* meq/l	16	110	15
[HCO_3^-], meq/l	14	3*	15
[H$^+$], neq/l	54		64
[H$^+$],* neq/l	55	345	62

La$^-$, lactate; [SID], strong ion difference; A$^-$, dissociated weak ion. * Calculated by *Eq. 1.* † Assumed equal to femoral venous value.

for by the change in plasma water volume. The ion concentrations gradually returned to preexercise levels over the following 5 min (Table 4, Fig. 1); similar changes occurred in the arterial and venous plasma. There were small increases in plasma [Cl$^-$], but these were transient (Table 4, Fig. 1). There were positive venoarterial differences for [K$^+$] and [Na$^+$] and a negative difference for [Cl$^-$] (Fig. 2). Changes in [Na$^+$] and [Cl$^-$] between venous and arterial blood were explained by changes in plasma volume (Table 4); although the venoarterial [K$^+$] differences were smaller in absolute terms, they were not due to volume changes alone and presumably indicated influx of K$^+$ into plasma from muscle.

Plasma [La$^-$] increased during exercise to reach 9.7 and 6.3 meq/l in venous and arterial samples, respectively, at the end of exercise; the concentrations continued to increase in the first 4 min of recovery, to reach peaks of 17.6 ± 2.2 and 13.9 ± 0.7 meq/l in venous and arterial blood, respectively (Table 4, Fig. 1); the venoarterial [La$^-$] difference was ~3.5 meq/l early after exercise but remained at 2–3 meq/l during the later part of recovery (Fig. 2).

The consequences of the individual plasma strong ion concentration changes were that femoral venous [SID] remained similar to resting values (42 ± 8 meq/l) during the 1st min postexercise (Fig. 1) but then decreased to 33 ± 2 meq/l at 1.5 min of recovery and remained relatively steady for the rest of recovery. In contrast, the arterial [SID] decreased below resting values (37 ± 1 meq/l) during exercise and reached relatively steady levels after 1 min of recovery (Fig. 1). The arterial and femoral venous [SID] values were similar after 2 min of recovery.

Femoral venous P_{CO_2} was 100 Torr immediately after exercise and continued to increase to 106 ± 18 Torr at 30 s postexercise (Table 3, Fig. 3), thereafter decreasing during the remainder of recovery to below resting values at 9.5 min postexercise. Arterial P_{CO_2} fell to 30 Torr within 2 min after exercise and remained at this level throughout the 10 min of recovery. The venoarterial P_{CO_2} difference increased from 5 ± 6 Torr at rest to 69 ± 16 Torr at 30 s postexercise, fell rapidly in the next 3 min, and fell more slowly to rach resting values after 9.5 min of recovery. Femoral venous P_{O_2} fell from 29.4 ± 10.3 Torr at rest to 18.7 ± 6.0 Torr immediately after exercise, thereafter returning rapidly to above resting values.

Femoral venous [H$^+$] increased from 42 ± 3 neq/l at rest to 95 ± 12 neq/l (pH 7.02) by 30 s postexercise (Table 3, Fig. 3); arterial [H$^+$] also increased but to a lesser degree (from 38 ± 2 to 47 ± 5 neq/l). The femoral

TABLE 4. *Plasma strong ion concentrations in femoral vein and artery at rest and during recovery from 30 s of maximal exercise*

	Rest	Time Postexercise, min								
		0	0.5	1.0	1.5	2.5	3.5	5.5	7.5	9.5
Femoral vein										
Na^+, meq/l	138±2	150±3*	149±2*	147±2*	145±1*	143±2	141±1	139±1	138±1	138±1
K^+, meq/l	5.4±1.1	7.8±1.2*	6.9±1.5*	6.1±1.6	5.7±2.4	5.3±1.4	5.2±1.3	5.2±1.2	5.3±1.1	5.2±1.0
Ca^{2+}, meq/l	1.1±0.0	1.4±0.0*	1.3±0.0*	1.3±0.0*	1.2±0.0*	1.2±0.0*	1.2±0.0	1.2±0.0	1.2±0.0	1.1±0.0
La^-, meq/l	1.0±0.5	9.7±0.5*	13.1±1.0*	14.8±1.4*	15.7±1.5*	16.9±1.3*	17.6±2.2*	15.5±1.3*	15.2±0.2*	14.2±0.8*
Cl^-, meq/l	102±6	108±3	105±4	102±5	104±7	101±5	101±6	100±5	99±6	101±3
Hb, g/100 ml	13.5±3.0	15.6±2.8*	14.8±2.3*	14.9±2.1*	14.6±2.8*	14.8±2.5*	14.7±2.4*	14.0±1.9*	14.5±2.5*	13.8±1.5
Artery										
Na^+, meq/l	137±1	144±2*	146±1*†	144±1*†	143±1*†	141±1*	141±1*	139±1*	138±1	138±1
K^+, meq/l	4.5±0.4	6.9±1.0*	6.3±0.9*	5.6±0.9*	5.2±0.8*	4.8±0.8	4.6±0.8	4.5±0.7	4.6±0.6	4.6±0.6
Ca^{2+}, meq/l	1.1±0.0	1.2±0.1*	1.2±0.1*	1.2±0.1*	1.2±0.1*	1.2±0.0*	1.2±0.0*	1.2±0.0	1.1±0.1*	1.1±0.0*
La^-, meq/l	0.9±0.5	6.3±3.0	9.6±1.4*	12.2±1.2*	13.5±1.0*	13.9±0.7*†	13.8±1.5*	13.8±1.2*	12.3±0.5*	12.3±0.7*
Cl^-, meq/l	105±2	112±2†	109±1†	109±1†	108±5	104±3	102±3	102±1	101±2	102±2
Hb, g/100 ml	12.9±1.6	14.3±1.4*†	14.3±1.8*†	14.1±1.8*†	14.1±1.7*†	14.1±1.8*†	14.0±1.8*†	14.3±1.5*	13.8±1.5*†	14.0±1.7

Values are means ± SE. La^-, lactate; Hb, hemoglobin. * Significantly different from rest ($P < 0.05$). † Significantly different from femoral vein ($P < 0.05$).

venous and arterial $[H^+]$ did not return to preexercise levels during the 10 min of recovery (Table 3, Fig. 3). Femoral venous $[HCO_3^-]$ remained near preexercise levels until 30 s of recovery and then fell to 15 meq/l by 3.5 min of recovery (Table 3, Fig. 3). Arterial plasma $[HCO_3^-]$ decreased during 30 s of exercise and continued to fall in the first 2.5 min of recovery; the femoral venoarterial $[HCO_3^-]$ difference increased from 1 ± 3 meq/l at rest to 9 ± 2 meq/l immediately after exercise and decreased to ~2 meq/l after 3.5 min of recovery (Fig. 2).

DISCUSSION

Previous studies of intense exercise established the importance of increases in $[La^-]$ as a contributor to the severe intracellular acidosis, which in turn may lead to inhibition of glycolysis and muscle fatigue. Heavy exercise of this type generates a severe acidosis in the working muscles, as indicated by marked elevations in intramuscular $[La^-]$ to as high as 40 meq/kg wet wt and in hexose phosphates to as high as 10 meq/kg wet wt (16) and decreases in muscle homogenate pH to ≤6.5 (9, 11, 29). By carrying out two groups of studies, one in which muscle biopsies were analyzed and one in which blood was sampled from an artery and the femoral vein draining the previously active muscle, we hoped to quantify the relative contributions and the time course of various ionic exchanges between muscle and plasma that occur in response to the intracellular acidosis. The physicochemical approach of Stewart (35, 36) was used to quantify the major factors influencing $[H^+]$ both intracellularly and in plasma.

Although there is controversy regarding the application of the physicochemical systems approach to acid-base regulation, we adopted it for several reasons. First, it offers an opportunity to clarify interrelationships between changes in muscle and plasma: other approaches are difficult to apply because of the large differences between the ionic composition in the two sites. Second, the approach employs a series of equations that are founded on classical physicochemical principles and may be validated independently by measurement of $[H^+]$ by other methods. Finally, the concept of dependent and independent variables inherent in Stewart's construct allows hypotheses that may explain regulatory mechanisms to be tested. For these reasons we would not agree with the views expressed by some critics that differences between approaches are merely semantic in origin.

The validation of the quantitative approach depends on the validity of the equations employed. Their basis in classical physicochemical laws or relationships is unquestioned—water dissociation, electrical neutrality, conservation of mass, and the Henderson-Hasselbalch equation (35). Thus the crucial variables and constants are the strong inorganic ions, P_{CO_2}, $[A_T]$, and K_A, within the constraints of electrical neutrality in aqueous solution. We may examine each of these variables to establish the effects of experimental error on the calculated $[H^+]$ by use of the measurements immediately after exercise. Also in the case of plasma we may compare calculated $[H^+]$ with measurements of $[H^+]$ to assess overall validity.

The standard deviation of individual strong ion measurements indicates a standard deviation for measurements of [SID] in plasma of 1.4 meq/l and of intramuscular [SID] of 11 meq/l. These errors will lead to potential errors of 2 (4%) and 5 neq/l (18%) for $[H^+]$ in plasma and resting muscle, respectively. Measurement of P_{CO_2} in plasma is accurate to ±1 Torr equivalent to ±1 neq/l in $[H^+]$. P_{CO_2} cannot be measured directly in muscle and was assumed to be equal to femoral venous P_{CO_2}: as such it may be an underestimate of muscle P_{CO_2}, but the effect of P_{CO_2} on intramuscular $[H^+]$ is much smaller than on plasma $[H^+]$. Whereas an increase of 10 Torr in plasma will increase $[H^+]$ by 10 neq/l (20%), a similar increase in muscle will be associated with an increase of only 5 neq/l (1.5%) in $[H^+]$.

$[A_T]$ in plasma is obtained from the total plasma protein concentration, as validated recently by Rossing

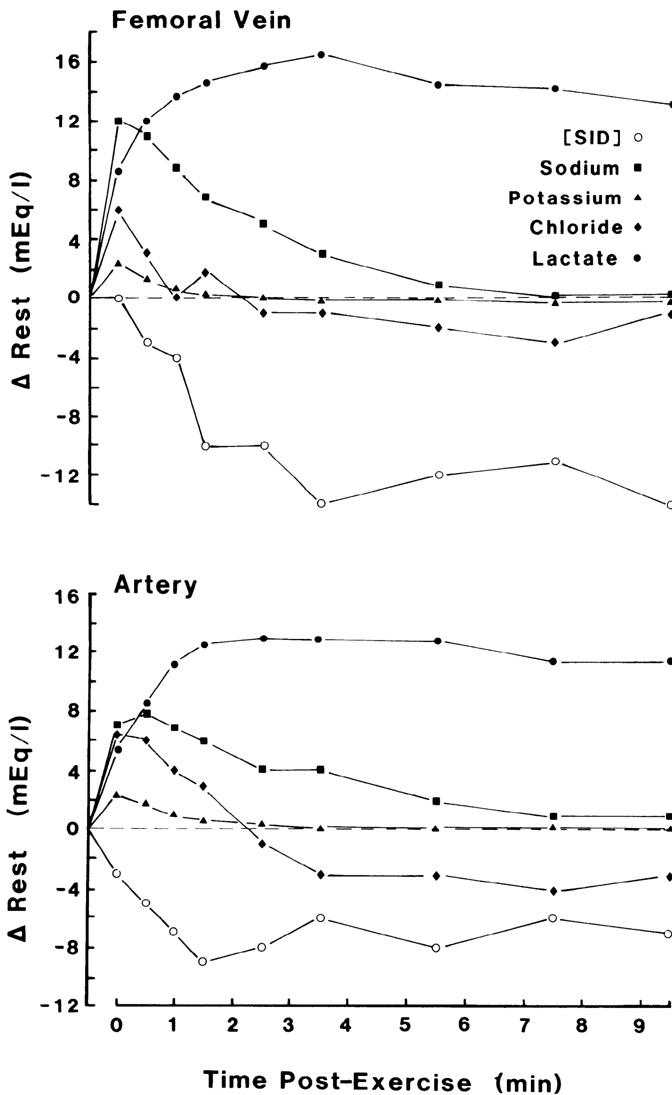

FIG. 1. Changes occurring in plasma concentrations of strong ions contributing to strong ion difference ([SID]) after exercise in femoral venous (*top*) and arterial (*bottom*) plasma. Values are derived from data in Table 4.

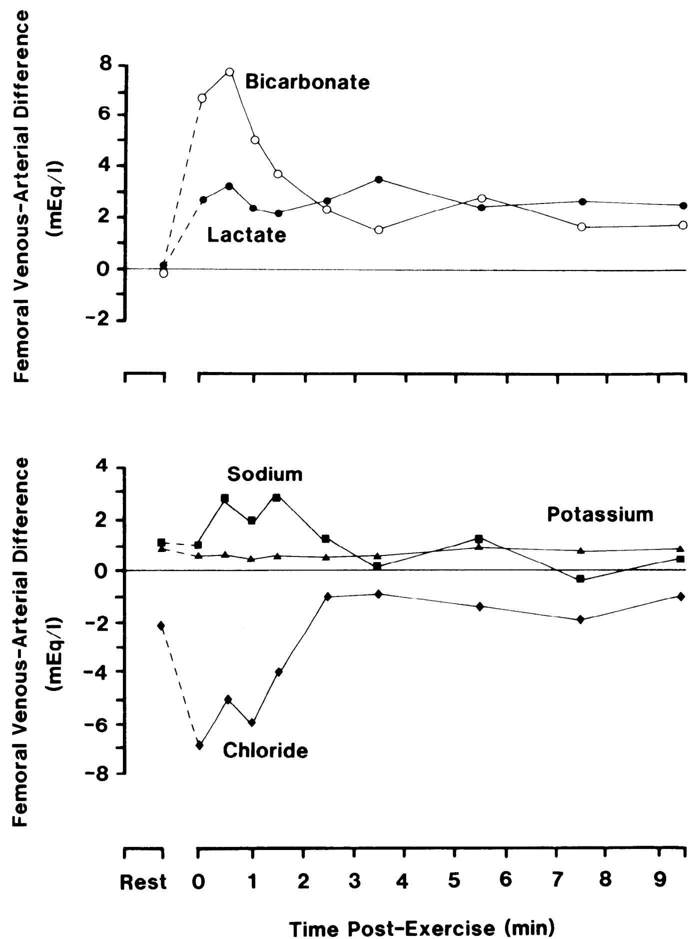

FIG. 2. Venoarterial differences in concentrations of La^- and HCO_3^- (*top*) and Na^+, K^+, and Cl^- (*bottom*). Values are derived from data in Table 4.

et al. (26). In muscle a value of 180 meq/kg was assumed, after measurements in rat muscle (18) in which acid titration was used to derive $[A_T]$ and K_A. A nominal 10% error in $[A_T]$ carries a potential error of 2 neq/l in $[H^+]$ in plasma and 10 neq/l in muscle $[H^+]$. K_A may be safely assumed to be 3.0×10^{-7} in plasma (26), but in muscle it will vary according to the state of dissociation of organic phosphate compounds. Although the K_A values for most high-energy phosphates, inorganic phosphates, and glycolytic pathway organic phosphates are reasonably uniform ($1.5–1.8 \times 10^{-7}$ eq/l), the K_A of creatine phosphate (CP) is widely different (3.16×10^{-5} eq/l). Thus the overall K_A is greatly influenced by the state of CP breakdown. The effects of variation in K_A on $[H^+]$ may be gauged from the values presented in Table 5.

The validation of the equations in plasma is straightforward, because calculated $[H^+]$ may be compared with measure $[H^+]$; in the present study they were closely comparable (Table 3), apart from arterial plasma at rest, where there was a marked variability between the subjects. For muscle, an uncertainty that we cannot resolve with presently available information is the relationship between ionic concentrations and ionic activities; concentration was measured, but activities determine $[H^+]$. We have attempted to resolve this question in validation studies that employed the isolated rat hindlimb, by comparing $[H^+]$ measured by two independent techniques (direct measurement of pH in homogenates and distribution of 5,5-dimethyl-2,4-oxazolidine-dione-2-^{14}C) with the value obtained by the methods of the present paper. Because acceptable comparisons were obtained between the methods, we do not believe that the distinction between concentration and activity detracts significantly from the findings. Further validation is clearly desirable, but at the present time the values for the constants appear to yield values for $[H^+]$ in plasma and muscle that are internally consistent and in agreement with previous studies (12, 28, 29).

A number of factors related to the studies carried out in human subjects make it difficult to obtain a precise description of the processes we wished to study. First, although the methods have a defined precision in the steady state, one must question the assumptions that were made in the situation of intense exercise. We assumed equilibration between muscle and femoral venous P_{CO_2} and that equilibrium conditions existed in ionic systems. Although both assumptions are questionable in the non-steady-state conditions immediately after exer-

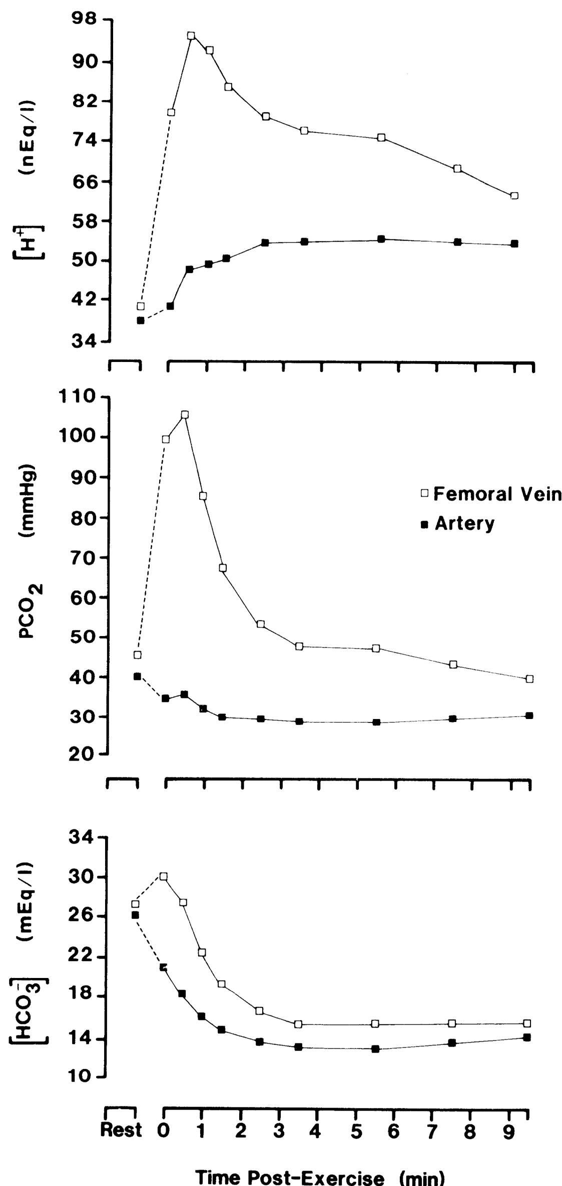

FIG. 3. Femoral venous and arterial plasma $[H^+]$, P_{CO_2}, and $[HCO_3^-]$ at rest and after exercise.

cise, it seems likely that, at rest and 3.5 and 9.5 min into recovery, equilibrium probably existed. A similar concern surrounds the choice of K_A in muscle, which exerts a considerable influence on intramuscular $[H^+]$ (Table 5). However, the dependence of K_A on [CP] allows us to be

TABLE 5. *Calculations of effects of changing K_A, P_{CO_2}, and SID on muscle $[H^+]$ and $[HCO_3^-]$*

	K_A, $\times 10^{-7}$ eq/l	P_{CO_2}, Torr	[SID], meq/l	$[A^-]$, meq/l	$[H^+]$, neq/l	$[HCO_3^-]$, meq/l
Rest	5.5	50	154	145	132	8.9
0 min	4.0	50	106	102	305	3.8
	4.0	100	106	98	328	7.1
	5.5	100	106	101	434	5.4
3.5 min	5.0	50	101	98	417	2.8
9.5 min	5.5	50	114	110	345	3.4

K_A, equilibrium constant for weak acid dissociation; [SID], strong ion difference; $[A^-]$, dissociated weak anion concentration.

fairly confident of the assumptions late in recovery. Second, the ion concentrations in muscle were calculated by assuming a distribution of water between the intracellular and extracellular spaces equal to that found by Sjøgaard and Saltin (34) in steady-state exercise of a lower intensity than that employed in the present study. However, it seems likely that errors inherent in these assumptions would lead to underestimates rather than overestimates of these changes. Third, the representativeness of the sampled femoral venous blood of effluent blood from the biopsied vastus lateralis may be questioned. We calculated that the tip of the venous catheter was ~8 cm from the inguinal ligament and thus close to the highest drainage tributary. Finally, the blood and muscle measurements were not carried out in the same subjects. We judged that an attempt to do so might impair the quality of individual studies and possibly the power output generated by the subjects, in addition to being unacceptably invasive. Since only small variations were found in both muscle and blood measurements, we believe that such comparisons are warranted; furthermore the results obtained in the muscle biopsies were vary similar to previous studies carried out in this laboratory (16). We might also be criticized for studying only three subjects with arterial and femoral venous catheters; again, however, the consistency in results and their close agreement with previous studies (10, 16, 24) were taken as arguments against femoral venous catheterizations in more subjects.

The main change in muscle at the end of maximal exercise was a dramatic increase in $[La^-]$ to 47 meq/kg wet wt, similar to or larger than found in previous studies (13, 14, 16, 29). The initial increase in $[La^-]$ accounted for an equivalent reduction in [SID]; if no other change had occurred we may calculate that there would be an associated increase in $[H^+]$ from the resting level of 132 to 225 neq/kg (pH 6.65) (Table 3), in agreement with measurements of homogenate pH obtained by Sahlin et al. (27–29). In addition, changes in other intracellular strong ions influence changes in $[H^+]$; the delayed decrease in intracellular $[K^+]$ contributed to the high $[H^+]$ later in recovery (Tables 2 and 3).

Our findings were in agreement with previous studies, which have demonstrated increases in intracellular $[Na^+]$ and decreases in intracellular $[K^+]$ (27, 28). It is well known that K^+ moves from muscle into the interstitial fluid (9, 20, 27, 33), leading to a reduction in intracellular [SID]; although in the present study the changes were not statistically significant, this change may be partially offset by an increase in muscle $[Na^+]$ and magnified by an increase in muscle $[Cl^-]$ (Table 2). The decrease in [SID] in the present study has the theoretical effect of increasing $[H^+]$ to 305 neq/kg (pH 6.52) and accounts for 93% of the estimated increase in muscle $[H^+]$ from 132 to 305 neq/l (Table 5). The decrease in [SID] is almost equally shared by the increase in $[La^-]$ and the decrease in $[K^+]$.

The changes in $[K^+]$, $[Na^+]$, and $[Cl^-]$ within intracellular and extracellular fluids may also contribute to changes in the membrane potential. Jennische (15) observed that during prolonged ischemia there was marked

depolarization of muscle membrane to an extent that was correlated ($r > 0.8$) with the increase in muscle [La−]. Also, Sjøgaard and co-workers (32, 33) demonstrated that the release of K^+ into the femoral venous blood was associated with a fall in muscle [K^+] and argued that there was incomplete reuptake of K^+ by the Na^+-K^+ adenosinetriphosphatase pump.

At the end of exercise, femoral venous P_{CO_2} increased to 106 Torr, reflecting a P_{CO_2} in muscle that must have been at least as high (Table 4). An increase in [H^+], secondary to a fall in [SID] and an increase in [A_T], even at a constant total CO_2 content, will increase P_{CO_2} transiently, and the marked increase in muscle metabolic CO_2 production also contributes to this dramatic increase in P_{CO_2}. We may calculate that such an increase in intracellular P_{CO_2} will be associated with an increase in [H^+] from the values noted above by a further 23 neq/kg to 328 neq/kg (pH 6.48) (Table 5). This calculation highlights the fact that increases in P_{CO_2} have a relatively small influence on intramuscular [H^+]. It should be noted that the intracellular P_{CO_2} may be an underestimate, at least to the extent that blood flow from less active tissue contributes to the total femoral venous flow after exercise.

The changes in femoral venous blood indicated the importance of the exchanges of CO_2 and ions between the muscle and perfusing blood in the control of intracellular [H^+]. A very large rise in P_{CO_2} occurred at the end of exercise, with peak efflux of CO_2 from muscle into venous blood taking place in the first 30 s postexercise. In contrast, [La−] did not peak in venous plasma until 3 min later. Accompanying the increase in venous [La−] were changes in the other strong ions, already discussed above, which resulted in [SID] showing no change at the end of exercise, in spite of the increase in [La−] to ~10 mmol/l. The result of these changes at the end of exercise was that venous [H^+] increased to 80 neq/l, almost entirely because of the increase in P_{CO_2}; as pointed out above, the increase in P_{CO_2} exerts a much larger relative effect on increasing plasma [H^+] than muscle [H^+]. Later in recovery the reversion of plasma strong inorganic ions toward their original resting values, with an increase in [La−] to >17 mmol/l, accounted for a reduction in [SID] of 13 meq/l. As P_{CO_2} also reverted to resting values after 5 min, the main change at this time was the reduction in [SID], accounting for a persistent elevation of [H^+] (75 neq/l at 5.5 min of recovery). The changes in femoral venous blood may be contrasted with the concurrent arterial changes. [L−] increased to 13.9 meq/l by 2.5 min postexercise, and changes in other strong ions, although directionally similar to venous blood, were not as great (Table 4, Fig. 1). The [SID] fell during the same time by 8 meq/l, and the arterial P_{CO_2} fell by 10 Torr (Fig. 3). Thus all these changes occurred in parallel, and the resultant peak increases in [H^+], to 54 neq/l, and fall in [HCO_3^-], to 14 meq/l, also occurred after 2.5 min.

HCO_3^- is often considered to play a role as a buffer during exercise and conceptually to act as an independent variable; however, [HCO_3^-] is a dependent variable that is influenced by changes in [SID], [A_T], and P_{CO_2}. Thus in considering changes in plasma [HCO_3^-] with exercise, the effect of [La−] cannot be considered in isolation, as reacting with HCO_3^- stoichiometrically, leading to an equimolar change in the two reactants. The findings in femoral venous plasma illustrate the fallacy of this concept; although [La−] rose by 10 meq/l immediately after exercise, [HCO_3^-] showed an increase of 3 meq/l rather than the expected 10-meq/l decrease (Fig. 3). We interpret the preserved [HCO_3^-] as due to the lack of change in [SID] and the large increase in P_{CO_2}; i.e., the 3-meq/l increase in [HCO_3^-] is to be expected from the increase in P_{CO_2} alone. Later in recovery both [SID] and P_{CO_2} fell in venous plasma, and the associated fall in [HCO_3^-] approached the magnitude of [La−] increase. Changes in intramuscular [HCO_3^-] are more complex; because of the larger [SID] and [A_T], [HCO_3^-] is only 8–10 meq/l at rest; at the end of exercise little change occurs in spite of the large reduction in [SID] (Table 3), because of the large rise in P_{CO_2} and the large change in [A^-]; a change in K_A is also important (Table 5). Later in recovery, a fall in [HCO_3^-] to 3 meq/l accompanied the fall in P_{CO_2}.

The effect of weak acids and bases differs greatly between cells and plasma. Within muscle, [A_T] represents the anionic equivalents of proteins and phosphates. During high-intensity exercise, large decreases occur in [CP], with increases in inorganic phosphate and creatine concentrations (7, 16). As pointed out above, these changes do not affect [A_T] (20) but reduce the apparent K_A for the equilibrium reaction from 5.5×10^{-7} at rest to 4.0×10^{-7} in heavy exercise, associated with decreases in [CP]. Such changes were accounted for in the calculations in the present study, and their effects may be seen in Table 5. These intracellular changes have important effects on [A^-]. Using the equations of Stewart (35), we calculated that [A^-] was 145 meq/kg at rest and 98 meq/kg at the end of exercise. This buffering effect reduced the impact of a decrease in [SID]. For example, as shown in Table 5, instead of [H^+] increasing to 325 neq/kg (pH 6.48), [H^+] would have increased to 434 neq/kg (pH 6.36), if K_A had remained at its resting value of 5.5×10^{-7}. Furthermore, as CP is rapidly resynthesized within the first few minutes of recovery, K_A rapidly reverts to its resting value; this increases [A^-] (Table 3), thus exerting an acidifying effect.

Because [A_T] in plasma is only one-tenth of the concentration in muscle, the buffering capacity of the plasma proteins is much less than in muscle. Plasma proteins do not move readily across the capillary membrane, and a decrease in plasma volume increases plasma protein concentration and thus plasma [A_T]. In the present study the plasma [A_T] was assumed to change in parallel with the hemoglobin concentration ([Hb]), increasing from 17 to 19 meq/l. Although there may be some uncertainty regarding changes in [Hb] as reflecting a loss of water from the intravascular space, this interpretation is supported by the increase in muscle tissue water (Table 2) and is consistent with the findings of Sjøgaard and Saltin (34) in exercising humans, using radioactive extracellular markers to document changes in intracellular water in exercise. As shown recently by Rossing et al. (23), the net effect of an increase in [A_T] is to increase [A^-]; at

given values of [SID] and P_{CO_2}, an increase of 2 meq/l in [A_T] will be accompanied by a 2-meq/l fall in [HCO_3^-] and a 3-neq/l increase in [H^+]. It is clear that changes occurring in plasma water during exercise, even in the absence of changes in other variables, will influence [H^+] and [HCO_3^-], through an effect on [A_T].

Control of intracellular [H^+] is largely accomplished by restoration of intracellular strong ion concentrations toward preexercise levels. The mechanisms responsible for [H^+] regulation include the movement of [Na^+] and [K^+] by active transport across the muscle membrane, metabolism of La^- to CO_2 or glycogen (11) within the muscle, and efflux of La^- from muscle (12). Boron (3) and Thomas (37) have reviewed evidence supporting the concept that the intracellular [H^+] is regulated by ion exchange mechanisms, examples of which include the Na^+-dependent Cl^--HCO_3^- exchange, Na^+-independent Cl^--HCO_3^- exchange, and Na^+-H^+ exchange. Aickin and Thomas (1) demonstrated in mouse soleus muscle fibers that recovery of intracellular [H^+] occurred by means of two ionic mechanisms, Na^+-H^+ exchange and a Cl^--HCO_3^- exchange. Whether actual translocation of H^+ and HCO_3^- occurs, or whether changes in [H^+] and [HCO_3^-] merely follow the changes in [SID] that accompany translocations of strong ions, at constant P_{CO_2}, is debatable. Nevertheless, transmembrane strong ion movements are important in the control of [H^+] both within and outside the muscle.

The total "acid load" in blood has traditionally been analyzed in terms of a respiratory (or CO_2) component and a metabolic (or non-CO_2) component. In the present study, CO_2 output from muscle contributed the major load in the first 1–2 min postexercise (Fig. 3), but later the fall in plasma [SID] (Fig. 1) and, to a lesser extent, the increase in [A_T] were important. The contribution of metabolic acid to blood [H^+] may be estimated by titrating whole blood with strong ions to obtain an equivalent H^+ addition, which may then be compared with increases in [La^-] (4). Barbee and co-workers (2) used this approach to study acid production across the electrically stimulated isolated gastrocnemius-plantaris muscle in the dog; they reported that H^+ addition to blood flowing through the preparation was 4.7 and 11 times greater than La^- output during steady-state and non-steady-state contractions, respectively. Medbö and Sejersted (24) reported that after exercise the acid load in the extracellular space exceeded La^- accumulation by 30–45% and concluded that H^+ was extruded from muscle independently of La^-. However, these reports of unaccounted H^+ efflux from muscle failed to take into account the accompanying changes in weak acids that could have contributed to changes in [H^+]. When data presented by Medbö and Sejersted are used in *Eq. 1* an excellent agreement between [H^+] calculated by the equation and the measured [H^+] is obtained.

During the 30 s of intense isokinetic cycle ergometry undertaken in the present study, power declines by 25% (22). A number of intracellular biochemical and ionic changes accompany this fatigue (7, 13, 14, 16), including a marked increase in [H^+] (10, 13, 25, 28, 29). The present study contributes to our understanding of the local mechanisms influencing intracellular [H^+] and emphasizes that accumulation of La^- may not be the only, or even the most important, factor. It is clear that efflux of CO_2 from muscle is very rapid but also falls off rapidly during recovery; the movement of K^+ from the muscle cell is also rapid, leading to a sustained fall in muscle [K^+]; La^- efflux increases gradually but is relatively slow, being dependent on the difference in [La^-] between muscle and arterial plasma. Other important factors that modulate [H^+] in plasma and muscle are changes in [A_T] and K_A. Although there are a number of uncertainties regarding the approach that we took, the findings indicate the extent of the contribution of the various ionic systems to [H^+] control after heavy exercise and pave the way for future work to quantify the effects more precisely, and in exercise of less severe intensity.

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